Design, synthesis, and evaluation of inhibitors of steroid sulfatase

by

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Author’s Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by examiners.

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Abstract

Steroid sulfatase (STS) catalyzes the desulfation of biologically inactive sulfated steroids to yield biologically active desulfated steroids and is currently being examined as a target for therapeutic intervention for the treatment of breast and other steroid-dependent cancers. A series of 17β-arylsulfonamides of 17β-aminoestra-1,3,5(10)-trien-3-ol were prepared and evaluated as inhibitors of STS. Introducing n-alkyl groups into the 4´-position of the 17β-benzenesulfonamide derivative resulted in an increase in potency with the n-butyl derivative exhibiting the best potency with an IC_{50} of 26 nM. A further increase in carbon units (to n-pentyl) resulted in a decrease in potency. Branching of the 4´-n-propyl group resulted in a decrease in potency while branching of the 4´-n-butyl group (to a *tert*-butyl group) resulted in a slight increase in potency (IC_{50} = 18 nM). Studies with 17β-benzenesulfonamides substituted at the 3´- and 4´-positions with small electron donating and electron withdrawing groups revealed the 3´-bromo and 3´-trifluoromethyl derivatives to be excellent inhibitors with IC_{50}'s of 30 and 23 nM respectively. The 17β-2´-naphthalenesulfonamide was also an excellent inhibitor (IC_{50} = 20 nM) while the 17β-4´-phenylbenzenesulfonamide derivative was the most potent inhibitor with an IC_{50} of 9 nM. Kinetic studies with 3´-bromo derivative revealed it to be a non-competitive inhibitor and so these types of inhibitors might be capable of binding at the active site and also at a secondary site outside the active site. The amide analogs of some of these compounds were found not to be as potent inhibitors as the sulfonamides. Introducing a nitro group or fluorine atom into the 4-position of the 17β-arylsulfonamide inhibitors resulted in an increase in potency. Some of these compounds are the most potent reversible STS inhibitors ever reported with apparent K_i's as low as 1 nM. 3-*O*-Sulfation of these compounds did not significantly alter their potency. It is not known if 3-*O*-sulfated derivatives were acting as

inhibitors or reversible suicide inhibitors. Docking studies were performed on selected inhibitors to gain insight into how they might interact with STS.

Selected 17β-arylsulfonamide inhibitors were sent to the NCI (USA) for in vitro screening with a panel of 60 human tumor cell lines (NCI-60 panel). Almost all of the compounds exhibited GI_{50}'s in the 1 to 10 μM range with all 60 cell lines and so were only moderately potent in terms of their ability to inhibit the growth. None of the compounds stood out in terms of their ability to inhibit the growth of any breast cancer, prostate cancer or any other cancer cell line studied.

The thiadiazolidinedione group was proposed as a sulfate mimic for obtaining STS inhibitors. A new approach to the synthesis of 3-aminoestrone was achieved as part of an attempt to prepare the thiadiazolidinedione target. 3-*O*-Sulfamoylation of one of the 17β-arylsulfonamide inhibitors was attempted using a variety of reaction conditions but was unsuccessful.

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To My Parents, My Lovely Wife Heba,

My Angel Malak and Mr. Mohammed

Table of Contents

List of Figures

- **Chapter 1 Figures**

List of Tables

List of Schemes

List of Abbreviations

ARSA	Aryl sulfatase A
ARSB	Aryl sulfatase B
ARSC (STS)	Aryl sulfatase C (Steroid sulfatase)
ARSD	Aryl sulfatase D
ARSE	Aryl sulfatase E
ARSF	Aryl sulfatase F
ARSG	Aryl sulfatase G
ARSH	Aryl sulfatase H
ARSI	Aryl sulfatase I
ARSJ	Aryl sulfatase J
ARSK	Aryl sulfatase K
GALNS	Galactosamine (*N*-acetyl)-6-sulfatase
G6S	Glucosamine (*N*-acetyl)-6-sulfatase
SGSH	*N*-sulfoglucosamine sulfohydroloase
IDS	Iduronate-2-sulfatase
Sulf 1	Endo sulfatase 1
Sulf 2	Endo sulfatase 2
pNPS	*p*-nitrophenol sulfate
4-MUS	4-methylumbelliferyl sulfate
ARSs	arylsulfatases
STS	Steroid Sulfatase
PAPS	3'-phosphoadenosine-5'-phosphosulfate
SULT	sulfotransferase
E1S	estrone sulfate
E2S	estradiol sulfate
DHEAS	dehydroepiandrosterone sulfate
PREGS	pregnenolone sulfate
CS	cholesterol sulfate
E1	Estrone
DHEA	dehydroepiandrosterone
FGE	formyl generating enzyme
FGly	Cα-formylglycine (2-amino-3-oxopropionic acid)
MSD	Multiple sulfatase deficiency
TM	transmembrane helices
SD1	subdomain 1
SD2	subdomain 2
MAL	Membrane-Associated Loops
FGS75	Formylglycine sulfate 75
ER+	estrogen receptor-positive
TNFα	Tumor Necrosis Factor
IL-6	Interleukin-6
EREs	estrogen responsive elements
SERM	selective estrogen receptor modulator

AIs	Aromatase inhibitors
SERDs	selective estrogen receptor down-regulators
HDBC	Hormone-Dependent Breast Cancer
MBC	metastatic breast cancer
EMATE	estrone-3-*O*-sulfamate
4-FE1	4-formyl estrone
NOMATE	17-deoxy analog of EMATE
DASIs	dual aromatase/sulfatase inhibitors
EC	endometrial cancer
MA	megestrol acetate
PFS	progression-free survival
HDC	hormone-dependent cancers
V_{max}	maximum theoretical velocity
K_{cat}	turnover rate of the enzyme
Triton X-100	*t*-octylphenoxypolyethoxyethanol
CMC	Critical Micelle Concentration
DEAE	Diethylaminoethyl cellulose
pI	isoelecterical point
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
BSA	bovine serum albumin
CAs	carbonic anhydrases
NCI60	National Cancer Institute 60 cells cancer panel
STAB-H	sodium triacetoxyborohydride
NDMBA	*N*,*N'*-dimethylbarbituric acid
K_i^{app}	apparent K_i
TBI	tight binding inhibitor
NBA	*N*-bromoacetamide
NBS	*N*-bromosuccinimide
SEAr	electrophilic aromatic substitution
NFSi	*N*-fluorodibenzenesulfonimide
NFPT	*N*-fluoropyridinium triflate
TCE	trichloroethane
MEM	2-methoxyethoxymethyl
E1P	estrone-1-phosphate
PTP	protein tyrosine phosphatase
Tf_2O	triflic anhydride
DMAP	4-dimethylaminopyridine
DPPA	diphenyl phosphoryl azide
Boc_2O	di-*t*-butyldicarbonate
DME	1,2-dimethoxy ethane
CSI	chlorosulfonyl isocyanate
BnBr	benzyl bromide

Chapter 1 – Steroid Sulfatase: Function, Localization, Structure, Mechanism, and Inhibitors

1 Introduction

1.1 Sulfatases

Sulfatases are esterases that catalyze sulfate ester hydrolysis of physiological substrates with diverse structure ranging from macromolecules such as sulfated proteoglycans to relatively small ones such as estrogen sulfate.[1] Seventeen sulfatases have been characterized in humans, with the majority functioning optimally at acidic pH (4-5) and are lysosomal enzymes (Table 1.1).[1,2] They are involved in numerous biological processes such as mediating signalling between cells and the activation of soluble sulfated steroids.[3]

Table 1.1. Known human sulfatases.

Sulfatase Name	Abbreviation	Location	Natural Substrate	Ref.
Aryl sulfatase A	ARSA	Lysosome	Cerebroside sulfate	4
Aryl sulfatase B	ARSB	Lysosome	Dermatan sulfate	5
Aryl sulfatase C (Steroid sulfatase)	ARSC (STS)	ER	Steroid sulfates	4
Aryl sulfatase D	ARSD	ER	Unknown	6
Aryl sulfatase E	ARSE	Golgi App.	Unknown	6
Aryl sulfatase F	ARSF	ER	Unknown	7
Aryl sulfatase G	ARSG	ER	Unknown	8
Aryl sulfatase H	ARSH	Unknown	Unknown	9
Aryl sulfatase I	ARSI	Unknown	Unknown	9
Aryl sulfatase J	ARSJ	Unknown	Unknown	9
Aryl sulfatase K	ARSK	Lysosomal	Glycosaminoglycans	10
Galactosamine (*N*-acetyl)-6-sulfatase	GALNS	Lysosome	Keratin and Chondroitin sulfate	11
Glucosamine (*N*-acetyl)-6-sulfatase	G6S	Lysosome	Heparan and Keratan sulfate	12
N-sulfoglucosamine sulfohydroloase	SGSH	Lysosome	Heparan sulfate	13
Iduronate-2-sulfatase	IDS	Lysosome	Dermatan and Heparan sulfate	14

Endo sulfatase 1	Sulf 1	ECM	Heparan sulfate	15
Endo sulfatase 2	Sulf 2	ECM	Heparan sulfate	15

Shortly after the discovery of the sulfatases it was found that their activity could be measured using small aromatic sulfated substrates like *p*-nitrophenol sulfate (pNPS, compound **1.1**, Fig. 1.1) and/ or 4-methylumbelliferyl sulfate (4-MUS, compound **1.2**, Fig. 1.1). For this reason human sulfatases have been historically classified as arylsulfatases (ARSs).[3]

1.1 (pNTS) **1.2 (4-MUS)**

Fig. 1.1. Common aromatic sulfated substrates for sulfatases

Subsequent studies on the sulfatases showed that they share common structural features such as their significant sequence homology over their entire length especially in the *N*-terminal region, in addition to having a similar size ranging from 500 to 800 amino acid residues. Moreover, they were found to be extensively glycosylated and have highly similar active sites which undergo a unique post-translational modification process.[16]

1.2 Steroid sulfatase (STS)

1.2.1 STS Substrates and Physiological function

Steroid sulfatase catalyzes the desulfation of steroidal sulfates to give unconjugated steroids (Fig. 1.2). Sulfated steroidal substrates are biosynthesized via the action of a sulfotransferase (SULT) using 3'-phosphoadenosine-5'-phosphosulfate (PAPS) as the sulfate donor. The best characterized steroidal substrates of STS are estrone sulfate (E1S), estradiol sulfate (E2S), dehydroepiandrosterone sulfate (DHEAS), pregnenolone sulfate (PREGS) and cholesterol sulfate (CS) (Fig. 1.2).[17] STS functions optimally between pH 7-8, and as all the

ARSs, STS can also act upon simple aryl sulfates such as pNPS and MUS although the K_m's for these substrates are more than 100 times higher than the K_m's of the natural steroidal substrates (0.6-2 μM).[17]

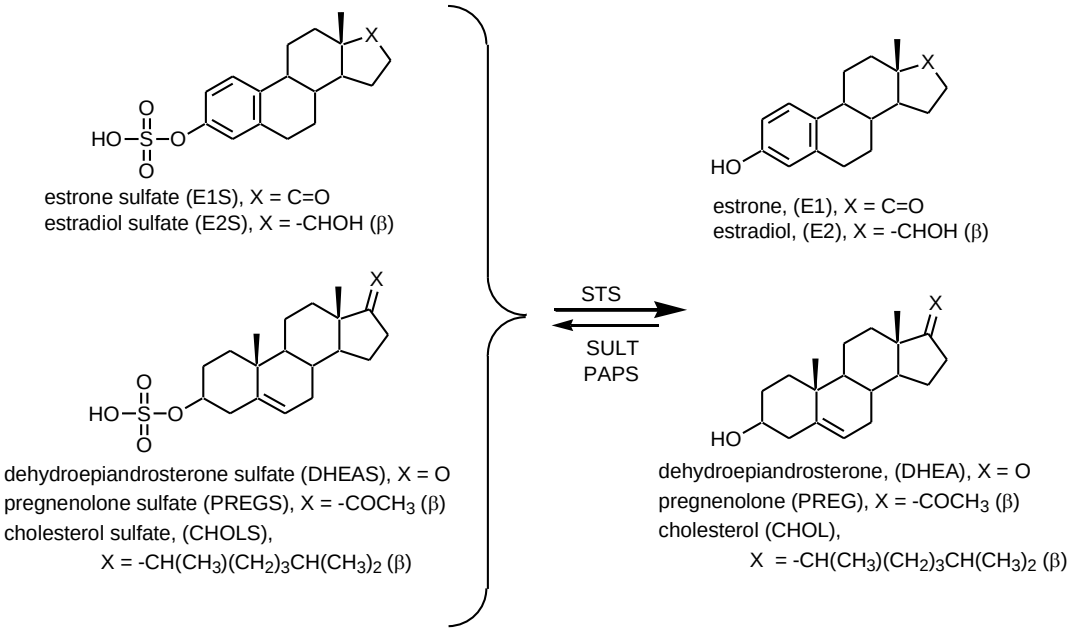


Fig. 1.2. The reaction catalyzed by STS. The best characterized steroidal STS substrates are shown.[17]

Sulfated steroids are unable to bind to steroid hormone receptors and are biologically inactive until removal of the sulfate group by STS. It has been proposed that the role of sulfated steroids is as a storage reservoir of water soluble and transportable steroids and so act as a source of biologically active steroid hormones when activated by STS. This is supported by the finding that circulating plasma concentrations of the sulfated steroids such as E1S, and DHEAS are significantly higher than those of their non-sulfated counterparts, E1 and DHEA.[18] Additionally, the half-life of E1S and DHEAS in plasma is about 10-12 hours, which is considerably longer than the 30-40 minutes half-life of E1 and DHEA.[19]

Immunohistochemical and biochemical localization assays of STS showed that it is an integral membrane protein tightly bound with the membrane of the endoplasmic reticulum of tissues of the reproductive system such as the endometrium, ovary, prostate, testis, and placenta. It is also found in the skin, brain, breast, bone, and blood. One of the richest sources of STS is the placental microsomal fraction.[20-22]

STS has a pseudoautosomal gene which is located on the distal short arm of X-chromosome at band Xp22.3, and consisting of 10 exons and spans 146 kb, with intron sizes ranging from 102 bp up to 35 kb. The cDNA for STS encodes a protein of 583 amino acid residues, with 4 glycosylation sites of which at least two are used (Asn 47 and Asn 259).[20-22]

1.2.2 STS: Post Translation Modification, Structure and Catalytic Mechanism

All sulfatases undergo a unique posttranslational modification in which an active site cysteine or serine (prokaryotes only) is oxidized by an enzyme, called the formyl generating enzyme (FGE), to give Cα-formylglycine (FGly; 2-amino-3-oxopropionic acid) (Fig. 1.3).[23] The discovery of this unique post-translational modification occurring was important in understanding the catalytic mechanism of this class of enzymes.[23]

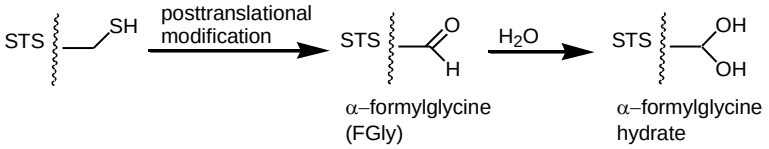


Fig. 1.3. The post-translational modification in eukaryotes that gives α-formylglycine (FGly) and the subsequent hydration of FGly.

This modified cysteine residue is highly conserved across the evolution of all human sulfatases. A lysosomal storage disease called multiple sulfatase deficiency (MSD) is

characterized by catalytically inactive sulfatases. Sulfatases derived from MSD cells were found not to have undergone the post-translational modification as a result of a mutation in a gene or genes encoding the FGE.[16,23-25]

The X-ray crystal structure of STS was resolved to 2.60 Å by Ghosh and coworkers in 2003. The topology of the overall fold of STS shows that the tertiary structure consists of two domains; a globular polar domain and a two transmembrane (TM) helices protruding on one side of the nearly spherical polar domain imparting the overall molecule a "mushroom-like" shape, as shown in Fig 1.4.[26,27]

The polar globular domain (55 × 60 × 70 Å) consists of two subdomains. Subdomain 1 (SD1) winds around a central 11-stranded mixed β-sheets (strands 1, 2, 4-11, and 17) flanked by 13 α-helical turns (helices 1-7, and 10-15). Inside SD1, the catalytic residue, FGly75 was found covalently bound to a sulfate group and a metal ion closely located near FGly75 was interpreted to be Ca^{+2}. The other subdomain, subdomain 2 (SD2) winds around a 4-stranded anti-parallel β-sheets (strands 13-16), flanked by α-helix 16, and packs against the turn and loop regions of the β-sheets of SD1.[26,27]

Even though the STS globular polar domain resembles ARSA and ARSB in shape, size, and folding, some of its loop regions (loop α4 and α5, and between β9 and α13), which approach the lipid bilayer and are proposed to associate with the membrane, have 4 to 7 residue peptide insertions which are not found in the structures of ARSA and ARSB. These insertions were found to be critical for anchoring its catalytic domain to the membrane surface.[26,27]

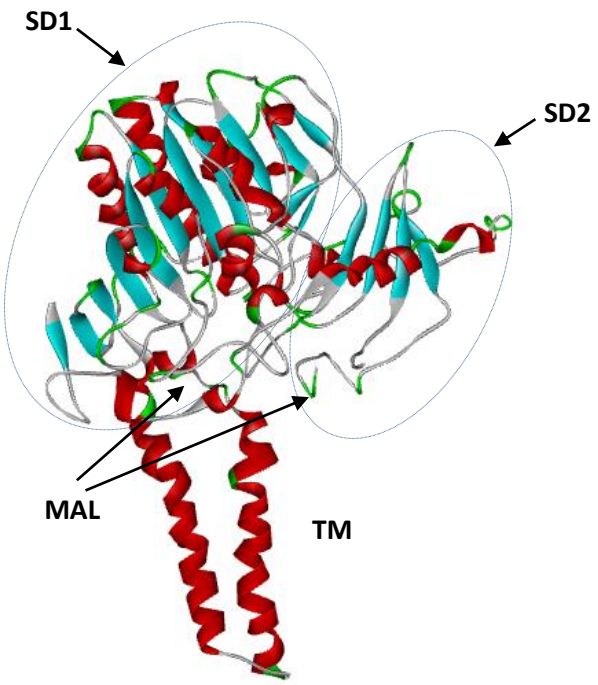


Fig. 1.4. A stereographic ribbon diagram of the STS crystal structure showing the secondary and tertiary structures. Sheets are drawn in green, and helices are in pink; Sub-Domains 1 and 2 (SD1 and SD2), Transmembrane domain (TM), Membrane-Associated Loops (MAL).[27]

A close look inside the STS crystal structure showed that the active site is located near the membrane surface of a cavity in the ‘‘gill’’ of the polar globular domain. Interestingly, the crucial catalytic residue, FGly75 was found as a sulfated hydrate form as shown in Fig. 1.5.[26,27] This is in contrast to ARSA whose crystal structure revealed a non-sulfated hydrate.[28] Although it is not known if the FGly75 hydrate is sulfated in STS's resting state in solution it is believed that this is not the case based upon the proposed mechanism (Fig.1.6).[27] It is believed that the enzyme crystalizes more efficiently when the hydrate in STS is sulfated with the sulfate coming from the crystallization buffer. The Ca^{+2} ion is found at the center of the catalytic site near the sulfated FGly75 residue, and is fixed there by a H-bonding interactions with oxygen atoms of various amino acid residues; Asp35, Asp36, Asp342, Gln343, and FGS75, with a distance ranging between 2.2 and 2.8Å.[27] Additionally, it was found that both Lys134 and Lys368 have

their positively charged amino group within contact distances (2.7-3.1 Å) of the sulfate oxygen atoms of FGS75, and in the same way another two sulfate oxygen atoms were found within a co-ordination distances of Ca^{+2} ion (2.6-2.7 Å).[27]

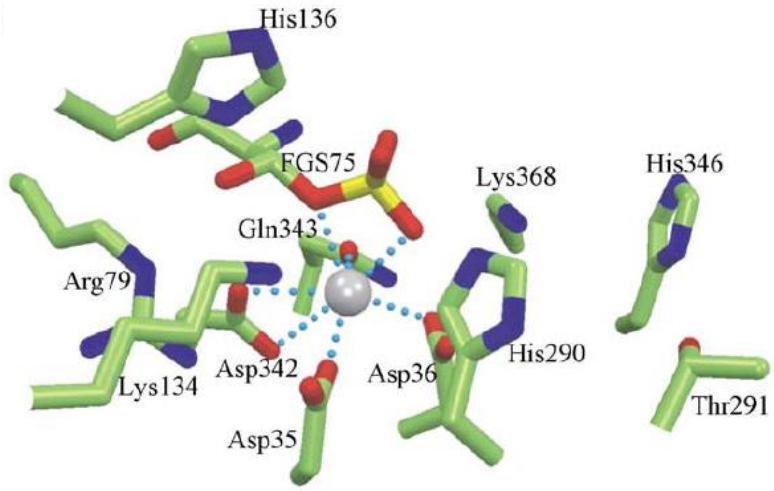


Fig. 1.5. Active site catalytic residues in STS and the coordination of Ca^{+2}.[26,27] Nitrogens are in blue, oxygens are in red and the sulfur atom is in yellow.

Von Figura and coworkers proposed a general mechanism for ARSs based on kinetic studies and the crystal structures of ARSs (Fig. 1.6).[3,26,29,30] The first step of the mechanism involves the activation of one of the oxygen atoms on the FGly75 hydrate by an aspartate residue acting as a general base. The oxygen performs a nucleophilic attack on the sulphur atom of the substrate, which consequently releases the desulfated product as well as forming a sulfated hydrate intermediate.[29,30] A histidine residue acting as a general acid aids the displacement of the desulfated product. The sulfated hydrate then undergoes a general-base catalyzed elimination reaction to release inorganic sulfate and forming formylglycine, which is then rehydrated to regenerate the initial formylglycine hydrate (Fig.1.6).[29,30]

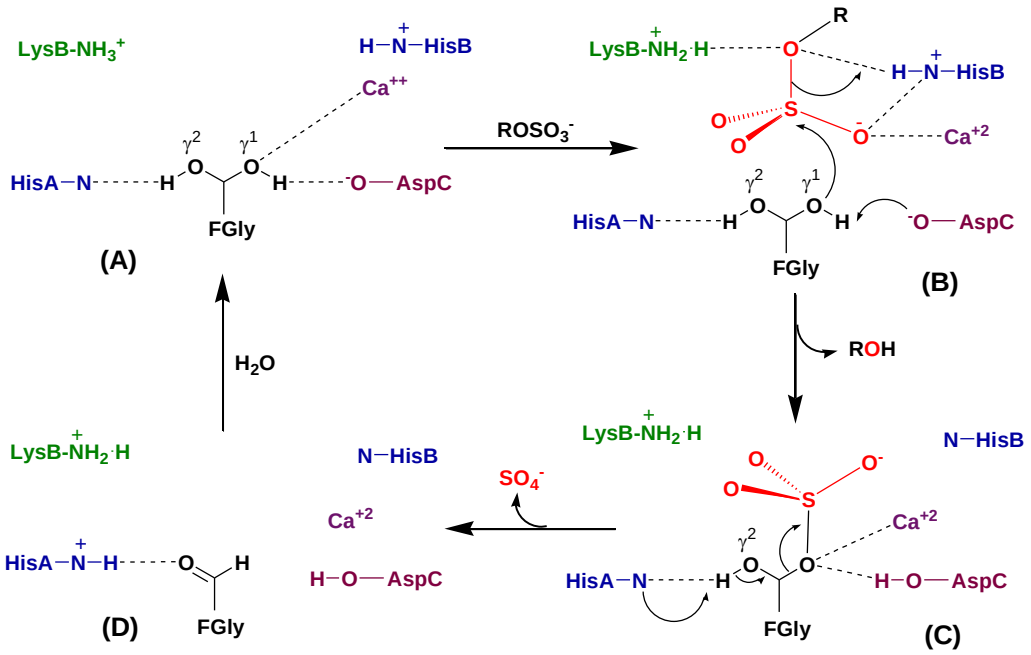


Fig. 1.6. A schematic diagram showing steps involved in the proposed reaction mechanism of steroid sulfatase.[3]

1.2.3 STS and Breast Cancer

The vast majority of research on STS inhibitors has been directed toward the development of drugs for treating estrogen receptor-positive (ER+) hormone-dependent breast cancer. This type of breast cancer occurs most frequently in post-menopausal women which is paradoxical as ovarian production of estrogen, which is the main, though not only source of estrogens has stopped.[17] Instead of local production of estrogens in tumors, it has been hypothesized that tumor growth is stimulated in part by estrogens derived from their sulfated precursors which are produced in peripheral tissues (Fig. 1.7). These soluble sulfated precursors are transported into the cancer cells by specific membrane transporters where they are then desulfated by STS.

Several lines of evidence suggest that STS plays an important role in the progression of steroid-dependent breast cancer.[31] The production of E1 from estrone sulfate (E1S) in breast cancer tissue is approximately 10 times greater than from androstenedione, which is converted to

E1 by aromatase (Fig. 1.7).[32] 90% of Adiol in post-menopausal women originates from DHEAS via desulfation of DHEAS by STS to give DHEA which is converted into Adiol by the dehydrogenase. There is approximately 50–200 times greater STS activity than aromatase activity in malignant breast tissues. Sulfatase activity in breast cancer cells is higher than that of normal breast cells.[33] Finally, STS expression in breast tissue is significantly higher than in normal tissue and STS expression is now used as a prognostic factor in human breast carcinoma.[34]

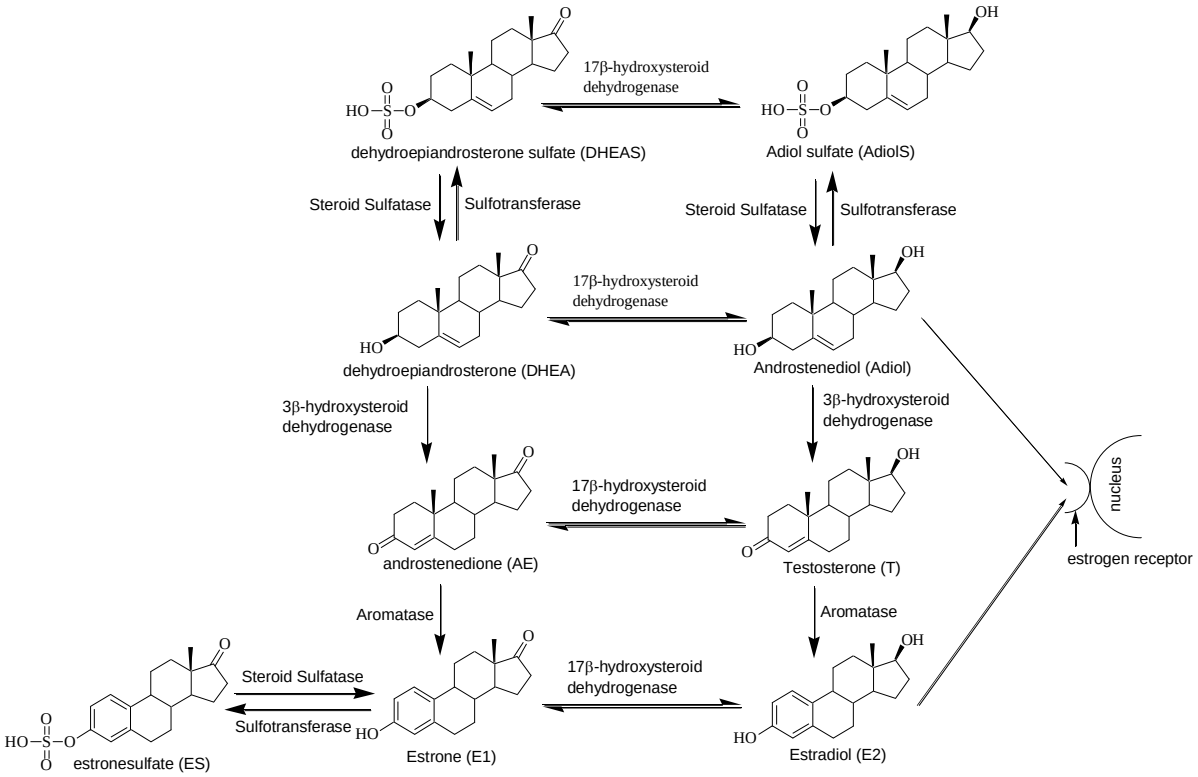


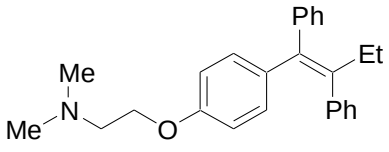
Fig. 1.7. The origin of estrogenic steroids in postmenopausal women with hormone-dependent breast cancer.

It is worth mentioning that there is little known in the literature about the control of STS expression, however both TNFα and IL-6 were found to up-regulate STS activity in MCF-7 breast cancer cells. Additionally, E2 was found to up-regulate STS expression through the

increased binding of the ER to estrogen responsive elements (EREs) located in the promoter regions of STS gene. Taken together, these findings could be used as strong evidence for the up-regulation of STS activity in hormone-dependent cancer, thus STS became a target for drug design.[35,36]

1.3 Current Therapies for Treating Hormone-Dependent Breast Cancer

Estrogenic steroids supporting the growth and development of hormone-dependent breast cancer represent the most common type of hormone-dependent malignancy. In postmenopausal HR+ breast cancer, therapies that block either the synthesis or action of estrogen are now established as first-line treatments for most postmenopausal women with estrogen receptor positive (ER+) breast cancer. Tamoxifen, (Fig. 1.8), is the first-generation selective estrogen receptor modulator (SERM), which has been the endocrine therapy of choice till the past 5 years or so. It is reported that tamoxifen has helped more than 400,000 women survive breast cancer; however, vaginal bleeding, thromboembolism, endometrial cancer, and therapeutic resistance were its long-term side-effects.[37] Consequently, looking for alternative therapeutic agents was very crucial. Aromatase inhibitors (AIs) and selective estrogen receptor down-regulators (SERDs) were used as alternative therapies for tamoxifen; AIs prevent estrogen synthesis by inhibiting the aromatase enzyme converting androgens to estrogen, whereas SERDs act by down-regulating ER expression.[37,38]



Tamoxifen

Fig. 1.8. Structure of Tamoxifen

Estrogen depletion achieved from the use of AIs in treating HDBC in ER postmenopausal early breast cancer has turned out to be in general a more effective way of treatment in comparison to blocking ER by SERMs. The most notable AIs are the third generation AI's; letrozole and anastrozole (Fig. 1.9), which have proven to be effective in the treatment of breast cancer and having greater benefits of reducing the risk of occurrence of contralateral breast cancer than tamoxifen.[37,38] Unfortunately, AIs share common side effects affecting both muscles and bones, and so tamoxifen is still used as a standard therapy or as an alternative to AI's. Moreover, many of breast cancer patients experience relapse of their breast cancer after treatment with AI's especially those with metastatic ER+ breast cancer.[37,38]

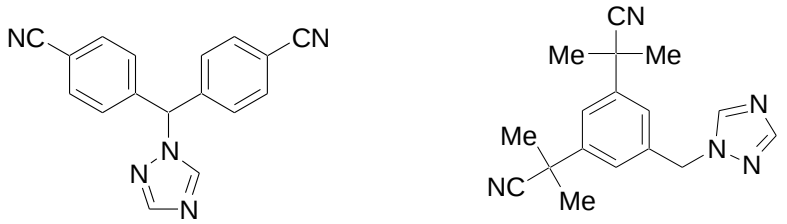
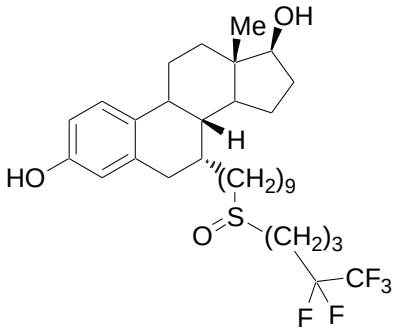
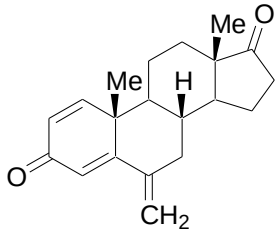


Fig. 1.9. Aromatase inhibitors letrozole and anastrozole

The 3rd generation non-steroidal AIs, fulvestrant and exemestane (Fig. 1.10), are currently used as second- or third-line endocrine therapies in selected patients with metastatic breast cancer (MBC) based on the studies that suggested dependency of tumors on estrogens following progression on the first-line and even second-line endocrine therapies.[37,38]



Fulvestrant



Exemestane

Fig. 1.10. Structures of Fulvestrant and Exemestrane

One of these major drawbacks of using AIs and SERD in treatment of different types of HDBC is the development of cross-resistance, thus new non-cross-resistant therapies are urgently needed to give patients with HDBC an alternative treatment option to prolong the time period without the use of the chemotherapy option. STS-inhibition represents such a novel approach which can be used to block the synthesis of a variety of steroids that have the potential to stimulate growth of HDBC.[38]

1.4 STS Inhibitors

STS inhibitors have been traditionally divided into two classes: steroidal and non-steroidal. These two classes have been divided into two subclasses: sulfamate and non-sulfamate-based.

1.4.1. Non-sulfamate Steroid-based Inhibitors

1.4.1.1 Early studies and Substrate Analogs

Initial reports on STS inhibitors, which appeared in the late 1960's and early 1970's, focussed on examining unconjugated steroids, both natural and synthetic, as potential STS inhibitors.[39-43] The ultimate objective of these studies was not the development of anticancer agents but rather to elucidate steroidal regulatory mechanisms and determine whether inhibition of STS by endogenous steroids could provide a mechanism for regulation of estrogen production

during human pregnancy. Crude preparations of STS (such as human placental or testicular homogenates) were used for the majority of these studies. None of the compounds studied proved to be potent STS inhibitors. Nevertheless, these studies revealed that inhibitory activity of these compounds is favored by planar Δ5- or 5α- structures unsubstituted except for oxygen functions at C-3 and C-20.[39-43]

Synthetic steroids such as danazol, and several progestins (Fig. 1.11), such as promegestone, tibolone, and medroxyprogesterone acetate, norelgestromin, and nomegestrol acetate, have been tested as potential STS inhibitors by incubating intact breast cancer cells with labeled E1S and the formation of E1 and E2 determined.[44-47] In these assays, the formation of E1 or E2 was reduced by varying degrees, however whether this was due to direct inhibition of STS was sometimes not ascertained. In cases where direct STS inhibition was determined (using purified enzyme or cell homogenates), the extent of inhibition was nil to moderate. This work has been reviewed and the reader is referred to these articles for a more in-depth discussion of these studies.[44-47]

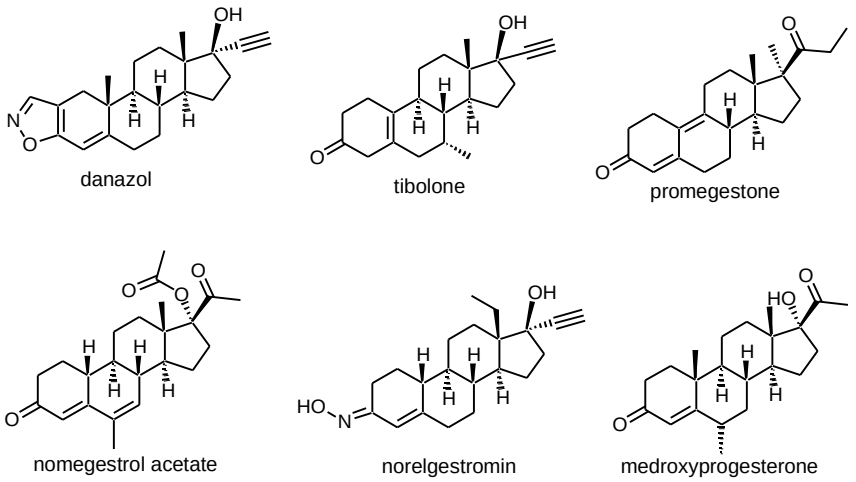
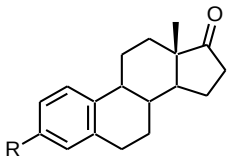


Fig. 1.11. Progestins that have been tested as potential STS inhibitors.

Efforts to develop STS inhibitors into drugs did not really begin in earnest until the early 1990's. Many of the studies from this time period centered upon finding substrate analogs in which the labile sulfate group was replaced with a sulfate mimic (Table 1.2). It was these studies which led to the discovery of estrone-3-*O*-sulfamate (EMATE, compound **1.36** in Table 1.2), a highly potent, irreversible STS inhibitor which is now the prototypical STS inhibitor and used as a standard when evaluating the in vitro potency of other STS inhibitors. As can be seen from Table 1.2, with the exception of EMATE, little success has been obtained using this approach to STS inhibitors as most of these compounds have proven to be moderate to weak reversible inhibitors. However, a few of these mimics are worthy of note. The methylthiophosphonate group (compound **1.3**) was one of the first sulfate mimics to be examined in detail.[48] It is a moderate, reversible, competitive STS inhibitor in both breast tumours and placental microsomes. The sulfur atom is not essential to activity as the oxygen analog was only 50% less potent. The S_p diastereomer is more potent than the R_p diastereomer. The methylthiophosphonate analogs of other natural substrates of STS such as DHEA-, cholesterol-, and pregnenolone-3-methylthiophosphonate were also reasonably good inhibitors with K_i's ranging from 1.4-6.2 μM in placental microsomes and inhibited STS activity in MCF-7 breast cancer cells by 31-85% at 1 μM. The *N*,*N*-dimethylated analog of EMATE (compound **1.38**) was a good reversible inhibitor though still considerably less potent than EMATE.[57] Formylation of E1 gave an irreversible inhibitor (compound **1.35**) though less potent than EMATE. This compound is labile and so is not suitable for as a lead for further drug development.[56] Surprisingly, a boronic acid group turned out to be a good sulfate mimic with compound **1.47** being a competitive inhibitor and exhibiting one of the lowest K_i's for all

substrate-based, reversible inhibitors bearing a sulfate mimic studied so far, though once again this compound was still considerably less potent than EMATE.[63]

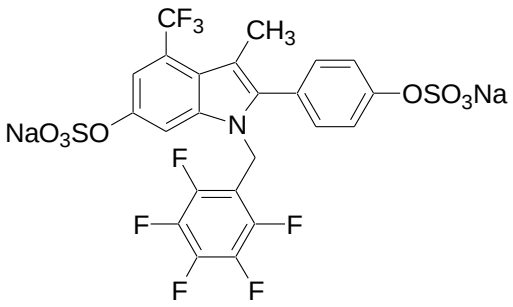
Table 1.2. Substrate analogs of E1S in which the labile sulfate group is replaced with a sulfate mimic.



Compound	R	% Inhibition, K_i or IC_{50} (μM)	Assay	Ref.
1.3	$-OPSCH_3$	43 (IC_{50})	Placental microsomes	48
		15 (K_i)	Placental microsomes	48
1.14	$-OPO_3^{-2}$	0.3 (K_i)	Purified STS	49
		0.17-52 (K_i)	Partially purified STS	50
1.15	$-OPO_2F$	14 (K_i)	Partially purified STS	50
1.16	$-SO_3^-$	40% at 300 μM	Placental microsomes	51
		40 (K_i)	Purified STS	52
1.17	$-SO_2Cl$	92% at 300 μM	Placental microsomes	51
		65% at 60 μM	Placental microsomes	51
		28 (K_i)	Purified STS	52
1.18	$-SO_2F$	44% at 300 μM	Placental microsomes	51
		35 (K_i)	Purified STS	52
1.19	$-SO_2NH_2$	45% at 300 μM	Placental microsomes	51
		35 (K_i)	Purified STS	52
1.20	$-SO_2CH_3$	36% at 300 μM	Placental microsomes	51
		130 (K_i)	Purified STS	52
1.21	-SH	10% at 10 μM	Placental microsomes	53
1.22	$-SSO_2NH_2$	12% at 50 μM	Placental microsomes	54
1.23	$-SSO_2N(CH_3)_2$	0% at 100 μM	Placental microsomes	54
1.24	$-SCON(CH_3)_2$	4% at 50 μM	Placental microsomes	54
1.25	$-NHSO_2NH_2$	53% at 50 μM	Placental microsomes	54
1.26	$-NHSO_2CF_3$	10.2 (IC_{50})	Placental microsomes	55
1.27	$-N(SO_2CF_3)_2$	14.6 (IC_{50})	Placental microsomes	55
1.28	$-NHCOCF_3$	8.7 (IC_{50})	Placental microsomes	55
1.29	$-NHCONH_2$	12.9 (IC_{50})	Placental microsomes	55
1.30	$-NH_2$	15% at 10 μM	Placental microsomes	53
1.31	$-OCONH_2$	>50 μM (IC_{50})	Purified STS	56
1.32	$-OCSNH_2$	>50 μM (IC_{50})	Purified STS	56
1.33	-OCN	>50 μM (IC_{50})	Purified STS	56
1.34	$-OCOCH_3$	>50 μM (IC_{50})	Purified STS	56
1.35	-OCHO	0.42 (IC_{50})	Purified STS	56
1.36	$-OSO_2NH_2$ (EMATE)	0.004 (IC_{50})	Placental microsomes	57
		0.056 (IC_{50})	Purified STS	58
		0.67 (K_i)	Placental microsomes	59
1.37	$-OSO_2NHCH_3$	87% at 1 μM	Intact MCF-7 cells	57
1.38	$-OSO_2NH(CH_3)_2$	79% at 0.1 μM	Intact MCF-7 cells	57
1.39	$-OSO_2CH_3$	28% at 10 μM	Intact MCF-7 cells	60
		23 (K_i)	Purified STS	50

1.40	$-OSO_2C_6H_4CH_3$	30% at 0.1 μM	Intact MCF-7 cells	60
1.41	$-CH_2SO_3H$	140 (K_i)	Purified STS	50
		600 (K_i)	Purified STS	62
1.42	$-CF_2SO_3H$	57 (K_i)	Purified STS	61
1.43	$-CH_2SO_2NH_2$	350 (K_i)	Purified STS	62
1.44	$-CF_2SO_2NH_2$	82 (K_i)	Purified STS	62
1.45	$-CH_2$tetrazole	72 (K_i)	Purified STS	61
1.46	$-CF_2$tetrazole	16 (K_i)	Purified STS	61
1.47	$-B(OH)_2$	2.8 (K_i)	Purified STS	63

Sulfated compounds like 2-(hydroxyphenyl) indole series have been examined as STS inhibitors, and one of which, compound **1.48** (Fig. 1.12), inhibited STS activity with an IC_{50} of 80 μM).[64]



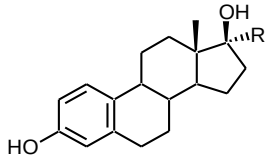
1.48

Fig. 1.12. Structure of 2-(hydroxyphenyl) indole sulfate **1.48**.[64]

1.4.1.2 Estradiol-based inhibitors

The Poirier group has examined a wide variety of 17α-substituted E2 derivatives of general structure **1.49** (Fig. 1.13) as STS inhibitors.[65-69] Many of these compounds proved to be highly potent reversible inhibitors of STS using JEG-3 cells as the source of STS. The potency of these compounds increased with the length of the alkyl substituent up to 8 carbon units whereas longer substituents led to a decrease in potency. Those bearing benzyl groups were found to be particularly good STS inhibitors with the most potent ones being 3´-bromobenzyl and 4´-*t*-butylbenzyl analogs (**1.51** and **1.52**, IC_{50}'s = 24 and 28 nM respectively). Compound **1.52** was found to be 7.5-fold less potent than EMATE using HEK-293 cells overexpressing

STS. The Poirier group performed an in-depth QSAR study on the benzylic derivatives and this led to the identification of compound **1.53** as a highly potent inhibitor (IC_{50} = 21 nM).[68,69]

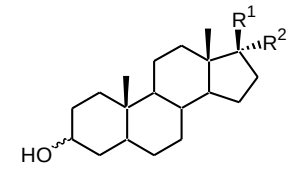


1.49, R = various alkyl, Bn or Ph substituted
1.50, R = Bn
1.51, R = CH_2(*m*-Br)Ph
1.52, R = CH_2 (p-*t*-Bu)Ph
1.53, R = CH_2(p-(o-CF_3)-Ph)-Ph
1.54, R = cyclohexylmethyl
1.55, R = $CH_2C_6F_5$

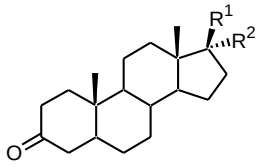
Fig. 1.13. 17α-benzyl E2 based inhibitors of STS.

Poirier and coworkers suggested that the high potency of these compounds is a result of the hydrophobic benzyl groups extending down into the tunnel that exists between the two hydrophobic alpha helices which are responsible for insertion of STS into the membrane of the endoplasmic reticulum. For compounds having a *t*-butyl, trifluoromethyl, or benzyloxy groups at the *meta* or *para* positions of the benzyl group, potency of the *m*-disubstituted benzyl derivatives is lower than both the single-substituted meta or para derivatives. The only exceptions were the derivatives substituted with bromine, a smaller substituent than the above mentioned groups, which had roughly the same potency. These results are consistent with the hydrophobic tunnel in STS being narrow and deep. These workers suggested that there may be a π–π interaction between the 17α-benzyl groups and three phenylalanines in the tunnel. However, the cyclohexylmethyl and pentafluorobenzyl derivatives, **1.54** and **1.55**, had similar potencies to the benzyl derivative **1.50**, suggesting that aromaticity is not necessary for high potency and that the interaction between the inhibitors and residues in the hydrophobic tunnel is not of the π-π type.[69]

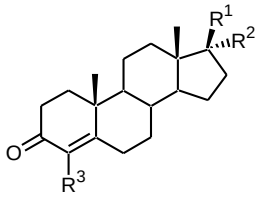
The benzyl pharmacophore was also examined in the context of 17α- and 20-substituted androstane and pregnane derivatives **1.56**-**1.62** (Fig. 1.14).[70] In general, lower potencies were found for the compounds of these series compared to the estrane derivative **1.50** (Fig. 1.14) with the exceptions of compounds **1.56** (3β) which was approximately equipotent to **1.50** when assayed using JEG-3 cell homogenates.[70]



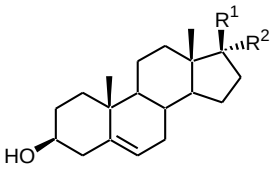
1.56 (3α and 3β), R^1 = OH, R^2 = Bn



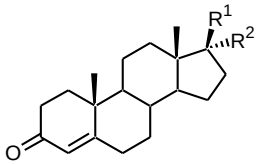
1.57, R^1 = OH, R^2 = Bn



1.58, R^1 = OH, R^2 = Bn, R^3 = Cl, OCH_3 or OH
R^1 = -COH(CH_3)Bn, R^2 = H, R^3 = Cl, OCH_3 or OH



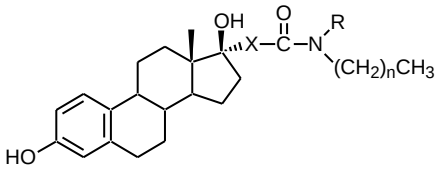
1.59 (3α and 3β), R^1 = OH, R^2 = Bn
1.60, R^1 = -COH(CH_3)Bn, R^2 = H



1.61, R^1 = OH, R^2 = Bn
1.62, R^1 = -COH(CH_3)Bn, R^2 = H

Fig. 1.14. 17α- and 20-Substituted androstane and pregnane derivatives as inhibitors of STS.

17α-Alkan and alkyn amide derivatives of E2 (compounds **1.63**) have also been examined as STS inhibitors using STS from homogenated JEG-3 cells.[71] The most potent inhibitor (**1.63**, X = $(CH_2)_2$, R^1 = Me, n = 7) had an IC_{50} of 80 nM. No estrogenic activity was observed for this compound at 30 nM in estrogen-sensitive ZR-75-1 cells (Fig. 1.15).[71]



1.63, X = $(CH_2)_2$ or C≡C
R = CH_3 or $(CH_2)_7CH_3$
n = 3, 5, or 7

Fig. 1.15. 17α-Alkan (or alkyn) amide derivatives of E2 as STS inhibitors.

The Taylor group has reported that 17α-benzyl E2 (compound **1.50**, Fig. 1.13) is a noncompetitive inhibitor of purified STS using MUS as substrate. Compound **1.50** had a K_i of 230 nM and αK_i of 420 nM respectively (αK_i is the dissociation constant for the inhibitor with the ES complex).[63] Interestingly, the boronic acid E2 derivative **1.64** (Fig. 1.16) was also a noncompetitive inhibitor with an almost identical affinity for STS. This is in contrast to aforementioned boronic acid inhibitor **1.47** (Table 1.2) which was a competitive inhibitor and was 20-fold more potent an STS inhibitor than estrone.[63] These studies suggest that there is an alternative binding site for the 17α-substituted inhibitors. This prompted Poirier and coworkers to examine E2 dimers (compounds **1.65** and **1.66**, Fig. 1.16), which could potentially occupy both binding sites, as STS inhibitors.[72] The best inhibitors were the C17-C17 dimers with an alkene or alkane spacer of four carbons and these compounds exhibited inhibitory potencies similar to compound **1.37** (56-62% inhibition at 1 μM) in an assay with homogenated HEK-293 cells overexpressing STS.[72]

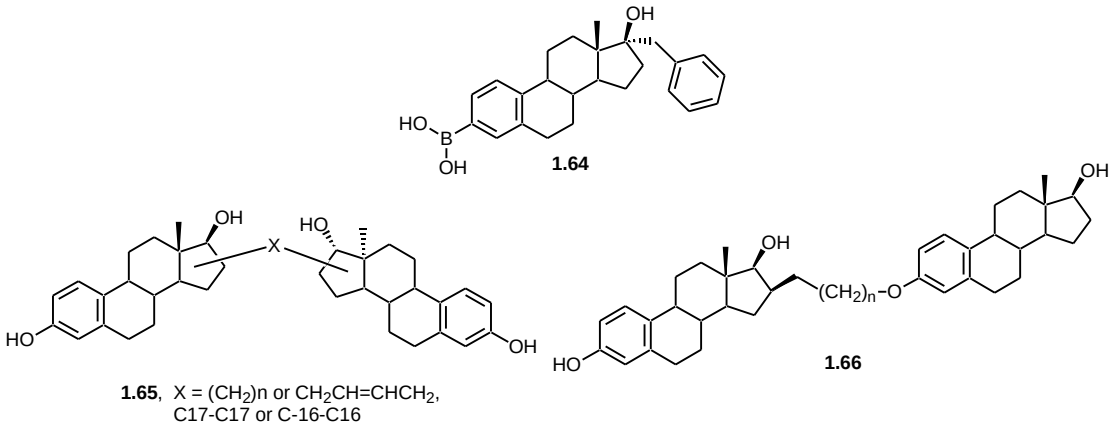


Fig. 1.16. A boronic acid and E2 dimers as STS inhibitors.

1.4.1.3 E1S-based Suicide Inhibitors (SIs)

The Taylor group has examined 2- and 4- mono- or difluoromethyl derivatives of E1S (compounds **1.67-1.70**, Fig. 1.17) as inhibitors of purified STS.[73]

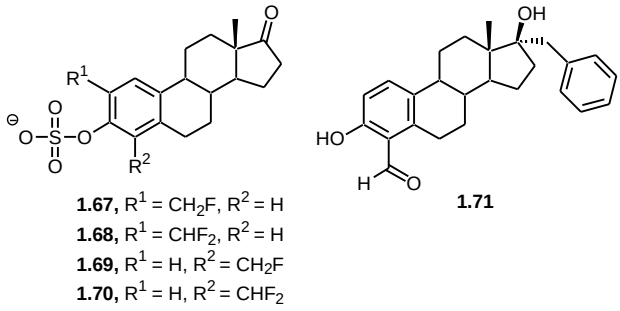


Fig. 1.17. 2- and 4- mono- or difluoromethyl derivatives of E1 (**1.67-1.70**) and 4-formyl-17β-benzyl E2 (**1.71**).

These compounds were designed to act as suicide inhibitors of STS by producing a reactive quinone methide in the active site which would then react with an active site nucleophile thus irreversibly inactivating STS (Fig. 1.18).[73]

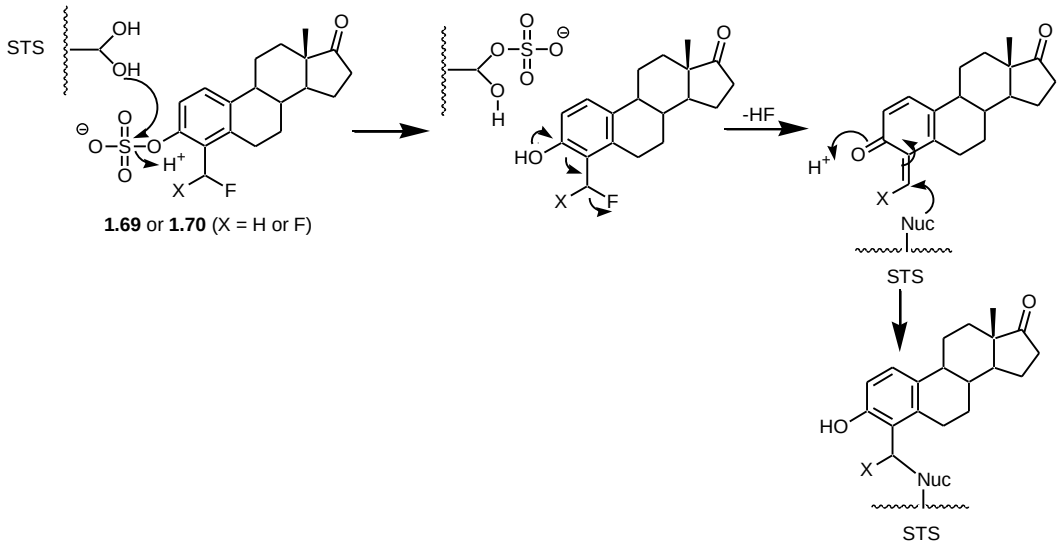


Fig. 1.18. Anticipated mechanism of inhibition of STS with compounds **1.69** and **1.70**. Compounds **1.67** and **1.68** were expected to inhibit by a similar mechanism.

The monofluoromethyl derivatives, **1.67** and **1.69**, were found to act as suicide inhibitors presumably by the mechanism outlined in Fig. 1.18. Interestingly, the 2-difluoromethyl derivative **1.68** was found to be a substrate but not an inhibitor while the 4-difluoromethyl derivative **1.70** exhibited time and concentration dependent inhibition. Detailed kinetic studies with **1.70** and STS suggested that this compound inactivates STS by multiple pathways. One route involves the process outlined in Fig. 1.19. The other route involves dissociation of the initial hydrolysis product from the active site where it undergoes decomposition to the quinone methide and subsequent reaction with water to give 4-formyl estrone (4-FE1). 4-FE1 then enters the active site and acts as an almost irreversible STS inhibitor (Fig. 1.19). Kinetic studies with purified STS and authentic, chemically synthesized 4-FE1 revealed 4-FE1 to be a time- and concentration-dependent inhibitor with a K_I of 1.5 μM and a k_{inact} of 0.13 min^{-1} and a k_{inact}/K_I = 1 x 10^5 M^{-1} min^{-1}. Interestingly, 2-formyl estrone, the ultimate product of the reaction of the 2-

difluoromethyl derivative, **1.68**, with STS, was found to not be a time- and concentration-dependent STS inhibitor up to a concentration of 10 μM.[73]

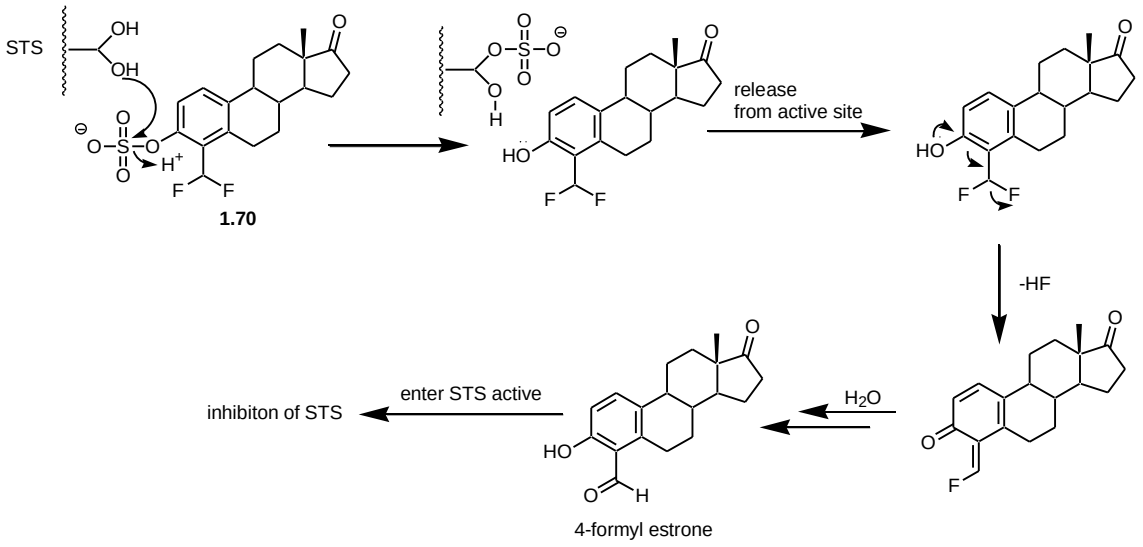


Fig. 1.19. Mechanism of inhibition of STS with compound **1.70**. The product of the reaction of STS with **1.70**, 4-formylestrone (4-FE1) enters the active site and inhibits STS in a time- and concentration-dependent manner.

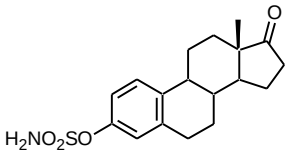
These results prompted the Taylor group to examine the 4-formyl-17α-benzyl E2 (**1.71**, Fig. 1.17) as an STS inhibitor.[74] Compound **1.71** turned out to be a potent time and conc-dependent STS inhibitor with a K_I of 85 nM and a K_{inact} of 0.021 min^{-1} (k_{inact}/K_I of 2.3 x 10^5 $M^{-1}min^{-1}$) with purified STS preparations.[74]

1.4.2 Steroidal Sulfamate-based STS Inhibitors

1.4.2.1 Estrone-3-O-Sulfamate (EMATE)

A breakthrough in the development of STS inhibitors was reported in 1994 when Potter and coworkers reported that the sulfamate analog of estrone sulfate (E1S), estrone-3-*O*-sulfamate (EMATE, **1.36**, Table 1.2, Fig. 1.20), was a potent, irreversible, dose and time-dependent inhibitor of STS.[57,59] Cleavage of the S-O bond was found to occur indicating that EMATE is a

suicide inhibitor of STS. EMATE exhibited an IC_{50} and K_I of 100 nM and 670 nM respectively using a placental microsome preparation of STS.[59] In intact MCF-7 cells EMATE exhibited 99% inhibition of STS at 100 nM and almost completely abolishes STS activity in rat tissues.[75] Unfortunately, EMATE is estrogenic and so is not a suitable candidate for further drug development.[76] Nevertheless, the discovery of EMATE spawned a plethora of work on sulfamate-based STS inhibitors.



1.36 (EMATE)

Fig. 1.20. Estrone-*O*-sulfamate (EMATE).

Early SAR studies on EMATE and other steroidal sulfamate-based inhibitors revealed the following:

(a) *N*-Alkylation of the sulfamate functionality in EMATE generally resulted in a significant decrease in activity.[57,59] Although good activity was obtained with the *N*,*N*-dimethyl derivative (Table 1.2, compound **1.38**) this compound was still considerably less potent than EMATE and, like other *N*-alkylated derivatives, was a reversible inhibitor.[57,59] *N*-acylation also resulted in a significant decrease in activity and a change in the mode of inhibition from irreversible to reversible. The *N*-acetyl analog of EMATE was reported to irreversibly inhibit STS although less efficiently than EMATE.[77] However, it was later demonstrated that this compound hydrolyzes to EMATE under the assay conditions and that its inhibitory activity as well as its supposed irreversible inhibition directly correlates with the amount of EMATE formed.[44]

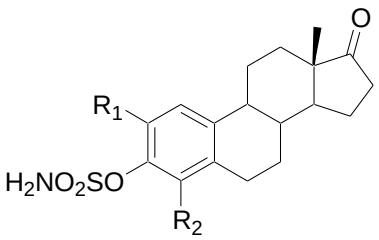
(b) Replacing the bridging oxygen of the sulfamate group in EMATE with a sulfur, nitrogen, methylene or difluoromethylene (Table 1.2, compounds **1.22**, **1.25**, **1.43** and **1.44**) moiety results in a considerable loss of potency and the mode of inhibition changes from irreversible to reversible.[54,62]

(c) Sulfamate derivatives of steroids having non-aromatic A-rings such as dehydroepiandrosterone (DHEA), cholesterol, pregnenolone, androsterone and epiandrosterone, are poor reversible inhibitors revealing that an aromatic A-ring is essential to potent activity.[58,78] This was somewhat surprising as STS accepts aromatic A-ring substrates (i.e. estrone sulfate) and non-aromatic A-ring substrates (i.e. cholesterol sulfate and DHEA sulfate). Examining a series of phenylsulfamates substituted at the 3- and 4-positions with various electron donating and electron withdrawing groups as STS inhibitors, it was shown that the inhibition of STS by these compounds depended upon the pK_a of the leaving group (phenol portion of the substrate): the lower the pK_a of the leaving group the lower the IC_{50} of the inhibitor.[79] Although rates of inactivation and a detailed kinetic study was not performed with these compounds and STS the results suggest that the lack of activity of the sulfamate derivatives of steroids having non-aromatic A-rings may be due in part to the poor leaving group ability of these steroids. Overall, the above studies suggest that the mechanism by which sulfamate-based inhibitors inhibit STS may be different from the mechanism by which STS hydrolyses its natural substrates.

Many modifications to the steroid skeleton of EMATE have been made. The main impetus behind such modifications has been the development of compounds with potencies similar to or greater than EMATE but with no estrogenic effects. The vast majority of these modifications have been on the A- and D-rings.

1.4.2.2 Modification of A-ring of EMATE

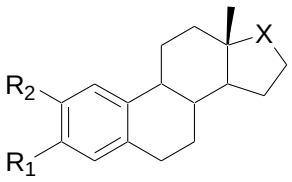
In 1998, the effect of modifying the 2- and/or 4-position of EMATE by either a small electron-donating or electron-withdrawing groups was examined by Purohit and co-workers. (Fig. 1.21, compounds **1.72a)**. They found that placing an allyl group at the 4-position of EMATE, compounds **1.72b** resulted in derivatives more potent inhibitors than their propyl analogues. However both of these classes of compounds were less potent than EMATE. On the other hand, nitro analogues were potent STS inhibitors than EMATE, especially those having the nitro group at 4-position of EMATE ring (5-fold more active than EMATE). Some of these new derivatives were non-estrogenic or having estrogenic activity less than EMATE.[80]



1.72a; R_1 and/or R_2= H, OCH_3, NO_2, n-Pr
1.72b; R_1 and/or R_2= H, allyl

Fig. 1.21. A-ring-modified EMATES

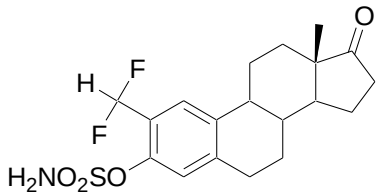
2-Methoxyestradiol (Fig. 1.22, **1.73a**, 2-MeOE2) is a natural metabolite of E2 but with a poor affinity for the ER. It is also an experimental drug candidate (under trade name of Panzem).[81] It induces apoptosis in some cancer cell lines and is undergoing clinical trials for treating breast cancer and ovarian cancer. This provided the impetus for the study of 2-methoxyestrone-3-*O*-sulfamate (2-MeOEMATE, compound **1.73c**) which was found to be equipotent to EMATE in inhibiting STS activity in placental microsomal preparation (IC_{50} = 30 nM), with the advantage of being non-estrogenic.[82,83]



1.73a, 2-MeOE$_2$; R_1= OH, R_2= OMe, X= CH(OH)(β)
1.73b, 2-MeOE$_1$; R_1= OH, R_2= OMe, X= C=O
1.73c, 2-MeOEMATE; R_1= OSO_2NH_2, R_2= OMe, X= C=O

Fig. 1.22. Structures of 2-MeOE1, 2-MeOE2 and 2-MeOEMATE.

The 2-difluoromethylestrone 3-O-sulfamate, compound **1.74** (Fig. 1.23) was found by Reed and co-workers to be a highly potent irreversible STS inhibitor with an IC_{50} of 100 pM in a placental microsomes preparation, which was 90-fold lower than EMATE in the same assay.[84]



1.74

Fig. 1.23. Structure of **1.74**.

1.4.2.3 Modification of the D-ring of EMATE

Modification of the B- and/or C-rings of EMATE has not been widely pursued possibly because modification of these two rings is more challenging from a synthetic point-of-view than modification of the A and D-rings. Most D-ring modifications were done to afford new classes of STS inhibitors and to supress the estrogenic side effects of the parent compounds.

Numerous D-ring modified EMATE derivatives have been prepared with the most common involving modification at the 17-position. Modification at this position not only provides a means of increasing potency but also as means of decreasing the estrogenicity of the

released steroidal portion upon inhibition as it has been shown that the estrogenicity of E1 and E2 can be abolished or significantly reduced by introducing substituents at this position.

Li et al. reported that 17β-(*N*-alkylcarbamoyl)-estra-1,3,5(10)-trien-3-O-sulfamates (**1.75**, Fig. 1.24) and 17β-(*N*-alkanoyl)-estra-1,3,5(10)-trien-3-O-sulfamates (**1.76**, Fig. 1.24) inhibited STS in intact MDA-MB-231 cells.[85] At 10 nM, the level of inhibition for all of them was similar to or exceeded that of EMATE. Some of these compounds (n = 5) exhibited IC_{50}'s as low as 0.5 nM and were not found to be estrogenic as determined by measuring the growth of estrogen-dependent MCF-7 human breast cancer cells at a concentration of 1 μM. Later Li et al. reported in a series of patents other 17-(*N*-alkylcarbamoyl)-estra-1,3,5(10)-triene-3-O-sulfamates (**1.77**) and the inverse amides are good inhibitors of STS with IC_{50} values ranging from the mid to low nanomolar using STS from CHO cells and E1S as substrate.[86-88]

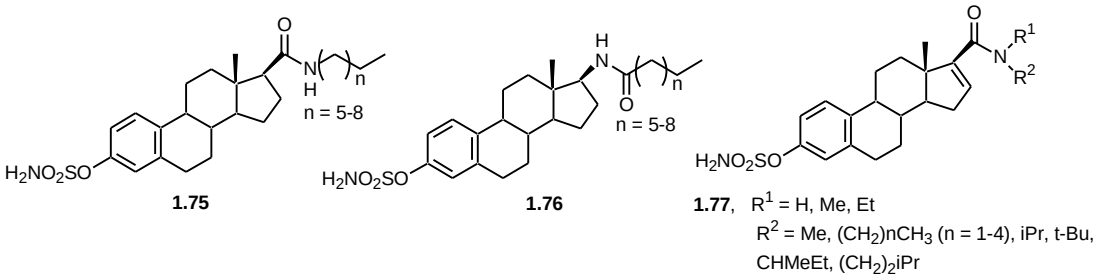


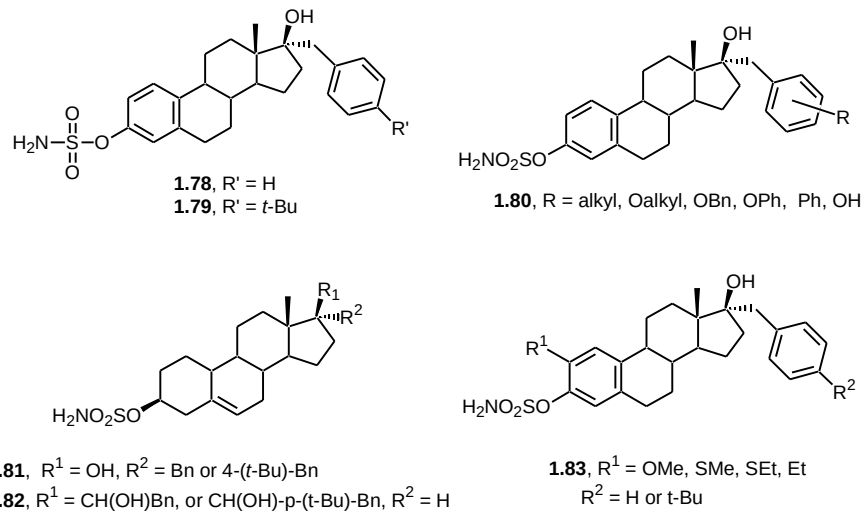
Fig. 1.24. 17β-(*N*-alkylcarbamoyl)-estra-1,3,5(10)-trien-3-*O*-sulfamates (**1.75** and **1.77**) and 17β-(*N*-alkanoyl)-estra-1,3,5(10)-trien-3-*O*-sulfamates (**1.76**).

The Li and Ishida groups have examined the bis-isopropyl derivative in more detail (**1.77**, $R^1 = R^2 = i$-Pr, also known as KW-2581, Fig.1. 24).[89,90] KW-2581, which exhibited an IC_{50} of 4.0 nM with crude human STS from transfected CHO cells and using MUS as substrate, was found to be non-estrogenic and inhibited tumour growth in a nitrosylmethylurea-induced rat mammary tumor model and a mouse xenograft model. It was also demonstrated that KW-2581

could inhibit the ability of AdiolS (see Fig. 1.7) to stimulate the in vivo growth of MCF-7 breast cancer cells overexpressing STS.

Several groups have examined 17α-benzyl derivatives of E2-3-*O*-sulfamate as well as other D-ring-benzylated steroidal sulfamates as STS inhibitors (Fig. 1.25). These compounds were based upon the observation by Poirier and coworkers that E2 derivatives bearing an α-benzylic group at the 17-position are potent STS inhibitors mentioned earlier.[65-69]

Poirier and coworkers examined compounds **1.78** and **1.79** (Fig. 1.25) as STS inhibitors in a homogenate of human embryonic kidney (HEK) cells transiently transfected with an STS expression vector and JEG-3 cells respectively.[66] Compounds **1.78** and **1.79** were 5- and 14-fold more potent than EMATE respectively for the transformation of DHEAS to DHEA. Compound **1.79** was examined in more detail and found to inhibit STS in a time and concentration-dependent manner. The same workers reported that **1.78** (IC_{50} = 85 nM) was 4-fold less potent than EMATE (IC_{50} = 20 nM) but considerably more potent than the corresponding non-sulfamoylated derivative **1.50** (Fig. 1.13) (IC_{50} = 6100 nM) in a placental microsomes assay using E1S as substrate. The antiproliferative activity for **1.78** was modest (10 μM gave 50% inhibition of basal MCF-7 cell growth in the absence of estrogen precursor E1S). Moreover, it was noted that the sulfamate group was not necessary for antiproliferative activity for **1.78** as the non-sulfamoylated analog **1.50** exhibited slightly superior antiproliferative activity.



1.78, R' = H
1.79, R' = *t*-Bu

1.80, R = alkyl, Oalkyl, OBn, OPh, Ph, OH

1.81, R^1 = OH, R^2 = Bn or 4-(*t*-Bu)-Bn
1.82, R^1 = CH(OH)Bn, or CH(OH)-p-(t-Bu)-Bn, R^2 = H

1.83, R^1 = OMe, SMe, SEt, Et
R^2 = H or t-Bu

Fig. 1.25. 17α-Benzyl derivatives of E2-3-*O*-sulfamate (**1.78**-**1.80**), 3-*O*-sulfamate derivatives of C19 and C21 steroids bearing *t*-butylbenzyl or benzyl groups (**1.82** and **1.82**) and 2-substituted-17α-benzyl-E2-3-*O*-sulfamates (**1.83**).

Nippon Organon reported in a patent that compounds of this type (compounds **1.80**, Fig 1.26) inhibited STS with IC_{50} values ranging from 30-160 nM and were more potent than EMATE under the same assay conditions (400 nM).[91]

The Poirier group has also examined 3-*O*-sulfamate derivatives of C19 and C21 steroids bearing a *t*-butylbenzyl or a benzyl group as steroid sulfatase inhibitors (**1.81** and **1.82**, Fig. 1.25) using crude STS obtained from HEK 293 homogenates and E1S as substrate.[58] No significant inhibition was found at a concentration of 3 μM when only a sulfamate group was added. With only a *t*-butylbenzyl or a benzyl group but no sulfamate group, good inhibition was obtained for pregn-5-ene series (IC_{50}'s 60-360 nM) but not the androst-5-ene series (IC_{50}'s > 1 μM). Addition of a sulfamate moiety to the *t*-butylbenzyl or benzyl-bearing compounds resulted in modest increases (1 to 2-fold) in potency when using E1S as substrate though more significant increases in potency were found when using DHEAS as substrate (up to 7.5-fold increase in potency). 3β-Sulfamoyloxy-17α-*t*-butylbenzyl-5-androsten-17β-ol (**1.81**, R^1 = OH, R^2 = 4-(*t*-Bu)-Bn) was the most potent compound with IC_{50} values of 46 and 14 nM; respectively for the

transformations of E1S to E1 and DHEAS to DHEA. However, in contrast to 17α-*t*-butylbenzyl-EMATE (**1.79**), this compound did not induce any proliferative effect on estrogen sensitive ZR-75-1 cells nor on androgen-sensitive Shionogi cells up to the highest concentration tested (1 μM).

Potter and coworkers have examined 2-substituted-17α-benzyl-E2-3-*O*-sulfamates (compounds **1.83**, Fig 1.25) as STS inhibitors. 2-MeO-17α-benzyl EMATE analogues (**1.83**, R^1 = OMe, R^2 = H or *t*-Bu, IC_{50}'s = 430 and 4300 nM respectively) were much less potent STS inhibitors than 2-MeOEMATE or EMATE in a placental microsome assay using E1S as substrate and did not exhibit antiproliferative activity at 10 μM in an MCF-7 assay.[92] This is in contrast to a report where Poirier and coworkers found that the same 2-MeO-17α-benzyl EMATE analogues (**1.83**, R^1 = OMe, R^2 = H or *t*-Bu) were found to be more potent (IC_{50}'s of 0.024 and 0.040 nM for the R^2 = H and R^2 = *t*-Bu derivatives respectively) than EMATE when assayed using STS obtained from HEK 293 homogenates and E1S as substrate.[93] Poirier and co-workers verified the activity of 2-MeO-17α-benzyl EMATE (**1.83**, R^1 = OMe, R^2 = H) in an animal model and found this inhibitor to block stimulation induced by E1S on the uterine weight of OVX mice.[93] Surprisingly, Potter and coworkers found that the 17α-benzyl derivative of 2-MeSEMATE (**1.83**, R^1 = SMe, R^2 = H) was a 3-fold more potent STS inhibitor than 2-MeSEMATE (IC_{50}s of 44 and 120 nM, respectively) in a placental microsomes assay and did exhibit some antiproliferative activity at 10 μM in the MCF-7 assay.[92] It is not yet clear why this effect is not seen with the 17α-benzyl derivatives of EMATE and 2-MeOEMATE under these conditions.

In addition to the benzylated derivatives discussed above, a variety of other C-17-modified steroidal sulfamates have been examined as STS inhibitors (Fig. 1.26). Potter and

coworkers have evaluated the antiproliferative activity of an array of 17-oxime derivatives of 2-MeOEMATE (compounds **1.84**, Fig. 1.26) in MCF-7 cells.[92] With the exception of the $NCH_2C_6F_5$ derivative, these compounds displayed equal or superior antiproliferative activity compared to 2-MeOEMATE and 2-EtEMATE (GI_{50} = 2.2 and 0.92 μM respectively). The sulfamoyl group was found to be necessary for good antiproliferative activity.

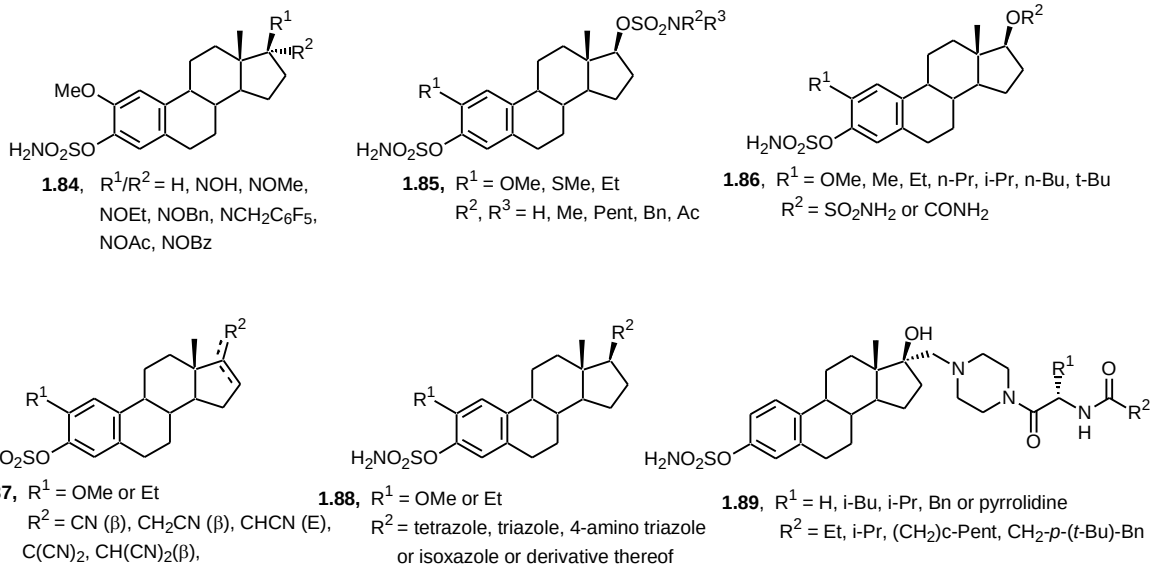


Fig. 1.26. 17-Oximo and 17-imino derivatives of 2-MeOEMATE (**1.84**), 3,17-*O*,*O*-bis-sulfamates (**1.85** and **1.86)** and estra-1,3,5(10)-triene-3-*O*-sulfamates bearing cyano (**1.87**) heterocyclic (**1.88**) and piperidinyl substituents (**1.89**) at C17.

The STS inhibitory and antiproliferative activity of 3,17-*O*,*O*-bis-sulfamates (E2bisMATEs, compounds **1.85**, Fig. 1.26) have been reported.[94,95] 3,17-*O*,*O*-bis-sulfamates bearing unsubstituted sulfamate groups (SO_2NH_2) at the 3- and 17-positions with or without a methoxy group at the 2-position were excellent irreversible STS inhibitors (IC_{50} 18-39 nM) and were much more effective than the corresponding 17-*O*-monosulfamate derivatives when assayed using placental microsomes and E1S as substrate. Bis-sulfamates bearing a methoxy or ethyl group at the 2-position exhibited potent antiproliferative activity with DU145, MDA-MB-231, and MCF7 cells and with mean graph midpoint values of 18-87 nM in the NCI 60-cell-line

panel. The 2-Et derivative dosed P.O. caused growth inhibition in a nude mouse xenograft tumor model.

A more extensive study of bis-sulfamoylated as well as 3-sulfamoyl-17α-carbamate derivatives was carried out (compounds **1.86**, Fig. 1.26).[96] Evaluation against human cancer cell lines (DU145, MDA-MB-231, and MCF-7) revealed the 2-methyl (DU145 GI_{50} = 0.38 μM) and 2-ethyl derivatives to be the most active novel bis-sulfamates (DU145 GI_{50} = 0.21 μM), while the 2-ethyl-17-carbamate derivative (GI_{50} = 0.22 μM) proved most active of its series. Larger C-2 substituents were deleterious to activity for both series.

17-Cyanated 2-substituted estra-1,3,5(10)-trienes (compounds **1.87**, Fig. 1.26) have been examined as STS inhibitors and as antiproliferative agents.[97] 2-Methoxy-17β-cyanomethyl-E2, but not the related 2-ethyl derivative, and the related 3-*O*-sulfamates displayed potent antiproliferative effects against human cancer cells in vitro (with MCF-7 cells, GI_{50} = 300, 60 and 70 nM, respectively). The 3-*O*-sulfamate of 2-methoxy-17β-cyanomethyl-E2 showed good activity in an athymic nude mouse MDA-MB-231 human breast cancer xenograft model when administered orally.

The anti-proliferative activities of a series of 2-substituted estra-1,3,5(10)-triene-3-*O*-sulfamates bearing heterocyclic substituents (oxazole, tetrazole, triazole) tethered to C-17 (compounds **1.88**, Fig. 1.26) has been reported.[98] *In vitro* evaluation of these molecules revealed that high anti-proliferative activity in breast and prostate cancer cells lines (GI_{50} of 340–850 nM with (DU145, MDA-MB-231 and MCF-7) could be retained when the heterocyclic substituent possesses H-bond acceptor properties. A good correlation was found between the calculated electron density of the heterocyclic ring and anti-proliferative activity.

Poirier and coworkers used a solid phase approach to synthesize a series of *N*-derivatized 17α-piperazinomethyl estradiol derivatives (compounds **1.89**, Fig. 1.26) which were subsequently evaluated for STS inhibitory activity using homogenized HEK 293 cells overexpressing STS and E1S as substrate.[99] Many of the compounds were more potent than EMATE. Those bearing a hydrophobic amino acid and carboxylic acid substituents were the most effective inhibitors with the most potent being as effective as 17α-*t*-butylbenzyl estradiol 3-*O*-sulfamate mentioned previously (**1.79**, Fig. 1.25). The corresponding non-sulfamoylated compounds were considerably less potent. The estrogenicity and antiproliferative ability of these compounds was not reported.

Peters et al have reported the evaluation of a large array of steroidal cyclic sulfamates bearing a wide variety of substituents at the 17-position as inhibitors of STS (compounds **1.90**, Fig. 1.27). These compounds exhibited IC_{50}'s in the low to high nM range in MCF-7 cells with compound **1.91** (Fig. 1.27) being the most potent (IC_{50} = 9 nM). Compound **1.91** was reported to be active in a breast cancer xenograft model in vivo.[100] The mode of inhibition (reversible or irreversible) of these compounds was not reported. Compounds **1.92** and **1.93** (Fig. 1.27) were also found to be good inhibitors with IC_{50} of 44 and 15 nM respectively. These results are in contrast to Woo et al. recent report that the five-membered cyclic sulfamates **1.94** and **1.95** (Fig. 1.27) are not inhibitors of STS up to concentrations of 10 μM when evaluated in a placental microsomes preparation of STS and in MCF-7 cells.[101] Interestingly, Hanson et al. reported that the non-steroidal cyclic sulfamates, **1.96** and **1.97** (Fig. 1.27), are irreversible, time and concentration dependent inhibitors of the aryl sulfatase from *Pseudomonas aeruginosa;* unfortunately, these compounds were not examined as STS inhibitors.[102]

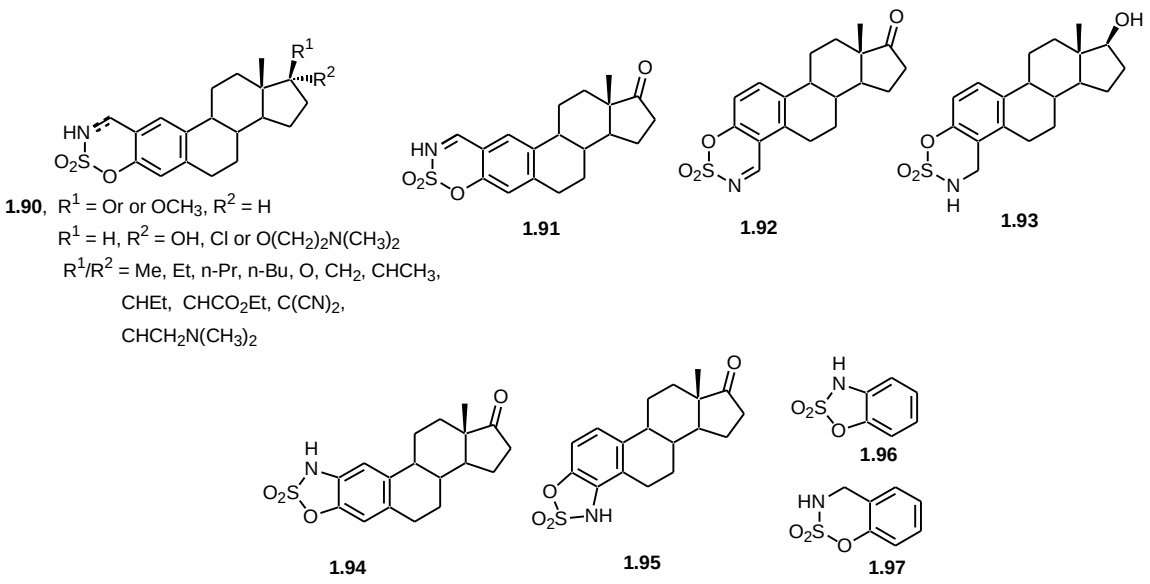


Fig. 1.27. Cyclic sulfamates.

The 17-deoxy analog of EMATE (**1.98**, NOMATE, Fig. 1.28) inhibited activity in MCF-7 cells by 97% at 10 nM, similar to the inhibition achieved with EMATE.[101,103] In contrast, the 17-deoxy analogue of 2-MeOEMATE (**1.99**, Fig. 1.28) and the related 2-ethyl and 2-methylsulfanyl compounds (**1.100** and **101**, Fig. 1.28) showed significantly reduced inhibition of MCF-7 proliferation.[92]

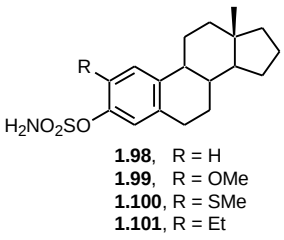


Fig. 1.28. 17-Deoxy analogs of EMATE and 2-substituted EMATE

There are only a handful of examples where analogs of EMATE have been developed that have been modified on the D-ring at positions other than or in addition to the 17-position or on the B or C rings. Poirier and coworkers have reported the solid phase synthesis of a library of 16β-aminopropyl estradiol derivatives (**1.102**, Fig. 1.29), which were subsequently evaluated for

STS inhibitory activity using homogenized HEK 293 cells overexpressing STS and E1S as substrate.[105] Several library members containing hydrophobic amino acids or substituents were more potent inhibitors than EMATE. The estrogenicity and antiproliferative ability of these compounds was not reported.

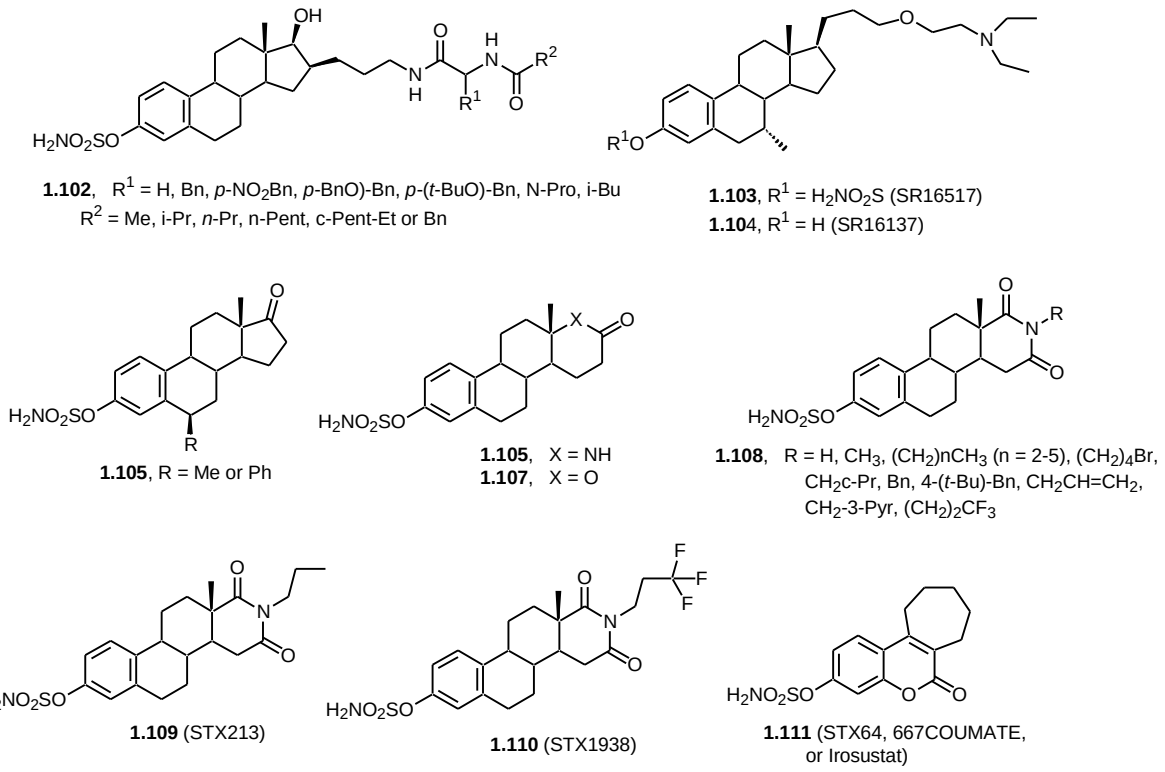


Fig. 1.29. 16β-Aminopropyl estradiol derivatives of E2EMATE, (**1.102**), SR 16517 (**1.103**) and SR 16137 (**1.104**), 6-substituted EMATE derivatives (**1.105**), cyclic amide (**1.106**), cyclic ester (**1.107**), and imide derivatives (**1.108-1.110**).

The Peters and Lykkesfeldt groups have reported compound **1.103** (Fig. 1.29), known as SR 16157, as a dual-action STS inhibitor and antiestrogen.[105] This compound is the sulfamate of the known antiestrogen **1.104** (Fig. 1.29). Upon inhibition of STS by **1.103**, the antiestrogen **1.104** would be produced and interact with the ER thus providing a dual mode of action. Compound **1.103** exhibited an IC_{50} of 100 nM when assayed using an MCF-7 extract and E1S as substrate. Compound **1.103** was found to bind poorly to the ER yet it was 10-fold more potent than **1.104** in inhibiting the growth of MCF-7 cells.

6-Me- and 6-phenyl-substituted EMATE (compounds **1.105**, Fig. 1.29) have been examined as STS inhibitors using placental microsomes preparations of STS and E1S as substrate.[106] These compounds were found to be much poorer inhibitors of STS than EMATE.

Potter and coworkers reported in a patent that cyclic amide **1.106** (Fig. 1.29) exhibited 91% inhibition of STS activity at 100 nM in MCF-7 cells.[107] In an independent patent, Koizumi et al. reported that the analogous ester **1.107** (Fig. 1.29) exhibited 97% and 78% inhibition at 10 nM and 1 nM respectively.[108] Both **1.106** and **1.107** blocked liver STS activity in rats at an oral dose of 2mg/kg/day over 5 days and both were found to be nonestrogenic.

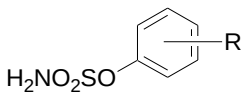
In a somewhat different approach to D-ring modification, Potter and coworkers described a series of imide derivatives of type **1.108** (Fig. 1.29) as STS inhibitors.[109-112] Several of these compounds (R = Me, n-Pr, Bn and (3-pyridyl)methyl, IC_{50}'s 1-12 nM) were more potent than EMATE (IC_{50} = 18 nM) using STS in placental microsomes and E1S as substrate. The two most potent derivatives, the *n*-propyl and (3-pyridyl)methyl compounds (IC_{50}'s = 1 nM) were found to almost completely inhibit rat liver STS in vivo and to be devoid of estrogenic activity in the uterine weight gain assay. The propyl derivative, (compound **1.109**, known as STX213, Fig. 29) was shown to reduce circulating E2 levels by >90% and arrest tumor progression stimulated by E2S in a MCF-7 xenograft breast cancer model. In this regard, STX213 was found to be superior to STX64 (compound **1.111**, also known as 667-COUMATE and by the generic name Irosustat, Fig. 1.29) a non-steroidal STS inhibitor discovered in the 1990's that has been undergoing evaluation in clinical trials (see section 1.5) It also had an improved duration of activity in vivo compared to STX64. To improve the pharmacokinetic profile of STX213, the *n*-propyl group was replaced with a 3,3,3-trifluoropropyl group (compound **1.110**, STX1938, Fig. 1.29). This resulted in a 5-fold improvement in in vitro activity using intact JEG-3 cells as the

source of STS. This compound completely inhibited the rat liver STS after a single dose of 0.5 mg/kg, and exhibited a significantly longer duration of action over the *n*-propyl derivative. The improved pharmacokinetic properties were attributed to an increase in metabolic stability and lipophilicity.[109-112]

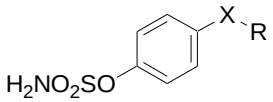
1.4.3 Non-Steroidal Sulfamate-based STS Inhibitors

1.4.3.1 Monocyclic Aryl Sulfamates

Shortly after the discovery of EMATE in 1994,[57] the sulfamate group became a clickable target for medicinal chemists who were looking for designing potent inhibitors of STS that did not have the estrogenic effects of EMATE. One of these approaches is to attach the sulfamate to a substituted aryl ring. The first such derivatives consisted of a sulfamoylated benzene ring substituted with small electron withdrawing groups (compounds **1.112**, Fig. 1.30). These compounds were far less potent STS inhibitors than EMATE.[79]



1.112, R= 3- or 4-NO_2, CN, F, Br, Cl



1.113, X = -O- or -C=O or -COO
R = alkyl or aryl

Fig. 1.30. Simple aryl sulfamates as STS inhibitors.

Attaching alkyl or aryl chains to the sulfamoylated benzene ring by a carbonyl, ether, or ester functional group was another approach examined in designing such type of inhibitors (compounds **1.113)**. Even though the new modifications enhanced the potency of such type of inhibitors compared to **1.112** they still considerably less potent than EMATE.[113-115]

Another approach involved the attachment of another aromatic ring, with or without a spacer, to the sulfamoylated benzene ring (compounds **1.114** and **1.115**, Fig. 1.31). Interestingly,

the benzophenone sulfamates showed moderate inhibitory activity against STS, while the reduced analog, the benzyl phenyl sulfamate, was lacking any activity up to 100 μM.[116] Moreover, Ahmed et al. in 2002 also reported another analog series of EMATE, the biphenyl series, compounds **1.115**, and the 2',4'-dicyano derivative (TZS-8478, compound **1.116**), was among the most potent inhibitors in vitro, in addition of being non-estrogenic.[117,118] Interestingly, it was found that daily administration of compound **1.116** (0.5 mg/kg/day) caused a marked suppression of the growth of breast tumors in rats stimulated by E1. Moreover, **1.116** completely inhibited STS activity in uterus and liver tumours in rats and reduced plasma concentrations of both E1 and E2.[119]

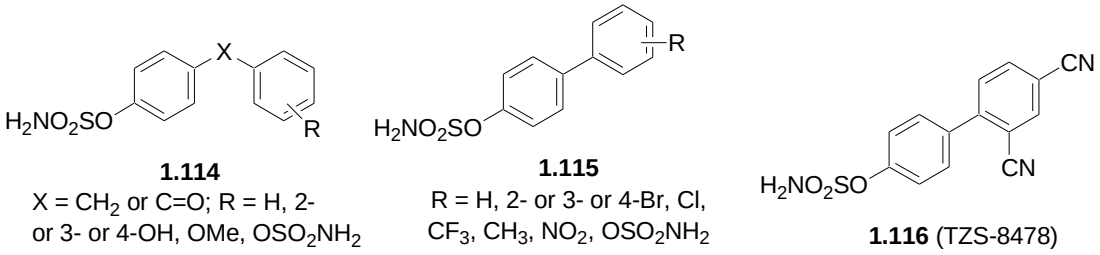
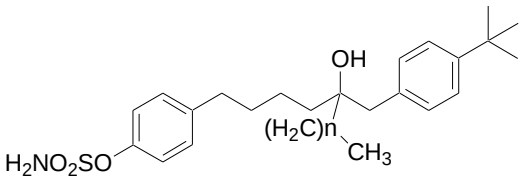


Fig. 1.31. Biphenyl and benzophenone sulfamates as STS inhibitors.

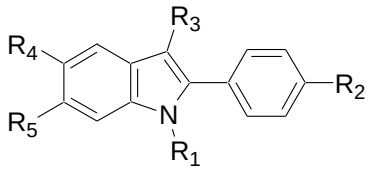
In 2002, Ciobanu and Poirier reported a new series of inhibitors, compounds **1.117** (Fig. 1.32), which has some of the structural features of the previously reported potent STS inhibitor compound **1.79** (Fig. 1.25). In this class of compounds both the C_{18}-Me and the hydrocarbon skeleton (rings B, C, and D), were replaced with an alkyl chain. Even though, compounds **1.117** were slightly less active than their steroidal analogues. Structural features required for STS inhibition by this class of compounds showed that the presence of six carbon atoms in the side chain is the optimal length mimic for the B, C and D steroid rings, between C_6 and C_{17}. The undecanol derivative showed the highest inhibitory potency of this class of compounds with an IC_{50} value of 0.4 nM (2-fold more potent than EMATE).[120]



1.117, n = 3,5,7,9

Fig. 1.32. Compounds **1.117**.

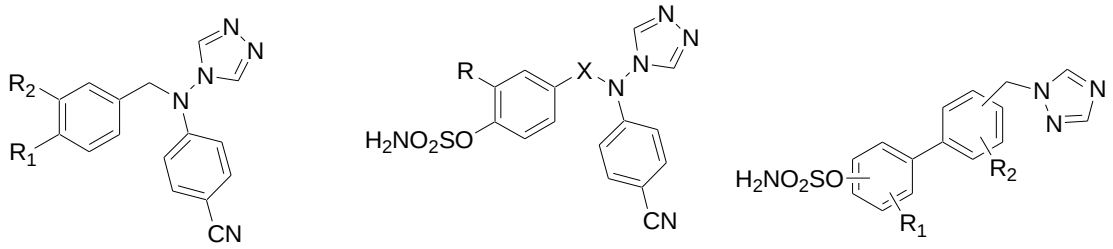
A series of 2-phenylindole sulfamates, compounds **1.118** (Fig. 1.33), with lipophilic side chains at either position 1 or 5 of the indole ring, were reported to be moderate to strong inhibitors of STS in MDA-MB-231 breast cancer cells (IC_{50}'s = 0.002-1 μM). Submicromolar concentrations of compounds **1.118** reduced proliferation of ER (+) MCF-7 breast cancer cells.[121]



1.118, R_1 = alkyl; R_2 = OH or OSO_2NH_2;
R_3 = H or Me; R_4 = H, CN, CO-
-NH(alkyl); R_5 = H or OSO_2NH_2

Fig. 1.33. 2-phenylindole sulfamates as STS inhibitors.

It has been proposed that dual aromatase/sulfatase (DASIs) inhibitors should provide a more effective endocrine therapy. Recently, a series of sulfamoylated derivatives, compounds **1.119b**, of the aromatase inhibitor YM511, compound **1.119a**, have been examined as DASIs and were found to strongly inhibit STS and aromatase in JEG-3 cells with IC_{50} values ranging from 20 to 227 nM and from 0.82 to 100 nM, respectively (Fig. 1.34).[122]



1.119a, R_1 = H, R_2 = Br (YM511)
1.119b, R_1 = OSO_2NH_2, R_2 = H, Br, F, Cl

1.120, X = (CH_2)n, $O(CH_2)$n $S(CH_2)$n, $CH_2S(CH_2)$n; R = H, F, Cl, Br

1.121

Fig. 1.34. Triazole-based DASIs.

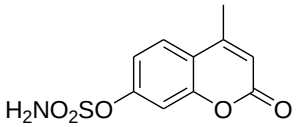
Later on, Woo and coworkers examined the effect of placing the sulfamate group at the *meta* position (**1.119b**) instead of the *para* one as in the previous series (**1.119a**). They found a marked improvement in the inhibitory activity on the aromatase enzyme, especially with derivatives bearing a fluorine ortho to the sulfamate group, which indicates that such motif is a good building block for a preparation of a potent DASI.[123] In 2008, the same group examined another approach by placing a different linker between the aryl sulfamate motif and the triazole-benzonitrile scaffold (compounds **1.120,** Fig. 1.34**)**. They found that nearly all derivatives show improved in vitro aromatase inhibition but with a marked decrease in STS inhibition.[124]

In 2010, Woo and coworkers published an article about joining their previously designed triazole-based DASIs into known biphenyl STS inhibitors. Their approach furnished a series of potent DASIs (compounds **1.121**), with some of them, at 1 mg/kg po, able to significantly reduce plasma E2 levels and strongly inhibit STS activity in the liver in rats. Moreover, these novel DASIs were found to be non-estrogenic.[125]

1.4.3.2 Bicyclic and Polycyclic Aryl Sulfamates

There are numerous studies concerning sulfamate-based inhibitors in which the sulfamate group has been attached to a scaffold that mimics the A and B rings of EMATE. Such scaffolds include naphthalenes, indanones, tetralones, 7-hydroxycoumarins, 4-(thio)chromenones,

benzoxazoles, and benzofurans.[58,126,127] To date, the most important is the coumarin scaffold. COUMATE, compound **1.122** (Fig. 1.35), is the corresponding sulfamate of 4-MUS. This compound inhibits STS in the micromolar range and is non-estrogenic in vivo. SAR studies showed that the optimal position for the sulfamate group, in terms of STS inhibition, is at C_7. Moreover, the inclusion of alkyl substituents in C_3 or C_4 enhances STS inhibition.[58]

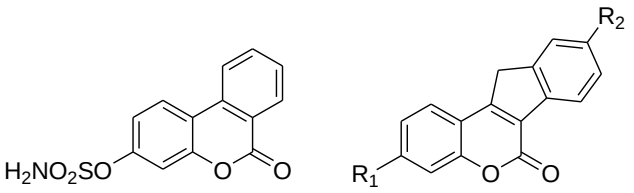


1.122 (COUMATE)

Fig. 1.35. Structure of COUMATE

Sterix Ltd, in the year 2000, reported one of the most potent irreversible STS inhibitors; 667COUMATE (compound **1.111**, STX-64 or Irusostat) which is a carbocyclic ring derivative of COUMATE as shown in Fig. 1.29. STX-64 efficiently inhibited STS in placental microsomal preparations (IC_{50} of 8 nM), without showing any estrogenic side effects. STX-64 is the first STS inhibitor to enter phase I clinical studies for treatment of HDBC.[128,129]

Interestingly, the benzocoumarin sulfamate **1.123,** which is structurally closely related to STX-64, (Fig. 1.36), was found to be 3-times less active than EMATE against purified STS (IC_{50} = 166 nM, vs. 56 nM for EMATE).[130]



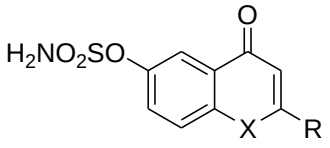
1.123

1.124, R_1= H, or OSO_2NH_2
R_2= H, OH, or OSO_2NH_2

Fig. 1.36. Benzocoumarin (**1.123**) and coumestane derivatives (**1.124**).

The same research group tested another approach in designing STS inhibitors, by preparing coumestane derivatives (Fig. 1.36, compounds **1.124)**. These derivatives exhibited quite divergent activity against STS, with highest potency achieved when R_1 is H and R_2 is equal to OSO_2NH_2 (IC_{50} = 280 nM, versus 18.5 μM when the R groups are inverted), and it was explained that the first regioisomer has much better resemblance to the steroid skeleton than the other isomer when the sulfamate groups were superimposed with that of EMATE.[128]

In 2002, Novartis examined a series of the 4-(thio)chromenone sulfamates, compounds **1.125** (**Fig. 1.37**), as STS inhibitors. Notably, this class demonstrated high inhibitory potency when both the sulfamate group and the side chain were placed in a diagonally opposite positions (i.e., 2,6- and 3,7-substitution pattern). The best inhibitory potency was achieved when fully branched, bulky aliphatic side chains were used in combination with the 4-thiochromenone scaffold. Compounds **1.125** were irreversible STS inhibitors with a biphasic time course of inactivation.[58]

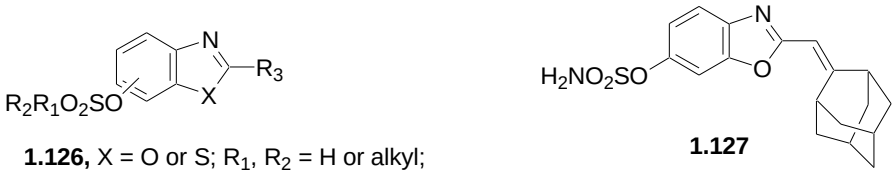


1.125, X= O, or S; R= (cyclo)alkyl, or 1-adamantyl

Fig. 1.37. Chromenone sulfamate STS inhibitors

Researchers at Novartis also published a number of articles about the use of sulfamoylated benzoxazoles and their sulfur analogs (compounds **1.126**) as inhibitors of STS (Fig. 1.38) as STS inhibitors. They preferred to use highly bulky substituents in their design in contrast to the previous chromenone series (**Fig.1.37**), in addition to substituting the bicyclic core with an adamantyl or an adamantylmethyl group. These structural modifications gave derivatives

with moderate inhibitory activity. However, switching from the saturated adamantylmethyl residue to the unsaturated adamantylidienemethyl residue, compound **1.127**, resulted in a derivative which is as efficient as EMATE in enzyme inactivation and was non-estrogenic in vitro. In a proof-of-concept study using rat models compound **1.127** was found to be able to inhibit STS activity in skin after both oral and topical administration. This provided support for STS inhibitors as potential therapeutics for treating acne.[131,132]

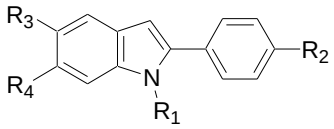


1.126, X = O or S; R_1, R_2 = H or alkyl; R_3 = (un)substituted alkyl

1.127

Fig. 1.38. Sulfamoylated benzoxazoles as STS inhibitors

In 2002, a series of sulfamoyloxy-substituted 2-phenylindoles, compounds **1.128**, were synthesized by a German group who were attempting to design STS inhibitors with no agonistic activity on the ER (**Fig. 1.39**). This series of compounds were found to be able to suppress STS gene expression in stably-transfected MCF-7/2a breast cancer cells via their enzyme inhibitory activity (IC_{50} values in the submicromolar range).[133]

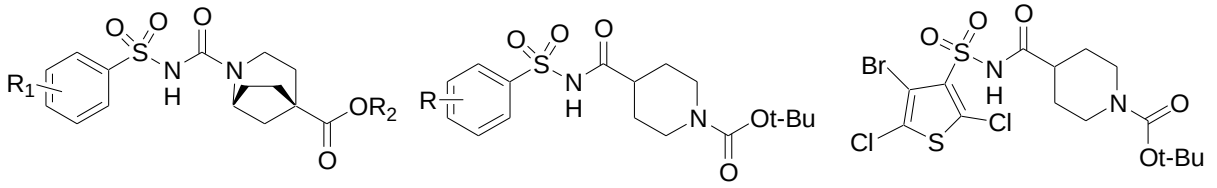


1.128, R_1 = Et, $(CH_2)_5CONC_4H_8$, $(CH_2)_6NC_4H_8$, or $(CH_2)_{10}SO_2C_5H_{10}$; R_2 = H, OH, or OSO_2NH_2; R_3 = R_4 = H, CN, OH, COOMe

Fig. 1.39. Sulfamoyloxy-substituted 2-phenylindoles as STS inhibitors.

1.4.4. Non-Steroidal, Non-Sulfamate-based STS Inhibitors

Nussbaumer and his co-workers at Novartis used high-throughput screening to find novel STS inhibitors (Fig. 1.40). These studies yielded the nortropinyl-arylsulfonylurea derivatives (compounds **1.129**) as moderate to a strong reversible inhibitors of purified STS using MUS as substrate (IC_{50} ranging from 84 nM to 100 μM).[134,135] The same group extended their SAR studies by modifying both the phenylacetyl side chain and nortropine groups. These modifications lead to the *N*-(Boc-piperidine-4-carbonyl)benzenesulfonamides (compounds **1.130**), which exhibited improved cellular potency, with the best inhibitory potency reported for compound **1.131** (IC_{50} of 270 nM using STS over-expressed in CHO-cells, Fig.1.40).



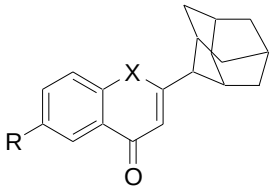
1.129, R_1 = 3- or 4-Cl, F, Br, Me CF_3, or 3,5-di-CF_3; R_2 = Bn

1.130, R = 2- or 4-Cl; 2, 6-di-Cl; 4-OMe 3, 5-di-Cl; 2, 5-di-OMe; or 3,5-di-CF_3

1.131

Fig. 1.40. Nortropinyl-arylsulfonylurea *and N*-(Boc-piperidine-4-carbonyl)benzenesulfonamides as STS inhibitors.

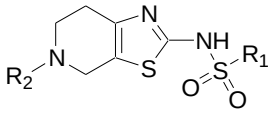
The Novartis group, on the basis of their previous studies on chromenone sulfamates (Fig. 1.37), extended these investigations to using sulfamate replacements. They discovered that 2-(1-adamantyl)-4-(thio)chromenone-6-carboxylic acids (Fig. 1.41, compounds **1.132**) are good reversible inhibitors of purified STS with the best K_i value for the 4-chromenone-6-carboxylic acid derivative (K_i = 0.50 μM). [136]



1.132, X = O or S; R = COOH, CH_2OH, CHO, COOMe, $CONH_2$, CN, $C(O)CH_2OH$, CH_2COOH, C(O)COOH

Fig. 1.41. 2-(1-adamantyl)-4-(thio)chromenone-6-carboxylic acids as STS inhibitors.

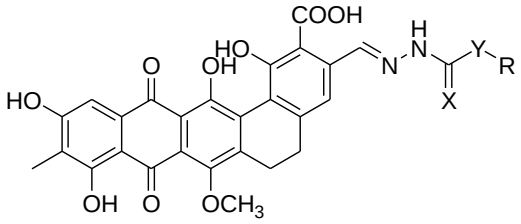
In 2004, Novartis reported novel STS inhibitors having a *N*-sulfonylaminothiazole scaffold (Fig. 1.42, compounds **1.133**), but unfortunately, such compounds were much less potent inhibitors of purified STS than EMATE.[137]



1.133, R_1 = Subst. aryl; R_2 = Subst. Ph or alkoxycarbonyl

Fig. 1.42. *N*-sulfonylaminothiazoles as STS inhibitors.

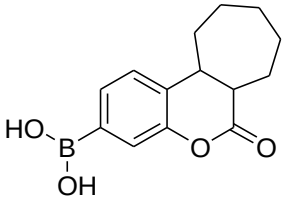
In 2002, Jütten et al. examined 32 thiosemicarbazone and *N*-acyl-hydrazone derivatives of madurahydroxylactone (Fig. 1.43, compounds **1.134**) as inhibitors of STS using E1S as substrate and the placental microsome fraction as a source of crude STS. Their IC_{50} values ranged from 0.35 to >100 μM, with a non-competitive mode of inhibition of STS.[138]



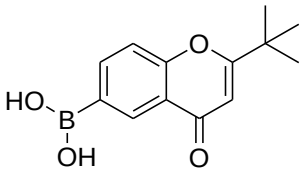
1.134, X = S, or O; Y = NH, NCH_3, or O; R = alkyl, c-alkyl, Ph derv. or piperidino

Fig. 1.43. Thiosemicarbazone and *N*-acyl-hydrazone derivatives of madurahydroxylactone as STS inhibitors.

In 2006 the Taylor group incorporated the boronic acid group into the chromenone and coumarin scaffolds (Fig. 1.44, compound **1.135** and **1.135**). However, these two compounds were moderate reversible inhibitors of purified STS (IC_{50} = 86 and 171 μM, respectively).[63]



Compound **1.59**



Compound **1.60**

Fig. 1.44. Boronic acids as STS inhibitors.

1.5 Clinical Studies with STS Inhibitors

The only STS inhibitor to have entered clinical trials is Irosustat or STX64 (compound **1.111**, Fig. 1.29).[139-141] The results of these trials are mixed; in a phase I clinical trial with postmenopausal women having HDBC, Irosustat to a great extent abolished STS activity in the peripheral blood lymphocytes and tumour tissue; however, reductions in serum E1 and E2 concentrations were still moderate. Nevertheless, five out of 14 patients (previously treated with AIs), showed evidence of stable disease. Interestingly, the concentration of androstenedione was reduced by 86%, which suggests that androstenedione is mainly derived from the peripheral conversion of DHEAS and not by direct secretion from the adrenal cortex. This supports the supposition that STS inhibitors may prove to be very effective in treating androgen-dependent cancers such as prostate cancer.

In a phase II endometrial cancer (EC) open-label trial, 17 out of 36 patients taking Irosustat (STX64) for 6 months showed more stable disease than did megestrol acetate (MA), a drug commonly administered to patients with advanced EC (only 12 out of 37 patients showed

stable disease). However, Irosustat did not show the desired effects on progression-free survival (PFS); for Irosustat the median PFS for 16 out of 36 patients ranged from 9 to 34 weeks, while for MA it was 16-63 weeks in 32 out of 37 patients. Hence development is now more directed to potential use of Irosustat as a combination therapy with AIs.

The somewhat promising results obtained in the initial breast cancer trial with STX64 and the potential of STS inhibitors as drugs for treating other HDCs, such as prostate and endometrial cancer, as well as non-oncological disorders, such as skin disorders, provides the impetus for the future development and study of new/better STS inhibitors.[142,143]

1.6 Thesis Objectives and Overview

The global objective of this thesis is to develop new classes of potent STS inhibitors. It is hoped that such inhibitors could be used as drugs or as lead structures for the development of therapeutics for treating hormone-dependent cancers (HDC).

We prefer to examine our inhibitors with purified enzyme as opposed to using crude enzyme preparations (i.e. placental microsome preparations), since this will enable us to more accurately obtain kinetic parameters and ascertain the mode of inhibition (competitive, noncompetitive, tight-binding, or irreversible). Hence, in *Chapter* 2, we briefly discuss the purification of STS from human placenta, which is one of the richest sources of STS.

In *Chapter* 3 we describe a new class of potent, reversible non-competitive STS inhibitors based on the steroidal scaffold bearing a sulfonamide group at the 17-position. Molecular modelling studies were performed on selected compounds to provide information on where and how they interact with STS.

In *Chapter* 4, we describe the most potent reversible STS inhibitors described to date. These compounds are analogs of our STS sulfonamide inhibitors described in *Chapter* 3 in that

they have a small electron-withdrawing group at the 2- and/or 4-positions. Some of these inhibitors, as well as some described in *Chapter* 3, were sent to the National Cancer Institute (Bethesda, Maryland, USA) where they were examined for their in vitro anti-proliferative activity against the NCI 60-cancer cell line screen.

In *Chapter* 5, initial efforts to introduce a sulfamate group into the 3-position of our sulfonamide-based inhibitors were done. We also describe our preliminary efforts to prepare an E1S analog in which the sulfate group is replaced with a 1,2,5-thiadiazolidin-3-one-1,1-dioxide ring. It is anticipated that this group will act as a novel hydrolytically stable sulfate mimic and will be useful in developing STS inhibitors.

Chapter 2 - Purification of Steroid Sulfatase

2.1. Introduction

2.1.1 Why Purify STS?

The vast majority of inhibitor studies on STS that have been reported have used impure STS either in the form of the microsomal fraction from human placenta or as cell lysates or homogenates from CHO, HEK-293, or JEG-3 cells expressing STS.[70,77,122,144-146] This makes performing detailed kinetic studies on the inhibitors challenging as contaminating enzymes can affect the results if using a general sulfatase substrate such as 4-MUS in the enzyme assays or time consuming if using radiolabelled substrates such as Tritiated E1S. Regardless of substrate, reproducibility can also be challenging when using impure enzyme.

The Taylor group performs their inhibition studies with purified STS since we believe that this provides more accurate and reproducible results. To our knowledge, with the exception of the Novartis group in Austria, the Taylor group is the only group that currently uses purified STS for their inhibition studies. STS is usually purified from human placenta. It does not express well in bacteria.[147] Although a report by Sterix Ltd. has appeared in which recombinant STS was overexpressed as a hexa-histidine tagged fusion protein in 293-EBNA cells (human, embryonic kidney cells expressing Epstein Barr virus nuclear antigen-1)[148] most researchers working with pure STS, such as ourselves and the Novartis group, still purify STS from human placenta mainly for reasons of ease and/or economy.

There are several published purifications of STS mainly from human placenta.[30,149,150] In all of these instances, the purification requires many columns, the yield is usually very low and the purity sometimes suspect. The purification that is used in the Taylor group was, in part,

developed by researchers at Novartis (never published by Novartis) and has since been modified and improved upon in the Taylor group. It makes use of an immunoaffinity column to purify STS from human placenta and is vastly superior to other published procedures in terms of time, purity, yield, and specific activity.

2.2. Objectives

The objective of the work described in this chapter is to purify STS using a procedure developed by researchers at Novartis and modified in the Taylor group.[151]

2.3. Results and Discussion

2.3.1. Placental Purification of STS

The extraction protocol of STS from human placenta was based on extraction procedures provided by Hernandez-Guzman et al.[152] Briefly, a number of homogenization and extraction steps of the placental tissue to obtain microsomal fraction were initially performed. This involved homogenization of the placental tissues in a 50 mM tris-HCl, pH 7, 0.25 M sucrose, pH 7.4 followed by centrifugation at 20,000 × *g* at 0^{o}C. The pellet was resuspended in the same buffer and centrifuged again. The pellet was suspended in an extraction buffer consisting of 20 mM tris-HCl, pH 7.4, and 0.3% *v*/*v* Triton X-100. This was subjected to ultracentrifugation at 100,000 × *g* and the supernatant was saved. This pellet was resuspended in the extraction buffer and the centrifugation repeated. The pellet was discarded and the two supernatants combined to give the solubilized microsomal fraction of the placenta in which STS resides.

Shankaran et al. reported that partial purification of STS can be achieved using an anion exchange column (DEAE, DiEthylAminoEthyl cellulose). A salt gradient is used to elute STS whose isoelecterical point (pI) is 6.9. This procedure has been widely used in many STS

purifications. Before we applied our material to a DEAE column it was necessary to dialyze the microsomal fraction into 20 mM tris-HCl, pH 7.4 but containing only 0.1% *v*/*v* Triton X-100 since high concentrations of Triton X-100 will not allow the STS protein to adhere to DEAE. After dialysis the solubilized microsomal fractions were applied to a DEAE column and STS was eluted with a salt gradient as shown in Fig.2.1.

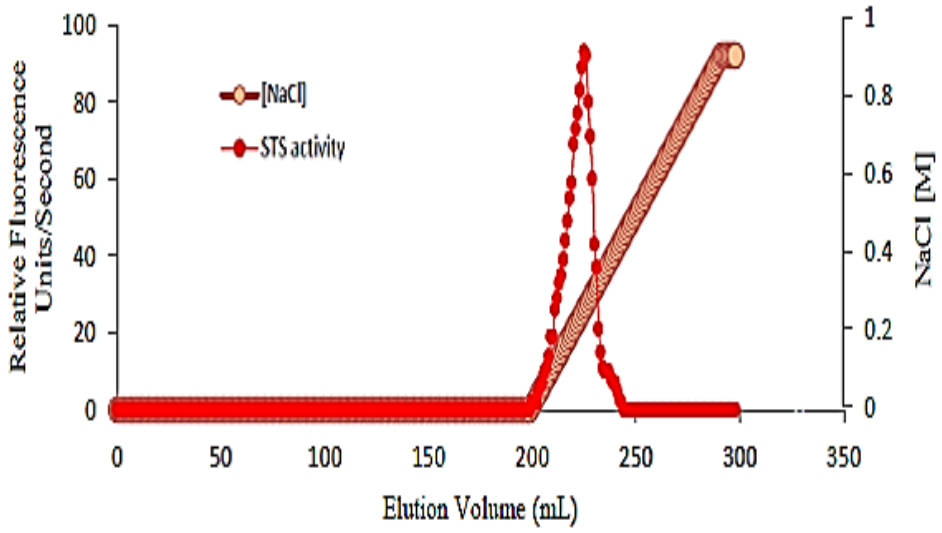


Fig. 2.1. Elution profile of STS activity by DEAE chromatography

Fractions from the DEAE column were assayed for STS activity using 4-methylumbelliferyl sulfate (4-MUS), a substrate that is widely used to assay STS and other sulfatases.[153] In this assay, the production of a fluorescent species called 4-methylumbelliferone (4-MU) is monitored using a fluorescence reading plate reader (Fig. 2.2). 4-MU is not highly fluorescent at λ_{ext} = 360 nm, however; when it is deprotonated it gives a highly fluorescent species with λ_{em} = 460 nm. Although the pK_a of 4-MU is 7.8, there is still enough of the fluorescent anion present at pH 7.0 such that the reaction can be easily followed continuously.[153]

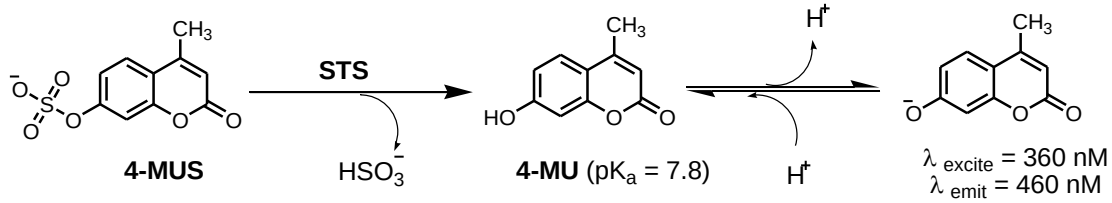


Fig. 2.2. The fluorogenic assay of STS using 4-MUS.

Fractions from the DEAE column containing sulfatase activity were pooled and then dialyzed into 20 mM Hepes buffer, pH 7.4, 1% v/v Triton X-100. These pooled fractions were applied to an immunoaffinity column consisting of anti-STS monoclonal antibodies covalently linked to Sepharose 4B. The column was washed with the same buffer and then 20 mM Hepes buffer containing, 100 mM NaCl, 0.1% v/v Triton X-100 at pH 7.4, and then STS was eluted with 50 mM citric acid, pH 2.7, 140 mM NaCl, 0.1% v/v Triton X-100 (fractions 49 to 53), as shown in Fig.2.3. Fractions containing STS activity were immediately pooled and then immediately neutralized by dialysis into 20 mM Tris, pH 7.4, 0.1% v/v Triton X-100 (STS storage buffer).

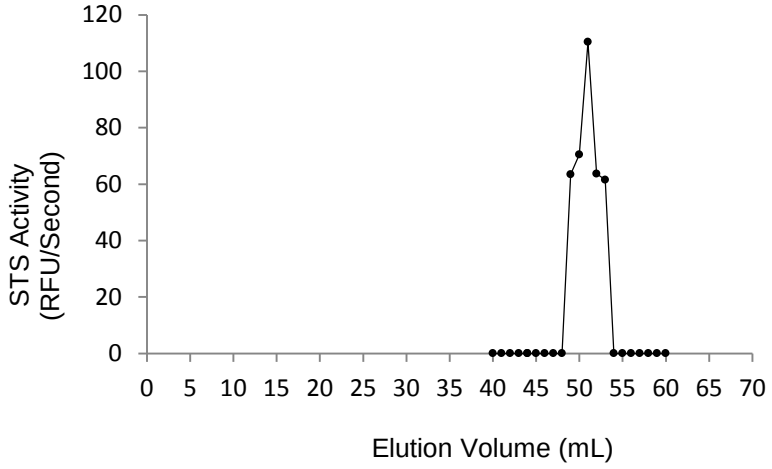


Fig. 2.3. Elution profile of STS activity from the anti-STS immunoaffinity column. The application of 50 mM citric acid, pH 2.7, 140 mM NaCl, 0.1% v/v Triton X-100 began after the column had been washed with 40 mL of 0 mM Hepes buffer containing, 100 mM NaCl, 0.1% v/v Triton X-100 at pH 7.4.

2.3.2 Evaluation of the Protein Concentration, Specific Activity and Molecular Weight of STS

Shankaran et al. and Vaccaro et al. have reported the purification of STS from human placenta by protocols different for the one reported here and both required four to five chromatographic steps after extraction from placenta. Their specific activities were 0.2 nmol 4-MU produced/mg/min in 0.25 M tris-HCl, pH 7.3 at 37^{o}C (Shankaran et al),[154] and 70 nmol 4-MU produced/mg/min in 0.02 M tris, pH 6.8, pH 7.0 (Vaccaro et al).[155] It appears that the specific activity of the protein purified in our lab is much higher than that of Shankaran et al., and Vaccaro et al, as seen in Table 2.1. The advantage of the method we used is that it employs a fewer number of chromatographic steps than those employed by both Shankaran and Vaccaro et al's.

Table 2.1. STS protein concentration and specific activity from three separate purifications.

Purification	Protein Conc. (mg/mL)	Specific Activity (μmol/mg/min)
1st	0.175	0.33
2nd	0.339	0.38
3rd	0.256	0.41

SDS-PAGE of our purified STS (Fig. 2.4) indicated a high degree of purity. The shadow bands that are present on our SDS-PAGE have also been reported by others and are attributed to *N*-glycosylation of STS.[52,152] It has been reported in the literature that STS has a wide range of molecular weights for its monomeric form with values ranging from 63-78 kDa.[149,150,155,156] The molecular mass of the major band detected by the SDS-PAGE as shown in Fig. 2.4 is approximately 68 kDa and 63 kDa for deglycosylated form, which is in a good agreement with

the results reported by Hernandez-Guzman *et al*., who reported a molecular mass of 65 kDa based on SDS-PAGE and whose placental extraction method we followed.[152]

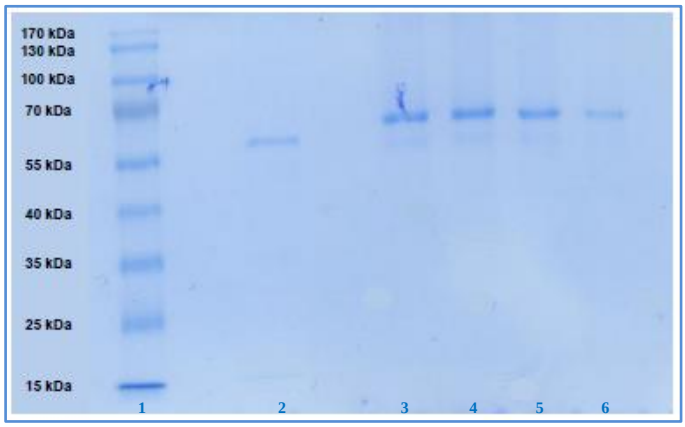


Fig. 2.4. SDS-PAGE of the purified STS. The gel was stained in Fermentas PageBlue™ Protein staining solution. lane 1 (protein Ladder), lane 2 (deglycosylated STS), lanes 3, 4, 5, and 6 (native STS at different concentrations).

There used to be conflicting evidence in the literature concerning the functional oligomer of STS, which had been reported as either trimeric,[155] tetrameric,[149] or hexameric,[156] according to SDS-PAGE. In fact these faint shadow bands were used later to grow diffraction-quality crystals to elucidate the first X-ray crystal structure of STS.[152] However, with the publication of the crystal structure in 2003,[30] there is now an agreement that the protein is a monomer and the previous reports of multimeric forms were caused by variations in detergent solubilization.[154,155]

2.4. Conclusions

The purification of STS is an important initial step for our inhibition studies as it allows for accurate kinetic analysis of our STS inhibitors. In the Taylor lab, we have developed a reliable method for the purification of STS from human placenta which yields the enzyme in very high purity and specific activity. This method represents an efficient adaptation of an established ion exchange chromatography method paired with an immunoaffinity column to

yield a large quantity of highly purified enzyme, essential for the detailed inhibitor studies we are performing.

2.5 Experimental

2.5.1 Materials

Biochemical reagents and buffers were obtained from Sigma-Aldrich® Chemicals Co. (St. Louis, Missouri, USA) unless stated otherwise. DEAE cellulose (DE-52) was obtained from Whatman® (Maidstone, UK). The STS Immunoaffinity column was prepared by Vanessa Ahmed, a previous PhD student in the Taylor group, by coupling an anti-STS monoclonal antibody, obtained as a gift from Novartis Austria GmbH, to a CNBr-activated Sepharose 4B obtained from Pharmacia (Sweden) at a density of 10 mg/ml resin. The procedure for purification of STS by immunoaffinity chromatography was provided by Dr. Andreas Billich at Novartis Austria GmbH. Protease inhibitor cocktail was obtained from Sigma-Aldrich® Chemicals Co. DC Protein Assay kit for Bradford protein determination was obtained from Bio-Rad Laboratories (Richmond, California). A gel electrophoresis silver-staining kit was obtained from InvitrogenTM (Carlsbad, California). Gel electrophoresis PageBlueTM Protein Staining Solution was obtained from Fermentas Life Science (Vilnius, Lithuania). Fluorometric assays were performed using a SpectraMax Gemini XS plate reader equipped with SOFTmax® Pro Version 3.1.1 software from Molecular Devices (Sunnyvale, California). Human placenta was obtained from Credit Valley Hospital, Mississauga, Ontario, shortly after birth, and immediately frozen at -80 ^{o}C until purification, for no longer than two weeks.

2.5.2 Methods

2.5.2.1 Activity Assay

Steroid sulfatase (STS) activity was assayed by the addition of 20 µL of sample to 180 µL of 0.1 M tris, pH 7.0, containing 200 µM 4-MUS**,** in a 96-well black microtiter plate (Corning), similar to the method reported by Roy, A. B. in 1971.[153] Production of fluorescent product (4-MU) was monitored for 10 minutes at an excitation λ of 360 nm and an emission λ of 460 nm, using a fluorescence plate reader (Gemini XS, Molecular Devices, Sunnyvale, CA). Enzyme activity was monitored in terms of relative fluorescence units per second (RFUs/Second) using a data acquisition software package, Softmax Pro 3.1.1.

2.5.2.2 Homogenization and Chromatography

Full-term human placenta was defrosted slowly at room temperature and a 200 g sample (excluding umbilical cord and surrounding membranes) was homogenized using a Brinkman polytron in 50 mM Tris HCl pH 7.5, 0.25 M sucrose, and 1g of protease inhibitor cocktail (Sigma-Aldrich®). The homogenate (400 mL) was centrifuged (20,000 × g, 30 min., 4° C), and the supernatant was discarded. The pellet was resuspended in the same buffer (300 mL) used in the homogenization and subjected to an additional centrifugation (20,000 × g, 30 min., 4°C). The supernatant was discarded and the pellet was resuspended in an extraction buffer of 20 mM Tris HCl pH 7.4, 0.3% v/v Triton X-100 (300 mL) and subjected to ultracentrifugation (100,000 × g, 70 min., 4°C). After centrifugation the supernatant was saved while the pellet was resuspended in the same extraction buffer (200 mL) and subjected to a second ultracentrifugation (100,000 × g, 70 min., 4°C). The resulting supernatant was pooled with that of the first ultracentrifugation and the pellet was discarded. This microsomal fraction (400 mL) was dialyzed into 20 mM tris HCl, pH 7.4, 0.1% v/v Triton X-100 (4 L × 3) and then subjected to a DEAE column (250 mL of DE-52, Whatman™) according to the procedure of Hernandez-Guzman et al.[30] After the dialysate was applied, the column was washed with 5 column volumes of 20 mM tris HCl, pH

7.4, 0.1% v/v Triton X-100, and eluted with a linear gradient of 10 column volumes of increasing NaCl concentration of up to 1 M. The pooled fractions containing STS (250 mL) were dialyzed into 20 mM Hepes buffer, pH 7.4, 1% v/v Triton X-100 (2 L × 3). To obtain pure STS, dialyzed fractions from the DEAE column were applied to a anti-STS immunoaffinity column (2.5 mL) that had been pre-equilibrated with 10 column volumes of the dialysis buffer based on a method provided to us by Novartis. The immunoaffinity column was prepared by coupling a purified monoclonal antibody raised against STS to a CNBr-activated Sepharose 4B at a concentration of 10 mg antibody per mL of resin according to the manufacturer's instructions (Pharmacia). The column was washed with 5 column volumes of the same buffer and then 10 column volumes of 20 mM Hepes, pH 7.4, 100 mM NaCl, 0.1% v/v Triton X-100 and then eluted with 10 column volumes of 50 mM citric acid, pH 2.7, 140 mM NaCl, 0.1% v/v Triton X-100. Fractions containing STS activity were immediately pooled and were neutralized by dialysis into 20 mM Tris, pH 7.4, 0.1% v/v Triton X-100 (STS storage buffer). The dialysed enzyme was then divided into aliquots of 40 µL each and flash frozen in N_2 (l) and stored at -80^{o}C until use. The purified STS homogeneity was > 95% as judged by 10% SDS-PAGE (stained with PageBlue^{TM} Protein Staining Solution, Fermentas Life Science).

2.5.2.3 Protein concentration determination

The protein concentration was determined according to DC Biorad Laboratories (Richmond, CA) protein concentration determination kit instructions using bovine serum albumin (BSA) as a standard. This colorimetric assay is for the determination of protein concentration following solubilization with a detergent such as Triton X-100. The assay is based on the reaction of protein with an alkaline copper tartrate solution and Folin reagent.[157]

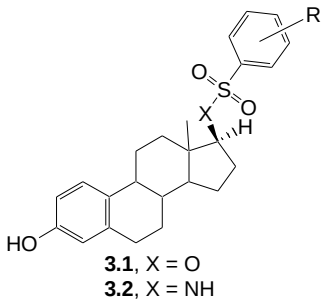
Chapter 3 – 17β-Arylsulfonamides of 17β-Aminoestra-1,3,5(10)-trien-3-ol as Highly Potent Inhibitors of Steroid Sulfatase.

3.1. Introduction

Although the vast majority of STS inhibitors are irreversible sulfamate-based inhibitors (see chapter 1), we decided to focus our efforts on developing reversible, non-sulfamate inhibitors. There are several reasons for this. First, although hundreds of aryl sulfamates have been examined as STS inhibitors over the previous 18 years, only one, STX64 (see chapter 1, section **1.5**), has made it to phase I clinical trials and with mixed results. Second, many aryl sulfamates, such as STX64 and EMATE, are very potent inhibitors of carbonic anhydrases (CAs).[158] Although binding to CAs protected STX64 against first pass metabolism,[159] the potential for side effects as a result of potent CA inhibition with this and other aryl sulfamate-based STS inhibitors is an issue for concern. Binding to CAs would also be expected to affect bioavailability.

As noted in *Chapter* 1, not many reversible inhibitors of STS have been developed. Perhaps the most notable are the E2 derivatives developed by Poirier and coworkers (compounds of type **1.49**, see *Chapter* 1, section 1.4.1.2, Fig. 1.13). Some of these compounds, such as **1.51** and **1.52**, exhibited IC_{50}'s as low as 24-28 nM when assayed using HEK-293 cells overexpressing STS and E1S as substrate. We decided to use these E2 derivatives as lead compounds for STS inhibitor development. However, there were some issues with these compounds. In our previous studies with these types of compounds (compound **1.50**)[63] we noted that they were very hydrophobic and so very poorly soluble in water (the log P coefficient has not been reported for these compounds). This made them difficult to assay in aqueous solution. Moreover, compounds of such hydrophobicity can sometimes be difficult to develop into drugs

due to their poor solubility, partitioning into cellular membranes and accumulation in adipose tissue. Finally, the synthesis of these compounds involved reacting estrone with a considerable excess of a Grignard reagent (sometimes 8-fold excess) and, even with this excessive amount of Grignard reagent, the yields were often very low. This made them inefficient and expensive to prepare. We decided to modify these compounds by introducing a different linker between appended aryl group and the carbon at the 17-position. This led us to design compounds such as sulfonates of type **3.1** and sulfonamides of type **3.2** as potential STS inhibitors (**Fig. 3.1**). It is expected that such compounds would be less hydrophobic than compounds of type **1.49**. Moreover, their synthesis would simply involve reacting 3-OH-protected E2 or the 17-amino analog of E2 with sulfonyl chlorides. Since a large number of sulfonyl chlorides are commercially available a large number of potential inhibitors could be made relatively quickly. Finally as far as the sulfonamides **3.2** are concerned, the sulfonamide group is very chemically and metabolically stable and is one of the most important pharmacophores in medicinal chemistry and numerous bioactive agents bear this functionality.



3.1, X = O
3.2, X = NH

Fig. 3.1. General structure of proposed STS inhibitors.

3.2. Objectives

These objectives of the work presented in this chapter were to synthesize a series of compounds of type **3.1** or **3.2** and evaluate these compounds, both in terms of potency and

modality of inhibition, as *in vitro* STS inhibitors using purified STS.

3.3. Results and Discussion

3.3.1 Studies with model sulfonates and sulfonamides

To determine which series of compounds (sulfonate or sulfonamide) that we would focus our efforts on we initiated these studies by synthesizing model sulfonates **3.3** and **3.4** and the analogous model sulfonamides **3.5** and **3.6** and then examined them as STS inhibitors (**Fig. 3.2**).

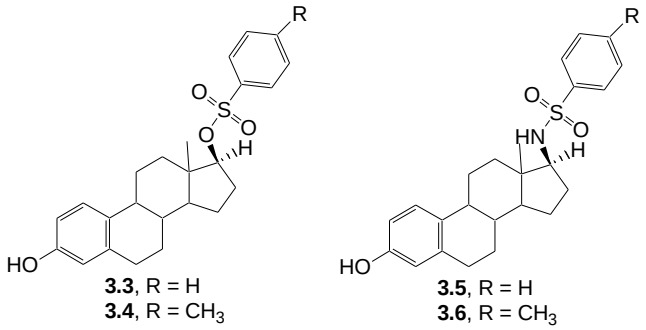
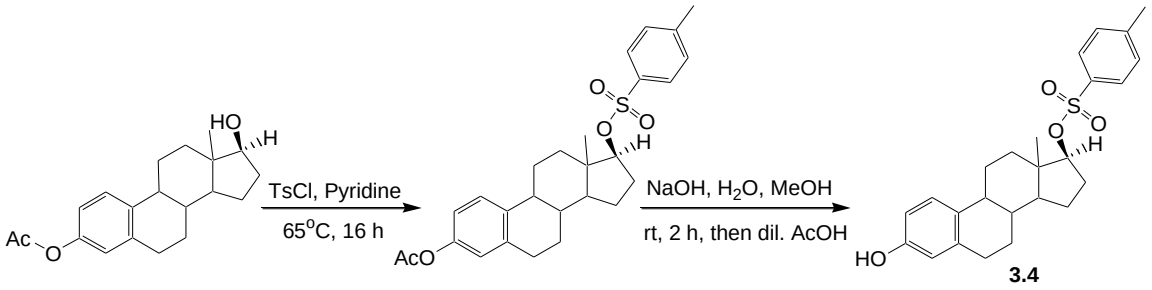


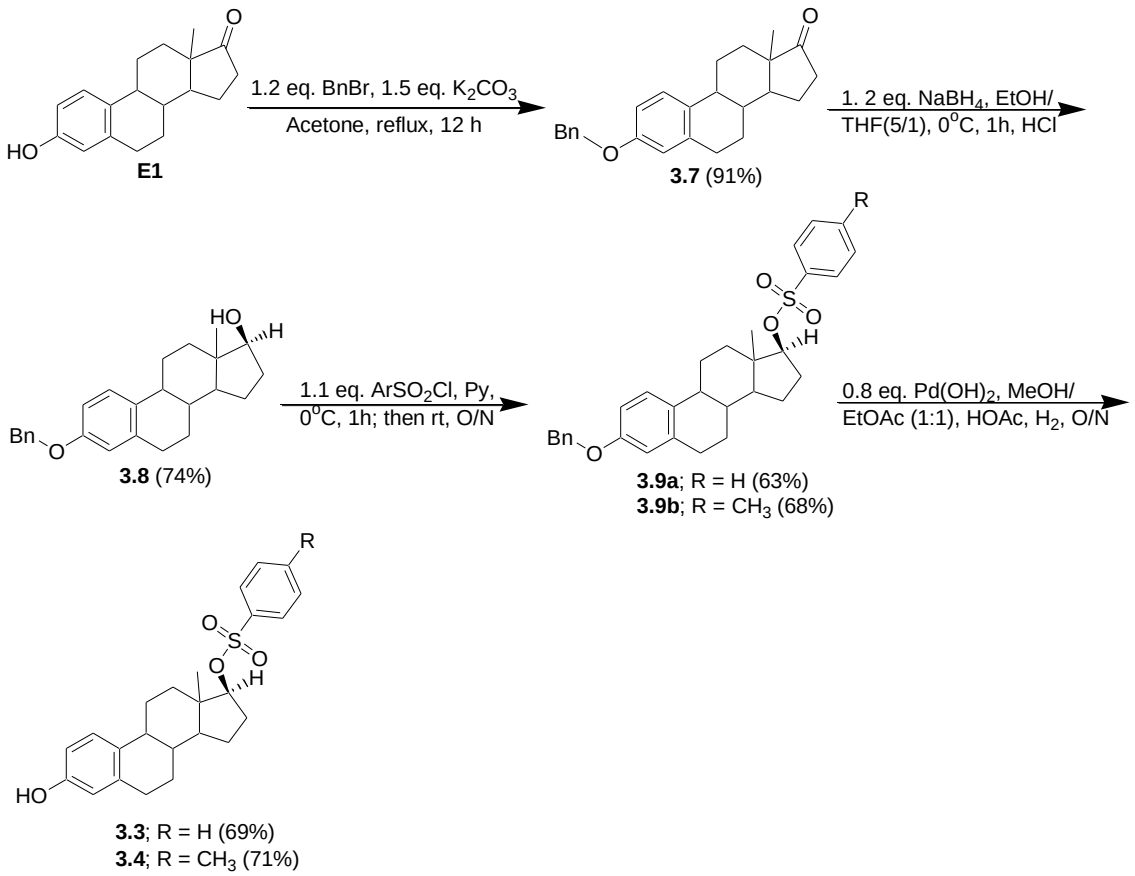
Fig. 3.2. Model sulfonates and sulfonamides.

Compound **3.4** was reported before in a US patent in 1958.[160] It was prepared from 3-acetoxy derivative of E2 according to Scheme 3.1. Unfortunately, they did not report any details including percent yield or spectral data.[160]



Scheme 3.1. Literature synthesis of compound **3.4**.

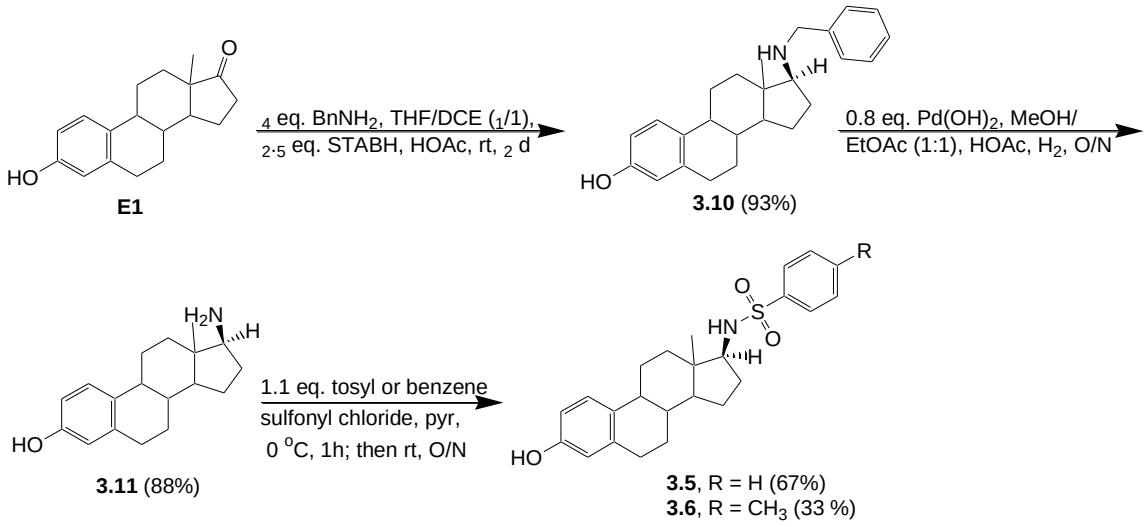
We decided to prepare compounds **3.3** and **3.4** from **E1** using the route shown in Scheme 3.2. Alkylation of **E1** by benzyl bromide in presence of potassium carbonate and acetone as a solvent afforded compound **3.7** in a 91% yield.[161] Reduction of the keto group in **3.3** using by $NaBH_4$ afforded the alcohol, **3.8**, in a 74% yield.[162] Reaction of **3.4** with a slightly excess of benzene sulfonyl chloride or tosyl chloride in anhydrous pyridine at room temperature for 18 h afforded the desired sulfonates **3.9a** and **3.9b** as white solids in 68 and 63% yield respectively. Finally, removal of the benzyl protecting group in **3.9a** and **3.9b** using H_2 and $Pd(OH)_2$ afforded the target compounds **3.3** and **3.4** in 69 and 71 % yield respectively.



Scheme 3.2. Synthesis of 17β-Sulfonate derivatives of E1

The analogous sulfonamide compounds **3.5** and **3.6** were prepared according to Scheme 3.3. Reductive amination of E1 using benzylamine and sodium triacetoxyborohydride (STAB-

H) gave compound **3.10** in a 93% yield after recrystallization from MeOH. Removal of the benzyl protecting group by hydrogenolysis with H_2 and $Pd(OH)_2$ gave amine **3.11** in good yield.[163] The appropriate sulfonyl chloride was then added dropwise as a solution in DCM via a syringe pump over 30 min to a solution of amine **3.11** in pyridine at 0 ^oC which gave sulfonamides **3.5** and **3.6** in good yield. By adding the sulfonyl chloride slowly we were able to minimize the amount of the disubstituted product which could happen from the presence of the unprotected phenolic 3-OH group.



Scheme 3.3. Synthesis of the 17β-Sulfonamides **3.5** and **3.6**.

The IC_{50}'s of compounds **3.3**-**3.6** were determined using purified STS in tris-HCl buffer at pH 7.0 containing 5% DMSO, 0.01% Triton X-100 with 200 μM 4-MUS as a substrate (K_m of 4-MUS). The results are given in Table 3.1. All four compounds were relatively good inhibitors of STS. The difference in inhibitory potency between the sulfonates and sulfonamides was relatively small. The best inhibitor of the series was the tosyl sulfonamide **3.6** with an IC_{50} of 207 nM which was about 30% lower than the IC_{50} of the analogous sulfonate **3.4**.[164] Although the difference in potency was relatively small between these sulfonates and sulfonamides we

decided to focus our future efforts on preparing and evaluating the sulfonamide series as these compounds were more readily prepared than the sulfonates and would be expected to be more stable than sulfonates.

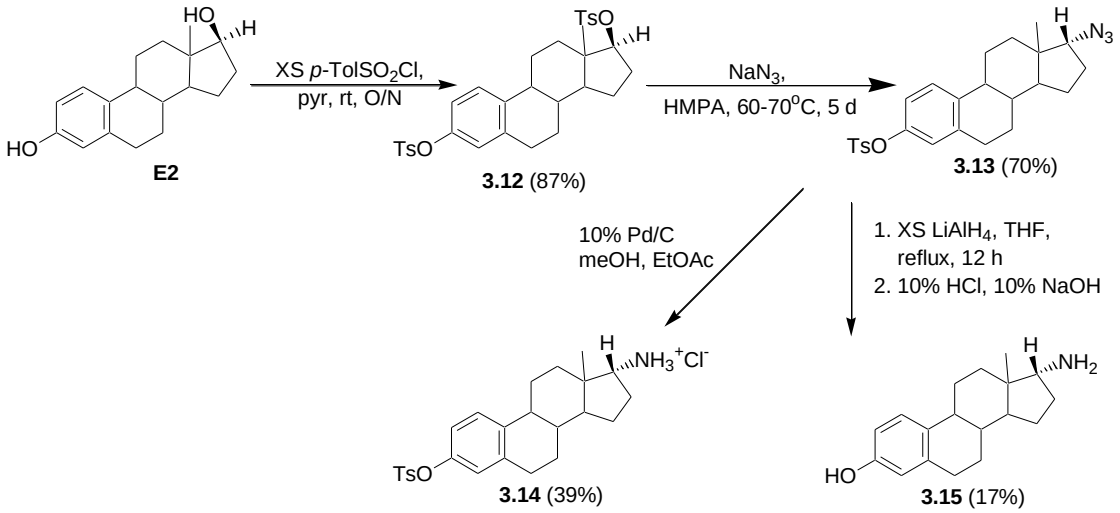
Table 3.1. IC_{50}'s of compounds **3.3-3.6.**[164]

Compound	IC_{50} (nM)[a]
3.3 benzenesulfoante	301
3.4 tosylsulfonate	293
3.5 benzenesulfamide	343
3.6 tosylamide	207

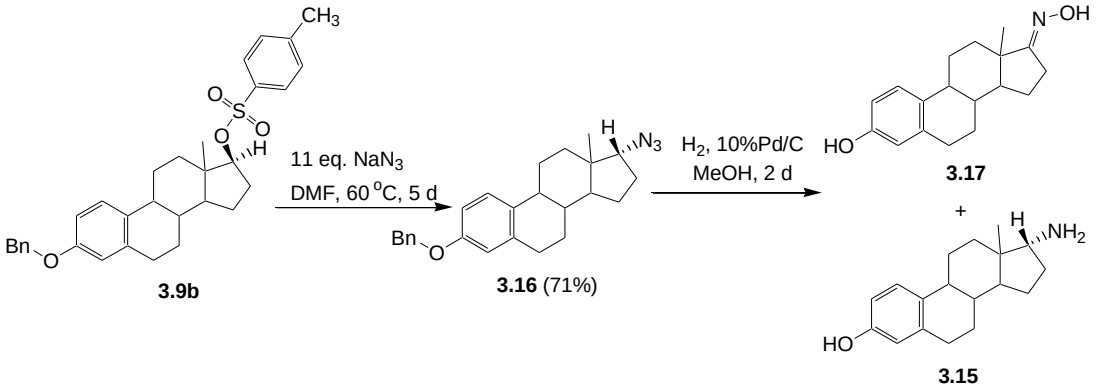
[a] Errors are within ± 5%

In Poirier's E2-based inhibitors (compounds **1.49**, Figure 1.13) the bulky benzyl group at the 17-position had the α-configuration. This is the inverse configuration of the compounds in Table 3.1. So before proceeding any further we decided to prepare the α-isomer of sulfonamides **3.5** and **3.6** and determine if the stereochemistry at the 17-position had a significant impact on inhibitory potency.

In 1998, Lemini et al. reported, albeit in low yield, the synthesis of the 17α-amino derivative of E1, compound **3.15**, via the route outlined in Scheme 3.4.[165] We decided to take a similar approach (Scheme 3.5) except starting from compound **3.9b** (Scheme 3.2) which we had made previously and had retained a small quantity from our synthesis of sulfonate **3.4**. Reacting **3.9b** with 11 equivalent of NaN_3 in DMF at 80 ^oC resulted in almost no consumption of **3.9b**. Adding another 5 equivalents of NaN_3 and increasing the temperature to 90^oC and reacting for 7 days also did not result in much consumption of **3.9b**. However, using HMPA as solvent and 11 eq. NaN_3 and heating for 6 days the desired azide **3.16** was obtained in a 71% yield.



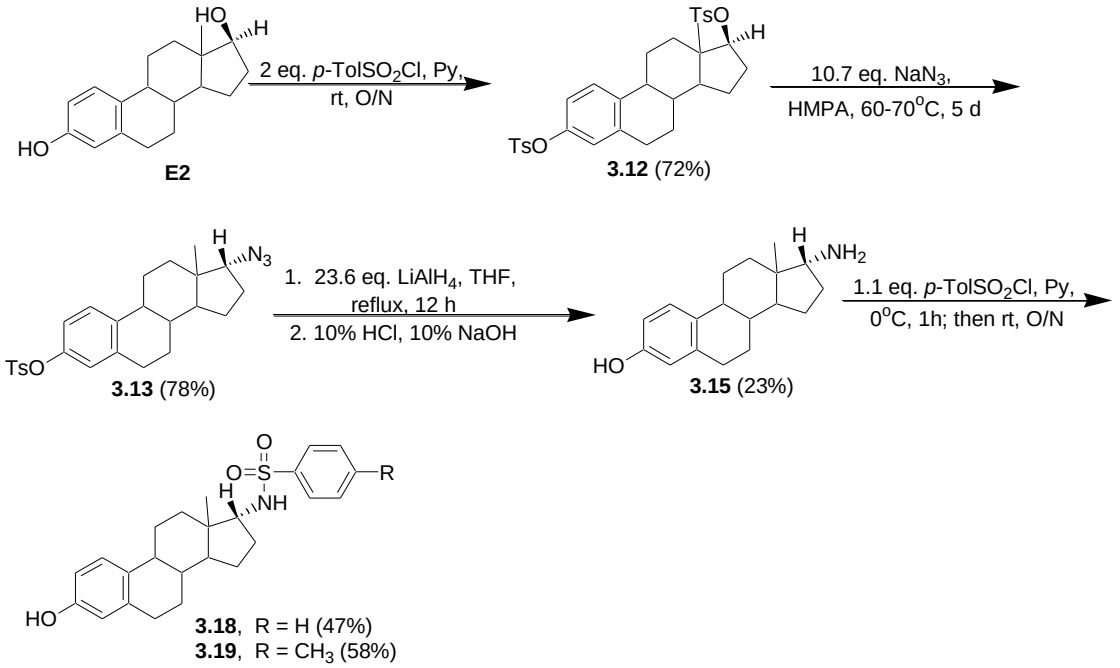
Scheme 3.4. Lemini's procedure for preparing the 17α-amino derivative of E1 (**3.15**).



Scheme 3.5. Initial route to compound **3.15**.

Reduction of compound **3.16** by hydrogenation over 10% Pd/C for 2 days gave a 1:1 mixture of our target compound and another compound with a molecular weight that was 14 mass units (MW 285) greater than X (MW 271) as determined by +EI-MS. It is possible that this impurity is ketoxime derivative **3.17**. Since $LiAlH_4$ is one of the best reagents for the reducing oximes to the corresponding amines,[166] we took this mixture and heated it under reflux with $LiAlH_4$ in THF for 6 hours. We noticed that the intensity of the peak at 285 decreased

while our target's peak increased in intensity suggesting to us that the impurity is indeed the ketoxime. Unfortunately, after these manipulations we had only a very small amount of impure **3.15** and that for our studies we needed to make more which meant that we would have to make more of the starting material **3.9b**. Although it looked like our route to **3.15** was promising in that it might produce **3.15** in a higher yield than Lemini's route it was longer than the one used by Lemini as the synthesis of **3.9b** requires three steps while the synthesis of **3.12** in Lemini's procedure only requires one. So we decided to pursue Lemini's route to **3.15**. We managed to obtain similar yields for most of the intermediate compounds (Scheme 3.6). Lemini and coworkers reported that the 17α (quasi-axial) hydrogen in **3.11** (17β-amino isomer) appears further upfield than the corresponding 17β (quasi-equatorial) hydrogen in **3.15** (17α-amino isomer). These signals exhibit also different splitting patterns; the 17α-hydrogen in **3.11** appears as a triplet, indicating couplings with both hydrogens at position 16, whereas the 17β-hydrogen in the isomeric 17α-isomer is coupled only with the 16β-H and so appears as a doublet. This is consistent with what we found with these two compounds. We also noted that the C-18 methyl group is further downfield in the 17α-isomer than the C18- methyl group in the 17β isomer.[165-168]



Scheme 3.6. Our synthesis of compound **3.15** using Lemini's route and the formation of sulfonamides **3.18** and **3.19**.

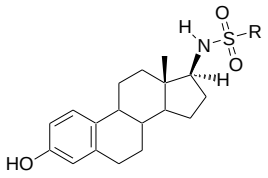
With sufficient quantities of **3.15** in hand we then subjected it to tosyl chloride or benzenesulfonyl chloride in pyridine which gave sulfonamides **3.18** and **3.19** in reasonable yields (Scheme 3.6). The IC_{50}'s of compounds **3.18** and **3.19** were determined to be 921 nM and 341 nM respectively which is greater than that of their corresponding β-isomers (**3.5** and **3.6**).

3.3.2. Inhibition of STS with 17β-arylsulfonamides.

In the light of these results we decided to focus on the preparation and evaluation of a collection of the 17β-sulfonamides. We initially focused on preparing arylsulfonamides substituted at the 4´-position with alkyl groups since Poirier had previously demonstrated that 17α-benzylE2 derivatives of type **1.49** bearing certain alkyl groups at the 4´-position of the benzyl moiety are potent STS inhibitors.[69] These sulfonamides were readily prepared using the same approach for the synthesis of **3.5** and **3.6** (reaction of **3.11** with the appropriate sulfonyl

chloride as shown in Scheme 3.3). The yields for these reactions are given in Table 3.2. The yields were not optimized. The IC_{50}'s of these compounds are shown in Table 3.3.[164]

Table 3.2. Yields of compounds **3.20**-**3.27**.

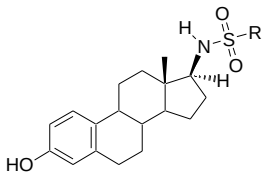


compound	R	% yield
3.20	Benzyl	26
3.21	4´-methylbenzyl	38
3.22	4´-chlorobenzyl	27
3.23	4´-n-propylbenzene	27
3.24	4´-n-butylbenzene	56
3.25	4´-n-pentylbenzene	21
3.26	4´-*iso*propylbenzene	43
3.27	4´-*tert*-butylbenzene	53

The unsubstituted benzyl derivative, compound **3.20**, exhibited a potency that was very close to its phenyl analog **3.5** (IC_{50} = 364 nM for **3.20**, 343 nM for **3.5**). Substituting the benzyl ring with hydrophobic groups such as CH_3 (electron donating) (**3.21**) or Cl (electron withdrawing) (**3.22**) resulted in an increase in potency compared to **3.20** but were poorer inhibitors than tosyl derivative **3.6**. Increasing the length of the n-alkyl group at the 4´-position in the benzenesulfonamide moiety from one (methyl, compound **3.6**) to four carbons (n-butyl derivative **3.24**) resulted in a significant increase in binding affinity with the n-butyl derivative **3.24** being a potent inhibitor with an IC_{50} of 26 nM. However, a decrease in affinity occurred when a fifth carbon unit was present (n-pentyl derivative **3.25**). Branching of the n-propyl group proved to be slightly detrimental to binding affinity (isopropyl derivative **3.26**) while the *tert*-butyl derivative **3.27** proved to be more potent than the n-butyl derivative with an IC_{50} of 18

nM.[164] It is worthy of note that among Poirier's 17α-benzylE2 inhibitors the 4'-tert-butyl derivative (**1.52**, Fig. 1.13) exhibited an IC_{50} (28 nM) that is remarkably similar to **3.27** suggesting that the sulfonamides studied here bind in a similar manner to the 17α-benzylE2 inhibitors.[69,164]

Table 3.3. Inhibition of STS with 4'-alkyl sulfonamide derivatives[164]

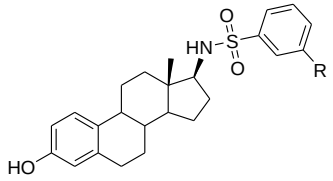


compound	R	IC_{50} (nM)[a]
3.5	Benzene	343
3.20	Benzyl	364
3.21	4´-methylbenzyl	261
3.22	4´-chlorobenzyl	291
3.6	4´-methylbenzene	207
3.23	4´-n-propylbenzene	44
3.24	4´-n-butylbenzene	26
3.25	4´-n-pentylbenzene	51
3.26	4´-*iso*propylbenzene	62
3.27	4´-*tert*-butylbenzene	18

[a]Errors are within ± 5%.

Since it has been demonstrated that among Poirier's 17α-benzylE2 derivatives certain groups/atoms (i.e. Br, compound **1.51**, Fig. 1.13) at the 3´-position significantly enhanced potency, the next series of compounds we prepared, using the usual procedure, were sulfonamides bearing small electron donating and electron withdrawing groups at the 3´-positon. The yields of these compounds are given in Table 3.4. No attempt was made to optimize the yields. The IC_{50}'s of these compounds are shown in Table 3.5.

Table 3.4. Yields of compounds **3.28**-**3.36**.

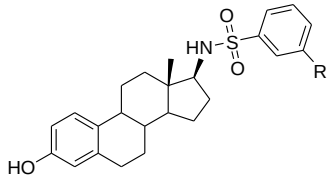


Compound	R	% yield
3.28	Br	82
3.29	Cl	73
3.30	F	65
3.31	NO_2	49
3.32	CN	53
3.33	CF_3	48
3.34	OCF_3	31
3.35	CH_3	43
3.36	OCH_3	70

Among the halobenzene derivatives (**3.28**-**3.30**), the 3'-Br derivative, compound **3.28**, was the most potent having an IC_{50} of 25 nM. Br, Cl and F are electron withdrawing with similar σ_m values though Br is the most hydrophobic of the three (largest positive π value) suggesting that hydrophobicity is important at this position.[169,170] The methyl derivative **3.35** is 2.5-fold less potent than the Br derivative. The methyl group is less hydrophobic than Br and more electron donating. One the other hand, the CF_3 derivative, **3.33**, was almost equipotent to the Br derivative. This compound is considerable more electron withdrawing than Br. The potency of the CF_3 derivative might not be due to the CF_3 group electron withdrawing ability as the introduction of polar and strongly electron withdrawing groups (at least by resonance) such as nitro and cyano groups (compounds **3.31** and **3.32**) at this position are less potent than the CF_3 derivative. The CF_3 group has almost an almost identical π value (same hydrophobicity) as a Br atom suggesting again that the presence of a hydrophobic group at the 3'-position may be important. We should also point out that the fluorines in **3.33** are also capable of acting as an H-

bond acceptor which may also contribute to the potency of **3.33**. The OCF_3 derivative, **3.34**, was 3-fold less potent than the Br and CF_3 derivatives. The OCF_3 group is slightly more hydrophobic than the Br or CF_3 groups and less electron withdrawing than the CF_3 group but more electron withdrawing than the Br group. Hence it is possible that the effect of the CF_3 group in **3.33** may in part be due to H-bonding interactions with the fluorines and that this effect is not as pronounced with the OCF_3 group. On the other hand the difference may be due to the larger size of the OCF_3 group. The electron donating OCH_3 derivative was the least potent of all of the 3'-derivatives studied suggesting that strongly electron donating groups at this position are detrimental to binding and also its greater size may also be a factor. The Br derivatives **3.28** was almost the most potent of all of the compounds studied in Table 3.5. The 3'-Br derivative was also among the most potent of Poirier's 17α-benzylE2 inhibitors again suggesting that our sulfonamide inhibitors and the 17α-benzylE2 inhibitors bind to STS in a similar manner.[69]

Table 3.5. Inhibition of STS with 3'-substituted benzene sulfonamide derivatives **3.28**-**3.36**.[164]



Compound	R	IC_{50} (nM)[a]
3.28	Br	25
3.29	Cl	67
3.30	F	112
3.31	NO_2	90
3.32	CN	137
3.33	CF_3	23
3.34	OCF_3	74
3.35	CH_3	65
3.36	OCH_3	192

[a] Error's are within ± 5%

A more detailed kinetic study was performed with compound **3.28** to determine the mode of inhibition of this class of compounds. It turns out that this compound exhibits a non-competitive mode of inhibition with a K_i of 23 nM and an αK_i of 108 nM (αKi being the dissociation constant for the inhibitor with the enzyme-substrate complex) (Figure 3.3). The fact that compound **3.8** is a non-competitive inhibitor of STS suggests that the inhibitor may also bind at a site besides the active site. Our previous studies with one of Poirier's compounds, compound **1.50** (Fig. 1.13), revealed that it too was a non-competitive inhibitor again supporting the supposition that our sulfonamides and Poirier's 17α-benzylE2 inhibitors may bind in a similar manner.[69]

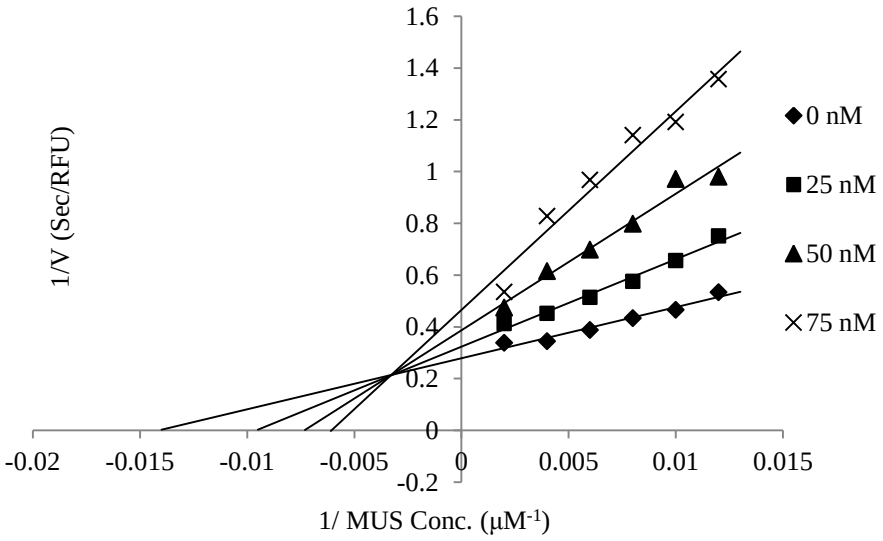


Fig. 3.3. Lineweaver-Burk plot of compound **3.28** (See **Fig. A.21** and **A.22** for the re-plot of this data that was used to determine both K_i and αK_i.[164]

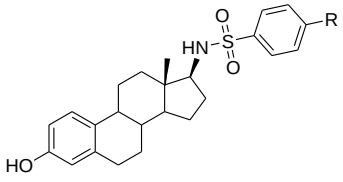
Several 4'-halo analogs, **3.28**-**3.30**, as well as the 4'-methyl, 4'-CF_3 and 4'-acetyl analogs **3.40**-**3.42**, were synthesized (see Table 3.6 for yields) and examined as STS inhibitors. Although the analogous 3'-substituted derivatives were highly potent (Table 3.5, IC_{50}'s ranging from 23

nM to 192 nM), the 4'-substituted halo-analogs were dramatically less active, as seen in Table 3.7. Moving the bromine atom and trifluoromethyl group from the 3'-position to the 4'-position, as in compounds **3.37** and **3.40**; resulted in a significant decrease in their inhibitory activity (IC_{50}'s of 492 nM and 503 nM, respectively). Poirier's noted a similar phenomenon with the 17α-benzylE2 inhibitors in that that the *meta*-substituted Br derivative was considerably more potent than either its *ortho* or *para* analogs.[68]

Table 3.6. Yields of 4´-derivatives **3.37-3.42**

Compound	R	% yield
3.37	Br	78
3.38	Cl	69
3.39	F	61
3.40	CF_3	34
3.41	CH_3	33
3.42	$COCH_3$	53

Table 3.7. Inhibition of STS with 4'-substituted benzene sulfonamide derivatives **3.37**-**3.42**.[164]



Compound	R	IC_{50} (nM)[a]
3.37	Br	190
3.38	Cl	271
3.39	F	479
3.40	CF_3	74
3.41	CH_3	207
3.42	$COCH_3$	204

[a] Error's are within ± 5%

Moving the bromine atom and trifluoromethyl group to the 2'-position, as in compounds **3.43** and **3.44** (Fig. 3.4), resulted in a significant decrease in their inhibitory activity (IC_{50}'s of 492 nM and 503 nM, respectively) compared to their 3' and 4' analogs.

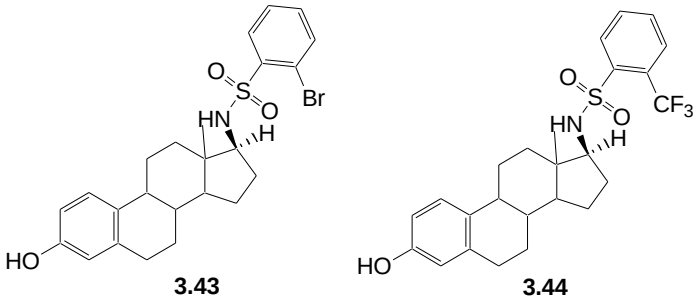
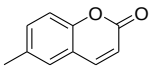
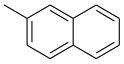
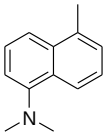
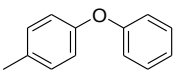
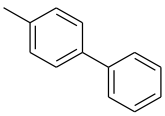
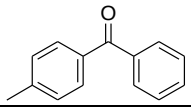
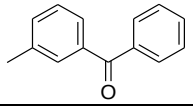


Fig. 3.4. Ortho-substituted analogs **3.43** and **3.44**.

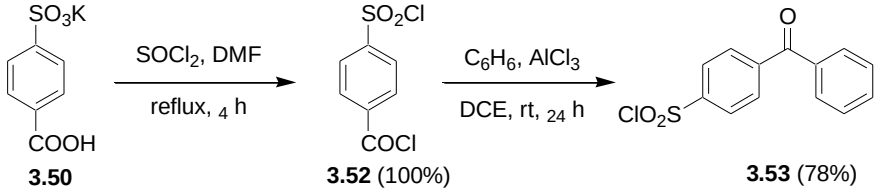
Finally, we prepared and examined bicyclic and biaryl sulfonamides as STS inhibitors **3.45**-**3.51**. These were prepared in the usual manner and the yields are given in Table 3.8. The

Table 3.8. Yields of 4´-derivatives **3.45-3.51**

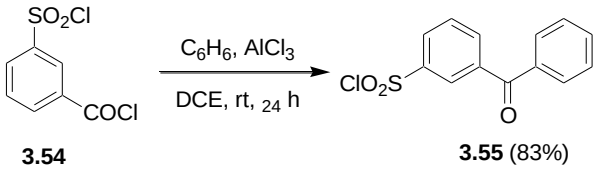
Compound	R	% yield
3.45		56
3.46		25
3.47		63
3.48		38
3.49		31
3.50		23

3.51		18

benzophenonesulfonyl chlorides (**3.53** and **3.55)** that used to prepare sulfonamides **3.50** and **3.51** were not commercially available and had to be prepared. This was readily achieved using the literature procedures outlined in Schemes 3.7 and 3.8.[171,172]



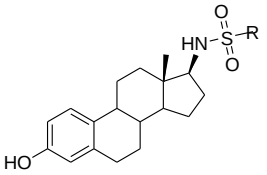
Scheme 3.7. Preparation of sulfonyl chloride **3.53**

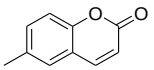
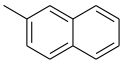
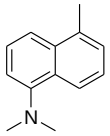
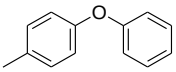
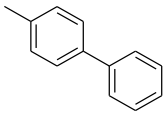
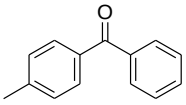
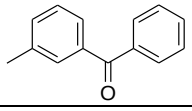


Scheme 3.8. Preparation of sulfonyl chloride **3.55**

Two of the bicyclic and biaryl sulfonamides, the naphthyl and biphenyl derivatives **3.43** and **3.49**, proved to be highly potent inhibitors (Table 3.9), and were even more potent than the 4'-t-butyl (**3.27**), 3'-Br (**3.28**) and 3'-CF_3 (**3.33**) derivatives. Placing an oxygen or carbonyl spacer between the two phenyl rings in **3.49** (compounds **3.48**, **3.50** and **3.51**) resulted in a decrease in potency. The dramatically lower potency of benzophenone derivatives was particularly surprising as the acetophenone derivative (**3.42** in Table 3.7) is considerably more potent.

Table 3.9. Inhibition of STS with bicyclic and biaryl sulfonamides **3.45**-**3.51.**[164]



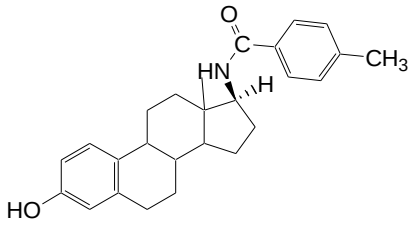
Compound	R	IC_{50} (nM)[a]
3.45 (Coumarin-6-yl)		185
3.46 (Naphthalen-2-yl)		20
3.47 (5'-(Dimethylamino)-naphthalen-2-yl (Dansyl))		113
3.48 (4'-Phenoxybenzen-1-yl)		39
3.49 (4'-Phenylbenzen-4-yl (Biphenyl))		9
3.50 (4'-Benzoylbenzen-4-yl)		1300
3.51 (3'-Benzoylbenzen-4-yl)		900

[a] Error's in IC_{50} are within ± 5%

3.3.3 Studies with 17β-Amides of E1

An obvious avenue for further extending our studies was to see if the sulfonamide group could be replaced with an amide which is considered to a sulfonamide biosteres (or vice versa). This could be important as it would be possible to test dozens of such amides as many acid chlorides are readily available. To determine this we prepared model amide **3.56** (Fig. 3.5). This compound was almost equipotent to the sulfonamide analog (with an IC_{50} value of 219 nM) and

so we decided to examine two other amides bearing a 3'-Br (**3.57**) or 3'-CF3 (**3.58**) group on the aryl ring.

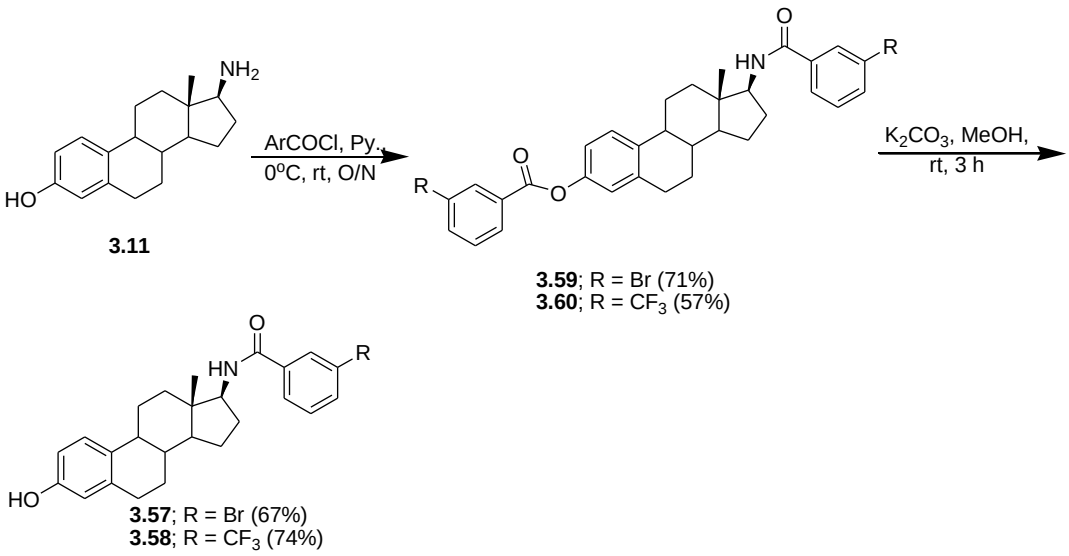


Compound **3.56**

Fig. 3.5. Model amide **3.56.**

The synthesis of amides **3.57** and **3.58** is given in Scheme 3.9. We found that reaction of **3.11** with one equiv of the appropriate acid chlorides gave a mixture of the two mono- and disubstituted products which were surprisingly difficult to separate by column. To get around this problem we reacted **3.11** with an excess of the acid chloride to get mainly disubstituted products **3.59** and **3.60** which was easily purified. These compounds were then subjected to K_2CO_3 in MeOH which resulted in exclusive hydrolysis of the ester group to give the desired compounds **3.57** and **3.58** in reasonable yields.

Much to our surprise, inhibition studies with compound **3.57** and **3.58** and STS revealed that these two amides were far less potent than their sulfonamide analogs: compound **3.57** was 10-times less potent (IC_{50} of 308 nM) than its sulfonamide analog, **3.28**, while compound **3.58** (IC_{50} of 705 nM) was 31-times less potent than its sulfonamide analog, **3.33**. On the basis of these finding we decided to abandon pursuing the amide-based inhibitors.



Scheme 3.9 Synthesis of 17β-amides, compounds **3.57** and **3.58**.

3.3.4 X-ray Crystallography of Compound 3.33

The X-ray crystal structure of one of our best inhibitors, the 3´-CF_3 derivative **3.33** was obtained. A colorless plate crystal of **3.33** with approximate dimension of 0.25 × 0.08 × 0.02 mm was used for data collection. An ORTEP plot of the asymmetric unit of **3.33** is shown in Figure 3.6. All four rings and the key features of the steroid are clearly visible. The asymmetric unit is seen to consist of two molecules of **3.33**. All C-C bond lengths were in the range 1.340-1.566 Å, the C-O bond lengths were between 1.358 and 1.40 Å, all S-O bond lengths were in the range 1.421-1.441 Å, and the C-N bond lengths were in the range 1.466-1.473 Å.

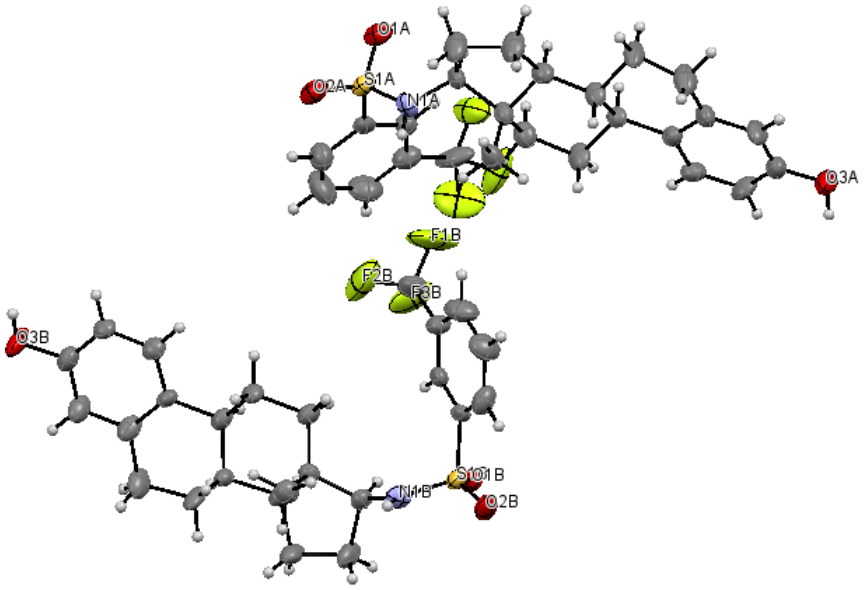


Fig. 3.6. ORTEP plot of the asymmetric unit in the X-ray crystal structure of sulfonamide **3.33** (thermal ellipsoids are shown at the 30% probability level).

As shown in Fig. 3.7 molecules of **3.33** interact via a network of intermolecular hydrogen bonds viewed along the *b* axis. In particular, the proton NH of the sulfonamide group (H1BA) interacts with oxygen atom (O2A) of the sulfonamide SO_2NH group in a proximate molecule, whereas the other oxygen atom (O1A) interacts with the proton of the 3-hydroxyl group (H3AA).

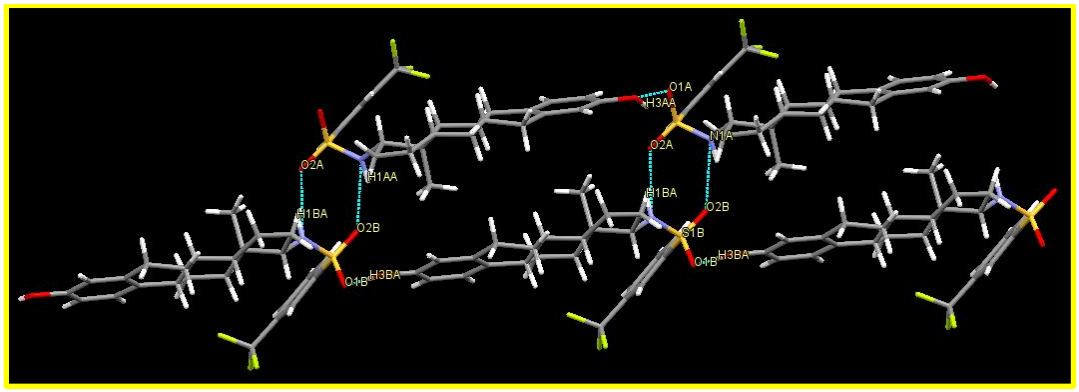
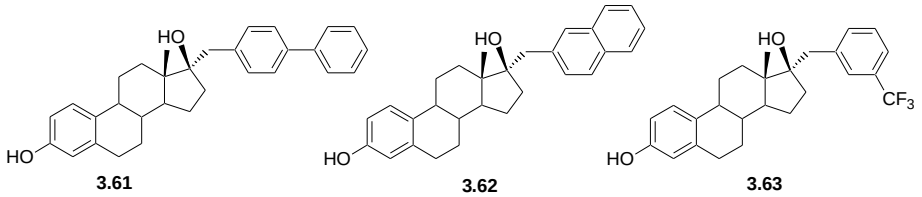


Fig. 3.7. Portion of extended structure present in **3.33** showing the network of intermolecular hydrogen bonding viewed along the *b* axis.

3.3.5 Molecular Modelling Studies

The finding that some of our sulfonamide inhibitors, such as the 4´-*tert*-butyl (**3.27**) and 3'-bromobenzene (**3.28**) derivatives, exhibit IC_{50}'s that are remarkably close to the analogous 17α-benzylE2 inhibitors reported by Poirier and coworkers suggests that these two classes of compounds may be binding in a similar fashion. This is somewhat surprising since the 17β-sulfonamide link between the aryl moieties and C-17 in the inhibitors described here is structurally, electronically and spatially (β versus α) very different from the 17α-CH_2 unit in the 17α-benzylestradiol inhibitors and lacks a 17-OH group. Moreover, the two sets of compounds were assayed under different conditions (the IC_{50}'s of 17α-benzyl estradiol inhibitors were determined using homogenates of JEG-3 cells in Tris-acetate buffer, 10% glycerol, pH 7.0 and [^{3}H]E1S as substrate[69] while our work was done with pure STS in Tris buffer, pH 7.0, 0.01% TritonX-100, and 5 % DMSO using 4-MUS as substrate). However, some differences in IC_{50}'s between the two sets of compounds do exist. For examples, the 4´-biphenyl derivative **3.49** (IC_{50} = 9 nM) and naphthyl derivative **3.46** (IC_{50} = 20 nM) are 4-6-fold more potent than 17α-4´-phenylbenzylestradiol (Fig. 3.8, **3.61**, IC_{50} of 35 nM) and the 17α-naphth-2´-ylmethylestradiol (Fig. 3.8, **3.62**, IC_{50} = 120 nM).[68,69] The 3´-trifluoromethylbenzenesulfonamide derivative **3.33** (IC_{50} = 23 nM) is almost six-fold more potent than 17β-3´-trifluoromethylbenzylestradiol (Fig. 3.8, **3.63**, IC_{50} = 126 nM).[68,69]



3.61 **3.62** **3.63**

Fig. 3.8 17α-benzylE2 derivatives **3.61-3.63**.

As discussed in *Chapter* 1, the X-ray crystal structure of STS has been reported by Ghosh and coworkers.[26,27] Over the last ten years the Ghosh group has attempted to obtain the X-ray crystal structure of STS complexed with inhibitors developed in the Taylor group and other groups. Unfortunately this has not been successful. Consequently we have turned to molecular modeling to learn more about how our sulfonamide inhibitors might interact with STS. However, without an x-ray crystal structure of one of our inhibitors bound to STS to guide these studies, assumptions must be made about how these compounds interact with STS to initiate the modeling studies. Under our assay conditions, sulfonamide inhibitor **3.28** and 17α-benzylE2 inhibitor **1.50** exhibit mixed inhibition and so these types of inhibitors may be capable of binding at the active site and also at a secondary site outside the active site (possibly in the hydrophobic channel between the two alpha helices as mentioned earlier). Since we do not know exactly where a possible secondary site might be, we focussed our modeling efforts on inhibitor-active site interactions. We had to assume a particular mode of binding in the active site to initiate these studies so we made the assumption that these compounds are capable of binding in the active site in a manner similar to what has been proposed for sulfamate-based inhibitors:[112] with the 3-OH on the A-ring occupying space that is in the proximity (facing) of the FGly75 hydrate. Selected inhibitors were docked into the crystal structure of STS (PDB ID: 1P49) after conversion of the sulfated Fgly75 hydrate to an Fgly75 hydrate, using the LibDock docking algorithm (see experimental **3.5.4** for details).

The docking results for the sulfonamide, **3.6** and its sulfonate analog **3.4** are shown in Fig. 3.9. In common with compounds **3.6**, **3.4**, and the rest of the compounds in the sulfonamide series studied in this section; the nonpolar rings B, C, and D of the steroid skeleton including the C_{18} methyl group were oriented in the centre of the active site and underwent non-polar

interaction with side chains of the hydrophobic pocket formed by Leu74, Arg98, Thr99, Val101, Leu103, Val177, phe178, Thr180, Gly181, Thr484, His485, Val486, phe488, and phe553 with a distance less than 5 Å. For reasons of clarity, not all of these hydrophobic interactions are indicated in Fig. 3.9 and subsequent docking figures. For both compounds, the bulky aromatic group at the 17-position was oriented closer to what is considered to be the entrance to the active site, and the aromatic A ring was oriented in the polar catalytic site at the apex comprised of FGly75, His290, and Lys368. For these two compounds and all other sulfonamide inhibitors studied in this section, the 3-OH is involved in an H-bond with the FGly75 hydrate.

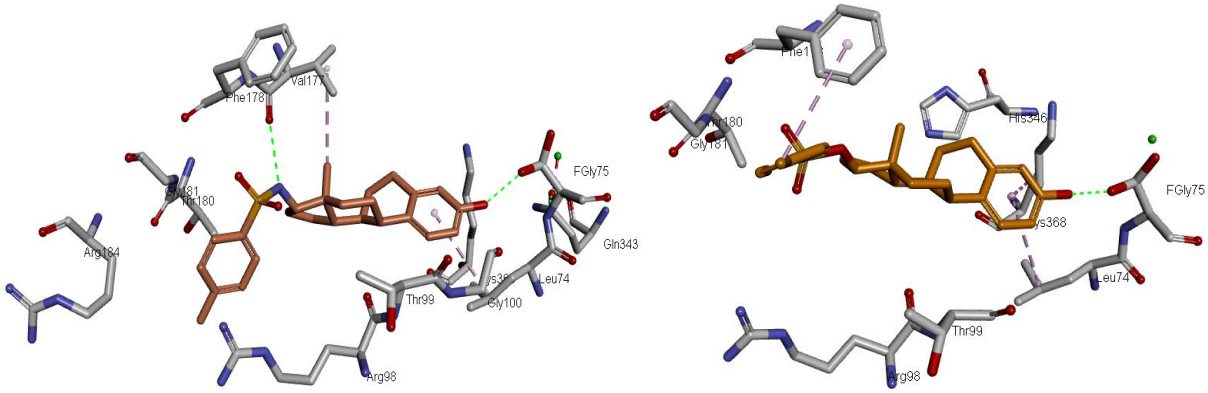


Fig. 3.9. The binding mode of compounds **3.6** (left) and **3.4** (right) with STS (green dotted lines indicate hydrogen bonding interactions, violet dotted lines indicate hydrophobic interactions; H-atoms were removed to increase clarity).

The N-H of the sulfonamide group in **3.6** is involved in an H-bond with the carbonyl oxygen of Phe178 and there is a hydrophobic interaction between the C-18 methyl group and the side chain of val177. No interactions between the sulfonate group and STS were evident. This may have been compensated somewhat by a hydrophobic interaction between the aromatic side chain of Phe178 and the aromatic group of the aryl sulfonate.

We then performed modeling studies on compound **3.19**, the α-isomer of compound **3.6.** These two compounds exhibited slightly different affinities for STS (IC_{50} = 207 nM for compound **3.6,** IC_{50} = 341 nM for compound **3.19**). The change in stereochemistry from the β to

the α configuration at the 17-position caused the NH of SO_2NH group to move away from Phe178 and the hydrophobic interactions between its C-18 methyl and Val177 were lost. The aryl group was bent away active site entrance (Fig. 3.10). Nevertheless, the N-H of the sulfonamide group in **3.19** was able to form a H-bond with the carbonyl oxygen of Arg98. A hydrophobic interaction with the Arg98 side chain and the aryl group of the sulfonamide moiety in **3.19** was also evident as was a Pi-alkyl interaction between his aliphatic skeleton and His 485.

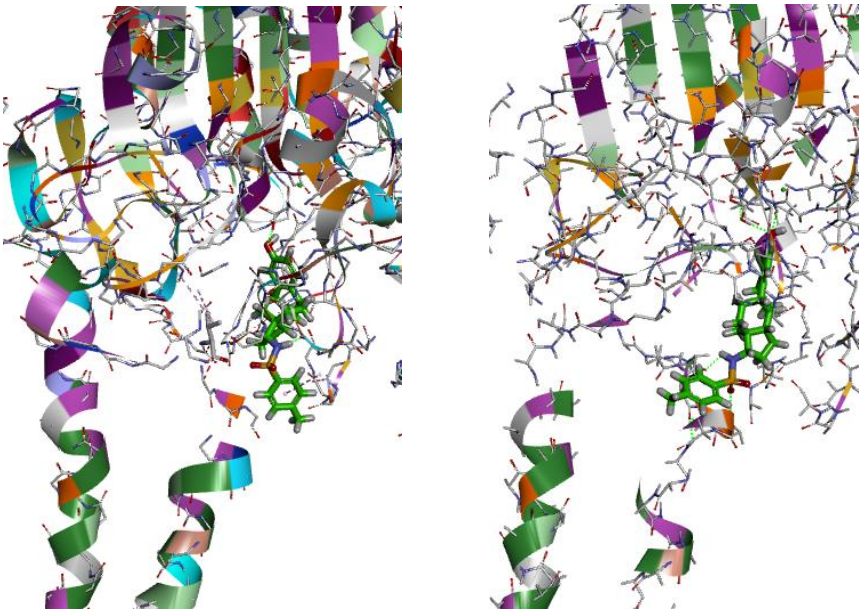


Fig. 3.10. The binding mode of compounds **3.19** (left) and **3.6** (right) with STS.

From our results in § **3.3.2**, we noticed that inclusion of alkyl chain at the 4´-position of our sulfonamide inhibitors caused a marked increase in potency till the chain length approaches 4 carbon atoms length (i.e. the n-pentyl derivative **3.25**) and then activity starts to decrease. The alkyl chain of the n-pentyl derivative **3.25** starts to bend away from the active site entrance (Fig. 3.11). The n-pentyl tail interacts through a network of hydrophobic and pi-alkyl interactions with Trp550, Phe553, and Leu554, while the n-butyl tail was having hydrophobic interactions

with Phe104 and Leu554. It is not clear from these studies as to why the n-pentyl derivative is a poorer inhibitor than the butyl derivative.

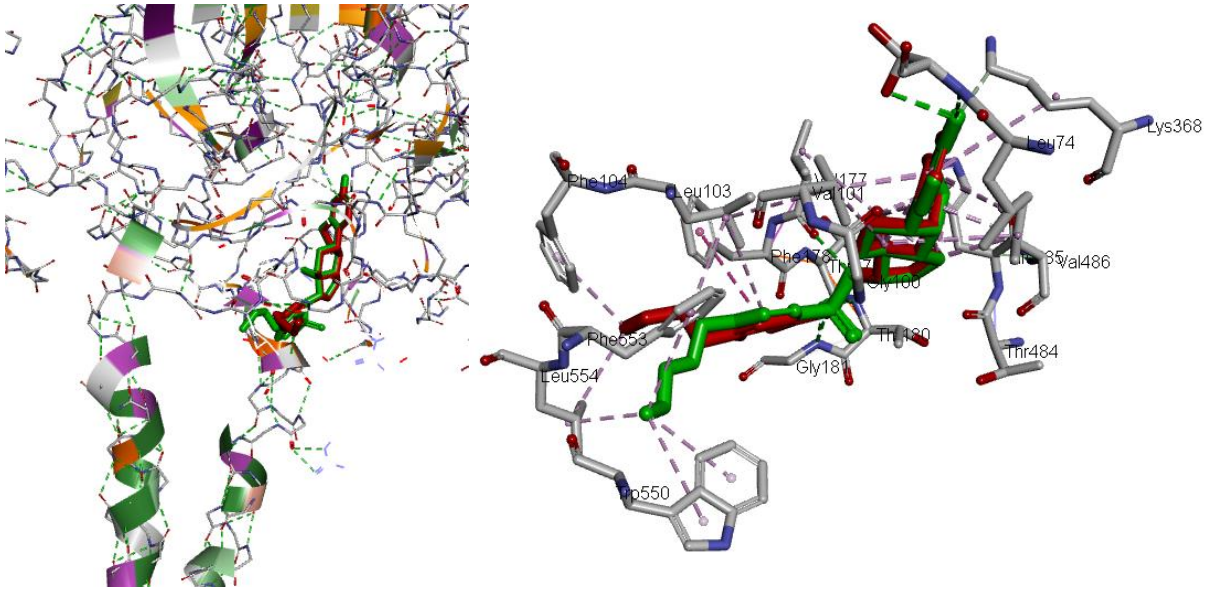


Fig. 3.11. The binding mode of compounds **3.24** (red) and **3.25** (green) with STS in its full shape (left Fig.) and zoomed active site (right Fig.)

We also investigated the binding interactions of one of our most potent inhibitors, the 3'-CF_3 sulfonamide (**3.33**, IC_{50} = 24 nM) and the corresponding compound reported by the Poirier group, (**3.63** in Fig. 3.8, IC_{50} = 126 nM),[68] as shown in Fig. 3.12.

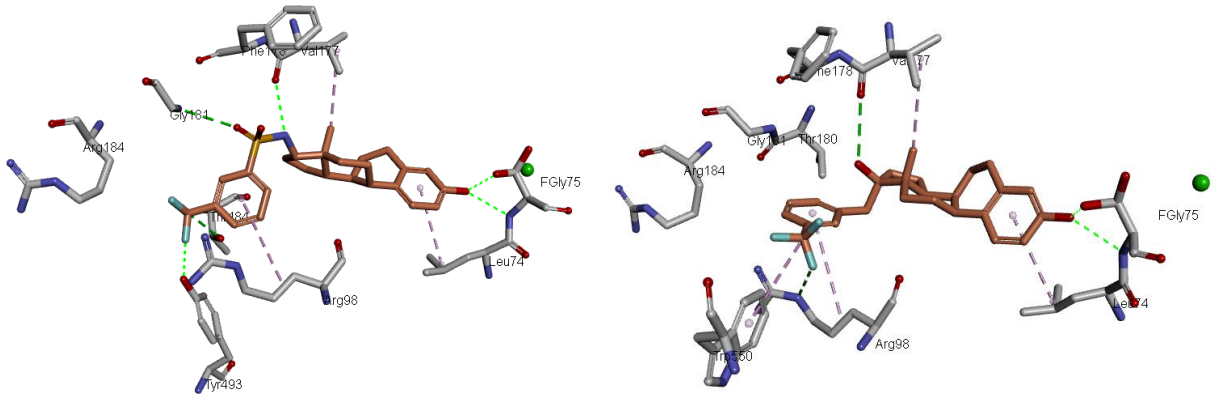


Fig. 3.12. The binding mode of compounds **3.33** (left) and **3.63** (right) with STS (green dotted lines indicate H-bonding interactions; light violet indicate non-polar interactions; H-atoms are removed to increase clarity).

The 3-OH in both compounds is involved in H-bond interactions with FGly75 (length ~ 3 Å). The N-H of the sulfonamide group in **3.33** was again involved in an H-bond with the carbonyl of Phe178 as was the 17-OH group in **3.63** (2.9 and 3.3 Å, respectively). One of the oxygens of the sulfonamide group in **3.33** was involved in an H-bond with the N-H of Gly181. Two of the fluorines of the CF_3 group of the sulfonamide were involved in H-bonding interactions with the side chains of Arg98 and Tyr 493 and these interactions were not evident in **3.63**. Moreover, multiple hydrophobic Pi-alkyl interactions with Leu74, Arg98, and Val177, however; the ones for the sulfonamide **3.33** were shorter than those of **3.63**.

We noted that the trifluoromethoxy derivative**, 3.34**, was 3-fold less potent than **3.33**. Docking **3.34** into the STS active revealed that the oxygen spacer of the CF_3O group caused a marked change in the orientation of the arylsulfonamide group which resulted in the loss of the H-bond interaction with Gly181 and the pi-alkyl hydrophobic interaction with as shown in Fig. 3.13.

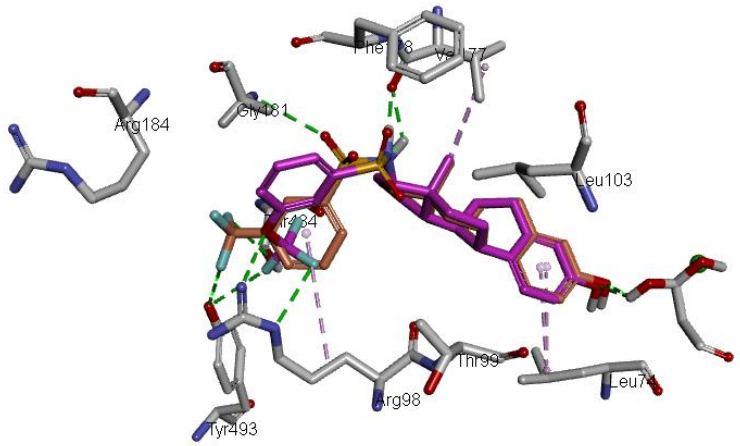


Fig. 3.13. The binding mode of compounds **3.33** (light brown) and **3.34** (yellow) with STS (green dotted lines indicate H-bonding interactions; H-atoms are removed to increase clarity).

One of the more dramatic changes in potency was when the sulfonamide group in **3.33** was replaced with an amide group (compound **3.58**): compound **3.58** was 31-times less potent than its sulfonamide analog, **3.33.** Docking of **3.58** into the STS active site revealed that it bound to STS in a very different manner than **3.33** (Fig. 3.14). One of the most significant changes compared to all of the compounds docked and mentioned above was the loss of the H-bond interaction between the 3-OH group and the catalytically crucial FGly75 residue. Moreover, the H-bonding interactions between the CF_3 group in **3.33** and STS are not evident in **3.58**. The carbonyl of the amide group in **3.58** is involved in H-bonding interactions with the side chain of Arg98 (3.19 and 3.2 A) but this does not seem to be sufficient to compensate for the loss of the key interactions that were evident in **3.33**.

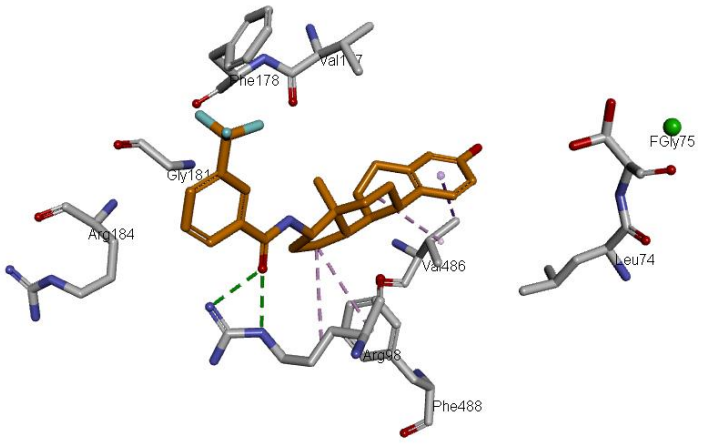


Fig. 3.14. The binding mode of compound **3.58** with STS (green dotted lines indicate H-bonding interactions, light violet dotted lines indicate hydrophobic interactions; H-atoms are removed to increase clarity).

3.4 Conclusions and Future Work

We have prepared and examined a library of 17β-arylsulfonamides of type **3.2** as STS inhibitors. Some of these compounds; such as the 4'-*t*-butyl derivative (**3.27**), 3'-Br derivative

(**3.28**), 3'-CF_3 derivative (**3.33**), 2'-naphthyl derivative (**3.46)**, and the 4'-biphenyl derivative (**3.49**), are among the most potent reversible STS inhibitors reported to date. Kinetic studies with compound **3.28** revealed it to be a non-competitive inhibitor and so these types of inhibitors might be capable of binding at the active site and also at a secondary site outside the active site, possibly in the hydrophobic channel between the two alpha helices as mentioned earlier. The amide analogs of some of these compounds (**3.57** and **3.58**) were found not to be as potent inhibitors as the sulfonamides. Modeling studies provided some information as to how certain inhibitors might interact with STS. In *Chapter* 4 we describe how a simple modification of these sulfonamide inhibitors resulted in the development of the most potent reversible inhibitors of STS reported to date.

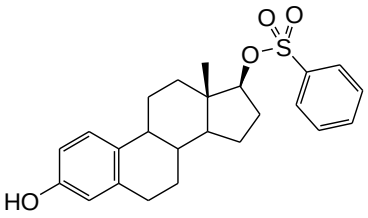
3.5 Experimental

3.5.1 General

All starting materials and reagents were obtained from Aldrich Chemical Company. THF was distilled from sodium-benzophenone, Pyridine was distilled from KOH pellets. DCE was dried by standing over activated type 4A molecular sieves. CH_2Cl_2 was distilled from calcium hydride under nitrogen. Silica gel chromatography was performed using silica gel (60Å, 230-400 mesh) obtained from Silicycle (Laval, Quebec, Canada). ^1H, ^{13}C, and ^{19}F NMR spectra were recorded on a Bruker Avance 300 spectrometer. For NMR spectra obtained using $CDCl_3$ as the solvent, chemical shifts (δ) for ^1H NMR spectra are reported relative to internal Me_4Si (δ 0.0 ppm), chemical shifts for ^{13}C spectra are relative to the residual solvent peak (δ 77.0 ppm, central peak), and chemical shifts for ^{19}F NMR are relative to a $CFCl_3$ (δ 0.0 ppm) external standard. Low-resolution (LRMS) and high-resolution (HRMS) electron impact (EI) and electrospray

ionization (ESI) mass spectra were obtained on a JEOL HX110 double focusing mass spectrometer. Electrospray (ESI) mass spectra were obtained with a Waters/Micromass QTOF Ultima Global mass spectrometer. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected.

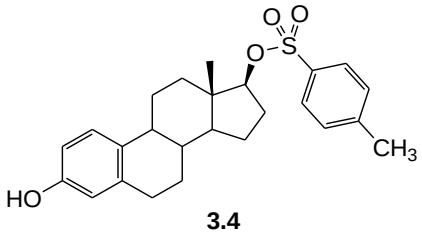
3.5.2 Syntheses



3.3

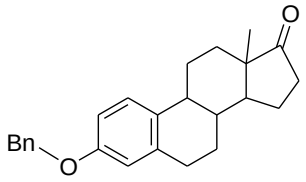
17β-Benzenesulfonyloxy-estra-1,3,5(10)-trien-3-ol (3.3). To a solution of compound **3.9a** (150 mg, 0.30 mmol) in a methanol/ethyl acetate (1:1, 10 mL) and a catalytic amount of acetic acid (150 µL) was added 20% $Pd(OH)_2$ (0.8 eq, 0.24 mmol). The mixture was stirred under H_2 gas for 16 h then filtered and concentrated. Purification of the residue by flash chromatography (ethyl acetate/hexane, 1:4) giving compound **3.3** as a white solid (85 mg, 69%). Mp 193-195 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.90 (dd, *J* = 7.1 and 1.4 Hz, 2H, ArH), 7.65-7.51 (m, 3H, ArH), 7.07 (d, *J* = 8.4 Hz, 1H, H-1), 6.58 (dd, *J* = 8.3 and 2.5 Hz, 1H, H-2), 6.51 (d, *J* = 2.4 Hz, 1H, H-4), 4.60 (s, 1H, ArOH), 4.35 (t, *J* = 7.8 Hz, 1H, H-17), 2.76 (m, 2H), 2.20-1.96 (m, 3H), 1.82-1.63 (m, 4H), 1.44-1.23 (m, 4H), 1.13-1.08 (m, 2H), 0.81 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.4 (C-3), 138.0 (C-SO_2O-), 137.2 (C-5), 133.5 (CH_{Ar}), 132.1 (C-10), 129.1 ($2CH_{Ar}$), 127.8 ($2CH_{Ar}$), 126.3 (CH_{Ar}), 115.2 (CH_{Ar}), 112.7 (CH_{Ar}), 90.1 (C-17), 49.0 (CH), 43.6 (CH), 43.3 (CH_2), 38.4 (CH), 36.0 (CH_2), 29.4 (CH_2), 27.7 (CH_2), 27.0 (CH_2), 25.9

(CH_2), 23.0 (CH_2), 11.7 (CH_3, C-18)); LRMS (ESI^-) *m/z* (%) 411 (M-H, 100); HRMS (ESI^-) calcd for $C_{24}H_{27}O_4S$ $(M-H)^-$ 411.1641; found 411.1641.



3.4

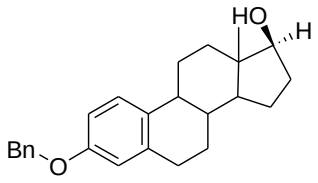
17β-Toluenesulfonyloxy-estra-1,3,5(10)-trien-3-ol (3.4). To a solution of compound **3.9b** (150 mg, 0.29 mmol) in a methanol/ethyl acetate (1:1, 10 mL) and a catalytic amount of acetic acid (150 µL) was added $Pd(OH)_2$ (0.8 eq., 0.23 mmol). The mixture was stirred under H_2 gas for 16 h then filtered and concentrated. Purification of the residue by flash chromatography (ethyl acetate/hexane, 1:9) afforded compound **3.4** as a white solid (88 mg, 71%). Mp 185-186 ^oC (lit. 186-187^oC)[160]; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.78 (dd, *J* = 6.6 and 1.7 Hz, 2H, ArH), 7.33 (d, *J* = 6.6 Hz, 2H, ArH), 7.08 (d, *J* = 8.5 Hz, 1H, H-1), 6.59 (dd, *J* = 8.4 and 2.7 Hz, 1H, H-2), 6.52 (d, *J* = 2.7 Hz, 1H, H-4), 4.59 (s, 1H, Ar-OH), 4.33 (q, *J* = 7.7 Hz, 1H, H-17), 2.76 (m, 2H), 2.43 (s, 3H, $OSO_2C_6H_4$-C<u>H</u>$_3$), 2.09-1.95 (m, 3H), 1.78-1.67 (m, 4H), 1.41-1.33 (m, 4H), 1.14-1.09 (m, 2H), 0.81 (s, 3H, CH_3, H-18).



3.7

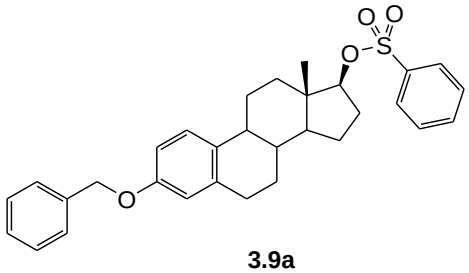
3-Benzyloxyestrone (3.7). Potassium carbonate (511 mg, 3.70 mmol) was added to a stirred solution of **E1** (500 mg, 1.85 mmol) in anhydrous acetone (25 mL), and the resulting suspension was stirred for 1 hour. Benzyl bromide (348 mg, 2.2 mmol) was added and the

mixture was refluxed for 4 hours. The mixture was poured into ice/water then extracted with ethyl acetate. The combined extracts were washed with water then dried by Na_2SO_4, and concentrated under vacuum. The resulting pale yellow crude solid was recrystallized from ethanol to yield compound **3.7** as white crystals (606 mg, 91%). Mp 132-133 ^oC (lit 132-134 ^oC)[161];^1H NMR ($CDCl_3$, 300 MHz) δ 7.42-7.29 (m 5H, $C_6\underline{H}_5$), 7.18 (d, *J* = 8.6 Hz, 1H, H-1), 6.77 (d, *J* = 8.5 Hz, 1H, H-2), 6.72 (br-s, 1H, H-4), 5.02 (s, 2H, $C_6H_5C\underline{H}_2$), 2.87 (d, *J* = 5.3 Hz, 2H), 2.53-2.35 (m, 2H), 2.24-1.88 (m, 4H), 1.63-1.41 (m, 7H), 0.89 (s, 3H, CH_3, H-18).



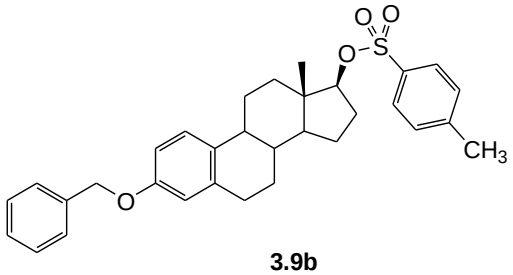
3.8

3-Benzyloxyestra-1,3,5(10)-trien-17β-ol (3.8). To a solution of compound **3.3** (500 mg, 1.38 mmol) in EtOH/THF (20 mL, 5:1) at 0^{o}C was added $NaBH_4$ (103 mg, 2.77 mmol). The resulting mixture was stirred for 1 h at 0^{o}C then the reaction was quenched with 1 M HCl. After extraction with ethyl acetate, the combined extracts were washed with water, brine, dried with Na_2SO_4, and finally concentrated under vacuum. Purification of the residue by flash chromatography (ethyl acetate/hexane, 1:4) gave compound **3.8** as a white solid (372 mg, 74%). Mp 62-63 ^oC (lit 61-63 ^oC);[173] ^1H NMR ($CDCl_3$, 300 MHz) δ 7.44-7.31 (m 5H, C_6H_5), 7.21 (d, *J* = 8.6 Hz, 1H, H-1), 6.78 (d, *J* = 8.5 Hz, 1H, H-2), 6.73 (brs, 1H, H-4), 5.03 (s, 2H, $C_6H_5C\underline{H}_2$), 3.72 (t, *J* = 7.8 Hz, 1H, H-17), 2.84 (m, 2H), 2.33-2.09 (m, 3H), 1.97-1.87 (m, 2H), 1.17-1.69 (m, 2H), 1.51-1.14 (m, 7H), 0.78 (s, 3H, CH_3, H-18).



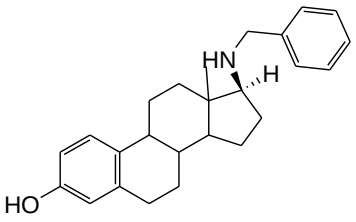
3.9a

3-Benzyloxy-17β-benzenesulfonyl-estra-1,3,5(10)-trien-17β-ol (3.9a). To a stirred solution of compound **3.8** (200 mg, 0.55 mmol) in anhydrous pyridine (5 mL) at 0^{o}C was added benzenesulfonyl chloride (106 mg, 0.60 mmol). The solution was stirred at room temperature for overnight, and then pyridine was azeotropically removed with toluene under vacuum. The residue was dissolved in ethyl acetate, then washed with 2 N HCl, water and brine then dried with Na_2SO_4, and concentrated. The residue was purified by flash chromatography (ethyl acetate/hexane, 3:7), to give **3.9a** as a white solid (175 mg, 63%). Mp 98-99^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.92 (d, *J* = 6.9 Hz, 2H, ArH), 7.63-7.51 (m, 3H, ArH), 7.39-7.25 (m, 5H, ArH), 7.13 (d, *J* = 8.3 Hz, 1H, H-1), 6.76-6.68 (m, 2H, H-2 and H-4), 5.00 (brs, 2H, $C_6H_5CH_2O$-), 4.34 (d, *J* = 8.2 Hz, 1H, H-17), 2.81 (brs, 2H, H-6), 2.22-1.97 (m, 3H), 1.83-1.72 (m, 4H), 1.42-1.05 (m, 6H), 0.82 (s, 3H, H-18). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 156.8 (C-3), 137.8 (C-SO_2O-), 137.3 (C-5), 133.5 (CH_{Ar}), 132.4 (C-10), 129.1 ($2CH_{Ar}$), 128.5 ($2CH_{Ar}$), 127.8 ($2CH_{Ar}$), 127.4 ($2CH_{Ar}$), 126.3 (CH_{Ar}), 114.8 (CH_{Ar}), 112.3 (CH_{Ar}), 90.1 (C-17), 69.9 (CH_2), 49.0 (CH), 43.7 (CH), 43.3 (CH_2), 38.4 (CH), 36.0 (CH_2), 29.6 (CH_2), 27.7 (CH_2), 27.0 (CH_2), 25.9 (CH_2), 23.0 (CH_2), 11.7 (CH_3, C-18)); LRMS (ESI^+) *m*/*z* (%) 503 (M+H, 100), 345 (40); HRMS (ESI^+) calcd for $C_{31}H_{35}O_4S$ $(M+H)^+$ 503.2256; found 503.2245.



3.9b

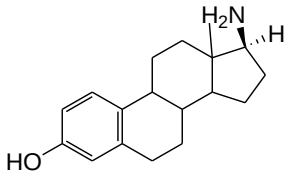
3-Benzyloxy-17β-toluenesulfonyl-estra-1,3,5(10)-trien-17β-ol (3.9b). To a stirred solution of compound **3.8** (200 mg, 0.55 mmol) in anhydrous pyridine (5 mL) at 0°C was added toluene-4-suflonylchloride (115 mg, 0.60 mmol). The solution was stirred at room temperature for overnight, and then pyridine was azeotropically removed with toluene under vacuum. The residue was dissolved in ethyl acetate, then washed with 2 N HCl, water and brine then dried with Na_2SO_4, and concentrated. The residue was purified by flash chromatography (ethyl acetate/hexane, 3:7), to give **3.9b** as a white solid (194 mg, 68%). Mp 115-117 °C (lit 115-117 °C); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.80 (d, *J* = 7.8 Hz, 2H, ArH), 7.42-7.32 (m, 7H, ArH), 7.15 (d, *J* = 8.5 Hz, 1H, H-1), 6.76 (d, *J* = 8.4 Hz, 1H, H-2), 6.70 (brs, 1H, H-4), 5.01 (s, 2H, $C_6H_5C\underline{H}_2O$-), 4.35 (t, *J* = 8.1 Hz, 1H, H-17), 2.81 (m, 2H), 2.45 (s, 3H, -$OSO_2C_6H_4C\underline{H}_3$), 2.24-2.10 (m, 2H), 1.99-1.92 (m, 1H), 1.85-1.61 (m, 4H), 1.43-1.26 (m, 4H), 1.16-1.08 (m, 2H), 0.83 (s, 3H, CH_3, H-18).



3.10

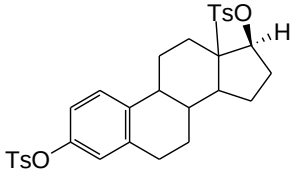
17β-Benzylamino-1,3,5(10)-estratrien-3-ol (3.10).[163] Estrone, **E1** (0.5 g, 1.85 mmol), and benzylamine (0.8 mL, 4 eq.) were mixed in 1,2-dichloroethane and THF mixture (1:1, 15 mL), and then treated with sodium triacetoxyborohydride (1 g, 2.5 eq.) and AcOH (0.5 mL, 4

eq.). The mixture was stirred at rt under a argon atmosphere for 48 h, then it was quenched by adding saturated solution of $NaHCO_3$, and the product was then extracted with EtOAc, washed with water (4×), brine (1×), then dried with Na_2SO_4, filtered and concentrated. The residue was then crystallized from methanol, giving white shiny crystals (92.6%): Mp 260-261°C. ^{1}H NMR ($CDCl_3$, 300 MHz), δ 7.34-7.20 (m, 5H, C_6H_5), 7.12 (d, *J* = 8.4 Hz, 1H, H-1), 6.58 (dd, *J* = 2.5 and 8.4 Hz, 1H, H-2), 6.51 (d, *J* = 2.5 Hz, 1H, H-4), 3.83 (AB system, overlapping dd, *J* = 13.4 Hz, 2H, $C_6H_5CH_2$), 2.87-2.76 (m, 2H), 2.65 (t, *J* = 8.4 Hz, 1H, NH), 2.26-1.99 (m, 5H), 1.86-1.80 (m, 1H), 1.72-1.64 (m, 1H), 1.46-1.15 (m, 8H), 0.77 (s, 3H, CH_3, C-18).



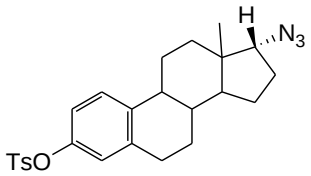
3.11

17β-Amino-1,3,5(10)-estratrien-3-ol (3.11).[163] To a solution of compound **3.10** (0.7 g, 1.9 mmol), in a methanol/ethyl acetate (6 mL, 1:1) mixture, was stirred with $Pd(OH)_2$ (0.8 eq.) in presence of catalytic amount of acetic acid (100 µL) under H_2 gas overnight. After that, it was filtered, concentrated under vacuum and flash chromatography (10% MeOH/1% aq. NH_4OH/ 89% $CHCl_3$), yielding **3.11** as a white solid (88%): Mp 232-33°C (lit. 235-37). ^{1}H NMR ($CDCl_3$, 300 MHz), δ 7.11 (d, *J* = 8.3 Hz, 1H, H-1), 6.59 (dd, *J* = 2.7 and 8.4 Hz, 1H, H-2), 6.52 (d, *J* = 2.6 Hz, 1H, H-4), 2.82-2.40 (m, 6H), 2.31-2.25 (m, 1H), 2.20-2.03 (m, 2H), 1.87-1.81 (m, 2H), 1.71-1.65 (m, 1H), 1.51-1.15 (m, 7H), 0.67 (s, 3H, CH_3, C-18).



3.12

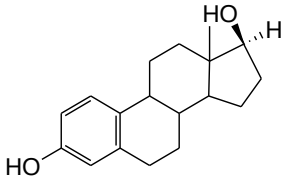
3,17β-bis(Toluene-4-sulfonyloxy)-1,3,5(10)-estratriene (3.12).[165] To a stirred solution of **E2** (350 mg, 0.52 mmol) in pyridine (5 ml) at room temperature, was added toluene-4-sulonyl chloride (217 mg, 1.13 mmol), and stirring was continued overnight. Pyridine was azeotropically removed with toluene under vacuum (2 × 5 ml), residue dissolved in ethyl acetate, washed with water, brine, and finally concentrated and dried with Na_2SO_4. The residue was purified by flash chromatography (ethyl acetate/hexane, 1:9) to give **3.12** as a white solid (537 mg, 72%). Mp: 155-156^{o}C (lit. 153-156); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.76 (d, J = 8.2 Hz, 2H, ArH), 7.69 (d, J = 8.2 Hz, 1H, ArH), 7.30 (t, J = 7.6 Hz, 4H, ArH), 7.05 (d, J = 8.6 Hz, 1H, H-1), 6.71 (d, J = 2.2 Hz, 1H, H-4), 6.62 (dd, J = 2.2 and 8.5 Hz 1H, H-2), 4.32 (t, J = 8.6 Hz, 1H, H-17), 2.73 (m, 2H, H-6), 2.42 (s, 6H, $SO_2C_6H_4C\underline{H}_3$), 2.15 (m, 2H), 1.93 (m, 1H), 1.79-1.62 (m, 4H), 1.42-1.25 (m, 4H), 1.15-1.05 (m, 2H), 0.79 (s, 3H, H-18).



3.13

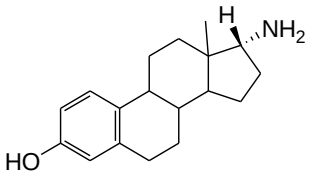
17α-Azido-3-(toluene-4-sulfonyloxy)-1,3,5(10)-estratriene (3.13).[165] To a stirred solution of **3.12** (500 mg, 0.87 mmol) in HMPA (10 mL), sodium azide (671 mg, 10.3 mmol) was added. Stirring was continued at 60-70^{o}C for 5 d, then reaction was poured on ice-water bath, precipitate filtered, dissolved in ethyl acetate, washed with water, brine, dried with Na_2SO_4, and finally concentrated under vacuum. The residue was purified by flash chromatography ($CH_3OH/CHCl_3$, 1:5) to yield compound **3.13** as a white solid (600 mg, 78%). Mp: 94-95^{o}C (lit. 93-95); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.71 (d, J = 8.0 Hz, 2H, ArH), 7.30 (d, J = 7.7 Hz, 2H, ArH), 7.13 (d, J = 8.4 Hz, 1H, ArH), 6.72 (brs, 1H, H-4), 6.63 (d, J = 8.2 Hz, 1H, H-2), 3.56 (d,

J = 6.0 Hz, 1H, H-17), 2.76 (m, 2H, H-6), 2.43 (s, 3H, $SO_2C_6H_4C\underline{H}_3$), 2.30-2.19 (m, 3H), 1.83-1.63 (m, 5H), 1.47-1.24 (m, 5H), 0.75 (s, 3H, H-18).



E2

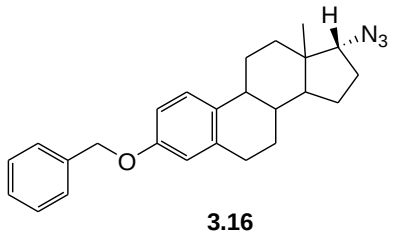
Estratrien-1,3,5(10)-3,17β-diol (E2). To a solution of **E1** (500 mg, 1.85 mmol) in EtOH/THF (150 mL, 2:1, heated to make a solution then cooled) at 0°C was added $NaBH_4$ (84 mg, 2.20 mmol, 1.2 equiv). The reaction was stirred 1 h at 0°C. The solvent was removed under vacuum and the residue was acidified with 1 N HCl and extracted with ethyl acetate. The combined extracts were washed with H_2O and brine then dried (Na_2SO_4), filtered and concentrated. The residue was purified by flash chromatography (ethyl acetate/hexane, 3:7) to give **E2** as a white solid (370 mg, 74%). Mp: 185-186°C (lit. 184-187);[174] ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.13 (d, *J* = 8.4 Hz, 1H, H-1), 6.60 (dd, *J* = 2.4 and 8.3 Hz, 1H, H-2), 6.54 (brs, 1H, H-4), 4.60 (brs, 1H, ArO<u>H</u>), 3.71 (t, *J* = 8.2 Hz, 1H, H-17), 2.81 (m, 2H, H-6), 2.25 (m, 1H), 2.12 (m, 2H), 1.91 (m, 2H), 1.67 (m, 1H), 1.55-1.16 (m, 8H), 0.76 (s, 3H, H-18).



3.15

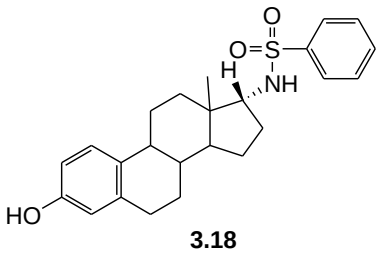
17α-Amino-1,3,5(10)-estratrien-3-ol (3.15).[165] To a stirred solution of **3.13** (500 mg, 1.11 mmol) in THF (10 mL), $LiAlH_4$ (1 g, 26.5 mmol) was added and stirring was continued for 24 h at room temperature. The reaction was quenched carefully with water (10 mL), and then acidified by HCl (10%, 10-12 mL) to pH of 1. The aqueous layer was then basified with NaOH

(10%, 15 mL), and extracted with ethyl acetate, washed with water, brine, dried with Na2SO4, and finally concentrated under vacuum. The residue was purified by flash chromatography ($CH_3OH/CHCl_3$, 1:5 then 1:1) to afford **3.15** as white solid (105 mg, 23%). Mp: 223-224^oC (lit. 226-227); ^1H NMR (DMSO-d_6, 300 MHz) δ 8.93 (brs, 1H, ArOH), 7.01 (d, J = 8.4 Hz, 1H, H-1), 6.46 (dd, J = 2.3 and 8.4 Hz, 1H, H-2), 6.39 (brs, 1H, H-4), 3.28 (s, 2H, NH_2 overlapping DMSO water), 2.80 (d, J = 6.9 Hz, 1H, H-17), 2.66 (m, 2H, H-6), 2.22 (m, 1H), 2.01 (m, 2H), 1.79-1.49 (m, 4H), 1.42-1.02 (m, 8H), 0.75 (s, 3H, H-18).



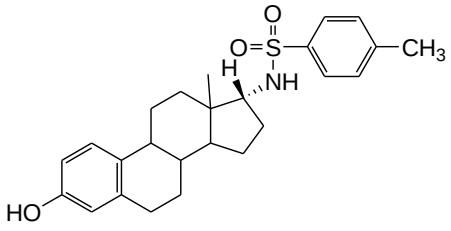
3.16

17α-Azido-3-Benzyloxy-1,3,5(10)-estratriene (3.16). To a stirred solution of **3.9b** (250 mg, 0.48 mmol) in HMPA (10 mL), sodium azide (500 mg, 7.7 mmol) was added. Stirring was continued at 90^oC for 2 d, then reaction was poured on ice-water bath, precipitate filtered, dissolved in ethyl acetate, washed with water, brine, dried with Na_2SO_4, and finally concentrated under vacuum. The residue was purified by flash chromatography ($CH_3OH/CHCl_3$, 1:5) to yield compound **3.16** as a white solid (133 mg, 71%). Mp: 77-78 ^oC (lit. 78-79^oC);[165] ^1H NMR ($CDCl_3$, 300 MHz) δ 7.43-7.28 (m, 5H, $C_6\underline{H}_5CH_2O$-), 7.20 (d, J = 8.6 Hz, 1H, H-1), 6.77 (d, J = 8.5 Hz, 1H, H-2), 6.71 (brs, 1H, H-4), 5.02 (brs, 2H, $C_6H_5C\underline{H}_2O$-), 3.58 (d, J = 6.5 Hz, 1H, H-17), 2.83 (m, 2H, H-6), 2.37-2.33 (m, 1H), 2.24-2.19 (m, 2H), 1.90-1.70 (m, 5H), 1.54-1.23 (m, 6H), 0.77 (s, 3H, H-18).



3.18

17α-Benzenesulfonamide-1,3,5(10)-estratrien-3-ol (3.18). To a stirred solution of **3.15** (100 mg, 0.37 mmol) in pyridine (2 mL) under Argon atmosphere, benzenesulfonyl chloride (71.5 mg, 0.40 mmol) in DCM (1 mL) was added portion-wise via a syringe pump at 0^{o}C. After complete addition of the benzenesulfonyl chloride, the reaction was left stirred overnight at room temperature, then pyridine was azeotropically removed under vacuum with toluene, and the residue was dissolved in ethyl acetate, washed with water, brine, and dried with Na_2SO_4, then filtered, and concentrated. The residue was purified by flash chromatography (ethyl acetate/hexane, 3:7) to afford **3.18** as white solid (71 mg, 47%). Mp: 118-119 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.87 (d, J = 7.7 Hz, 2H, ArH), 7.58-7.47 (m, 3H, ArH), 7.08 (d, J = 8.4 Hz, 1H, H-1), 6.58 (dd, J = 2.4 and 8.4 Hz, 1H, H-2), 6.52 (brs, 1H, H-4), 4.49 (s, 1H, ArOH), 4.38 (d, J = 9.2 Hz, 1H, NH), 3.17 (d, J = 8.6 Hz, 1H, H-17), 2.80 (d, J = 6.9 Hz, 1H, H-17), 2.76 (m, 2H, H-6), 2.22-2.07 (m, 2H), 1.85-1.58 (m, 5H), 1.38-1.10 (m, 7H), 0.68 (s, 3H, H-18). ^{13}C NMR ($CDCl_3$, 75 MHz), δ 153.3 (C-3), 141.1 (C-SO_2NH-), 138.1 (C-5), 132.5 (CH_{Ar}), 132.4 (C-6), 129.0 ($2CH_{Ar}$), 127.1 ($2CH_{Ar}$), 126.5 (C-1), 115.2 (C-4), 112.7 (C-2), 63.4 (C-17), 51.1 (C-14), 43.7 (CH), 42.9 (C-13), 38.8 (CH), 36.3 (CH_2), 29.5 ($2CH_2$ overlapping), 27.1 (CH_2), 26.0 (CH_2), 23.1 (CH_2), 11.8 (CH_3, C-18); LRMS (ESI$^+$) m/z (%) 412 (M+H, 100), 325 (20); HRMS (ESI$^+$) calcd for $C_{24}H_{30}NO_3S$ $(M+H)^+$ 412.19409; found 412.19379.

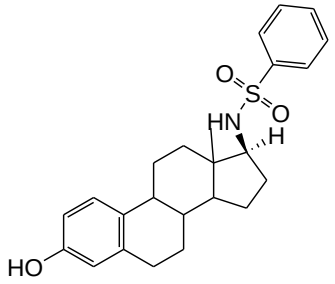


3.19

17α-Toluenesulfonamide-1,3,5(10)-estratrien-3-ol (3.19). To a stirred solution of **3.15** (100 mg, 0.37 mmol) in pyridine (2 mL) under Argon atmosphere, toluene-4-sulfonyl chloride (78 mg, 0.40 mmol) in DCM (1 mL) was added portion-wise via a syringe pump at 0^{o}C. After complete addition of the benzenesulfonyl chloride, the reaction was left stirred overnight at room temperature, then pyridine was azeotropically removed under vacuum with toluene, and the residue was dissolved in ethyl acetate, washed with water, brine, and dried with Na_2SO_4, then filtered, and concentrated under vacuum. Purification was achieved using flash chromatography (ethyl acetate/hexane, 1:9) which provided **3.19** as a white solid (88mg, 56%). Mp 131-132 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz), δ 7.72 (d, *J* = 8.2 Hz, 2H, ArH-a), 7.28 (d, *J* = 8.0 Hz, 2H, ArH-b), 7.10 (d, *J* = 8.4 Hz, 1H, H-1), 6.64 (dd, *J* = 8.4 and 2.7 Hz, 1H, H-2), 6.53 (d, *J* = 2.5 Hz, 1H, H-4), 5.03 (br-s, 1H, Ar-OH), 4. 50 (d, *J* = 9.1 Hz, 1H, NH), 3.29 (t, *J* = 8.3 Hz, 1H, H-17), 2.75 (br-s, 2H, H-6), 2.41 (s, 3H, $C_6H_4C\underline{H}_3$), 2.25-2.20 (m, 1H), 2.08-2.03 (m, 1H), 1.79-1.58 (m, 3H), 1.51 (m, 1H), 1.41-1.05 (m, 8H), 0.69 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.3 (C-3), 143.1 ($\underline{C}H_3$-$C_6H_4SO_2NH$-), 138.3 ($\underline{C}$-SO_2NH-), 138.0 (C-5), 132.4 (C-6), 129.0 (2ArCH), 126.9 (2ArCH), 126.5 (C-1), 115.2 (C-4), 112.8 (C-2), 63.5 (C-17), 49.6 (C-14), 44.9 (CH_2), 43.2 (C-13), 39.0 (CH), 32.7 (CH_2), 31.2 (CH_2), 29.6 (CH_2), 27.8 (CH_2), 26.0 (CH_2), 24.0 (CH_2), 21.5 ($\underline{C}H_3$-C_6H_4-SO_2NH-), 18.2 (CH_3, C-18); LRMS (ESI^+) *m*/*z* (%) 426 (M+H, 22), 255 (100); HRMS (ESI^+) calcd for $C_{25}H_{32}NO_3S$ $(M+H)^+$ 426.2103; found 426.2104.

General procedure for synthesis of sulfonamides of17β-aminoestra-1,3,5(10)-trien-3-ol (3.11).

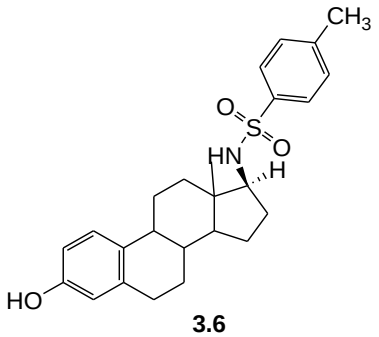
To a stirred solution of 17β-amino-1,3,5(10)-estratrien-3-ol, compound **3.11** (0.38 mmol), in dry pyridine (3 mL) at 0^{o}C was added a solution of the sulfonyl chlorides (0.40 mmol) in dichloromethane (1 mL) via a syringe pump for 30 min. After addition, the reaction was stirred for 16 h at room temperature, then pyridine was azeotropically removed with toluene under vacuum, the residue was dissolved in ethyl acetate, washed with water and brine then dried (Na_2SO_4), filtered, and concentrated under vacuum.[164]



3.5

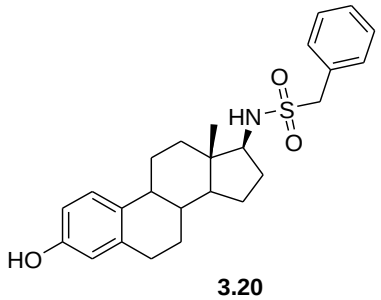
17β-Benzenesulfonamide-1,3,5(10)-estratrien-3-ol (3.5). Purification was achieved using flash chromatography (ethyl acetate/hexane, 2:8) which provided compound **3.5** as a white solid (67%). Mp 234-235 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.88 (d, *J* = 8.1 Hz, 2H, H-a & a` ), 7.58-7.47 (m, 3H, H-b, b` & c), 7.09 (d, *J* = 8.4 Hz, 1H, H-1), 6.59 (d, *J* = 8.4 Hz, 1H, H-2), 6.52 (s, 1H, H-4), 4.60 (s, 1H, Ar-OH), 4.48 (d, *J* = 9.2 Hz, 1H, NH), 3.15 (q, *J* = 8.9 Hz, 1H, H-17), 2.75 (br-s, 2H), 2.21-2.16 (m, 2H), 1.88-1.60 (m, 4H), 1.42-1.07 (m, 7H), 0.68 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.3 (C-3), 141.1 (C-SO_2NH-), 138.1 (C-5), 132.5 (CH_{Ar}), 132.4 (C-6), 129.0 ($2CH_{Ar}$), 127.1 ($2CH_{Ar}$), 126.5 (C-1), 115.2 (C-4), 112.7 (C-2), 63.4 (C-17), 51.1 (C-14), 43.7 (CH), 42.9 (C-13), 38.8 (CH), 36.3 (CH_2), 29.5 ($2CH_2$ overlapping), 27.1

(CH_2), 26.0 (CH_2), 23.1 (CH_2), 11.8 (CH_3, C-18); LRMS (ESI^+) *m*/*z* (%) 412 (M+H, 100), 255 (18); HRMS (ESI^+) calcd for $C_{24}H_{30}NO_3S$ $(M+H)^+$ 412.1946; found 412.1950.



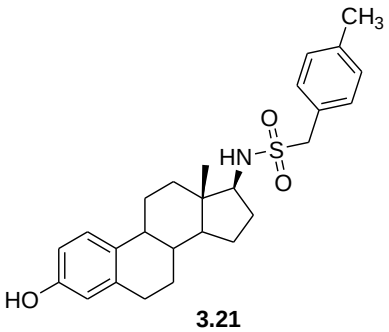
3.6

17β-(4´-Methylbenzene)sulfonamide-1,3,5(10)-estratrien-3-ol, (3.6). Purification was done by flash chromatography (methanol/chloroform, 1:9), yielding (33%) of compound **3.6** as a white solid: Mp 179-180^{o}C. ^{1}H NMR ($CDCl_3$, 300 MHz), δ 7.75 (d, *J* = 8.2 Hz, 2H, H-a), 7.28 (d, *J* = 8.0 Hz, 2H, H-b), 7.08 (d, *J* = 8.4 Hz, 1H, H-1), 6.58 (dd, *J* = 8.3 and 2.7 Hz, 1H, H-2), 6.52 (d, *J* = 2.6 Hz, 1H, H-4), 4.73 (br-s, 1H, Ar-OH), 4. 50 (d, *J* = 9.2 Hz, 1H, NH), 3.13 (q, *J* = 8.9 Hz, 1H, H-17), 2.76 (br-s, 2H), 2.41 (s, 3H, C_6H_4-CH_3), 2.23-2.10 (m, 2H), 1.83-1.71 (m, 3H), 1.62-1.60 (m, 1H), 1.39-1.08 (m, 7H), 0.68 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.3 (C-3), 140.9 (C-SO_2NH-), 139.1 (C-CH_3), 138 (C-5), 133.2 (ArCH), 132.4 (C-6), 129.0 (2Ar-CH's-b), 127.1 (2Ar-CH's-a), 115.2 (C-4), 112.7 (C-2), 63.3 (C-17), 51.1 (C-14), 43.7 (CH), 42.9 (C-13), 38.8 (CH), 36.3 (CH_2), 29.5 ($2CH_2$), 27.1 (CH_2), 26 (CH_2), 23.1 (CH_2), 21.3 (Ar-CH_3), 11.8 (CH_3, C-18); LRMS (EI) *m*/*z* (%) 425 (M^+, 100), 270 (60), 253 (25), 213 (20); HRMS (EI) calcd for $C_{25}H_{31}NO_3S$ 425.2025; found 425.2031.



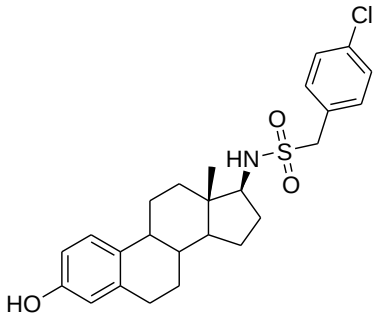
3.20

17β-Benzylsulfonamide-1,3,5(10)-estratrien-3-ol (3.20). Purification was achieved using flash chromatography (methanol/chloroform, 1:9) which provided **3.20** as a white solid (26%). Mp 202-203^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.40-7.35 (m, 5H, ArH's), 7.11 (d, *J* = 8.4 Hz, 1H, H-1), 6.60 (dd, *J* = 8.4 and 2.6 Hz, 1H, H-2), 6.53 (d, *J* = 2.5 Hz, 1H, H-4), 4.79 (br-s, 1H, Ar-OH), 4.23 (AB system, 2H, *J* = 13.9 and 13.9 Hz, 2H, Ar-CH_2), 4.09 (d, *J* = 9.2 Hz, 1H, NH), 3.23 (q, *J* = 8.4 Hz, 1H, H-17), 2.77 (br-s, 2H), 2.28-2.09 (m, 3H), 1.94-1.68 (m, 3H), 1.42-1.18 (m, 7H), 0.65 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.4 (C-3), 138.1 (C-5), 132.4 (C-6), 130.7 (2Ar-CH), 129.4, 128.7 (2Ar-CH), 126.5 (C-1), 115.2 (C-4), 112.7 (C-2), 63.8 (C-17), 59.7 ($ArCH_2SO_2NH$), 51.1 (C-14), 43.7 (CH), 42.9 (C-13), 38.9 (CH), 36.5 (CH_2), 29.9 (CH_2), 29.5 (CH_2), 27.1 (CH_2), 26.1 (CH_2), 23.1 (CH_2), 11.8 (CH_3, C-18); LRMS (EI) *m*/*z* (%) 425 (M^+, 100), 270 (35), 213, 91; HRMS (EI) calcd for $C_{25}H_{31}NO_3S$ 425.2025; found 425.2018.



3.21

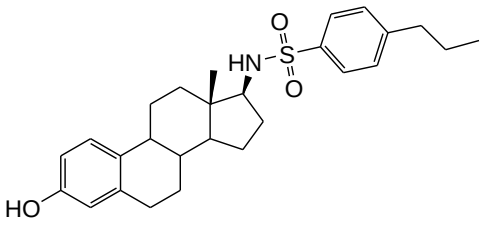
17β-(4´-Methylbenzylsulfonamide-1,3,5(10)-estratrien-3-ol (3.21). Purification was achieved using flash chromatography (methanol/chloroform, 1:9) which provided **3.21** as a white solid (38%). Mp 140-141 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.27 (d, *J* = 7.9 Hz, 2H, ArH-a), 7.17 (d, *J* = 7.9 Hz, 2H, ArH-b), 7.11 (d, *J* = 8.4 Hz, 1H, H-1), 6.60 (dd, *J* = 8.4 and 2.5 Hz, 1H, H-2), 6.53 (d, *J* = 2.2 Hz, 1H, H-4), 4.66 (br-s, 1H, Ar-OH), 4.18 (AB system, 2H, *J* = 13.9 and 13.9 Hz, 2H, Ar-CH_2), 3.99 (d, *J* = 9.2 Hz, 1H, NH), 3.27 (q, *J* = 8.9 Hz, 1H, H-17), 2.78 (br-s, 2H), 2.34 (s, 3H, Ar-CH_3), 2.30-2.25 (m, 1H), 2.15-2.10 (m, 2H), 1.95-1.69 (m, 3H), 1.43-1.13 (m, 7H), 0.65 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.4 (C-3), 138.6, 138.1 (C-5), 132.4 (C-6), 130.6 (2Ar-CH), 129.4 (2Ar-CH), 126.5 (C-1), 126.4, 115.2 (C-4), 112.7 (C-2), 63.8 (C-17), 59.3 (CH_2SO_2NH), 51.1 (C-14), 43.7 (CH), 42.9 (C-13), 38.9 (CH), 36.5 (CH_2), 30.0 (CH_2), 29.5 (CH_2), 27.1 (CH_2), 26.1 (CH_2), 23.1 (CH_2), 21.2 (Ar-CH_3), 11.8 (CH_3, C-18); LRMS (ESI$^+$) *m/z* (%) 440 (M+H, 31), 377 (28), 376 (100), 270 (28); HRMS (ESI$^+$) calcd for $C_{26}H_{34}NO_3S$ $(M+H)^+$ 440.2259; found 440.2265.



3.22

17β-(4´-Chlorobenzylsulfonamide-1,3,5(10)-estratrien-3-ol (3.22). Purification was achieved using flash chromatography (ethyl acetate/hexane, 1:4) which provided **3.22** as a white solid (27%). Mp 178-179 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.34 (m, 4H, ArH), 7.12 (d, *J* = 8.3 Hz, 1H, H-1), 6.60 (dd, *J* = 2.5 and 8.3 Hz, 1H, H-2), 6.53 (brs, 1H, H-4), 4.50 (brs, 1H, ArOH),

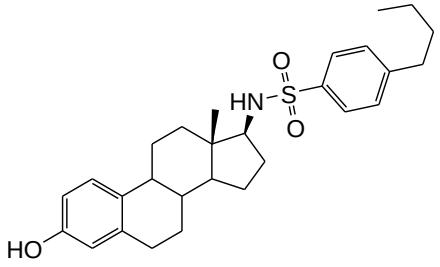
4.19 (AB system, 2H, *J* = 13.9 and 13.9 Hz, 2H, Ar-CH_2), 3.98 (d, *J* = 9.4 Hz, 1H, NH), 3.26 (q, *J* = 8.8 Hz, 1H, H-17), 2.78 (brs, 2H), 2.31-2.26 (m, 1H), 2.17-2.06 (m, 2H), 1.94-1.68 (m, 3H), 1.48-1.15 (m, 7H), 0.67 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.3 (C-3), 138.1 (C-5), 134.9 (C-Cl), 132.4 (C-6), 132.0 (2 CH_{Ar}), 128.9 (2 CH_{Ar}), 127.9 (CH), 126.5 (C-1), 115.2 (C-4), 112.7 (C-2), 63.8 (C-17), 59.0 (CH_2SO_2NH), 51.1 (C-14), 43.7 (CH), 42.9 (C-13), 38.9 (CH), 36.5 (CH_2), 30.0 (CH_2), 29.5 (CH_2), 27.1 (CH_2), 26.1 (CH_2), 23.1 (CH_2), 11.8 (CH_3, C-18); LRMS (ESI^+) *m/z* (%) 460 (M+H, 35), 396 (100); HRMS (ESI^+) calcd for $C_{25}H_{31}NO_3SCl$ $(M+H)^+$ 460.1713; found 460.1708.



3.23

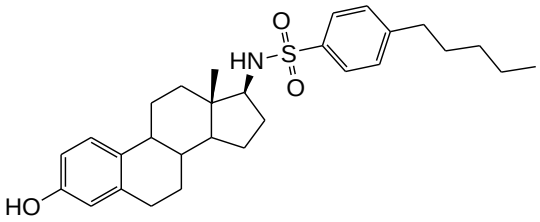
17β-(4´-n-Propylbenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (3.23). Purification was achieved using flash chromatography (methanol/chloroform, 1:9) which provided compound **3.23** as a white solid (27%). Mp 217-218 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.76 (d, *J* = 8.1 Hz, 2H, ArH-a), 7.27 (d, *J* = 8.1 Hz, 2H, ArH-b), 7.08 (d, *J* = 8.4 Hz, 1H, H-1), 6.58 (d, *J* = 8.4 Hz, 1H, H-2), 6.52 (br-s, 1H, H-4), 4.59 (br-s, 1H, Ar-OH), 4.39 (d, *J* = 9.2 Hz, 1H, NH), 3.15 (q, *J* = 8.7 Hz, 1H, H-17), 2.76 (br-s, 2H), 2.64 (t, *J* = 7.6 Hz, 2H, $C\underline{H}_2$-CH_2-CH_3), 2.20-2.07 (m, 2H), 1.89-1.61 (m, 6H), 1.42-1.06 (m, 7H), 0.92 (t, *J* = 7.3 Hz, 3H, CH_2-CH_2-$C\underline{H}_3$), 0.68 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.3 (C-3), 147.9 (C-propyl), 138.3 (C-SO_2NH-), 138.0 (C-5), 132.4 (C-6), 129.0 (2Ar-CH's-b), 127.1 (2Ar-CH's-a), 126.5 (C-1), 115.2 (C-4), 112.7 (C-2), 63.3 (C-17), 51.1 (C-14), 43.7 (CH), 42.9 (C-13), 38.8 (CH), 37.8 ($\underline{C}H_2$-CH_2-CH_3), 36.3 (CH_2), 29.5 ($2CH_2$), 27.1 (CH_2), 26.0 (CH_2), 24.2 (CH_2-$\underline{C}H_2$-CH_3), 23.1 (CH_2), 13.6 (CH_2-CH_2-

CH_3), 11.8 (CH_3, C-18); LRMS (ESI^+) *m/z* (%) 454 (M+H, 72), 256 (20), 255 (100); HRMS (ESI^+) calcd for $C_{27}H_{36}NO_3S$ $(M+H)^+$ 454.2416; found 454.2410.



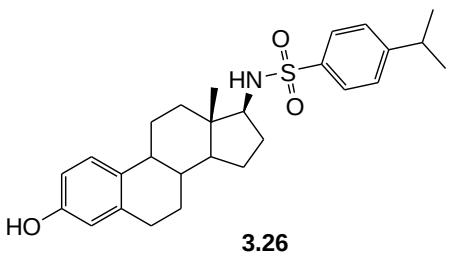
3.24

17β-(4'-*n*-Butylbenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (3.24). Purification was achieved using flash chromatography (methanol/chloroform, 1:9) which provided compound **3.24** as a white solid (56%). Mp 221-222 ^oC; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.76 (d, *J* = 8.3 Hz, 2H, ArH-a), 7.28 (d, *J* = 8.3 Hz, 2H, ArH-b), 7.09 (d, *J* = 8.4 Hz, 1H, H-1), 6.58 (dd, *J* = 8.3 and 2.6 Hz, 1H, H-2), 6.52 (d, *J* = 2.6 Hz, 1H, H-4), 4.50 (br-s, 1H, Ar-OH), 4.32 (d, *J* = 9.2 Hz, 1H, NH), 3.15 (q, *J* = 8.9 Hz, 1H, H-17), 2.76 (br-s, 2H), 2.67 (t, *J* = 7.5 Hz, 2H, CH_2-CH_2-CH_2-CH_3), 2.20-2.11 (m, 2H), 1.83-1.58 (m, 6H), 1.37-1.10 (m, 9H), 0.92 (t, *J* = 7.3 Hz, 3H, CH_2-CH_2-CH_2-CH_3), 0.68 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.3 (C-3), 148.1 (C-Butyl), 138.3 (C-SO_2NH-), 138.1 (C-5), 132.5 (C-6), 129.0 (2Ar-CH's-b), 127.1 (2Ar-CH's-a), 126.5 (C-1), 115.2 (C-4), 112.7 (C-2), 63.3 (C-17), 51.1 (C-14), 43.7 (CH), 42.9 (C-13), 38.8 (CH), 36.3 (CH_2), 35.5 (CH_2- CH_2-CH_2-CH_3), 33.2 (CH_2-CH_2-CH_2-CH_3), 29.5 ($2CH_2$), 27.1 (CH_2), 26.0 (CH_2), 23.1 (CH_2), 22.2 (CH_2-CH_2-CH_2-CH_3), 13.9 (CH_2-CH_2-CH_2-CH_3), 11.8 (CH_3, C-18); LRMS (ESI^+) *m/z* (%) 468 (M+H, 100), 255 (74), 219 (29), 152 (31); HRMS (ESI^+) calcd for $C_{28}H_{38}NO_3S$ $(M+H)^+$ 468.2572; found 468.2559.



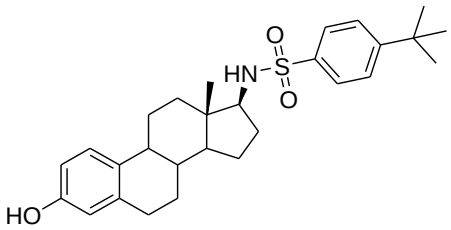
3.25

17β-(4'-*n*-Pentylbenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (3.25). Purification was achieved using flash chromatography (methanol/chloroform, 1:4), which provided compound **3.25** as a white solid (21%). Mp 168-169 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.76 (d, *J* = 7.8 Hz, 2H, ArH-a), 7.28 (d, *J* = 8.0 Hz, 2H, ArH-b), 7.09 (d, *J* = 8.4 Hz, 1H, H-1), 6.58 (dd, *J* = 8.2 and 2.2 Hz, 1H, H-2), 6.52 (br-s, 1H, H-4), 4.50 (br-s, 1H, Ar-OH), 4.35 (d, *J* = 9.2 Hz, 1H, NH), 3.15 (q, *J* = 8.7 Hz, 1H, H-17), 2.76 (br-s, 2H), 2.66 (t, *J* = 7.4 Hz, 2H, $C\underline{H}_2$-CH_2- CH_2-CH_2-CH_3), 2.21-2.11 (m, 2H), 1.85-1.80 (m, 2H), 1.77-1.59 (m, 4H), 1.38-1.09 (m, 11H), 0.87 (t, *J* = 6.3 Hz, 3H, CH_2-CH_2- CH_2-CH_2-$C\underline{H}_3$), 0.68 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.3 (C-3), 148.2 (C-pentyl), 138.3 (C-SO_2NH-), 138.1 (C-5), 132.5 (C-6), 129.0 (2Ar-CH's-b), 127.1 (2Ar-CH's-a), 126.5 (C-1), 115.2 (C-4), 112.7 (C-2), 63.3 (C-17), 51.1 (C-14), 43.7 (CH), 42.9 (C-13), 38.8 (CH), 36.3 (CH_2), 35.8 ($\underline{C}H_2$-CH_2-CH_2-CH_2-CH_3), 31.3 (CH_2-$\underline{C}H_2$- CH_2-CH_2-CH_3), 30.7 (CH_2-CH_2- $\underline{C}H_2$-CH_2-CH_3), 29.5 ($2CH_2$), 27.1 (CH_2), 26.0 (CH_2), 23.1 (CH_2), 22.4 (CH_2-CH_2-CH_2-$\underline{C}H_2$-CH_3), 13.9 (CH_2-CH_2-CH_2-CH_2-$\underline{C}H_3$), 11.8 (CH_3, C-18); LRMS (ESI^+) *m*/*z* (%) 482 (M+H, 100), 255 (80); HRMS (ESI^+) calcd for $C_{29}H_{40}NO_3S$ $(M+H)^+$ 482.2729; found 482.2721.



3.26

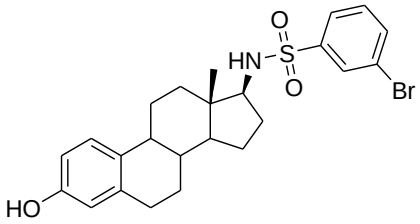
17β-(4'-*i*-Propylbenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (3.26). Purification was achieved using flash chromatography (methanol/chloroform, 1:9) which provided **3.26** as a white solid (43%). Mp 206-207 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.78 (d, *J* = 8.4 Hz, 2H, ArH-a), 7.33 (d, *J* = 8.3 Hz, 2H, ArH-b), 7.08 (d, *J* = 8.4 Hz, 1H, H-1), 6.58 (dd, *J* = 8.4 and 2.7 Hz, 1H, H-2), 6.52 (d, *J* = 2.6 Hz, 1H, H-4), 4.61 (br-s, 1H, Ar-OH), 4.42 (d, *J* = 9.2 Hz, 1H, NH), 3.15 (q, *J* = 8.8 Hz, 1H, H-17), 3.01-2.92 (m, 1H), 2.76 (br-s, 2H), 2.20-2.11 (m, 2H), 1.82-1.61 (m, 4H), 1.38-1.10 (m overlapping d of –CH($C\underline{H}_3$)$_2$ with *J* = 7.0 Hz, 13H), 0.68 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 154.0 (C-isopropyl), 153.3 (C-3), 138.4 (C-SO_2NH-), 138.1 (C-5), 132.4 (C-6), 127.1 (4Ar-CH's-b & a overlapping), 126.5 (C-1), 115.2 (C-4), 112.7 (C-2), 63.3 (C-17), 51.1 (C-14), 43.7 (CH), 42.9 (C-13), 38.8 (CH), 36.3 (CH_2), 34.1 ($\underline{C}H(CH_3)_2$), 29.5 ($2CH_2$), 27.1 (CH_2), 26.0 (CH_2), 23.7 ($2CH_3$, $CH(\underline{C}H_3)_2$), 23.1 (CH_2), 11.8 (CH_3, C-18); LRMS (EI) *m*/*z* (%) 453 (M^+, 100), 270 (90), 253 (30); HRMS (ESI^+) calcd for $C_{27}H_{36}NO_3S$ $(M+H)^+$ 454.2416; found 454.2403.



3.27

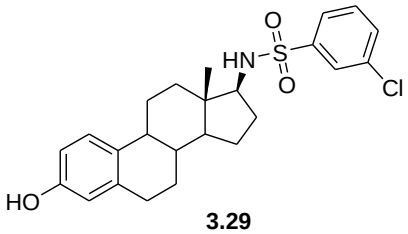
17β-(4´-t-Butylbenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (3.27). Purification was achieved by flash chromatography (methanol/chloroform, 1:9) which provided **3.27** as a white solid (53%). Mp 218-219 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.78 (d, *J* = 8.4 Hz, 2H, ArH-a), 7.48 (d, *J* = 8.5 Hz, 2H, ArH-b), 7.09 (d, *J* = 8.4 Hz, 1H, H-1), 6.58 (dd, *J* = 8.3 and 2.5 Hz, 1H, H-2), 6.52 (d, *J* = 2.5 Hz, 1H, H-4), 4.56 (br-s, 1H, Ar-OH), 4.32 (d, *J* = 9.2 Hz, 1H, NH), 3.15 (q, *J* = 8.7 Hz, 1H, H-17), 2.76 (br-s, 2H), 2.15-2.03 (m, 2H), 1.86-1.61 (m, 4H), 1.39-1.11 (m,

16H), 0.68 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 156.3 (C-*t*-butyl), 153.3 (C-3), 148.1 (C-*t*-butyl), 138.1 (C-SO_2NH-), 138.0 (C-5), 132.5 (C-6), 126.9 (2Ar-CH's-b), 126.5 (C-1), 125.9 (2Ar-CH's-a), 115.2 (C-4), 112.7 (C-2), 63.3 (C-17), 51.1 (C-14), 43.7 (CH), 42.9 (C-13), 38.8 (CH), 36.3 (CH_2), 35.1 ($\underline{C}(CH_3)_3$), 31.1 ($C(\underline{C}H_3)_3$), 29.5 ($2CH_2$), 27.1 (CH_2), 26.0 (CH_2), 23.1 (CH_2), 11.8 (CH_3, C-18); LRMS (EI) *m*/*z* (%) 467 (M^+, 91), 270 (100), 253 (32); HRMS (ESI^+) calcd for $C_{28}H_{38}NO_3S$ $(M+H)^+$ 468.2572; found 468.2566.



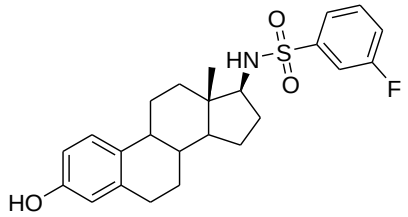
3.28

17β-(3'-Bromobenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (3.28). Purification was achieved using flash chromatography (ethyl acetate/hexane, 1:9) which provided compound **3.28** as a white solid (82%). Mp 182-183 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.03 (dd overlapping, *J* = 1.7 and 1.6 Hz, 1H, H-d), 7.80 (d, *J* = 7.9 Hz, 1H, H-a), 7.67 (dd, *J* = 7.7 and 6.9 Hz, 1H, H-c), 7.37 (dd, *J* = 7.9 and 7.9 Hz, 1H, H-b), 7.09 (d, *J* = 8.4 Hz, 1H, H-1), 6.60 (dd, *J* = 8.3 and 2.6 Hz, 1H, H-2), 6.52 (d, *J* = 2.5 Hz, 1H, H-4), 4.76 (s, 1H, Ar-OH), 4.63 (d, *J* = 9.4 Hz, 1H, NH), 3.17 (q, *J* = 8.8 Hz, 1H, H-17), 2.76 (br-s, 2H), 2.23-2.12 (m, 2H), 1.84-1.65 (m, 4H), 1.39-1.13 (m, 7H), 0.69 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.3 (C-3), 143 (C-SO_2NH-), 138 (C-5), 135.5 (C-c), 132.3 (C-6), 130.5 (C-b), 130 (C-d), 126.5 (C-1), 125.5 (C-a), 122.9 (C-Br), 115.2 (C-4), 112.7 (C-2), 63.5 (C-17), 51 (C-14), 43.7 (CH), 42.9 (C-13), 38.8 (CH), 36.3 (CH_2), 29.5 ($2CH_2$), 27.1 (CH_2), 26 (CH_2), 23.1 (CH_2), 11.8 (CH_3, C-18); LRMS (ESI^-) *m*/*z* (%) 491 (M+2, 27), 490 (M-H+2, 98), 489 (M^+, 28), 488 (M-H, 97), 255 (100), HRMS (ESI^-) calcd for $C_{24}H_{27}BrNO_3S$ $(M-H)^-$ 488.0895; found 488.0908.



3.29

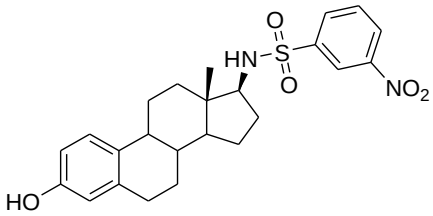
17β-(3'-Chlorobenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (3.29). Purification was achieved using flash chromatography (ethyl acetate/hexane, 1:9) which provided **3.29** as a white solid (73%). Mp 186-187 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.87 (dd overlapping, *J* = 1.8 and 1.7 Hz, 1H, H-d), 7.75 (ddd, *J* = 7.7, 1.6, and 1.2 Hz, 1H, H-a), 7.53 (dddd, *J* = 8.02, 2.0, 1.9, and 1.2 Hz, 1H, H-c), 7.43 (dd overlapping, *J* = 7.9 and 7.9 Hz, 1H, H-b), 7.09 (d, *J* = 8.4 Hz, 1H, H-1), 6.59 (dd, *J* = 8.4 and 2.7 Hz, 1H, H-2), 6.52 (d, *J* = 2.6 Hz, 1H, H-4), 4.53 (s, 1H, Ar-OH), 4.50 (d, , *J* = 9.5 Hz, 1H, NH), 3.18 (q, *J* = 8.9 Hz, 1H, H-17), 2.76 (br-s, 2H), 2.24-2.12 (m, 2H), 1.85-1.69 (m, 4H), 1.40-1.13 (m, 7H), 0.69 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.3 (C-3), 142.9 (C-SO_2NH-), 138 (C-5), 135.1 (C-6), 132.6 (C-c), 132.3 (C-Cl), 130.3 (C-b), 127.2 (C-d), 126.5 (C-1), 125.1 (C-a), 115.2 (C-4), 112.6 (C-2), 63.5 (C-17), 51 (C-14), 43.7 (CH), 42.9 (C-13), 38.8 (CH), 36.3 (CH_2), 29.4 ($2CH_2$), 27.1 (CH_2), 26 (CH_2), 23.1 (CH_2), 11.8 (CH_3, C-18); LRMS (EI) *m*/*z* (%) 447 (M+2, 40), 445 (M^+, 100), 270 (43), 253 (25), 213 (30), HRMS (EI) calcd for $C_{24}H_{28}ClNO_3S$ 445.1478; found 445.1474.



3.30

17β-(3'-Fluorobenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (3.30). Purification was achieved using flash chromatography (ethyl acetate/hexane, 3:7) which provided **3.30** as a white

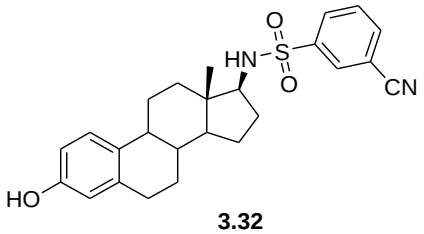
solid (65%). Mp 182-183 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.67 (d, *J* = 7.8 Hz, 1H, H-a), 7.58 (d, *J* = 8.2 Hz, 1H, H-d), 7.48 (m, 1H, H-b), 7.26 (m, overlapped with $CDCl_3$, 1H, H-c), 7.09 (d, *J* = 8.4 Hz, 1H, H-1), 6.59 (dd, *J* = 8.4 and 2.5 Hz, 1H, H-2), 6.52 (d, *J* = 2.3 Hz, 1H, H-4), 4.60 (s, 1H, Ar-OH), 4.56 (d, , *J* = 9.3 Hz, 1H, NH), 3.18 (q, *J* = 8.8 Hz, 1H, H-17), 2.76 (br-s, 2H), 2.22-2.12 (m, 2H), 1.88-1.63 (m, 4H), 1.39-1.12 (m, 7H), 0.69 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 162.3 (d, *J* = 250 Hz, C-F), 153.3 (C-3), 143.2 (d, *J* = 6.6 Hz, C-SO_2NH-), 138 (C-5), 132.3 (C-6), 130.8 (d, *J* = 7.6 Hz, C-b), 126.5 (C-1), 122.7 (d, *J* = 3.3 Hz, C-a), 119.6 (d, *J* = 21.0 Hz, C-c), 115.2 (C-4), 114.4 (d, *J* = 24.1 Hz, C-d), 112.7 (C-2), 63.5 (C-17), 51 (C-14), 43.7 (CH), 42.9 (C-13), 38.8 (CH), 36.3 (CH_2), 29.4 ($2CH_2$), 27.1 (CH_2), 26 (CH_2), 23.1 (CH_2), 11.8 (CH_3, C-18); ^{19}F NMR ($CDCl_3$, 282 MHz), δ -109; LRMS (EI) *m*/*z* (%) 429 (M^+, 100), 270 (25), 213 (22); HRMS (EI) calcd for $C_{24}H_{28}FNO_3S$ 429.1774; found 429.1782.



3.31

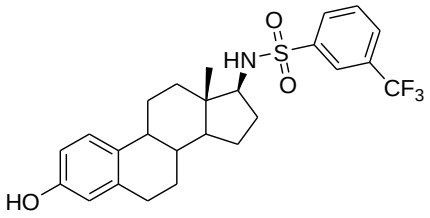
17β-(3'-Nitrobenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (3.31). Purification was achieved using flash chromatography (ethyl acetate/hexane, 3:2) which provided **3.31** as a yellow solid (49%). Mp 200-201 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.72 (br-s, 1H, H-d), 8.41 (m, 1H, H-c), 8.20 (m, 1H, H-a), 7.72 (m, 1H, H-b), 7.09 (d, *J* = 8.4 Hz, 1H, H-1), 6.58 (dd, *J* = 8.3 and 2.4 Hz, 1H, H-2), 6.52 (br-s, 1H, H-4), 4.53 (d, , *J* = 9.5 Hz, 1H, NH), 4.45 (s, 1H, Ar-OH), 3.25 (q, *J* = 8.6 Hz, 1H, H-17), 2.76 (br-s, 2H), 2.24-2.13 (m, 2H), 1.92-1.66 (m, 4H), 1.40-1.14 (m, 7H), 0.71 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.3 (C-3), 148.2 (C-NO_2), 143.5 (C-SO_2NH-), 138 (C-5), 132.5 (ArCH), 132.2 (C-6), 130.4 (ArCH), 127 (ArCH),

126.5 (C-1), 122.3 (ArCH), 115.2 (C-4), 112.7 (C-2), 63.6 (C-17), 51 (C-14), 43.7 (CH), 43 (C-13), 38.7 (CH), 36.3 (CH_2), 29.4 ($2CH_2$), 27.1 (CH_2), 26 (CH_2), 23.1 (CH_2), 11.9 (CH_3, C-18); LRMS (EI) *m*/*z* (%) 456 (M^+, 100), 270 (18), 213 (20); HRMS (ESI^+) calcd for $C_{24}H_{29}N_2O_5S$ $(M+H)^+$ 457.1797; found 457.1807.



3.32

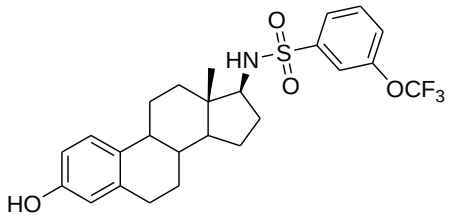
17β-(3'-Cyanobenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (3.32). Purification was achieved using flash chromatography (ethyl acetate/hexane, 2:3) which provided **3.32** as a white solid (53%). Mp 127-128 ^oC; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.16 (s, 1H, H-a), 8.10 (d, *J* = 7.9 Hz, 1H, H-d), 7.84 (d, *J* = 7.8 Hz, 1H, H-c), 7.64 (dd, *J* = 7.7 and 7.9 Hz, 1H, H-b), 7.09 (d, *J* = 8.4 Hz, 1H, H-1), 6.59 (dd, *J* = 8.4 and 2.5 Hz, 1H, H-2), 6.53 (br-s, 1H, H-4), 4.48-4.46 (s and d overlapping, 2H, Ar-OH and NH), 3.20 (q, *J* = 8.9 Hz, 1H, H-17), 2.77 (br-s, 2H), 2.25-2.13 (m, 2H), 1.89-1.79 (m, 2H), 1.70-1.66 (m, 2H), 1.40-1.09 (m, 7H), 0.70 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.3 (C-3), 143 (C-SO_2NH-), 138 (C-5), 135.6 (C-d), 132.2 (C-6), 130.9 (CH_{Ar}), 130.6 (CH_{Ar}), 130.1 (CH_{Ar}), 126.4 (C-1), 117.1 (CN), 115.2 (C-4), 113.6 (C-CN), 112.7 (C-2), 63.5 (C-17), 51 (C-14), 43.7 (CH), 42.9 (C-13), 38.8 (CH), 36.3 (CH_2), 29.6 (CH_2), 29.6 (CH_2), 27 (CH_2), 26 (CH_2), 23.1 (CH_2), 11.8 (CH_3, C-18); LRMS (EI) *m*/*z* (%) 436 (M^+, 100), 253 (15), 213 (22); HRMS (EI) calcd for $C_{25}H_{28}N_2O_3S$ 436.1821; found 436.1811.



3.33

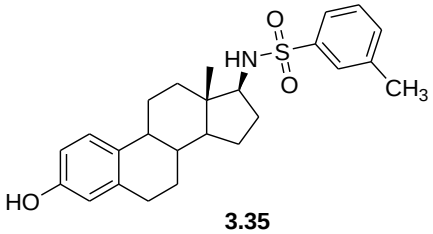
17β-(3´-Trifluoromethylbenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (3.33).

Purification was achieved using flash chromatography (ethyl acetate/hexane, 1:4) which provided **3.33** as a white solid (48%). Mp 196-197 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz), δ 8.14 (br-s, 1H, ArH), 8.07 (d, *J* = 7.9 Hz, 1H, ArH), 7.82 (d, *J* = 7.8 Hz, 1H, ArH), 7.65 (dd overlapping, *J* = 7.8 and 7.8 Hz, 1H, H-b), 7.09 (d, *J* = 8.4 Hz, 1H, H-1), 6.59 (dd, *J* = 8.3 and 2.5 Hz, 1H, H-2), 6.52 (d, *J* = 2.5 Hz, 1H, H-4), 4.47 (s overlapping d, *J* = 8.8 Hz, 2H, Ar-OH and NH), 3.21 (q, *J* = 8.8 Hz, 1H, H-17), 2.76 (br-s, 2H), 2.22-2.12 (m, 2H), 1.86-1.65 (m, 4H), 1.39-1.12 (m, 7H), 0.69 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.4 (C-3), 142.5 (C-SO_2NH-), 138.0 (C-5), 132.3 (C-6), 131.7 (q, *J* = 33.2 Hz C-CF_3), 130.2 (d, *J* =1.0 Hz, Ar-CH), 129.8 (Ar-CH), 129.1 (q, *J* = 3.7 Hz, , Ar-CH), 126.5 (C-1), 124.1 (q, *J* = 3.9 Hz, Ar-CH), 123.2 (q, *J* = 271.3 Hz, -CF_3), 115.2 (C-4), 112.7 (C-2), 63.5 (C-17), 51.0 (C-14), 43.7 (CH), 42.9 (C-13), 38.8 (CH), 36.3 (CH_2), 29.4 ($2CH_2$), 27.1 (CH_2), 26.0 (CH_2), 23.1 (CH_2), 11.8 (CH_3, C-18); ^{19}F NMR ($CDCl_3$, 282 MHz), δ -62.8; LRMS (EI) *m*/*z* (%) 479 (M^+, 100), 270 (21), 213 (22); HRMS (EI) calcd for $C_{25}H_{28}F_3NO_3S$ 479.1742; found 479.1732.



3.34

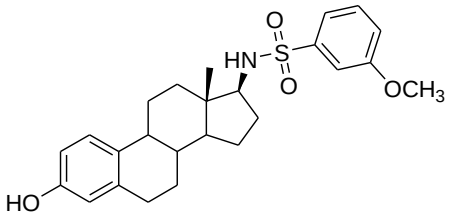
17β-(3'-Trifluoromethoxybenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (3.34). Purification was achieved using flash chromatography (ethyl acetate/hexane, 1:1) which provided **3.34** as a white solid (31%). Mp 152-153 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.84 (d, *J* = 7.8 Hz, 1H, H-a), 7.75 (br-s, 1H, ArH), 7.55 (dd overlapped, *J* = 8.0 and 7.9 Hz, 1H, H-b), 7.41 (d, *J* = 8.1 Hz, 1H, ArH), 7.07 (d, *J* = 8.4 Hz, 1H, H-1), 6.59 (d, *J* = 8.4 Hz, 1H, H-2), 6.53 (br-s, 1H, H-4), 4.98 (s, 1H, Ar-OH), 4.92 (d, *J* = 9.3 Hz, 1H, NH), 3.18 (q, *J* = 8.9 Hz, 1H, H-17), 2.75 (br-s, 2H), 2.20-2.06 (m, 2H), 1.89-1.69 (m, 2H), 1.68-1.59 (m, 2H), 1.42-1.07 (m, 7H), 0.68 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.4 (C-3), 149.3 (q, *J* = 2.0 Hz, C-OCF_3), 143.3 (C-SO_2NH-), 138.0 (C-5), 132.3 (C-6), 130.7 (ArCH), 126.5 (C-1), 120.3 (q, *J* = 275.2 Hz, OCF_3), 125.3 (ArCH), 124.9 (ArCH), 119.7 (d, *J* = 0.8 Hz, ArCH), 115.2 (C-4), 112.7 (C-2), 63.5 (C-17), 51.0 (C-14), 43.7 (CH), 42.9 (C-13), 38.8 (CH), 36.3 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 27.1 (CH_2), 26.0 (CH_2), 23.1 (CH_2), 11.8 (CH_3, C-18); ^{19}F NMR ($CDCl_3$, 282 MHz) δ -57.6; LRMS (ESI^+) *m/z* (%) 496 (M+H, 32), 255 (41); HRMS (ESI^+) calcd for $C_{25}H_{29}F_3NO_4S$ $(M+H)^+$ 496.1769; found 496.1758.



3.35

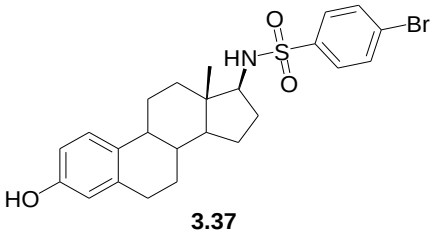
17β-(3'-Methylbenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (3.35). Purification was achieved using flash chromatography (ethyl acetate/hexane, 1:9) which provided **3.35** as a white solid (43%). Mp 202-203 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.67 (pseudo t, 2H, ArH), 7.36 (pseudo q, 2H, ArH), 7.09 (d, *J* = 8.5 Hz, 1H, H-1), 6.59 (dd, *J* = 8.4 and 2.7 Hz, 1H, H-2), 6.51 (d, *J* = 2.5 Hz, 1H, H-4), 4.47 (s, 1H, Ar-OH), 4.34 (d, *J* = 9.3 Hz, 1H, NH), 3.16 (q, *J* = 8.7 Hz,

1H, H-17), 2.76 (br-s, 2H), 2.4 (s, 3H, Ar-CH_3), 2.23-2.11 (m, 2H), 1.82-1.72 (m, 4H), 1.35-1.12 (m, 7H), 0.68 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.3 (C-3), 140.9 (C-SO_2NH-), 139.1 (C-CH_3), 138 (C-5), 133.2 (ArCH), 132.4 (C-6), 128.8 (ArCH), 127.4 (ArCH), 126.5 (C-1), 124.1 (ArCH), 115.2 (C-4), 112.7 (C-2), 63.3 (C-17), 51.1 (C-14), 43.7 (CH), 42.9 (C-13), 38.8 (CH), 36.3 (CH_2), 29.5 ($2CH_2$), 27.1 (CH_2), 26 (CH_2), 23.1 (CH_2), 21.3 (Ar-CH_3), 11.8 (CH_3, C-18); LRMS (EI) *m*/*z* (%) 425 (M^+, 100), 270 (60), 253 (25), 213 (20); HRMS (EI) calcd for $C_{25}H_{31}NO_3S$ 425.2025; found 425.2035.



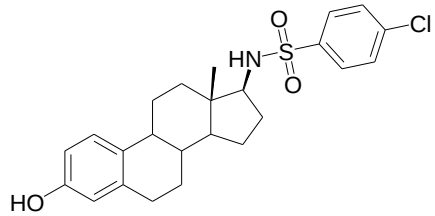
3.36

17β-(3'-Methoxybenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (3.36). Purification was achieved using flash chromatography (ethyl acetate/hexane, 2:3) which provided **3.36** as a white solid (70%). Mp 198-199 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.43 (pseudo q, 3H, ArH), 7.09 (pseudo d, 2H, ArH overlapping H-1), 6.58 (d, *J* = 8.3 Hz, 1H, H-2), 6.52 (s, 1H, H-4), 4.49 (s, 1H, Ar-OH), 4.39 (d, *J* = 9.1 Hz, 1H, NH), 3.85 (s, 3H, $ArOCH_3$), 3.16 (pseudo q, 1H, H-17), 2.76 (br-s, 2H), 2.23-2.11 (m, 2H), 1.91-1.62 (m, 4H), 1.43-1.14 (m, 7H), 0.69 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 159.8 (C-OCH_3), 153.3 (C-3), 142.3 (C-SO_2NH-), 138.1 (C-5), 132.4 (C-6), 130 (ArCH), 126.5 (C-1), 119.2 (ArCH), 118.8 (ArCH), 115.2 (C-4), 112.7 (C-2), 111.8 (C-d), 63.4 (C-17), 55.6 (OCH_3), 51.1 (C-14), 43.7 (CH), 42.9 (C-13), 38.8 (CH), 36.3 (CH_2), 29.5 ($2CH_2$), 27.1 (CH_2), 26 (CH_2), 23.1 (CH_2), 11.8 (CH_3, C-18); LRMS (EI) *m*/*z* (%) 441 (M^+, 100), 270 (70), 253 (26), 213 (20); HRMS (EI) calcd for $C_{25}H_{31}NO_4S$ 441.1974; found 441.1979.



3.37

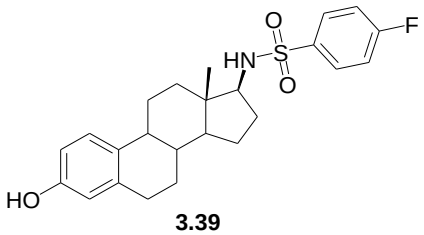
17β-(4'-Bromobenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (3.37). Purification was achieved using flash chromatography (ethyl acetate/hexane, 1:9) which provided **3.37** as a white solid (78%). Mp 115-116 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.74 (d, *J* = 8.7 Hz, 2H, ArH-a), 7.64 (d, *J* = 8.7 Hz, 2H, ArH-b), 7.09 (d, *J* = 8.4 Hz, 1H, H-1), 6.58 (dd, *J* = 8.5 and 2.5 Hz, 1H, H-2), 6.52 (d, *J* = 2.5 Hz, 1H, H-4), 4.54 (br-s, 1H, Ar-OH), 4.42 (d, *J* = 9.4 Hz, 1H, NH), 3.15 (q, *J* = 8.7 Hz, 1H, H-17), 2.76 (br-s, 2H), 2.24-2.08 (m, 2H), 1.88-1.60 (m, 4H), 1.40-1.12 (m, 7H), 0.68 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.3 (C-3), 140.2 (C-SO_2NH-), 138.1 (C-5), 132.3 (C-6), 132.2 (2Ar-CH's), 128.6 (2Ar-CH's), 127.4 (C-Br), 126.5 (C-1), 115.2 (C-4), 112.7 (C-2), 63.4 (C-17), 51.1 (C-14), 43.7 (CH), 43.0 (C-13), 38.8 (CH), 36.3 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 27.1 (CH_2), 26.0 (CH_2), 23.1 (CH_2), 11.8 (CH_3, C-18); LRMS (ESI^+) *m*/*z* (%) 492 (M+H+2, 75), 490 (M+H, 72), 256 (20), 255 (100); HRMS (ESI^+) calcd for $C_{24}H_{29}BrNO_3S$ $(M+H)^+$ 490.1052; found 490.1046.



3.38

17β-(4'-Chlorobenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (3.38). Purification was achieved using flash chromatography (ethyl acetate/hexane, 1:9) which provided **3.38** as a white solid (69%). Mp 124-125 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.82 (d, *J* = 8.4 Hz, 2H, ArH-a),

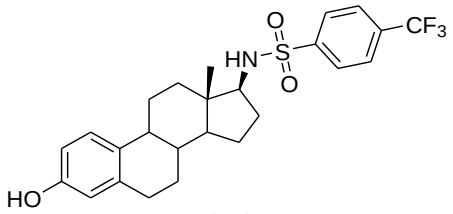
7.45 (d, *J* = 8.4 Hz, 2H, ArH-b), 7.07 (d, *J* = 8.5 Hz, 1H, H-1), 6.60 (d, *J* = 8.4 Hz, 1H, H-2), 6.53 (br-s, 1H, H-4), 5.29 (br-s, 1H, Ar-OH), 4.98 (d, *J* = 9.2 Hz, 1H, NH), 3.13 (q, *J* = 8.6 Hz, 1H, H-17), 2.74 (br-s, 2H), 2.21-2.05 (m, 2H), 1.87-1.60 (m, 4H), 1.42-1.07 (m, 7H), 0.67 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.3 (C-3), 139.7 (C-SO_2NH-), 138.9 (C-Cl), 138.0 (C-5), 132.3 (C-6), 129.3 (2Ar-CH's-b), 128.5 (2Ar-CH's-a), 126.5 (C-1), 115.2 (C-4), 112.7 (C-2), 63.4 (C-17), 51.1 (C-14), 43.7 (CH), 43.0 (C-13), 38.8 (CH), 36.3 (CH_2), 29.4 ($2CH_2$), 27.1 (CH_2), 26.0 (CH_2), 23.1 (CH_2), 11.8 (CH_3, C-18); LRMS (ESI^+) *m*/*z* (%) 448 (M+H+2, 38), 447 (M+2, 30), 446 (M+H, 100), 255 (18), 239 (38); HRMS (ESI^+) calcd for $C_{24}H_{29}ClNO_3S$ $(M+H)^+$ 446.1557; found 446.1542.



3.39

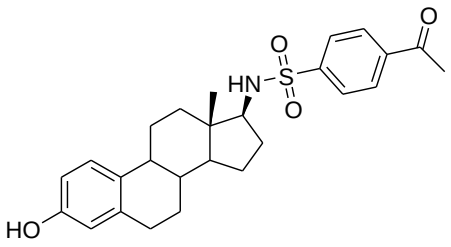
17β-(4'-Fluorobenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (3.39). Purification was achieved using flash chromatography (ethyl acetate/hexane, 3:7) which provided **3.39** as a white solid (61%). Mp 140-141 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.89 (m, 2H, ArH-a), 7.16 (dd overlapping, *J* = 8.5 and 8.5 Hz, 2H, ArH-b), 7.08 (d, *J* = 8.4 Hz, 1H, H-1), 6.59 (dd, *J* = 8.4 and 2.5 Hz, 1H, H-2), 6.53 (d, *J* = 2.5 Hz, 1H, H-4), 4.89 (br-s, 1H, Ar-OH), 4.71 (d, *J* = 9.2 Hz, 1H, NH), 3.13 (q, *J* = 8.8 Hz, 1H, H-17), 2.75 (br-s, 2H), 2.22-2.10 (m, 2H), 1.85-1.61 (m, 4H), 1.38-1.09 (m, 7H), 0.68 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 166.6-163.3 (d, *J* = 253.0 Hz, C-F), 153.4 (C-3), 138.0 (C-5), 137.2 (d, *J* = 3.2 Hz, C-SO_2NH-), 132.3 (C-6), 129.7 (d, *J* = 9.9 Hz, 2Ar-CH's-a), 126.5 (C-1), 116.1 (d, *J* = 22.4 Hz, 2Ar-CH's-b), 115.2 (C-4), 112.7 (C-2), 63.4 (C-17), 51.1 (C-14), 43.7 (CH), 43.0 (C-13), 38.8 (CH), 36.3 (CH_2), 29.4 ($2CH_2$), 27.1

(CH_2), 26.0 (CH_2), 23.1 (CH_2), 11.8 (CH_3, C-18); ^{19}F NMR ($CDCl_3$, 282 MHz) δ -105; LRMS (ESI^+) *m*/*z* (%) 430 (M+H, 100), 255 (13), 223 (15); HRMS (ESI^+) calcd for $C_{24}H_{29}FNO_3S$ $(M+H)^+$ 430.1852; found 430.1847.



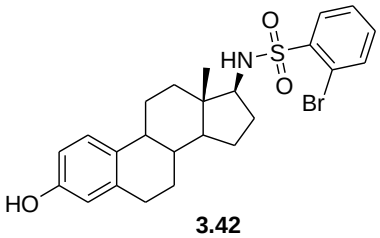
3.40

17β-(4'-Trifluoromethylbenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (3.40). Purification was achieved using flash chromatography (ethyl acetate/hexane, 1:1) which provided **3.40** as a white solid (34%). Mp 138-139 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.00 (d, *J* = 8.1 Hz, 2H, ArH-a), 7.76 (d, *J* = 8.0 Hz, 2H, ArH-b), 7.09 (d, *J* = 8.4 Hz, 1H, H-1), 6.58 (d, *J* = 8.8 Hz, 1H, H-2), 6.53 (br-s, 1H, H-4), 4.51 (pseudo d, 2H, Ar-OH and NH), 3.20 (q, *J* = 8.6 Hz, 1H, H-17), 2.77 (br-s, 2H), 2.23-2.09 (m, 2H), 1.92-1.61 (m, 4H), 1.44-1.10 (m, 7H), 0.69 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.4 (C-3), 144.8 (d, *J* = 1.2 Hz, C-SO_2NH-), 138.0 (C-5), 134.2 (d, *J* = 33.0 Hz, C-CF_3), 132.3 (C-6), 127.5 (2Ar-CH's-a), 126.5 (C-1), 126.2 (q, *J* = 3.7 Hz, 2Ar-CH's-b), 123.0 (q, *J* = 271.2 Hz, CF_3), 115.2 (C-4), 112.7 (C-2), 63.5 (C-17), 51.1 (C-14), 43.7 (CH), 43.0 (C-13), 38.8 (CH), 36.3 (CH_2), 29.4 ($2CH_2$), 27.1 (CH_2), 26.0 (CH_2), 23.1 (CH_2), 11.8 (CH_3, C-18); ^{19}F NMR ($CDCl_3$, 282 MHz) δ -62.5; LRMS (ESI^+) *m*/*z* (%) 480 (M+H, 43), 256 (19), 255 (100); HRMS (ESI^+) calcd for $C_{25}H_{29}F_3NO_3S$ $(M+H)^+$ 480.1820; found 480.1813.



3.41

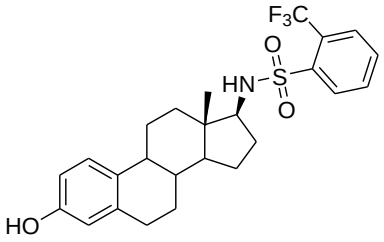
17β-(4'-Acetylbenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (3.41). Purification was achieved using flash chromatography (methanol/chloroform, 1:9) which provided **3.41** as a white solid (53%). Mp 118-119 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.05 (d, *J* = 8.5 Hz, 2H, ArH), 7.97 (d, *J* = 8.2 Hz, 2H, ArH), 7.08 (d, *J* = 8.5 Hz, 1H, H-1), 6.59 (dd, *J* = 2.6 and 8.4 Hz, 1H, H-2), 6.52 (d, *J* = 2.4 Hz, 1H, H-4), 4.59 (brs, 1H, ArOH), 4.53 (d, *J* = 9.4 Hz, 1H, NH), 3.19 (q, *J* = 8.7 Hz, 1H, H-17), 2.76 (m, 2H), 2.64 (s, 3H, -$COCH_3$), 2.23-2.11 (m, 2H), 1.86-1.62 (m, 4H), 1.39-1.12 (m, 7H), 0.69 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 196.8 (CO), 153.3 (C-3), 145.2 (C-$COCH_3$), 139.3 (C-SO_2NH-), 138.0 (C-5), 132.3 (C-6), 128.9 (2 CH_{Ar}), 127.3 (2 CH_{Ar}), 126.5 (C-1), 115.2 (C-4), 112.7 (C-2), 63.5 (C-17), 51.1 (C-14), 43.7 (CH), 42.9 (C-13), 38.8 (CH), 36.3 (CH_2), 29.5 (2 CH_2), 27.1 (CH_2), 26.9 (CH_3), 26.0 (CH_2), 23.1 (CH_2), 11.8 (CH_3, C-18); LRMS (ESI$^+$) *m/z* (%) 454 (M+H, 40), 255 (100); HRMS (ESI$^+$) calcd for $C_{26}H_{32}NO_4S$ $(M+H)^+$ 454.2052; found 454.2050.



3.42

17β-(2´-Bromobenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (3.42). Purification was achieved using flash chromatography (ethyl acetate/hexane, 1:4) which provided **3.42** as a white

solid (67%): Mp 225-226 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.14 (dd, *J* = 8.9 and 1.3 Hz 1H, H-a), 7.72 (d, *J* = 7.4 Hz, 1H, H-d), 7.42 (m, 2H, H-c & b), 7.07 (d, *J* = 8.4 Hz, 1H, H-1), 6.58 (d, *J* = 8.3 Hz, 1H, H-2), 6.52 (br-s, 1H, H-4), 5.06 (d, *J* = 8.9 Hz, 1H, NH), 4.64 (s, 1H, Ar-OH), 3.12 (q, *J* = 8.8 Hz, 1H, H-17), 2.75 (br-s, 2H), 2.20-2.05 (m, 2H), 1.80-1.58 (m, 6H), 1.39-0.82 (m, 6H), 0.74 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.3 (C-3), 139.9 (C-SO_2NH-), 138.0 (C-5), 134.9 (Ar-CH), 133.5 (Ar-CH), 132.3 (C-6), 131.4 (Ar-CH), 127.8 (Ar-CH), 126.5 (C-1), 119.9 (C-Br), 115.2 (C-4), 112.7 (C-2), 63.7 (C-17), 51 (C-14), 43.7 (CH), 43.0 (C-13), 38.7 (CH), 36.2 (CH_2), 29.4 (CH_2), 28.7 (CH_2), 27.1 (CH_2), 26 (CH_2), 23.1 (CH_2), 11.9 (CH_3, C-18); LRMS (ESI^+) *m*/*z* (%) 492 (M+H+2, 28), 491 (M+2, 9), 490 (M+H, 28), 256 (20), 255 (100); HRMS (ESI^+) calcd for $C_{24}H_{29}BrNO_3S$ $(M+H)^+$ 490.1052; found 490.1053.

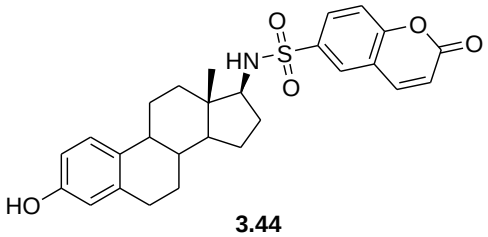


3.43

17β-(2´-Trifluoromethylbenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (3.43).

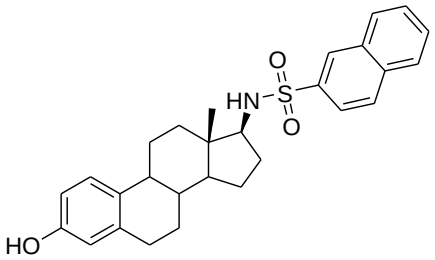
Purification was achieved using flash chromatography (ethyl acetate/hexane, 3:7) which provided **3.43** as a white solid (51%). Mp 244-245 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz), δ 8.27-8.25 (m, 1H, ArH), 7.89-7.86 (m, 1H, ArH), 7.72-7.69 (m,2H, ArH), 7.09 (d, *J* = 8.5 Hz, 1H, H-1), 6.59 (d, *J* = 8.2 Hz, 1H, H-2), 6.53 (brs, 1H, H-4), 4.60 (d, *J* = 8.8 Hz, 1H, NH), 4.53 (s, 1H, ArOH), 3.21 (q, *J* = 9.4 Hz, 1H, H-17), 2.76 (m, 2H), 2.21-2.08 (m, 2H), 1.81-1.63 (m, 4H), 1.43-1.05 (m, 8H), 0.69 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.3 (C-3), 139.8 (C-SO_2NH-), 138.0 (C-5 and C-6), 132.3 (d, *J* = 2.5 Hz, CH_{Ar}), 131.6 (CH_{Ar}), 128.4 (q, *J* = 32.5 Hz, <u>C</u>-CF_3), 124.8 (q, *J* = 272.2 Hz, C-CF_3), 115.2 (C-4), 112.7 (C-2), 63.6 (C-17), 51.0 (C-14),

43.7 (CH), 42.9 (C-13), 38.8 (CH), 36.3 (CH_2), 29.2 ($2CH_2$), 27.1 (CH_2), 26.0 (CH_2), 23.1 (CH_2), 11.8 (CH_3, C-18); ^{19}F NMR ($CDCl_3$, 282 MHz), δ -58.2; LRMS (ESI^+) *m*/*z* (%) 480 (M+H, 40), 255 (100); HRMS (ESI^+) calcd for $C_{25}H_{29}F_3NO_3S$ 480.1820; found 480.1823.



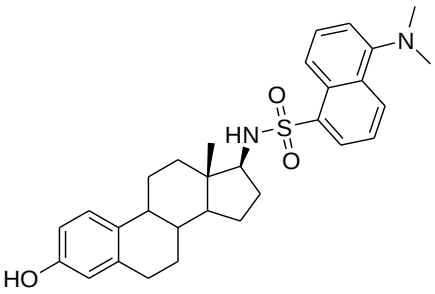
3.44

17β-(Coumarin-6-yl)sulfonamide-1,3,5(10)-estratrien-3-ol (3.44). Purification was achieved using flash chromatography (ethyl acetate/hexane, 1:4) which provided **3.44** as a white solid (56%). Mp 178-179 ^oC; ^1H NMR ($CDCl_3$, 300 MHz), δ 8.06 (d, *J* = 1.5 Hz, 1H, ArH), 8.00 (dd, *J* = 1.8 and 8.2 Hz, 1H, ArH), 7.73 (d, *J* = 8.6 Hz, 1H, ArH), 7.42 (d, *J* = 8.6 Hz, 1H, ArH), 7.06 (d, *J* = 8.4 Hz, 1H, H-1), 6.61-6.51 (m, 2H, H-2 and H-4), 5.09 (brs, 1H, ArOH), 4.80 (brs, 1H, NH), 3.17 (q, *J* = 8.8 Hz, 1H, H-17), 2.75 (m, 2H), 2.21-2.06 (m, 2H), 1.86-1.59 (m, 4H), 1.43-1.07 (m, 7H), 0.69 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 159.3 (C(O)O), 156.2, 153.3 (C-3), 142.4 (CH_{Ar}), 138.0 (C-5), 137.6, 132.2 (C-6), 130.0 (CH_{Ar}), , 127.4 (CH_{Ar}), 126.5 (C-1), 118.7, 118.4, 117.9, 115.2 (C-4), 112.7 (C-2), 63.5 (C-17), 51.1 (C-14), 43.7 (CH), 42.9 (C-13), 38.8 (CH), 36.4 (CH_2), 29.5 ($2CH_2$), 27.1 (CH_2), 26.0 (CH_2), 23.1 (CH_2), 11.8 (CH_3, C-18); LRMS (ESI^+) *m*/*z* (%) 480 (M+H, 100), 256 (20), 255 (50); HRMS (ESI^+) calcd for $C_{27}H_{30}NO_5S$ $(M+H)^+$ 480.1845; found 480.1820.



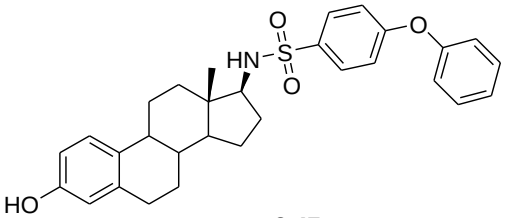
3.45

17β-Naphthalen-2-ylsulfonamide-1,3,5(10)-estratrien-3-ol (3.45). Purification was achieved using flash chromatography (ethyl acetate/hexane, 2:3) which provided **3.45** as a white solid (25%). Mp 136-137 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.45 (br-s, 1H, ArH), 7.96-7.82 (m, 4H, ArH's), 7.66-7.57 (m, 2H, ArH), 7.06 (d, *J* = 8.3 Hz, 1H, H-1), 6.58 (d, *J* = 8.4 Hz, 1H, H-2), 6.51 (br-s, 1H, H-4), 4.54 (s overlapping d, *J* = 8.8 Hz, 2H, Ar-OH and NH), 3.20 (q, *J* = 8.6 Hz, 1H, H-17), 2.74 (br-s, 2H), 2.18-2.02 (m, 2H), 1.83-1.73 (m, 2H), 1.60-1.55 (pseudo d, 1H), 1.41-1.12 (m, 7H), 0.70 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.3 (C-3), 138.1 (C-5), 137.8, 134.7, 132.5 (C-6), 132.1, 129.4, 129.2, 128.7, 128.3, 127.9, 127.5, 126.5 (C-1), 122.4, 115.1 (C-4), 112.6 (C-2), 63.4 (C-17), 51.1 (C-14), 43.7 (CH), 42.9 (C-13), 38.8 (CH), 36.3 (CH_2), 29.5 ($2CH_2$), 27.1 (CH_2), 26 (CH_2), 23.1 (CH_2), 11.8 (CH_3, C-18); LRMS (ESI^+) *m*/*z* (%) 462 (M+H, 100), 256 (20), 255 (97); HRMS (ESI^+) calcd for $C_{28}H_{32}NO_3S$ $(M+H)^+$ 462.2103; found 462.2102.



3.46

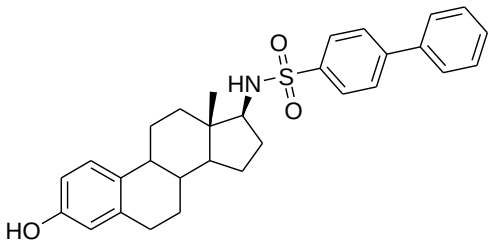
17β-(5'-Dimethylamino)naphthalen-2-ylsulfonamide-1,3,5(10)-estratrien-3-ol (3.46). Purification was achieved using flash chromatography (ethyl acetate/hexane, 2:7) which provided **3.46** as a white solid (63%). Mp 206-207 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.52 (d, *J* = 8.5 Hz, 1H, ArH), 8.30-8.26 (m, 2H, ArH), 7.58-7.48 (m, 2H, ArH), 7.17 (d, *J* = 7.5 Hz, 1H,ArH), 7.01 (d, *J* = 8.5 Hz, 1H, H-1), 6.56 (dd, *J* = 2.6 and 8.4 Hz, 1H, H-2), 6.49 (d, *J* = 2.5 Hz, 1H, H-4), 4.91 (s 1H, ArOH), 4.68 (d, *J* = 9.3 Hz, 1H, NH), 3.09 (q, *J* = 8.6 Hz, 1H, H-17), 2.88 (s, 6H, -N($C\underline{H}_3$)$_2$), 2.70 (m, 2H), 2.07-1.98 (m, 2H), 1.74-1.47 (m, 4H), 1.40-1.36 (m, 1H), 1.30-1.01 (m, 6H), 0.92-0.84 (m, 1H), 0.61 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.4 (C-3), 151.9 ($\underline{C}$-N(CH_3)$_2$), 138.0 (C-5), 135.6 (C-SO_2NH), 132.3 (C-6), 130.3, 129.7 (2 C), 129.6, 128.3, 126.4 (C-1), 123.2, 118.8, 115.1 (2 CH_{Ar}), 112.6 (C-2), 63.5 (C-17), 51.0 (C-14), 45.4 (2 CH_3, -N(CH_3)$_2$), 43.7 (CH), 42.8 (C-13), 38.7 (CH), 36.0 (CH_2), 29.3 (2 CH_2), 27.1 (CH_2), 26.0 (CH_2), 23.1 (CH_2), 11.8 (CH_3, C-18); LRMS (ESI$^+$) *m/z* (%) 505 (M+H, 100); HRMS (ESI$^+$) calcd for $C_{30}H_{37}N_2O_3S$ (M+H)$^+$ 505.2525; found 505.2518.



3.47

17β-(4'-Phenoxybenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (3.47). Purification was achieved using flash chromatography (methanol/chloroform, 2:3) which provided **3.47** as a white solid (38%). Mp 184-185 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.81 (d, *J* = 8.5 Hz, 2H, ArH-a), 7.39 (dd overlapping, *J* = 7.7 and 7.7 Hz, 2H, ArH-b`), 7.18 (d, *J* = 7.1 Hz, 1H, Ar-H-c`), 7.06 (d overlapping dd, *J* = 8.4 Hz, 8.2 and 8.6 Hz respectively, 5H, H-1 and 4 Ar-H-a` & b), 6.58 (d, *J* = 8.5 Hz, 1H, H-2), 6.52 (br-s, 1H, H-4), 4.49 (br-s, 1H, Ar-OH), 4.35 (d, *J* = 9.3 Hz,

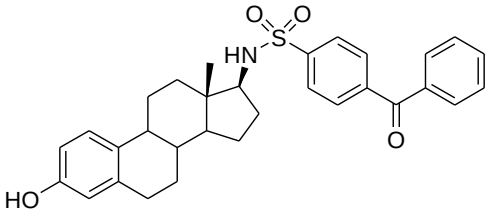
1H, NH), 3.15 (q, *J* = 8.7 Hz, 1H, H-17), 2.76 (br-s, 2H), 2.23-2.09 (m, 2H), 1.90-1.61 (m, 2H), 1.40-1.09 (m, 7H), 0.69 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 161.3 (C-Phenyl), 155.3 (O-C-Phenyl), 153.3 (C-3), 138.1 (C-5), , 134.8 (C-SO_2NH-), 132.4 (C-6), 130.1 (2Ar-CH-b`), 129.3 (2Ar-CH-a), 126.5 (C-1), 124.8 (Ar-CH-c`), 120.1 (2Ar-CH-b), 117.7 (2Ar-CH-a`), 115.2 (C-4), 112.7 (C-2), 63.3 (C-17), 51.1 (C-14), 43.8 (CH), 42.9 (C-13), 38.8 (CH), 36.3 (CH_2), 29.5 (2CH_2 overlapping), 27.1 (CH_2), 26.1 (CH_2), 23.2 (CH_2), 11.8 (CH_3, C-18); LRMS (ESI^+) *m/z* (%) 504 (M+H, 100), 256 (15), 255 (75); HRMS (ESI^+) calcd for $C_{30}H_{34}NO_4S$ $(M+H)^+$ 504.2209; found 504.2207.



3.48

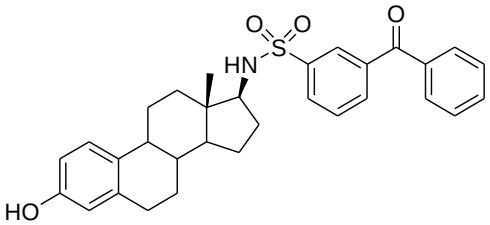
17β-(4'-Phenylbenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (3.48). Purification was achieved using flash chromatography (methanol/chloroform, 1:4) which provided **3.48** as a white solid (31%). Mp 223-224 ^oC; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.92 (d, *J* = 8.4 Hz, 2H, ArH-a), 7.70 (d, *J* = 8.3 Hz, 2H, ArH-b), 7.62 (pseudo t, 2H, Ar-H-a`), 7.50-7.38 (m, 3H, Ar-H-b` and c`), 7.09 (d, *J* = 8.3 Hz, 1H, H-1), 6.58 (dd, *J* = 8.4 and 2.4 Hz, 1H, H-2), 6.52 (br-s, 1H, H-4), 4.45 (br-s, 1H, Ar-OH), 4.40 (d, *J* = 9.3 Hz, 1H, NH), 3.20 (q, *J* = 8.5 Hz, 1H, H-17), 2.76 (br-s, 2H), 2.23-2.12 (m, 2H), 1.91-1.63 (m, 2H), 1.40-1.12 (m, 7H), 0.70 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.3 (C-3), 145.4 (C-phenyl), 139.7 (C-SO_2NH-), 139.3 (C-C-phenyl), 138.1 (C-5), 132.5 (C-6), 129.0 (2Ar-CH's), 128.5 (Ar-CH-c`), 127.6 (4Ar-CH's overlapping), 127.3 (2Ar-CH's), 126.5 (C-1), 115.2 (C-4), 112.7 (C-2), 63.4 (C-17), 51.1 (C-14), 43.7 (CH), 42.9 (C-13), 38.8 (CH), 36.3 (CH_2), 29.5 (2CH_2 overlapping), 27.1 (CH_2), 26.0

(CH_2), 23.1 (CH_2), 11.8 (CH_3, C-18); LRMS (ESI^+) *m*/*z* (%) 488 (M+H, 17), 391 (47), 323 (40), 219 (100), 173 (48); HRMS (ESI^+) calcd for $C_{30}H_{34}NO_3S$ $(M+H)^+$ 488.2259; found 488.2262.



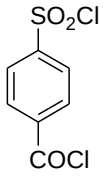
3.49

17β-(4'-Benzoylbenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (3.49). Purification was achieved using flash chromatography (ethyl acetate/hexane, 1:9) which provided **3.49** as a white solid (65 mg, 23%). Mp 145-147 ^oC; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.99 (d, *J* = 8.5 Hz, 2H, ArH), 7.87 (d, *J* = 8.3 Hz, 2H, ArH), 7.78 (d, *J* = 7.4 Hz, 2H, ArH), 7.65-7.60 (m, 1H), 7.52-7.47 (m, 1H), 7.07 (d, *J* = 8.5 Hz, 1H, H-1), 6.60 (dd, *J* = 2.3 and 8.3 Hz, 1H, H-2), 6.53 (brs, 1H, H-4), 5.12 (brs, 1H, ArOH), 4.81 (d, *J* = 9.2 Hz, 1H, NH), 3.21 (q, *J* = 8.7 Hz, 1H, H-17), 2.75 (m, 2H), 2.22-2.03 (m, 2H), 1.89-1.60 (m, 5H), 1.39-1.09 (m, 8H), 0.70 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 195.5 (CO), 153.5 (C-3), 144.5 (C-COC_6H_5), 141.0 (C-SO_2NH-), 138.0 (C-5), 136.6 (C-6), 133.3 (CH_{Ar}), 132.2, 130.4 (2 CH_{Ar}), 130.1 (2 CH_{Ar}), 128.6 (2 CH_{Ar}), 127.0 (2 CH_{Ar}), 126.5 (C-1), 115.2 (C-4), 112.7 (C-2), 63.5 (C-17), 51.1 (C-14), 43.7 (CH), 43.0 (C-13), 38.8 (CH), 36.4 (CH_2), 29.5 (2 CH_2), 27.1 (CH_2), 26.0 (CH_2), 23.1 (CH_2), 11.9 (CH_3, C-18); LRMS (ESI^+) *m*/*z* (%) 516 (M+H, 100); HRMS (ESI^+) calcd for $C_{31}H_{34}NO_4S$ $(M+H)^+$ 516.22031; found 516.22007.



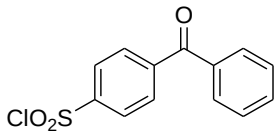
3.50

17β-(3'-Benzoylbenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (3.50). Purification was achieved using flash chromatography (ethyl acetate/hexane, 3:7) which provided **3.50** as a white solid (20 mg, 18%). Mp 177-179 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.27 (brs, 1H, ArH), 8.09 (d, *J* = 7.7 Hz, 1H, ArH), 7.97 (d, *J* = 7.6 Hz, 1H, ArH), 7.77 (d, *J* = 7.7 Hz, 2H, ArH), 7.66-7.60 (m, 2H), 7.52-7.47 (m, 2H), 7.08 (d, *J* = 8.5 Hz, 1H, H-1), 6.58 (d, *J* = 8.2 Hz, 1H, H-2), 6.52 (brs, 1H, H-4), 4.68 (brs, 1H, ArOH), 4.54 (d, *J* = 9.5 Hz, 1H, NH), 3.19 (q, *J* = 8.3 Hz, 1H, H-17), 2.76 (m, 2H), 2.22-2.11 (m, 2H), 1.91-1.59 (m, 5H), 1.42-1.09 (m, 8H), 0.69 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 195.1 (CO), 153.5 (C-3), 141.8 (C-COC_6H_5), 138.5 (C-SO_2NH-), 138.0 (C-5), 136.5 (C-6), 133.5 (CH_{Ar}), 133.2 (CH_{Ar}), 132.2, 130.4 (CH_{Ar}), 130.1 (2 CH_{Ar}), 129.3 (CH_{Ar}), 128.6 (2 CH_{Ar}), 128.3 (CH_{Ar}), 126.4 (C-1), 115.3 (C-4), 112.7 (C-2), 63.5 (C-17), 51.1 (C-14), 43.7 (CH), 43.0 (C-13), 38.8 (CH), 36.4 (CH_2), 29.5 (2 CH_2), 27.1 (CH_2), 26.0 (CH_2), 23.1 (CH_2), 11.8 (CH_3, C-18); LRMS (ESI^+) *m*/*z* (%) 516 (M+H, 60), 371 (100); HRMS (ESI^+) calcd for $C_{31}H_{34}NO_4S$ $(M+H)^+$ 516.22031; found 516.22043.



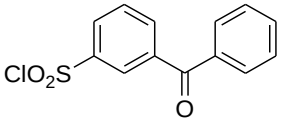
3.52

4'-Chlorosulfonylbenzoyl chloride (3.52).[171] A suspension of 4-sulfobenzoic acid potassium salt, compound **3.51** (2.0 g, 8.2 mmol) and thionyl chloride (20 mL) in DMF (two drop), was refluxed for 4 h., after that, the precipitate formed was filtered out, and filtrate was azeotropically evaporated with toluene, and formed precipitate was filtered and washed three times toluene to afford the acid chloride **3.52** as white solid (1.8 g, 100%). Mp 53-55^{o}C (lit. 53-57); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.15 (d, *J* = 8.1 Hz, 2H, ArH), 7.96 (d, *J* = 8.1 Hz, 2H, ArH).



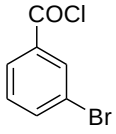
3.53

4'-Benzoylbenzenesulfonyl chloride (3.53).[172] To a solution of **3.52** (1.4 g, 5.85 mmol) in 1,2-DCE (40 mL), was added benzene (1.0 mL, 12 mmol) and $AlCl_3$ (1.66 g, 12.5 mmol) at room temperature. The stirring was continued for 24 h, after that, it was quenched by ice-water mixture, and the organic layer was evaporated under vacuum, to afford pale yellow oil, which was crystallized from hexane to afford **3.53** as white crystals (1.3 g, 78%). Mp 85-87^{o}C (lit. 88-90); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.15 (d, *J* = 8.1 Hz, 2H, ArH), 7.96 (d, *J* = 8.1 Hz, 2H, ArH), 7.78 (d, *J* = 7.7 Hz, 2H, ArH), 7.67-7.62 (m, 1H), 7.54-7.49 (m, 2H).



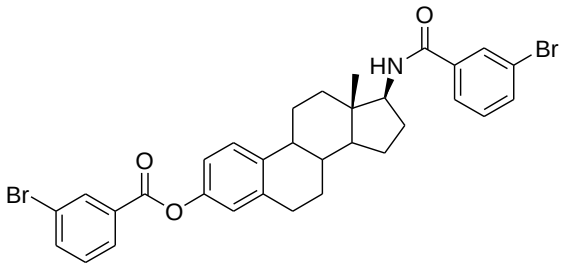
3.55

3'-Benzoylbenzenesulfonyl chloride (3.55).[172] To a solution of **3.54** (140 mg, 0.6 mmol) in 1,2-DCE (5 mL), was added benzene (0.1 mL, 1.2 mmol) and $AlCl_3$ (0.16 mg, 1.2 mmol) at room temperature. The stirring was continued for 24 h, after that, it was quenched by ice-water mixture, and the organic layer was evaporated under vacuum, to afford **3.55** in an 83% yield as pale yellow oil, which is used without further purification. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.41 (brs, 2H, ArH), 9.16-8.93 (m, 4H, ArH), 8.54-8.45 (m, 3H, ArH).



3.58

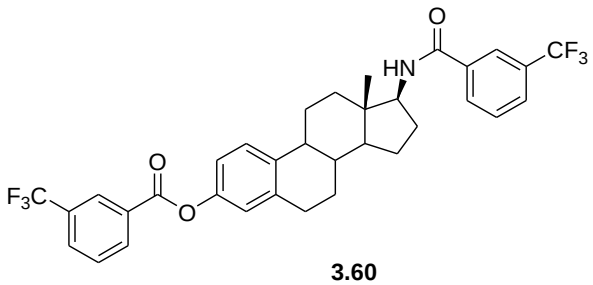
3'-Bromobenzoyl chloride (3.58). 3-Bromobenzoic acid, compound **3.57** (1 g, 5 mmol) in toluene (20 mL) was heated under reflux together with thionyl chloride (10 mL) for 6 h, cooled and solvent removed under reduced pressure. The residue was dried azeotropically with toluene (2 x 15mL) to give **3.58** as pale yellow oil (1.1 g, 100%). ^{1}H NMR ($CDCl_3$, 300 MHz), δ 8.65 (brs, 1H, ArH), 8.56 (d, *J* = 7.8 Hz, 1H, ArH), 7.89 (brs, 1H, ArH), 7.77 (d, *J* = 8.0 Hz, 1H, ArH).



3.59

17β-(3'-Bromo)benzamido-estra-1,3,5(10)-trien-3-yl-(3'-bromo)benzoate (3.59). To a stirred solution of 17β-amino-1,3,5(10)-estratrien-3-ol, compound **3.11** (150 mg, 0.55 mmol) in dry pyridine (3 mL) at 0 ^{o}C was added the 3'-bromobenzoyl chloride, compound **3.58** (241 mg, 1.10 mmol). The reaction was stirred overnight at room temperature, then pyridine was azeotropically removed with toluene under vacuum, and the residue was dissolved in chloroform, washed with water, dil. HCl, water, $NaHCO_3$, water, and finally brine then dried (Na_2SO_4), and concentrated. Purification was achieved using flash chromatography (ethyl acetate/hexane, 1:9) which provided **3.59** as a white solid (250 mg, 71%). Mp 143–144 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz), δ 8.30 (brs, 1H, ArH), 8.09 (d, *J* = 7.8 Hz, 1H, ArH), 7.87 (brs, 1H, ArH), 7.73 (d, *J* = 8.0 Hz, 1H, ArH), 7.66 (d, *J* = 7.8 Hz, 1H, ArH), 7.60 (d, *J* = 8.1 Hz, 1H, ArH), 7.38–7.26 (m, 3H, ArH), 6.92 (d overlapping brs, *J* = 8.9 Hz, 2H, H-2 and H-4, respectively), 5.96 (d, *J* = 8.9 Hz, 1H, NH), 4.18 (q, *J* = 8.9 Hz, 1H, H-17), 2.88 (m, 2H), 2.34-2.20 (m, 3H), 1.91–1.83 (m, 3H),

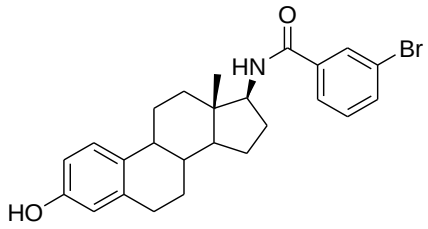
1.60-1.23 (m, 7H), 0.83 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 166.0 (C(O)NH), 164.1 (C(O)O), 148.5 (C-3), 138.3 (C-5), 138.2 (C-6), 136.9 (C-C(O)NH-),136.3 (CH_{Ar}), 134.3 (CH_{Ar}), 133.0 (CH_{Ar}), 131.6 (C-C(O)O-), 130.6 (3 CH_{Ar}), 128.7 (CH_{Ar}), 126.6 (CH_{Ar}), 125.4 (CH_{Ar}), 122.7 (C), 122.6 (C), 121.4 (C-4), 118.5 (C-2), 59.4 (C-17), 51.7 (C-14), 44.0 (C-13), 43.8 (CH), 38.6 (CH), 37.0 (CH_2), 29.5 (CH_2), 28.7 (CH_2), 27.1 (CH_2), 26.1 (CH_2), 23.4 (CH_2), 12.2 (CH_3, C-18); LRMS (ESI^+) m/z (%) 636 (M+H, 38), 622 (100%); HRMS (ESI^+) calcd for $C_{32}H_{32}NO_3Br_2$ $(M+H)^+$ 636.0749; found 636.0760.



3.60

17β-(3'-Trifluoromethyl)benzamido-estra-1,3,5(10)-trien-3-yl-(3'-trifluoromethyl)-benzoate (3.60). To a stirred solution of 17-amino-1,3,5(10)-estratrien-3-ol, compound **3.11** (150 mg, 0.55 mmol) in dry pyridine (3 mL) at 0 ^oC was added the 3-trifluoromethylbenzoyl chloride (230 mg, 1.10 mmol). The reaction was stirred overnight at room temperature. The pyridine was azeotropically removed with toluene under vacuum, the residue was dissolved in chloroform, washed with water, dil. HCl, water, $NaHCO_3$, water, and finally brine then dried (Na_2SO_4), and concentrated. Purification was achieved using flash chromatography (ethyl acetate/ hexane, 3:7) which provided **3.60** as a white solid (194 mg, 57%). Mp 118–119 ^oC; ^1H NMR ($CDCl_3$, 300 MHz), δ 8.43 (s, 1H, ArH), 8.35 (d, *J* = 8.1 Hz, 1H, ArH), 8.00 (s, 1H, ArH), 7.93 (d, *J* = 7.7 Hz, 1H, ArH), 7.87 (d, *J* = 7.4 Hz, 1H, ArH), 7.74 (d, *J* = 7.6 Hz, 1H, ArH), 7.66–7.54 (m, 2H, ArH), 7.33 (d, J = 8.7 Hz, 1H, H-1), 6.94 (d overlapping brs, *J* = 8.9 Hz, 2H, H-2 and H-4, respectively), 5.97 (d, *J* = 8.7 Hz, 1H, NH), 4.22 (q, *J* = 8.9 Hz, 1H, H-17), 2.89 (m, 2H), 2.33-

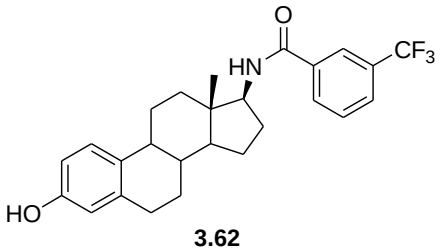
2.26 (m, 3H), 1.94-1.82 (m, 3H), 1.63-1.40 (m, 7H), 0.83 (s, 3H, CH3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 166.1 (C(O)NH), 164.2 (C(O)O), 148.4 (C-3), 138.3 (2 C, C-5 and C-6), 135.8 (C-C(O)NH-),133.3 (CH_{Ar}), 131.6 (q, *J* = 33 Hz, 2 C, C-CF3), 130.6 (C-C(O)O-), 130.1 (CH_{Ar}), 130.0 (d, *J* = 3.5 Hz, CH_{Ar}), 129.2 (d, *J* = 3.4 Hz, 2 CH_{Ar}), 127.9 (d, *J* = 3.5 Hz, CH_{Ar}), 126.9 (d, *J* = 3.7 Hz, CH_{Ar}), 126.6 (C-1), 123.8 (q, *J* = 3.7 Hz, CH_{Ar}), 123.6 (q, *J* = 270.9 Hz, 2 C, C-CF_3), 121.4 (C-4), 118.5 (C-2), 59.5 (C-17), 51.7 (C-14), 44.0 (C-13), 43.8 (CH), 38.6 (CH), 37.0 (CH_2), 29.5 (CH_2), 28.7 (CH_2), 27.1 (CH_2), 26.1 (CH_2), 23.4 (CH_2), 12.2 (CH_3, C-18); ^{19}F NMR ($CDCl_3$, 282 MHz) δ -62.5 (2 CF_3 overlapping); LRMS (ESI^+) m/z (%) 616 (M+H, 100); HRMS (ESI^+) calcd for $C_{34}H_{32}F_6NO_3$ $(M+H)^+$ 616.2286; found 616.2296.



3.61

17β-(3'-Bromo)benzamido-estra-1,3,5(10)-trien-3-ol (3.61). To a stirred solution of **3.59** (100 mg, 0.15 mmol) in methanol (10 mL), was added a solution of K_2CO_3 (21 mg, 0.15 mmol) in H_2O (100 μL). The resultant solution was stirred for 3 h, then diluted with water, neutralized with dil. HCl, and extracted with chloroform. The extract was then washed with water, brine, dried with Na_2SO_4, and concentred. Purification was achieved using flash chromatography (ethyl acetate/hexane, 1:9) which provided **3.61** as a white solid (48 mg, 67%). Mp 166-167^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz), δ 7.86 (t, *J* = 1.7 Hz, 1H, ArH), 7.67-7.58 (m, 2H, ArH), 7.28 (m, 1H, ArH), 7.11 (d, *J* = 8.4, 1H, ArH), 7.12 (d, *J* = 8.3 Hz, 1H, H-1), 6.61 (dd, *J* = 2.7 and 8.3 Hz, 1H, H-2), 6.55 (d, *J* = 2.6 Hz, 1H, H-4), 5.94 (d, *J* = 9.0 Hz, 1H, NH), 5.00 (brs, 1H, OH), 4.15 (q, *J* = 8.8 Hz, 1H, H-17), 2.80 (m, 2H), 2.30-2.19 (m, 3H), 1.88-1.83 (m, 3H),

1.56-1.23 (m, 7H), 0.79 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 166.1 (C(O)NH), 153.5 (C-3), 138.1 (C-5), 136.9 (C-C(O)NH), 134.3 (CH_{Ar}), 132.4 (C-6), 130.0 (2 CH_{Ar}), 126.5 (CH_{Ar}), 125.4 (CH_{Ar}), 122.7 (C), 115.3 (C-4), 112.7 (C-2), 59.5 (C-17), 51.6 (C-14), 43.8 (C-13), 43.7 (CH), 38.9 (CH), 37.0 (CH_2), 29.6 (CH_2), 28.8 (CH_2), 27.3 (CH_2), 26.2 (CH_2), 23.4 (CH_2), 12.3 (CH_3, C-18); LRMS (ESI^+) m/z (%) 454 (M+H, 100); HRMS (ESI^+) calcd for $C_{25}H_{29}NO_2Br$ $(M+H)^+$ 454.1382; found 454.1370.



3.62

17β-(3'-Trifluoromethyl)benzamido-estra-1,3,5(10)-trien-3-ol (3.62). To a stirred solution of **3.60** (120 mg, 0.27 mmol) in methanol (15 mL), was added a solution of K_2CO_3 (35 mg, 0.25 mmol) in H_2O (120 μL). The resultant solution was stirred for 3 h, then diluted with water, neutralized with dil. HCl, and extracted with chloroform. The extract was then washed with water, brine, dried with Na_2SO_4, and concentred. Purification was achieved using flash chromatography (ethyl acetate/hexane, 3:7) which provided **3.62** as a white solid (123 mg, 74%). Mp 135-136°C; ^1H NMR ($CDCl_3$, 300 MHz), δ 7.99 (s, 1H, ArH), 7.92 (d, *J* = 7.7 Hz, 1H, ArH), 7.74 (d, *J* = 7.8 Hz, 1H, ArH), 7.56 (dd overlapping, *J* = 7.7 and 7.8 Hz, 1H, ArH), 7.12 (d, *J* = 8.3 Hz, 1H, H-1), 6.61 (d, *J* = 8.3 Hz, 1H, H-2), 6.56 (brs, 1H, H-4), 6.01 (d, *J* = 8.7 Hz, 1H, NH), 5.07 (brs, 1H, OH), 4.19 (q, *J* = 9.0 Hz, 1H, H-17), 2.80 (m, 2H), 2.29-2.21 (m, 3H), 1.89-1.85 (m, 3H), 1.58-1.39 (m, 7H), 0.81 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 166.1 (C(O)NH), 153.3 (C-3), 138.1 (C-5), 135.7 (C-C(O)NH-), 132.4 (C-6), 131.0 (d, *J* = 32.5 Hz, C-CF_3), 130.0 (d, *J* = 1.3 Hz, CH_{Ar}), 129.2 (d, *J* = 2.4 Hz, CH_{Ar}), 127.9 (q, *J* = 3.7 Hz, CH_{Ar}), 126.5

(C-1), 123.8 (q, J = 3.8 Hz, CH_{Ar}), 123.7 (q, J = 271.0 Hz, C-CF3), 115.3 (C-4), 112.7 (C-2), 59.5 (C-17), 51.6 (C-14), 43.8 (C-13), 43.7 (CH), 38.9 (CH), 37.0 (CH_2), 29.6 (CH_2), 28.8 (CH_2), 27.3 (CH_2), 26.2 (CH_2), 23.4 (CH_2), 12.3 (CH_3, C-18); ^{19}F NMR ($CDCl_3$, 282 MHz) δ -62.9; LRMS (ESI^+) m/z (%) 444 (M+H, 100); HRMS (ESI^+) calcd for $C_{26}H_{29}F_3NO_2$ $(M+H)^+$ 444.2150; found 444.2135.

3.5.3 Inhibition Studies

3.5.3.1 General

STS was purified as previously described in *Chapter* 2. All buffers and assay reagents were purchased form Sigma Aldrich (Milwaukee, WI, USA). All fluorescent measurements were carried out on a SpectraMax GeminiXS® fluorimeter (Molecular Devices, Sunnyvale, CA, USA) at 24 ^oC in 96-well black microtiter plates from Corning (Corning, MA, USA). All determinations were carried out in quadruplicate.

3.5.3.2 Determination of IC_{50}s

An inhibitor stock solution in DMSO/0.1 M Tris-HCl, pH 7.0 (1:1), 20 µL weas added to the wells of a 96-well microtiter plate containing 140 µL of 0.1 M Tris, pH 7.0. After that, 20 µL of a 2.0 mM MUS stock solution in 0.1 M Tris–HCl, pH 7.0, was added. The assay was initiated by adding 20 µL STS (100 nM stock solution in 20 mM Tris–HCl, pH 7.4, 0.1% Triton X-100). The final concentration of inhibitor ranged from 5 nM to 5 µM. The final concentration of 4-MUS was 200 µM. The reactions was followed were followed by detection of fluorescent product, 4-methylumbelliferone (excitation 360 nm, emission, 460 nm), over 10 min at 24^oC. Each reaction was performed in quadruplicate. Additional controls were performed in an identical manner but did not contain STS. Eleven concentrations of inhibitor bracketing the IC_{50} value were used for each compound. The initial rates of enzyme activity in relative fluorescence

units per second (RFU/s) were used to determine the IC_{50}. The ratio of the initial rate in the presence of inhibitor (V_i) to that in the absence of inhibitor (V_o) was calculated and plotted as a semi-log curve in Grafit (Erithacus Software, Surrey, U.K.), from which the IC_{50} value was calculated based on the following equation: $V_i = V_o/[1 + ([I]/IC_{50})S] + B$, where: V_i is the initial rate of reaction at an inhibitor concentration of [I]; V_o is the velocity in the absence of inhibitor; *B* is background and *s* is the slope factor (see **Appendix A** for IC_{50} plots).

3.5.3.3 Determination of K_i and αK_i values for compound 3.28.

20 µL of a MUS stock solution in 0.1 M Tris-HCl of pH 7.0 was added to the wells of a 96-well microtiter plate containing 140 µL 0.1 M Tris-HCl buffer of the same pH such that the total volume was 160 µL. To the wells was added 20 µL of a stock solution of inhibitor in 50% DMSO, (for a control, 20 µL of 50% DMSO was added instead). The assay was initiated by the addition of 20 µL STS (100 nM stock solution in 20 mM Tris-HCl, pH 7.4, 0.1% Triton X-100). To detect non-enzymatic hydrolysis of the substrate 20 µL of 20 mM Tris-HCl, pH 7.4, 0.1% Triton X-100 was added instead. The final volume of the assay was 200 µL. The final concentration of buffer was 184 mM Tris-HCl, 0.01% Triton X-100, and 5% DMSO. The final enzyme concentration was 10 nM.

For studies with compound **3.28** at pH 7.0, the final concentration of MUS was 83.3–500 µM, the final concentration of inhibitor was 1-3 times IC_{50}. The reactions were followed by detection of fluorescent product, 4-methylumbelliferone (excitation 360 nm, emission, 460 nm), over 10 min at 24^{o}C. Each reaction was performed in quadruplicate. Additional controls were performed in an identical manner but did not contain STS. Initial rates (*v*) were determined by taking the slopes of plots of the change in relative fluorescence units with time. These data were plotted as Lineweaver–Burk graphs and K_i and αK_i values were calculated from re-plots of the

slopes or intercepts of the Lineweaver–Burk graphs according to the equations for mixed and competitive inhibition (see **Appendix A** for re-plots of these data).

3.5.4 Molecular Modeling (Docking) Experiments

Docking experiments were performed using Discovery Studio Client v2.5.0.9164 (2005-09), Accelrys Software Inc. running on a HP xw4600 workstation (Processor x86 family 6 model 23 stepping 10 Genuine Intel 2999 ~ MHz). The coordinates for the X-ray crystal structure of human steroid sulfatase enzyme were obtained from RCSB Protein Data Bank (PDB file: 1P49). The ligand molecules were constructed using the Build Fragment tool and energy minimized for 1000 iterations reaching a convergence of 0.01 kcal/mol Å. The steroid sulfatase enzyme was prepared for docking experiments first by deleting water molecules and then by using the prepare protein tool in Discovery studio. The formylglycine (FGly75), present in the catalytic site was modified to the *gem*-diol using the build fragment tools. Subsequently, the enzyme was energy minimized by steepest descent method for 10,000 steps reaching a convergence of 0.1 kcal/mol Å and further by conjugate gradient method for 10,000 steps reaching a convergence of 0.01 kcal/mol Å. The binding site of the enzyme was defined by generating a 10 Å radius sphere, after selecting using the amino acid Thr99. The test compounds were docked in the active site of steroid sulfatase enzyme using the Libdock command under the receptor-ligand protocol in Discovery Studio using the CHARMM force field. The quality of ligand-enzyme complex obtained was evaluated using the Libdock scoring function (kcal/mol) and by considering various intermolecular polar and nonpolar interactions between the ligand and the enzyme.

3.5.5 X-ray Crystallography of Compound 3.33.

Crystal data: $C_{25}H_{28}F_3NO_3S$, MW = 479.54, Monoclinic; a = 9.611 (3) Å, b = 13.461 (4) Å, c = 17.786 (6) Å, β = 96.239 (18)^o, V = 2287.5 (12) A^3; space group $P2_1$, Z = 4, Dc = 1.392 g

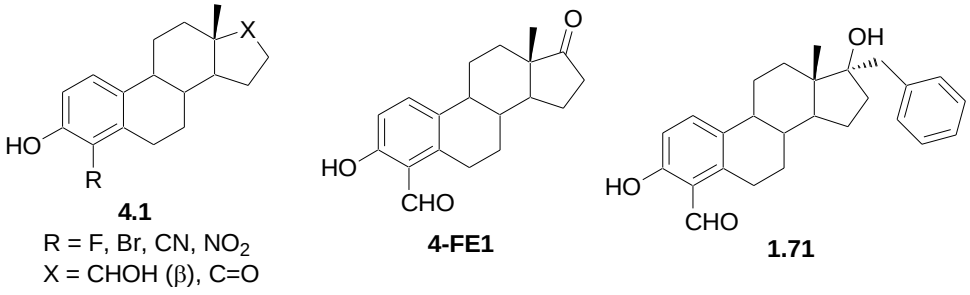
cm^{-3}; μ (Mo Kα) = 0.19 mm^{-1}, *F*(000) = 1008. Crystallographic measurements were made at 200 K on a Bruker Kappa APEX II diffractometer in the range $3.0^{o} < \theta < 26.0^{o}$.Data (6503 reflections) were corrected for absorption using intensity measurements (SADABS).

The structure was solved by direct methods and refined using the Flack parameter measurement.[175] Tables of geometric data, indicating bond angels, are available in **Appendix C** (for X-ray crystal data).

Chapter 4 – Steroid Sulfatase Inhibitors: A-Ring Modification of 17β-arylsulfonamide E1 Derivatives.

4.1. Introduction

The Taylor Group has shown that the affinity of estrogens for STS can be increased by introducing certain groups at the 4-position of the A-ring.[63,73,74] For example, E1 was found to be a reversible non-competitive inhibitor of STS with IC_{50} of 51 μM, however; introducing a small electron-withdrawing group or atom, such as a F, Br, CN, and NO_2 group at the 4-position of the A-ring of E2 or E1 (compounds **4.1**, Fig. 4.1), resulted in reversible inhibitors with IC_{50}'s in the 2-7 micromolar range. The 4-nitroE1 derivative proved to be the most potent of this series with an IC_{50} of 2.4 μM.[74] The reason for this increase in potency is not known though studies showed that it was not due to the decrease in pK_a of the 3-OH group (there was no correlation between the pK_a of the steroid and IC_{50}).



4.1
R = F, Br, CN, NO_2
X = CHOH (β), C=O

4-FE1

1.71

Fig. 4.1. Structures of compounds **4.1**, 4-FE1 and **1.71**.

Additionally, as mentioned before in *Chapter 1* (see section 1.4.1.3), the Taylor group reported that 4-formyl estrone (**4-FE1**) acts as an almost irreversible inhibitor of STS, with a K_I of 1.5 μM.[73] This finding prompted Taylor group to examine 4-formyl-17α-benzyl E2, compound **1.71** (see section 1.4.1.3), as an STS inhibitor. This compound turned out to be a potent concentration and time-dependent STS inhibitor with a K_I of 85 nM and a k_{inact} of 0.021 min^{-1} (k_{inact}/K_I of 2.3×10^5 M^{-1} min^{-1}).[74]

4.2. Objectives

In *Chapter* 3 we developed some highly potent inhibitors of STS (of type **3.2**, Fig. 3.1) with one of these inhibitors, compound **3.49** (Fig. 4.2), exhibiting an IC_{50} as low as 9 nM. Based upon our previous results on the inhibition of STS with 4-substituted estrogens mentioned in section 4.1 we reasoned that the potency of compounds of type **3.2** could be further increased by introducing a small group or atom, such as NO_2, F, Br, or CHO, at C-4. The objective of the work described in this chapter is to synthesize such compounds and determine if this modification does indeed increase the potency of the parent molecules.

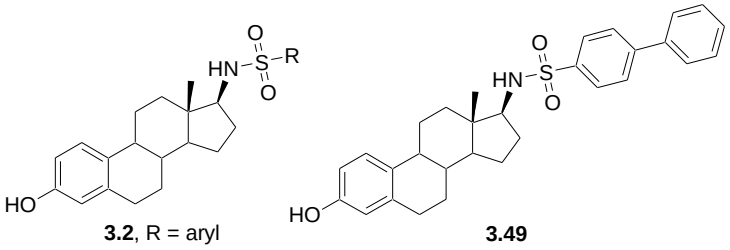


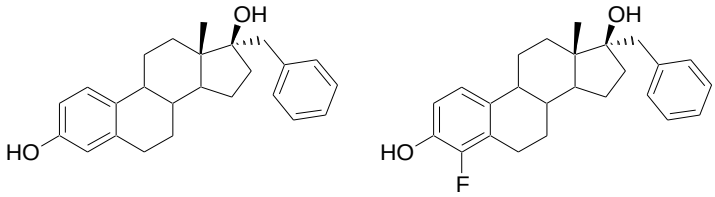
Fig. 4.2. General structure of 17β-arylsulfonamide E1 derivatives **3.2** and the structure of compound **3.49**.

4.3. Results and Discussion

4.3.1 Studies with 4-fluoro-17β-E2 (compound 4.2)

Previous to our work on compounds of type **4.1**, Yong Liu, a former graduate student in the Taylor group and Prof. Scott D. Taylor had prepared 4-fluoro-17β-benzylE2 (**4.2**, Fig. 4.3).[74] The purpose of making this compound was to determine if the introduction of a fluorine at the 4-position of compound **1.50** would enhance its potency as an STS inhibitor. We (Y. Mostafa) found that its IC_{50} was 40 nM, which is approximately six-fold lower than that of its parent compound **1.18a**.[74] Moreover, compound **4.2** exhibited mixed-type inhibition (Fig. 4.4) with K_i

of 40 nM and an αK_i of 90 nM, which was the same inhibition mode shown by both **E1** and its parent compound **1.50**. So the introduction of the fluorine atom at the 4-position improved the potency of its non-fluorinated derivative without affecting its mode of inhibition.[74] These results provided further impetus to pursue compounds of type **4.1**.



1.50
noncompetitive STS inhibitor
K_i = 230 nM, αK_i = 420 nM

4.2
noncompetitive STS inhibitor
K_i = 40 nM, αK_i = 90 nM

Fig. 4.3. STS inhibitors **1.50** and **4.2**.[74]

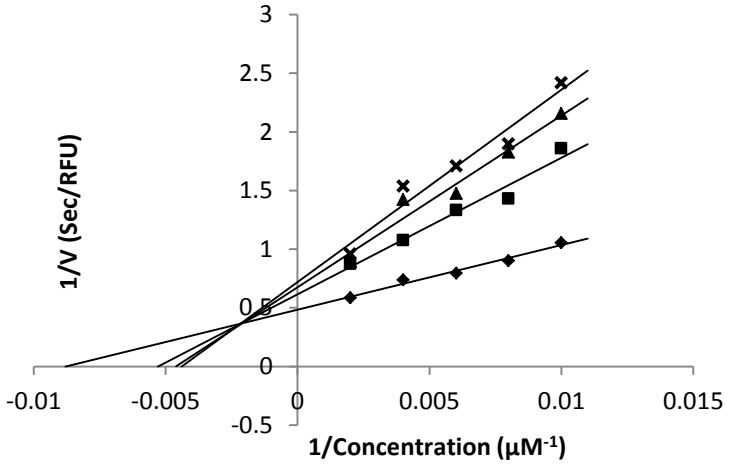


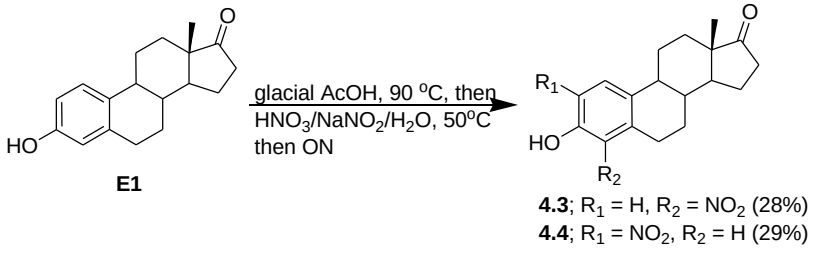
Fig. 4.4. Lineweaver-Burk plot of compound **4.2 (**◆ 0 nM ■ 10 nM ▲ 20 nM ×40 nM) [See **Appendix B** for the re-plot of this data that was used to determine both K_i and αK_i].[74]

4.3.2. 2- and 4-Nitro-17β-arylsulfonamide E1 derivatives as STS inhibitors.

These studies were initiated by preparing the sulfonamide derivatives bearing a NO_2 group at 2- and/or 4-position. Upon examining the literature about different methods for

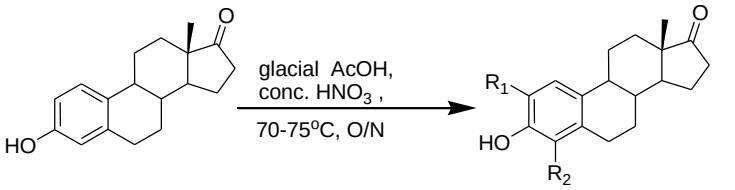
nitration of the A-ring of estrogens we concluded that direct nitration of arylsulfonamides of type **3.2** would not be a suitable approach to these compounds since nitration will most likely occur not only occur at 2- and/or 4-position but also occur on the aromatic ring of the arylsulfonamide moiety. So we decided to prepare 2- and 4-nitroE1 first and then convert these into the desired sulfonamides.

Nitration of the A-ring of estrogens always results in the formation of a mixture of the 2- and 4-NO_2 isomers and the 2,4-dinitro compound with the major product being the 2-NO_2 isomer for steric reasons.[176-179] The yields of the individual isomers are usually quite low especially for the 4-isomer. One of these reports described the synthesis of 4-NO_2E1 (**4.3**) and 2-NO_2E1 (**4.4**) in low yields (isolated) but in almost equimolar amounts using HNO_3/$NaNO_2$/glacial AcOH (Scheme 4.1).[176] Although the amount of $NaNO_2$ was unspecified, we attempted this reaction using a cat. amount of $NaNO_2$. We obtained a 1:1 mixture of **4.3** and **4.4** plus other side products. Several attempts to separate and purify the 2- and 4-isomers by flash or gravity column chromatography were unsuccessful. However, recrystallization from hot glacial acetic acid gave pure **4.3** in a 12% yield as yellow crystals.



Scheme 4.1. Nitration of **E1** using a HNO_3/$NaNO_2$/glacial AcOH mixture.[176]

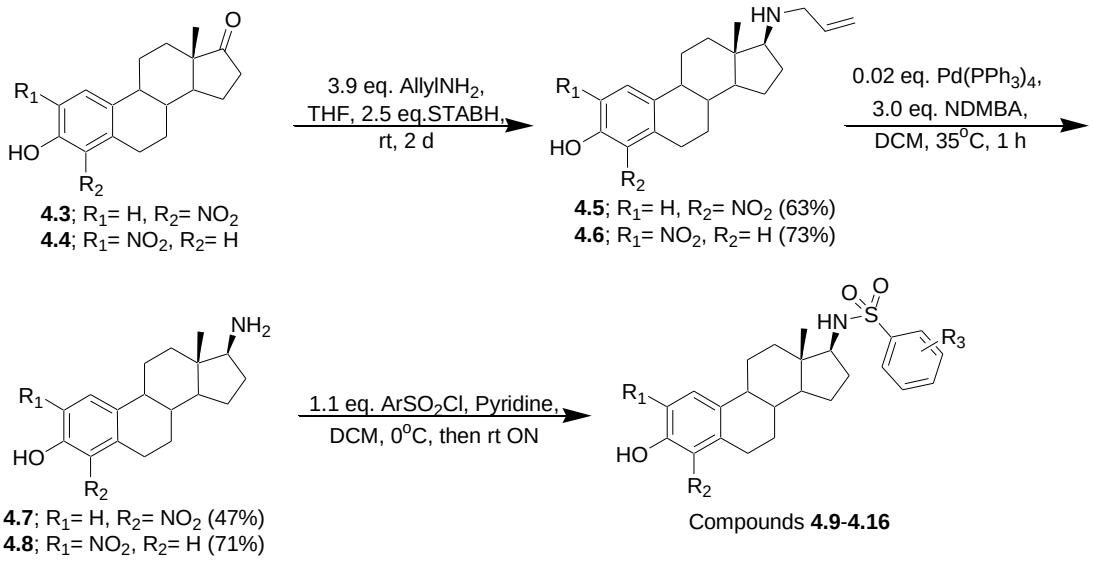
Due to the low yields of the above procedure we decided to try another older procedure which uses conc. HNO_3 alone without use of $NaNO_2$ again in glacial AcOH but at 70-75 ^oC (Scheme 4.2).[179] During the reaction we noticed the formation of a yellow precipitate. After stirring overnight (O/N) the mixture was filtered (while still hot) and the precipitate was washed with hot glacial AcOH. The precipitate turned out to be pure 4-NO_2E1, **4.3** (20% yield). The filtrate, after work-up, afforded the 2-NO_2 isomer, **4.4**, in a 23% yield. This was easily performed on a multigram scale. This represents a significant improvement in the synthesis and isolation of these compounds.



Scheme 4.2. Nitration of **E1** using conc. HNO_3 in glacial AcOH at 70-75^oC.[179]

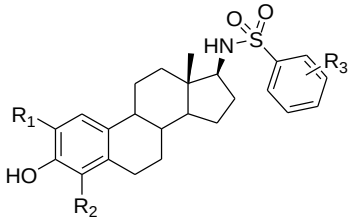
With the nitrated E1 isomers in hand, we then subjected these to the same reductive amination procedure that we used for the preparation of 17β-aminoE1 (compound **3.11**, see chapter 3, section **3.3.1)** except that instead of doing the reductive amination with benzyl amine we used allylamine since the nitro groups would not survive the removal of the benzyl group by hydrogenolysis (Scheme 4.3). The reductive amination proceeded well with compounds **4.5** and **4.6** being obtained in good yields. Pd-catalyzed removal of the allyl group was achieved in reasonable to good yields using *N*,*N'*-dimethylbarbituric acid (NDMBA) as a carbon nucleophile scavenger for the allyl group. The resulting 2- or 4-NO_2-17β-amine derivatives of E1, compounds **4.7** and **4.8**, were reacted with sulfonyl chlorides using the same general method we

reported in section **3.3.1** to give the desired nitrate sulfonamides **4.9**-**4.16**. The yields for the final step (not optimized) are shown in Table 4.1.



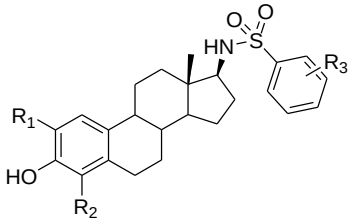
Scheme 4.3. Synthesis of nitro sulfonamides **4.9**-**4.16.**

Table 4.1. Yields of nitrated sulfonamides **4.9**-**4.16**.



Compound	R_1	R_2	R_3	Yield (%)
4.9	H	NO_2	3'-Br	48
4.10	H	NO_2	3'-CF_3	53
4.11	H	NO_2	4'-*t*-Bu	40
4.12	H	NO_2	4'-Phenyl	27
4.13	NO_2	H	3'-Br	66
4.14	NO_2	H	3'-CF_3	67
4.15	NO_2	H	4'-*t*-Bu	26
4.16	NO_2	H	4'-Phenyl	43

Table4.2. IC_{50}'s of nitrated sulfonamides **4.9**-**4.16** and their non-nitrated analogs (**3.27**, **3.28**, **3.33**, and **3.49**)



Compound	R_1	R_2	R_3	IC_{50} (nM)[a]
3.28	H	H	3'-Br	25
3.33	H	H	3'-CF_3	23
3.27	H	H	4'-*t*-Bu	18
3.49	H	H	4'-Phenyl	9
4.9	H	NO_2	3'-Br	12
4.10	H	NO_2	3'-CF_3	11
4.11	H	NO_2	4'-*t*-Bu	8[b]
4.12	H	NO_2	4'-Phenyl	1[b]
4.13	NO_2	H	3'-Br	50
4.14	NO_2	H	3'-CF_3	67
4.15	NO_2	H	4'-*t*-Bu	81
4.16	NO_2	H	4'-Phenyl	33

[a] Errors are within ±5%; [b] Apparent K_i

The IC_{50}'s or apparent K_i's (K_i^{app}) for compounds **4.9**-**4.16** and their A-ring unsubstituted analogs (**3.27**, **3.28**, **3.33**, and **3.49**) are shown in Table 4.2. Introduction of a nitro group at the 2-position resulted in a marked decrease in potency while substitution of a nitro group at the 4-position resulted in an increase in potency. To the best of our knowledge, these 4-substituted derivatives are the most potent reversible inhibitors ever reported for STS.

Some of the inhibition data presented in Table 4.2 are given as IC_{50}'s while some are given as apparent K_i's (K_i^{app}). The reason for this has to do with the potency of some of the inhibitors. When one determines the IC_{50} of an inhibitor the concentration of the inhibitor ([I]) required to achieve 50% inhibition (IC_{50}) is usually far in excess of the enzyme concentration since most inhibitors do not exhibit IC_{50}'s that are below the concentration of enzyme used in the

assay. So the amount of inhibitor sequestered in formation of the enzyme-inhibitor complex is a small fraction of the total concentration of inhibitor. Therefore, the amount of free inhibitor ($[I]_F$) is approximately the same as the total concentration of inhibitor ($[I]_T$). However, with a tight binding inhibitor (TBI), which is an inhibitor with a K_i is in the low nM region or lower which is usually less than the total concentration of enzyme ($[E]_T$), then $[I]_F \neq [I]_T$ and the equilibrium assumptions used to derive the classic equations used for calculating K_i no longer hold.[180]

The relationship between IC_{50} and the apparent K_i (for a competitive inhibitor $K_i^{app} = K_i(1 + [S]/K_m)$ while for a non-competitive inhibitor $K_i^{app} = K_i(K_m + [S])/Km/K_{iE} + [S]/K_{iES})$ is shown in Eqn. 4.1.

$$IC_{50} = K_i^{app} + \tfrac{1}{2}[E]_T \quad \text{(Eqn. 4.1)}$$

From this eqn. we can see that:

(1) When $K_i^{app} >> [E]_T$ then IC_{50} is approximately equal to K_i^{app}

(2) When $K_i^{app} = [E]_T$ then IC_{50} is approximately equal to $K_i^{app} + \tfrac{1}{2}[E]_T$

(3) When $K_i^{app} << [E]_T$ then $IC_{50} = \tfrac{1}{2}[E]_T$

Eqn. 4.1 shows that if one determines the IC_{50}'s of a series TBIs (when $K_i^{app} << [E]_T$) then the results can be misleading as the IC_{50}'s for all of the TBI's will be the same (equal to half the concentration of the enzyme used in the assay). Whether or not a compound is a TBI can be ascertained by the dependence of its IC_{50} on enzyme concentration. If the inhibitor is not tight binding then there is no dependence of IC_{50} on $[E]_T$. If the inhibitor is a TBI then IC_{50} depends upon $[E]_T$ and a plot of IC_{50} vs. $[E]_T$ should be a straight line with the Y-intercept equal to K_i^{app}.[180]

In our standard STS assay the concentration of STS was 10 nM and so we suspected that some of the compounds we examined in Table 4.2 might be TBIs and, therefore, their IC_{50}'s should depend on $[E]_T$. We examined the effect of enzyme concentration on the IC_{50}'s for two of the best inhibitors in Table 4.2, the 4'-*t*-butyl compound **4.11**, and 4'-biphenyl compound **4.12**. The IC_{50}'s of both of these inhibitors showed a dependence on enzyme concentration (see Figs 4.5 and 4.6). Their K_i^{app}'s were obtained from the Y-intercept of these plots.

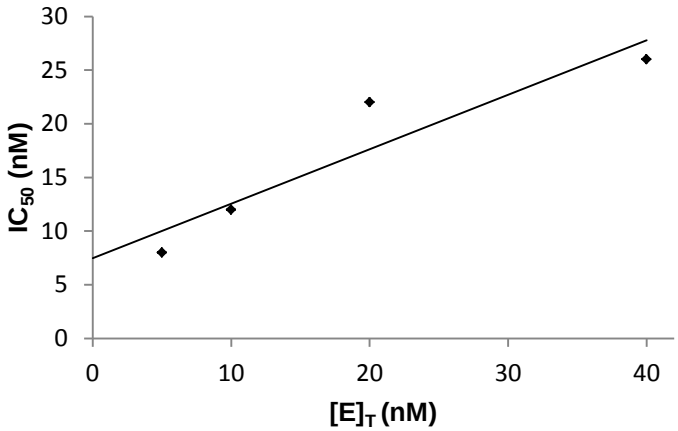


Fig. 4.5. A plot of IC_{50}'s for compound **4.11** as a function of $[E]_T$.

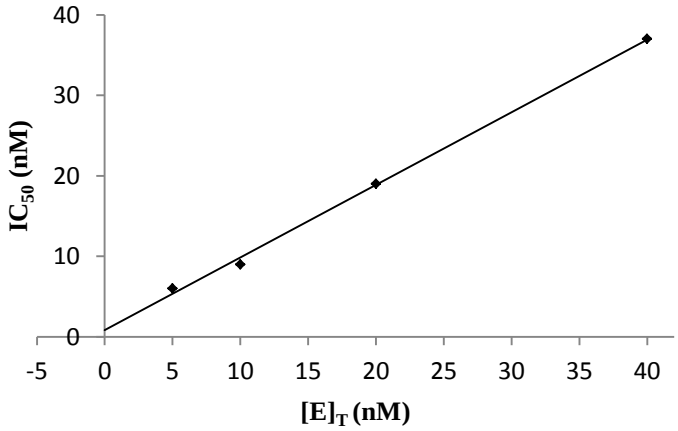
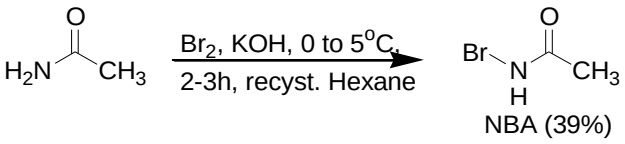


Figure 4.6. A plot of IC_{50}'s for compound **4.12** as a function of $[E]_T$.

It is interesting that we noticed that in the ^{1}H-NMR of all 2-nitro arylsulfonamide derivatives, compounds **4.13**-**4.16**; their phenolic 3-OH proton was strongly downfield shifted (δ = 10.4 ppm), while the one for 4-nitro arylsulfonamides, compounds **4.9**-**4.12**, was less downfield shifted (δ = 9.4 ppm), suggesting the presence of a stronger interaction between the 2-NO_2 group and 3-OH in all the 2-nitro arylsulfonamides, than for 4-nitro derivatives. How the nitro group interacts with the 3-OH may somehow affect the potency of these compounds.

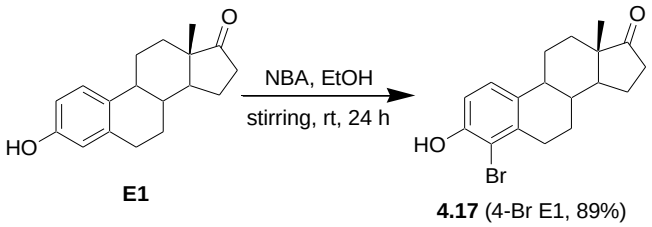
4.3.3 2- and 4-Bromo-17β-arylsulfonamide E1 derivatives as STS inhibitors.

For the 2- and 4-bromo series we focussed our efforts on just the 2- and 4-bromo 3'-trifluoromethylbenzensulfonamide derivatives. We first prepared 2- and 4-bromoestrone first rather than trying to brominate our sulfonamide inhibitors to avoid brominating the aromatic ring of the arylsulfonamide group. Bromination of the A-ring of an estrogen using common electrophilic brominating agents such as $Br_2/FeBr_3$ or NBS always results in the formation of a mixture of 2- and 4- bromoestrogen, with the 2-isomer as the major product, and are very challenging to separate.[181,182] However, in 1968 Utne et al reported that if *N*-bromoacetamide (NBA) is used as the brominating agent with EtOH as solvent then 4-bromoestradiol (4-BrE2) could be obtained in reasonable yield (25-40%).[183] This unusual selectivity for the 4-position for an electrophilic aromatic substitution (SEAr) is unprecedented for any other SEAr reaction on an estrogen and no explanation for this has been presented. We decided to attempt the synthesis of 4-BrE1 using this procedure. The brominating agent, NBA, was prepared according to a literature procedure from acetamide and bromine in the presence of KOH. This afforded NBA in a 39% yield after recrystallization from hexane (Scheme 4.4).[184]



Scheme 4.4. Preparation of **NBA** from acetamide and bromine.[184]

The bromination of E1was achieved using equivalent amounts of E1 and NBA in ethanol with stirring at rt for 24 h (Scheme 4.5).[183] The crude product was recrystallized from ethanol to afford 4-BrE1 (**4.17**) in an 89% yield as white crystalline plates.

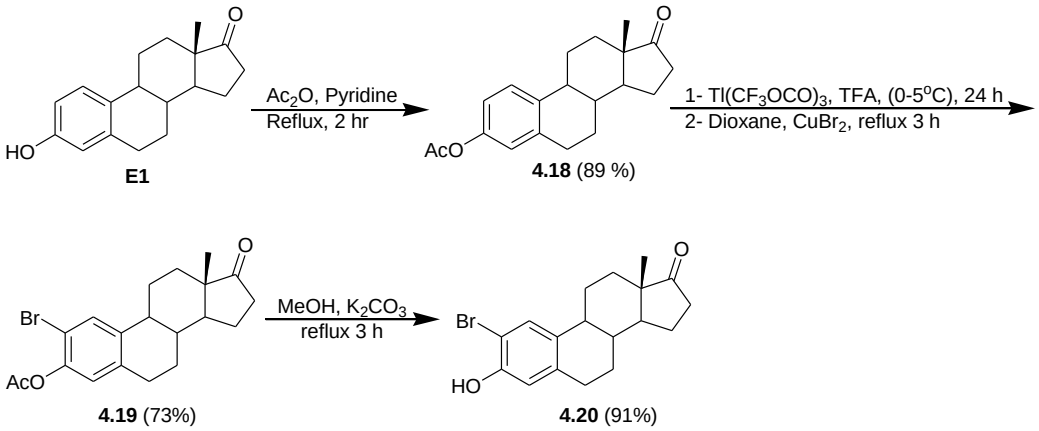


E1

4.17 (4-Br E1, 89%)

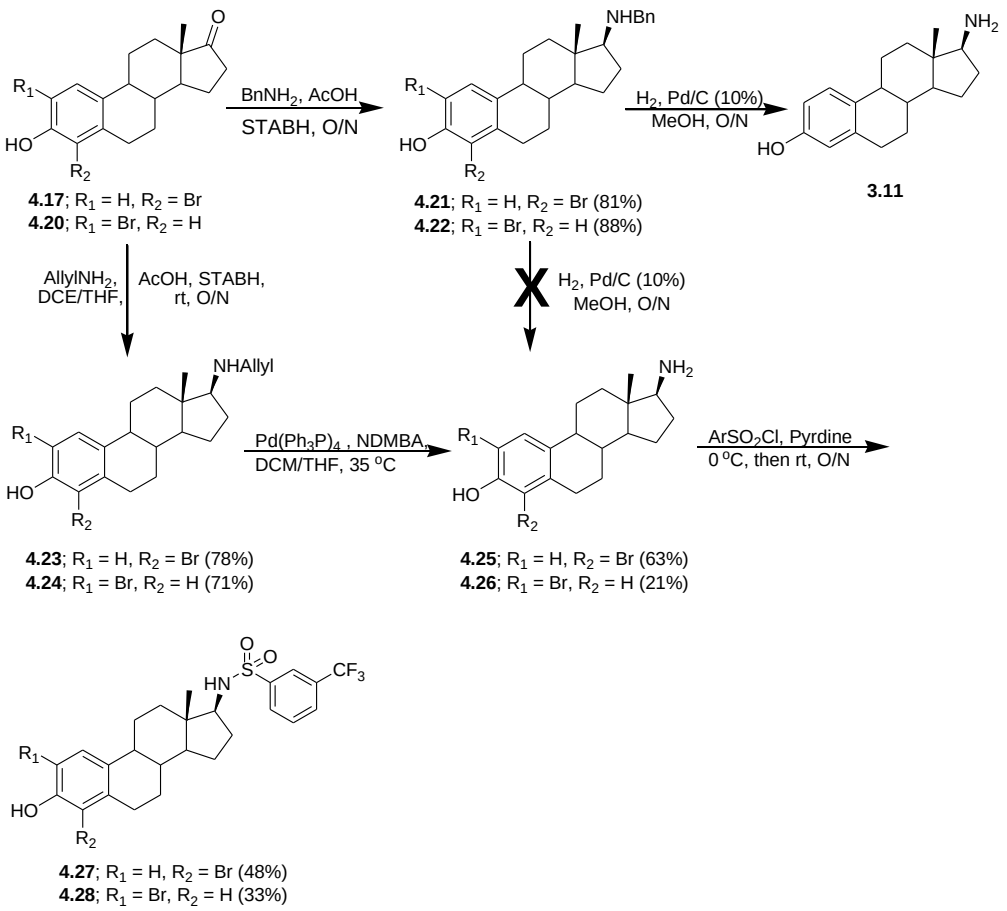
Scheme 4.5. Bromination of E1 using **NBA** to give 4-BrE1 (**4.17**) in high yield.

Selective bromination at the 2-position was not as easy as bromination at the 4-position. We tried two different methods; the first one involved the use of Br_2 in presence of a catalytic amount of powdered Fe^o and glacial AcOH as a solvent, but we obtained a mixture of 2-, 4-, and 2,4-diBrE1.[182] We tried to purify the two isomers by chromatography but we failed because they had very similar R_f's. The other method was a 3-step reaction scheme, but was reported to exhibit high selectivity and produce the 2-Br derivative in high yield (Scheme 4.6).[185] This method involved regioselective thallation of the A-ring of acetylated E1 (**4.18**) with thallic trifluoroacetate, $Tl(OCOCF_3)_3$, in trifluoroacetic acid (TFA), and subsequent displacement of the thallium moiety with bromide anion to afford the 3-acetyl-2-bromo derivative, **4.19**. Deprotection of the 3-OH gave 2-BrE1 (**4.20**) in good yield.



Scheme 4.6. Synthesis of 2-BrE1 (**4.20**).

2- and 4-BrE1 were subjected to our usual reductive amination conditions using benzylamine and STAB-H to give compounds **4.21** and **4.22** in good yield (Scheme 4.7). However, during the subsequent hydrogenolysis step the debrominated amine, compound **3.11**, rather than the brominated ones, compounds **4.25**, and **4.26**, was formed (Scheme 4.7). Numazawa et al., reported that hydrodebromination of 2-, 4-mono- and/or 2,4-di-bromo estrogens is possible during catalytic hydrogenation using Pd over charcoal if there is acidic impurities in the reaction media.[186] So we decided to use allylamine instead of benzylamine in the reductive amination. The corresponding allyl-protected amines (**4.23** and **4.24**) were obtained in better than a 70% yield for each compound. Deprotection of the amine using the Pd-catalyzed deallylation method gave the desired amines, (**4.25** and **4.26**) in low to reasonable yields.

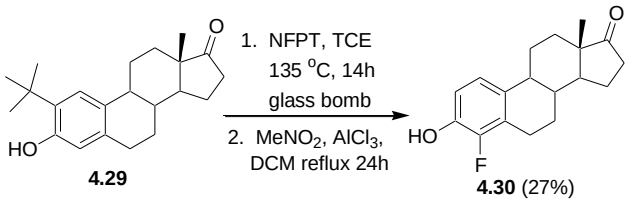


Scheme 4.7. Synthesis of bromosulfonamides, **4.27** and **4.28**.

Compounds **4.27** and **4.28** were less potent than their non-brominated parent sulfonamide, compound **3.33** with their IC_{50}'s equal to 95 nM and 49 nM respectively (c.f. 23 nM for compound **3.33**). This was somewhat unexpected, especially with the 4-bromo derivative, **4.28**, because the Taylor group had previously demonstrated that placing a Br at the 4-position of E1 enhanced its inhibitor potency of STS.[74] It appears that **4.27** and **4.28** interact with STS in a different manner from 2- or 4-BrE1. The 4-NO_2 derivative, **4.10**, is 4-fold more potent than the 4-Br derivative **4.28**. It is unlikely this difference is due to sterics as the Br atom is smaller than a NO_2 group (smaller A value). The greater electron withdrawing ability of the nitro group may be important here and also the nitro group can act as an H-bond acceptor while the Br atom cannot and this might be important for binding.

4.3.4 A 4-fluoro-17β-arylsulfonamide E1 derivative as an STS inhibitor.

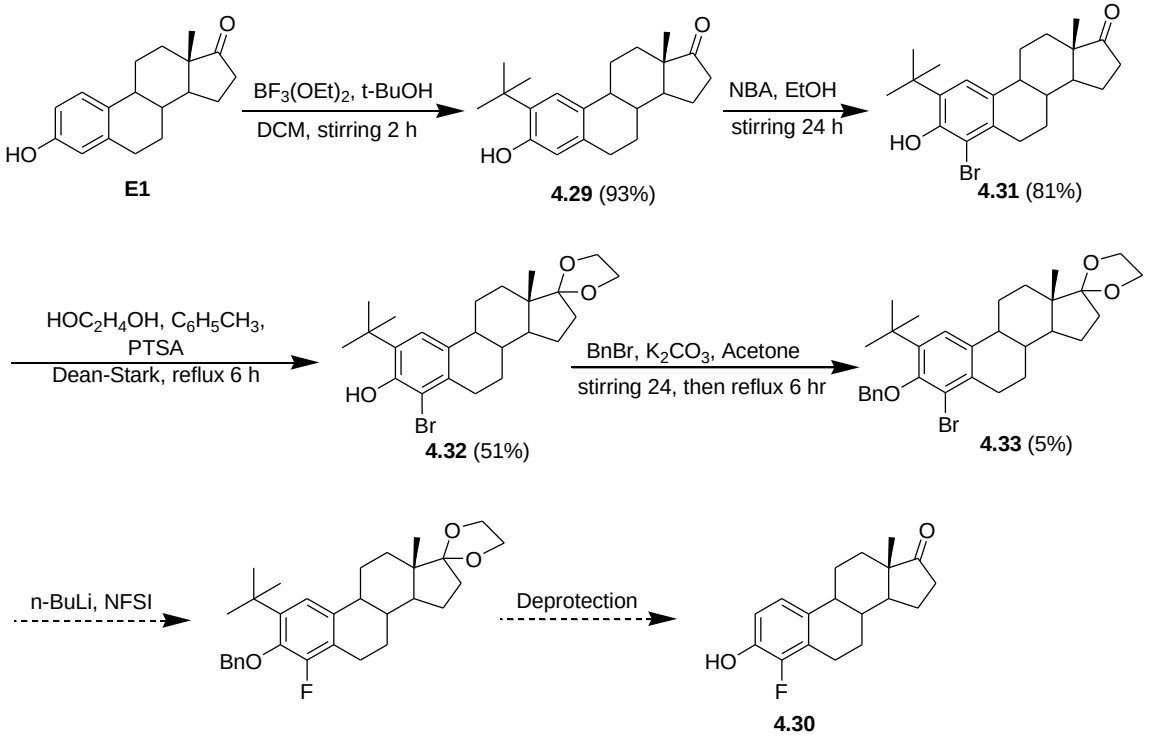
Our approach to the synthesis of compounds of type **4.1** with a fluorine at the 4-position was to prepare 4-fluoroestrone (4-FlE1, **4.30**) and then proceed with the introduction of the sulfonamide group in the usual manner. Yong Liu in the Taylor group prepared 4-FlE1 by electrophilic fluorination of the *tert*-butyl derivative **4.29** with *N*-fluoropyridinium triflate (NFPT)[187] followed by removal of the *tert*-butyl protecting group (Scheme 4.8). Although this gave the desired compound the yield was very low, the purification was very challenging and NFPT is very expensive. So we decided to look into alternative approaches to 4-FlE1.



Scheme 4.8. Liu's synthesis of 4-FlE1.

4-FlE1 and similar compounds have been prepared in a variety of ways. The 3-O-methyl ether of 4-FlE1 has been prepared in low yield by thermal decomposition of estrone 3-*O*-methyl ether 4-diazonium fluoroborate.[188] 4FlE1 has been prepared via oxidation of 4-F-19-nortesterone,[189] this is a multi-step procedure requiring expensive starting materials. (4) Electrophilic fluorination of E1 or E2 using Selectfluor™ in ionic liquids like 1-butyl-3-methylimidazolium tetrafluoroborate (bmim-BF_4),[190] or using *N*-fluoropyridinium triflate (NFPT) in chlorinated solvents like trichloroethane (TCE) has been used to prepare 4-FlE1 or 4-FlE2 albeit in low yields.. These procedures gave mixtures of the 2- and 4-isomers with the 2-isomer as the major product by far and the isomers were very challenging to separate.

Our first approach is shown in Scheme 4.9. The idea was to introduce the fluorine regioselectively by metal halogen exchange as this has been used previously to prepare aryl fluorides.[191] Compound **4.29** was prepared in 93 % yield using *t*-BuOH and boron trifluoride diethyl etherate ($BF_3.OEt_2$).[192,193] Bromination at the 4-position with NBA in EtOH gave compound **4.31** in good yield. The ketone in **4.31** was protected as a ketal using ethylene glycol and *p*-toluene sulfonic acid (PTSA) and a Dean-Stark trap to give **4.32** in a moderate yield. The point from this last protection step was to avoid fluorination at the 16-position. However, attempts to protect the 3-OH group with a benzyl group turned out to be challenging. We were only able to obtain very small amounts of the desired compound **4.33** using XS BnBr in the presence of a base such as K_2CO_3 or NaH. Attempts to introduce the benzyl group into **4.29** were also unsuccessful. We think that this is due to steric hindrance by the bulky *t*-butyl group at the 2-position.



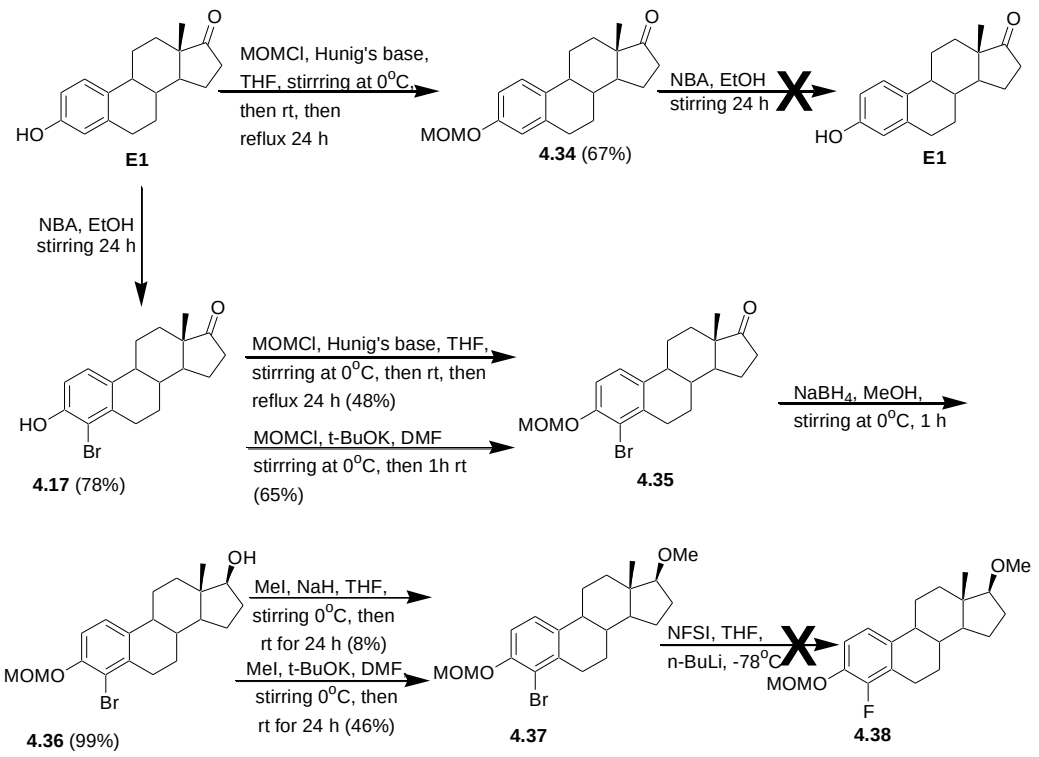
Scheme 4.9. First attempted route to **4.30**.

We also attempted to introduce the 2-methoxyethoxymethyl (MEM) protecting group into compound **4.32**, but unfortunately, this also did not work (Scheme 4.10).

4.32

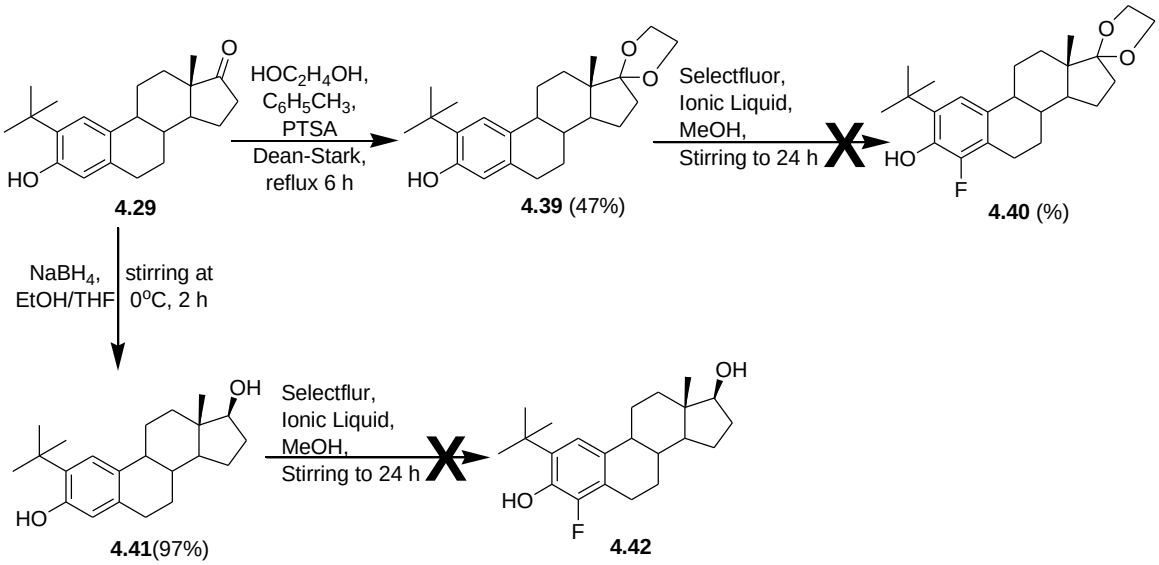
Scheme 4.10. Attempted MEM-protection of compound **4.32.**

Since the introduction of the *t*-butyl group first into **E1** prevented subsequent protection of the 3-OH we changed the order of protection (Scheme 4.11). The 3-OH was protected with the MOM group (methoxymethyl) using MOMCl in presence of *N*,*N*-diisopropyl ethylamine (DIPEA, Hunig's base) to afford compound **4.34** in a 67% yield. However, reaction of **4.34** with NBA in EtOH resulted in complete loss of the MOM group. So instead we reacted 4-BrE1 (**4.17**) with MOMCl in presence of either Hunig's base or *t*-BuOK base to give the desired compound **4.35** in reasonable yield with *t*-BuOK. The 17-keto group was then reduced and then methylated to give compound **4.37.** We reasoned that it should be possible to introduce the fluorine into the 4-position without protection of the 3-position. However, attempts to introduce the fluorine via metal halogen exchange followed by reaction with *N*-fluorobenzenesulfonimide were unsuccessful.



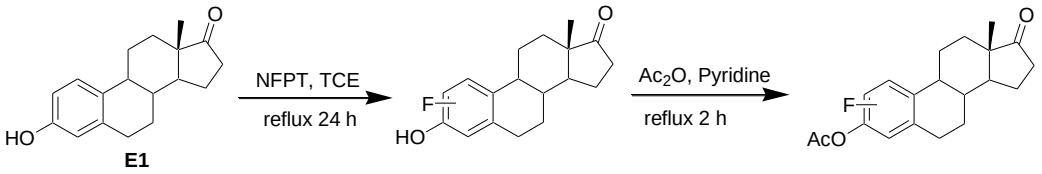
Scheme 4.11. Attempted synthesis of **4.38**.

Our alternative strategy was to try fluorinating the 4-position of protected E1 using F-TEDA-BF_4 (Selectfluor^{TM}) as a fluorinating agent in 1-butyl-3-methylimidazolium tetrafluoroborate (bmim-BF_4), an ionic liquid (Scheme 4.12) since it has been reported that phenols undergo aromatic electrophilic fluorination very readily in this ionic liquid using Selectfluor.[190] First we protected the ketone at the 17-position of **4.29** as a ketal to give **4.39** then subjected **4.39** to Selectfluor^{TM} in bmim BF_4. After 1 hour a major spot on the TLC was evident running slower than the starting material spot, and this increased in intensity over 24 h. Upon workup we found that the fluorination did not happen but loss of the ketal occurred. So, we reduced the ketone group of compound **4.29** using $NaBH_4$, and subjecting the resulting alcohol **4.41** to Selectfluor^{TM} in bmim BF_4 but no reaction occurred.



Scheme 4.12. Attempted synthesis of compounds **4.40** and **4.42**.

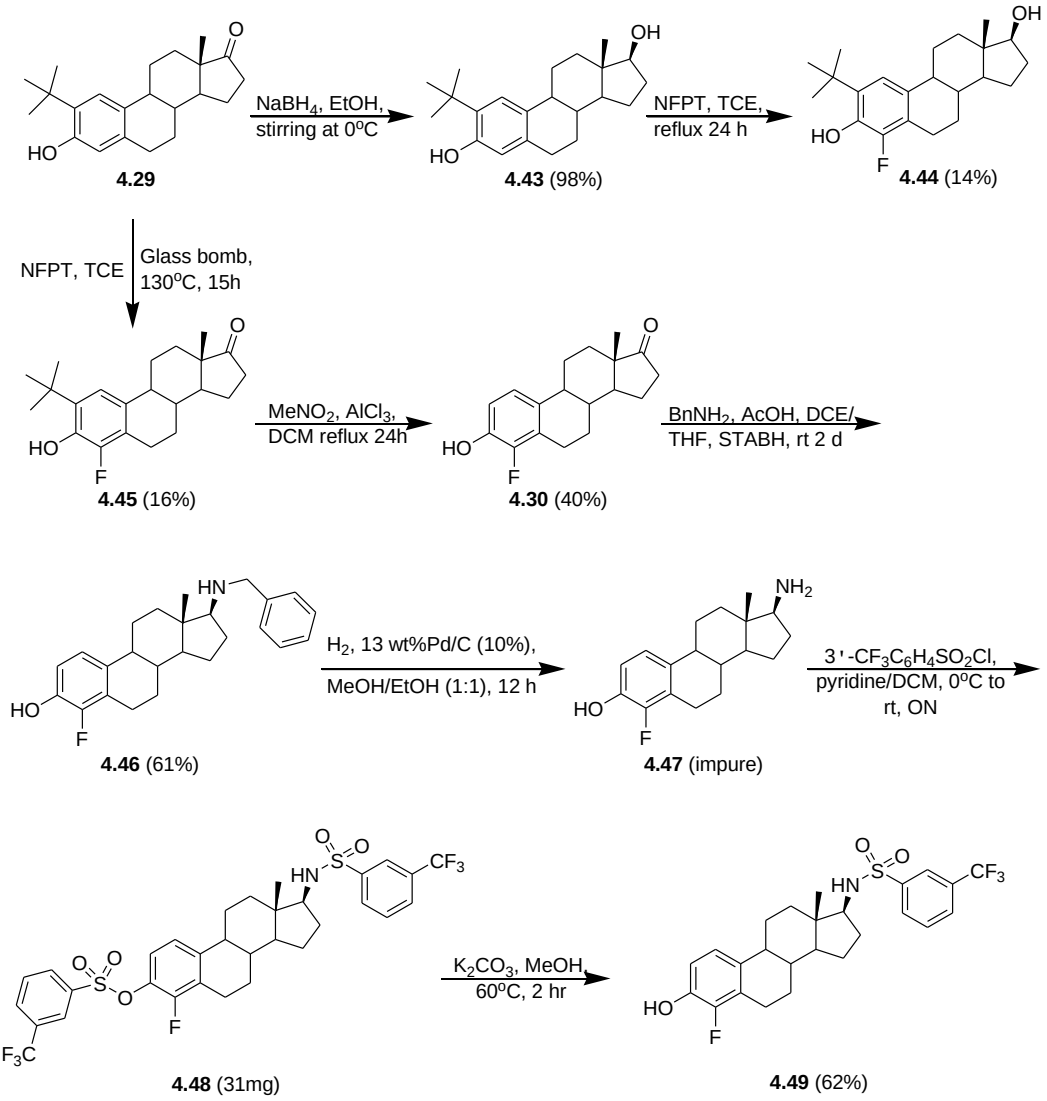
The approach used by Liu to prepare **4.30** (Scheme 4.7) was based upon a journal article published in 1990 which used NFPT to prepare 2- and 4-fluoroestrone (Scheme 4.13).[185] The procedure involves refluxing E1 and NFPT in 1,1,2-trichloroethane (TCE) for 24 h. This gives a mixture of the 2- and 4-fluoro products which were not separated until after acylation by flash chromatography followed by fractional recrystallization of the co-eluted isomers. The yield was 53% for 2-F-E1 acetate and 20% for 4-F-E1 acetate. We followed their approach exactly but were unable to separate the two isomers.



Scheme 4.13. Attempted synthesis of synthesis of 2- and 4-fluoroestrone using NFPT.

Finally, we resorted back to the procedure developed by Liu. The fluorination of **4.29** followed by removal of the *t*-butyl group gave **4.30** in a 6 % yield for the two steps (Scheme

4.14). We thought that perhaps the fluorination reaction might proceed better on 2-*t*-butylE2 (**4.41**) than on 2-t-butylE1 (**4.29**) since a possible side reaction was fluorination α to the carbonyl at C-16 of **4.29** but this yield was equally poor with **4.41** (Scheme 4.14). Reductive amination of **4.30** with $BnNH_2$ and STABH gave benzyl protected 17β-amine derivative, compound **4.46** in 61% yield. Hydrogenolysis of **4.46** gave the desired 17β-amino derivative, **4.47** together with another unknown compound which we could not remove. Reacting impure **4.47** with excess of 3'-CF_3-benzenesulfonyl chloride gave pure **4.48** (7% yield over 2 steps). Methanolysis of the sulfonate ester moiety in **4.48** with methanolic K_2CO_3 afforded our desired final 4-fluorosulfonamide derivative, compound **4.59**, in a 62% yield.



Scheme 4.14. Synthesis of **4.49**.

The IC_{50} of **4.49** was found to be dependent upon the concentration of enzyme indicating that this compound was a TBI (Fig. 4.7). From this data an apparent K_i of 2.5 nM was obtained. So introduction of a fluorine at the 4 position of inhibitor **3.33** resulted in an increase in potency by almost 10-fold. The F atom has a σ_m value similar to that of Br (0.34 and 0.39 respectively) and a σ_p value that is less than Br (0.06 vs. 0.23). So it looks like electron-withdrawing ability is

not important here. The ability of the F atom to act as an H-bond acceptor may be important for binding.

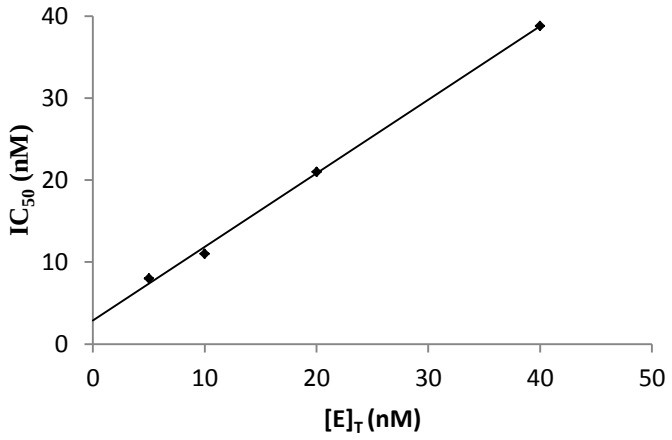


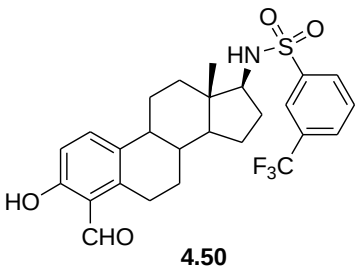
Figure 4.7. A plot of the IC_{50}'s for compound **4.49** as a function of $[E]_T$.

4.3.5 Inhibition studies with a 4-formyl 17β-arylsulfonamide derivative of E1

As we mentioned in § 1.4.1.3 and § 4.1, 4-formyl estrone (4-FE1) and the 4-formyl-17α-benzyl E2, compound **1.71**, were concentration and time-dependent inhibitors of STS.[73,74] The mechanism by which these compounds inhibit STS is still unknown. It is known that most aldehyde-based enzyme inhibitors function by forming Schiff bases (imine-formation) with residues such as lysine and arginine.[194] This has been demonstrated by reducing the imine adducts to stable amines with borohydride followed by proteolytic digest of the inactivated enzyme and then sequencing of the modified peptides. It is very possible that 4-FE1 and **1.71** inhibit STS by forming relatively stable Schiff bases with active site residues such as Lys134, Lys368 and Arg79.

If the 17β-arylsulfonamide inhibitors reported here bind in manner similar to the 17α-benzylE2 inhibitors (of type **1.49**) reported by Poirier then one would expect that introducing a formyl group to the 4-position of the 17β-arylsulfonamide inhibitors would yield time- and

concentration-dependent STS inhibitors. To determine if this is indeed the case compound **4.50** (Fig. 4.8) was prepared (by Prof. Scott D. Taylor) and then examined as an STS inhibitor (by Y. Mostafa). Compound **4.50** is derived from one of our most potent inhibitors (compound **3.33**, IC_{50} = 23 nM) and so we anticipate that if **4.50** is capable of forming a covalent adduct with STS then it should prove to be a very potent time- and concentration-dependent inhibitor of STS.



4.50

Fig. 4.8. Proposed inhibitors **4.50**.

We examined **4.50** for time- and concentration-dependent inhibition of STS by incubating it with STS at pH 7.0 in 100 mM tris buffer containing 0.01% Triton X-100, and aliquots were withdrawn at various time intervals and diluted 50-fold into a solution of a high concentration (4 M, 20 × K_m) of 4-MUS in the same buffer and STS activity was determined by following the production of 4-MU by fluorometry. The results were somewhat puzzling (Fig. 4.9). There was no inhibition up to 200 nM inhibitor. At 250 nM inhibitor we see 55% loss of activity after 10 minutes and as the concentration of inhibitor increased greater loss of activity after 10 minutes was observed but no real further loss of activity occurred after 10 minutes. It is possible that **4.50** is a very potent reversible inhibitor and MUS is able to compete for active site binding as the concentration of inhibitor decreases (i.e. 4 M MUS is able to completely displace the inhibitor when its concentration is 200 nM or less but not when the concentration of inhibitor is greater than 200 nM. In any case, the kinetics of inhibition seen with **4.50** are very different from those seen with **1.71** where loss of activity was linear over the entire time course of the

experiment which suggests that the formyl groups in compound **1.71** and compound **4.50** interact with the enzyme differently.

We also examined whether STS activity could be restored by dialysis after it had been incubated with 1 μM **4.50** for 1 h. After extensive dialysis (10^{12}-fold dilution over a 24-hour period) we were able to restore 70% of STS activity. This is in contrast to **1.71** where only 14% of the activity could be restored. Nevertheless, if **4.50** was a reversible inhibitor like its non-formylated analog **3.33**, then all activity should have been restored and so it appears that **4.50** may indeed be capable of forming some kind of covalent (though reversible) adduct with STS. More detailed studies will have to be performed to ascertain this such as looking at the time-dependence of inhibition over the first 10 minutes of the reaction.

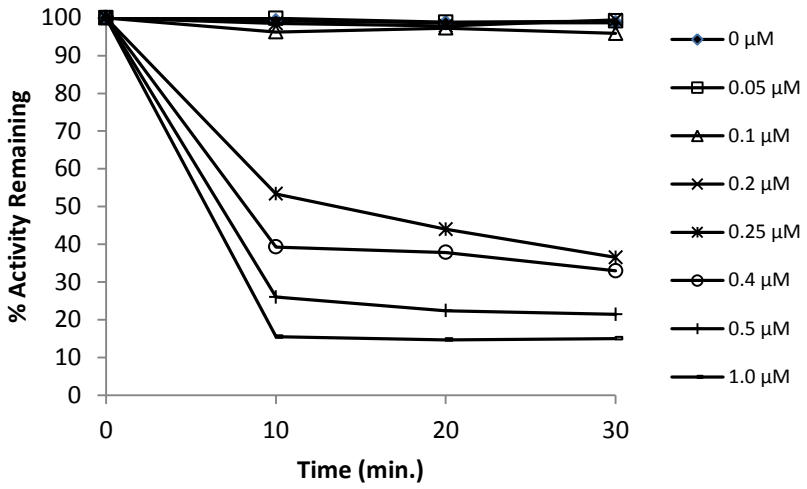


Fig. 4.9. Concentration-dependent inhibition of STS by compound **4.50**.

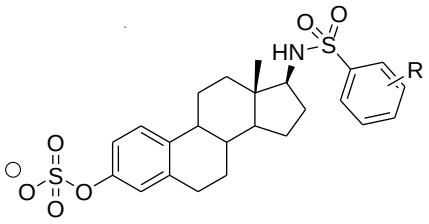
4.3.6 Inhibition studies with 3-*O* sulfated 17β-arylsulfonamide derivative of E1

Although P values have not been determined for the 17α-benzylE2 inhibitors developed by Poirier or for the inhibitors described here, it is likely that our sulfonamide-based inhibitors

are more hydrophilic than their 17α-benzylE2 counterparts. Nevertheless, our sulfonamide inhibitors are still quite hydrophobic. We reasoned that introduction of a polar group at the 3-OH would increase the hydrophilicity of these compounds. This could readily be accomplished by sulfation of the 3-OH. Such compounds could turn out to be inhibitors of STS or they could be substrates for STS and so act as prodrugs (in this case they would be acting as reversible suicide inhibitors). Although the cell permeability of our inhibitors may be compromised by sulfation, there is evidence that E1S is transported into cells and such transporters might be capable of transporting other sulfated steroids or steroid derivatives into cells.[31]

To determine if 3-OH sulfation affects the potency of our sulfonamide inhibitors, Jason Tao (a former undergraduate student in the Taylor group) prepared four sulfated sulfonamides (compounds **4.51**-**4.54**, Table 4.3) which were examined as STS inhibitors (by Y. Mostafa). Surprisingly, the introduction of the sulfate group did not significantly affect the potency of these compounds in that three of these sulfated derivatives exhibited potencies comparable to their non-sulfated counterparts. The exception was the biphenyl derivative, compound **4.54**, which was *more* potent than its parent compound. This was not due to an increase in solubility: all of the inhibitors described in this thesis were soluble under our assay conditions. The IC_{50} of **4.54** was dependent upon enzyme concentration and a K_i^{app} was obtained from a plot of IC_{50} vs. enzyme concentration (Fig. 4.10).

Table 4.3. Inhibition studies with sulfated sulfonamides **4.51**-**4.54**



Compound	R	IC_{50} (nM)[a]
4.51	3'-Br	18
4.52	3'-CF_3	22
4.53	4'-*t*-Butyl	19
4.54	4'-Phenyl	4[b]

[a] Errors in IC_{50}'s are within ±5%; [b] Apparent K_i.

We do not know if these sulfated inhibitors are substrates for STS and therefore acting as reversible suicide STS inhibitors. This is difficult to ascertain since the products of such a reaction are very potent STS inhibitors themselves. One would expect that if they are substrates then STS would only be capable of only one or two turnovers before being completely or significantly inhibited by the product. This makes it very difficult to determine if these compounds are substrates as the amount of product formed will be very small (app. equal to the concentration of enzyme used in the assay). Mass spectrometry might be the best method for detecting any products formed from the reaction of STS with these sulfated inhibitors though quantification of product would be challenging.

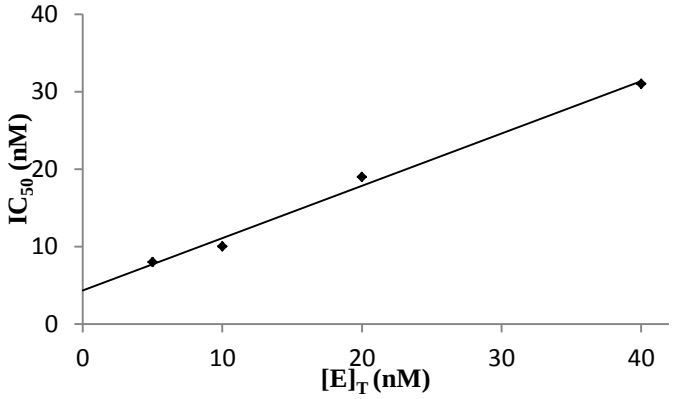


Figure 4.10. A plot of IC_{50}'s for compound **4.54** as a function of $[E]_T$.

4.3.7 Molecular Modelling Studies

Modelling studies were performed to gain some insight into possible modes of binding of some of the new inhibitors described above. We started by examining the effect of placing the NO_2 group at the 4-position (as in compound **4.10** and **4.4**, Fig. 4.11). Compounds **4.10** and **4.4** share common hydrophobic alkyl or mixed pi-alkyl interactions with Val486, His485, and Val 171. The sulfonamide group in compound **4.10** as with most of our sulfonamide inhibitors is involved in H-bond interactions with Phe178 and Gly181 (2.8 and 2.4 Å, respectively), as shown in Fig. 4.11. In contrast to inhibitor **3.33** (see Fig. 3.14), which lacks a 4-NO_2 group as is less potent than **4.10**, the CF_3-group in **4.10** lost its H-bond interactions with both Arg98 and Tyr493. However, the introduction of the nitro group at the 4-position resulted in the formation of an electrostatic charge attractive interaction with the side chain nitrogen of Lys 368. There is also a very strong H-bond interaction between the 3-OH and one of the hydroxyl groups of FGly75 (2.0Å). These additional interactions may account for the greater potency of **4.10** compared to **3.33**.

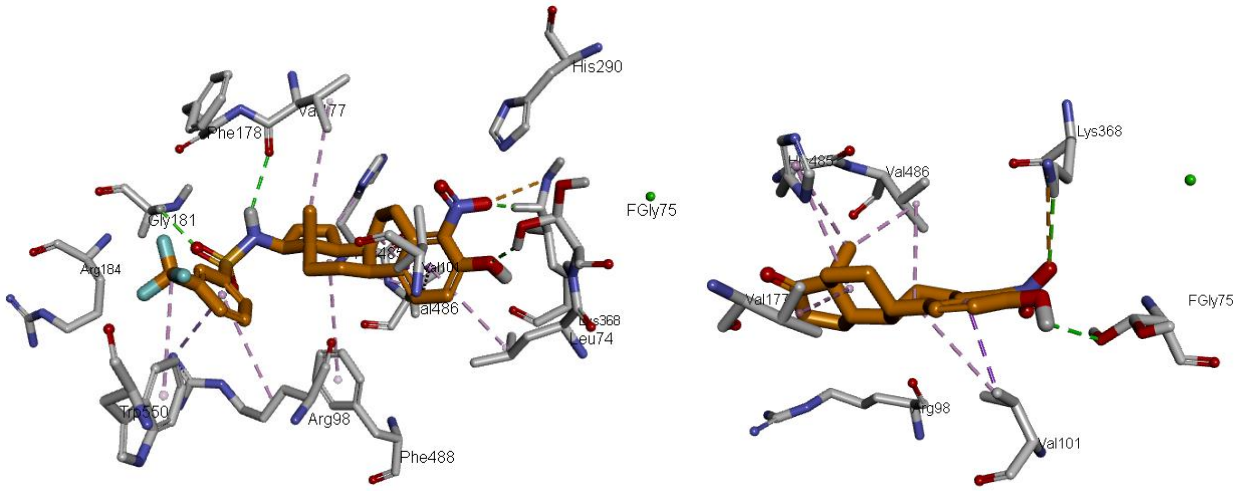


Fig. 4.11. The binding mode of **4.10** (left) and **4.4** (right) with STS (green dotted lines indicate H-bonding interactions, violet dotted lines indicate hydrophobic interactions, and orange dotted lines indicate electrostatic charge interactions; some of H-atoms were removed to increase clarity).

The introduction of a bromine at the 4-position in **3.33** resulted in a decrease in potency (compound **4.27**). Upon examining the docking poses for **4.27** and **4.17** we found that both of them share the same interactions with the FGly75 hydrate (H-bonding), and the hydrophobic interactions with Lys368, Val486, His485, Val177, Leu74, Val101 (as shown in Fig. 4.12), however; the 17β-sulfonamide scaffold of **4.29** added new bonding: two H-bonding interactions (one between NH of the SO_2NH and oxygen atom of Val177, and another one between one of the oxygen atoms of SO_2NH group and a H of Phe178) and three hydrophobic pi-alkyl or pi-cation interactions (as seen in Fig. 4.12). The bromine is involved in a hydrophobic pi-alkyl interaction (4.49 Å). Inhibitor **3.33** (Fig. 3.14) is involved in similar interactions between 3-OH and sulfonamide group NH and the amino acid residues FGgly75 and Phe178, respectively. However, the introduction of the bromine atom at 4-position caused a marked shift in the position of the whole molecule within active site compared to **3.33** as shown in Fig. 4.12. This shift resulted in losing all the H-bond interactions between the CF_3-group and Tyr493 and

Thr484 and the loss of an H-bond between one of oxygens of the sulfonamide group with the N-H of Gly181 that were found in **3.33**. We think that the loss of such interactions could be one of the factors responsible for the lower potency of **4.27** compared to **3.33**.

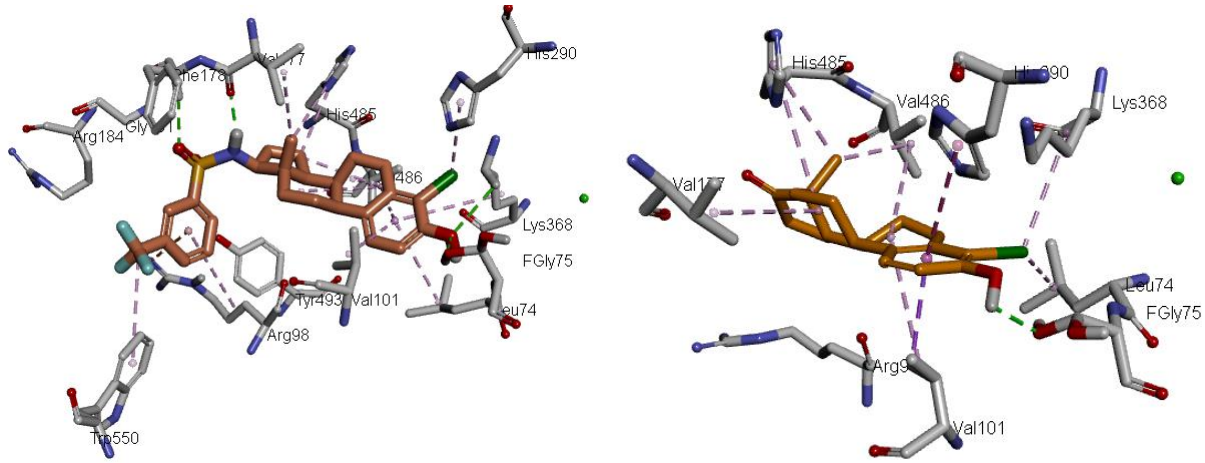


Fig. 4.12. The binding mode of compounds **4.27** (left) and **4.17** (right) with STS (Green dotted lines indicate H-bonding interactions; violet lines indicate hydrophobic interactions; some of H-atoms were removed to increase clarity).

Docking studies were also performed on the 4-fluoro sulfonamide derivative, **4.49** and 4-F-E1, **4.30.** As seen in Fig. 4.13; the 3-OH group was interacting with FGly75 via a strong H-bond interaction (distance 2.22 Å), one of the oxygen atoms of the $–SO_2NH-$ group was interacting with Phe178 residue, and finally, its 3´-CF_3-benzene scaffold was interacting via a network of hydrophobic interactions with both Phe178 and Phe553 as seen in Fig. 4.13. The fluorine at the 4-position does not seem to be involved in any significant interactions beyond a H-bond with a C-H of the Lys368 side chain. Such H-bonds are not considered to be very strong. On the other hand, **4.30** lacks the key interactions found with **4.49**, and hence its lower potency.

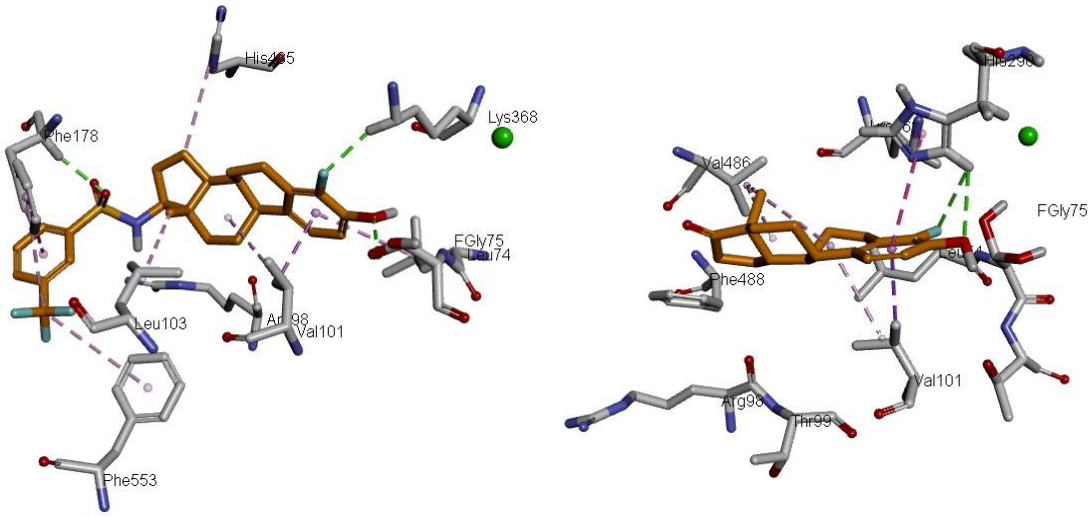


Fig. 4.13. The binding mode of compounds **4.49** (left) and **4.30** (right) with STS (green dotted lines indicate H-bond interactions; violet lines indicate hydrophobic interactions; some of hydrogen atoms were removed to increase clarity).

It is difficult to rationalize the increased potency of inhibitor **4.49** compared to inhibitor **3.33** based upon the docking studies. Upon comparing the docking poses for both **4.49** and **3.33** we found that even with the loss of the characteristic H-bond interactions of the CF_3-group seen in **3.33** (Fig.3.14) compound **4.49** was more potent than **3.33**. Upon visualizing both of these two compounds in the context of almost the entire enzyme we noticed that the introduction of the fluorine atom at 4-position changed the orientation such that the arylsulfonamide moiety of **4.49** was oriented almost in between the two alpha helices (Fig. 4.14). This may somehow result in an increase in potency.

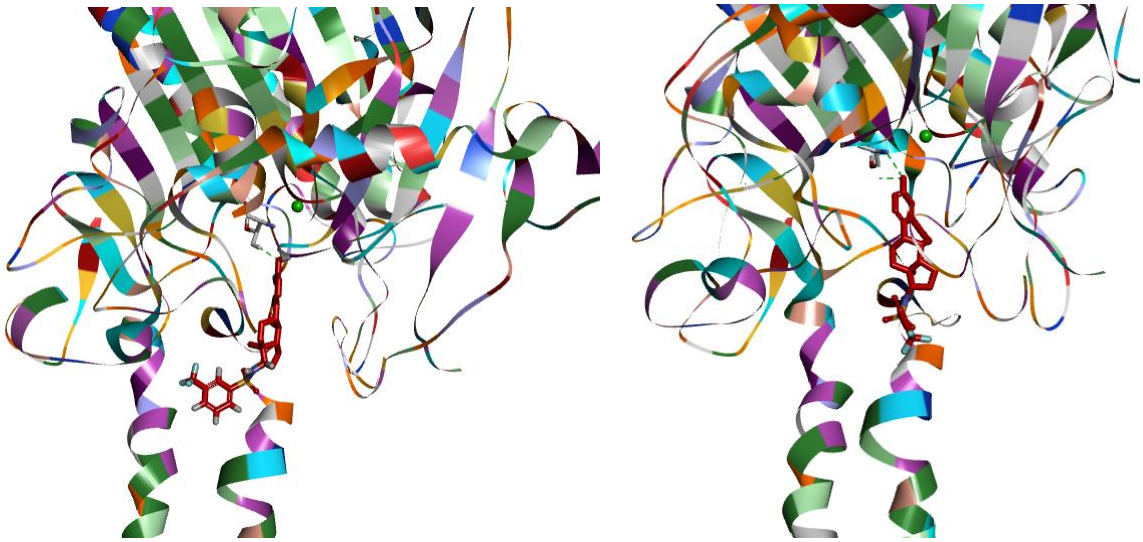


Fig. 4.14. The binding mode of compounds **4.49** (left) and **3.33** (right) with STS in its complete form.

4.3.8 Anti-proliferative effect of the 17β-arylsulfonamide E1 derivatives with the NCI-60 panel

To determine the anti-proliferative activity of our compounds, we submitted 29 of our sulfonamides to the Developmental Therapeutics Program (DTP) at the National Cancer Institute (NCI, USA) for in vitro primary screening in a diverse panel consisting of 60 human tumor cell lines (NCI-60 panel). These 29 compounds were initially assessed by subjecting the cell lines to a one-dose screen at 10 μM inhibitor. From these studies 17 of the 29 compounds exhibited sufficient antiproliferative activity in some or all of the cell lines to warrant further studies which consisted of a five-dose screen with each cell line. These compounds were: **3.22**, **3.23**, **3.27**, **3.28**, **3.29**, **3.30**, **3.33**, **3.34**, **3.37**, **3.38, 3.40, 3.45**, **3.46**, **3.48**, **4.09, 4.10,** and **4.53**. GI_{50}'s, TGI's and LC_{50}'s were obtained from these studies and are given in Appendix A and B . The GI_{50} is the concentration of inhibitor that causes 50% growth inhibition. The TGI is the concentration of

inhibitor that signifies a cytostatic effect. The LC_{50} is the concentration of drug that is lethal to 50% of the cells. Original reports of GI_{50}'s, TGIs and LC_{50}'s are in **Appendix A** and **B**.

There was surprisingly little variation in GI_{50}'s, TGI's and LC_{50}'s for all 17 compounds, for example, almost all of the compounds exhibited GI_{50}'s in the 1-10 μM range with all 60 cell lines which included two prostate cancer cell lines (PC-3 and DU-45) and six breast cancer cell lines (MCF7, MDA-MB-23, HS578T, BT-549, T-47D and MDA-MB-468). Interestingly, there was no correlation between the STS inhibitory potency of the compounds (IC_{50}'s) and GI_{50}'s, TGIs and LC_{50}'s reported in the NCI60 panel. Since all compounds moderately inhibited the growth of all of the cell lines it is possible that they are all affecting a common biological process/pathway that is necessary to the survival of all of the cell lines.

4.4 Conclusions and Future work

A series of 4-substituted 17β-arylsulfonamide estrogen derivatives that were designed to act as potent inhibitors of STS were synthesized. Inhibition studies revealed that the introduction of a NO_2 group or F atom to the 4-position increased the potency of these compounds. Some of these compounds are the most potent reversible STS inhibitors ever reported. Introduction of a Br at this position resulted in a decrease in potency. Modeling studies were performed on these compounds in an attempt to determine the origins of the effect of these C-4 substituents. In general, 3-*O*-sulfation of these compounds did not affect potency. It is not known if 3-O-sulfated derivatives were acting as inhibitors or reversible suicide inhibitors (by acting as substrates).

Future inhibitor design based upon the compounds presented in this thesis would benefit significantly from knowing where and how they interact with STS. The modeling studies provided some guidance on this matter; however, in the absence of an X-ray crystal structure of

one of our inhibitors bound to STS the modeling studies must be interpreted with caution as we have assumed active site binding even though our kinetic studies indicate that these inhibitors bind at more than one site (i.e. perhaps at the active site and in the hydrophobic tunnel between the two α-helices) or bind only to one site outside the active site (i.e. possibly just in the hydrophobic tunnel between the two α-helices). Photoaffinity labels, such as compounds **4.55** and **4.56** (Fig. 4.15), might be useful for determining where the aryl sulfonamide group is binding to STS. These compounds should be readily accessible. Inhibitor **3.47**, which contains a fluorescent dansyl group, may also be useful in shedding some light on this matter. The fluorescence of the dansyl group is known to be very sensitive to the polarity of its environment in that its fluorescence increases and undergoes a blue shift when going from a polar environment to a hydrophobic environment. Compounds containing this group and other similar environment-sensitive fluorophores have been used to examine protein-protein interactions,[195,196] protein conformation changes,[196] and for structural characterization of ligand-binding domains.[197-200] If our inhibitors are indeed binding in the hydrophobic tunnel then one would expect to see a large increase in fluorescence and a blue shift upon binding to STS. However, it is also possible that such a change in fluorescence might also result from active site binding and so the results of such studies would have to be interpreted with caution.

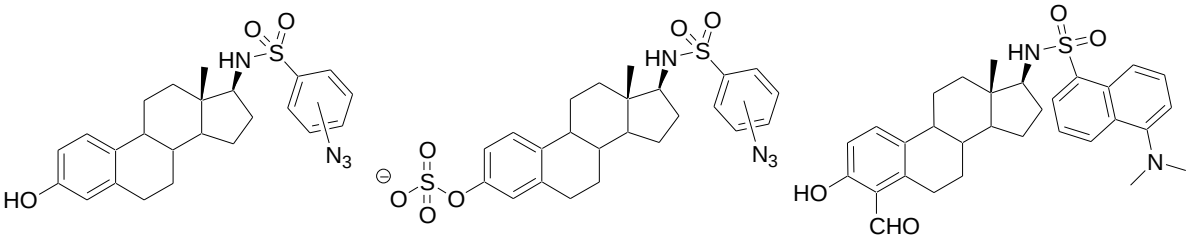


Fig. 4.15. Structures of potential photoaffinity labels **4.55** and **4.56**.

To determine if 4-formyl derivatives such as **4.50** are capable of forming Schiff bases with active site residues compound **4.57** (Fig. 4.15) will be examined as a time- and concentration-dependent inhibitor (Fig. 4.16). Should this compounds exhibit time- and concentration-dependent inhibition then the imine adduct(s) will be reduced to a stable amine with sodium borohydride followed by proteolytic digestion of the inactivated enzyme and then sequencing of the modified peptides. The advantage of using **4.57** instead of **4.50** for these studies is that any labelled peptide fragments will be fluorescent and therefore easy to detect and isolate using an HPLC equipped with a fluorescence detector.

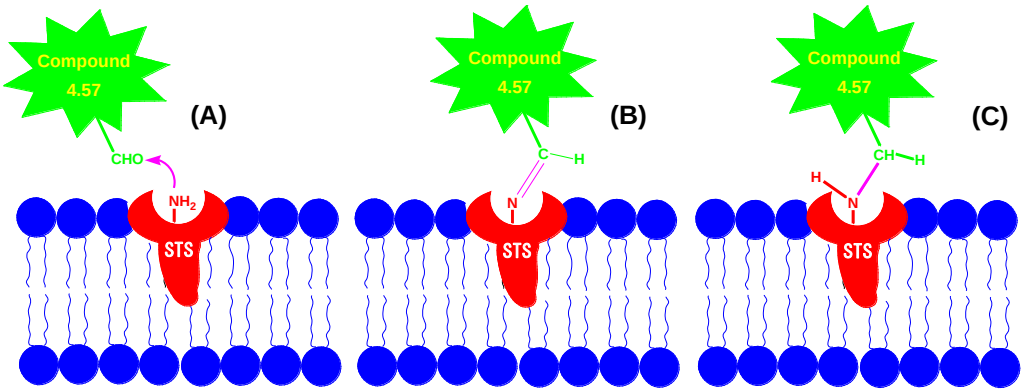


Fig. 4.16. Schematic presentation of the proposed fluorescence-tagging of STS by **4.57**; (A) Nucleophilic attack of basic amino acids (lysine, or arginine) on CHO-group of **4.57**, (B) Imine-formation, (C) Borohydride reduction of unstable imine to corresponding stable amine.

Selected inhibitors developed in *Chapters* 3 and 4 were sent to the NCI (USA) for in vitro screening with a panel of 60 human tumor cell lines (NCI-60 panel). Almost all of the compounds exhibited GI_{50}'s in the 1-10 μM range with all 60 cell lines and so were only moderately potent in terms of their ability to inhibit the growth. None of the compounds stood out in terms of their ability to inhibit the growth of any breast cancer, prostate cancer or any other cancer cell line studied. Many of the sulfamate-based inhibitors discussed in chapter 1 are highly active in cell assays. It would be interesting to see if the introduction of a sulfamate

group or a non-hydrolyzable sulfate mimic at the 3-OH group in the inhibitors described here would result in more cell active compounds. Our preliminary work on such compounds is described in *Chapter* 5.

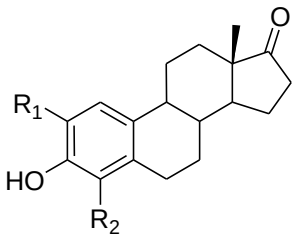
4.5 Experimental

4.5.1 General

All starting materials and reagents were obtained from Aldrich Chemical Company. THF was distilled from sodium-benzophenone, Pyridine was distilled from KOH pellets, 1,2-DCE and 1,1,2-TCE were dried by standing over activated type 4A molecular sieves, CH_2Cl_2 was distilled from calcium hydride under nitrogen. Both benzylamine and allylamine were dried by distillation from KOH pellets and stored in dark over type 4A molecular sieves. Tetrakis (triphenylphosphine)palladium (0), [$Pd(Ph_3)_4$] was prepared according to literature procedure from triphenylphosphine (PPh_3) and palladium chloride ($PdCl_2$). Dioxane was distilled from Na^o and stored over type 4A molecular sieves. Both ethylene glycol and *t*-Butyl alcohol were dried by distillation and standing over type 3A molecular sieves. Silica gel chromatography was performed using silica gel (60Å, 230-400 mesh) obtained from Silicycle (Laval, Quebec, Canada). ^1H, ^{13}C, and ^{19}F NMR spectra were recorded on a Bruker Avance 300 spectrometer. For NMR spectra obtained using $CDCl_3$ as the solvent, chemical shifts (δ) for ^1H NMR spectra are reported relative to internal Me_4Si (δ 0.0 ppm), chemical shifts for ^{13}C spectra are relative to the residual solvent peak (δ 77.0 ppm, central peak), and chemical shifts for ^{19}F NMR are relative to a $CFCl_3$ (δ 0.0 ppm) external standard. Both Electron Impact-Low Resolution (EI-LRMS) and Electro-Spray Ionization-Low Resolution (ESI) Mass Spectra were obtained on a JEOL HX110 double focusing mass spectrometer. Electro-Spray Ionization High Resolution

(ESI-HRMS) Mass Spectra were obtained with a Waters/Micromass QTOF Ultima Global mass spectrometer. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Cellular studies were done using human tumor cell lines of the cancer screening panel (NCI60) at the U.S. NCI research centre.

4.5.2 Syntheses

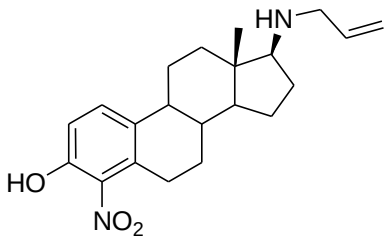


4.3; R_1= H, R_2= NO_2
4.4; R_1= NO_2, R_2= H

Nitroestrones, 4.3 and 4.4.[179] To a stirred solution of **E1** (1.73 g, 6.4 mmol) in glacial acetic acid (90 mL) at 70-75^{o}C, was added a mixture of concentrated nitric acid (0.4 mL) and glacial acetic acid (10 mL), stirring was continued at room temperature for 18 h. The precipitate was filtered and washed with hot glacial acetic acid to afford (200 mg, 20%) of 4-NO_2-E1, **4.3** as a pale yellow powder, while filtrate was concentrated, dissolved in benzene (30 mL), and stirred with $NaHCO_3$ (2%, 20 mL) for 6 h, then aqueous layer was acidified with conc. HCl (10 mL), and the filtrate was concentrated and recrystallized from ethanol (95%, 10 mL) to afford 2-NO_2-E1, **4.4** as bright yellow crystals (250 mg, 23%). 4-NO_2-E1, **4.3**, Mp: 277-280^{o}C;[179] ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.40 (brs, 1H, ArOH), 7.44 (d, *J* = 8.8 Hz, 1H, H-1), 6.94 (d, *J* = 8.8 Hz, 1H, H-2), 3.20-3.14 (m, 1H), 3.02-2.94 (m, 1H), 2.55-2.46 (m, 1H), 2.35-1.94 (m, 6H), 1.67-1.23 (m, 8H), 0.90 (s, 3H, H-18). 2-NO_2-E1, **4.4,** Mp: 181-183^{o}C;[179] ^{1}H NMR ($CDCl_3$, 300

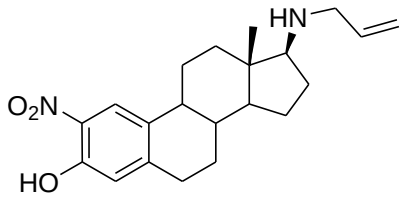
MHz) δ 10.32 (brs, 1H, ArOH), 7.90 (s, 1H, H-1), 6.80 (s, 1H, H-4), 2.95-2.92 (m, 2H, H-6), 2.54-2.39 (m, 2H), 2.17-1.96 (m, 5H), 1.61-1.41 (m, 7H), 0.89 (s, 3H, H-18).

General method for reductive amination of nitroestrones 4.3 and 4.4. To a stirred solution of nitroestrones, **4.3** or **4.4** (500 mg, 1.60 mmol) in THF (10 mL) was added allylamine (360 mg, 6.30 mmol), glacial acetic acid (375 μL, 6.30 mmol), and STABH (850 mg, 3.92 mmol). The mixture was stirred for 2 d, after that the reaction was quenched with aq. saturated $NaHCO_3$ and stirring was continued for additional 10 min. The reaction mixture was extracted with DCM, and the organic layer was washed with aq. saturated $NaHCO_3$, water, brine, dried with Na_2SO_4, and finally concentrated under vacuum.



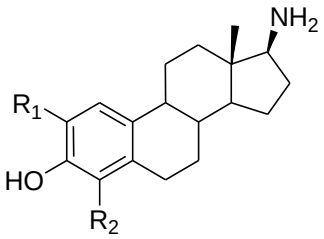
4.5

17β-Allylamino-4-nitro-estratrien-1,3,5(10)-3-ol (4.5). Purification was done by flash chromatography (methanol/chloroform, 1:9) to afford **4.5** as a yellow solid (350 mg, 63%); Mp 150-151^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.43 (d, *J* = 8.9 Hz, 1H, H-1), 6.94 (d, *J* = 8.2 Hz, 1H, H-2), 5.90 (m, 1H, $NHCH_2C\underline{H}CH_2$), 5.15 (m, 2H, $NHCH_2CHC\underline{H_2}$), 3.33 (m, 1H), 3.15 (m, 1H), 2.95 (m, 1H), 2.77-2.70 (m, 1H), 2.22-1.90 (m, 6H), 1.70-1.20 (m, 6H), 0.76 (s, 3H, H-18); LRMS (ESI^{+}) *m*/*z* (%) 357 (M+H, 100); HRMS (ESI^{+}) calcd for $C_{21}H_{29}N_2O_3$ $(M+H)^{+}$ 357.2178; found 357.2186.



4.6

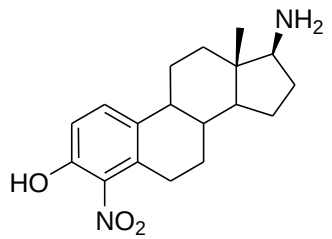
17β-Allylamino-2-nitro-estratrien-1,3,5(10)-3-ol (4.6). Purification was done by flash chromatography (ethyl acetate/hexane, 3:7) to afford **4.6** as a yellow solid (415 mg, 73%); Mp 163-164^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.95 (s, 1H, H-1), 6.81 (s, 1H, H-4), 5.90 (dddd, *J* = 6.0, 10.2, 11.9, 16.3 Hz, 1H, $NHCH_2C\underline{H}CH_2$), 5.51 (brs, 1H, ArOH), 5.09 (ddd, *J* = 1.7, 17.2, 18.8 Hz, 2H, $NHCH_2CHC\underline{H}_2$), 3.27 (dd, *J* = 1.3 and 6.0 Hz, 2H, $NHC\underline{H}_2CHCH_2$), 2.92-2.82 (m, 2H, H-6), 2.67-2.61 (m, 1H), 2.30-2.26 (m, 1H), 2.15-1.98 (m, 3H), 1.89 (m, 1H), 1.71-1.20 (m, 8H), 0.73 (s, 3H, H-18). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 152.8 (C-3), 149.2, 137.4, 133.9 (C-5), 131.7 (C-6), 121.4, 118.8, 115.5, 68.3 (C-17), 52.2 ($NH\underline{C}H_2$), , 51.1 (C-14), 43.5 (CH), 42.9 (C-13), 38.1 (CH), 37.6 (CH_2), 29.7 (2 CH_2), 26.7 (CH_2), 26.2 (CH_2), 23.4 (CH_2), 11.8 (CH_3, C-18); LRMS (ESI^+) *m*/*z* (%) 357 (M+H, 100); HRMS (ESI^+) calcd for $C_{21}H_{29}N_2O_3$ $(M+H)^+$ 357.2178; found 357.2176.



4.7; R_1= H, R_2= NO_2
4.8; R_1= NO_2, R_2= H

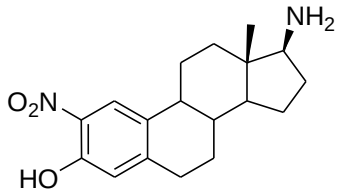
General method for deallylation of 17β-Allyl-amino nitroestrones 4.5 and 4.6. To a stirred solution of compounds **4.5** or **4.6 (**350 mg, 0.98 mmol) in dry DCM (20 mL) was added to a solution of *N*,*N*-dimethylbarbituric acid (NDMBA, 254 mg, 2.97 mmol) and $Pd(Ph_3)_4$ (23.5

mg, 0.02 mmol) in dry DCM (2 mL). The mixture was stirred for 3 h at 35°C under an Argon atmosphere, and then diluted with DCM (20 mL). The mixture was washed with sat. $NaHCO_3$, water, brine, and finally organic layer was dried with Na_2SO_4, and concentrated under vacuum.



4.7

17β-Amino-4-nitro-estratrien-1,3,5(10)-3-ol (4.7). Purification was done by flash chromatography (methanol/chloroform/NH_4OH, 4.5:95:0.5) to afford **4.7** as a yellow solid (150 mg, 47%). Mp 167-169°C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.44 (d, J = 8.8 Hz, 1H, H-1), 6.92 (d, J = 9.0 Hz, 1H, H-2), 3.20 (m, 2H, H-6), 2.96-2.78 (m, 3H, NH_2 and H-17), 2.23-2.15 (m, 4H), 1.97-1.92 (m, 2H), 1.70 (m, 1H), 1.46-1.17 (m, 8H), 0.72 (s, 3H, H-18); LRMS (ESI^+) *m*/*z* (%) 317 (M+H, 10); HRMS (ESI^+) calcd for $C_{18}H_{25}N_2O_3$ $(M+H)^+$ 317.18597; found 317.18601.

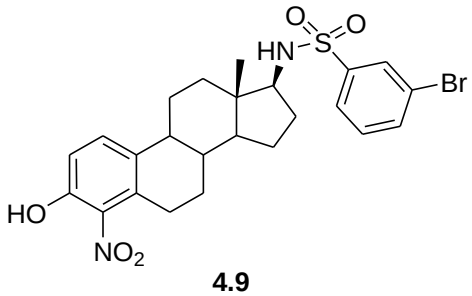


4.8

17β-Amino-2-nitro-estratrien-1,3,5(10)-3-ol (4.8). Purification was done by flash chromatography (methanol/chloroform/NH_4OH, 4.5:95:0.5) to afford **4.8** as an orange solid (220 mg, 71%). Mp 133-135°C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.95 (s, 1H, H-1), 6.81 (s, 1H, H-4), 3.77 (brs, 2H, NH_2), 2.92-2.70 (m, 3H, H-6 and H-17), 2.33-2.28 (m, 1H), 2.14-2.00 (m, 2H), 1.87 (m, 2H), 1.70-1.64 (m, 1H), 1.53-1.48 (m, 1H), 1.38-1.15 (m, 6H), 0.65 (s, 3H, H-18). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 152.8 (C-3), 149.2, 133.8 (C-5), 131.7 (C-6), 121.4, 118.8, 62.7 (C-

17), 51.9 (C-14), 43.4 (CH), 42.8 (C-13), 38.4 (CH), 36.3 (CH_2), 30.9 (CH_2), 29.8 (CH_2), 26.7 (CH_2), 26.1 (CH_2), 23.3 (CH_2), 11.1 (CH_3, C-18); LRMS (ESI^+) *m*/*z* (%) 317 (M+H, 100); HRMS (ESI^+) calcd for $C_{18}H_{25}N_2O_3$ $(M+H)^+$ 317.1865; found 317.1853.

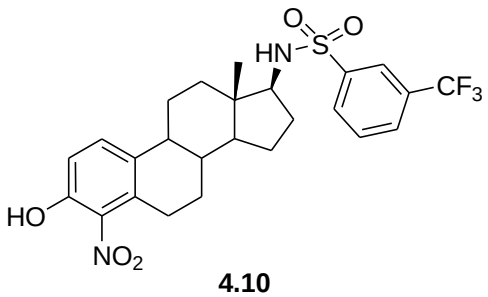
General procedure for synthesis of sulfonamides of 2-nitro or 4-nitro-17β-amino-1,3,5(10)-estratrien-3-ol. To a stirred solution of **4.7** and/or **4.8** (50 mg, 0.16mmol) in dry pyridine (1 mL) at 0 ^oC was added a solution of the appropriate sulfonyl chloride (0.17 mmol) in dichloromethane (1 mL) drop-wise via a syringe pump over 10 min. The reaction was stirred for 16 h at room temperature, and pyridine was azeotropically removed with toluene, and the residue was dissolved in chloroform, washed with water and brine, then dried with anhydrous Na_2SO_4, and finally concentrated under vacuum.



4.9

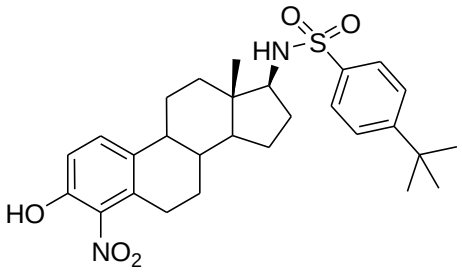
4-Nitro-17β-(3'-bromobenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (4.9). Purification was done by flash chromatography (ethyl acetate/hexane, 3:7) to afford **4.9** as a yellow solid (41 mg, 48%). Mp 230-231^oC; ^1H NMR ($CDCl_3$, 300 MHz) δ 9.40 (s, 1H, ArOH), 8.02 (s, 1H, ArH), 7.80 (d, *J* = 6.9 Hz, 1H, ArH), 7.68 (d, *J* = 7.4 Hz, 1H, ArH), 7.44-7.35 (m, 2H, ArH), 6.92 (d, *J* = 8.7 Hz, 1H, ArH), 4.55 (d, *J* = 9.2 Hz, 1H, NH), 3.21-3.09 (m, 2H, H-17 and H-6), 2.94-2.88 (m, 1H), 2.23-2.15 (m, 2H), 1.89-1.65 (m, 4H), 1.44-1.19 (m, 8H), 0.72 (s, 3H, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 152.1 (C-3), 143.1, 136.1, 135.6 (CH_{Ar}), 134.0 (C-5), 133.6 (C-6), 132.8 (CH_{Ar}), 130.6 (CH_{Ar}), 130.0 (CH_{Ar}), 125.5 (CH_{Ar}), 122.9, 121.5, 116.5 (CH_{Ar}), 63.3 (C-17), 50.8 (C-14), 44.2, 42.9 (C-13), 37.6 (CH), 36.3 (CH_2), 29.5 (CH_2), 27.8

(CH_2), 26.5 (2 CH_2), 22.9 (CH_2), 11.8 (CH_3, C-18); LRMS (ESI^+) *m/z* (%) 537 (M+H+2, 90), 535 (M+H, 80), 300 (100); HRMS (ESI^+) calcd for $C_{24}H_{28}N_2O_5SBr$ $(M+H)^+$ 535.0902; found 535.0909.



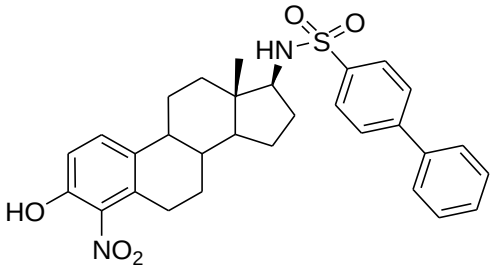
4.10

4-Nitro-17β-(3'-trifluoromethylbenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (4.10). Purification was done by flash chromatography (ethyl acetate/hexane, 1:4) to afford **4.10** as a yellow solid (44 mg, 53%). Mp 263-264^oC; ^1H NMR ($CDCl_3$, 300 MHz) δ 9.42 (s, 1H, ArOH), 8.15 (s, 1H, ArH), 8.07 (d, *J* = 7.7 Hz, 1H, ArH), 7.83 (d, *J* = 7.8 Hz, 1H, ArH), 7.66 (dd, *J* = 7.9 and 8.0 Hz, 1H, ArH), 7.43 (d, *J* = 8.9 Hz, 1H, ArH), 6.94 (d, *J* = 8.9 Hz, 1H, ArH), 4.47 (d, *J* = 9.5 Hz, 1H, NH), 3.25-3.08 (m, 2H), 2.96-2.89 (m, 1H), 2.18 (m, 2H), 1.94-1.87 (m, 4H), 1.36-1.18 (m, 8H), 0.73 (s, 3H, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 152.2 (C-3), 142.5, 136.1 (C-5), 133.5 (q., *J* = 38.2 Hz, 1 C, $\underline{C}$-CF_3), 132.9 (CH_{Ar}), 132.0 (C-6), 130.2 (CH_{Ar}), 129.1 (apparent d, *J* = 3.6 Hz, 1 C, CH_{Ar}), 124.1 (apparent d, *J* = 3.7 Hz, 1 C, CH_{Ar}), 116.6 (CH_{Ar}), 63.4 (C-17), 51.0 (C-14), 44.2 (CH), 42.8 (C-13), 37.5 (CH), 36.3 (CH_2), 29.5 (CH_2), 27.8 (CH_2), 26.4 (2 CH_2), 22.9 (CH_2), 11.8 (CH_3, C-18); ^{19}F NMR ($CDCl_3$, 282 MHz), δ -63.1; LRMS (ESI^+) *m/z* (%) 525 (M+H, 100); HRMS (ESI^+) calcd for $C_{25}H_{28}N_2O_5F_3S$ $(M+H)^+$ 525.1671; found 525.1667.



4.11

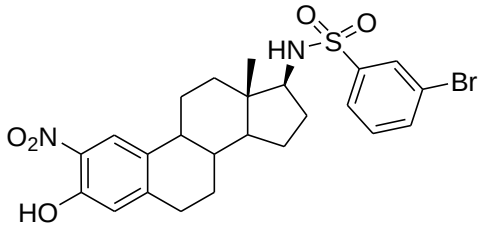
4-Nitro-17β-(4'-*t*-butylbenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (4.11). Purification was done by flash chromatography (ethyl acetate/hexane, 2.5:7.5) to afford **4.11** as a yellow solid (32 mg, 40%). Mp 228-229^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.40 (s, 1H, ArOH), 7.77 (d, *J* = 8.4 Hz, 1H, ArH), 7.48 (d, *J* = 8.4 Hz, 1H, ArH), 7.41 (d, *J* = 8.4 Hz, 1H, ArH), 6.92 (d, *J* = 8.6 Hz, 1H, ArH), 4.35 (d, *J* = 9.3 Hz, 1H, NH), 3.22-3.06 (m, 2H, H-17 and H-6), 2.95-2.87 (m, 1H, H-6), 2.20-2.11 (m, 2H), 1.90-1.77 (m, 3H), 1.67-1.59 (m, 1H), 1.43-1.12 (m, 17 H), 0.71 (s, 3H, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 156.4 (C-$C(CH_3)_3$, 152.2 (C-3), 138.0, 136.1, 134.1 (C-5), 133.7 (C-6), 132.9 (CH_{Ar}), 126.9 (2 CH_{Ar}), 125.9 (2 CH_{Ar}), 116.5 (CH_{Ar}), 63.1 (C-17), 50.9 (C-14), 44.3 (CH), 42.8 (C-13), 37.6 (CH), 36.2 (CH_2), 35.1 (CH_2), 31.1 (2 CH), 29.5 (CH_2), 27.8 (CH_2), 26.5 (2 CH_2), 22.9 (CH_2), 11.8 (CH_3, C-18); LRMS (ESI^+) *m*/*z* (%) 513 (M+H, 100); HRMS (ESI^+) calcd for $C_{28}H_{37}N_2O_5S$ $(M+H)^+$ 513.2423; found 513.2432.



4.12

4-Nitro-17β-4'-biphenylsulfonamide-1,3,5(10)-estratrien-3-ol (4.12). Purification was done by flash chromatography (methanol/chloroform, 1:8) to afford **4.12** as a yellow solid (23 mg, 27%). Mp 270-271^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.40 (s, 1H, ArOH), 7.93 (d, *J* = 8.2

Hz, 2H, ArH), 7.70 (d, *J* = 8.3 Hz, 2H, ArH), 7.61 (d, *J* = 6.8 Hz, 2H, ArH), 7.49-7.38 (m, 4H), 6.80 (d, *J* = 8.6 Hz, 1H, ArH), 4.51 (d, *J* = 8.9 Hz, 1H, NH), 3.47-3.17 (m, *2*H, H-17 and H-6), 3.12-3.06 (m, 1H), 2.22-2.15 (m, 3H), 1.91-1.80 (m, 3H), 1.66-1.60 (m, 2H), 1.44-1.15 (m, 8H), 0.73 (s, 3H, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 152.1 (C-3), 145.4, 139.7, 139.2, 136.1, 134.0 (C-5), 133.7 (C-6), 132.7 (CH_{Ar}), 129.0 (2 CH_{Ar}), 128.5 (CH_{Ar}), 127.6 (4 CH_{Ar}), 127.3 (2 CH_{Ar}), 116.5 (CH_{Ar}), 63.3 (C-17), 51.1 (C-14), 44.3 (CH), 42.8 (C-13), 37.6 (CH), 36.3 (CH_2), 29.5 (CH_2), 27.8 (CH_2), 26.5 (2 CH_2), 23.0 (CH_2), 11.8 (CH_3, C-18); LRMS (ESI^+) *m*/*z* (%) 533 (M+H, 100); HRMS (ESI^+) calcd for $C_{30}H_{33}N_2O_5S$ $(M+H)^+$ 533.2110; found 533.2098.

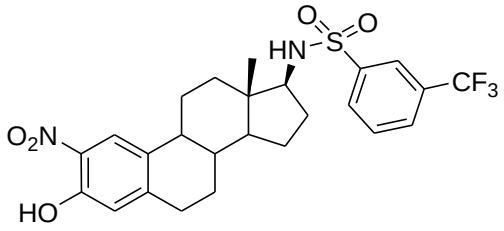


4.13

2-Nitro-17β-(3'-bromobenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (4.13).

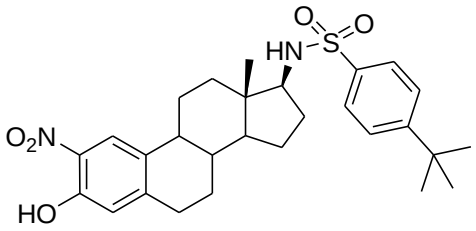
Purification was done by flash chromatography (ethyl acetate/hexane, 3:7) to afford **4.13** as a yellow solid (56 mg, 66%). Mp 242-243^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 10.40 (s, 1H, ArOH), 8.03 (s, 1H, ArH), 7.91 (s, 1H, ArH), 7.80 (d, *J* = 7.9 Hz, 1H, ArH), 7.68 (d, *J* = 7.9 Hz, 1H, ArH), 7.38 (dd, *J* = 7.9 and 7.9 Hz, 1H, ArH), 6.80 (s, 1H, ArH), 4.68 (d, *J* = 9.3 Hz, 1H, NH), 3.19 (q, *J* = 8.7 Hz, 1H, H-17), 2.86-2.80 (m, 2H), 2.27-2.23 (m, 1H), 2.11 (m, 1H), 1.91-1.65 (m, 4H), 1.46-1.14 (m, 7H), 0.70 (s, 3H, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 152.8 (C-3), 148.9, 143.0, 135.6 (CH_{Ar}), 133.3 (C-5), 131.7 (C-6), 130.6 (CH_{Ar}), 130.0 (CH_{Ar}), 125.5 (CH_{Ar}), 122.9, 121.5, 118.8, 63.3 (C-17), 51.0 (C-14), 43.2 (CH), 42.8 (C-13), 38.2 (CH), 35.9 (CH_2), 29.5 (2 CH_2), 26.5 (CH_2), 25.8 (CH_2), 23.1 (CH_2), 11.8 (CH_3, C-18); LRMS (ESI^+) *m*/*z* (%) 537

(M+H+2, 95), 535 (M+H, 90), 300 (100); HRMS (ESI^+) calcd for $C_{24}H_{28}N_2O_5SBr$ $(M+H)^+$ 535.0902; found 535.0902.



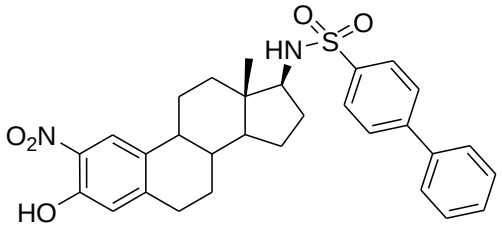
4.14

2-Nitro-17β-(3'-trifluoromethylbenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (4.14). Purification was done by flash chromatography (methanol/chloroform, 0.5:9.5) to afford **4.14** as a yellow solid (55 mg, 67%). Mp 278-279°C; ^1H NMR ($CDCl_3$, 300 MHz) δ 10.40 (s, 1H, ArOH), 8.15 (s, 1H, ArH), 8.07 (d, *J* = 8.07 Hz, 1H, ArH), 7.91 (s, 1H, ArH), 7.83 (d, *J* = 7.8 Hz, 1H, ArH), 7.66 (dd, *J* = 7.4 and 7.9 Hz, 1H, ArH), 6.80 (s, 1H, ArH), 4.59 (d, *J* = 9.6 Hz, 1H, NH), 3.22 (q, *J* = 8.5 Hz, 1H, H-17), 2.89 (m, 2H, H-6), 2.26-2.22 (m, 1H), 2.11 (m, 1H), 1.91-1.85 (m, 2H), 1.73-1.68 (m, 2H), 1.49-1.10 (m, 7H), 0.70 (s, 3H, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 152.8 (C-3), 148.9, 142.5, 133.3 (C-5), 131.8 (q., *J* = 33.3 Hz, 1 C, C-CF_3), 131.7 (C-6), 130.2 (d, *J* = 1.0 Hz, 1 C, CH_{Ar}), 129.8 (d, *J* = 2.5 Hz, 1 C, CH_{Ar}), 129.1 (q, *J* = 3.6 Hz, 1 C, CH_{Ar}), 124.1 (q, *J* = 3.8 Hz, 1 C, CH_{Ar}), 123.2, (q, *J* = 271.3 Hz, 1 C, C-CF_3), 121.5 (CH_{Ar}), 118.8 (CH_{Ar}), 63.4 (C-17), 51.0 (C-14), 43.2 (CH), 42.8 (C-13), 38.2 (CH), 35.9 (CH_2), 29.5 (2 CH_2), 26.5 (CH_2), 25.8 (CH_2), 23.0 (CH_2), 11.8 (CH_3, C-18); ^{19}F NMR ($CDCl_3$, 282 MHz), δ -63.1; LRMS (ESI^+) *m*/*z* (%) 525 (M+H, 89), 300 (100%); HRMS (ESI^+) calcd for $C_{25}H_{28}N_2O_5F_3S$ $(M+H)^+$ 525.1671; found 525.1656.



4.15

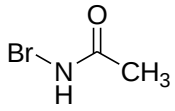
2-Nitro-17β-(4'-*t*-butylbenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (4.15). Purification was done by flash chromatography (ethyl acetate/hexane, 1:4) to afford **4.15** as a yellow solid (21 mg, 26%). Mp 212-213°C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 10.37 (app. d, 1H, ArOH), 7.91 (s, 1H, ArH), 7.80-7.77 (2 overlapping d, *J* = 8.4 Hz, 2H, ArH), 7.50-7.47 (2 overlapping d, *J* = 8.4 Hz, 2H, ArH), 6.80 (s, 1H, ArH), 4.57 (d, J = 9.2 Hz, 1H, NH), 3.15 (apparent t., J = 8.6 Hz, 1H, H-17), 2.85 (m, 2H, H-6), 2.24-2.20 (m, 1H), 2.09-2.01 (m, 1H), 1.84-1.75 (m, 3H), 1.65-1.62 (m, 1H), 1.49-1.14 (m, 17 H), 0.69 (s, 3H, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 156.4 (C-$C(CH_3)_3$, 152.8 (C-3), 148.9, 137.9, 133.5 (C-5), 131.7 (C-6), 126.9 (2 CH_{Ar}), 125.9 (2 CH_{Ar}), 121.5, 118.8, 63.1 (C-17), 51.1 (C-14), 43.2 (CH), 42.8 (C-13), 38.2 (CH), 35.9 (CH_2), 35.1 (CH_2), 31.1 (CH), 29.5 (2 CH_2), 26.5 (CH_2), 25.8 (CH_2), 23.1 (CH_2), 11.7 (CH_3, C-18); LRMS (ESI^+) *m*/*z* (%) 513 (M+H, 100); HRMS (ESI^+) calcd for $C_{28}H_{37}N_2O_5S$ $(M+H)^+$ 513.2423; found 513.2424.



4.16

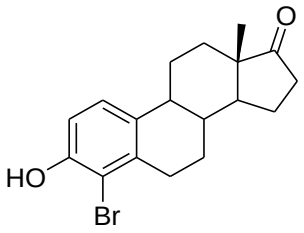
2-Nitro-17β-4'-biphenylsulfonamide-1,3,5(10)-estratrien-3-ol (4.16). Purification was done by flash chromatography (ethyl acetate/hexane, 1:9) to afford **4.16** as a yellow solid (36

mg, 43%). Mp 265-266°C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 10.40 (s, 1H, ArOH), 7.96-7.91 (m, 3H, ArH), 7.71 (d, *J* = 8.3 Hz, 2H, ArH), 7.61 (d, *J* = 7.7 Hz, 2H, ArH), 7.49-7.38 (m, 3H), 6.80 (s, 1H, ArH), 4.67 (d, *J* = 9.2 Hz, 1H, NH), 3.21 (q, *J* = 8.7 Hz, 1H, H-17), 2.90-2.77 (m, 2H), 2.26-2.21 (m, 2H), 1.90-1.63 (m, 4H), 1.40-1.12 (m, 8H), 0.71 (s, 3H, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 152.8 (C-3), 149.2, 145.5, 139.7, 139.2, 133.8 (C-5), 131.7 (C-6), 129.1 (2 CH_{Ar}), 128.5 (CH_{Ar}), 127.6 (4 CH_{Ar}), 127.3 (2 CH_{Ar}), 121.5, 118.9, 63.3 (C-17), 51.1 (C-14), 43.3 (CH), 42.8 (C-13), 38.1 (CH), 36.0 (CH_2), 29.5 (2 CH_2), 26.5 (CH_2), 25.8 (CH_2), 23.1 (CH_2), 11.8 (CH_3, C-18); LRMS (ESI^+) *m*/*z* (%) 533 (M+H, 100); HRMS (ESI^+) calcd for $C_{30}H_{33}N_2O_5S$ $(M+H)^+$ 533.2110; found 533.2103.



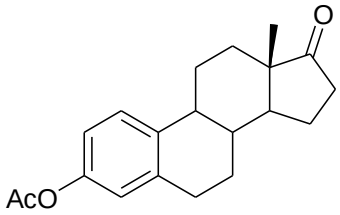
NBA

***N*-Bromoacetamide (NBA).**[184] To a solution of acetamide (2.0 g, 34 mmol), and bromine (1.7 mL, 68 mmol) at 0-5°C, a 50% aqu. KOH solution (4 mL) was added drop-wise. After complete addition, the solution was left at 0-5°C for 2-3 h to complete precipitation, then NaCl (4 g) and $CHCl_3$ (50 mL) were added, and the reaction was heated on a hot water bath to dissolve precipitate with vigorous stirring. The organic layer was dried with Na_2SO_4, then hexane (50 mL) was added and mixture was left in the fridge overnight. The formed needles of NBA were filtered, washed with cold hexane, and dried under vacuum (1.8 g, 39%). Mp: 103-105°C.



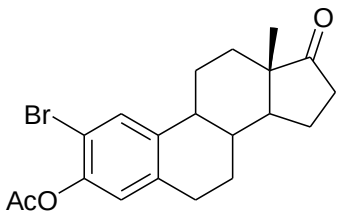
4.17

4-Bromo-estratrien-1,3,5(10)-3-ol (4.17). To a stirred solution of **E1** (500 mg, 1.8 mmol) in ethanol (50 mL), was added NBA (255 mg, 1.8 mmol), and stirring was continued for 24 h. The precipitate formed was filtered and washed with cold ethanol. Purification was done by recrystallization from ethanol to afford **4.17** as white solid (575 mg, 89%). Mp: 264-265^{o}C;[185] ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.80 (s, 1H, ArOH), 7.07 (d, *J* = 8.8 Hz, 1H, H-1), 6.72 (d, *J* = 8.5 Hz, 1H, H-2), 2.87-2.79 (m, 1H), 2.63-2.51 (m, 1H), 2.42-1.92 (m, 6H), 1.72-1.65 (m, 1H), 1.55-1.27 (m, 5H), 0.77 (s, 3H, H-18).



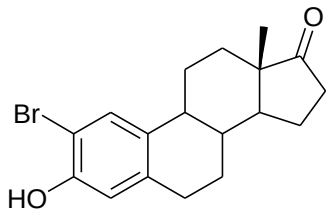
4.18

Estratrien-1,3,5(10)-3-acetate (4.18). To a stirred solution of estrone (1 g, 3.7 mmol) in pyridine (7 mL, 8.6 mmol), was added acetic anhydride (1.7 mL, 18 mmol). Stirring was continued under reflux for 2 h, and then pyridine was azeotropically removed with toluene. Residues was dissolved in ethyl acetate (10 mL), washed with water, brine, and dried with Na_2SO_4. Recrystallization from 95% ethanol afforded **4.18** as white plates (1.3 g, 89%). Mp: 115-116^{o}C;[185] ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.25 (d, *J* = 8.3 Hz, 1H, H-1), 6.83-6.78 (m, 2H, H-2 and H-4), 2.01 (m, 2H, H-6), 2.51-2.34 (m, 2H), 2.24 (m, 4H), 2.16-1.90 (m, 4H), 1.61-1.33 (m, 6H), 0.86 (s, 3H, H-18).



4.19

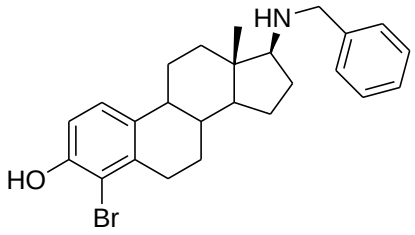
2-Bromo-estratrien-1,3,5(10)-3-acetate (4.19). To a stirred solution of **4.18** (1 g, 3.2 mmol) in TFA (20 mL) at 0-5^{o}C, was added TTFA (3.5 g, 6.4 mmol), and stirring was continued for 24 h. TFA was removed under vacuum, and then residue was washed with DCE (2x10 mL), dried under high vacuum for 6 h. The residue was then dissolve in 1,4-Dioxane (50 mL), and $CuBr_2$ (1.14 g, 7.9 mmol) was added and mixture was refluxed for 3 h, after that, the solvent was removed, and residue was dissolved in DCM (25 mL), washed with water (2x20 mL), brine, dried by Na_2SO_4, and finally concentrated under vacuum. Purification was done by flash chromatography (ethyl acetate/hexane, 4:1) to afford **4.19** as white solid (0.9 g, 73%). Mp: 166-167^{o}C;[185] ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.46 (s, 1H, H-1), 6.82 (s, 1H, H-4), 2.85-2.82 (m, 2H, H-6), 2.53-2.44 (m, 1H), 2.31-1.93 (m, 9H), 1.59-1.39 (m, 6H), 0.88 (s, 3H, H-18).



4.20

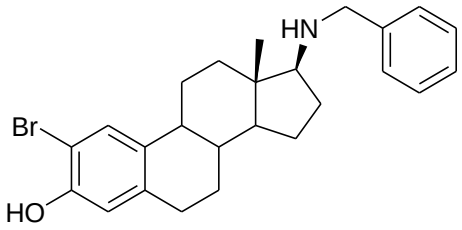
2-Bromo-estratrien-1,3,5(10)-3-ol (4.20). To a stirred solution of **4.19** (0.5 g, 1.3 mmol) in methanol (20 mL), was added potassium carbonate (0.8 g, 6.3 mmol), and stirring was continued under reflux for 3 h. The solvent was then removed under vacuum, and then residue was dissolved in water (20 mL), extracted with DCM (20 mL), washed with brine, dried by Na_2SO_4, and finally concentrated under vacuum. Purification was done by flash chromatography

(ethyl acetate/hexane, 3:2) to afford **4.20** as white solid (0.45 g, 91%). Mp: 194-196°C;[185] ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.32 (s, 1H, H-1), 6.74 (s, 1H, H-4), 5.26 (s, 1H, ArOH), 2.83-2.80 (m, 2H, H-6), 2.53-2.44 (m, 1H), 2.32-1.93 (m, 6H), 1.59-1.39 (m, 7H), 0.88 (s, 3H, H-18).



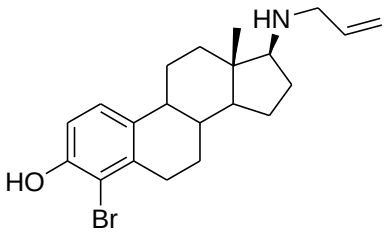
4.21

4-Bromo-17β-benzylamino-estratrien-1,3,5(10)-3-ol (4.21). To a stirred solution of **4.17** (200 mg, 0.56 mmol) in DCE (10 mL), benzylamine (0.25 mL, 2.24 mmol), and glacial acetic acid (0.14 mL, 2.24 mmol) were added. After that, STABH (300 mg, 1.4 mmol) was added and stirring was continued for 48 h. The reaction was then quenched with sat. $NaHCO_3$ solution (10 mL), extracted with DCM (2x10 mL), washed with water, brine, dried with Na_2SO_4, and finally concentrated under vacuum. Purification was done by flash chromatography (methanol/chloroform, 1:9) to afford **4.21** as white solid (204 mg, 81%). Mp: 188-189°C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.34-7.23 (m, 5H, ArH), 7.13 (d, *J* = 8.5 Hz, 1H, H-1), 6.78 (d, *J* = 8.4 Hz, 1H, H-4), 3.87 (brs, *4*H, NH, ArOH, and $C_6H_5C\underline{H}_2$), 2.92-2.87 (m, 1H), 2.71-2.66 (m, 2H), 2.27-1.92 (m, 5H), 1.73-1.70 (m, 1H), 1.49-1.17 (m, 7H), 0.78 (s, 3H, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 150.4 (C-3), 140.7, 136.5 (C-5), 134.3 (C-6), 128.4 (2 CH_{Ar}), 128.1 (2 CH_{Ar}), 126.9 (CH_{Ar}), 125.3 (CH_{Ar}), 113.9, 112.9 (CH_{Ar}), 68.2 (C-17), 52.7 (CH_2), 52.1 (C-14), 44.2 (CH), 43.1 (C-13), 38.0 (CH), 37.9 (CH_2), 31.2 (CH_2), 29.6 (CH_2), 27.5 (CH_2), 26.7 (CH_2), 23.5 (CH_2), 11.9 (CH_3, C-18); LRMS (ESI^+) *m/z* (%) 442 (M+H+2, 99), 440 (M+H, 100); HRMS (ESI^+) calcd for $C_{25}H_{31}NOBr$ $(M+H)^+$ 440.1589; found 440.1591.



4.22

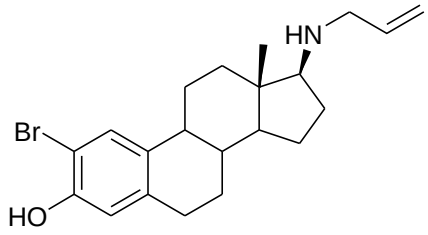
2-Bromo-17β-benzylamino-estratrien-1,3,5(10)-3-ol (4.22). Compound **4.20** (250 mg, 0.7 mmol), DCE (10mL), benzylamine (0.3 mL, 2.8 mmol), Gl. AcOH (0.17 mL, 2.8 mmol), and STABH (375 mg, 1.4 mmol). Purification was done by flash chromatography (methanol/chloroform, 1:9) to afford **4.22** as white solid (277 mg, 88%). Mp: 156-157^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.33-7.23 (m, 7H, ArH, ArOH, and H-1), 6.70 (s, 1H, H-4), 3.83 (AB system, *J* = 13.5 and 13.3 Hz, 2H, $C_6H_5CH_2$), 3.40 (brs, 1H, NH), , 2.76-2.74 (m, 2H, H-6), 2.65 (t, *J* = 8.2 Hz, 1H, H-17), 2.20-2.01 (m, 4H), 1.86-1.82 (m, 1H), 1.69-1.66 (m, 1H), 1.49-1.13 (m, 7H), 0.77 (s, 3H, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 149.8 (C-3), 138.2 (2 C), 134.6 (C-6), 128.6 (2 CH_{Ar}), 128.3 (2 CH_{Ar}), 128.0 (2 CH_{Ar}), 126.8 (CH_{Ar}), 115.8 (CH_{Ar}), 107.3, 68.2 (C-17), 52.7 (CH_2), 52.1 (C-14), 43.8 (CH), 43.1 (C-13), 38.4 (CH), 37.8 (CH_2), 29.1 (CH_2), 27.2 (CH_2), 26.5 (CH_2), 23.5 (CH_2), 11.9 (CH_3, C-18); LRMS (ESI^+) *m*/*z* (%) 442 (M+H+2, 98), 440 (M+H, 100); HRMS (ESI^+) calcd for $C_{25}H_{31}NOBr$ $(M+H)^+$ 440.1589; found 440.1600.



4.23

4-Bromo-17β-allylamino-estratrien-1,3,5(10)-3-ol (4.23). To a stirred solution of **4.17** (200 mg, 0.5 mmol) in DCE (10 mL), allylamine (0.17 mL, 2.3 mmol), and glacial acetic acid

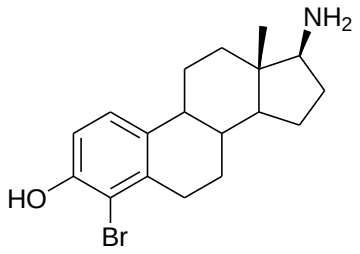
(0.13 mL, 2.3 mmol) were added. After that, STABH (280 mg, 1.3 mmol) was added and stirring was continued for 24 h. The reaction was then quenched with sat. $NaHCO_3$ solution (15 mL), extracted with DCM (2x15 mL), washed with water, brine, dried with Na_2SO_4, and finally concentrated under vacuum. Purification was done by flash chromatography (ethyl acetate/hexane, 1:4) to afford **4.23** as white solid (160 mg, 71%). Mp: 145-147°C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.14 (d, *J* = 8.5 Hz, 1H, H-1), 6.81 (d, *J* = 8.5 Hz, 1H, H-2), 5.96-5.83 (m, 1H, $NHCH_2C\underline{H}CH_2$), 5.18-5.04 (m, *2*H, $NHCH_2CHC\underline{H}_2$), 3.46-3.27 (brs overlapped by an AB system, 3H, $NHC\underline{H}_2CHCH_2$, and ArOH), 2.93-2.85 (m, 1H), 2.72-2.62 (m, 2H), 2.28-1.91 (m, 5H), 1.72-1.70 (m, 1H), 1.47-1.23 (m, 7H), 0.72 (s, 3H, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 150.1 (C-3), 137.4 ($NHCH_2\underline{C}HCH_2$), 136.5 (C-5), 134.4 (C-6), 125.4 (CH_{Ar}), 115.6 ($NHCH_2CH\underline{C}H_2$), 113.7 (C-Br), 112.6 (CH_{Ar}), 68.3 (C-17), 52.1 (CH_2), 51.4 (C-14), 44.1 (CH), 42.9 (C-13), 38.0 (CH), 37.9 (CH_2), 31.1 (CH_2), 29.7 (CH_2), 27.5 (CH_2), 26.7 (CH_2), 23.4 (CH_2), 11.8 (CH_3, C-18); LRMS (ESI^+) *m/z* (%) 392 (M+H+2, 85), 390 (M+H, 90); HRMS (ESI^+) calcd for $C_{21}H_{29}NOBr$ $(M+H)^+$ 390.1433; found 390.1443.



4.24

2-Bromo-17β-allylamino-estratrien-1,3,5(10)-3-ol (4.24). Compound **4.20** (150 mg, 0.4 mmol), DCE (8 mL), allylamine (0.13 mL, 1.7 mmol), glacial acetic acid (0.10 mL, 1.7 mmol), and STABH (210 mg, 1.0 mmol). Purification was done by flash chromatography (ethyl acetate/hexane, 1:4) to afford **4.24** as white solid (120 mg, 71%). Mp: 173-175°C; ^{1}H NMR

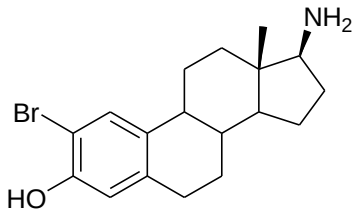
($CDCl_3$, 300 MHz) δ 7.30 (s, 1H, H-1), 6.65 (s, 1H, H-4), 5.94-5.87 (m, 1H, $NHCH_2C\underline{H}CH_2$), 5.2-5.05 (m, 2H, $NHCH_2CHC\underline{H}_2$), 4.10 (brs, 2H, NH and ArOH), 3.86 (t, J = 5.7 Hz, 1H, H-17), 3.30-3.28 (m, 2H, $NHC\underline{H}_2CHCH_2$), 2.75-2.63 (m, 3H), 2.20-1.97 (m, 5H), 1.84-1.81 (m, 1H), 1.70-1.67 (m, 1H), 1.43-1.23 (m, 7H), 0.72 (s, 3H, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 150.4 (C-3), 137.7, 136.8 (CH, $NHCH_2\underline{C}HCH_2$), 134.0 (C-6), 128.9 (CH_{Ar}), 116.1 (CH_{Ar}), 116.0 ($NHCH_2CH\underline{C}H_2$), 107.4, 68.2 (C-17), 52.1 (CH_2), 51.2 (C-14), 43.7 (CH), 43.0 (C-13), 38.4 (CH), 37.8 (CH_2), 29.2 (2 CH_2), 27.1 (CH_2), 26.4 (CH_2), 23.4 (CH_2), 11.7 (CH_3, C-18); LRMS (ESI⁻) *m*/*z* (%) 390 (M-H+2, 84), 388 (M-H, 100); HRMS (ESI⁻) calcd for $C_{21}H_{27}NOBr$ $(M-H)^-$ 388.1276; found 388.1272.



4.25

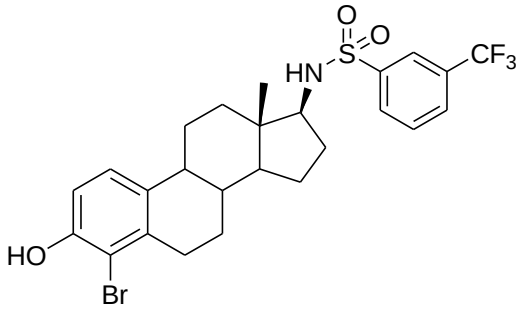
4-Bromo-17β-amino-estratrien-1,3,5(10)-3-ol (4.25). To a stirred solution of **4.23** (150 mg, 0.4 mmol) in DCM (8 mL), was added was added to a solution of dimethylbarbituric acid (177 mg, 1.14 mmol) and $Pd(Ph_3)_4$ (9 mg, 0.007 mmol) in dry DCM (1 mL). The mixture was stirred for 3 h at 35°C under argon atmosphere, and then diluted with DCM (10 mL), and the mixture was washed with sat. $NaHCO_3$, water, brine, and finally organic layer was dried with Na_2SO_4, and concentrated under vacuum. Purification was done by flash chromatography (methanol/chloroform/NH_4OH, 4.5:95:0.5) to afford **4.25** as white solid (58 mg, 63%). Mp: 163-165°C; ^1H NMR (CD_3OD, 300 MHz) δ 7.28 (s, 1H, H-1), 6.57 (s, 1H, H-4), 2.73 (m, 3H), 2.26-

1.73 (m, 7H), 1.35-1.28 (m, 9H), 0.73 (s, 3H, H-18); LRMS (ESI^+) *m/z* (%) 352 (M+H+2, 100), 350 (M+H, 98); HRMS (ESI^+) calcd for $C_{18}H_{25}NOBr$ $(M+H)^+$ 350.1120; found 350.1127.



4.26

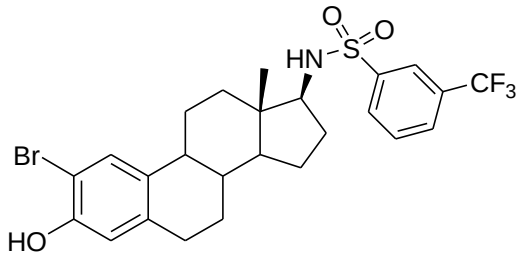
2-Bromo-17β-amino-estratrien-1,3,5(10)-3-ol (4.26). Compound **4.24** (100 mg, 0.25 mmol), DCM (5 mL), NDMBA (118 mg, 0.76 mmol), $Pd(Ph_3)_4$ (6 mg, 0.005 mmol), DCM (1 mL). Purification was done by flash chromatography (methanol/chloroform/NH_4OH, 4.5:95:0.5) to afford **4.26** as a white solid (20 mg, 21%). Mp: 190-191^oC; ^1H NMR (CD_3OD, 300 MHz) δ 7.28 (s, 1H, H-1), 6.57 (s, 1H, H-4), 2.73 (m, 3H), 2.26-1.73 (m, 7H), 1.35-1.28 (m, 9H), 0.73 (s, 3H, H-18); LRMS (ESI^+) *m/z* (%) 352 (M+H+2, 98), 350 (M+H, 100); HRMS (ESI^+) calcd for $C_{18}H_{25}NOBr$ $(M+H)^+$ 350.1120; found 350.1111.



4.27

4-Bromo-17β-(3'-trifluoromethylbenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (4.27). To a stirred solution of **4.25** (50 mg, 0.12 mmol) in dry pyridine (2 mL) at 0 ^oC was added a solution of the 3'-CF_3-benzenesulfonyl chloride (39 mg, 0.15 mmol) in DCM (1 mL) drop-wise via a syringe pump. The reaction was stirred for overnight at room temperature, then pyridine was removed under vacuum, and the residue was dissolved in ethyl acetate (5 mL),

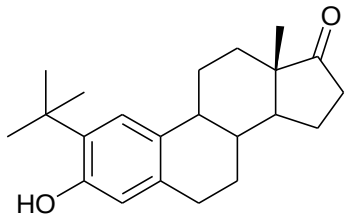
washed with water (2×2.5 mL) and brine (5 mL), dried with Na_2SO_4, and concentrated under vacuum. Purification was done by flash chromatography (ethyl acetate/hexane, 1:9) to afford **4.27** as a white solid (15 mg, 48%). Mp 215-217^oC; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.15 (s, 1H, ArH), 8.07 (d, *J* = 7.9 Hz, 1H, ArH), 7.82 (d, *J* = 7.9 Hz, 1H, ArH), 7.65 (dd, *J* = 7.8 and 7.8 Hz, 1H, ArH), 7.12 (d, *J* = 8.5 Hz, 1H, ArH), 6.81 (d, *J* = 8.5 Hz, 1H, ArH), 5.49 (brs, 1H, ArOH), 4.72 (d, *J* = 9.4 Hz, 1H, NH), 3.20 (q , *J* = 8.6 Hz, 1H, H-17), 2.91-2.83 (m, 1H), 2.69-2.60 (m, 1H), 2.24-2.15 (m, 2H), 1.92-1.83 (m, 2H), 1.72-1.62 (m, 3H), 1.37-1.08 (m, 8H), 0.69 (s, 3H, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 150.1 (C-3), 142.5 (C-SO_2NH), 136.2 (C-5), 133.9 (C-6), 131.5 (q, *J* = 33.2 Hz, C-CF_3), 130.2 (CH_{Ar}), 129.8 (CH_{Ar}), 129.1 (q, *J* = 3.4 Hz, CH_{Ar}), 125.5 (CH_{Ar}), 124.1 (q, *J* = 3.7 Hz, CH_{Ar}), 123.4 (q, *J* = 306.7 Hz, CF_3), 113.6 (C-Br), 112.7 (CH_{Ar}), 63.5 (C-17), 50.9 (C-14), 43.8 (CH), 42.9 (C-13), 38.0 (CH), 36.3 (CH_2), 30.9 (CH_2), 29.5 (CH_2), 27.2 (CH_2), 26.2 (CH_2), 11.8 (CH_3, C-18); ^{19}F NMR ($CDCl_3$, 282 MHz), δ -63.1; LRMS (ESI^-) *m*/*z* (%) 558 (M-H+2, 100%), 556 (M-H, 98%); HRMS (ESI^-) calcd for $C_{25}H_{26}NO_3F_3SBr$ $(M-H)^-$ 556.0769; found 556.0756.



4.28

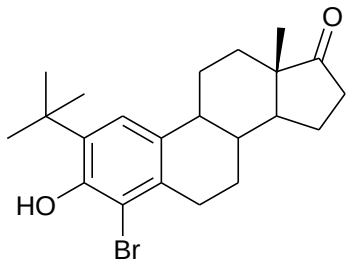
2-Bromo-17β-(3'-trifluoromethylbenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (4.28). Compound **4.26** (20 mg, 0.05 mmol), dry pyridine (1 mL), 3'-CF_3-benzenesulfonyl chloride (15.5 mg, 0.06 mmol), and DCM (0.5 mL). Purification was done by flash chromatography (ethyl acetate/hexane, 0.5:9.5) to afford **4.28** as a white solid (10 mg, 33%). Mp

233-234°C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.14 (s, 1H, ArH), 8.06 (d, *J* = 7.7 Hz, 1H, ArH), 7.82 (d, *J* = 8.0 Hz, 1H, ArH), 7.65 (dd, *J* = 7.7 and 8.0 Hz, 1H, ArH), 7.27 (s, 1H, ArH), 6.70 (s, 1H, ArH), 5.23 (brs, 1H, ArOH), 4.45 (d, *J* = 9.2 Hz, 1H, NH), 3.20 (app. t , *J* = 9.0 Hz, 1H, H-17), 2.74 (m, 2H, H-6), 2.21-2.12 (m, 2H), 1.90-1.79 (m, 2H), 1.70-1.62 (m, 2H), 1.40-1.12 (m, 8H), 0.69 (s, 3H, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 149.9 (C-3), 142.5 (C-SO_2NH), 138.0 (C-5), 134.1 (C-6), 132.0 (C-CF_3), 130.3 (CH_{Ar}), 129.9 (CH_{Ar}), 129.2 (CH_{Ar}), 128.7 (CH_{Ar}), 124.2, (CH_{Ar}), 107.4 (C-Br), 63.5 (C-17), 51.0 (C-14), 43.5 (CH), 42.9 (C-13), 38.5 (CH), 36.2 (CH_2), 26.9 (CH_2), 26.0 (CH_2), 11.8 (CH_3, C-18); ^{19}F NMR ($CDCl_3$, 282 MHz), δ -63.1; LRMS (ESI^+) *m*/*z* (%) 560 (M+H+2, 72), 558 (M+H, 70%), 335 (99%), 333 (100%); HRMS (ESI^+) calcd for $C_{25}H_{28}NO_3F_3SBr$ $(M+H)^+$ 558.0925; found 558.0927.



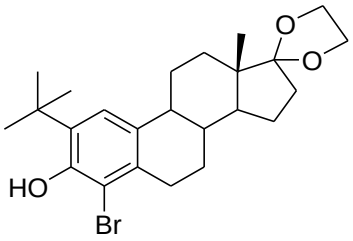
4.29

2-*t*-Butyl-estra-1,3,5(10)-triene-17-one (4.29). To a suspension of **E1** (0.7 g, 2.6 mmol) in DCM (30 mL), *t*-butyl alcohol (0.5 mL, 5 mmol) was added, after that $BF_3.OEt_2$ (1 mL, 7.4 mmol) was added dropwise via a syringe pump, and stirring was continued for 2 h. The reaction mixture was then quenched by sat. $NaHCO_3$ (15 mL) and organic layer was washed by water (2×10 mL), brine (15 mL), dried by Na_2SO_4, and finally concentrated under vacuum. Purification was done by flash chromatography (ethyl acetate/hexane, 1:4) to afford **4.29** as a white solid (0.78 g, 93%). Mp: 242-244°C;[192] ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.17 (s, 1H, H-1), 6.41 (s, 1H, H-4), 4.81 (brs, 1H, ArOH), 2.80-2.78 (m, 2H), 2.47-2.39 (m, 2H), 2.35-1.93 (m, 5H), 1.63-1.38 (m, 16H), 0.89 (s, 3H, H-18).



4.31

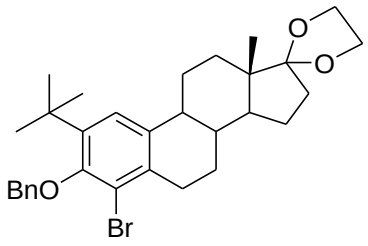
2-*t*-Butyl-4-bromoestra-1,3,5(10)-triene-17-one (4.31). A mixture of **4.29** (200 mg, 0.6 mmol) and NBA (84.5 mg, 0.6 mmol) in EtOH (10 mL) was stirred at room temperature for 12 h. The solvent was then removed, and residue was dissolved in DCM (20 mL), washed with water (2×5 mL), dried with Na_2SO_4, and concentrated under vacuum. Purification was done by flash chromatography (ethyl acetate/hexane, 1:9) to afford **4.31** as a white solid (203 mg, 81%). Mp: 171-172^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.19 (s, 1H, H-1), 5.86 (s, 1H, ArOH), 2.95-2.87 (m, 1H), 2.71-2.68 (m, 1H), 2.54-2.38 (m, 1H), 2.26-1.94 (m, 5H), 1.67-1.35 (m, 16H), 0.89 (s, 3H, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 220.7 (C=O), 148.3, 134.1, 133.6, 132.4, 122.9 (CH_{Ar}), 115.8, 50.3, 47.9, 44.4, 37.7 (CH), 35.8, 35.3, 31.6 (CH_2), 30.9, 29.5 ($C(\underline{C}H_3)_3$, 26.2, 26.7 (CH_2), 21.5 (CH_2), 13.8 (CH_3, C-18); LRMS (ESI$^+$) *m/z* (%) 407 (M+H+2, 100), 405 (M+H, 98); HRMS (ESI$^+$) calcd for $C_{22}H_{30}O_2Br$ $(M+H)^+$ 405.1429; found 405.1434.



4.32

2-*t*-Butyl-4-bromo-17,17-ethylenedioxyestra-1,3,5(10)-trien-3-ol (4.32). To A stirred solution of **4.31** (180 mg, 0.4 mmol) in toluene (2.5 mL) and ethylene glycol (0.15 mL, 1.0 mmol), was added PTSA (11.2 mg, 0.04 mmol) and the reaction under a Dean-Stark conditions

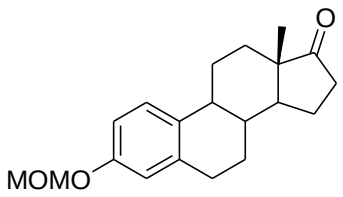
for 6 h. After that, the reaction was poured into sat. $NaHCO_3$ solution (5 mL), diluted with ethyl acetate (5 mL), then organic layer was washed with water, brine, dried with Na_2SO_4, and concentrated under vacuum. Purification was done by flash chromatography (ethyl acetate/hexane, 1:9) to afford **4.32** as a white solid (101 mg, 51%). Mp: 163-164^oC; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.19 (s, 1H, H-1), 5.82 (s, 1H, ArOH), 3.96-3.85 (m, 4H, OCH_2CH_2O), 2.89-2.81 (m, 1H), 2.65-2.63 (m, 1H), 2.35-2.24 (m, 2H), 2.02-1.92 (m, 2H), 1.85-1.80 (m, 2H), 1.79-1.75 (m, 3H), 1.74-1.71 (m, 3H), 1.54-1.27 (m, 13H), 0.86 (s, 3H, H-18); ^{13}C NMR (CD_3OD, 75 MHz) δ 149.1, 134.6, 133.5, 132.5, 122.3 (CH_{Ar}), 119.0, 115.5, 64.8, 64.2, 50.1, 45.8, 44.1, 38.5, 34.8, 33.7, 30.8, 30.5, 28.7 ($C(\underline{C}H_3)_3$, 27.1, 26.2, 21.8 (CH_2), 13.4 (CH_3, C-18); LRMS (ESI^+) *m/z* (%) 449 (M+H+2, 40), 447 (M+H, 30), 415 (100); HRMS (ESI^+) calcd for $C_{24}H_{32}O_3Br$ $(M+H)^+$ 447.1535; found 447.1534.



4.33

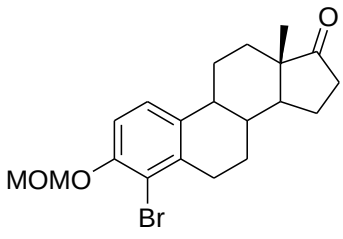
3-Benzyloxy-4-bromo-2-*t*-Butyl-17,17-ethylenedioxyestra-1,3,5(10)-triene (4.33). To a stirred solution of **4.32** (50 mg, 0.11 mmol) in acetone (5 mL), was added K_2CO_3 (17 mg, 0.12 mmol), and the mixture was stirred for 30 min., after that, BnBr (16 uL, 0.13 mmol) was added and stirring was continued overnight. Solvent was then removed under vacuum, and residue was dissolved in water (10 mL), extracted by DCM (2×5 mL), washed with water, dried with Na_2SO_4, and concentrated under vacuum. Purification was done by flash chromatography (methanol/chloroform, 0.5:9.5) to afford **4.33** as a pale yellow solid (5 mg, 8%). ^1H NMR

($CDCl_3$, 300 MHz) δ 7.46 (d, *J* = 7.5 Hz, 2H, ArH), 7.42-7.30 (m, 4H, ArH and H-1), 5.08 (s, 1H, $C_6H_5C\underline{H}_2O$), 3.97-3.89 (m, 4H, OCH_2CH_2O), 2.93-2.92 (m, 1H), 2.67 (m, 1H), 2.32-2.28 (m, 2H), 2.03-1.99 (m, 2H), 1.82-1.77 (m, 3H), 1.61-1.30 (m, 13H), 0.88 (s, 3H, H-18).



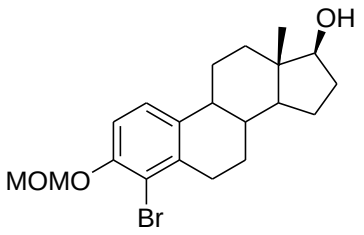
4.34

Estra-1,3,5(10)-triene-3-(methoxymethyl)ether-17-one (4.34). To a stirred mixture of **E1** (250 mg, 0.9 mmol) in THF (4 mL) at 0°C, was added DIPEA (0.25 mL, 1.4 mmol), and continue stirring at 0°C for 30 min., then MOMCl (0.1 mL, 1.4 mmol) was added. The reaction mixture was allowed to warm up to room temperature and stirring was continued for another 1 h, then refluxed for 24 h. The reaction was then cooled to room temperature, and quenched with NH_4Cl, extracted with ether (2×15 mL), washed with brine, dried with Na_2SO_4, and finally concentrated under vacuum. Purification was done by flash chromatography (ethyl acetate/hexane, 1:4) to afford **4.34** as a white solid (195 mg, 67%). Mp: 97-99°C;[201] ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.18 (d, *J* = 8.6 Hz, 1H, H-1), 6.81 (dd, *J* = 2.4 and 8.8 Hz, 1H, H-2), 6.77 (brs, 1H, H-4), 5.13 (s, 2H, $CH_3OC\underline{H}_2O$), 3.45 (s, 3H, $C\underline{H}_3OCH_2O$), 2.88-2.85 (m, 2H), 2.46-2.36 (m, 2H), 2.15-1.95 (m, 5H), 1.60-1.41 (m, 7H), 0.88 (s, 3H, H-18).



4.35

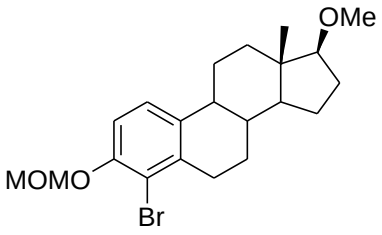
4-Bromo-estra-1,3,5(10)-triene-3-(methoxymethyl)ether-17-one (4.35). ***Method A*** To a stirred solution of **4.17** (100 mg, 0.25 mmol) in THF (5 mL), was added DIPEA (76 µL, 0.8 mmol, Hungi's base) and stirring was continued at 0^{o}C for 30 min. MOMCl (33 µL, 0.8 mmol) was then added and the reaction was allowed to warm up to room temperature for 1 h, then refluxed for 24 h. After that, reaction was cooled to room temperature, and quenched with NH_4Cl, extracted with ether (2×10 mL), dried with Na_2SO_4. Purification was achieved by flash chromatography (ethyl acetate/hexane, 1:9) to afford **4.35** as a white solid (54 mg, 48%). Mp: 171-173^{o}C;[202] ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.18 (d, *J* = 8.6 Hz, 1H, H-1), 6.94 (d, *J* = 8.6 Hz, 1H, H-2), 5.19 (s, 2H, $CH_3OC\underline{H}_2O$), 3.48 (s, 3H, $C\underline{H}_3OCH_2O$), 2.99-2.97 (m, 1H), 2.46-2.34 (m, 2H), 2.15-1.91 (m, 7H), 1.60-1.40 (m, 6H), 0.86 (s, 3H, H-18). ***Method B*** To a stirred solution of **4.17** (100 mg, 0.25 mmol) in DMF (5 mL), was added *t*-BuOK (40 mg, 0.37 mmol) and stirring was continued at 0^{o}C for 10 min. MOMCl (26 µL, 0.37 mmol) was then added and the reaction was allowed to warm up to room temperature for 1 h, then refluxed for 24 h. After that, reaction was cooled to room temperature, and quenched with sat. NH_4Cl solution, dilute with water (5 mL), filter the formed precipitate, recrystallize from ethanol, and finally dried under high vacuum for overnight to obtain **4.35** as a white solid (110 mg, 65%).



4.36

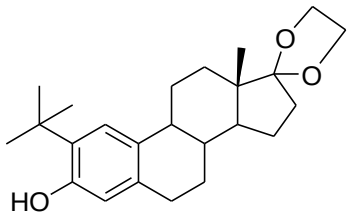
4-Bromo-estra-1,3,5(10)-triene-3-(methoxymethyl)ether-17β-ol (4.36). To a stirred solution of **4.35** (100 mg, 0.25 mmol) in methanol (5 mL) at 0^{o}C, was added $NaHB_4$ (54 mg, 0.07 mmol), and stirring was continued for 1.5 h. at the same temperature. Quenched with sat.

NH_4Cl solution, extracted with chloroform (2×7.5 mL), washed with water, dried with Na_2SO_4, and concentrated under vacuum to afford **4.36** as a white solid which used without further purification (100 mg, 99%). Mp: 155-157^oC;[202] ^1H NMR ($CDCl_3$, 300 MHz) δ 7.19 (d, *J* = 8.5 Hz, 1H, H-1), 6.94 (d, *J* = 8.6 Hz, 1H, H-2), 5.20 (s, 2H, $CH_3OC\underline{H}_2O$), 3.70 (app. q, *J* = 8.7 Hz, 1H, H-17), 3.50 (s, 3H, $C\underline{H}_3OCH_2O$), 2.99-2.93 (m, 1H), 2.68 (m, 1H), 2.31-1.94 (m, 5H), 1.92-1.91 (m, 1H), 1.49-1.27 (m, 7H), 0.75 (s, 3H, H-18).



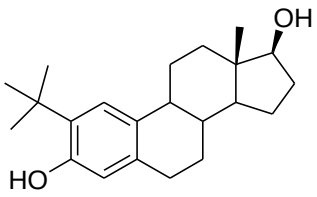
4.37

4-Bromo-17β-methoxy-estra-1,3,5(10)-triene-3-(methoxy methoxy)ether (4.37). To a stirred solution of **4.36** (100 mg, 0.25 mmol) in DMF (5 mL) at 0^oC, was added *t*-BuOK (40 mg, 0.37 mmol) and stirring was continued at 0^oC for 5 min. A solution of MeI (21 µL, 0.32 mmol) in DMF (1 mL) was then added and the reaction was stirred at room temperature for 15 h, then the reaction was quenched with sat. NH_4Cl solution, diluted with water (10 mL), and the formed precipitate was filtered, and recrystallized from ethanol obtain **4.37** as a white solid (80 mg, 46%). Mp: 155-157^oC;[202] ^1H NMR ($CDCl_3$, 300 MHz) δ 7.19 (d, *J* = 8.6 Hz, 1H, H-1), 6.95 (d, *J* = 8.6 Hz, 1H, H-2), 5.22 (s, 2H, $CH_3OC\underline{H}_2O$), 3.51 (s, 3H, $C\underline{H}_3OCH_2O$), 3.37 (s, 3H, OCH_3), 3.30 (t, *J* = 8.1 Hz, 1H, H-17), 3.01-2.93 (m, 1H), 2.75-2.68 (m, 1H), 2.29-1.92 (m, 5H), 1.71-1.16 (m, 9H), 0.77 (s, 3H, H-18).



4.39

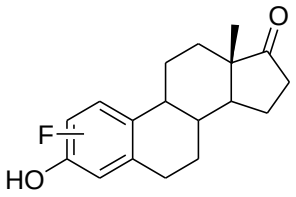
2-*t*-Butyl-17,17-ethylenedioxyestra-1,3,5(10)-trien-3-ol (4.39). To A stirred solution of **4.29** (250 mg, 0.76 mmol) in toluene (5 mL) and ethylene glycol (0.20 mL, 1.9 mmol), was added PTSA (13 mg, 0.07 mmol) and the reaction under a Dean-Stark conditions for 6 h. After that, the reaction was quenched with sat. $NaHCO_3$ solution (10 mL), diluted with ethyl acetate (5 × 2 mL), then organic layer was washed with water, brine, dried with Na_2SO_4, and concentrated under vacuum. Purification was done by flash chromatography (ethyl acetate/hexane, 0.5:99.5) to afford **4.39** as a white solid (133 mg, 47%). Mp: 144-146^oC; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.14 (s, 1H, H-1), 6.38 (s, 1H, H-4), 3.96-3.87 (m, 5H, 3-OH and OCH_2CH_2O), 2.73 (m, 2H), 2.66-2.62 (m, 1H), 2.31 (m, 3H), 2.00-1.72 (m, 10H), 1.51-1.22 (m, 24H), 0.86 (s, 3H, H-18).



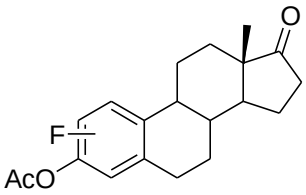
4.41

2-*t*-Butyl-estra-1,3,5(10)-triene-3,17β-diol (4.41). To a stirred mixture solution of **4.29** (500 mg, 1.53 mmol) in ethanol/THF (30 mL, 1:1) mixture, was added sodium borohydride (250 mg, 6.1 mmol), and stirring was continued for 2 h, after that it was quenched with aqu. NH_4Cl solution, extracted with ethyl acetate, washed with water, brine, dried with Na_2SO_4, and finally concentrated under vacuum to afford **4.41** as white solid (500 mg, 97%). The compound was used in the next step without further purification. Mp: 174-176^oC;[193] ^1H NMR ($CDCl_3$, 300

MHz) δ 7.18 (s, 1H, H-1), 6.39 (s, 1H, H-4), 3.72 (t, *J* = 8.2 Hz, 1H, H-17), 2.78-2.73 (m, 2H), 2.30 (m, 1H), 2.17-2.12 (m, 3H), 1.96-1.92 (m, 2H), 1.51-1.16 (m, 19H), 0.76 (s, 3H, H-18).

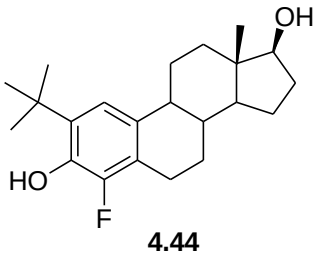


2- and 4-Fluoroestra-1,3,5(10)triene-17-one.[185] A stirred mixture of **E1** (250 mg, 0.9 mmol), and NFPT (460 mg, 1.85 mmol) in 1,1,2-TCE (10 mL) was refluxed for 24 h, then solvent was removed under vacuum, and mixture was poured into water (20 mL), extracted with DCM (2x10 mL), dried with Na_2SO_4, and finally concentrated. Purification by different flash chromatography systems failed completely to separate two isomers a part, and gave a brown solid (360 mg). ^{19}F NMR ($CDCl_3$, 282 MHz) δ -145 and -146.



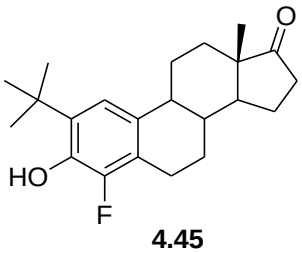
2- and 4-Fluoro-3-acetyloxy-estra-1,3,5(10)triene-17-one.[185] To a stirred solution of previous mixture (360 mg) in pyridine (2.5 mL), was added acetic anhydride (0.7 mL), and mixture was refluxed for 2 h. The solvent was then removed under vacuum, residue was poured into ice-water mixture (15 mL), and precipitate formed was filtered, washed with water, and finally dried under high vacuum for overnight. Purification by different flash chromatography

systems failed completely to separate two isomers a part, and gave a white solid (400 mg). ^{19}F NMR ($CDCl_3$, 282 MHz) δ -134 and -135.



4.44

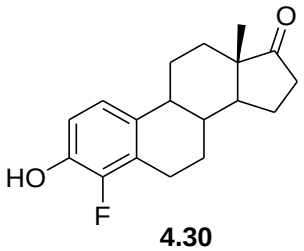
4-Fluoro-2-*t*-butyl-estra-1,3,5(10)-triene-3,17β-diol (4.44). To a stirred solution of **4.43** (250 mg, 0.76 mmol) in 1,1,2-TCE (10 mL), was added NFPT (207 mg, 0.84 mmol), then the mixture was refluxed for 24 h. after that, it was cooled down, and solvent was azeotropically removed with toluene (10 mL), then residue was mixed with water (10 mL), extracted by DCM (2x10 mL). The organic layer was washed with water (2x5 mL), dried with Na_2SO_4, and finally concentrated under vacuum. Purification was achieved by flash chromatography (ethyl acetate/hexane, 1:9) to afford **4.44** as a pale brown solid (36 mg, 14%). Mp: 213-214^oC; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.96 (s, 1H, H-1), 5.28 (d, *J* = 7.6 Hz, 1H, ArOH), 3.73 (t, *J* = 8.0 Hz, 1H, H-17), 2.86-2.84 (m, 1H), 2.62 (m, 1H), 2.30 (m, 1H), 2.16-2.04 (m, 2H), 1.97-1.93 (m, 2H), 1.50-1.16 (m, 22H), 0.77 (s, 3H, H-18); ^{19}F NMR ($CDCl_3$, 282 MHz) δ -147.



4.45

2-*t*-Butyl-4-fluoroestra-1,3,5(10)-triene-17-one (4.45). A stirred mixture of **4.29 (**1.9 g, 6.0 mmol) and NFPT (2.9 g, 12.0 mmol) in DCE (50 mL) in a glass pump under Argon

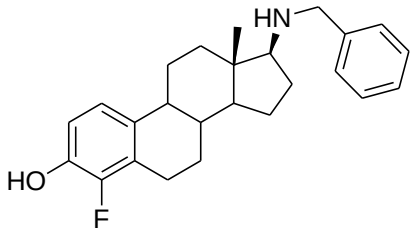
atmosphere was heated at 135°C for 15 h. DCE was then removed under high vacuum and water (90 mL) was added and reaction mixture was extracted with DCM (2×30 mL). The combined organic extracts were washed with water, brine, dried with Na_2SO_4, and finally concentrated under vacuum. Purification was achieved by flash chromatography (ethyl acetate/hexane, 1:5) to afford **4.45** as a pale brown solid (330 mg, 16%). Mp: 181-183°C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.96 (s, 1H, H-1), 5.29 (d, *J* = 7.5 Hz, 1H, ArOH), 2.97 (m, 1H), 2.91-2.89 (m, 1H), 2.49-2.46 (m, 2H), 2.21-1.95 (m, 5H), 1.62-1.34 (m, 16H), 0.90 (s, 3H, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 220.9 (C=O), 151.1, 148.0, 140 (d, *J* = 15.2 Hz, C-2), 134.9, 131 (d, *J* = 3.9 Hz), 121.3 (d, *J* = 15.7 Hz, C-6), 117.9 (CH_{Ar}), 50.3, 47.9, 44.0 (d, *J* = 2.1Hz), 38.0 (CH), 35.8, 34.9, 31.5 (CH_2), 29.5, 25.7 (2 CH_2), 22.0 (d, *J* = 3.9 Hz), 21.5 (CH_2), 13.8 (CH_3, C-18); ^{19}F NMR ($CDCl_3$, 282 MHz) δ -147; LRMS (ESI^+) *m/z* (%) 345 (M+H, 100); HRMS (ESI^+) calcd for $C_{22}H_{30}O_2F$ $(M+H)^+$ 345.22243; found 345.22235.



4.30

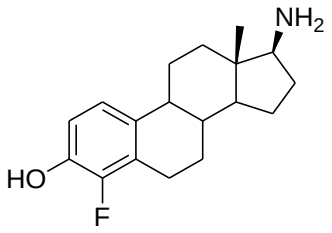
4-Fluoroestra-1,3,5(10)-triene-17-one (4.30). To a stirred solution of **4.45** (325 mg, 0.9 mmol) in DCM (40 mL), was added nitromethane (1.1 mL, 21.7 mmol), the mixture was then cooled to 0°C before addition of $AlCl_3$ (1.26 g, 9.4 mmol). Stirring was continued at 0°C for 2 h, and then quenched by ice, acidified by HCl (1 M), extracted by ethyl acetate (2×10 mL), washed with water, brine, dried with Na_2SO_4, and finally concentrated under vacuum. Purification was done by flash chromatography (ethyl acetate/hexane, 3:7) to afford **4.30** as pink solid (108 mg, 40%). Mp: 221-222°C;[185] ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.92 (d, J = 8.6 Hz, 1H, H-1), 6.78 (2

overlapping d, J = 8.8 Hz, 1H, H-2), 4.90 (d, *J* = 4.4 Hz, 1H, ArOH), 3.00-2.92 (m, 1H), 2.73-2.69 (m, 1H), 2.54-2.45 (m, 1H), 2.36-2.33 (m, 1H), 2.22-1.91 (m, 5H), 1.61-1.24 (m, 7H), 0.89 (s, 3H, H-18); ^{19}F NMR ($CDCl_3$, 282 MHz) δ -146.



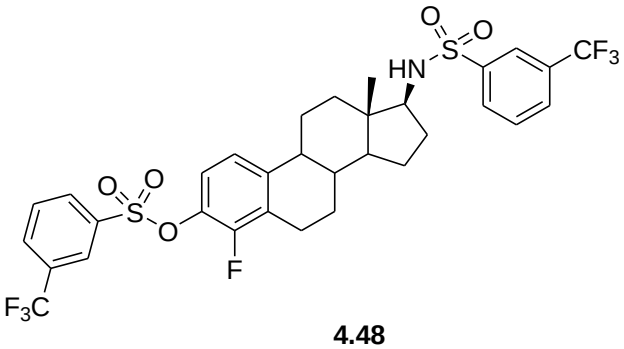
4.46

4-Fluoro-17β-benzylamino-estra-1,3,5(10)-triene (4.46). To a stirred solution of **4.30** (100 mg, 0.35 mmol) in DCE/THF (10 mL, 1:1) mixture, was added benzylamine (150 mg, 1.4 mmol), glacial acetic acid (0.1 mL, 1.4 mmol), and STABH (185.4 mg, 0.9 mmol). Stirring was continued for 48 h, and then quenched with sat. $NaHCO_3$ solution, extracted by DCM (20 mL), washed with water, brine, dried with Na_2SO_4, and concentrated. Purification by flash chromatography (methanol/chloroform, 1:9) to give **4.46** as a white solid (80 mg, 61%). Mp: 211-213^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.32-7.25 (m, 5H, ArH), 6.91 (d, *J* = 8.6 Hz, 1H, H-1), 6.75 (2 overlapped d, *J* = 8.6 Hz, 1H, H-2), 3.99-3.71 (m, 4H, Ar$\underline{C}H_2$, NH, ArOH), 2.87-2.85 (m, 1H), 2.70-2.64 (m, 2H), 2.22-2.02 (m, 5H), 1.90 (m, 1H), 1.72-1.69 (m, 1H), 1.49-1.21 (m, 8H), 0.78 (s, 3H, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 150.6, 147.5, 140.1 (d, *J* = 14.8 Hz, C-2), 140.7, 133.6 (d, *J* = 3.1 Hz), 128.3 (2 CH_{Ar}), 128.0 (2 CH_{Ar}), 126.8 (CH_{Ar}), 124.1 (d, *J* = 14.1 Hz, C-6), 120.7 (d, *J* = 3.8 Hz, CH_{Ar}), 113.8 (d, *J* = 2.2 Hz, CH_{Ar}), 68.1 (CH-17), 52.6 ($\underline{C}H_2C_6H_5$), 52.1, 43.8 (d, *J* = 2.1 Hz), 43.1, 38.2 (CH), 37.9, 29.5, 26.4 (2 CH_2), 23.5, 22.3 (d, *J* = 4.3 Hz), 11.8 (CH_3, C-18); ^{19}F NMR ($CDCl_3$, 282 MHz) δ -144; LRMS (ESI$^+$) *m*/*z* (%) 380 (M+H, 100); HRMS (ESI$^+$) calcd for $C_{25}H_{31}NOF$ $(M+H)^+$ 380.2390; found 380.2380.



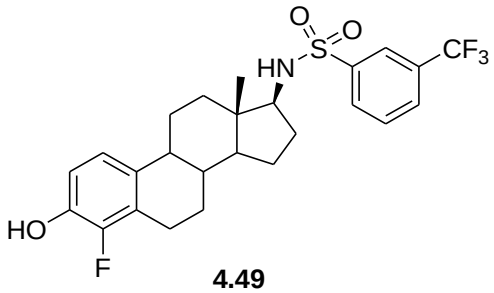
4.47

4-Fluoro-17β-amino-estra-1,3,5(10)-triene (4.47). To a stirred solution of **4.46** (80 mg, 0.2 mmol) in methanol (10 mL), 10% Pd/C (13 wt%, 15 mg) was added, and the system was kept under H_2 atmosphere for overnight. After that, the reaction mixture was filtered through Celite, and concentrated under vacuum. Purification was done by flash chromatography (methanol/chloroform/NH_4OH, 4:95:1%) to afford **4.47** as a pale brown solid (42 mg), which turned out to be a mixture of two compounds, one of them is our target compound and this was clear from ^{1}H NMR, ^{19}F NMR, and LRMS, so it was used in the next step without further trials to separate it from the other compound. Here we are listing the ^{1}H NMR (CD_3OD, 300 MHz) δ 6.84 (d, *J* = 8.3 Hz, 2H, ArH), 6.67 (2 overlapping d, J = 8.9 Hz, 2H, ArH), 2.90-2.62 (m, 6 H), 2.47 (brs, 2H), 2.29-2.04 (m, 7H), 1.92-1.88 (m, 2H), 1.77-1.74 (m, 2H), 1.45-1.19 (m, 15H), 0.76 (s, 3H), 0.71 (s, 3H, H-18); ^{19}F NMR ($CDCl_3$, 282 MHz) δ -144; LRMS (ESI^+) *m*/*z* (%) 304 (100%), 290 (M+H, 78).



4.48

4-Fluoro-17β-(3'-trifluoromethylbenzene)sulfonamide-estra-1,3,5(10)-trien-3-yl-(3'-tirfluoromethyl)benzene sulfonate (4.48). To a solution of **4.47** (42 mg) in pyridine (1 mL), was added a solution of 3'-CF_3-benzenesulfonyl chloride in DCM (0.5), and stirring was continued for overnight. Pyridine was azeotropically removed with toluene, residue was dissolved in ethyl acetate (5 mL), washed with water, brine, dried with Na_2SO_4, and concentrated under vacuum. Purification was achieved by flash chromatography (methanol/chloroform, 0.5:9.5) to afford **4.48** as a white solid (31 mg). Mp: 221-222^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.14-8.05 (m, 4H, ArH), 7.91 (d, *J* = 7.8 Hz, 1H, ArH), 7.81 (d, *J* = 7.8 Hz, 1H, ArH), 7.72-7.63 (m, 2H, ArH), 7.00-6.90 (m, 2H, ArH), 4.58 (d, *J* = 9.6 Hz, 1H, NH), 3.20 (q, *J* = 8.5 Hz, 1H, H-17), 2.79-2.71 (m, 1H), 2.53-2.50 (m, 1H), 2.22-2.15 (m, 2H), 1.88-1.82 (m, 3H), 1.74-1.53 (m, 5H), 1.37-1.09 (m, 7H), 0.70 (s, 3H, H-18); ^{19}F NMR ($CDCl_3$, 282 MHz) δ -133.0 (F-C-4), -63.0 (-$NHSO_2C_6H_4CF_3$).



4.49

4-Fluoro-17β-(3'-trifluoromethylbenzene)sulfonamide-estra-1,3,5(10)-trien-3-ol (4.49). To a stirred solution of **4.48** (31 mg, 0.04 mmol) in methanol (2 mL), was added a solution of potassium carbonate (6 mg, 0.04 mmol) in water (0.1 mL), and stirring was continued for 2 h. after that the reaction mixture was diluted with water (2 mL), neutralized with dil. HCl, extracted with DCM, washed with brine, dried with Na_2SO_4, and finally concentrated. Purification was done by flash chromatography (ethyl acetate/hexane, 1:4) to afford **4.49** as a

white solid (13 mg, 62%). Mp: 253-255^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.14 (s, 1H, ArH), 8.06 (d, *J* = 7.4 Hz, 1H, ArH), 7.81 (d, *J* = 7.5 Hz, 1H, ArH), 7.65 (2 overlapping d, *J* = 7.7 Hz, 1H, ArH), 6.89 (d, *J* = 8.2 Hz, 1H, H-1), 6.76 (2 overlapping d, *J* = 8.5 Hz, 1H, H-2), 4.86 (brs, 1H, ArOH), 4.46 (d, *J* = 9.3 Hz, 1H, NH), 3.21 (m, 1H, H-17), 2.91-2.85 (m, 1H), 2.62-2.58 (m, 1H), 2.22-1.65 (m, 7H), 1.42-1.13 (m, 7H), 0.70 (s, 3H, H-18); ^{19}F NMR ($CDCl_3$, 282 MHz) δ -146 (F-C-4), -63 (2 $C_6H_4CF_3$); LRMS (ESI^+) *m/z* (%) 498 (M+H, 58), 273 (100); HRMS (ESI^+) calcd for $C_{25}H_{28}NO_3F_4S$ $(M+H)^+$ 498.1726; found 498.1723.

4.5.3 Inhibition Studies

4.5.3.1 General

STS was purified as previously described in *Chapter* 2. All buffers and assay reagents were purchased form Sigma Aldrich (Milwaukee, WI, USA). All fluorescent measurements were carried out on a SpectraMax GeminiXS® fluorimeter (Molecular Devices, Sunnyvale, CA, USA) at 24 ^{o}C in black microtiter plates from Corning (Corning, MA, USA). All determinations were carried out in triplicate and errors reported as ± 5% of obtained results.

4.5.3.2 Determination of IC_{50} for compounds 4.9, 4.10, 4.13-4.16, 4.27, 4.28, 4.51-4.53

20 µL of inhibitor stock solution in DMSO/0.1 M Tris-HCl, pH 7.0 (1:1), were added to the wells of a 96-well microtiter plate containing 140 µL of 0.1 M Tris, pH 7.0. After that, 20 µL of a 2 mM MUS stock solution in 0.1 M Tris–HCl, pH 7.0, was added. The assay was initiated by adding 20 µL STS (100 nM stock solution in 20 mM Tris–HCl, pH 7.4, 0.1% Triton X-100). The final concentration of inhibitor ranged from 5 nM to 5 µM. The final concentration of 4-MUS was 200 µM, and 10 nM for STS. The reactions were followed as described before in § **3.5.3.2**. Each reaction was performed in quadruplicate. Additional controls were performed in an

identical manner but did not contain STS. Eleven concentrations of inhibitor bracketing the IC_{50} value were used for each compound. The initial rates of enzyme activity in relative fluorescence units per second (RFU/s) were used to determine the IC_{50}. The ratio of the initial rate in the presence of inhibitor (V_i) to that in the absence of inhibitor (V_o) was calculated and plotted as a semi-log curve in Grafit (Erithacus Software, Surrey, U.K.), from which the IC_{50} value was calculated based on the following equation: $V_i = V_o/[1 + ([I]/IC_{50})S] + B$, where: V_i is the initial rate of reaction at an inhibitor concentration of [I]; V_o is the velocity in the absence of inhibitor; *B* is background and *s* is the slope factor.

4.5.3.3 Determination of IC_{50} for Tight-Binding Inhibitors, compounds 4.11, 4.12, 4.49, and 4.54.

The IC_{50} was be determined for each compound as described in § **4.5.3.2** at a number of different enzyme concentrations (stock solutions of 50, 100, 200, and 400 nM). A plot of IC_{50} as a function of $[E]_T$ was constructed. The y-intercept provided the apparent K_i. The final concentration of STS in the plate will be 5, 10, 20, and 40 nM, (See Appendix **B** for IC_{50} plots used for construction of each compound apparent K_i plot).

4.5.3.4 Determination of K_i and αK_i of compound 4.2

20 mL of MUS stock solution in 0.1 M Tris-HCl of pH 7.0 was added to the wells of a 96-well microtiter plate containing 140 mL 0.1 M Tris-HCl buffer of the same pH such that the total volume was 160 µL. To the wells was added 20 µL of a stock solution of inhibitor in 50% DMSO, (for a control, 20 µL of 50% DMSO was added instead). The assay was initiated by the addition of 20 µL STS (100 nM stock solution in 20 mM Tris-HCl, pH 7.4, 0.1% Triton X-100). To detect non-enzymatic hydrolysis of the substrate 20 µL of 20 mM Tris-HCl, pH 7.4, 0.1%

Triton X-100 was added instead. The final volume of the assay was 200 μL. The final concentration of buffer was 184 mM Tris-HCl, 0.01% Triton X-100, and 5% DMSO. The final enzyme concentration was 10 nM.

For studies with compound **4.2** at pH 7.0, the final concentration of MUS was 100–500 μM, the final concentration of inhibitor was 1-3 times IC_{50}. The reactions were followed by detection of fluorescent product, 4-methylumbelliferone (excitation 360 nm, emission, 460 nm), over 10 min at 24^{o}C. Each reaction was performed in quadruplicate. Additional controls were performed in an identical manner but did not contain STS. Initial rates (v) were determined by taking the slopes of plots of the change in relative fluorescence units with time. These data were plotted as Lineweaver–Burk graphs and K_i and aK_i values were calculated from re-plots of the slopes or intercepts of the Lineweaver–Burk graphs according to the equations for mixed and competitive inhibition.

4.5.3.5 Examining Time- and/or Concentration-dependent Inhibition of STS with compound 4.50

Compound **4.50** was screened for time- and/or conc.-dependent inhibition by incubating 180 μL solution of various concentrations of compound **4.50**, in buffer containing 0.1 M tris, pH 7.0, a 20 μL solution of 400 nM STS in 20 mM tris, pH 7.4, 0.1% Triton X-100 was added. Controls which did not contain inhibitor were performed for all experiments. These mixtures were allowed to incubate at 24 ^{o}C and 4 μL aliquots were removed at various time intervals and added to the wells of a 96-well microtiter plate containing 196 μL of 4 mM of 4-MUS sulfate in assay buffer. The final concentrations of compound **4.50** were ranging from 50 nM to 1 μM. The production of the fluorescent product, 4-methylumbelliferone (4-MU), was followed for 10

minutes (λ_{ex} = 360 nm, λ_{em} = 460 nm) at 24 ^{o}C. The percent activity of STS in the presence of inhibitor after each time interval was calculated as a percentage of activity in the absence of inhibitor.

4.5.3.6 Dialysis Experiment

STS (400 nM) was incubated with compound **4.50** (1 μM) in assay buffer (200 μL) for 1 hour. A control was also performed in an identical manner except that it did not contain inhibitor. 4 μL aliquots were withdrawn and STS activity was determined as described in § **4.5.3.2**. After no more than 15% of activity was remaining, the remaining incubation mixture was dialyzed in micro-dialysis units into 1 L of 0.1 M tris, pH 7, 0.1 % Triton at 1-4°C. The dialysis proceeded for 24 hours with the dialysis buffer changed after 3, 6, 9, and 12 hours. After 24 h, aliquots (4 μL) were withdrawn from the incubation mixture and diluted into 196 μL of 4 mM MUS in 0.1 M tris, pH 7 and STS activity followed as described in § **4.5.3.2**. More than 70% of STS activity was recovered with compound **4.50**.

4.5.4 Molecular Modeling (Docking) Experiments

Docking experiments were performed on compounds **4.3**, **4.10**, **4.17**, **4.27**, **4.30**, and **4.49** using Discovery Studio Client v2.5.0.9164 (2005-09), Accelrys Software Inc. as described in § **3.5.4.**

4.5.5 In Vitro Screening with the NCI-60 panel

4.5.5.1 General Method

The NCI screen is performed as follows. Cell suspensions are added by pipet (100 μL) into 96-well microtiter plates. Inoculates were allowed a preincubation period of 24 h at 37 °C

for stabilization. Dilutions at twice the intended inhibitor concentration were added at t = 0 in 100 µL aliquots to the microtiter plate wells. Incubations lasted for 48 h in a 5% CO_2 atmosphere and 100% humidity. The cells were assayed by using a sulforhodamine B assay. A plate reader was used to read the optical densities. From this data GI_{50}, TGI or LC_{50} values are calculated.

The GI_{50} is the concentration of inhibitor that causes 50% growth inhibition or the concentration of inhibitor where 100 × (T - T0)/(C - T0) = 50. The optical density of the test well after a 48-h period of exposure to test drug is T, the optical density at time zero is T0, and the control optical density is C. The ``50" is called the GI50PRCNT that can have values from +100 to -100. The TGI is the concentration of test drug where 100 × (T - T0)/(C - T0) = 0. Thus, the TGI signifies a cytostatic effect. The LC_{50}, which signifies a cytotoxic effect, is the concentration of drug where 100 × (T - T0)/T0 = -50. The control optical density is not used in the calculation of LC_{50}. It is the concentration of inhibitor that causes 50% cell death.

4.5.5.2 The In-Vitro Testing Results Data Sheet

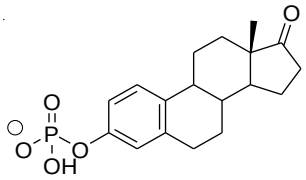
This page of the data package presents the experimental data collected against each cell line. The first two columns describe the subpanel (e.g. leukemia) and cell line (e.g. CCRF-CEM) involved. The next two columns list the Mean OD_{tzero} and Mean OC_{ctrl}: the next five columns list the Mean OD_{test} for each of five different concentrations. Each concentration is expressed as the $\log_{10}$ (molar or µg/ml). The next five columns list the calculated PGs for each concentration. The response parameters GI_{50}, TGI, and LC_{50} are interpolated values representing the concentrations at which the PG is +50.0, and -50, respectively. Sometimes these response parameters cannot be obtained by interpolation. If, for instance, all of the PGs in a given row exceed +50, then none of the three parameters can be obtained by interpolation. In such a case, the value given for each response parameter is the highest concentration tested and is preceded by a “>” sign. This

practice is extended similarly to the other possible situations where a response parameter cannot be obtained by interpolation, as shown in Appendix **A** and **B**.

Chapter 5 - Towards the Synthesis of an E1S Analog Bearing a Thiadiazolidinedione Sulfate Mimic and a 3-*O*-Sulfamate of a 17β-Arylsulfonamide STS Inhibitor

5.1. Introduction

In *Chapter* 1 (§ **1.3.1.1**, Table 1.2) we discussed **E1S** derivatives bearing non-hydrolyzable sulfate mimics as inhibitors of STS. With the exception of the sulfamate group (H_2NO_2SO) and a few other mimics, this was not a particularly effective approach for obtaining STS inhibitors. The phosphate group proved to be one of the more effective mimics in that the monoanionic form of estrone-1-phosphate (**E1P**, **1.14**, Fig. 5.1) was a reversible competitive inhibitor with a K_i = 2-4 μM at pH 7 and exhibited an affinity for STS that was similar to that of the natural substrate, **E1S**. However, the use of this group as a sulfate mimic is very limited in terms of drug development as it would be labile to phosphatases and might prevent movement of such inhibitors across cell membranes.

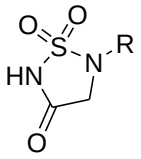


1.14 (**E1P**)

Fig. 5.1 Structure of **E1P**.

It is possible that a good phosphate mimic that is less highly charged than a phosphate group may act as a good sulfate mimic for STS inhibition. The question is how does one evaluate and chose a phosphate mimic for a sulfatase? One approach is to look at inhibitors of phosphatases. Perhaps it is not particularly surprising that the phosphate group is a good sulfate mimic for STS inhibition as it has been recently reported that arylsulfatases and phosphatases are

evolutionarily related and that arylsulfatases have evolved from phosphatases.[203] There are multiple phosphate mimics that have been examined in the context of protein tyrosine phosphatase (PTP) inhibition.[204] Among the most effective are specific five-membered ring heterocycles, such as the thiadiazolidinedione group (**5.1**, Fig. 5.2). Compounds bearing this group exhibit higher affinity for PTP's and have better cell permeability than the analogous compounds bearing a phosphate group.[205] Since the phosphate group is a good sulfate mimic in the context of STS inhibition then it is possible that the thiadiazolidinedione group may also be a good sulfate mimic.



5.1

Fig. 5.2. Structure of a thiadiazolidinedione group, **5.1**

As mentioned above, one of the few other effective sulfate mimics (in the context of STS inhibition), besides the phosphate group, is the sulfamate group. Compounds bearing this group, such as EMATE, are usually irreversible inhibitors of STS. However, whether the sulfamate group is a true sulfate mimic is debatable since the mechanism by which the sulfamates inhibit STS and other sulfatases is still not known. Cleavage of the S-O bond in the sulfamates by sulfatases is necessary for irreversible inhibition and biphasic kinetics are always observed. Using Tritiated EMATE (labeled on the steroid portion), it has been shown that no association of the labeled inhibitor with inactivated STS was detected indicating that the estrone portion is released after cleavage of the ArO-S bond.[44] The stoichiometry of the inactivation process was reported to be 3-6 as determined by quantifying the release of the parent phenol with the highest values being observed for the most potent inactivators.[206] This result implies that multiple

sulfamoylation events occur during the inactivation process. On the basis of these studies an inactivation mechanism has been proposed in which elimination of the phenolic portion occurs, assisted by active site acid and base residues, and the amino sulfene, NH_2SO_2, is formed (Fig. 5.3). The reactive amino sulfene then reacts non-specifically with active site residues. Further support for the elimination mechanism shown in **Fig. 5.3** is that *N*,*N*-dialkyl-substituted sulfamates are reversible inhibitors of STS.[57]

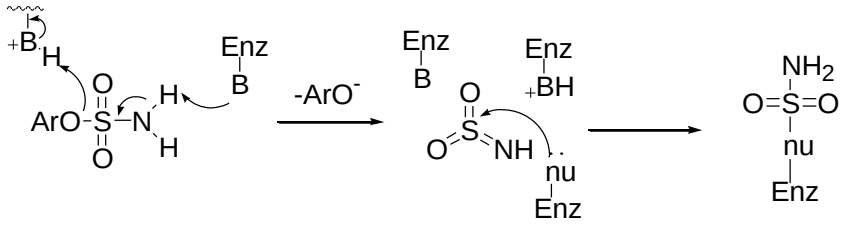


Fig. 5.3. Proposed mechanism for the inactivation of sulfatases by sulfamates.

We wish to learn more about how our 17β-arylsulfonamide inhibitors, described in *Chapters* **3** and **4**, interact with STS in comparison to sulfamoylated inhibitors such as EMATE. Would 3-*O*-sulfamoylation of our 17β-arylsulfonamide inhibitors yield irreversible inhibitors or would the sulfamoyl group have little or no effect on inhibition as was the case when we prepared the 3-*O*-sulfated 17β-arylsulfonamide inhibitors (compounds **4.54**-**4.57**, Table 4.2). Should 3-*O*-sulfamoylation have no little or no effect on our 17β-arylsulfonamide inhibitors then it would suggest that these inhibitors are not occupying any or, only part of, the active site.

5.2. Objectives

The objectives of the work in this chapter were two-fold. One was to evaluate the thiadiazolidinedione group **5.1** as a sulfate mimic for STS inhibition. This was to be achieved by synthesizing compound **5.2** and evaluating it as an STS inhibitor (Fig. 5.4). The other was to evaluate the sulfamate group as a sulfate mimic in the context of our 17β-arylsulfonamide

inhibitors described in *Chapters* 3 and 4 (compounds of type **5.3**). Below we report our preliminary work towards attaining these objectives.

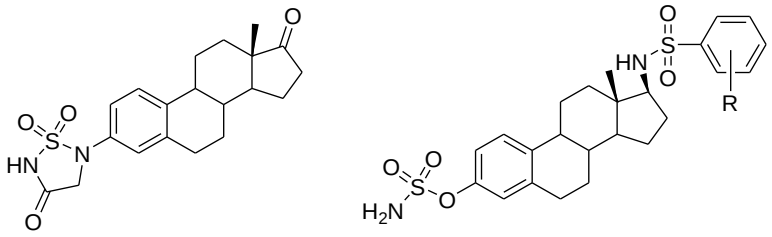
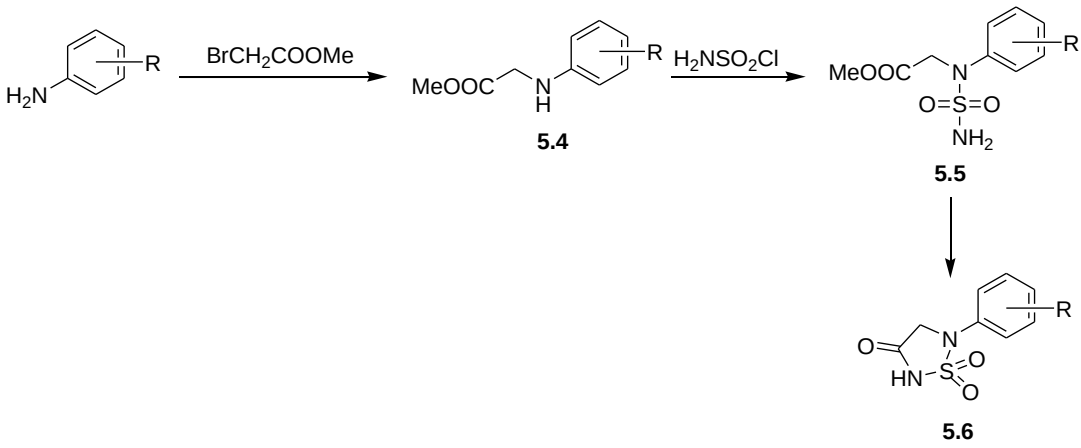


Fig. 5.4. Compounds **5.2** and the general structure of sulfamates **5.3**.

5.3. Results and Discussion

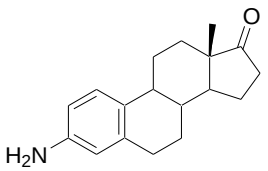
5.3.1 Towards the synthesis of 5.2

Aryl thiadiazolidinediones are prepared by the route outlined in Scheme 5.1. An aniline derivative is alkylated with the methyl ester of α-bromoacetate to give compound **5.4**. Reaction of **5.4** with sulfamoyl chloride gives compound **5.5,** Scheme 5.1.



Scheme 5.1. General route to the synthesis of aryl thiadiazolidinediones

Treatment of **5.5** with base results in cyclization and formation of the desired aryl thiadiazolidinedione **5.6**. Consequently, to prepare compound **5.2** the first step was to prepare 3-aminoestrone (**5.7**, Fig. 5.5).



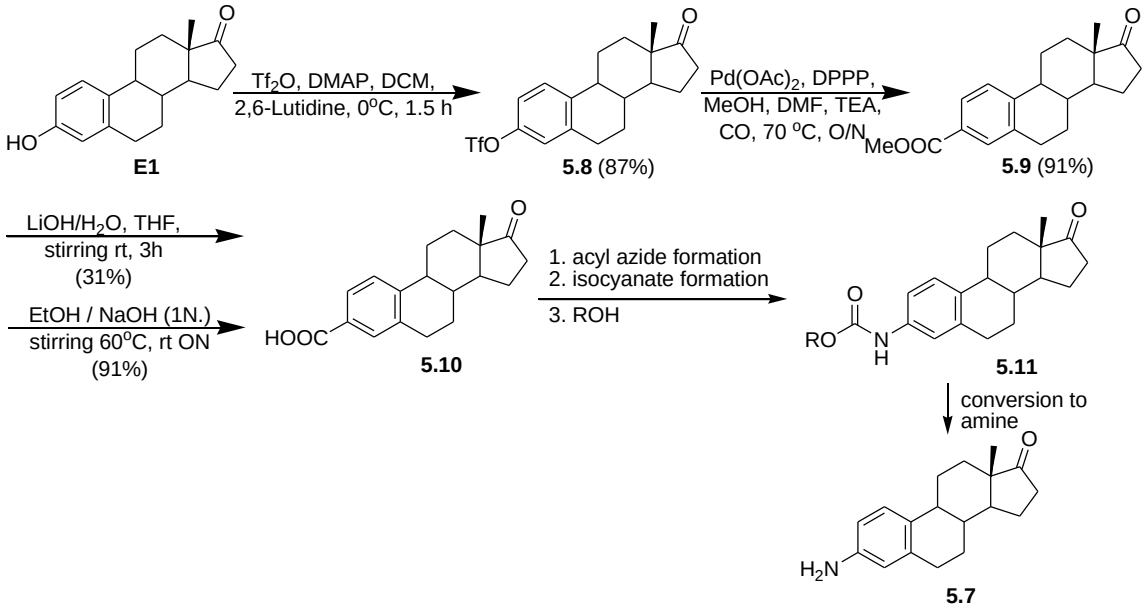
5.7

Fig. 5.5. Structure of 3-amino estrone **5.7**.

3-Aminoestrone is a non-natural C-18 steroid and has been used as a key intermediate for the synthesis of biologically active steroid derivatives. Unfortunately it is not commercially available. After examining literature syntheses of **5.7** we concluded that both the classical and recently described synthetic methods had some serious drawbacks such as poor yields or requiring expensive catalysts and/or ligands.[53,207-212]

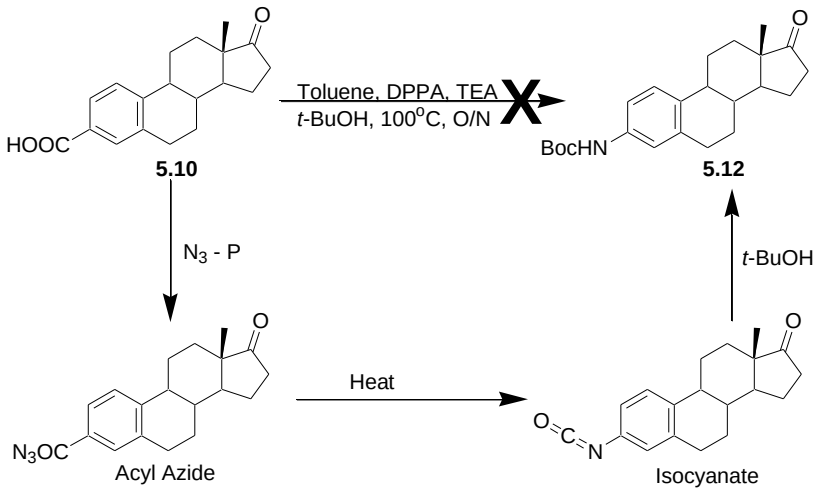
We decided to prepare **5.7** by a new approach in which acid **5.10** would be converted to an acyl azide followed by a Curtius rearrangement (Scheme 5.2). The resulting isocyanate would be trapped with an alcohol to give a carbamate (**5.11**) which would be converted into **5.7** (Scheme 5.2). The synthesis began with the construction of triflate **5.8** using a literature procedure.[49] Thus, estrone was treated with triflic anhydride (Tf_2O), 4-dimethylaminopyridine (DMAP), and 2,6-lutidine to give triflate **5.8** in a 87% yield. Palladium catalyzed carboxylation of **5.8** using $Pd(OAc)_2$/DPPP and CO gave the corresponding methyl ester **5.9** in 91% yield.[49] Our first attempt to hydrolyze ester **5.9** using aqueous LiOH (2N) solution resulted in just a 31%

conversion to the corresponding acid, **5.10**, however; using a stronger base, ethanolic NaOH (1N), we obtained a 91% yield of **5.10**.



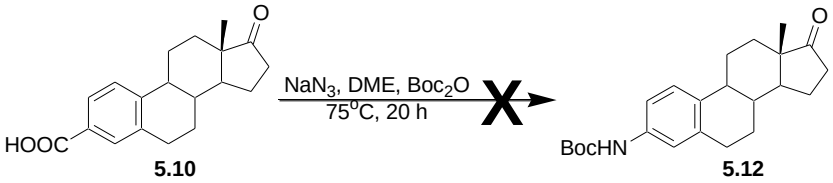
Scheme 5.2. Synthesis of acid **5.10** and its proposed conversion to **5.7**.

We anticipated that the conversion of the carboxylic acid **5.10** to the corresponding 3-amino derivative **5.7** would be a straightforward. We first tried to prepare the Boc-protected amine, **5.12**, from acid **5.10**. Acid **5.10** was reacted with diphenyl phosphoryl azide (DPPA) to give the acyl azide (Scheme 5.3). However, we did not obtain compound **5.12**, after heating of the acyl azide followed by trapping of the resulting isocyanate with *t*-BuOH in presence of toluene and TEA.



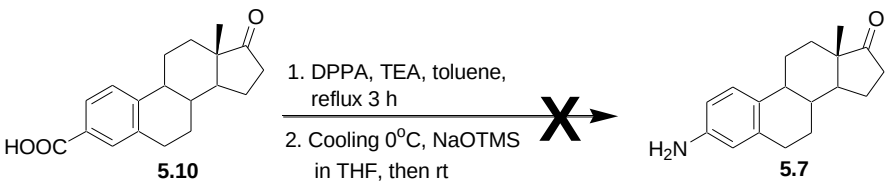
Scheme 5.3. First attempt to prepare Boc-protected amine **5.12**.

We tried another approach to **5.12** by reacting **5.10** using NaN_3 and di-*t*-butyldicarbonate (Boc_2O) in 1,2-dimethoxy ethane (DME) but still none of the desired carbamate was formed (Scheme 5.4).[213]



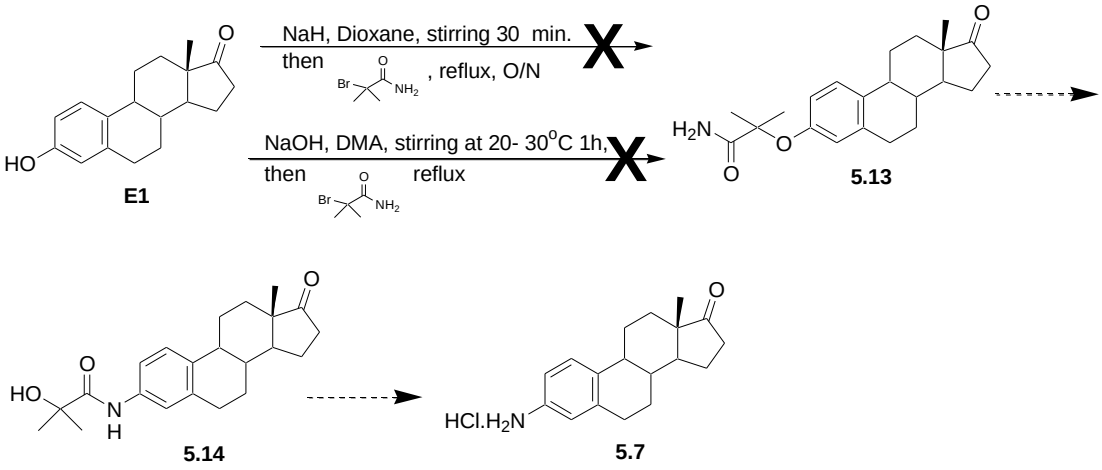
Scheme 5.4. Second attempt to prepare **5.12**.

We also tried trapping the isocyanate with sodium trimethylsilanolate (NaOTMS, prepared from hexamethyldisiloxane)[214] as this tactic has been used as a one-pot synthesis of free amines from carboxylic acids (Scheme 5.5),[215] however; this also did not work.



Scheme 5.5. Attempted preparation of **5.7** by trapping the isocyanate with NaOTMS.

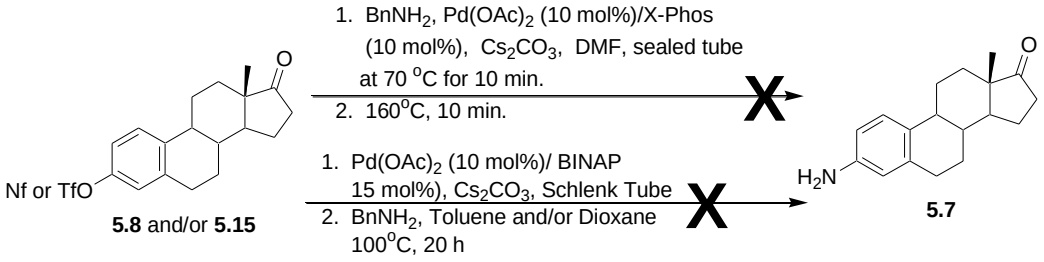
Peet and coworkers reported the synthesis of the hydrochloride salt of **5.7** in an unspecified yield from **E1** via Smiles rearrangement of ether **5.13** followed by hydrolysis of the resulting amide **5.14** (Scheme 5.6).[216] We decided to attempt to prepare **5.7** using this approach. Although Peet and coworkers reported a one-pot synthesis of ether **5.14** in a 59% yield,[216] we were unable to obtain any significant quantities of this compound.



Scheme 5.6. Attempted synthesis of **5.7** via a Smiles rearrangement.

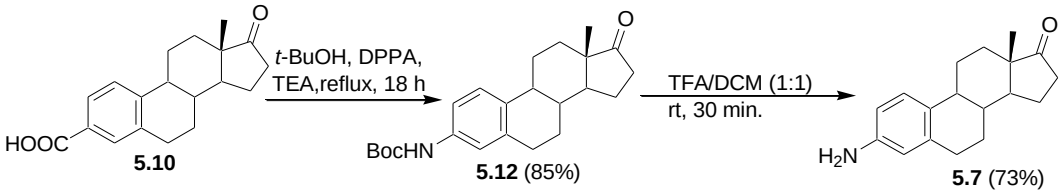
Our next approach to **5.7** was to use a Pd(0)-catalyzed amination reaction as this approach was reported by several groups as an efficient route to **5.7** (Scheme 5.7).[210,211] One of these reports used triflate **5.8** as substrate. In our hands this reaction resulted mainly in the formation of **E1**, generated by the hydrolysis of triflate **5.8** under the alkaline reaction

conditions, and only trace amounts of **5.7** were obtained. Another report claimed that nonaflate **5.15** gave better yields due to its greater hydrolytic stability.[217] However, as with the triflate **5.8**, very little of the desired product was obtained.



Scheme 5.7. Attempted synthesis of **5.6** via a Pd(0)-catalyzed amination reaction.

We then turned our attention back to the acyl azide/Curtius rearrangement approach. We found a procedure which reported the synthesis of Boc carbamates from carboxylic acids in high yields by forming the acyl azides and performing the Curtius rearrangements in *t*-BuOH without the use of other solvents.[218] Using this procedure carbamate **5.12** was obtained in a 85% yield (Scheme 5.8). Deprotection of **5.12** was achieved in a 73% yield using TFA in DCM.[219] The overall yield of **5.7** from E1 was 53%.

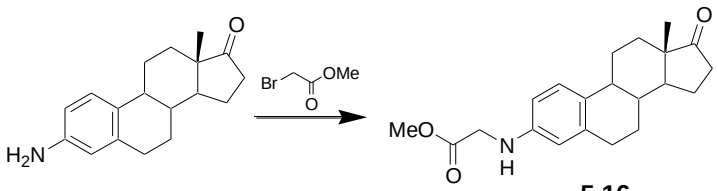


Scheme 5.8. Synthesis of carbamate **5.12** and the target amine **5.7**.

With a good synthesis of **5.7** in hand we then proceeded to the alkylation step using the methyl ester of α-bromoacetate. Unfortunately, we have not been able to get this reaction to proceed in a significant yield under a variety of reaction conditions (Table 5.1). We attempted

this reaction using aniline as a model substrate and the conditions outlined in entry 6 in Table 5.1. In this case we obtained a 62% yield of the alkylated product. This suggests that steric hindrance might be affecting our ability to alkylate **5.7**. The synthesis of compound **5.16** is still in progress in the Taylor group

Table5.1. Alkylation of **5.7** under different conditions.



5.7 **5.16**

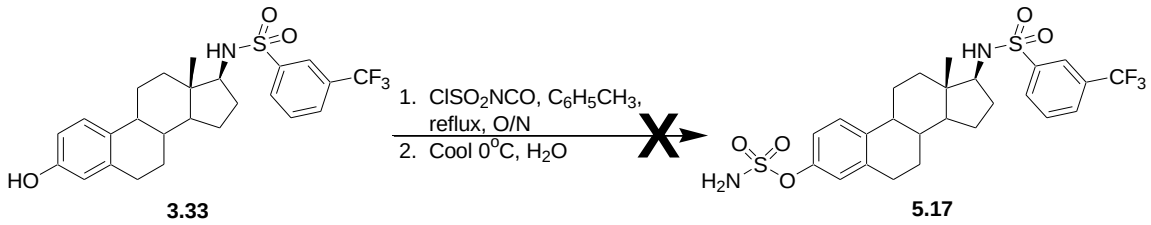
Entry	Base	Equiv	Equiv. ($BrCH_2COOMe$)	Solvent	Temp (^oC)	Time (h)	% Yield
1	NaH	1.5	1.2	DMF	rt	48	-
2	NaH	1.5	1.2	DMF	reflux	6	11.5
3	NaH	1.5	1.2	DMSO	rt	96	-
4	NaH	1.5	1.2	DMSO	50	48	-
5	K_2CO_3	1.2	1.5	MeOH	rt	20	11.0
6	DIPEA	2.5	1.0	DMF	60	24	7.0

5.3.2 Attempts to prepare a sulfamate derivative of a 17β-arylsulfonamide inhibitor.

Sulfamoylation of phenolic hydroxyl groups is achieved using either sulfamoyl chloride ($ClSO_2NH_2$), with or without the aid of a base, or by reaction with chlorosulfonyl isocyanate (CSI, $ClSO_2NCO$) followed by hydrolysis of the isocyanate group. Although sulfamoylation with sulfamoyl chloride is the more direct approach, sulfamoyl chloride is very expensive. Although it can be readily prepared it does not store well and so it is usually not isolated and is

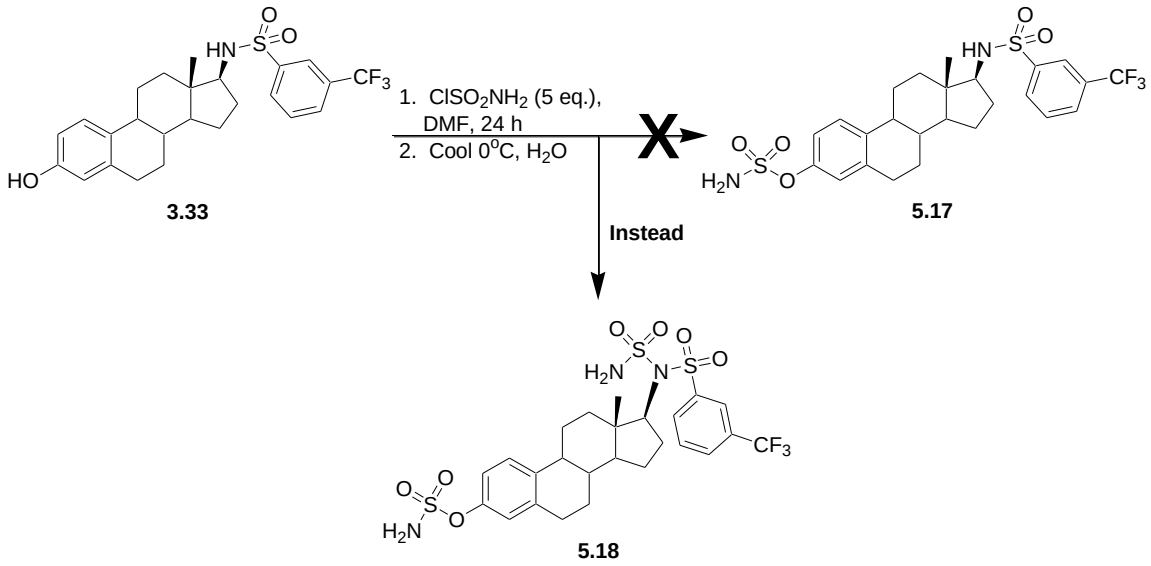
used immediately after its preparation. The CSI approach tends to be more widely used as CSI is commercially available and much less expensive and more stable than sulfamoyl chloride.

We started with the sulfamoylation of one of our most potent sulfonamide inhibitors, the 3-CF_3-benzene sulfonamide **3.33** using CSI as shown in Scheme 5.9. Unfortunately, the reaction gave a mixture of many products, which were difficult to separate and none of them were our desired sulfamate. It appeared that some sulfamoylation was occurring at the 2-position of the A-ring and none of the desired product, **5.17**, was obtained.



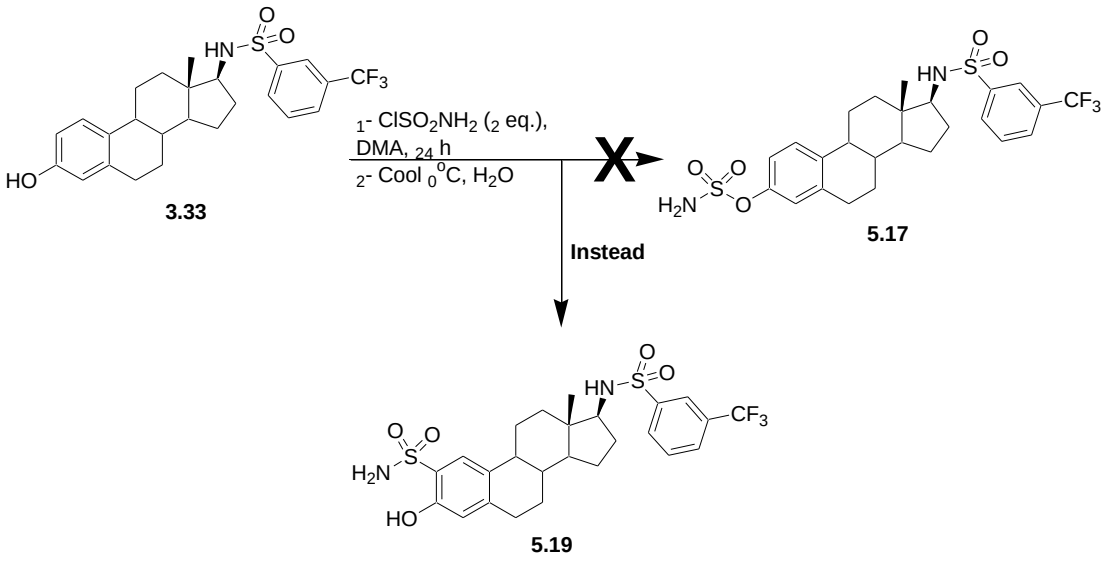
Scheme 5.9. First approach to the synthesis of the sulfamate derivative of compound **3.33**.

Our next approach was to use sulfamoyl chloride itself which we generated in situ from CSI and formic acid in DCM.[220] A solution of sulfonamide **3.33** in DMF was added drop-wise to the sulfamoyl chloride solution in presence of TEA at 0 ^oC. Since sulfamoylation of phenols is usually carried out with a considerable excess of sulfamoyl chloride, due to reaction of sulfamoyl chloride with DMF, we also used an excess of this reagent (5-fold excess over phenolic derivative). Not surprisingly sulfamoylation occurred at two positions; the 3-OH and at the NH of the sulfonamide group, to give compound **5.18** (Scheme 5.10).



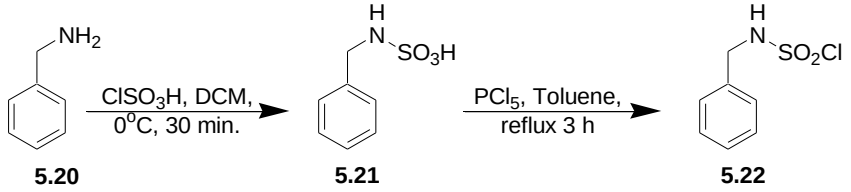
Scheme 5.10. Sulfamoylation of **3.33** with sulfamoyl chloride.

Okada and coworkers reported that *N*,*N*-dimethylacetamide (DMA) is a better solvent than DMF for carrying out sulfamoylation reactions claiming that sulfamoyl chloride is more stable in DMA than DMF and so the amount of sulfamoyl chloride can be reduced.[221] We used their procedure using 2 equivalents of sulfamoyl chloride. This time we obtained a product with a sulfamoyl group at the 2-position of A-ring (**Scheme 5.11**).

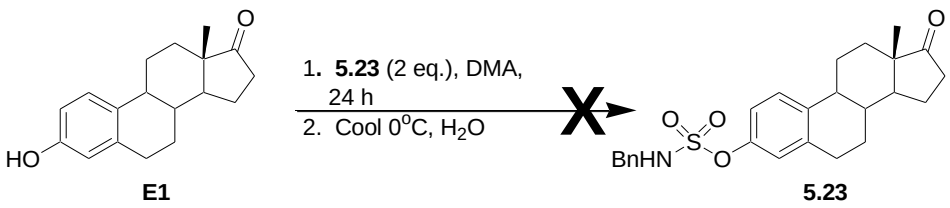


Scheme 5.11. Sulfamoylation of **3.33** by sulfamoyl chloride (2 equiv) in DMA.

We reasoned that a sterically hindered form of sulfamoyl chloride; such as benzyl derivative **5.22**, would be more selective for the less sterically hindered 3-OH group. After sulfamoylation the benzyl group would then be removed by hydrogenolysis. Compound **5.22** was prepared by a literature route (**Scheme 5.12**).[222] However, using **E1** as a model substrate; we were unable to obtain any sulfamoylated product (no reaction occurred) using this reagent (**Scheme 5.13**). The synthesis of compound **5.17** is still in progress in the Taylor group.



Scheme 5.12. Preparation of benzyl sulfamoyl chloride, compound **5.22**.



E1 **5.23**

Scheme 5.13. Attempted sulfamoylation of **E1** using **5.23** in DMA.

5.3. Conclusions and Future Work

The thiadiazolidinedione group was proposed as a sulfate mimic for obtaining STS inhibitors. A new approach to the synthesis of 3-amino estrone (**5.7**) was achieved as part of an attempt to prepare the thiadiazolidinedione target, compound **5.2**. This new approach made use of a Curtius rearrangement of an acyl azide precursor followed by trapping of the resulting isocyanate with t-butyl alcohol to give a t-butyl carbamate which was then converted to **5.7** using TFA. Unfortunately, conversion of compound **5.7** into compound **5.2** was not achieved. This is due to difficulties in achieving reasonable yields for the alkylation of **5.7** with the methyl ester of α-bromoacetate. We believe that this reaction can accomplished in reasonable yield though it will require more screening of alternate reaction conditions such as using other bases, solvents, reaction temperatures etc. These studies are in progress in the Taylor group.

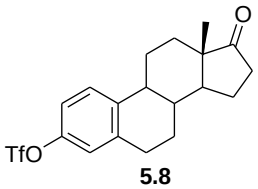
3-*O*-Sulfamoylation of sulfonamide **3.33** was attempted using a variety of reaction conditions. Unfortunately, side reactions have prevented us from achieving the synthesis of the target compound **5.17**. Nevertheless, we believe that this reaction can be accomplished in reasonable yield although it will require more screening of alternate reaction conditions such as using other bases, solvents, reaction temperatures etc. The use of sterically hindered organic base such as 2,6-lutidine may be useful here as the N-H of the sulfonamide group is more sterically hindered than the 3-OH of **3.33**. These studies are in progress in the Taylor group.

5.4. Experimental

5.4.1 General

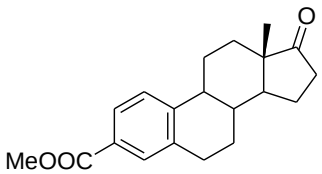
All starting materials and reagents were obtained from Aldrich Chemical Company. THF was distilled from sodium-benzophenone, DCM was distilled from calcium hydride under nitrogen. Benzylamine was dried by distillation from KOH pellets and stored in dark over type 4A molecular sieves. Dioxane was distilled from Na^o and stored over type 4A molecular sieves. *t*-Butyl alcohol was dried by distillation and standing over type 3A molecular sieves. Silica gel chromatography was performed using silica gel (60Å, 230-400 mesh) obtained from Silicycle (Laval, Quebec, Canada). ^1H, ^{13}C, and ^{19}F NMR spectra were recorded on a Bruker Avance 300 spectrometer. For NMR spectra obtained using $CDCl_3$ as the solvent, chemical shifts (δ) for ^1H NMR spectra are reported relative to internal Me_4Si (δ 0.0 ppm), chemical shifts for ^{13}C spectra are relative to the residual solvent peak (δ 77.0 ppm, central peak), and chemical shifts for ^{19}F NMR are relative to a $CFCl_3$ (δ 0.0 ppm) external standard. Low-resolution (LRMS) and high-resolution (HRMS) electron impact (EI) and electrospray ionization (ESI) mass spectra were obtained on a JEOL HX110 double focusing mass spectrometer. Electrospray (ESI) mass spectra were obtained with a Waters/Micromass QTOF Ultima Global mass spectrometer. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected.

5.4.2 Syntheses



5.8

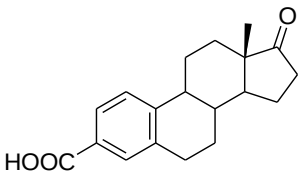
Estra-1,3,5(10)-trien-17-one-3-trifluoromethanesulfonate (5.8). To a solution of **E1** (5.0 g, 18.4 mmol), DMAP (0.6 g, 4.6 mmol, 0.25 equiv) and 2,6-lutidine (4.6 mL, 4.0 mmol, 2.1 equiv) in DCM (140 mL) at 0 ^{o}C was added triflic anhydride (3.5 mL, 22.1 mmol, 1.2 equiv) slowly. After addition, the reaction mixture was stirred for 70 min at 0 ^{o}C before quenching with 2 M HCl (30 mL) at 0 ^{o}C. The organic layer was separated and washed with 2N HCl, 7.5% $NaHCO_3$, and brine, then dried with Na_2SO_4 and concentrated. Purification by flash chromatography (ethyl acetate/hexane, 1:9 to 1:5), afforded **5.8** as a white solid, which was recrystallized from hexane to give E1-3-triflate, **5.8** as white crystals (6.4 g, 87%). Mp 77-78^{o}C (lit. 75-77^{o}C);[49] ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.33 (d, *J* = 8.7 Hz, 1H, H-1), 7.01 (d, *J* = 8.7 Hz, 1H, H-2), 6.99 (m, 1H, H-4), 2.95-2.91 (m, 2H), 2.55-1.95 (m, 7H), 1.69-1.57 (m, 7H), 0.91 (s, 3H, CH_3, H-18); ^{19}F NMR (CDCl3, 282 MHz) δ -73.2**.**



5.9

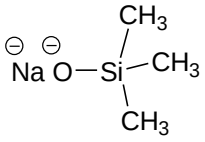
3-Methoxycarbonylestra-1,3,5(10)-trien-17-one (5.9). To a mixture of E1-3-triflate **5.8** (1.0 g, 3.2 mol), $Pd(OAc)_2$ (35.5 mg, 0.2 mmol) and 1,3-bis(diphenylphosphino) propane (DPPP, 60 mg, 0.14 mmol) in a 250 mL round bottom flask under argon was added DMF (5 mL), MeOH (5 mL) and TEA (1 mL, 7.6 mmol, 2.4 equiv) successively. After purging with CO, the reaction mixture was heated at 70 ^{o}C under CO (balloon) overnight (16 h) and then cooled to rt. The mixture was diluted with water, extracted with ether (2×5ml), the combined extracts were washed with 2N HCl (10 mL), sat. $NaHCO_3$ (10 mL), and brine, then dried with Na_2SO_4) and concentrated. Purification by recrystallization from MeOH afforded **5.9** as a white solid (91%

yield). Mp 134-136°C (lit. 134-135 °C);[49] ^{1}H NMR (CDCl3, 300 MHz) δ 7.70 (d, *J* = 9.3 Hz, 2H, H-2 and H-4), 7.25 (m, 1H, H-1), 3.80 (s, 3H, CO_2CH_3), 2.87-2.84 (m, 2H), 2.47-1.86 (m, 7H), 1.60-1.38 (m, 6H), 0.87 (s, 3H, CH_3, H-18).



5.10

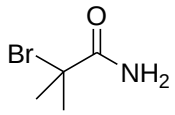
3-Carboxy-1,3,5(10)-estratrien-17-one (5.10). ***Method A*** To a stirred solution of **5.9** (500 mg, 1.6 mmol) in THF (15 mL), was added a solution of LiOH (677 mg, 16 mmol) in water (10 mL), and stirring was continued at room temperature for 3 h. Then the reaction was neutralized by 1N HCl, the precipitate formed was filtered, washed twice with water to afford **5.10** as a white solid (148 mg, 31%). ***Method B*** To a stirred solution of **5.9** (1 g, 3.2 mmol) in ethanol (150 mL), was added aqueous solution of 1N NaOH (42 mL), and stirring was continued at 60 °C for overnight. After that, the reaction was cooled to 0 °C, and neutralized by 1N HCl, and the precipitate formed was filtered, washed with water to afford **5.10** as a white solid (870 mg, 91%). Mp: 318-320 °C;[223] ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.87-7.83 (m, 2H, ArH), 7.38 (d, J = 7.2 Hz, 1H, ArH), 2.96 (m, 2H, H-6), 2.55-2.34 (m, 3H), 2.18-1.96 (m, 4H), 1.60-1.52 (m, 6H), 0.91 (s, 3H, H-18).



NaOTMS

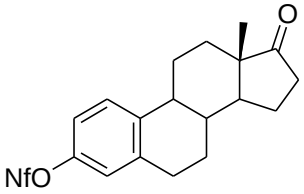
Preparation of Sodium Trimethylsilanolate (NaOTMS).[214] To a stirred solution of hexamethyldisiloxane (4 g, 0.02 mol) in 1,2-dimethoxyethane (25 mL), NaOH (2.0 g, 0.05 mol)

was added. The reaction mixture was vigorously stirred at reflux temperature for 72 h. After that, the mixture was filtered and the crude residue was washed with boiling DME (2×50 mL). The filtrate was evaporated to dryness. Crude product was azeotropically dried with toluene (2 x 100 mL) affording NaOTMS as white crystals (2 g, 37% yield). Mp: > 300^{o}C (lit. > 395^{o}C).



2-Br-2-methyl propionamide

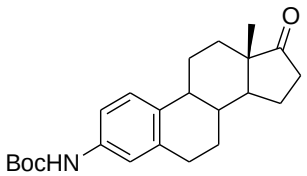
2-Bromo-2-methylpropionamide (5.12).[216] To a stirred solution of bromoisobutyryl bromide, **5.11** (2.6 g, 40 mmol) in hexane (30 mL) at 0 ^{o}C, was added conc NH_4OH solution portion-wise over 30 min, and stirring was continued for additional 30 min at the same temperature. The resulting white precipitate was filtered, washed with cold water, and dried under high vacuum to afford **5.12** as a white solid (1.8 g, 100%). Mp 146-147 ^{o}C (lit. 146-148 ^{o}C); ^{1}HNMR ($CDCl_3$, 300 MHz) δ 6.50 (brs, 1H, NH_2), 5.65 (brs, 1H, NH_2), 1.95 (s, 6H, 2 CH_3).



5.15

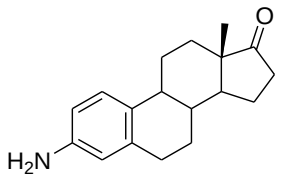
Estra-1,3,5(10)-trien-17-one-3-nonafluorobutane-1-sulfonate (5.15).[217] To a solution of **E1** (2.1 g, 7.8 mmol) in DCM (100 mL), was added TEA (0.25 mL, 11.6 mmol), then nonafluorobutanesulfonyl fluoride (0.6 mL, 11.6 mmol) was added, and stirring was continued for 20h. After that, the reaction was washed twice NaOH (5%, 10 mL) and water, then, the organic layer was dried with Na_2SO_4, and finally concentrated under vacuum. Purification was done by flash chromatography (ethyl acetate/hexane, 1:4) to afford **5.15** as white plates (1.0 g,

23%). Mp: 177-178°C ; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.31 (d, *J* = 8.6 Hz, 1H, H-1), 7.03-6.97 (m, 2H, H-2 and H-4), 2.94-2.90 (m, 2H, H-6), 2.47-1.94 (m, 7H), 1.61-1.44 (m, 6H), 0.89 (s, 3H, H-18); ^{19}F NMR ($CDCl_3$, 282 MHz) δ -80.5, -108.8, -120.8, -125.7.



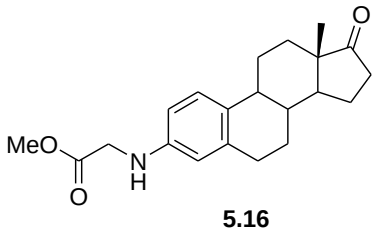
5.12

3-(*t*-Butyloxycarbonyl)-amino-1,3,5(10)-estratrien-17-one (5.12). To a stirred solution of **5.10** (750 mg, 2.5 mmol) in *t*-BuOH (60 mL), was added TEA (75 µL, 5.4 mmol), and stirring was continued for 30 min., after that, DPPA (60 µL, 2.7 mmol) was added, and the mixture was refluxed for 18-20 h. The solvent was then removed under vacuum, and the residue was dissolved in ethyl acetate, washed with water (50 mL), dried with Na_2SO_4, and finally concentrated under vacuum. Purification was done by flash chromatography (ethyl acetate/hexane, 1:4) to afford **5.12** as a white solid (790 mg, 85%). Mp: 199-201°C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.18-7.15 (m, 2H, ArH), 7.01 (d, J = 8.4 Hz, 1H, ArH), 6.45 (s, 1H, NH), 2.87-2.85 (m, 2H, H-6), 2.46-2.34 (m, 2H), 2.14-1.91 (m, 5H), 1.59-1.25 (m, 6H), 0.88 (s, 3H, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 220.9 (C-17), 152.8 (NHCO), 137.3 (C-5), 136.0 (C-3), 134.6 (C-6), 125.8 (CH_{Ar}), 118.8 (CH_{Ar}), 116.2 (CH_{Ar}), 50.4, 47.9 ($\underline{C}(CH_3)_3$), 44.1, 38.2, 35.8, 31.5, 29.5, 28.3 ($C(\underline{C}H_3)_3$), 26.5, 25.8, 22.1, 21.5, 13.8 (CH_3, C-18); LRMS (ESI^+) *m*/*z* (%) 370 (M+H, 42), 369 (M, 94), 367 (100%); HRMS (ESI^+) calcd for $C_{23}H_{32}NO_3$ $(M+H)^+$ 370.23767; found 370.23757.



5.7

3-Amino-1,3,5(10)-estratrien-17-one (5.7). ***Method A*** A stirred solution of **5.12** (100 mg, 0.27 mmol) in ethanol/(6 M) HCl mixture (5 mL, 1:1) was heated under reflux for 6 h, then solvent was removed under vacuum, and residue was dissolved in water (5 mL), neutralized with 2 M NaOH, and precipitate formed was filtered and washed twice with water giving **5.7** as a white solid (25 mg, 11%). ***Method B*** To a stirred solution of **5.12** (300 mg, 0.8 mmol) in DCM (5 mL), was added TFA (5 mL) and stirring was continued for 0.5 h, then solvent was removed, and residue was dissolved in ethyl acetate, washed with sat. $NaHCO_3$ solution, water, and brine, dried by Na_2SO_4, and finally concentrated under vacuum. Purification was done by flash chromatography (methanol/chloroform, 3:7) to afford **5.7** as a white solid (160 mg, 73%). Mp: 197-199^{o}C (lit. 199-200);[212] ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.06 (d, *J* = 8.2 Hz, 1H, H-1), 6.50 (dd, *J* = 2.3 and 8.2 Hz, 1H, H-2), 6.44 (brs, 1H, H-4), 3.49 (brs, 2H, NH_2), 2.84-2.79 (m, 2H, H-6), 2.52-2.44 (m, 1H), 2.36-2.33 (m, 1H), 2.20-1.90 (m, 5H), 1.60-1.36 (m, 6H), 0.89 (s, 3H, H-18).



5.16

(Estra-1,3,5(10)-trien-17-on-3-ylamino)acetic acid methyl ester (5.16). ***Method A*** To a stirred solution of **5.7** (100 mg, 0.37 mmol) in acetone (10 mL) was added K_2CO_3 (102.3 mg,

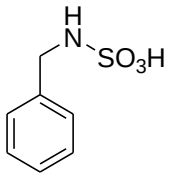
0.74 mmol), and stirring was continued for 30 min., after that methyl bromoacetate (53 µL, 0.55 mmol) was added. Stirring was continued for another 30 min at room temperature, and under reflux for 2 h, and then left at room temperature for overnight. The solvent was then removed, water (10 mL) was added, and the mixture was extracted with DCM (2x5 mL), dried with Na_2SO_4, and finally concentrated. ***Method B*** To a stirred solution of **5.7** (250 mg, 0.9 mmol) in DMF (20 mL) was added NaH (33 mg, 1.4 mmol) at 0 ^oC, and stirring was continued for 30 min., after that methyl bromoacetate (0.25 mL, 1.1 mmol) was added. Stirring was continued for another 30 min at room temperature, and under reflux for 6 h. after that the reaction mixture was cooled to room temperature and poured into ice-water mixture, extracted with ethyl acetate (2×10 mL), washed with brine, dried with Na_2SO_4, and finally concentrated under vacuum. ***Method C*** to a stirred solution of **5.7** (50 mg, 0.18 mmol) in DMF (5 mL), was added DIPEA (78 µL, 0.45 mmol) at 0 ^oC, and stirring was continued at oC for an hour, after that it was heated at 60 ^oC for 40 h. the reaction was then cooled to room temperature, and poured into an ice-water mixture, washed with sat. NH_4Cl, brine, dried with Na_2SO_4, and concentrated under vacuum. Purification by flash chromatography (ethyl acetate/hexane, 3:7) afforded **5.16** as a white solid. Mp: 189-191^oC; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.11 (d, J = 8.5 Hz, 1H, H-1), 6.45 (dd, J = 2.3 and 8.3 Hz, 1H, H-2), 6.37 (brs, 1H, H-4), 4.24 (s, 1H, NH), 3.89 (s, 2H, $NHC\underline{H}_2COOCH_3$), 3.76 (s, 3H, $NHCH_2COOC\underline{H}_3$), 2.85-2.82 (m, 2H, H-6), 2.47-1.91 (m, 7H), 1.57-1.44 (m, 6H), 0.88 (s, 3H, H-18).

NH_2SO_2Cl

Sulfamoyl Chloride

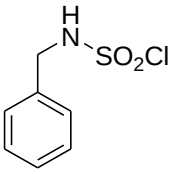
Sulfamoyl chloride.[220] To an ice-cooled solution of chlorosulfonyl isocyanate (1.2 mL, 14.1 mmol) in DCM (7 mL), was added a solution of formic acid (97%, 0.54 mL, 5 eq.) in DCM

(3 mL) drop-wise under argon atmosphere for 10 min. after that, the reaction was warmed to room temperature with stirring for additional hour, before being used in the sulfamoylation reaction.



5.21

***N*-Benzyl sulfonic acid (5.21)**.[222] To an ice-cooled solution of benzylamine **5.20** (2.2 mL, 0.02 mmol) in DCM (20 mL), was cautiously added chlorosulfonic acid (0.4 mL, 6×10^{-3} mmol). After complete addition, the resultant suspension was stirred at room temperature for 30 min., and then filtered. The solid was dried by leaving under vacuum overnight, and used in the next step without further purification (3.0 g, 90% yield).



5.22

***N*-Benzyl sulfamoyl chloride (5.22)**.[222] To a stirred solution of **5.21** (3.0 g, 0.02 mmol) in toluene (25 mL), was added phosphorous pentachloride (3.32 g, 0.02 mmol). Stirring was continued under reflux for 3 h, and then solvent was removed under vacuum, and dried under vacuum overnight to afford **5.22** as a syrupy liquid (2.8 g, 85% yield) which was used for the sulfamoylation without further purification.

References

1. Khurana, A.; Beleford, D.; He, X.; Chien, J.; Shridhar, V. *Am J. Cancer Res.* **2013**, 3(1), 34-45.
2. Obaya, A. J. *Gene* **2006**, 372, 110-117.
3. Hanson, S.R.; Best, M.D.; Wong, C.H. *Angew. Chem. Int. Ed*., **2004**, 43, 5736-5763.
4. Stein, C.; Heille, A.; Seidel, J.; Rijnboutz, S.; Waheed, A.; Schmidt, B.; Geuze, H.; von Figura, K. *J. Biol. Chem.* **1989**, 264(23), 13865-13872.
5. Anson, D. S.; Taylor, J. A.; Bielicki, J.; Harper, G. S.; Peters, C.; Gibson, G. J.; Hopwood, J. J. *Biochem. J.* **1992**, 284, 789-794.
6. Franco, B.; Meroni, G.; Parenti, G.; Levilliers, J.; Bernard, L.; Gebbia, M.; Cox, L.; Maroteaux, P.; Sheffield, L.; Rappold, G. A.; Andria, G.; Petit, C.; Ballabio, A. *Cell* **1995**, 81, 15-25.
7. Puca, A. A.; Zollo, M.; Repetto, M.; Andolfi, G.; Guffanti, A.; Simon, G.; Ballabio, A.; Franco, B. *Genomics* **1997**, 42, 192-199.
8. Ferrante, P.; Messali, S.; Meroni, G.; Ballabio, A. *Eur. J. Hum. Genet.* **2002**, 10, 813-818.
9. Sardiello, M.; Annunziata, I.; Roma, G.; Ballabio, A. *Hum. Mol. Genet.* **2005**, 14, 3203-3217.
10. Wiegmann, E. M.; Westendorf, E.; Kalus, I.; Pringle, T. H.; Lübke, T.; Dierks, T. *J. Biol. Chem.* **2013**, 288, 30019-30028.
11. Tomatsu, S.; Fukuda, S.; Masue, M.; Sukegawa, K.; Fukao, T.; Yamagishi, A.; Horil, T.; Iwata, H.; Ogawa, T.; Nakashima, Y.; Hanyn, Y.; Hashimoto, T.; Titani, K.; Oyama, R.; Suzuki, M.; Yagi, K.; Hayashi, Y.; Orii, T. *Biochem. Bioph. Res. Co.* **1991**, 181, 677-683.
12. Scott, H. S.; Blanch, L.; Guo, X.-H.; Freeman, C.; Orsborn, A.; Baker, E.; Sutherland, G. R.; Morris, C. P.; Hopwood, J. J. *Nat. Genet.* **1995**, 11, 465-467.
13. Freeman, C.; Hopwood, J. J. *Biochem. J.* **1991**, 279, 399-405.
14. Bielicki, J.; Hopwood, J. J.; Melville, E. L.; Anson, D. S. *Biochem. J.* **1998**, 329, 145-150.
15. Morimoto-Tomita, M.; Uchimura, K.; Werb, Z.; Hemmerich, S.; Rosen, S. D. *J. Biol. Chem.* **2002**, 277, 49175-49185.
16. Diez-Roux, G.; Ballabio, A. *Annu. Rev. Genomics Hum. Genet.* **2005**, 6, 355-379.
17. Mostafa, Y. A.; Taylor, S. D. *J. Steroid Biochem. Mol. Biol.* **2013**, 137, 183-198.

18. Pasqualini, J. R.; Chetrite, G.; Blacker, C.; Feinstein, M.-C.; Delalonde, L.; Talbi, M.; Maloche, C. *J. Clin. Endocr. Metab.* **1996**, 81(4), 1460-1464.
19. Ruder, H.J.; Loriaux, D.L.; Lipsett, M.B. *J. Clin. Invest.* **1972**, 51, 1020-1023.
20. Matsumoto, J.; Ariyoshi, N.; Ishii, I.; Kitada, M. *Drug Metab. Pharmacokinet.* **2010**, 25, 403-407.
21. Miech, C.; Dierks, T.; Selmer, T.; von Figura, K.; Schmidt B. *J. Biol. Chem.* **1998**, 273, 4835-4837.
22. Bond, C. S.; Clements, P. R.; Ashby, S. J.; Collyer, C. A.; Harrop, S. J.; Hopwood, J. J.; Guss, J. M. *Structure* **1997**, 5(2), 277-289.
23. Miech, C.; Dierks, T.; Selmer, T.; von Figura, K.; Schmidt B. *J. Biol. Chem.* **1998**, 273, 4835-4837.
24. Schmidt, B.; Selmer, T.; Ingendoh, A.; von Figura, K. *Cell* **1995**, 82, 271-278.
25. Horwitz, A. L. *Proc. Natl. Acad. Sci. USA.* **1979**, 76(12), 6496-6499.
26. Ghosh, D. *Cell. Mol. Life Sci.* **2007**, 64, 2013-2022.
27. Ghosh. D. *Method Enzymol.* **2005**, 400, 273-293.
28. Waldow, A.; Schmidt, B.; Dierks, T.; von Bülow, R.; von Figura, K. *J. Biol. Chem.* **1999**, 274 (18), 12284-12288.
29. Lukatela, G.; Krauss, N.; Theis, K.; Selmer, T.; Gieselmann, V.; von Figura, K.; Saenger, W. *Biochemistry* **1998**, 37, 3654-3664.
30. Hernandez-Guzman, F. G.; Higashiyama, T.; Pangborn, W.; Osawa, Y.; Ghosh, D. *J. Biol. Chem.* **2003**, 278, 22989-22997.
31. Maeda, T.; Irokawa, M.; Arakawa, H.; Kuraoka, E.; Nozawa, T.; Tateoka, R.; Itoh, Y.; Nakanishi, T.; Tamai, I. *J. Steroid Biochem. Mol. Biol.* **2010**, 122, 180-185.
32. Santner, S.J.; Feil, P.D.; Santen, R.J. *J. Clin. Endocrin. Metab.* **1984**, 59, 29-33.
33. Utsumia, T.; Yoshimurab, N.; Takeuchic, S.; Marutaa, M.; Maedaa, K.; Haradab, N. *J. Steroid Biochem. Mol. Biol.* **2000**, 73, 141-145.
34. Suzuki, T.; Nakata, T.; Miki, Y.; Kaneko, C.; Moriya, T.; Shida, T.; Akinaga, S.; Hirakawa, H.; Kimura, M.; Sasano, H. *Cancer Res.* **2003**, 63, 2762-2770.
35. Zaichuk, T.; Ivancic, D.; Scholtens, D.; Schiller, C.; Khan, S. A. *J. Steroid Biochem. Mol. Biol.* **2007**, 105, 76-84.

36. Newman, S.P.; Purohit, A.; Ghilchik, M.W.; Potter, B.V.L.; Reed, M.J. *J. Steroid Biochem. Mol. Biol.* **2000**, 75, 259-264.
37. Chia, S.; Gradishar, W.; Mauriac, L.; Bines, J.; Amant, F.; Federico, M.; Fein, L.; Romieu, G.; Buzdar, A.; Robertson, J. F. R.; Brufsky, A.; Possinger, K.; Rennie, P.; Sapunar, F.; Lowe, E.; Piccart, M. *J. Clin. Oncol.* **2008**, 26(10), 1664-1670.
38. Miller, W. R.; Bartlett, J. M. S.; Canney, P.; Verrill, M. *Breast Cancer Res. Tr.* **2007**, 103,149–160.
39. Payne, A.H.; Mason, M.; Jaffe, J.B. *Steroids* **1969**, 14 (5), 685-704.
40. Townsley, J.D.; Scheel, D.A.; Rubin, E.J. *J. Clin. Endocrin. Metab.* **1970**, 31, 670-678.
41. Payne, A.H. *Biochim. Biophy. Acta* **1972**, 258, 473-483.
42. Farnsworth, W.E. *Steroids* **1973**, 21, 647-664.
43. Townsley, J.D. *Endocrin.* **1973**, 923, 172-181.
44. Nussbaumer, P.; Billich, A. *Med. Res. Rev.* **2004**, 24, 529-576.
45. Pasqualini, J.R. *Biochim. Biophy. Acta* **2004**, 1654 (2), 123-143.
46. Pasqualini, J.R.; Paris, J. ; Sitruk-Ware, R.; Chetrite, G.; Botell, J. *J. Steroid Biochem. Mol. Biol.* **1998**, 65, 225-235.
47. Pasqualini, J.R.; Chetrite, G.; Nguyen, B.L.; Maloche, C.; Delalonde, L.; Talbi, M.; Feinstei, M.C.; Blacker, C.; Botella, J.; Paris, J. *J. Steroid Biochem. Mol. Biol.* **1995**, 53, 407-412.
48. Duncan, L.; Purohit, A.; Howarth, N. M.; Potter, B. V. L.; Reed, M. J. *Cancer Res.* **1993**, 53, 298-303.
49. Li, P.K.; Pillai, R.; Dibbelt, L. *Steroids* **1995**, 60, 299-306.
50. Anderson, C.; Freeman, J.; Lucas, L. H.; Farley, M.; Dalhoumi, H.; Widlanski, T. S. *Biochemistry* **1997**, 36, 2586-2594.
51. Li, P.-K.; Pillai, R.; Young, B. L.; Bender, W. H.; Martino, D. M.; Lint, F. U. *Steroids* **1993**, 58,106-111.
52. Dibbelt, L.; Li, P.K.; Pillai, R.; Knuppen, R. *J. Steroid Biochem. Mol. Biol.* **1994**, 50, 261-266.
53. Selcer, K.W.; Li, P.K. *J. Steroid Biochem. Mol. Biol.* **1995**, 52, 281-286.
54. Woo, L. W. L.; Lighttowler, M.; Purohit, A.; Reed, M. J.; Potter, B. V. L. *J. Steroid Biochem. Mol. Biol.* **1996**, 57, 79-88.

55. Selcer, K.W.; Jagannathan, S.; Rhode, M.E.; Li, P.K. *J. Steroid Biochem. Mol. Biol.* **1996**, 59, 83-91.
56. Schreiner, E.P.; Billich, A. *Bioorg. Med. Chem. Lett.* **2004**, 14, 4999-5002.
57. Howarth, N. M.; Purohit, A.; Reed, M. J.; Potter, B. V. L. *J. Med. Chem.* **1994**, 37, 219-221.
58. Nussbaumer, P.; Lehr, P.; Billich, A. *J. Med. Chem.* **2002**, 45, 4310-4320.
59. Purohit, A.; Williams, G. J.; Howarth, N. M.; Potter, B. V. L.; Reed, M. J. *Biochemistry* **1995**, 34, 11508-11514.
60. Howarth, N. M.; Purohit, A.; Reed, M. J.; Potter, B. V. L. *Steroids* **1997**, 62, 346-350.
61. Lapierre, J.; Ahmed, V.; Chen, V.M.-J.; Ispahany, I.; Guillemette, J.G.; Taylor, S.D. *Bioorg. Med. Chem. Lett.* **2004**, 14, 151-155.
62. Liu, Y.; Ahmed, V.; Hill, B.; Taylor, S. D. *Org. Biomol. Chem.* **2005**, 3, 3329-3335.
63. Ahmed, V.; Liu, Y.; Silvestro, C.; Taylor, S. D. *Bioorg. Med. Chem.* **2006**, 14, 8564-8573.
64. Birnek, H.; Angerer, E. V. *Biochem. Pharmaco.* **1990**, 39(11), 1709-1713.
65. Poirier, D.; Boivin, R. P. *Bioorg. Med. Chem. Lett.* **1998**, 8, 1891-1896.
66. Ciobanu, L.C.; Boivin, R.P.; Luu-The, V.; Labrie, F.; Poirier, D. *J. Med. Chem.* **1999**, 42, 2280-2286.
67. Boivin, R. P.; Luu-The, V.; Lachance, R.; Labrie, F.; Poirier, D. *J. Med. Chem.* **2000,** 43, 4465-4478.
68. Maltais, R.; Fournier, D.; Poirier, D. *QSAR Comb. Sci.* **2009**, 28, 1284-1299.
69. Fournier, D.; Poirier, D. *Eur. J. Med. Chem.* **2011**, 46, 4227-4237.
70. Ciobanu, L.C.; Boivin, R.P.; Luu-The, V.; Poirier, D. *Eur. J. Med. Chem.* **2001**, 36, 659-671.
71. Boivin, R.P.; Labrie, F.; Poirier, D. *Steroids* **1999**, 64, 825-833.
72. Fournier, D.; Poirier, D. *Bioorg. Med. Chem. Lett.* **2009**, 19, 693-696.
73. Ahmed, V.; Liu, Y.; Taylor, S. D. *ChemBioChem* **2009**, 10, 1457-1461.
74. Phan, C.-M.; Liu, Y.; Kim, B.-M.; Mostafa, Y.; Taylor, S. D. *Bioorg. Med. Chem.* **2011**, 19, 5999–6005.
75. Purohit, A.; Williams, G.J.; Roberts, C.J.; Potter, B.V.L.; Reed, M.J. *Int. J. Cancer* **1995**, 63, 106–111.

76. Elger, W.; Schwarz, S.; Hedden, A.; Reddersen, G.; Schneider, B. *J. Steroid Biochem. Mol. Biol.* **1995**, 55, 395–403.
77. Woo, L. W. L.; Purohit, A.; Reed, M. J.; Potter, B. V. L. *Bioorg. Med. Chem. Lett.* **1997**, 7, 3075-3080.
78. Ciobanu, L. C.; Boivin, R. P.; Luu-the, V.; Poirier, D. *J. Enz. Inh. Med. Chem.* **2003**, 18 (1), 15-26.
79. Ahmed, S.; Owen, C. P.; James, K.; Patel, C. K.; Patel, M. *Bioorg. Med. Chem. Lett.* **2001**, 11, 899-902.
80. Purohit, A.; Vernon, K. A.; Hummelinck, K. A. W.; Woo, L. W. L.; Hejaz, H. A. M.; Potter, B. V. L.; Reed, M. J. *J. Steroid Biochem. Mol. Biol.* **1998**, 64, 269-275.
81. Geisler, J.; Lonning, P.E. *Crit. Rev. Oncol. Hematol.* **2006**, 57, 53-61.
82. Purohit, A.; Hejaz, H.A.M.; Woob, L.W.L.; van Striena, A. E.; Potter, B. V. L.; Reed, M.J. *J. Steroid Biochem. Mol. Biol.* **1999**, 69, 227-238.
83. Tevaarwerk, A. J.; Holen, K. D.; Alberti, D. B.; Sidor, C.; Arnott, J.; Quon, C.; Wilding, G.; Liu, G. *Clin. Cancer Res.* **2009**, 15, 1460-1465.
84. Reed, J. E.; Woo, L. W. L.; Robinson, J. J.; Leblond, B.; Leese, M. P.; Purohit, A.; Reed, M. J.; Potter, B. V. L. *Biochem. Bioph. Res. Co.* **2004**, 317, 169-175.
85. Li, P.-K.; Chu, G.-H.; Guo, J. P.; Peters, A.; Selcer, K. W. *Steroids* **1998**, 63, 425–432.
86. Li, P.-K.; Akinaga, S.; Murakata, C. PCT Int. Appl. WO 2000043408 **2000**.
87. Li, P.-K.; Murakata, C.; Akinaga, S. US Patent 6,288,050 **2001**.
88. Li, P.-K.; Murakata, C.; Akinaga, S. US Patent 6,376,687 **2002**.
89. Ishida, H.; Nakata, T.; Suzuki, M.; Shiotsu, Y.; Tanaka, H.; Sato, N.; Terasaki, Y.; Takebayashi, M.; Anazawa, H.; Murakata, C.; Li, P.-K.; Kuwabara, T.; Akinaga, S. *Breast Cancer Res. Tr.* **2007**, 106, 215-217.
90. Ishida, H.; Nakata, T.; Sato, N.; Li, P.-K.; Kuwabara, T.; Akinaga, S. *Breast Cancer Res. Tr.* **2005**, 104, 211-219.
91. Y. Jinbo, Y. Inoue, Novel estradiol derivatives, PCT Int. Appl. WO 2000053620 **2000**.
92. Leese, M.P.; Leblond, B.; Newman, S.P.; Purohit, A.; Reed, M.J.; Potter, B.V.L. *J. Steroid Biochem. Mol. Biol.* **2005**, 94, 239-251.
93. Ciobanu, L. C.; Luu-the, V.; Martel, C.; Labrie, F.; Poirier, D. *Cancer Res.* **2003**, 63, 6442-6446.

94. Potter, B.V.L.; Reed, M.J. PCT Int. Appl. WO 200201639 **2002**.
95. Leese, M.P.; Leblond, B.; Smith, A.; Newman, S.P.; Di Fiore, A.; Simone, G. De; Supuran, C.T.; Purohit, A.; Reed, M.J.; Potter, B.V.L. *J. Med. Chem.* **2006**, 49, 7683-7696.
96. Bubert, C.; Leese, M.P.; Mahon, M.F.; Ferrandis, E.; Regis-Lydi, S.; Kasprzyk, P.G.; Newman, S.P.; Ho, Y.T.; Purohit, A.; Reed, M.J.; Potter, B.V.L. *J. Med. Chem.* **2007**, 50, 4431-4443.
97. Leese, M.P.; Jourdan, F.L.; Gaukroger, K.; Mahon, M.F.; Newman, S.P.; Foster, P.A.; Stengel, C.; Regis-Lydi, S.; Ferrandis, E.; Di Fiore, A.; De Simone, G.; Supuran, C.T.; Purohit, A.; Reed, M.J.; Potter, B.V.L. *J. Med. Chem.* **2008**, 51, 1295-1308.
98. Jourdan, F.; Bubert, C.; Leese, M.P.; Smith, A.; Ferrandis, E.; Regis-Lydi S, S.; Newman, S.P.; Purohit, A.; Reed, M.J.; Potter, B.V.L. *Org. Biomol. Chem.* **2008**, 6, 4108-4119.
99. Ciobanu, L. C.; Poirier, D. *J. Comb. Chem.* **2003**, 5, 429-440.
100. Peters, R.H.; Chao, W.R.; Sato, B.; Shigeno, K.; Zaveri, N.T.; Tanabe, M.; *Steroids* **2003**, 68, 97-110.
101. Woo, L.W.L.; Leblond, B.; Purohit, A.; Potter, B.V.L. *Bioorg. Med. Chem.* **2012**, 20, 2506-2519.
102. Hanson, S.R.; Whalen, L.J.; Wong, C.-H. *Bioorg. Med. Chem.* **2006**, 14, 8386–8395.
103. Purohit, A.; Hejaz, H.A.M.; Woo, L.W.L.; Van Stien, A.E.; Potter, B.V.L.; Reed, M.J. *J. Steroid Biochem. Mol. Biol.* **1999**, 69, 227-238.
104. Ciobanu, L.C.; Poirier, D. *ChemMedChem* **2006**, 1, 1249-1259.
105. Rasmussen, L.M.; Zaveri, N.T.; Stenvang, J.; Peters, R.H.; Lykkesfeldt, A.E. *Breast Cancer Res. Tr.* **2007**, 106, 191-203.
106. Numazawa, M.; Tominaga, T.; Watari, Y.; Tada, Y. *Steroids* **2006**, 71, 371-379.
107. Reed, M.J.; Potter, B.V.L. WO 9927935 **1999**.
108. Koizumi, N.; Takegawa, S.; Iwashita, S.; Kawachi, T.; Mieda, M.; Fujii, T. WO 9811124 **1998**.
109. Fischer, D. S.; Woo, L.W. L.; Mahon, M. F.; Purohit, A.; Reed, M. J.; Potter, B. V. L. *Bioorg. Med. Chem.* **2003**, 11, 1685-1700.
110. Fischer, D.S.; Chander, S.K.; Woo, L.W.L.; Fenton, J.C.; Purohit, A.; Reed, M.J.; Potter, B.V.L. *J. Steroid Biochem. Mol. Biol.* 2003, 84, 343-349.

111. Foster, P.A.; Newman, S.P.; Chander, S.K.; Stengel, C.; Jhalli, R.; Woo, L.W.L.; Potter, B.V.L.; Reed, M.J.; Purohit, A. *Clin. Cancer Res.* **2006**, 12, 5543-5549.
112. Woo, L. W. L.; Fischer, D. S.; Sharland, C. M.; Trusselle, M.; Foster, P. A.; Chander, S. K.; Fiore, A. D.; Supuran, C. T.; Simone, G. D.; Purohit, A.; Reed, M. J.; Potter, B. V. L. *Mol. Cancer Ther.* **2008**, 7, 2435-2444.
113. Ahmed, S.; James, K.; Owen, C. P.; Patel, C. K.; Patel, M. *Bioorg. Med. Chem. Lett.* **2001** 11, 2525–2528.
114. Purohit, A.; Hejaz, H.A.M.; Woo, L.W.L.; van Strien, A.E.; Potter, B.V.L.; Reed, M.J. *J. Steroid Biochem. Mol. Biol.* **1999**, 69, 227-238.
115. Ahmed, S.; James, K.; Owen, C. P. *Bioorg. Med. Chem. Lett.* **2002**, 12, 2391-2394.
116. Nussbaumer, P.; Bilban, M.; Billich, A. *Bioorg. Med. Chem. Lett.* **2002**, 12, 2093-2095.
117. Ahmed, S.; James, K.; Owen, C. P.; Patel, C. K. *Bioorg. Med. Chem. Lett.* **2002**, 12, 1343-1346.
118. Okada, M.; Nakagawa, T.; Iwashita, S.; Takegawa, S.; Fujii, T.; Koizumi, N. *J. Steroid Biochem. Mol. Biol.* **2003**, 87, 141-148.
119. Saito, T.; Kinoshita, S.; Fujii, T.; Bandoh, K.; Fuse, S.; Yamauchi, Y.; Koizumi, N.; Horiuchi, T. *J. Steroid Biochem. Mol. Biol.* **2004**, 88, 167-173.
120. Ciobanu, L. C.; Luu-The, V.; Poirier, D. *J. Steroid Biochem. Mol. Biol.* **2002**, 80, 339-353.
121. Walter, G.; Liebl, R.; von Angerer, E. *J. Steroid Biochem. Mol. Biol.* **2004**, 88, 409-420.
122. Woo, L. W. L.; Sutcliffe, O. B.; Bubert, C.; Grasso, A.; Chander, S. K.; Purohit, A.; Reed, M. J.; Potter, B. V. L. *J. Med. Chem.* **2003**, 46, 3193-3196.
123. Woo, L. W. L.; Bubert, C.; Sutcliffe, O. B.; Smith, A.; Chander, S. K.; Mahon, M. F.; Purohit, A.; Reed, M. J.; Potter, B. V. L. *J. Med. Chem.* **2007**, 50, 3540-3560.
124. Bubert, C.; Woo, L. W. L.; Sutcliffe, O. B.; Mahon, M. F.; Chander, S. K.; Purohit, A.; Reed, M. J.; Potter, B. V. L. *ChemMedChem* **2008**, 3, 1708-1730.
125. Woo, L. W. L.; Jackson, T.; Putey, A.; Cozier, G.; Leonard, P.; Acharya, K. R.; Chander, S. K.; Purohit, A.; Reed, M. J.; Potter, B. V. L. *J. Med. Chem.* **2010**, 53, 2155-2170.
126. Woo, L. W. L.; Howarth, N. M.; Purohit, A.; Hejaz, H. A. M.; Reed, M. J.; Potter, B. V. L. *J. Med. Chem.* **1998**, 41, 1068-1083.
127. Woo, L. W. L.; Purohit, A.; Reed, M. J.; Potter, B. V. L. *J. Med. Chem.* **1996**, 39, 1349-1351.

128. Woo, L. W. L.; Purohit, A.; Malini, B.; Reed, M. J.; Potter, B. V. L. *Chem. Biol.* **2000**, 7(10), 773-791.
129. Purohit, A.; Woo, L. W. L.; Singh, A.; Winterborn, C. J.; Potter, B. V. L.; Reed, M. J. *Cancer Res*. **1996**, 56, 4950-4955.
130. Bilban, M.; Billich, A.; Auer, M. Nussbaumer, P. *Bioorg. Med. Chem. Lett.* **2000**, 10, 967-969.
131. Schreiner, E. P.; Wolff, B.; Winiski, A. P.; Billich, A. *Bioorg. Med. Chem. Lett.* **2003**, 13, 4313-4316.
132. Billich, A.; Meingassner, J. G.; Nussbaumer, P.; Desrayaud, S.; Lam, C.; Winiski, A.; Schreiner, E. *J. Steroid Biochem. Mol. Biol.* **2004**, 92, 29-37.
133. Golob, T.; Liebl, R.; von Angerer, E. *Bioorg. Med. Chem.* **2002**, 10, 3941-3953.
134. Nussbaumer, P.; Geyl, D.; Horvath, A.; Lehr, P.; Wolff, B.; Billich, A.; *Bioorg. Med. Chem. Lett.* **2003**, 13, 3673-3677.
135. Lehr, P.; Billich, A.; Wolff, B.; Nussbaumer, P. *Bioorg. Med. Chem. Lett.* **2005**, 15, 1235-1238.
136. Horvath, A.; Nussbaumer, P.; Wolff, B.; Billich, A. *J. Med. Chem.* **2004**, 47, 4268-4276.
137. Schreiner, E.P. PCT Int. Appl. WO 2004043968, **2004**.
138. Jütten, P.; Schumann, W.; Härtl, A.; Heinisch, L.; Gräfe, U.; Werner, W.; Ulbricht, H. *Bioorg. Med. Chem. Lett.* **2002**, 12, 1339-1342.
139. Stanway, S.J.; Purohit, A.; Woo, L.W.L.; Sufi, S.; Vigushin, D.; Ward, R.; Wilson, R.H.; Stanczyk, F.Z.; Dobbs, N.; Kulinskaya, E. *Clin. Cancer Res.* **2006**, 12, 1585-1592.
140. Stanway, S.J.; Delavault, P.; Purohit, A.; Woo, L.W.L.; Thurieau, C.; Potter, B.V.L.; Reed, M.J. *Oncologist* **2007**, 12, 370-374.
141. Palmieri, C.; Januszewski, A.; Stanway, S.; Coombes, R.C. *Expert Rev. Antican. Ther.* **2011**, 11, 179-183.
142. Reed, M.J.; Purohit, A.; Woo, L.W.L.; Potter, B.V.L. *Drugs Future* **2008**, 33, 597-606.
143. Pautier, P.; Lobbedez, F.J.; Melichar, B.; Kutarska, E.; Hall, G.; Reed, N. *Annals Oncol.* **2012**, 23 (9), 329.
144. Billich, A., Nussbaumer, P., Lehr, P. *J. Steroid Biochem. Mol. Biol.* **2000**, 73, 225-235.

145. Wolff B., Billich A., Brunowsky W., Herzig G., Lindley I., Nussbaumer P., Pursch E., Rabeck C., Winkler G. *Anal. Biochem.* **2003**, 318, 276–284.

146. Wood, P. M.; Woo, L. W. L.; Thomas, M. P.; Mahon, M. F.; Purohit, A.; and Potter, B. V. L., *ChemMedChem.* **2011**, 6, 1423-1438.

147. Yen, P.H.; Allen, E.; Marsh, B.; Mohandas, T.; Wang, N.; Taggart, N.; Shapiro, L.J. *Cell* **1987**, 49, 443-454.

148. Stengel, C.; Simon, P.; Newman, J.; Day, M.; Tutill, H.J.; Reed, M.J.; Purohit, A. *Mol. Cell. Endocrinol*. **2008**, 283, 76-82.

149. Van der Loos, C.M., Van Breda, A.J., Van den Berg, F.M., Walboomers J.M.M., Jobsis A.C. *J. Inher. Metab. Dis.* **1984**, 7, 97-103.

150. Noel, H., Plante, L., Bleau, G., Chapdelaine, A., Roberts, K.D., *J. Steroid Biochem.* **1983**, 19, 1591-1598.

151. Ahmed, V.; Ispahany, M.; Ruttgaizer, S.; Guillemette, J. G.; Taylor, S. D. *Anal. Biochem.* **2005**, 340(1), 80-88.

152. Hernandez-Guzman, F.G., Higashiyama, T., Osawa, Y., Ghosh, D. J. Steroid Biochem. Mol. Biol. 2001, 78, 441-450.

153. Roy, A. B., Methods in Enzymology 1971, 143, 207-217.

154. Shankaran, R., Ameen, M., Daniel, W., Davidson, R.G., Chang, P.L., Biochem. Biophys. Acta. 1991, 1078, 251-257.

155. Vaccaro, A.M., Salvioli, R., Muscillo, M., Renola, L., Enzyme. 1987, 37, 115-126.

156. Burns, G.R.J., Biochim. Biophys. Acta. 1983, 759, 199-204.

157. Bradford, M. M. Anal. Biochem. 1976, 72, 248-254.

158. Ho, Y.T.; Purohit, A.; Vicker, N.; Newman, S.P.; Robinson, J.J.; Leese, M.P.; Ganeshapillai, D.; Woo, L.W.L.; Potter, B.V.L.; Reed, M.J. *Biochem. Biophys. Res. Commun.* **2003**, 305, 909-914.

159. Elger, W.; Palme, H.-J.; Schwarz, S. *Exp. Opin. Invest. Drugs* **1998**, 7(4), 575-589.

160. Dodson, Raymond M.; Sollman, Paul B. US 2840577 **1958**.

161. Prokai, L.; Oon, S.-M.; Prokai-Tatrai, K. ; Abboud, K. A. ; Simpkins, J. W. *J. Med. Chem.* **2001,** 44, 110-114.

162. Frank, E.; Molnar, J.; Zupko, I.; Kadar, Z.; Wolfling, J. *Steroids* **2011**, 76, 1141-1148.

163. Taylor, S. D.; Harris, J. *Steroids* **2011**, 76, 1098-1102.

164. Mostafa, A.Y.; Taylor, S.D. *Bioorg. Med. Chem.* **2012**, 20, 1535-1544.

165. Lemini, C.; Cruz–Ramos, E.; Toscano, R. A.; Cruz–Almanza, R. *Steroids* **1998**, 63, 556-564.

166. Smith, D. R.; Maiesthal, M.; Tipton, J. *J. Org. Chem.* **1951**, 294-297.

167. Szendi, Z.; Dombi, G.; Vincze, I. *Monatshefte für Chemie* **1996**, 127, 1189-1196.

168. Robinson, C. H.; Ermann, C. *Steroids* **1965**, 6 (5), 509-518.

169. Foye, W. O.; Lemke, T. L. Foye's Principles of Medicinal Chemistry, 6th ed., **2008**, Lippincott Williams and Wilkins.

170. Nogrady, T.; Weaver, D. F. Medicinal Chemistry: A Molecular and Biochemical Approach, 3rd ed., **2005**, Oxford University Press.

171. McGeary, R. P.; Bennett, A. J.; Tran, Q. B.; Prins, J.; Ross, B. P. *Tetrahedron* **2009**, 65, 3990-3997.

172. Dart, E. C.; Holt, G. *J. Chem. Soc., Perkin Trans.* 1 **1974**, 1403-1406.

173. Page, P. C. B.; Moore, J. P. G.; Mansfield, I.; McKenzie, M. J.; Bowler, W. B.; Gallagher, J. A. *Tetrahedron* **2001**, 57, 1837-1848.

174. Kabasakalian, P.; McGlotten, J.; Basch, A.; Yudis, M. D. *J. Org. Chem.* **1961**, 26, 1738-1744.

175. Flack, H. D. *Acta Cryst.* **1983**, A39, 876-881.

176. Labrie, F.; Gauthier, S.; Cloutier, J.; Mailhot, J. ; Potvin, S. ; Dion, S. ; Sanceau, J.-Y. USP 2009 0042844 A1.

177. Katzenellenbogen, J.; Miyers, H. N.; Johnson, H. J. *J. Org. Chem*. **1973**, 38(20), 3525-3533.

178. Kraychy, S.; Gallagher, T. F. *J. Biol. Chem.* **1957**, 229, 519-526.

179. Tomsoan, A. J.; Horwitz. J. P. *J. Org. Chem.* **1959**, 24(12), 2056–2059.

180. Copeland, R. A. *Evaluation of enzyme inhibitors in drug discovery: a guide for medicinal chemists and pharmacologists*. **2005**, Wiley, Hoboken, N.J.

181. Numazawa, M.; Osawa, Y. *Steroids* **1979**, 34(3), 347-360.

182. Slaunwhite Jr., W. R.; Neely, L. *J. Org. Chem.* **1962**, 27 (5), 1749-1752.

183. Utne, T.; Jobson, R. B.; Landgraf, F. W. *J. Org. Chem.* **1968**, 33 (4), 1654-1656.

184. Oliveto, O. P.; Gerold, C. *Org. Synth. Coll. Vol.* **1963**, 4, 104-105.

185. Page, P. C. B.; Hussain, F.; Maggs, J. L.; Morgan, P.; Park, K. B. *Tetrahedron* **1990**, 46 (6), 2059-2068.

186. Numazawa, M.; Kimura, K.; Ogata, M.; Nagaoka, M. *J. Org. Chem.* **1985**, 50 (25), 5421-5423.

187. Liu, Y; Taylor, S.D. unpublished results.

188. Utne, T,; Jobson, R.B.; Babson, R.D. *J. Org. Chem.* **1968**, 33(6), 2469-2473.

189. Njar, V.C.O.; Arunachalam, T.; Caspi, E. *J. Org. Chem.* **1983**, 48(7), 1007-1011.

190. Heravi, M.R.P. *J. Fluorine Chem.* **2008**, 129, 217-221.

191. Snieckus, V.; Beaulieu, F. ; Mohri, K. ; Han, W.; Murphy, C.K.; Davis, F.A. *Tetrahedron Lett.* **1994**, 35 (21), 3465-3468.

192. Liu, Y.; Kim, B.; Taylor, S.D. *J. Org. Chem.* **2007**, 72, 8824-8830.

193. Miller, C.P.; Jirkovsky, I.; Hayhurst, D.A.; Adelman, S.J. *Steroids* **1996**, 61, 305-308.

194. Gefflaut, T.; Blonski, C.; Perie, J. ; Willson, M. *Prog. Biophys. Mol. Biol.* **1995**, 63, 301-340.

195. Loving, G.; Imperiali, B. *J. Am. Chem. Soc*. **2008**, 130(41), 13630-13638.

196. Loving, G.; Imperiali, B. *Bioconjug. Chem*. **2009**, 20(11), 2133-2141.

197. Toutchkine, A.; Kraynov, V.; Hahn, K. *J. Am. Chem. Soc*. **2003**, 125(14), 4132-4145.

198. Dunn, A.R.; Hays, A.M.; Goodin, D.B.; Stout, C.D.; Chiu, R.; Winkler, J.R.; Gray, H.B. *J. Am. Chem. Soc.* **2002**, 124(35), 10254-10255.

199. Cohen, B.E.; McAnaney, T.B.; Park, E.S.; Jan, Y.N.; Boxer, S.G.; Jan, L.Y. *Science* **2002**, 296(5573), 1700-1703.

200. Green, A.M.; Marshall, J.S.; Pensky, J.; Stanbury, J.B. *Science* **1972**, 175(4028), 1378-1380.

201. Edsall, A.B.; Mohanakrishnan, A.K.; Yang, D.; Fanwick, P.E.; Hamel, E.; Hanson, A.D.; Agoston, G.E.; Cushman, M. *J. Med. Chem.* **2004,** *47*, 5126-5139.

202. Mohr, J.-T. EP 0849274 A1

203. O'Brien, P.J.; Herschlag, D. *J. Am. Chem. Soc.* **1998**, 120, 12369-12370.

204. Burke, T.R., Lee, K. *Acc. Chem. Res.* **2003**, 36, 426.

205. Black, E.; Breed, J.; Breeze, A.L.; Embrey, K.; Garcia, R.; Gero, T.W.; Godfrey, L.; Kenny, P.W.; Morley, A.D.; Minshull, C.A.; Pannifer, A.D.; Read, J.; Rees, A.; Russell; D.J.; Toader, D.; Tucker, J. *Bioorg. Med. Chem. Lett.* **2005**, 15, 2503-2507.

206. Bojarova, P., and Williams, S.J. *Curr. Opin. Chem. Biol.* **2008**, 12, 573-581.

207. Gold, A.M.; Schwenk, E. *J. Am. Chem. Soc.* **1959**, 81, 2198-2000.

208. Conrow, R.B.; Bernstein, S. *Steroids* **1968**, 11 (2), 151-64.

209. Sadek, S.A.; Shaw, S.M.; Kessler, W.V.; Wolf, G.C. *J. Org. Chem.* **1981**, 46 (16), 3259-3262.

210. Radu, I.-I.; Poirier, D.; Provencher, L. *Tetrahedron Lett.* **2002**, 43 (42), 7617-7619.

211. Zhang, X. ; Sui, Z. *Tetrahedron Lett.* **2003**, 44 (15), 3071-3073.

212. Schön, U.; Messinger, J.; Buchholz, M.; Reinecker, M.; Thole, H.; Prabhu, M.K.S.; Konda, A. *Tetrahedron Lett.* **2005**, 46 (42), 7111-7115.

213. Lebel, H.; Leogane, O. *Org. Lett.* **2006**, 8 (25), 5717-5720.

214. Lovrić, M.; Cepanec, I.; Litvić, M.; Bartolinčić, A.; Vinković, V. *Croat. Chem. Acta* **2007**, 80 (1), 109-115.

215. Ma, B.; Lee, W.-C. *Tetrahedron Lett.* **2010**, 51, 385-386.

216. Weidner, J.J.; Weintraub, P.M.; Schnettler, R.A.; Peet, N.P. *Tetrahedron* **1997**, 53 (18), 6303-6312.

217. Horwitz, J.P.; Iyer, V.K.; Vardhan, H.B.; Corombos, J.; Brooks, S.C. *J. Med. Chem.* **1986**, 29, 692-698.

218. Manna, S.; Falck, J. R.; Mioskowski, C. *J. Org. Chem.* **1982**, 47, 5021-5023.

219. Shendage, D.M.; Fröhlich, R.; Haufe, G. *Org. Lett.* **2004**, 6 (21), 3675-3678.

220. Hanson, S.R.; Whalen, L.J.; Wong, C.-H. *Bioorg. Med. Chem.* **2006**, 14, 8386-8395.

221. Okada, M.; Iwashita, S.; Koizumi, N. *Tetrahedron Lett.* **2000**, 41, 7047-7051.

222. Kloek, J.A.; Leschinsky, K.L. *J. Org. Chem.* **1976**, 41 (25), 4028-4029.

223. Birch, A.J.; Hughes, G.A.; Kruger, G.; Rao, G.S.R.S. *J. Chem. Soc.* **1964**, 12, 5889-5891.

Appendix A – Supplementary Figures and Tables for Chapter 3

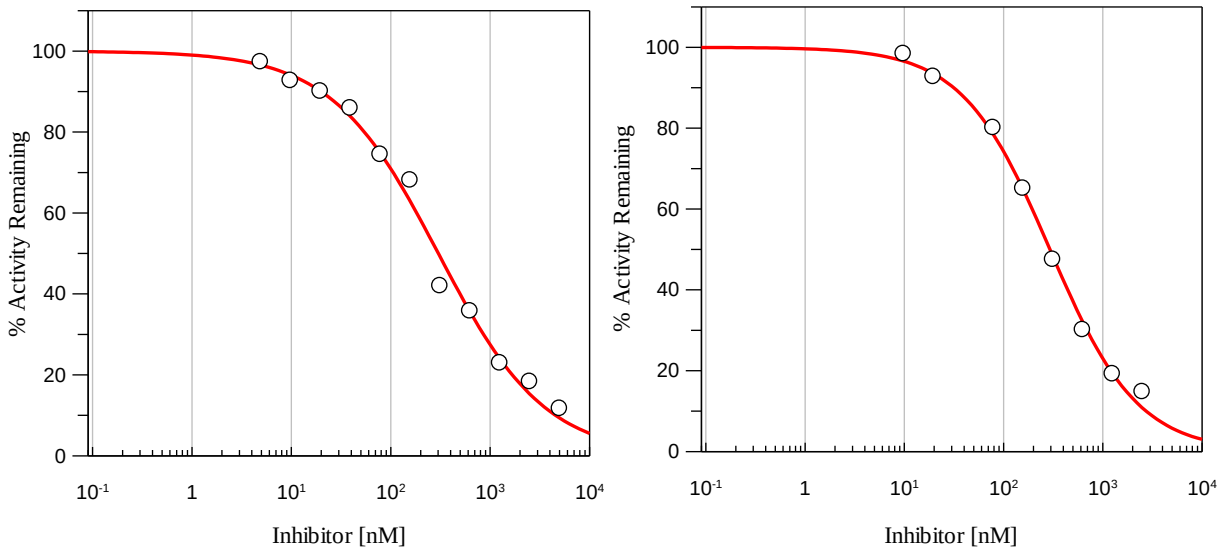


Figure A.1. (a) IC_{50} plot for **3.3** (left). **(b)** IC_{50} plot for **3.4** (right).

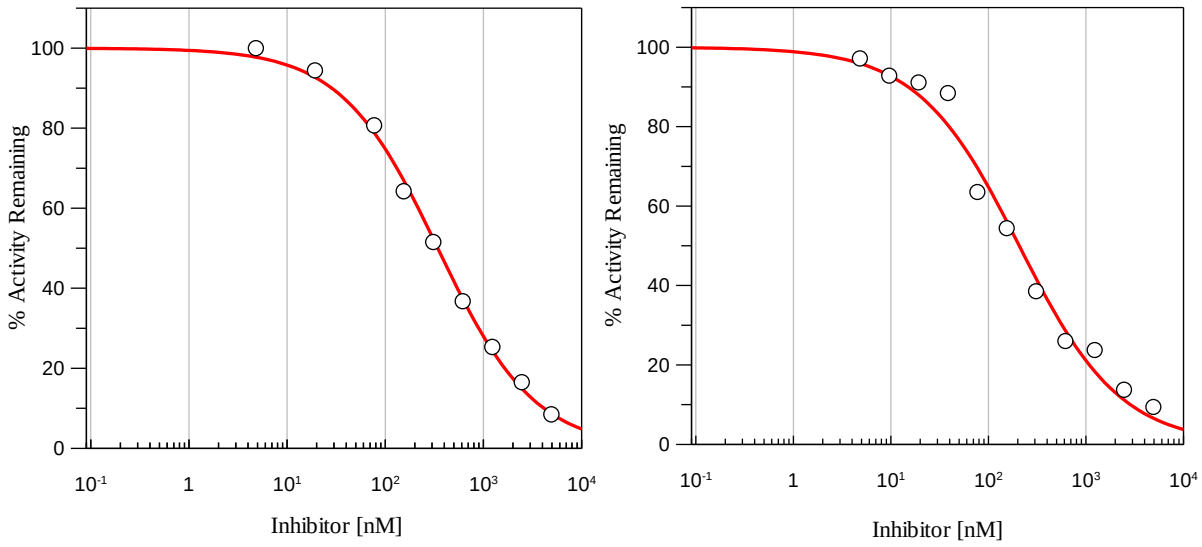


Figure A.2. (a) IC_{50} plot for **3.5** (left). **(b)** IC_{50} plot for **3.6** (right).

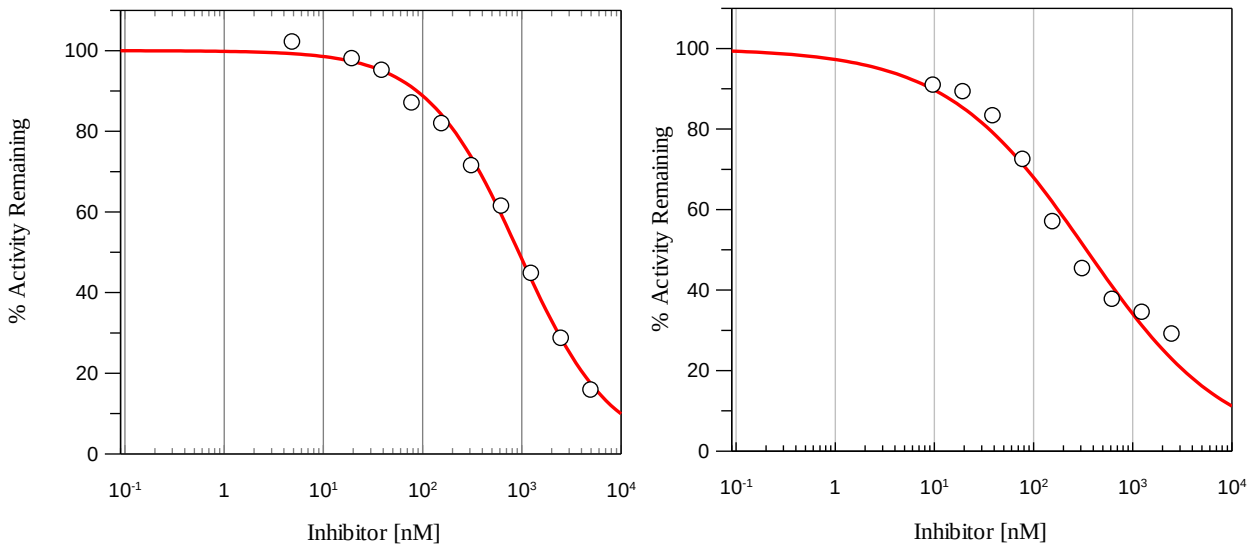


Figure A.3. (a) IC_{50} plot for **3.18** (left). **(b)** IC_{50} plot for **3.19** (right).

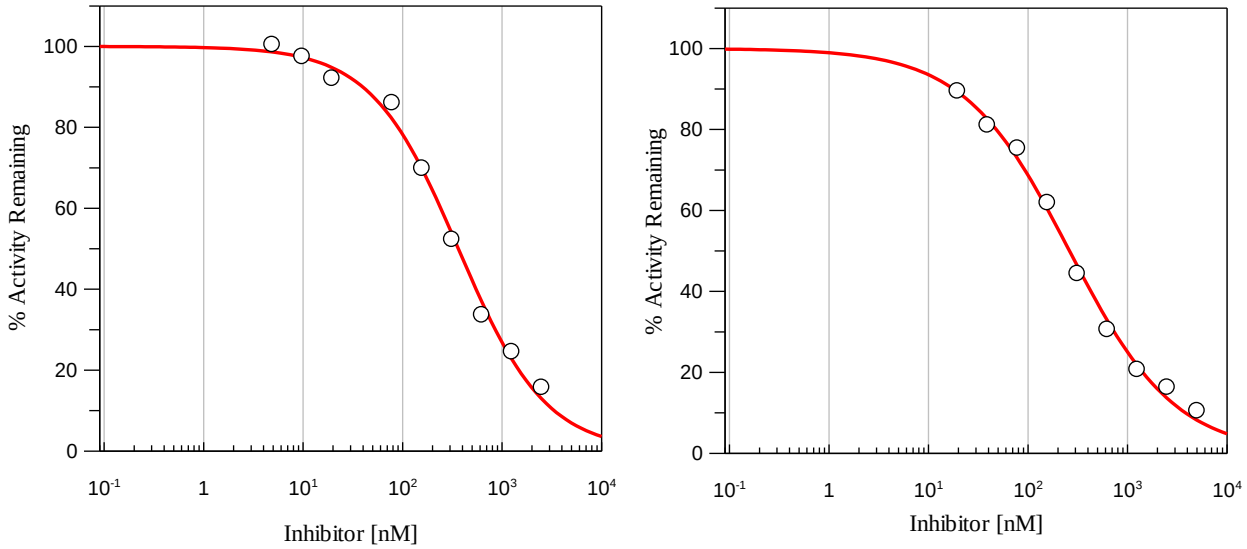


Figure A.4. (a) IC_{50} plot for **3.20** (left). **(b)** IC_{50} plot for **3.21** (right).

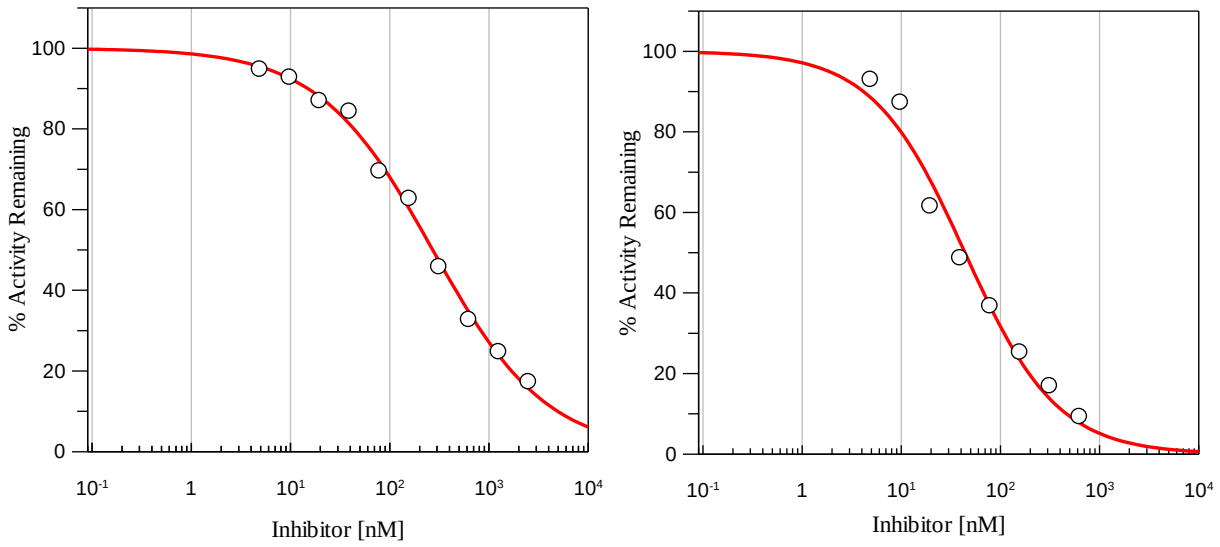


Figure A.5. (a) IC_{50} plot for **3.22** (left). **(b)** IC_{50} plot for **3.23** (right).

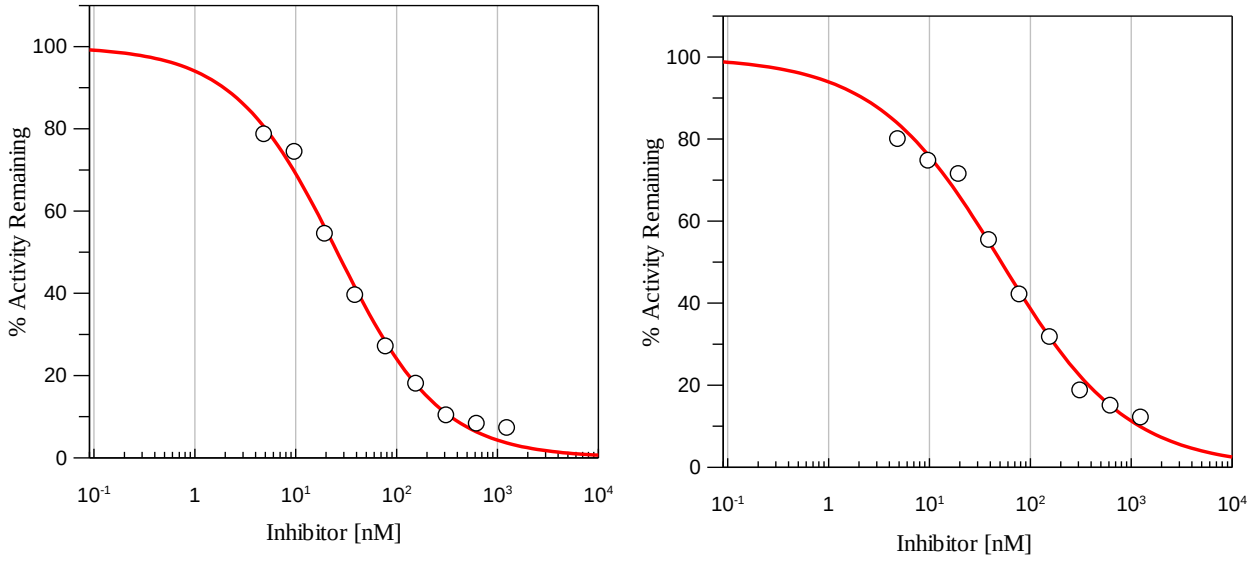


Figure A.6. (a) IC_{50} plot for **3.24** (left). **(b)** IC_{50} plot for **3.25** (right).

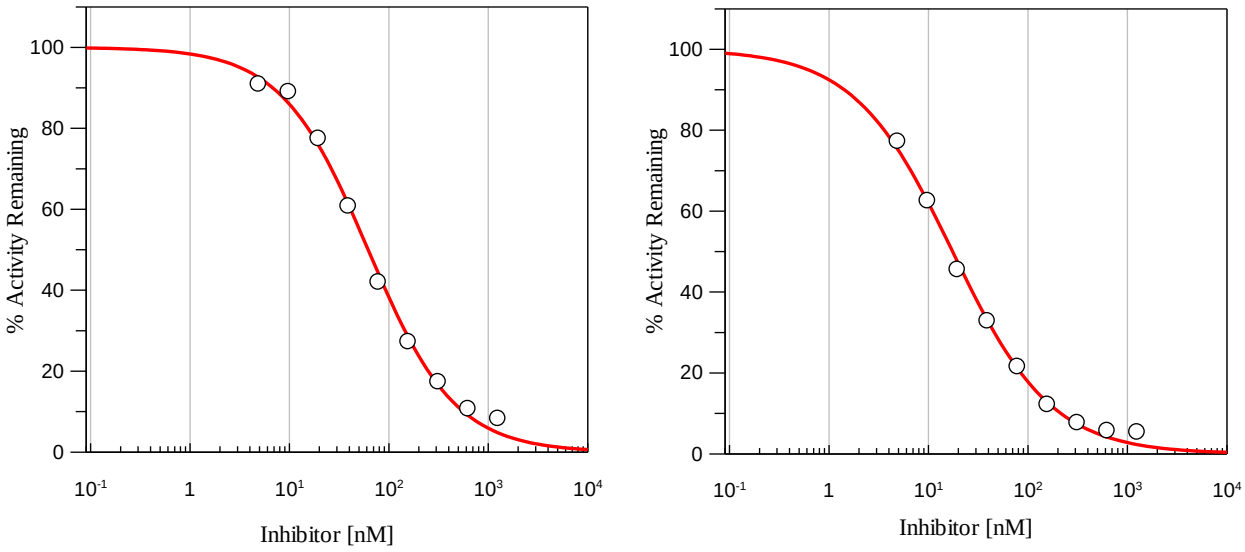


Figure A.7. (a) IC_{50} plot for **3.26** (left). **(b)** IC_{50} plot for **3.27** (right).

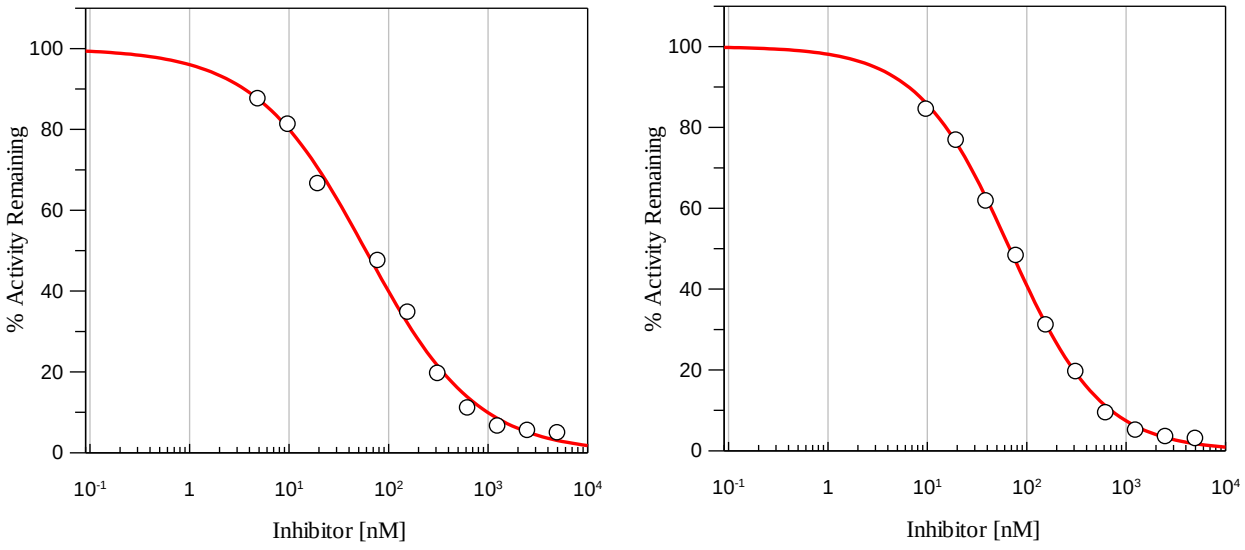


Figure A.8. (a) IC_{50} plot for **3.28** (left). **(b)** IC_{50} plot for **3.29** (right).

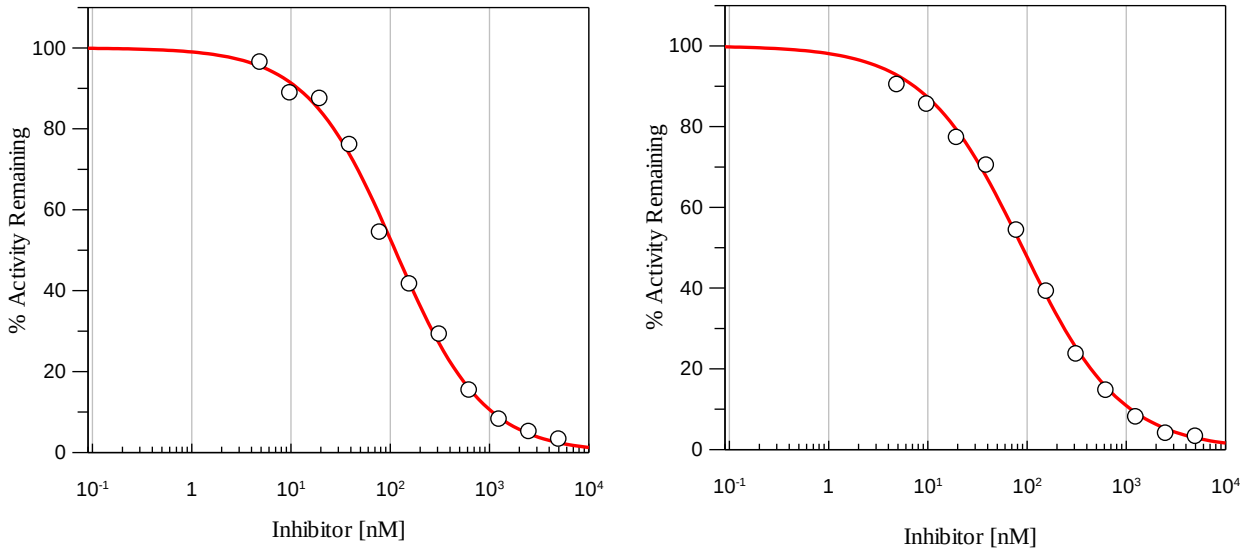


Figure A.9. (a) IC_{50} plot for **3.30** (left). **(b)** IC_{50} plot for **3.31** (right).

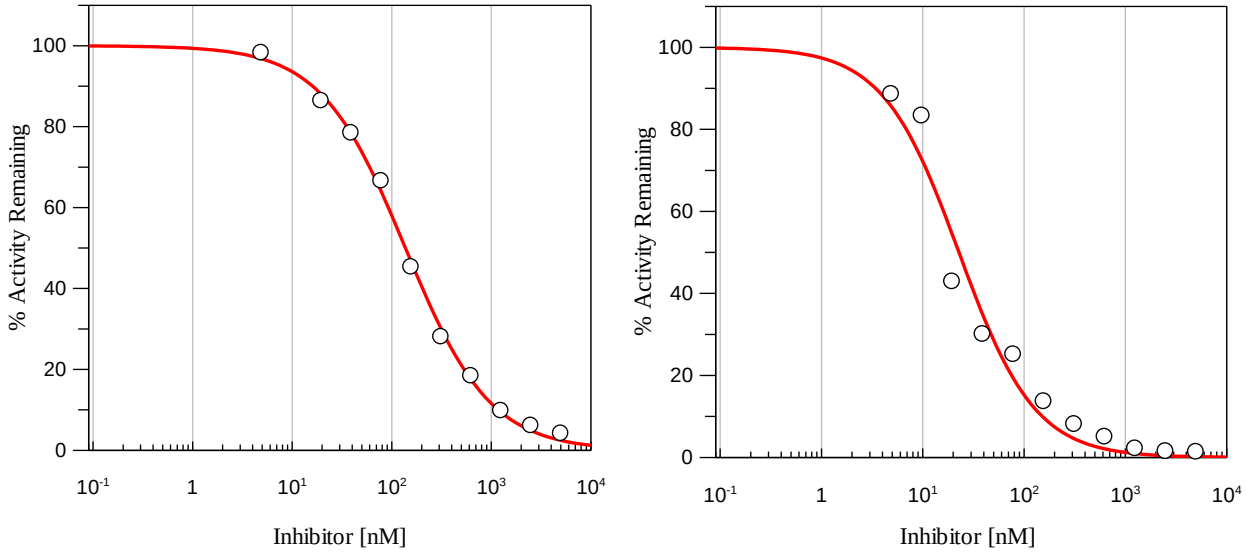


Figure A.10. (a) IC_{50} plot for **3.32** (left). **(b)** IC_{50} plot for **3.33** (right).

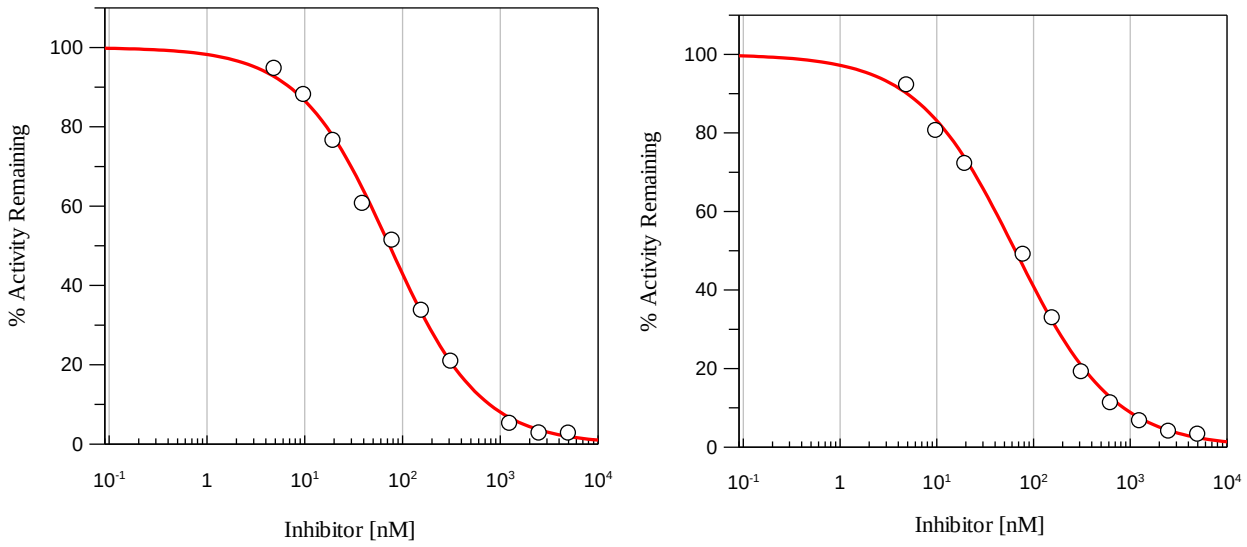


Figure A.11. (a) IC_{50} plot for **3.34** (left). **(b)** IC_{50} plot for **3.35** (right).

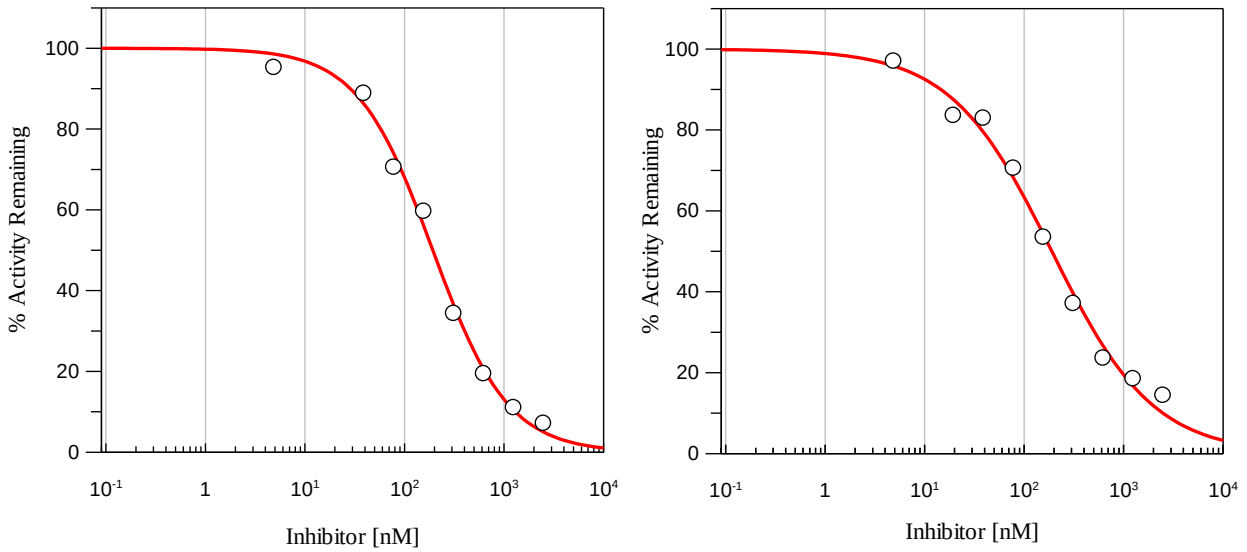


Figure A.12. (a) IC_{50} plot for **3.36** (left). **(b)** IC_{50} plot for **3.37** (right).

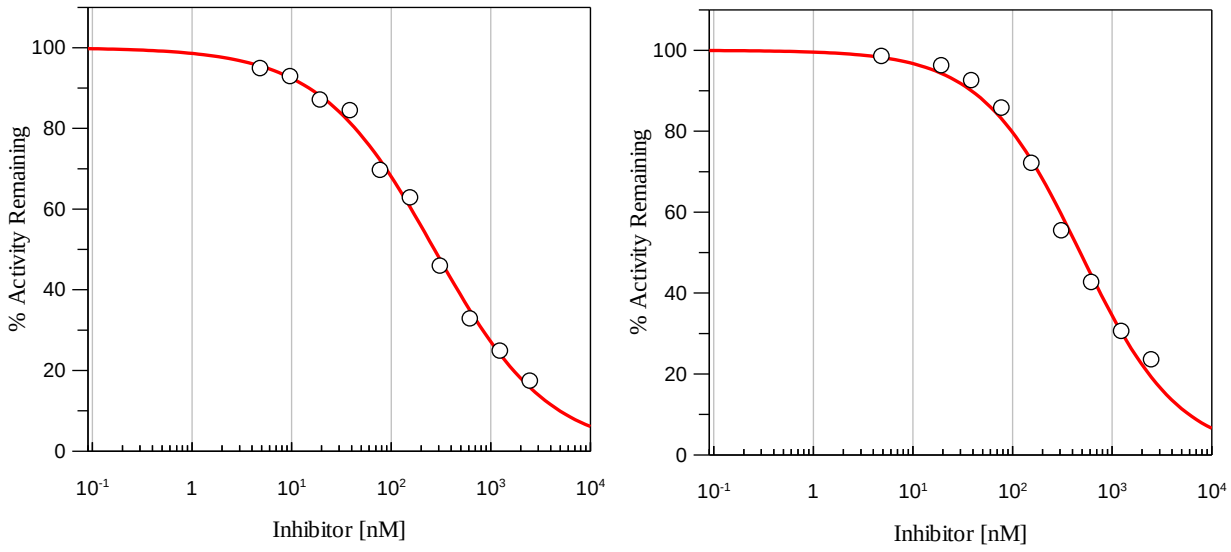
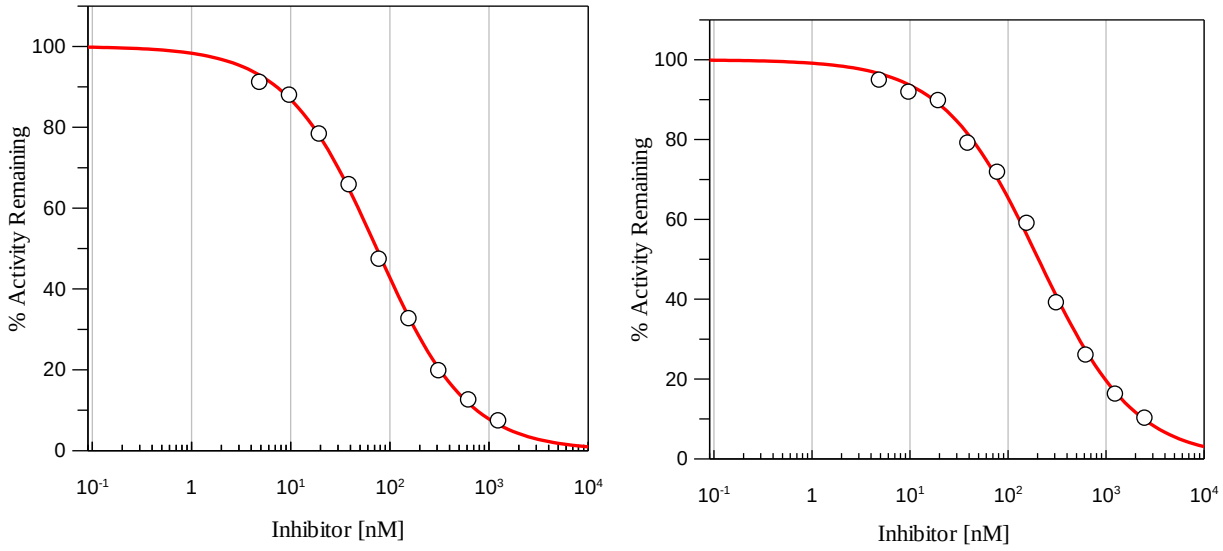


Figure A.13. (a) IC_{50} plot for **3.38** (left). **(b)** IC_{50} plot for **3.39** (right).



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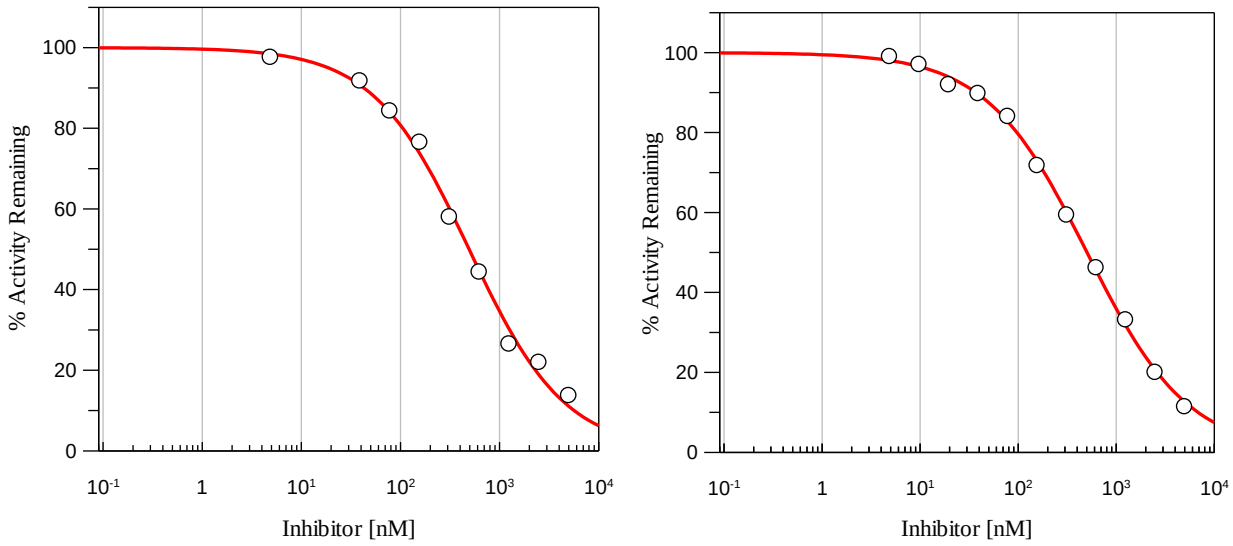

Figure A.15. (a) IC_{50} plot for **3.43** (left). **(b)** IC_{50} plot for **3.44** (right).

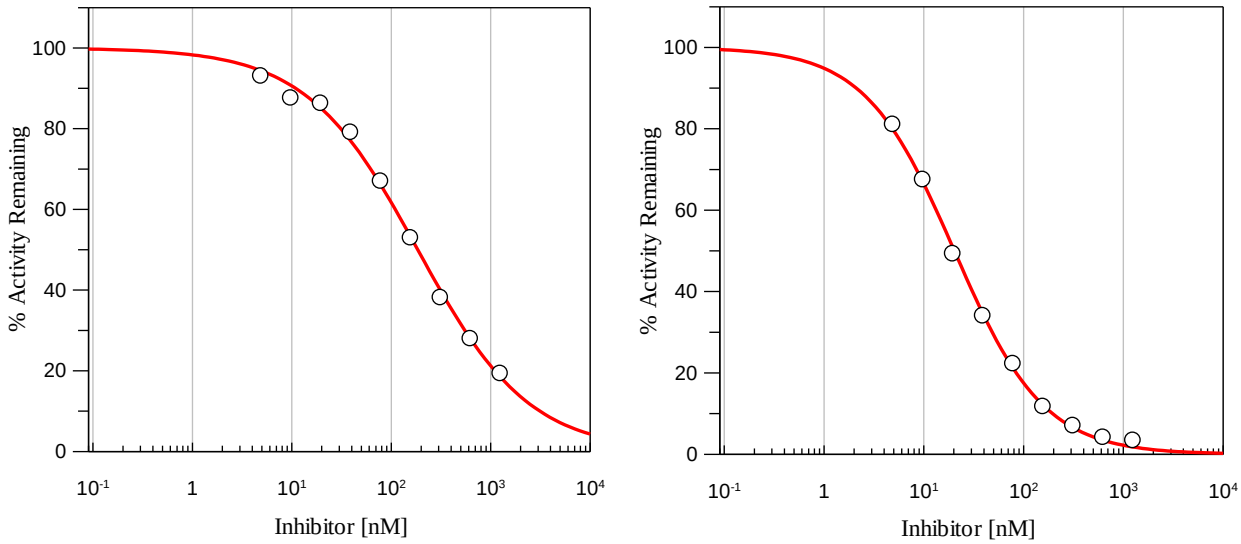

Figure A.16. (a) IC_{50} plot for **3.45** (left). **(b)** IC_{50} plot for **3.46** (right).

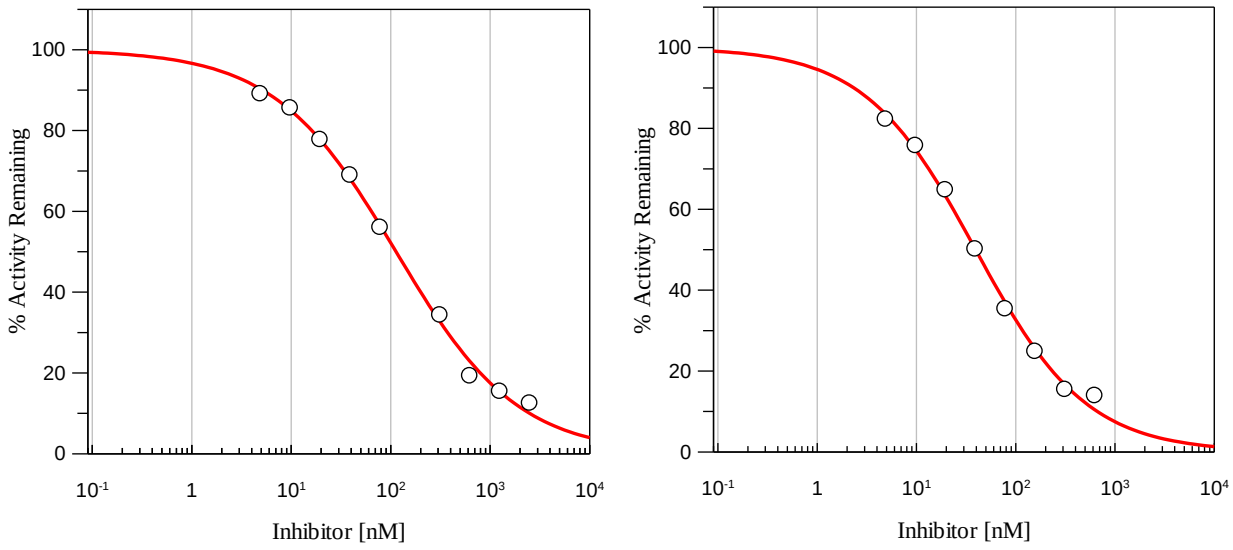

Figure A.17. (a) IC_{50} plot for **3.47** (left). **(b)** IC_{50} plot for **3.48** (right).

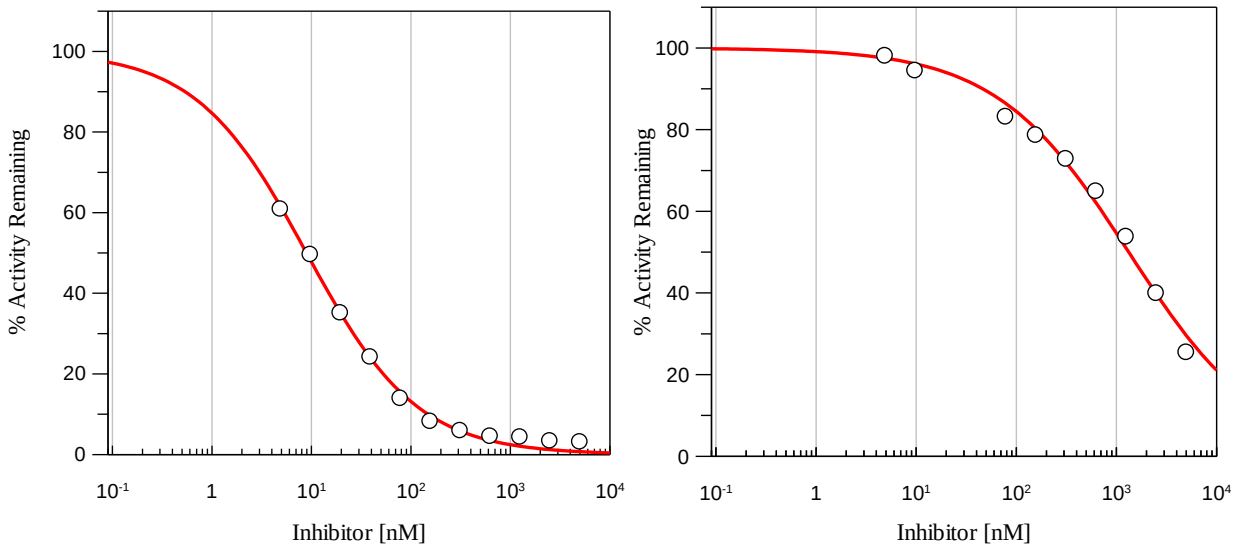

Figure A.18. (a) IC_{50} plot for **3.49** (left). **(b)** IC_{50} plot for **3.50** (right).

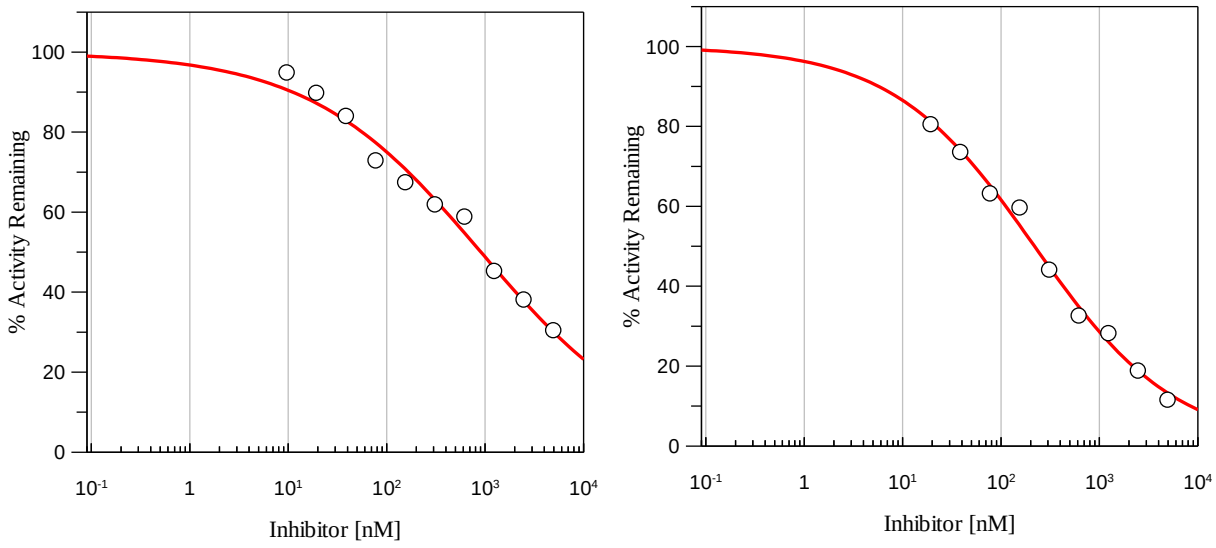

Figure A.19. (a) IC_{50} plot for **3.51** (left). **(b)** IC_{50} plot for **3.56** (right).

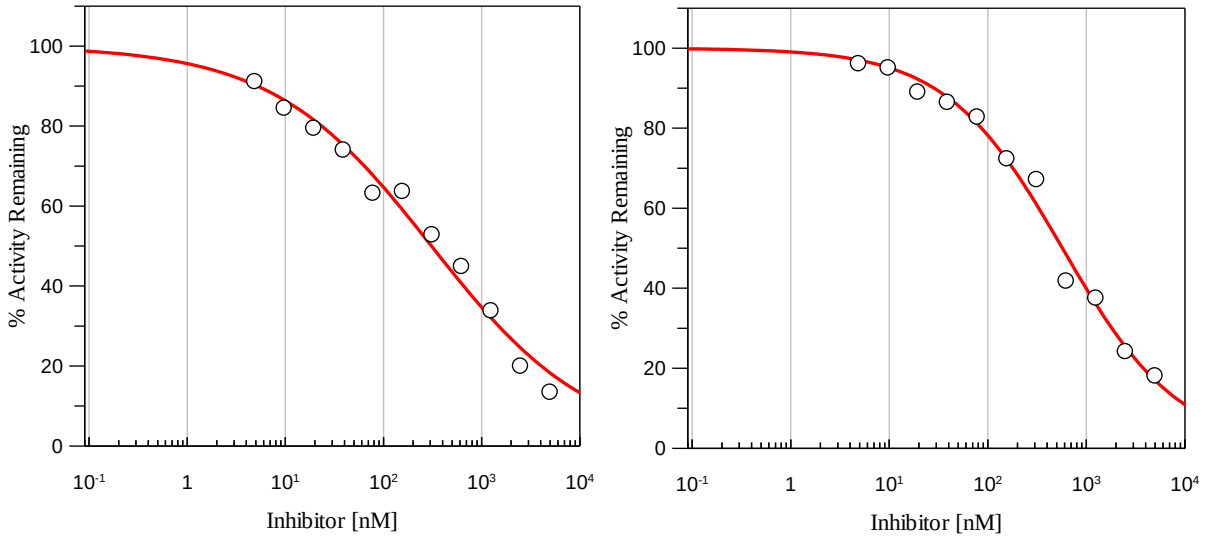

Figure A.20. (a) IC_{50} plot for **3.57** (left). **(b)** IC_{50} plot for **3.58** (right).

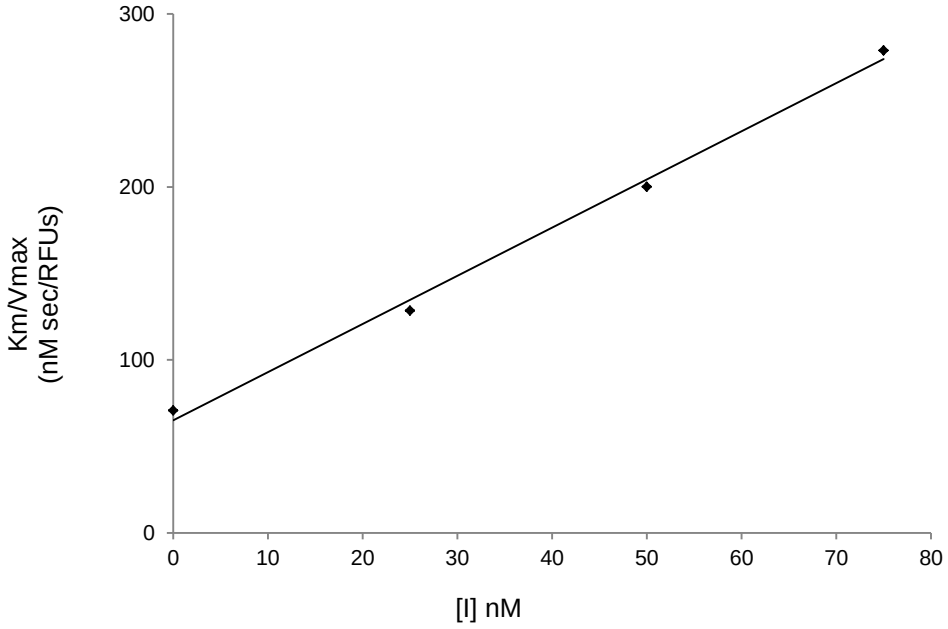

Figure A.21. Replot of the data from **Fig. A.8 (a)** to determine K_i of **3.28**.

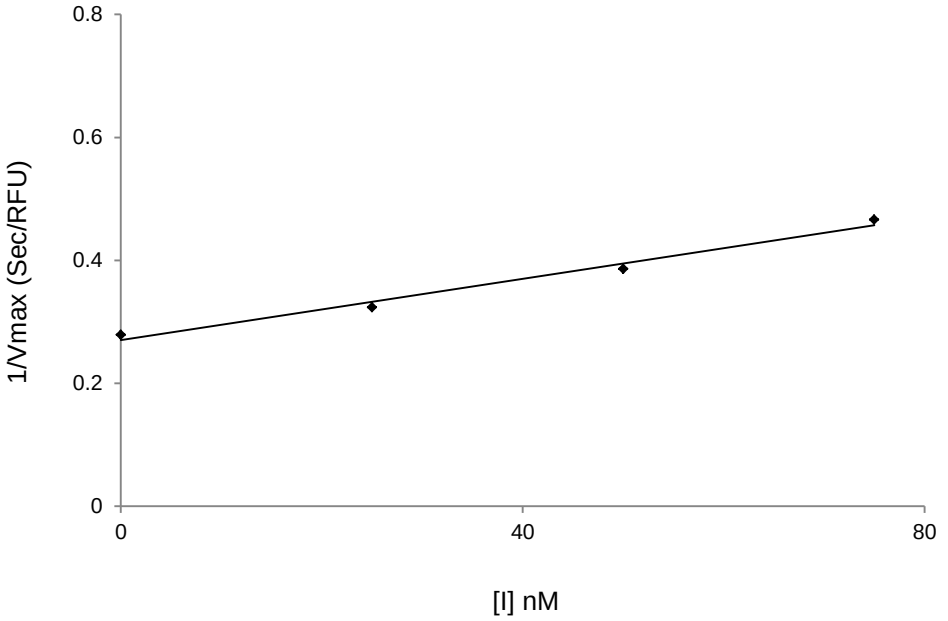

Figure A.22. Replot of the data from **Fig. A.8 (a)** to determine αK_i of **3.28**.

National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

NSC : 772960 / 1	Experiment ID : 1307NS20	Test Type : 08	Units : Molar
Report Date : November 05, 2013	Test Date : July 02, 2013	QNS :	MC :
COMI : ST-17 (127021)	Stain Reagent : SRB Dual-Pass Related	SSPL : 0YWM	

			Log10 Concentration												
	Time		Mean Optical Densities					Percent Growth							
Panel/Cell Line	Zero	Ctrl	-8.0	-7.0	-6.0	-5.0	-4.0	-8.0	-7.0	-6.0	-5.0	-4.0	GI50	TGI	LC50
Leukemia															
CCRF-CEM	0.264	1.288	1.218	1.212	1.069	0.371	0.285	93	93	79	10	2	2.63E-6	> 1.00E-4	> 1.00E-4
HL-60(TB)	1.348	3.406	3.348	3.351	3.331	1.474	0.921	97	97	96	6	-32	3.26E-6	1.45E-5	> 1.00E-4
K-562	0.194	1.874	1.852	1.840	1.710	0.288	0.172	99	98	90	6	-11	2.99E-6	2.13E-5	> 1.00E-4
MOLT-4	0.729	3.067	2.963	2.964	2.669	0.827	0.698	96	96	83	4	-4	2.62E-6	3.13E-5	> 1.00E-4
RPMI-8226	0.748	2.327	2.350	2.308	2.200	0.868	0.508	101	99	92	8	-32	3.14E-6	1.55E-5	> 1.00E-4
SR	0.292	1.044	1.041	1.008	1.022	0.398	0.309	100	95	97	14	2	3.69E-6	> 1.00E-4	> 1.00E-4
Non-Small Cell Lung Cancer															
A549/ATCC	0.343	1.766	1.757	1.788	1.650	0.394	0.072	99	102	92	4	-79	2.98E-6	1.10E-5	4.44E-5
HOP-62	0.402	1.301	1.351	1.280	1.337	0.361	0.048	106	98	104	-10	-88	2.97E-6	8.14E-6	3.24E-5
HOP-92	1.215	1.705	1.686	1.636	1.612	1.055	0.170	96	86	81	-13	-86	2.13E-6	7.24E-6	3.20E-5
NCI-H226	0.592	1.698	1.588	1.616	1.608	0.688	0.137	90	93	92	9	-77	3.19E-6	1.26E-5	4.85E-5
NCI-H23	0.923	2.717	2.661	2.639	2.502	0.958	0.386	97	96	88	2	-58	2.76E-6	1.08E-5	7.31E-5
NCI-H322M	0.774	1.963	1.896	1.868	1.896	1.033	0.154	94	92	94	22	-80	4.08E-6	1.63E-5	5.06E-5
NCI-H460	0.330	2.799	2.876	2.851	2.702	0.210	0.020	103	102	96	-36	-94	2.23E-6	5.31E-6	1.72E-5
Colon Cancer															
COLO 205	0.392	1.797	1.799	1.832	1.798	0.022	0.029	100	102	100	-95	-93	1.81E-6	3.27E-6	5.90E-6
HCC-2998	1.001	3.105	3.137	3.170	3.206	0.898	0.115	102	103	105	-10	-89	2.99E-6	8.14E-6	3.22E-5
HCT-116	0.219	2.048	2.072	1.978	1.955	0.080	0.066	101	96	95	-64	-70	1.92E-6	3.97E-6	8.20E-6
HCT-15	0.271	1.727	1.702	1.728	1.510	0.243	0.037	98	100	85	-11	-87	2.33E-6	7.76E-6	3.31E-5
HT29	0.223	1.110	1.113	1.065	1.130	0.246	0.050	100	95	102	3	-78	3.34E-6	1.08E-5	4.53E-5
KM12	0.671	2.748	2.685	2.688	2.684	0.309	0.055	97	97	97	-54	-92	2.05E-6	4.39E-6	9.41E-6
SW-620	0.397	2.462	2.439	2.437	2.352	0.657	0.237	99	99	95	13	-40	3.50E-6	1.73E-5	> 1.00E-4
CNS Cancer															
SF-268	0.487	1.672	1.612	1.594	1.566	0.594	0.082	95	93	91	9	-83	3.16E-6	1.25E-5	4.37E-5
SF-295	0.897	2.734	2.633	2.617	2.494	0.264	0.058	95	94	87	-71	-94	1.72E-6	3.56E-6	7.40E-6
SF-539	0.826	2.403	2.452	2.334	2.204	0.424	0.058	103	96	87	-49	-93	1.88E-6	4.39E-6	1.07E-5
SNB-19	1.039	2.501	2.377	2.310	2.262	1.388	0.919	92	87	84	24	-12	3.66E-6	4.71E-5	> 1.00E-4
SNB-75	0.860	1.644	1.526	1.517	1.437	0.853	0.169	85	84	74	-1	-80	2.08E-6	9.75E-6	4.15E-5
U251	0.634	2.330	2.373	2.349	2.275	0.759	0.046	103	101	97	7	-93	3.33E-6	1.18E-5	3.74E-5
Melanoma															
MALME-3M	0.499	1.138	1.113	1.067	1.086	0.498	0.027	96	89	92	.	-95	2.85E-6	9.95E-6	3.37E-5
M14	0.465	1.903	1.855	1.821	1.894	0.347	0.130	97	94	99	-25	-72	2.49E-6	6.25E-6	3.35E-5
MDA-MB-435	0.437	2.013	1.976	1.951	1.910	0.418	0.059	98	96	93	-4	-86	2.78E-6	9.03E-6	3.60E-5
SK-MEL-2	1.008	2.127	2.115	2.074	2.134	0.759	0.118	99	95	101	-25	-88	2.54E-6	6.35E-6	2.50E-5
SK-MEL-28	0.614	1.974	1.979	1.918	1.937	0.256	0.113	100	96	97	-58	-82	2.01E-6	4.22E-6	8.84E-6
SK-MEL-5	0.567	2.871	2.752	2.765	2.714	0.112	0.005	95	95	93	-80	-99	1.77E-6	3.45E-6	6.69E-6
UACC-257	0.681	1.525	1.481	1.505	1.457	0.617	0.208	95	98	92	-9	-70	2.59E-6	8.08E-6	4.73E-5
UACC-62	0.795	2.486	2.408	2.375	2.279	0.433	0.105	95	93	88	-46	-87	1.92E-6	4.55E-6	1.28E-5
Ovarian Cancer															
IGROV1	0.661	2.103	2.146	2.077	2.019	0.680	0.146	103	98	94	1	-78	2.99E-6	1.04E-5	4.44E-5
OVCAR-3	0.750	1.852	1.879	1.857	1.817	0.232	0.046	102	100	97	-69	-94	1.91E-6	3.83E-6	7.67E-6
OVCAR-4	0.517	0.946	0.894	0.881	0.856	0.566	0.136	88	85	79	11	-74	2.69E-6	1.36E-5	5.26E-5
OVCAR-5	0.517	1.766	1.779	1.767	1.730	0.708	0.079	101	100	97	15	-85	3.76E-6	1.42E-5	4.50E-5
OVCAR-8	0.432	1.849	1.869	1.865	1.737	0.378	0.217	101	101	92	-13	-50	2.52E-6	7.58E-6	> 1.00E-4
NCI/ADR-RES	1.003	2.992	3.047	3.002	2.730	1.028	0.581	103	101	87	1	-42	2.69E-6	1.07E-5	> 1.00E-4
SK-OV-3	0.779	1.840	1.831	1.877	1.928	0.676	0.136	99	103	108	-13	-83	3.02E-6	7.78E-6	3.39E-5
Renal Cancer															
786-0	0.762	2.585	2.505	2.585	2.613	0.612	0.304	96	100	102	-20	-60	2.66E-6	6.87E-6	5.62E-5
A498	1.469	2.149	2.009	1.922	1.887	0.835	0.103	79	67	61	-43	-93	1.29E-6	3.87E-6	1.37E-5
ACHN	0.397	1.862	1.894	1.828	1.727	0.452	0.163	102	98	91	4	-59	2.94E-6	1.15E-5	7.20E-5
CAKI-1	0.438	2.105	2.080	2.098	1.995	0.507	0.092	98	100	93	4	-79	3.06E-6	1.12E-5	4.47E-5
RXF 393	0.868	1.589	1.469	1.471	1.464	0.669	0.092	83	84	83	-23	-89	2.04E-6	6.06E-6	2.55E-5
SN12C	0.737	2.444	2.360	2.357	2.256	0.148	0.015	95	95	89	-80	-98	1.70E-6	3.36E-6	6.65E-6
TK-10	0.571	1.413	1.395	1.400	1.398	0.653	0.305	98	98	98	10	-47	3.50E-6	1.49E-5	> 1.00E-4
UO-31	0.718	2.211	2.023	1.981	1.654	0.534	0.065	87	85	63	-26	-91	1.39E-6	5.13E-6	2.36E-5
Prostate Cancer															
PC-3	0.555	1.653	1.604	1.571	1.424	0.666	0.294	96	93	79	10	-47	2.64E-6	1.50E-5	> 1.00E-4
DU-145	0.348	1.548	1.586	1.536	1.570	0.535	0.162	103	99	102	16	-54	3.99E-6	1.68E-5	8.87E-5
Breast Cancer															
MCF7	0.486	2.355	2.219	2.274	2.263	0.478	0.351	93	96	95	-2	-28	2.92E-6	9.62E-6	> 1.00E-4
MDA-MB-231/ATCC	0.611	1.361	1.382	1.314	1.207	0.358	0.058	103	94	79	-41	-91	1.75E-6	4.54E-6	1.49E-5
HS 578T	1.495	2.402	2.326	2.331	2.298	1.302	0.616	92	92	89	-13	-59	2.40E-6	7.46E-6	6.43E-5
BT-549	0.888	1.920	1.915	1.913	1.899	0.612	0.163	100	99	98	-31	-82	2.35E-6	5.74E-6	2.36E-5
T-47D	0.405	0.956	0.935	0.928	0.956	0.365	0.316	96	95	100	-10	-22	2.85E-6	8.11E-6	> 1.00E-4
MDA-MB-468	0.721	1.270	1.244	1.248	1.249	0.797	0.376	95	96	96	14	-48	3.63E-6	1.67E-5	> 1.00E-4

Table A.1. In-Vitro Testing Results (GI_{50}, TGI, and LC_{50}) of Compound **3.22.**

National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

NSC : 772956 / 1	Experiment ID : 1306NS09	Test Type : 08	Units : Molar
Report Date : January 30, 2014	Test Date : June 17, 2013	QNS :	MC :
COMI : ST-13 (126747)	Stain Reagent : SRB Dual-Pass Related	SSPL : 0YWM	

		Log10 Concentration													
	Time	Mean Optical Densities						Percent Growth							
Panel/Cell Line	Zero	Ctrl	-8.0	-7.0	-6.0	-5.0	-4.0	-8.0	-7.0	-6.0	-5.0	-4.0	GI50	TGI	LC50
Leukemia															
CCRF-CEM	0.434	2.034	1.990	1.944	1.807	0.490	0.256	97	94	86	3	-41	2.72E-6	1.20E-5	> 1.00E-4
K-562	0.223	1.490	1.362	1.537	1.411	0.270	0.127	90	104	94	4	-43	3.06E-6	1.20E-5	> 1.00E-4
MOLT-4	0.780	2.728	2.661	2.339	2.369	0.756	0.531	97	80	82	-3	-32	2.36E-6	9.18E-6	> 1.00E-4
RPMI-8226	1.046	2.853	2.859	2.858	2.725	1.047	0.604	100	100	93	.	-42	2.90E-6	1.00E-5	> 1.00E-4
SR	0.596	2.098	2.061	1.971	1.950	0.541	0.311	98	92	90	-9	-48	2.53E-6	8.06E-6	> 1.00E-4
Non-Small Cell Lung Cancer															
A549/ATCC	0.458	2.315	2.236	2.306	2.150	0.649	0.070	96	99	91	10	-85	3.22E-6	1.28E-5	4.30E-5
HOP-62	0.667	1.730	1.683	1.676	1.772	0.913	0.050	96	95	104	23	-93	4.65E-6	1.58E-5	4.29E-5
HOP-92	1.173	1.702	1.669	1.628	1.595	0.865	0.093	94	86	80	-26	-92	1.91E-6	5.65E-6	2.29E-5
NCI-H226	0.682	1.821	1.768	1.734	1.623	0.116	0.135	95	92	83	-83	-80	1.57E-6	3.15E-6	6.32E-6
NCI-H23	0.924	2.577	2.551	2.494	2.407	1.146	0.300	98	95	90	13	-68	3.31E-6	1.46E-5	6.07E-5
NCI-H322M	0.984	2.412	2.282	2.282	2.328	1.410	0.115	91	91	94	30	-88	4.85E-6	1.79E-5	4.74E-5
NCI-H460	0.305	2.924	3.009	3.016	2.928	0.232	0.050	103	103	100	-24	-84	2.54E-6	6.41E-6	2.73E-5
NCI-H522	0.876	2.089	1.967	1.918	1.855	0.846	0.196	90	86	81	-3	-78	2.32E-6	9.09E-6	4.24E-5
Colon Cancer															
COLO 205	0.569	2.320	2.271	2.269	2.372	0.020	0.009	97	97	103	-97	-99	1.84E-6	3.28E-6	5.84E-6
HCC-2998	1.045	3.137	3.254	3.233	3.211	1.353	0.079	106	105	104	15	-92	4.00E-6	1.37E-5	4.01E-5
HCT-116	0.288	2.217	2.162	2.169	2.166	0.024	0.011	97	97	97	-92	-96	1.78E-6	3.27E-6	6.01E-6
HCT-15	0.342	2.203	2.032	2.007	1.993	0.271	0.117	91	89	89	-21	-66	2.26E-6	6.45E-6	4.43E-5
HT29	0.313	1.746	1.729	1.721	1.749	0.321	0.082	99	98	100	1	-74	3.19E-6	1.02E-5	4.78E-5
KM12	0.561	2.364	2.305	2.311	2.297	0.496	0.094	97	97	96	-12	-83	2.68E-6	7.81E-6	3.43E-5
SW-620	0.383	2.462	2.423	2.379	2.377	0.659	0.101	98	96	96	13	-74	3.60E-6	1.42E-5	5.33E-5
CNS Cancer															
SF-268	0.722	2.030	1.983	1.999	1.935	0.894	0.070	96	98	93	13	-90	3.44E-6	1.34E-5	4.08E-5
SF-295	0.992	3.031	2.973	2.997	2.915	1.054	0.204	97	98	94	3	-79	3.06E-6	1.09E-5	4.40E-5
SF-539	1.032	2.747	2.813	2.788	2.757	0.471	0.098	104	102	101	-54	-91	2.12E-6	4.46E-6	9.37E-6
SNB-19	0.857	2.102	2.003	1.995	2.049	1.136	0.095	92	91	96	22	-89	4.20E-6	1.59E-5	4.47E-5
SNB-75	0.860	1.608	1.477	1.503	1.434	0.860	0.031	83	86	77	.	-96	2.23E-6	9.98E-6	3.30E-5
U251	0.895	2.686	2.626	2.654	2.554	0.405	0.040	97	98	93	-55	-96	1.95E-6	4.25E-6	9.28E-6
Melanoma															
LOX IMVI	0.493	2.815	2.779	2.777	2.683	0.030	0.054	98	98	94	-94	-89	1.72E-6	3.17E-6	5.84E-6
MALME-3M	0.818	1.650	1.627	1.569	1.645	0.735	0.213	97	90	99	-10	-74	2.82E-6	8.07E-6	4.21E-5
M14	0.549	1.929	1.862	1.853	1.934	0.563	0.044	95	94	100	1	-92	3.21E-6	1.03E-5	3.53E-5
MDA-MB-435	0.672	2.832	2.806	2.852	2.750	0.737	0.266	99	101	96	3	-60	3.13E-6	1.11E-5	6.83E-5
SK-MEL-2	1.330	2.767	2.694	2.672	2.795	1.096	0.214	95	93	102	-18	-84	2.72E-6	7.12E-6	3.08E-5
SK-MEL-28	0.712	2.024	2.009	2.071	1.985	0.541	0.048	99	104	97	-24	-93	2.45E-6	6.33E-6	2.37E-5
SK-MEL-5	0.909	3.066	3.037	3.026	2.844	0.021	0.020	99	98	90	-98	-98	1.63E-6	3.01E-6	5.56E-6
UACC-257	1.080	2.250	2.190	2.221	2.170	1.305	0.229	95	97	93	19	-79	3.83E-6	1.57E-5	5.08E-5
UACC-62	0.911	2.408	2.354	2.367	2.254	0.453	0.064	96	97	90	-50	-93	1.92E-6	4.37E-6	9.95E-6
Ovarian Cancer															
IGROV1	0.583	2.149	2.183	2.092	2.172	0.770	0.204	102	96	101	12	-65	3.76E-6	1.43E-5	6.38E-5
OVCAR-4	0.702	1.415	1.359	1.369	1.255	0.692	0.006	92	93	77	-1	-99	2.23E-6	9.57E-6	3.14E-5
OVCAR-5	0.595	1.697	1.704	1.673	1.663	0.281	0.139	101	98	97	-53	-77	2.06E-6	4.44E-6	9.57E-6
OVCAR-8	0.504	2.390	2.351	2.312	2.282	0.666	0.178	98	96	94	9	-65	3.29E-6	1.31E-5	6.29E-5
NCI/ADR-RES	0.763	2.494	2.527	2.503	2.283	0.795	0.196	102	101	88	2	-74	2.75E-6	1.06E-5	4.79E-5
SK-OV-3	0.770	1.880	1.780	1.849	1.890	0.884	0.013	91	97	101	10	-98	3.64E-6	1.24E-5	3.59E-5
Renal Cancer															
786-0	0.899	2.676	2.669	2.635	2.673	0.864	0.060	100	98	100	-4	-93	3.02E-6	9.16E-6	3.27E-5
A498	1.565	2.239	2.039	2.030	2.076	1.141	0.074	70	69	76	-27	-95	1.78E-6	5.45E-6	2.17E-5
ACHN	0.515	1.978	2.046	1.927	1.851	0.467	0.025	105	96	91	-9	-95	2.57E-6	8.06E-6	2.97E-5
CAKI-1	1.002	3.035	3.019	3.021	3.050	1.131	0.215	99	99	101	6	-79	3.45E-6	1.19E-5	4.61E-5
RXF 393	1.073	1.863	1.822	1.879	1.677	0.624	0.175	95	102	76	-42	-84	1.67E-6	4.42E-6	1.56E-5
SN12C	0.977	2.820	2.637	2.655	2.660	0.206	0.113	90	91	91	-79	-88	1.75E-6	3.44E-6	6.76E-6
TK-10	0.712	1.616	1.557	1.523	1.615	0.838	0.081	93	90	100	14	-89	3.80E-6	1.37E-5	4.20E-5
UO-31	0.881	2.397	2.151	2.150	2.083	0.839	0.068	84	84	79	-5	-92	2.23E-6	8.78E-6	3.28E-5
Prostate Cancer															
PC-3	0.519	2.283	2.213	2.178	1.917	0.633	0.052	96	94	79	6	-90	2.52E-6	1.17E-5	3.85E-5
DU-145	0.415	1.596	1.622	1.622	1.600	0.619	-0.002	102	102	100	17	-100	4.04E-6	1.40E-5	3.75E-5
Breast Cancer															
MCF7	0.726	2.836	2.754	2.826	2.804	0.613	0.110	96	100	98	-16	-85	2.66E-6	7.29E-6	3.13E-5
MDA-MB-231/ATCC	0.847	2.005	2.001	1.937	1.909	0.644	0.107	100	94	92	-24	-87	2.29E-6	6.21E-6	2.57E-5
HS 578T	1.662	2.677	2.653	2.668	2.658	1.724	0.846	98	99	98	6	-49	3.33E-6	1.29E-5	> 1.00E-4
BT-549	1.004	2.005	1.935	1.960	2.027	0.698	0.130	93	95	102	-30	-87	2.47E-6	5.89E-6	2.21E-5
T-47D	0.572	1.231	1.215	1.235	1.277	0.593	0.049	97	101	107	3	-91	3.54E-6	1.08E-5	3.65E-5
MDA-MB-468	0.821	1.385	1.324	1.350	1.271	0.807	0.230	89	94	80	-2	-72	2.32E-6	9.51E-6	4.86E-5

Table A.2. In-Vitro Testing Results (GI_{50}, TGI, and LC_{50}) of Compound **3.23.**

National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

NSC : 772958 / 1	Experiment ID : 1306NS17	Test Type : 08	Units : Molar
Report Date : January 30, 2014	Test Date : June 24, 2013	QNS :	MC :
COMI : ST-15 (126749)	Stain Reagent : SRB Dual-Pass Related	SSPL : 0YWM	

		Log10 Concentration													
	Time	Mean Optical Densities						Percent Growth							
Panel/Cell Line	Zero	Ctrl	-8.0	-7.0	-6.0	-5.0	-4.0	-8.0	-7.0	-6.0	-5.0	-4.0	GI50	TGI	LC50
Leukemia															
CCRF-CEM	0.443	2.060	2.049	2.031	1.972	0.392	0.267	99	98	95	-12	-40	2.63E-6	7.79E-6	> 1.00E-4
HL-60(TB)	0.773	2.444	2.491	2.485	2.331	0.577	0.423	103	102	93	-25	-45	2.31E-6	6.11E-6	> 1.00E-4
K-562	0.197	1.422	1.395	1.481	1.248	0.186	0.099	98	105	86	-6	-50	2.46E-6	8.64E-6	> 1.00E-4
MOLT-4	0.729	2.531	2.609	2.579	2.231	0.716	0.501	104	103	83	-2	-31	2.46E-6	9.53E-6	> 1.00E-4
RPMI-8226	0.654	2.139	2.114	2.109	1.931	0.357	0.306	98	98	86	-45	-53	1.88E-6	4.51E-6	3.87E-5
SR	0.281	0.891	0.819	0.903	0.816	0.212	0.155	88	102	88	-25	-45	2.17E-6	6.04E-6	> 1.00E-4
Non-Small Cell Lung Cancer															
A549/ATCC	0.375	1.722	1.639	1.561	1.468	0.326	0.073	94	88	81	-13	-81	2.14E-6	7.24E-6	3.51E-5
HOP-62	0.655	2.050	2.019	2.124	2.125	0.194	0.100	98	105	105	-70	-85	2.07E-6	3.98E-6	7.66E-6
HOP-92	1.382	1.891	1.814	1.790	1.745	0.787	0.117	85	80	71	-43	-92	1.53E-6	4.20E-6	1.39E-5
NCI-H226	0.693	1.887	1.839	1.830	1.768	0.173	0.136	96	95	90	-75	-80	1.75E-6	3.51E-6	7.05E-6
NCI-H23	0.309	0.929	0.947	0.946	0.914	0.133	0.058	103	103	98	-57	-81	2.03E-6	4.28E-6	9.01E-6
NCI-H322M	0.854	2.064	2.146	2.113	2.030	1.160	0.051	107	104	97	25	-94	4.53E-6	1.63E-5	4.27E-5
NCI-H460	0.342	2.938	2.917	2.939	2.833	0.140	0.035	99	100	96	-59	-90	1.98E-6	4.16E-6	8.74E-6
NCI-H522	0.685	1.618	1.521	1.563	1.560	0.575	0.257	90	94	94	-16	-63	2.50E-6	7.13E-6	5.36E-5
Colon Cancer															
COLO 205	0.675	2.725	2.736	2.767	2.779	0.033	0.160	101	102	103	-95	-76	1.85E-6	3.30E-6	5.91E-6
HCC-2998	0.751	2.557	2.635	2.630	2.650	0.613	0.113	104	104	105	-18	-85	2.80E-6	7.10E-6	2.98E-5
HCT-116	0.164	1.598	1.610	1.624	1.459	-0.002	-0.025	101	102	90	-100	-100	1.63E-6	2.98E-6	5.46E-6
HCT-15	0.281	1.704	1.633	1.608	1.548	0.083	0.148	95	93	89	-71	-47	1.76E-6	3.61E-6	
HT29	0.186	1.151	1.116	1.156	1.171	0.045	0.058	96	101	102	-76	-69	1.96E-6	3.74E-6	7.14E-6
KM12	0.586	2.410	2.455	2.485	2.362	0.180	0.032	102	104	97	-68	-94	1.93E-6	3.87E-6	7.76E-6
SW-620	0.386	2.430	2.379	2.349	2.313	0.511	0.090	97	96	94	6	-77	3.18E-6	1.19E-5	4.75E-5
CNS Cancer															
SF-268	0.747	2.147	2.078	2.162	2.122	0.988	-0.002	95	101	98	17	-100	3.94E-6	1.40E-5	3.74E-5
SF-295	0.994	2.845	2.703	2.712	2.649	0.103	0.144	92	93	89	-90	-86	1.66E-6	3.16E-6	6.01E-6
SF-539	0.773	2.324	2.318	2.274	2.221	0.044	-0.023	100	97	93	-94	-100	1.70E-6	3.14E-6	5.81E-6
SNB-19	0.896	2.286	2.209	2.104	2.081	1.188	0.346	94	87	85	21	-61	3.54E-6	1.80E-5	7.27E-5
SNB-75	0.795	1.497	1.421	1.383	1.376	0.636	0.040	89	84	83	-20	-95	2.08E-6	6.38E-6	2.51E-5
U251	0.508	2.193	2.080	2.031	1.976	0.268	0.030	93	90	87	-47	-94	1.89E-6	4.45E-6	1.14E-5
Melanoma															
LOX IMVI	0.613	3.020	2.990	3.009	3.003	0.009	0.160	99	100	99	-99	-74	1.77E-6	3.17E-6	5.68E-6
MALME-3M	0.910	1.798	1.869	1.851	1.835	0.747	0.237	108	106	104	-18	-74	2.78E-6	7.13E-6	3.73E-5
M14	0.447	1.857	1.881	1.892	1.862	0.156	0.102	102	102	100	-65	-77	2.02E-6	4.04E-6	8.10E-6
MDA-MB-435	0.559	2.327	2.295	2.238	2.264	0.067	0.052	98	95	96	-88	-91	1.79E-6	3.33E-6	6.22E-6
SK-MEL-2	0.936	2.070	2.122	2.198	2.236	0.452	0.215	105	111	115	-52	-77	2.45E-6	4.89E-6	9.76E-6
SK-MEL-28	0.406	1.375	1.315	1.311	1.306	0.034	-0.017	94	93	93	-92	-100	1.71E-6	3.18E-6	5.94E-6
SK-MEL-5	0.850	2.958	2.897	2.883	2.769	0.024	0.123	97	96	91	-97	-86	1.65E-6	3.05E-6	5.61E-6
UACC-257	0.777	1.586	1.515	1.514	1.536	0.629	0.157	91	91	94	-19	-80	2.45E-6	6.78E-6	3.23E-5
UACC-62	0.883	2.578	2.559	2.560	2.380	0.652	0.191	99	99	88	-26	-78	2.16E-6	5.91E-6	2.86E-5
Ovarian Cancer															
IGROV1	0.786	2.259	2.467	2.398	2.459	0.825	0.174	114	109	114	3	-78	3.74E-6	1.08E-5	4.50E-5
OVCAR-3	0.744	1.713	1.787	1.807	1.788	0.202	-0.054	108	110	108	-73	-100	2.09E-6	3.95E-6	7.47E-6
OVCAR-4	0.855	1.520	1.534	1.456	1.472	0.860	0.039	102	90	93	1	-95	2.91E-6	1.02E-5	3.37E-5
OVCAR-5	0.598	1.675	1.745	1.709	1.699	0.228	0.187	107	103	102	-62	-69	2.08E-6	4.20E-6	8.47E-6
OVCAR-8	0.451	1.988	1.976	1.911	1.874	0.238	0.200	99	95	93	-47	-56	2.02E-6	4.59E-6	2.07E-5
NCI/ADR-RES	0.712	2.275	2.311	2.341	2.232	0.439	0.317	102	104	97	-38	-55	2.23E-6	5.21E-6	4.78E-5
SK-OV-3	0.875	2.105	2.100	2.245	2.235	0.772	-0.005	100	111	111	-12	-100	3.12E-6	8.00E-6	2.71E-5
Renal Cancer															
786-0	0.684	2.459	2.458	2.566	2.413	0.399	-0.011	100	106	97	-42	-100	2.19E-6	5.02E-6	1.39E-5
A498	1.633	2.147	2.034	2.012	1.985	0.185	-0.015	78	74	68	-89	-100	1.31E-6	2.72E-6	5.67E-6
ACHN	0.313	1.520	1.544	1.515	1.465	0.216	-0.028	102	100	95	-31	-100	2.28E-6	5.67E-6	1.88E-5
CAKI-1	0.706	2.611	2.519	2.518	2.435	0.543	0.177	95	95	91	-23	-75	2.28E-6	6.26E-6	3.30E-5
RXF 393	0.973	1.642	1.611	1.561	1.514	0.303	0.170	95	88	81	-69	-83	1.61E-6	3.46E-6	7.48E-6
SN12C	0.576	2.111	2.050	2.011	1.965	0.151	0.244	96	94	90	-74	-58	1.76E-6	3.55E-6	7.16E-6
TK-10	0.698	1.547	1.492	1.600	1.620	0.761	0.114	94	106	109	7	-84	3.79E-6	1.21E-5	4.27E-5
UO-31	0.888	2.435	2.305	2.339	2.218	0.409	0.106	92	94	86	-54	-88	1.81E-6	4.11E-6	9.36E-6
Prostate Cancer															
PC-3	0.530	2.085	1.982	1.950	1.758	0.473	0.096	93	91	79	-11	-82	2.10E-6	7.57E-6	3.56E-5
DU-145	0.493	1.753	1.841	1.850	1.819	0.648	-0.048	107	108	105	12	-100	3.93E-6	1.29E-5	3.59E-5
Breast Cancer															
MCF7	0.452	2.215	2.076	2.105	2.017	0.128	0.212	92	94	89	-72	-53	1.74E-6	3.57E-6	7.33E-6
MDA-MB-231/ATCC	0.567	1.341	1.360	1.312	1.280	0.242	0.210	102	96	92	-57	-63	1.91E-6	4.13E-6	8.92E-6
HS 578T	1.118	2.282	2.273	2.213	2.171	0.931	0.777	99	94	90	-17	-31	2.38E-6	6.98E-6	> 1.00E-4
BT-549	0.854	1.899	1.873	1.892	1.836	0.117	0.063	98	99	94	-86	-93	1.75E-6	3.32E-6	6.29E-6
T-47D	0.771	1.755	1.704	1.717	1.649	0.729	0.145	95	96	89	-5	-81	2.59E-6	8.76E-6	3.87E-5
MDA-MB-468	0.950	1.603	1.518	1.527	1.540	0.935	0.332	87	88	90	-2	-65	2.74E-6	9.60E-6	5.78E-5

Table A.3. In-Vitro Testing Results (GI_{50}, TGI, and LC_{50}) of Compound **3.27.**

National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

NSC : 772948 / 1	Experiment ID : 1306NS09	Test Type : 08	Units : Molar
Report Date : January 30, 2014	Test Date : June 17, 2013	QNS :	MC :
COMI : ST-3 (126737)	Stain Reagent : SRB Dual-Pass Related	SSPL : 0YWM	

			Log10 Concentration											
	Time		Mean Optical Densities					Percent Growth						
Panel/Cell Line	Zero	Ctrl	-8.0	-7.0	-6.0	-5.0	-4.0	-8.0	-7.0	-6.0	-5.0	-4.0	GI50	TGI	LC50
Leukemia															
CCRF-CEM	0.434	2.103	2.085	2.044	1.844	0.660	0.302	99	96	84	14	-31	3.06E-6	2.03E-5	> 1.00E-4
K-562	0.223	1.538	1.613	1.634	1.565	0.332	0.133	106	107	102	8	-41	3.59E-6	1.48E-5	> 1.00E-4
MOLT-4	0.780	2.580	2.669	2.655	2.267	0.850	0.516	105	104	83	4	-34	2.59E-6	1.27E-5	> 1.00E-4
RPMI-8226	1.046	2.972	2.952	2.929	2.788	1.325	0.562	99	98	90	14	-46	3.41E-6	1.73E-5	> 1.00E-4
SR	0.596	2.028	1.981	1.974	1.839	0.527	0.337	97	96	87	-12	-43	2.37E-6	7.63E-6	> 1.00E-4
Non-Small Cell Lung Cancer															
A549/ATCC	0.458	2.349	2.282	2.237	2.119	0.667	0.073	96	94	88	11	-84	3.11E-6	1.31E-5	4.38E-5
HOP-62	0.667	1.734	1.726	1.786	1.780	1.230	0.018	99	105	104	53	-97	1.04E-5	2.25E-5	4.84E-5
HOP-92	1.173	1.663	1.607	1.575	1.539	0.966	0.064	89	82	75	-18	-95	1.85E-6	6.44E-6	2.63E-5
NCI-H226	0.682	1.772	1.700	1.675	1.608	0.852	0.239	93	91	85	16	-65	3.19E-6	1.56E-5	6.52E-5
NCI-H23	0.924	2.536	2.418	2.388	2.388	1.273	0.253	93	91	91	22	-73	3.89E-6	1.70E-5	5.75E-5
NCI-H322M	0.984	2.196	2.244	2.178	2.146	1.466	0.057	104	99	96	40	-94	6.56E-6	1.98E-5	4.68E-5
NCI-H460	0.305	2.908	2.887	2.849	2.848	0.242	0.081	99	98	98	-21	-74	2.53E-6	6.67E-6	3.57E-5
NCI-H522	0.876	2.007	1.853	1.842	1.745	0.931	0.144	86	85	77	5	-84	2.36E-6	1.13E-5	4.17E-5
Colon Cancer															
COLO 205	0.569	2.286	2.396	2.429	2.367	0.038	0.001	106	108	105	-93	-100	1.89E-6	3.38E-6	6.04E-6
HCC-2998	1.045	3.038	3.113	3.102	3.063	1.454	0.197	104	103	101	21	-81	4.31E-6	1.59E-5	4.94E-5
HCT-116	0.288	2.201	2.277	2.274	2.115	0.307	0.006	104	104	96	1	-98	3.03E-6	1.02E-5	3.28E-5
HCT-15	0.342	2.074	1.931	1.920	1.842	0.411	0.063	92	91	87	4	-82	2.77E-6	1.11E-5	4.26E-5
HT29	0.313	1.800	1.822	1.827	1.860	0.462	0.043	102	102	104	10	-86	3.76E-6	1.27E-5	4.19E-5
KM12	0.551	2.381	2.328	2.413	2.178	0.459	0.063	97	102	89	-18	-89	2.31E-6	6.76E-6	2.82E-5
SW-620	0.383	2.457	2.359	2.307	2.287	0.865	0.123	95	93	92	23	-68	4.07E-6	1.80E-5	6.35E-5
CNS Cancer															
SF-268	0.722	2.083	2.057	2.007	1.981	1.016	0.171	98	94	93	22	-76	3.98E-6	1.66E-5	5.38E-5
SF-295	0.992	2.972	2.916	2.883	2.854	1.125	0.102	97	95	94	7	-90	3.19E-6	1.17E-5	3.87E-5
SF-539	1.032	2.749	2.694	2.673	2.564	0.978	0.038	97	96	89	-5	-96	2.60E-6	8.80E-6	3.10E-5
SNB-19	0.857	2.135	2.113	2.119	2.111	1.388	0.060	98	99	98	42	-93	7.09E-6	2.04E-5	4.79E-5
SNB-75	0.860	1.652	1.540	1.488	1.505	0.967	0.087	86	79	81	14	-90	2.90E-6	1.35E-5	4.11E-5
U251	0.895	2.603	2.558	2.570	2.565	0.964	0.032	97	98	98	4	-96	3.23E-6	1.10E-5	3.45E-5
Melanoma															
LOX IMVI	0.493	2.768	2.708	2.655	2.620	0.621	0.091	97	95	93	6	-82	3.13E-6	1.16E-5	4.35E-5
MALME-3M	0.818	1.619	1.619	1.573	1.492	0.724	0.103	100	94	84	-11	-87	2.27E-6	7.58E-6	3.22E-5
M14	0.549	1.940	1.919	1.965	1.907	0.741	0.070	98	102	98	14	-87	3.70E-6	1.37E-5	4.27E-5
MDA-MB-435	0.672	2.753	2.735	2.665	2.550	0.650	0.135	99	96	90	-3	-80	2.69E-6	9.21E-6	4.06E-5
SK-MEL-2	1.330	2.758	2.786	2.860	2.831	1.440	0.143	102	107	105	8	-89	3.68E-6	1.20E-5	3.93E-5
SK-MEL-28	0.712	2.080	2.043	1.971	2.013	1.026	0.067	97	92	95	23	-91	4.21E-6	1.59E-5	4.39E-5
SK-MEL-5	0.909	3.083	3.077	3.004	2.986	0.107	0.089	100	96	96	-88	-90	1.77E-6	3.31E-6	6.19E-6
UACC-257	1.080	2.249	2.145	2.144	2.104	1.483	0.214	91	91	88	34	-80	5.10E-6	2.00E-5	5.45E-5
UACC-62	0.911	2.375	2.390	2.405	2.220	1.074	0.098	101	102	89	11	-89	3.19E-6	1.29E-5	4.06E-5
Ovarian Cancer															
IGROV1	0.583	1.936	2.036	1.996	1.876	0.860	0.153	107	104	96	20	-74	4.04E-6	1.65E-5	5.60E-5
OVCAR-4	0.702	1.428	1.393	1.390	1.297	0.702	0.018	95	95	82	.	-98	2.45E-6	9.98E-6	3.25E-5
OVCAR-5	0.595	1.688	1.658	1.593	1.589	0.858	0.063	97	91	91	24	-89	4.09E-6	1.63E-5	4.49E-5
OVCAR-8	0.504	2.380	2.365	2.297	2.271	0.890	0.143	99	96	94	21	-72	3.98E-6	1.67E-5	5.83E-5
NCI/ADR-RES	0.763	2.478	2.437	2.407	2.290	0.944	0.237	98	96	89	11	-69	3.14E-6	1.36E-5	5.77E-5
SK-OV-3	0.770	1.805	1.876	1.971	1.951	1.154	-0.004	107	116	114	37	-100	6.80E-6	1.86E-5	4.32E-5
Renal Cancer															
786-0	0.899	2.714	2.770	2.787	2.724	0.931	0.072	103	104	101	2	-92	3.25E-6	1.04E-5	3.56E-5
A498	1.565	2.197	2.124	2.053	2.001	1.480	0.051	88	77	69	-5	-97	1.80E-6	8.45E-6	3.08E-5
ACHN	0.515	1.922	1.920	1.925	1.910	0.480	0.005	100	100	99	-7	-99	2.91E-6	8.61E-6	2.93E-5
CAKI-1	1.002	2.993	2.930	2.832	2.833	1.077	0.125	97	92	92	4	-88	2.99E-6	1.10E-5	3.88E-5
RXF 393	1.073	1.824	1.816	1.739	1.720	0.831	0.159	99	89	86	-23	-85	2.15E-6	6.20E-6	2.74E-5
SN12C	0.977	2.754	2.753	2.804	2.637	0.686	0.103	100	103	93	-30	-89	2.25E-6	5.73E-6	2.18E-5
TK-10	0.712	1.539	1.529	1.517	1.522	0.782	0.035	99	97	98	8	-95	3.43E-6	1.21E-5	3.67E-5
UO-31	0.881	2.289	2.144	2.163	1.928	0.840	0.043	90	91	74	-5	-95	2.03E-6	8.72E-6	3.17E-5
Prostate Cancer															
PC-3	0.519	2.186	2.148	2.057	1.919	0.666	0.035	98	92	84	9	-93	2.83E-6	1.22E-5	3.77E-5
DU-145	0.415	1.548	1.616	1.614	1.560	0.755	-0.009	106	106	101	30	-100	5.22E-6	1.70E-5	4.12E-5
Breast Cancer															
MCF7	0.726	2.829	3.083	3.105	3.086	0.802	0.077	112	113	112	4	-89	3.74E-6	1.09E-5	3.77E-5
MDA-MB-231/ATCC	0.847	1.984	2.076	2.039	1.999	1.009	0.121	108	105	101	14	-86	3.88E-6	1.39E-5	4.39E-5
HS 578T	1.662	2.662	2.603	2.626	2.566	1.900	1.057	94	96	90	24	-36	4.04E-6	2.48E-5	> 1.00E-4
BT-549	1.004	1.995	1.976	2.091	1.954	1.016	0.107	98	110	96	1	-89	3.05E-6	1.03E-5	3.67E-5
T-47D	0.572	1.254	1.317	1.366	1.341	0.733	0.157	109	116	113	24	-73	5.05E-6	1.76E-5	5.82E-5
MDA-MB-468	0.821	1.367	1.325	1.295	1.251	0.845	0.260	92	87	79	4	-68	2.43E-6	1.15E-5	5.59E-5

Table A.4. In-Vitro Testing Results (GI_{50}, TGI, and LC_{50}) of Compound **3.28.**

National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

NSC : 772949 / 1	Experiment ID : 1306NS09	Test Type : 08	Units : Molar
Report Date : January 30, 2014	Test Date : June 17, 2013	QNS :	MC :
COMI : ST-4 (126738)	Stain Reagent : SRB Dual-Pass Related	SSPL : 0YWM	

		Log10 Concentration												
	Time	Mean Optical Densities					Percent Growth							
Panel/Cell Line	Zero	Ctrl	-8.0	-7.0	-6.0	-5.0	-4.0	-8.0	-7.0	-6.0	-5.0	-4.0	GI50	TGI	LC50
Leukemia															
CCRF-CEM	0.434	2.017	2.044	2.023	1.825	0.545	0.271	102	100	88	7	-38	2.94E-6	1.44E-5	> 1.00E-4
K-562	0.223	1.601	1.657	1.618	1.543	0.273	0.120	104	101	96	4	-46	3.14E-6	1.18E-5	> 1.00E-4
MOLT-4	0.780	2.688	2.658	2.469	2.242	0.751	0.451	98	88	77	-4	-42	2.14E-6	8.97E-6	> 1.00E-4
RPMI-8226	1.046	2.891	2.869	2.971	2.752	1.094	0.561	99	104	92	3	-46	2.97E-6	1.13E-5	> 1.00E-4
SR	0.596	2.125	2.080	1.841	1.884	0.486	0.310	97	81	84	-19	-48	2.15E-6	6.60E-6	> 1.00E-4
Non-Small Cell Lung Cancer															
A549/ATCC	0.458	2.212	2.142	2.176	2.060	0.631	0.066	96	98	91	10	-86	3.22E-6	1.27E-5	4.24E-5
HOP-62	0.667	1.768	1.696	1.723	1.820	0.893	0.066	93	96	105	20	-90	4.46E-6	1.53E-5	4.34E-5
HOP-92	1.173	1.757	1.715	1.692	1.679	0.844	0.094	93	89	87	-28	-92	2.08E-6	5.69E-6	2.20E-5
NCI-H226	0.682	1.801	1.743	1.760	1.649	0.383	0.214	95	96	86	-44	-69	1.90E-6	4.61E-6	1.77E-5
NCI-H23	0.924	2.584	2.491	2.441	2.358	0.969	0.193	94	91	86	3	-79	2.72E-6	1.08E-5	4.41E-5
NCI-H322M	0.984	2.440	2.331	2.352	2.281	1.426	0.084	92	94	89	30	-91	4.62E-6	1.77E-5	4.57E-5
NCI-H460	0.305	2.900	3.015	3.001	2.899	0.175	0.048	104	104	100	-43	-84	2.24E-6	5.02E-6	1.50E-5
NCI-H522	0.876	2.070	1.875	1.851	1.766	0.765	0.204	84	82	74	-13	-77	1.91E-6	7.15E-6	3.83E-5
Colon Cancer															
COLO 205	0.569	2.416	2.411	2.407	2.401	0.048	0.049	100	99	99	-92	-91	1.81E-6	3.31E-6	6.05E-6
HCC-2998	1.045	3.150	3.151	3.193	3.282	1.077	0.094	100	102	106	2	-91	3.45E-6	1.04E-5	3.60E-5
HCT-116	0.288	2.153	2.086	2.132	2.010	0.014	0.003	96	99	92	-95	-99	1.68E-6	3.11E-6	5.74E-6
HCT-15	0.342	2.148	2.107	2.006	1.893	0.293	0.058	98	92	86	-14	-83	2.28E-6	7.20E-6	3.30E-5
HT29	0.313	1.696	1.649	1.716	1.699	0.226	0.048	97	101	100	-28	-85	2.47E-6	6.07E-6	2.46E-5
KM12	0.561	2.346	2.265	2.314	2.230	0.250	0.064	95	98	93	-56	-89	1.96E-6	4.24E-6	9.18E-6
SW-620	0.383	2.401	2.351	2.453	2.378	0.712	0.089	98	103	99	16	-77	3.91E-6	1.50E-5	5.16E-5
CNS Cancer															
SF-268	0.722	2.137	2.018	2.046	1.977	0.904	0.114	92	94	89	13	-84	3.23E-6	1.36E-5	4.44E-5
SF-295	0.992	3.054	2.972	3.032	2.969	1.045	0.270	96	99	96	3	-73	3.10E-6	1.08E-5	4.98E-5
SF-539	1.032	2.715	2.695	2.698	2.581	0.214	0.007	99	99	92	-79	-99	1.76E-6	3.45E-6	6.75E-6
SNB-19	0.857	2.083	1.966	1.988	2.039	1.072	0.054	90	92	96	18	-94	3.87E-6	1.44E-5	4.05E-5
SNB-75	0.860	1.667	1.576	1.560	1.500	0.828	0.069	89	87	79	-4	-92	2.25E-6	9.01E-6	3.34E-5
U251	0.895	2.642	2.608	2.592	2.571	0.592	0.026	98	97	96	-34	-97	2.26E-6	5.48E-6	1.80E-5
Melanoma															
LOX IMVI	0.493	2.814	2.730	2.738	2.633	0.093	0.038	96	97	92	-81	-92	1.75E-6	3.40E-6	6.61E-6
MALME-3M	0.818	1.649	1.648	1.576	1.573	0.665	0.249	100	91	91	-19	-70	2.36E-6	6.75E-6	4.12E-5
M14	0.549	1.863	1.834	1.802	1.764	0.425	0.049	98	95	92	-23	-91	2.34E-6	6.35E-6	2.51E-5
MDA-MB-435	0.672	2.853	2.793	2.912	2.646	0.633	0.270	97	103	90	-6	-60	2.63E-6	8.70E-6	6.58E-5
SK-MEL-2	1.330	2.674	2.582	2.611	2.720	0.891	0.150	93	95	103	-33	-89	2.46E-6	5.73E-6	2.02E-5
SK-MEL-28	0.712	2.027	1.970	2.043	1.913	0.522	0.037	96	101	91	-27	-95	2.24E-6	5.93E-6	2.19E-5
SK-MEL-5	0.909	3.089	3.077	3.046	2.947	0.088	0.109	99	98	93	-90	-88	1.72E-6	3.22E-6	6.03E-6
UACC-257	1.080	2.204	2.134	2.153	2.045	1.173	0.186	94	95	86	8	-83	2.90E-6	1.23E-5	4.36E-5
UACC-62	0.911	2.456	2.369	2.401	2.184	0.565	0.126	94	96	82	-38	-86	1.86E-6	4.84E-6	1.78E-5
Ovarian Cancer															
IGROV1	0.583	2.143	2.203	2.184	2.191	0.870	0.214	104	103	103	18	-63	4.23E-6	1.68E-5	6.86E-5
OVCAR-4	0.702	1.437	1.412	1.376	1.253	0.719	0.039	97	92	75	2	-95	2.20E-6	1.05E-5	3.47E-5
OVCAR-5	0.595	1.693	1.667	1.657	1.584	0.556	0.035	98	97	90	-7	-94	2.59E-6	8.54E-6	3.13E-5
OVCAR-8	0.504	2.368	2.316	2.390	2.229	0.686	0.153	97	101	93	10	-70	3.26E-6	1.33E-5	5.66E-5
NCI/ADR-RES	0.763	2.486	2.490	2.469	2.212	0.717	0.126	100	99	84	-6	-83	2.39E-6	8.57E-6	3.70E-5
SK-OV-3	0.770	1.945	1.948	2.039	1.994	0.894	0.048	100	108	104	11	-94	3.79E-6	1.26E-5	3.81E-5
Renal Cancer															
786-0	0.899	2.707	2.639	2.616	2.701	0.657	0.057	96	95	100	-27	-94	2.47E-6	6.13E-6	2.22E-5
A498	1.565	2.186	2.030	2.059	1.991	1.137	0.031	75	80	69	-27	-98	1.56E-6	5.19E-6	2.09E-5
ACHN	0.515	1.945	1.957	1.924	1.852	0.453	0.005	101	98	93	-12	-99	2.58E-6	7.69E-6	2.73E-5
CAKI-1	1.002	3.017	2.992	3.079	2.875	1.046	0.215	99	103	93	2	-79	2.97E-6	1.06E-5	4.43E-5
RXF 393	1.073	1.906	1.890	1.883	1.776	0.667	0.160	98	97	84	-38	-85	1.91E-6	4.90E-6	1.81E-5
SN12C	0.977	2.800	2.679	2.700	2.611	0.341	0.139	93	95	90	-65	-86	1.80E-6	3.80E-6	7.99E-6
TK-10	0.712	1.568	1.512	1.522	1.593	0.753	0.050	93	95	103	5	-93	3.46E-6	1.12E-5	3.63E-5
UO-31	0.881	2.410	2.158	2.189	2.010	0.841	0.046	84	86	74	-5	-95	2.01E-6	8.75E-6	3.19E-5
Prostate Cancer															
PC-3	0.519	2.211	2.166	2.190	2.021	0.639	0.062	97	99	89	7	-88	2.98E-6	1.19E-5	3.98E-5
DU-145	0.415	1.624	1.634	1.659	1.597	0.667	0.002	101	103	98	21	-100	4.17E-6	1.49E-5	3.87E-5
Breast Cancer															
MCF7	0.726	2.833	2.824	2.955	2.980	0.613	0.113	100	106	107	-16	-85	2.92E-6	7.46E-6	3.16E-5
MDA-MB-231/ATCC	0.847	2.098	2.102	2.059	1.956	0.754	0.168	100	97	89	-11	-80	2.44E-6	7.75E-6	3.66E-5
HS 578T	1.662	2.626	2.530	2.586	2.546	1.727	0.828	90	96	92	7	-50	3.09E-6	1.31E-5	9.92E-5
BT-549	1.004	2.026	1.953	1.915	1.951	0.642	0.059	93	89	93	-36	-94	2.15E-6	5.25E-6	1.74E-5
T-47D	0.572	1.258	1.257	1.279	1.296	0.594	0.233	100	103	106	3	-59	3.49E-6	1.13E-5	7.09E-5
MDA-MB-468	0.821	1.401	1.368	1.355	1.252	0.761	0.256	94	92	74	-7	-69	1.98E-6	8.12E-6	4.93E-5

Table A.5. In-Vitro Testing Results (GI_{50}, TGI, and LC_{50}) of Compound **3.29.**

National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

NSC : 772950 / 1	Experiment ID : 1306NS09	Test Type : 08	Units : Molar
Report Date : January 30, 2014	Test Date : June 17, 2013	QNS :	MC :
COMI : ST-5 (126739)	Stain Reagent : SRB Dual-Pass Related	SSPL : 0YWM	

			Log10 Concentration												
	Time		Mean Optical Densities					Percent Growth							
Panel/Cell Line	Zero	Ctrl	-8.0	-7.0	-6.0	-5.0	-4.0	-8.0	-7.0	-6.0	-5.0	-4.0	GI50	TGI	LC50
Leukemia															
CCRF-CEM	0.434	2.063	2.050	2.047	1.871	0.664	0.267	99	99	88	14	-38	3.28E-6	1.86E-5	> 1.00E-4
K-562	0.223	1.467	1.579	1.502	1.391	0.318	0.146	109	103	94	8	-35	3.22E-6	1.52E-5	> 1.00E-4
MOLT-4	0.780	2.467	2.412	2.261	2.198	0.768	0.385	97	88	84	-2	-51	2.50E-6	9.59E-6	9.70E-5
RPMI-8226	1.046	2.918	2.919	3.017	2.901	1.268	0.745	100	105	99	12	-29	3.65E-6	1.96E-5	> 1.00E-4
SR	0.596	2.043	1.912	1.800	1.809	0.552	0.356	91	83	84	-7	-40	2.35E-6	8.28E-6	> 1.00E-4
Non-Small Cell Lung Cancer															
A549/ATCC	0.458	2.333	2.251	2.272	2.129	0.650	0.031	96	97	89	10	-93	3.13E-6	1.26E-5	3.82E-5
HOP-62	0.667	1.833	1.692	1.772	1.790	1.168	0.114	88	95	96	43	-83	7.38E-6	2.19E-5	5.48E-5
HOP-92	1.173	1.798	1.742	1.713	1.705	1.106	0.133	91	86	85	-6	-89	2.43E-6	8.64E-6	3.42E-5
NCI-H226	0.682	1.834	1.764	1.755	1.720	0.910	0.212	94	93	90	20	-69	3.72E-6	1.67E-5	6.12E-5
NCI-H23	0.924	2.511	2.501	2.448	2.336	1.110	0.192	99	96	89	12	-79	3.19E-6	1.35E-5	4.77E-5
NCI-H322M	0.984	2.360	2.328	2.269	2.355	1.639	0.108	98	93	100	48	-89	9.00E-6	2.23E-5	5.18E-5
NCI-H460	0.305	2.914	2.990	3.092	2.955	0.253	0.037	103	107	102	-17	-88	2.72E-6	7.18E-6	2.91E-5
NCI-H522	0.876	2.040	1.879	1.875	1.725	0.790	0.083	86	86	73	-10	-91	1.89E-6	7.60E-6	3.14E-5
Colon Cancer															
COLO 205	0.569	2.549	2.505	2.449	2.478	0.133	0.081	98	95	96	-77	-86	1.85E-6	3.61E-6	7.02E-6
HCC-2998	1.045	2.864	3.002	3.004	3.106	1.511	0.135	108	108	113	26	-87	5.27E-6	1.69E-5	4.68E-5
HCT-116	0.288	2.134	2.162	2.138	2.080	0.245	0.017	102	100	97	-15	-94	2.63E-6	7.33E-6	2.76E-5
HCT-15	0.342	2.159	2.127	2.097	1.918	0.428	0.059	98	97	87	5	-83	2.80E-6	1.13E-5	4.21E-5
HT29	0.313	1.659	1.670	1.650	1.667	0.317	0.030	101	99	101	.	-90	3.20E-6	1.01E-5	3.58E-5
KM12	0.561	2.369	2.303	2.306	2.240	0.431	0.051	96	97	93	-23	-91	2.34E-6	6.31E-6	2.48E-5
SW-620	0.383	2.466	2.402	2.458	2.382	0.886	0.068	97	100	96	24	-82	4.36E-6	1.68E-5	4.97E-5
CNS Cancer															
SF-268	0.722	2.128	2.060	2.047	2.031	1.043	0.082	95	94	93	23	-89	4.10E-6	1.60E-5	4.50E-5
SF-295	0.992	3.021	2.956	2.870	2.933	1.140	0.113	97	93	96	7	-89	3.29E-6	1.19E-5	3.96E-5
SF-539	1.032	2.733	2.777	2.694	2.696	1.324	0.033	103	98	98	17	-97	3.91E-6	1.41E-5	3.88E-5
SNB-19	0.857	2.095	2.001	1.977	2.029	1.282	0.035	92	91	95	34	-96	5.49E-6	1.83E-5	4.44E-5
SNB-75	0.860	1.760	1.630	1.645	1.571	1.012	0.188	85	87	79	17	-78	2.93E-6	1.50E-5	5.05E-5
U251	0.895	2.678	2.617	2.601	2.547	1.055	0.010	97	96	93	9	-99	3.23E-6	1.21E-5	3.52E-5
Melanoma															
LOX IMVI	0.493	2.865	2.868	2.822	2.748	0.566	0.047	100	98	95	3	-90	3.09E-6	1.08E-5	3.69E-5
MALME-3M	0.818	1.634	1.603	1.594	1.562	0.726	0.327	96	95	91	-11	-60	2.52E-6	7.75E-6	6.23E-5
M14	0.549	1.838	1.784	1.796	1.816	0.647	0.030	96	97	98	8	-95	3.41E-6	1.19E-5	3.66E-5
MDA-MB-435	0.672	2.785	2.743	2.724	2.548	0.636	0.157	98	97	89	-5	-77	2.58E-6	8.77E-6	4.22E-5
SK-MEL-2	1.330	2.652	2.574	2.622	2.629	1.202	0.105	94	98	98	-10	-92	2.80E-6	8.14E-6	3.08E-5
SK-MEL-28	0.712	2.143	2.135	2.176	2.031	1.147	0.068	99	102	92	30	-90	4.81E-6	1.78E-5	4.63E-5
SK-MEL-5	0.909	3.075	3.020	3.053	2.851	0.088	0.010	97	99	90	-90	-99	1.66E-6	3.15E-6	5.97E-6
UACC-257	1.080	2.208	2.120	2.131	2.067	1.374	0.029	92	93	88	26	-97	4.08E-6	1.63E-5	4.13E-5
UACC-62	0.911	2.445	2.373	2.355	2.274	0.994	0.059	95	94	89	5	-94	2.92E-6	1.13E-5	3.63E-5
Ovarian Cancer															
IGROV1	0.583	2.097	2.146	2.183	2.156	1.044	0.237	103	106	104	30	-59	5.42E-6	2.18E-5	7.87E-5
OVCAR-4	0.702	1.512	1.495	1.449	1.341	0.803	0.235	98	92	79	12	-67	2.72E-6	1.44E-5	6.18E-5
OVCAR-5	0.595	1.695	1.713	1.748	1.648	0.860	0.063	102	105	96	24	-89	4.35E-6	1.63E-5	4.49E-5
OVCAR-8	0.504	2.342	2.388	2.349	2.270	0.821	0.155	102	100	96	17	-69	3.84E-6	1.58E-5	5.98E-5
NCI/ADR-RES	0.763	2.589	2.566	2.548	2.368	0.864	0.623	99	98	88	6	-18	2.88E-6	1.70E-5	> 1.00E-4
SK-OV-3	0.770	1.983	1.986	2.052	2.143	1.169	0.052	100	106	113	33	-93	6.12E-6	1.82E-5	4.54E-5
Renal Cancer															
786-0	0.899	2.702	2.667	2.639	2.702	1.000	0.034	98	96	100	6	-96	3.38E-6	1.13E-5	3.51E-5
A498	1.565	2.228	2.135	2.166	2.104	1.295	0.023	86	91	81	-17	-99	2.07E-6	6.68E-6	2.53E-5
ACHN	0.515	1.952	2.023	1.973	1.853	0.483	0.001	105	101	93	-6	-100	2.71E-6	8.66E-6	2.93E-5
CAKI-1	1.002	2.966	2.968	3.021	2.852	1.219	0.255	100	103	94	11	-75	3.40E-6	1.35E-5	5.17E-5
RXF 393	1.073	1.872	1.846	1.854	1.790	0.929	0.090	97	98	90	-13	-92	2.42E-6	7.40E-6	2.93E-5
SN12C	0.977	2.810	2.698	2.665	2.679	0.758	0.068	94	92	93	-22	-93	2.35E-6	6.39E-6	2.46E-5
TK-10	0.712	1.530	1.506	1.491	1.579	0.837	0.045	97	95	106	15	-94	4.14E-6	1.38E-5	3.97E-5
UO-31	0.881	2.418	2.130	2.089	1.985	0.901	0.066	81	79	72	1	-93	2.04E-6	1.03E-5	3.52E-5
Prostate Cancer															
PC-3	0.519	2.327	2.339	2.307	2.071	0.698	0.216	101	99	86	10	-58	2.96E-6	1.40E-5	7.52E-5
DU-145	0.415	1.606	1.577	1.609	1.581	0.807	0.009	98	100	98	33	-98	5.45E-6	1.79E-5	4.31E-5
Breast Cancer															
MCF7	0.726	2.858	2.829	2.948	2.977	0.797	0.194	99	104	106	3	-73	3.49E-6	1.10E-5	4.97E-5
MDA-MB-231/ATCC	0.847	2.028	2.062	2.016	1.989	1.006	0.098	103	99	97	13	-88	3.64E-6	1.36E-5	4.20E-5
HS 578T	1.662	2.646	2.675	2.677	2.588	1.851	0.849	103	103	94	19	-49	3.88E-6	1.91E-5	> 1.00E-4
BT-549	1.004	1.994	1.987	1.957	1.966	0.928	0.094	99	96	97	-8	-91	2.82E-6	8.46E-6	3.24E-5
T-47D	0.572	1.211	1.168	1.243	1.242	0.641	0.272	93	105	105	11	-52	3.83E-6	1.48E-5	9.15E-5
MDA-MB-468	0.821	1.335	1.292	1.310	1.247	0.797	0.194	92	95	83	-3	-76	2.42E-6	9.25E-6	4.37E-5

Table A.6. In-Vitro Testing Results (GI_{50}, TGI, and LC_{50}) of Compound **3.30.**

National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

NSC : 772952 / 1	Experiment ID : 1307NS20	Test Type : 08	Units : Molar
Report Date : November 05, 2013	Test Date : July 02, 2013	QNS :	MC :
COMI : ST-8 (126742)	Stain Reagent : SRB Dual-Pass Related	SSPL : 0YWM	

			Log10 Concentration												
	Time		Mean Optical Densities					Percent Growth							
Panel/Cell Line	Zero	Ctrl	-8.0	-7.0	-6.0	-5.0	-4.0	-8.0	-7.0	-6.0	-5.0	-4.0	GI50	TGI	LC50
Leukemia															
CCRF-CEM	0.264	1.157	1.153	1.132	1.068	0.406	0.197	99	97	90	16	-26	3.46E-6	2.41E-5	> 1.00E-4
HL-60(TB)	1.348	3.380	3.368	3.441	3.320	1.579	0.702	99	103	97	11	-48	3.54E-6	1.55E-5	> 1.00E-4
K-562	0.194	1.911	2.023	2.065	1.936	0.357	0.113	107	109	101	9	-42	3.63E-6	1.53E-5	> 1.00E-4
MOLT-4	0.729	3.001	3.038	3.118	2.798	0.948	0.484	102	105	91	10	-34	3.19E-6	1.67E-5	> 1.00E-4
RPMI-8226	0.748	2.221	2.270	2.238	2.151	0.852	0.453	103	101	95	7	-39	3.26E-6	1.42E-5	> 1.00E-4
SR	0.292	1.057	1.042	1.071	1.055	0.420	0.229	98	102	100	17	-22	3.98E-6	2.72E-5	> 1.00E-4
Non-Small Cell Lung Cancer															
A549/ATCC	0.343	1.761	1.709	1.804	1.647	0.479	0.071	96	103	92	10	-79	3.23E-6	1.28E-5	4.68E-5
HOP-62	0.402	1.289	1.256	1.309	1.330	0.652	0.067	96	102	105	28	-83	5.18E-6	1.79E-5	5.02E-5
HOP-92	1.215	1.662	1.611	1.577	1.545	1.107	0.299	89	81	74	-9	-75	1.94E-6	7.81E-6	4.15E-5
NCI-H226	0.592	1.629	1.551	1.611	1.612	0.762	0.280	92	98	98	16	-53	3.88E-6	1.72E-5	9.14E-5
NCI-H23	0.923	2.775	2.696	2.668	2.675	1.238	0.288	96	94	95	17	-69	3.76E-6	1.58E-5	6.03E-5
NCI-H322M	0.774	1.891	1.907	1.938	1.886	1.338	0.014	101	104	100	50	-98	1.01E-5	2.18E-5	4.74E-5
NCI-H460	0.330	2.828	2.915	2.842	2.819	0.264	0.065	103	101	100	-20	-80	2.60E-6	6.79E-6	3.13E-5
Colon Cancer															
COLO 205	0.392	1.931	1.983	2.013	2.026	0.133	0.066	103	105	106	-66	-83	2.12E-6	4.13E-6	8.07E-6
HCC-2998	1.001	3.106	3.146	3.087	3.180	1.475	0.336	102	99	104	23	-66	4.58E-6	1.79E-5	6.54E-5
HCT-116	0.219	1.944	1.936	1.993	1.906	0.182	0.029	100	103	98	-17	-87	2.61E-6	7.12E-6	2.98E-5
HCT-15	0.271	1.751	1.655	1.799	1.663	0.171	0.089	93	103	94	-37	-67	2.17E-6	5.23E-6	2.69E-5
HT29	0.223	1.169	1.176	1.211	1.221	0.230	0.033	101	104	106	1	-85	3.39E-6	1.02E-5	3.89E-5
KM12	0.671	2.826	2.882	3.003	2.811	0.813	0.102	103	108	99	7	-85	3.40E-6	1.18E-5	4.16E-5
SW-620	0.397	2.497	2.372	2.386	2.316	0.794	0.082	94	95	91	19	-79	3.72E-6	1.56E-5	5.02E-5
CNS Cancer															
SF-268	0.487	1.671	1.728	1.715	1.616	0.797	0.137	105	104	95	26	-72	4.52E-6	1.85E-5	5.97E-5
SF-295	0.897	2.734	2.637	2.718	2.670	0.216	0.101	95	99	97	-76	-89	1.86E-6	3.63E-6	7.07E-6
SF-539	0.826	2.290	2.230	2.262	2.205	1.169	0.093	96	98	94	23	-89	4.21E-6	1.62E-5	4.51E-5
SNB-19	1.039	2.415	2.313	2.210	2.198	1.491	0.063	93	85	84	33	-94	4.64E-6	1.82E-5	4.50E-5
SNB-75	0.860	1.615	1.487	1.466	1.432	1.023	0.066	83	80	76	22	-92	2.98E-6	1.55E-5	4.25E-5
U251	0.634	2.349	2.296	2.243	2.268	0.914	0.066	97	94	95	16	-90	3.75E-6	1.43E-5	4.23E-5
Melanoma															
MALME-3M	0.499	1.080	1.087	1.108	1.059	0.467	0.052	101	105	96	-7	-90	2.83E-6	8.64E-6	3.34E-5
M14	0.465	1.842	1.819	1.934	1.859	0.660	0.072	98	107	101	14	-85	3.88E-6	1.39E-5	4.47E-5
MDA-MB-435	0.437	1.938	1.919	1.879	1.877	0.408	0.042	99	96	96	-7	-91	2.80E-6	8.62E-6	3.29E-5
SK-MEL-2	1.008	2.282	2.374	2.397	2.312	1.139	0.204	107	109	102	10	-80	3.70E-6	1.30E-5	4.67E-5
SK-MEL-28	0.614	1.884	1.880	1.894	1.886	1.057	0.065	100	101	100	35	-89	5.86E-6	1.91E-5	4.81E-5
SK-MEL-5	0.567	2.822	2.822	2.886	2.768	0.051	0.031	100	103	98	-91	-95	1.79E-6	3.29E-6	6.06E-6
UACC-257	0.681	1.502	1.458	1.441	1.374	0.860	0.076	95	93	84	22	-89	3.54E-6	1.57E-5	4.45E-5
UACC-62	0.795	2.423	2.338	2.319	2.178	0.840	0.055	95	94	85	3	-93	2.66E-6	1.07E-5	3.55E-5
Ovarian Cancer															
IGROV1	0.661	2.071	2.181	2.235	2.121	1.065	0.109	108	112	104	29	-84	5.19E-6	1.80E-5	5.03E-5
OVCAR-3	0.750	1.795	1.908	1.887	1.802	0.697	0.023	111	109	101	-7	-97	2.95E-6	8.60E-6	3.00E-5
OVCAR-4	0.517	0.893	0.826	0.850	0.841	0.552	0.052	82	88	86	9	-90	2.95E-6	1.24E-5	3.96E-5
OVCAR-5	0.517	1.719	1.710	1.687	1.656	0.838	0.080	99	97	95	27	-85	4.54E-6	1.74E-5	4.89E-5
OVCAR-8	0.432	1.830	1.720	1.792	1.844	0.776	0.166	92	97	101	25	-62	4.65E-6	1.93E-5	7.34E-5
NCI/ADR-RES	1.003	2.991	2.990	2.993	2.854	1.357	0.397	100	100	93	18	-60	3.73E-6	1.69E-5	7.36E-5
SK-OV-3	0.779	1.864	1.895	1.958	1.965	1.019	0.023	103	109	109	22	-97	4.78E-6	1.53E-5	4.03E-5
Renal Cancer															
786-0	0.762	2.478	2.545	2.542	2.515	0.723	0.065	104	104	102	-5	-91	3.06E-6	8.95E-6	3.31E-5
A498	1.469	2.072	2.051	2.063	2.024	1.386	0.066	97	99	92	-6	-96	2.70E-6	8.75E-6	3.11E-5
ACHN	0.397	1.829	1.822	1.823	1.760	0.481	0.025	100	100	95	6	-94	3.20E-6	1.14E-5	3.64E-5
CAKI-1	0.438	2.061	2.055	2.040	1.970	0.557	0.023	100	99	94	7	-95	3.23E-6	1.18E-5	3.64E-5
RXF 393	0.868	1.451	1.426	1.499	1.452	0.681	0.139	96	108	100	-22	-84	2.58E-6	6.65E-6	2.86E-5
SN12C	0.737	2.431	2.302	2.314	2.249	0.547	0.128	92	93	89	-26	-83	2.19E-6	5.97E-6	2.66E-5
TK-10	0.571	1.392	1.410	1.441	1.459	0.673	0.069	102	106	108	12	-88	4.05E-6	1.33E-5	4.18E-5
UO-31	0.718	2.231	2.097	2.086	1.832	0.819	0.005	91	90	74	7	-99	2.25E-6	1.16E-5	3.43E-5
Prostate Cancer															
PC-3	0.555	1.589	1.501	1.497	1.377	0.691	0.121	92	91	80	13	-78	2.78E-6	1.39E-5	4.90E-5
DU-145	0.348	1.525	1.573	1.596	1.509	0.762	0.010	104	106	99	35	-97	5.83E-6	1.84E-5	4.40E-5
Breast Cancer															
MCF7	0.486	2.325	2.143	2.253	2.213	0.451	0.191	90	96	94	-7	-61	2.72E-6	8.49E-6	6.29E-5
MDA-MB-231/ATCC	0.611	1.287	1.304	1.281	1.233	0.497	0.115	103	99	92	-19	-81	2.39E-6	6.77E-6	3.17E-5
HS 578T	1.495	2.242	2.293	2.228	2.163	1.586	0.737	107	98	89	12	-51	3.24E-6	1.56E-5	9.75E-5
BT-549	0.888	1.865	1.824	1.843	1.851	0.762	0.126	96	98	99	-14	-86	2.70E-6	7.48E-6	3.16E-5
T-47D	0.405	0.973	0.975	0.990	0.983	0.461	0.132	100	103	102	10	-67	3.65E-6	1.34E-5	5.95E-5
MDA-MB-468	0.721	1.271	1.244	1.290	1.261	0.827	0.249	95	104	98	19	-65	4.08E-6	1.69E-5	6.57E-5

Table A.7. In-Vitro Testing Results (GI_{50}, TGI, and LC_{50}) of Compound **3.33.**

National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

NSC : 772951 / 1	Experiment ID : 1306NS17	Test Type : 08	Units : Molar
Report Date : January 30, 2014	Test Date : June 24, 2013	QNS :	MC :
COMI : ST-7 (126741)	Stain Reagent : SRB Dual-Pass Related	SSPL : 0YWM	

			Log10 Concentration												
	Time		Mean Optical Densities					Percent Growth							
Panel/Cell Line	Zero	Ctrl	-8.0	-7.0	-6.0	-5.0	-4.0	-8.0	-7.0	-6.0	-5.0	-4.0	GI50	TGI	LC50
Leukemia															
CCRF-CEM	0.443	1.925	1.858	1.991	1.845	0.522	0.209	95	104	95	5	-53	3.16E-6	1.23E-5	8.90E-5
HL-60(TB)	0.773	2.608	2.523	2.696	2.299	0.658	0.320	95	105	83	-15	-59	2.18E-6	7.04E-6	6.34E-5
K-562	0.197	1.466	1.459	1.582	1.497	0.229	0.059	99	109	102	2	-70	3.35E-6	1.08E-5	5.29E-5
MOLT-4	0.729	2.518	2.401	2.443	2.209	0.816	0.334	93	96	83	5	-54	2.63E-6	1.21E-5	8.47E-5
RPMI-8226	0.654	2.049	2.081	2.130	1.965	0.507	0.293	102	106	94	-23	-55	2.38E-6	6.40E-6	6.90E-5
SR	0.281	0.920	0.883	1.085	0.959	0.280	0.147	94	126	106	.	-48	3.37E-6	9.92E-6	> 1.00E-4
Non-Small Cell Lung Cancer															
A549/ATCC	0.375	1.564	1.544	1.537	1.497	0.343	0.062	98	98	94	-9	-84	2.70E-6	8.24E-6	3.56E-5
HOP-62	0.655	1.913	1.969	2.004	2.021	0.086	0.092	104	107	109	-87	-86	1.99E-6	3.59E-6	6.47E-6
HOP-92	1.382	1.870	1.787	1.765	1.714	0.746	0.208	83	78	68	-46	-85	1.44E-6	3.95E-6	1.27E-5
NCI-H226	0.693	1.792	1.727	1.737	1.677	0.115	0.288	94	95	89	-83	-58	1.69E-6	3.29E-6	6.40E-6
NCI-H23	0.309	0.989	0.976	1.004	0.943	0.178	0.039	98	102	93	-42	-88	2.08E-6	4.87E-6	1.47E-5
NCI-H322M	0.854	2.081	2.058	2.121	2.079	1.131	0.028	98	103	100	23	-97	4.42E-6	1.55E-5	4.06E-5
NCI-H460	0.342	2.960	2.993	2.996	2.886	0.091	0.042	101	101	97	-74	-88	1.89E-6	3.71E-6	7.28E-6
NCI-H522	0.685	1.556	1.581	1.609	1.512	0.450	0.109	103	106	95	-34	-84	2.23E-6	5.42E-6	2.06E-5
Colon Cancer															
COLO 205	0.675	2.738	2.765	2.819	2.824	0.021	0.127	101	104	104	-97	-81	1.86E-6	3.30E-6	5.84E-6
HCC-2998	0.751	2.727	2.712	2.755	2.784	0.729	0.154	99	101	103	-3	-80	3.16E-6	9.37E-6	4.11E-5
HCT-116	0.164	1.651	1.672	1.623	1.601	0.033	0.038	101	98	97	-80	-77	1.84E-6	3.53E-6	6.77E-6
HCT-15	0.281	1.715	1.652	1.706	1.438	0.114	0.070	96	99	81	-60	-75	1.65E-6	3.76E-6	8.54E-6
HT29	0.186	1.115	1.173	1.213	1.229	0.044	0.018	106	111	112	-76	-90	2.14E-6	3.94E-6	7.25E-6
KM12	0.566	2.457	2.518	2.525	2.442	0.208	0.041	103	104	99	-63	-93	2.01E-6	4.08E-6	8.28E-6
SW-620	0.386	2.454	2.454	2.411	2.344	0.579	0.065	100	98	95	9	-83	3.34E-6	1.26E-5	4.38E-5
CNS Cancer															
SF-268	0.747	2.115	2.109	2.085	1.947	0.919	0.145	100	98	88	13	-81	3.18E-6	1.36E-5	4.69E-5
SF-295	0.994	2.893	2.794	2.857	2.614	0.068	0.086	95	98	85	-93	-91	1.58E-6	3.00E-6	5.73E-6
SF-539	0.773	2.212	2.167	2.142	2.113	0.023	0.033	97	95	93	-97	-96	1.69E-6	3.09E-6	5.66E-6
SNB-19	0.896	2.294	2.157	2.134	2.061	1.197	0.155	90	89	83	21	-83	3.46E-6	1.61E-5	4.85E-5
SNB-75	0.795	1.563	1.374	1.388	1.386	0.444	0.053	75	77	77	-44	-93	1.67E-6	4.32E-6	1.32E-5
U251	0.508	2.028	1.957	1.949	1.975	0.416	0.079	95	95	96	-18	-85	2.54E-6	6.94E-6	3.01E-5
Melanoma															
LOX IMVI	0.613	3.075	3.044	3.090	3.051	0.010	0.057	99	101	99	-98	-91	1.77E-6	3.17E-6	5.68E-6
MALME-3M	0.910	1.833	1.832	1.837	1.744	0.503	0.195	100	100	90	-45	-79	1.99E-6	4.67E-6	1.43E-5
M14	0.447	1.904	1.924	1.951	1.898	0.246	0.119	101	103	100	-45	-73	2.20E-6	4.88E-6	1.50E-5
MDA-MB-435	0.559	2.309	2.235	2.242	2.148	0.008	0.013	96	96	91	-99	-98	1.64E-6	3.01E-6	5.54E-6
SK-MEL-2	0.936	2.078	2.151	2.191	2.142	0.387	0.117	106	110	106	-59	-88	2.18E-6	4.39E-6	8.85E-6
SK-MEL-28	0.406	1.286	1.248	1.253	1.255	0.029	0.019	96	96	97	-93	-95	1.76E-6	3.23E-6	5.93E-6
SK-MEL-5	0.850	3.042	2.973	2.949	2.818	0.150	0.066	97	96	90	-82	-92	1.70E-6	3.32E-6	6.49E-6
UACC-257	0.777	1.545	1.510	1.485	1.431	0.625	0.066	95	92	85	-20	-92	2.16E-6	6.49E-6	2.65E-5
UACC-62	0.883	2.551	2.553	2.386	2.425	0.486	0.174	100	90	92	-45	-80	2.04E-6	4.70E-6	1.38E-5
Ovarian Cancer															
IGROV1	0.786	2.322	2.455	2.398	2.520	0.752	0.215	109	105	113	-4	-73	3.44E-6	9.19E-6	4.66E-5
OVCAR-3	0.744	1.726	1.791	1.807	1.756	0.236	-0.005	107	108	103	-68	-100	2.04E-6	3.99E-6	7.81E-6
OVCAR-4	0.855	1.505	1.451	1.446	1.394	0.508	0.041	92	91	83	-41	-95	1.85E-6	4.69E-6	1.48E-5
OVCAR-5	0.598	1.575	1.549	1.557	1.524	0.044	0.026	97	98	95	-93	-96	1.73E-6	3.20E-6	5.92E-6
OVCAR-8	0.451	1.858	1.826	1.820	1.765	0.432	0.153	98	97	93	-4	-66	2.78E-6	9.05E-6	5.50E-5
NCI/ADR-RES	0.712	2.267	2.314	2.379	2.170	0.627	0.168	103	107	94	-12	-76	2.59E-6	7.70E-6	3.88E-5
SK-OV-3	0.875	2.104	2.133	2.239	2.257	0.748	-0.003	102	111	112	-15	-100	3.10E-6	7.68E-6	2.60E-5
Renal Cancer															
786-0	0.684	2.503	2.448	2.577	2.425	0.237	0.027	97	104	96	-65	-96	1.92E-6	3.93E-6	8.03E-6
A498	1.633	2.177	1.976	1.990	1.975	0.358	0.028	63	66	63	-78	-98	1.23E-6	2.79E-6	6.32E-6
ACHN	0.313	1.451	1.448	1.447	1.390	0.251	0.001	100	100	95	-20	-100	2.45E-6	6.70E-6	2.38E-5
CAKI-1	0.706	2.599	2.544	2.516	2.331	0.531	0.049	97	96	86	-25	-93	2.11E-6	5.97E-6	2.34E-5
RXF 393	0.973	1.598	1.544	1.527	1.484	0.339	0.147	91	89	82	-65	-85	1.64E-6	3.60E-6	7.88E-6
SN12C	0.576	2.056	1.881	1.878	1.859	0.103	0.152	88	88	87	-82	-74	1.65E-6	3.26E-6	6.45E-6
TK-10	0.698	1.570	1.584	1.582	1.635	0.703	0.043	102	101	107	1	-94	3.45E-6	1.01E-5	3.43E-5
UO-31	0.888	2.408	2.316	2.297	2.069	0.642	0.041	94	93	78	-28	-95	1.83E-6	5.45E-6	2.13E-5
Prostate Cancer															
PC-3	0.530	1.942	1.894	1.777	1.634	0.491	0.053	97	88	78	-7	-90	2.13E-6	8.18E-6	3.28E-5
DU-145	0.493	1.760	1.879	1.857	1.853	0.608	-0.011	109	108	107	9	-100	3.83E-6	1.21E-5	3.48E-5
Breast Cancer															
MCF7	0.452	2.272	2.112	2.117	1.789	0.153	0.154	91	91	73	-66	-66	1.47E-6	3.36E-6	7.66E-6
MDA-MB-231/ATCC	0.567	1.267	1.238	1.197	1.147	0.236	0.074	96	90	83	-58	-87	1.71E-6	3.86E-6	8.72E-6
HS 578T	1.118	2.363	2.306	2.271	2.181	1.130	0.662	95	93	85	1	-41	2.62E-6	1.05E-5	> 1.00E-4
BT-549	0.854	1.828	1.958	1.950	1.870	0.164	0.111	114	113	104	-81	-87	1.97E-6	3.66E-6	6.82E-6
T-47D	0.771	1.810	1.754	1.819	1.739	0.484	0.230	95	101	93	-37	-70	2.14E-6	5.18E-6	2.44E-5
MDA-MB-468	0.950	1.563	1.541	1.515	1.519	0.856	0.330	96	92	93	-10	-65	2.61E-6	8.01E-6	5.29E-5

Table A.8. In-Vitro Testing Results (GI_{50}, TGI, and LC_{50}) of Compound **3.34.**

National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

NSC : 772953 / 1	Experiment ID : 1307NS20	Test Type : 08	Units : Molar
Report Date : November 05, 2013	Test Date : July 02, 2013	QNS :	MC :
COMI : ST-9 (126743)	Stain Reagent : SRB Dual-Pass Related	SSPL : 0YWM	

			Log10 Concentration												
	Time		Mean Optical Densities					Percent Growth							
Panel/Cell Line	Zero	Ctrl	-8.0	-7.0	-6.0	-5.0	-4.0	-8.0	-7.0	-6.0	-5.0	-4.0	GI50	TGI	LC50
Leukemia															
CCRF-CEM	0.264	1.224	1.216	1.189	1.131	0.351	0.289	99	96	90	9	3	3.14E-6	> 1.00E-4	> 1.00E-4
HL-60(TB)	1.348	3.440	3.423	3.472	3.346	1.251	0.957	99	102	95	-7	-29	2.77E-6	8.50E-6	> 1.00E-4
K-562	0.194	1.922	2.011	2.077	1.830	0.252	0.204	105	109	95	3	1	3.08E-6	> 1.00E-4	> 1.00E-4
MOLT-4	0.729	3.083	3.115	3.163	2.941	0.925	0.658	101	103	94	8	-10	3.26E-6	2.87E-5	> 1.00E-4
RPMI-8226	0.748	2.296	2.320	2.343	2.276	0.635	0.594	102	103	99	-15	-21	2.68E-6	7.37E-6	> 1.00E-4
SR	0.292	1.098	1.103	1.140	1.092	0.341	0.280	101	105	99	6	-4	3.37E-6	3.93E-5	> 1.00E-4
Non-Small Cell Lung Cancer															
A549/ATCC	0.343	1.730	1.696	1.662	1.584	0.395	0.080	98	95	89	4	-77	2.89E-6	1.11E-5	4.66E-5
HOP-62	0.402	1.295	1.258	1.407	1.421	0.279	0.111	96	113	114	-31	-73	2.77E-6	6.14E-6	2.89E-5
HOP-92	1.215	1.790	1.699	1.684	1.661	0.797	0.265	84	82	78	-34	-78	1.76E-6	4.93E-6	2.27E-5
NCI-H226	0.592	1.678	1.636	1.679	1.679	0.229	0.170	96	100	100	-61	-71	2.04E-6	4.17E-6	8.50E-6
NCI-H23	0.923	2.761	2.668	2.628	2.636	1.157	0.426	95	93	93	13	-54	3.44E-6	1.55E-5	8.75E-5
NCI-H322M	0.774	2.070	2.065	2.088	2.047	1.102	0.096	100	101	98	25	-88	4.58E-6	1.67E-5	4.64E-5
NCI-H460	0.330	2.923	2.941	2.964	2.853	0.181	0.067	101	102	97	-45	-80	2.15E-6	4.81E-6	1.37E-5
Colon Cancer															
COLO 205	0.392	1.920	1.959	1.989	2.045	0.118	0.096	103	104	108	-70	-76	2.12E-6	4.05E-6	7.72E-6
HCC-2998	1.001	3.101	3.122	3.111	3.092	1.036	0.229	101	100	100	2	-77	3.21E-6	1.05E-5	4.52E-5
HCT-116	0.219	2.007	1.977	2.107	2.024	0.130	0.127	98	106	101	-41	-42	2.29E-6	5.16E-6	> 1.00E-4
HCT-15	0.271	1.735	1.704	1.719	1.673	0.117	0.203	98	99	96	-57	-25	2.00E-6	4.24E-6	
HT29	0.223	1.143	1.163	1.209	1.239	0.099	0.051	102	107	110	-56	-77	2.31E-6	4.63E-6	9.25E-6
KM12	0.671	2.770	2.847	2.879	2.766	0.206	0.140	104	105	100	-69	-79	1.97E-6	3.89E-6	7.69E-6
SW-620	0.397	2.553	2.469	2.460	2.452	0.592	0.127	96	96	95	9	-68	3.35E-6	1.31E-5	5.82E-5
CNS Cancer															
SF-268	0.487	1.766	1.740	1.785	1.683	0.644	0.074	98	101	94	12	-85	3.43E-6	1.34E-5	4.38E-5
SF-295	0.897	2.787	2.581	2.722	2.688	0.103	0.244	89	97	95	-89	-73	1.75E-6	3.29E-6	6.16E-6
SF-539	0.826	2.326	2.271	2.261	2.390	0.235	0.119	96	96	104	-72	-86	2.04E-6	3.92E-6	7.54E-6
SNB-19	1.039	2.516	2.391	2.325	2.340	1.395	0.189	92	87	88	24	-82	3.94E-6	1.69E-5	5.00E-5
SNB-75	0.860	1.703	1.542	1.516	1.494	0.834	0.088	81	78	75	-3	-90	2.10E-6	9.15E-6	3.48E-5
U251	0.634	2.401	2.371	2.264	2.235	0.680	0.053	98	92	91	3	-92	2.89E-6	1.06E-5	3.61E-5
Melanoma															
MALME-3M	0.499	1.180	1.182	1.179	1.114	0.245	0.070	100	100	90	-51	-86	1.93E-6	4.36E-6	9.84E-6
M14	0.465	1.928	1.936	1.972	1.916	0.236	0.179	101	103	99	-49	-62	2.14E-6	4.66E-6	1.15E-5
MDA-MB-435	0.437	2.069	1.970	1.947	1.906	0.173	0.068	94	92	90	-60	-84	1.84E-6	3.97E-6	8.53E-6
SK-MEL-2	1.008	2.300	2.390	2.344	2.332	0.615	0.153	107	103	102	-39	-85	2.35E-6	5.30E-6	1.74E-5
SK-MEL-28	0.614	1.957	1.928	1.855	1.912	0.368	0.114	98	92	97	-40	-81	2.19E-6	5.09E-6	1.73E-5
SK-MEL-5	0.567	2.917	2.895	2.949	2.852	0.033	0.073	99	101	97	-94	-87	1.76E-6	3.22E-6	5.88E-6
UACC-257	0.681	1.512	1.448	1.412	1.435	0.620	0.217	92	88	91	-9	-68	2.56E-6	8.12E-6	4.92E-5
UACC-62	0.795	2.486	2.439	2.384	2.285	0.258	0.085	97	94	88	-68	-89	1.76E-6	3.68E-6	7.71E-6
Ovarian Cancer															
IGROV1	0.661	2.245	2.333	2.407	2.411	0.774	0.146	106	110	110	7	-78	3.85E-6	1.21E-5	4.69E-5
OVCAR-3	0.750	1.906	1.979	2.026	1.965	0.319	0.039	106	110	105	-57	-95	2.18E-6	4.43E-6	9.00E-6
OVCAR-4	0.517	0.944	0.899	0.906	0.892	0.555	0.067	89	91	88	9	-87	3.01E-6	1.24E-5	4.11E-5
OVCAR-5	0.517	1.780	1.741	1.697	1.699	0.381	0.152	97	93	94	-26	-71	2.31E-6	6.03E-6	3.43E-5
OVCAR-8	0.432	1.848	1.865	1.795	1.791	0.482	0.166	101	96	96	3	-62	3.14E-6	1.13E-5	6.64E-5
NCI/ADR-RES	1.003	3.035	3.013	3.040	2.937	0.947	0.297	99	100	95	-6	-70	2.81E-6	8.79E-6	4.84E-5
SK-OV-3	0.779	2.062	2.195	2.286	2.293	0.963	0.047	110	117	118	14	-94	4.53E-6	1.36E-5	3.93E-5
Renal Cancer															
786-0	0.762	2.620	2.580	2.668	2.673	0.719	0.186	98	103	103	-6	-76	3.07E-6	8.86E-6	4.30E-5
A498	1.469	2.210	2.103	2.063	2.050	0.595	0.076	86	80	78	-59	-95	1.61E-6	3.70E-6	8.53E-6
ACHN	0.397	1.819	1.840	1.798	1.761	0.458	0.044	102	99	96	4	-89	3.17E-6	1.11E-5	3.82E-5
CAKI-1	0.438	2.229	2.132	2.178	2.199	0.449	0.043	95	97	98	1	-90	3.12E-6	1.02E-5	3.60E-5
RXF 393	0.868	1.542	1.572	1.594	1.622	0.449	0.113	104	108	112	-48	-87	2.43E-6	4.99E-6	1.11E-5
SN12C	0.737	2.454	2.373	2.406	2.336	0.163	0.104	95	97	93	-78	-86	1.79E-6	3.50E-6	6.87E-6
TK-10	0.571	1.450	1.440	1.457	1.524	0.645	0.073	99	101	108	8	-87	3.84E-6	1.22E-5	4.08E-5
UO-31	0.718	2.341	2.189	2.194	2.035	0.388	0.020	91	91	81	-46	-97	1.76E-6	4.35E-6	1.20E-5
Prostate Cancer															
PC-3	0.555	1.753	1.663	1.624	1.498	0.668	0.234	93	89	79	9	-58	2.60E-6	1.38E-5	7.65E-5
DU-145	0.348	1.588	1.661	1.677	1.675	0.482	0.025	106	107	107	11	-93	3.91E-6	1.27E-5	3.86E-5
Breast Cancer															
MCF7	0.486	2.358	2.251	2.540	2.478	0.228	0.206	94	110	106	-53	-58	2.26E-6	4.64E-6	9.55E-6
MDA-MB-231/ATCC	0.611	1.352	1.351	1.334	1.292	0.328	0.089	100	98	92	-46	-86	2.01E-6	4.62E-6	1.24E-5
HS 578T	1.495	2.439	2.351	2.393	2.275	1.165	0.797	91	95	83	-22	-47	2.05E-6	6.15E-6	> 1.00E-4
BT-549	0.888	1.948	2.025	2.013	2.009	0.433	0.306	107	105	106	-51	-66	2.27E-6	4.72E-6	9.81E-6
T-47D	0.405	1.010	1.017	1.073	1.038	0.409	0.208	101	110	105	1	-49	3.35E-6	1.03E-5	> 1.00E-4
MDA-MB-468	0.721	1.301	1.294	1.335	1.339	0.746	0.136	99	106	107	4	-81	3.57E-6	1.12E-5	4.32E-5

Table A.9. In-Vitro Testing Results (GI_{50}, TGI, and LC_{50}) of Compound **3.37.**

National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

NSC : 772954 / 1	Experiment ID : 1307NS20	Test Type : 08	Units : Molar
Report Date : November 05, 2013	Test Date : July 02, 2013	QNS :	MC :
COMI : ST-10 (126744)	Stain Reagent : SRB Dual-Pass Related	SSPL : 0YWM	

		Log10 Concentration												
	Time	Mean Optical Densities					Percent Growth							
Panel/Cell Line	Zero	Ctrl	-8.0	-7.0	-6.0	-5.0	-4.0	-8.0	-7.0	-6.0	-5.0	-4.0	GI50	TGI	LC50
Leukemia															
CCRF-CEM	0.264	1.194	1.133	1.138	1.096	0.422	0.231	93	94	89	17	-13	3.50E-6	3.76E-5	> 1.00E-4
HL-60(TB)	1.348	3.384	3.358	3.404	3.381	1.953	0.805	99	101	100	30	-40	5.14E-6	2.66E-5	> 1.00E-4
K-562	0.194	1.697	1.775	1.661	1.785	0.373	0.171	105	98	106	12	-12	3.93E-6	3.17E-5	> 1.00E-4
MOLT-4	0.729	3.012	2.975	2.931	2.874	1.014	0.585	98	96	94	12	-20	3.46E-6	2.43E-5	> 1.00E-4
RPMI-8226	0.748	2.309	2.263	2.302	2.225	1.050	0.503	97	100	95	19	-33	3.92E-6	2.35E-5	> 1.00E-4
SR	0.292	1.027	1.014	0.985	1.018	0.467	0.263	98	94	99	24	-10	4.47E-6	5.08E-5	> 1.00E-4
Non-Small Cell Lung Cancer															
A549/ATCC	0.343	1.786	1.806	1.810	1.740	0.614	0.060	101	102	97	19	-83	3.98E-6	1.53E-5	4.78E-5
HOP-62	0.402	1.302	1.315	1.267	1.464	0.864	0.075	101	96	118	51	-81	1.02E-5	2.44E-5	5.80E-5
HOP-92	1.215	1.767	1.749	1.690	1.688	1.228	0.293	97	86	86	2	-76	2.68E-6	1.07E-5	4.67E-5
NCI-H226	0.592	1.782	1.710	1.713	1.707	1.064	0.118	94	94	94	40	-80	6.43E-6	2.14E-5	5.60E-5
NCI-H23	0.923	2.838	2.842	2.840	2.726	1.604	0.349	100	100	94	36	-62	5.67E-6	2.31E-5	7.50E-5
NCI-H322M	0.774	2.008	1.951	1.955	1.996	1.410	0.058	95	96	99	52	-93	1.02E-5	2.28E-5	5.06E-5
NCI-H460	0.330	2.918	2.993	2.960	2.941	0.259	0.039	103	102	101	-22	-88	2.60E-6	6.66E-6	2.67E-5
Colon Cancer															
COLO 205	0.392	1.874	1.837	1.875	2.033	0.117	0.134	98	100	111	-70	-66	2.17E-6	4.09E-6	7.74E-6
HCC-2998	1.001	3.157	3.142	3.232	3.280	1.906	0.306	99	103	106	42	-69	7.48E-6	2.38E-5	6.69E-5
HCT-116	0.219	1.962	2.012	1.943	2.016	0.486	0.153	103	99	103	15	-30	4.03E-6	2.17E-5	> 1.00E-4
HCT-15	0.271	1.785	1.611	1.663	1.468	0.053	0.140	89	92	79	-81	-49	1.52E-6	3.13E-6	
HT29	0.223	1.144	1.152	1.157	1.160	0.202	0.036	101	101	102	-10	-84	2.92E-6	8.19E-6	3.48E-5
KM12	0.671	2.821	2.774	2.737	2.688	0.657	0.121	98	96	94	-2	-82	2.86E-6	9.51E-6	3.98E-5
SW-620	0.397	2.531	2.455	2.396	2.375	0.847	0.064	96	94	93	21	-84	3.95E-6	1.59E-5	4.75E-5
CNS Cancer															
SF-268	0.487	1.745	1.685	1.712	1.687	0.931	0.084	95	97	95	35	-83	5.69E-6	1.99E-5	5.28E-5
SF-295	0.897	2.740	2.553	2.545	2.265	0.031	0.271	90	89	74	-97	-70	1.39E-6	2.72E-6	5.34E-6
SF-539	0.826	2.303	2.321	2.182	2.211	1.325	0.112	101	92	94	34	-86	5.36E-6	1.91E-5	4.98E-5
SNB-19	1.039	2.459	2.409	2.283	2.256	1.835	0.092	96	88	86	56	-91	1.10E-5	2.40E-5	5.25E-5
SNB-75	0.860	1.695	1.575	1.514	1.482	0.835	0.186	86	78	74	-3	-78	2.07E-6	9.17E-6	4.20E-5
U251	0.634	2.435	2.418	2.411	2.319	1.258	0.035	99	99	94	35	-94	5.49E-6	1.85E-5	4.52E-5
Melanoma															
MALME-3M	0.499	1.128	1.123	1.092	1.125	0.516	0.060	99	94	99	3	-88	3.25E-6	1.07E-5	3.81E-5
M14	0.465	1.947	1.901	1.870	1.956	1.176	0.174	97	95	101	48	-63	9.14E-6	2.71E-5	7.69E-5
MDA-MB-435	0.437	2.057	2.055	2.009	1.896	0.169	0.011	100	97	90	-61	-97	1.84E-6	3.93E-6	8.42E-6
SK-MEL-2	1.008	2.280	2.281	2.252	2.310	1.392	0.082	100	98	102	30	-92	5.31E-6	1.77E-5	4.54E-5
SK-MEL-28	0.614	1.976	2.014	1.966	1.934	1.202	0.117	103	99	97	43	-81	7.45E-6	2.23E-5	5.62E-5
SK-MEL-5	0.567	2.901	2.785	2.787	2.728	0.457	0.023	95	95	93	-19	-96	2.40E-6	6.71E-6	2.51E-5
UACC-257	0.681	1.492	1.480	1.516	1.456	1.059	0.145	98	103	95	47	-79	8.49E-6	2.35E-5	5.89E-5
UACC-62	0.795	2.467	2.366	2.339	2.239	1.302	0.048	94	92	86	30	-94	4.46E-6	1.75E-5	4.43E-5
Ovarian Cancer															
IGROV1	0.661	2.173	2.239	2.231	2.298	1.137	0.083	104	104	108	31	-87	5.74E-6	1.84E-5	4.84E-5
OVCAR-3	0.750	1.920	1.942	1.923	1.945	0.882	0.039	102	100	102	11	-95	3.75E-6	1.28E-5	3.78E-5
OVCAR-4	0.517	0.931	0.904	0.875	0.821	0.492	0.030	94	86	73	-5	-94	1.99E-6	8.65E-6	3.20E-5
OVCAR-5	0.517	1.777	1.770	1.776	1.724	0.984	0.093	99	100	96	37	-82	6.02E-6	2.05E-5	5.38E-5
OVCAR-8	0.432	1.908	1.906	1.988	1.776	0.893	0.137	100	105	91	31	-68	4.86E-6	2.06E-5	6.55E-5
NCI/ADR-RES	1.003	3.112	3.155	3.169	3.059	1.396	0.357	102	103	98	19	-64	4.00E-6	1.68E-5	6.70E-5
SK-OV-3	0.779	2.076	2.010	2.037	2.200	1.382	0.115	95	97	110	46	-85	8.79E-6	2.25E-5	5.40E-5
Renal Cancer															
786-0	0.762	2.615	2.549	2.589	2.691	1.289	0.246	96	99	104	28	-68	5.19E-6	1.97E-5	6.53E-5
A498	1.469	2.223	2.100	2.083	2.111	1.432	0.024	84	81	85	-3	-98	2.52E-6	9.36E-6	3.13E-5
ACHN	0.397	1.839	1.869	1.875	1.791	0.554	0.045	102	102	97	11	-89	3.50E-6	1.29E-5	4.08E-5
CAKI-1	0.438	2.210	2.227	2.297	2.244	0.469	0.015	101	105	102	2	-97	3.30E-6	1.04E-5	3.36E-5
RXF 393	0.868	1.553	1.557	1.495	1.562	0.821	0.149	101	91	101	-5	-83	3.02E-6	8.89E-6	3.76E-5
SN12C	0.737	2.450	2.300	2.328	2.248	0.886	0.076	91	93	88	9	-90	3.02E-6	1.23E-5	3.95E-5
TK-10	0.571	1.417	1.371	1.374	1.484	0.820	0.056	94	95	108	29	-90	5.46E-6	1.76E-5	4.61E-5
UO-31	0.718	2.366	2.113	2.090	2.031	0.849	0.011	85	83	80	8	-99	2.59E-6	1.19E-5	3.50E-5
Prostate Cancer															
PC-3	0.555	1.808	1.732	1.697	1.607	0.674	0.203	94	91	84	9	-63	2.86E-6	1.35E-5	6.54E-5
DU-145	0.348	1.596	1.639	1.587	1.615	0.882	0.052	103	99	102	43	-85	7.53E-6	2.16E-5	5.32E-5
Breast Cancer															
MCF7	0.486	2.310	2.013	2.048	2.033	0.298	0.125	84	86	85	-39	-74	1.91E-6	4.86E-6	2.08E-5
MDA-MB-231/ATCC	0.611	1.318	1.341	1.297	1.267	0.724	0.073	103	97	93	16	-88	3.60E-6	1.42E-5	4.31E-5
HS 578T	1.495	2.619	2.546	2.490	2.368	1.612	0.689	94	89	78	10	-54	2.58E-6	1.45E-5	8.68E-5
BT-549	0.888	1.947	1.942	1.883	1.939	1.246	0.328	100	94	99	34	-63	5.65E-6	2.23E-5	7.32E-5
T-47D	0.405	0.964	0.918	0.903	0.948	0.481	0.161	92	89	97	14	-60	3.66E-6	1.53E-5	7.24E-5
MDA-MB-468	0.721	1.332	1.307	1.325	1.354	0.882	0.112	96	99	104	26	-84	4.94E-6	1.73E-5	4.88E-5

Table A.10. In-Vitro Testing Results (GI_{50}, TGI, and LC_{50}) of Compound **3.38.**

National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

NSC : 772957 / 1	Experiment ID : 1306NS09	Test Type : 08	Units : Molar
Report Date : January 30, 2014	Test Date : June 17, 2013	QNS :	MC :
COMI : ST-14 (126748)	Stain Reagent : SRB Dual-Pass Related	SSPL : 0YWM	

			Log10 Concentration											
	Time		Mean Optical Densities					Percent Growth						
Panel/Cell Line	Zero	Ctrl	-8.0	-7.0	-6.0	-5.0	-4.0	-8.0	-7.0	-6.0	-5.0	-4.0	GI50	TGI	LC50
Leukemia															
CCRF-CEM	0.434	2.017	2.032	2.013	1.865	0.431	0.302	101	100	90	-1	-31	2.77E-6	9.80E-6	> 1.00E-4
K-562	0.223	1.601	1.619	1.639	1.695	0.208	0.131	101	103	107	-7	-41	3.16E-6	8.69E-6	> 1.00E-4
MOLT-4	0.780	2.688	2.733	2.716	2.382	0.830	0.604	102	101	84	3	-23	2.61E-6	1.27E-5	> 1.00E-4
RPMI-8226	1.046	2.891	2.884	2.920	2.820	0.843	0.832	100	102	96	-19	-20	2.51E-6	6.79E-6	> 1.00E-4
SR	0.596	2.125	2.111	2.054	1.963	0.520	0.360	99	95	89	-13	-40	2.43E-6	7.50E-6	> 1.00E-4
Non-Small Cell Lung Cancer															
A549/ATCC	0.458	2.212	2.172	2.162	2.044	0.279	0.070	98	97	90	-39	-85	2.05E-6	4.98E-6	1.73E-5
HOP-62	0.667	1.768	1.770	1.773	1.842	0.125	0.017	100	100	107	-81	-97	2.00E-6	3.70E-6	6.82E-6
HOP-92	1.173	1.757	1.677	1.644	1.621	0.331	0.054	86	81	77	-72	-95	1.51E-6	3.28E-6	7.13E-6
NCI-H226	0.682	1.801	1.754	1.703	1.692	0.080	0.134	96	91	90	-88	-80	1.68E-6	3.20E-6	6.10E-6
NCI-H23	0.924	2.584	2.408	2.448	2.390	0.630	0.230	89	92	88	-32	-75	2.08E-6	5.43E-6	2.63E-5
NCI-H322M	0.984	2.440	2.571	2.475	2.388	1.233	0.192	109	102	96	17	-80	3.84E-6	1.50E-5	4.87E-5
NCI-H460	0.305	2.900	2.890	2.922	2.815	0.119	0.056	100	101	97	-61	-82	1.98E-6	4.11E-6	8.52E-6
NCI-H522	0.876	2.070	1.980	2.030	1.856	0.367	0.136	92	97	82	-58	-84	1.69E-6	3.85E-6	8.75E-6
Colon Cancer															
COLO 205	0.569	2.416	2.518	2.467	2.395	0.069	0.055	105	103	99	-88	-90	1.83E-6	3.38E-6	6.26E-6
HCC-2998	1.045	3.150	3.087	3.122	3.174	0.453	0.058	97	99	101	-57	-94	2.11E-6	4.37E-6	9.07E-6
HCT-116	0.288	2.153	2.186	2.197	2.072	0.017	0.008	102	102	96	-94	-97	1.74E-6	3.19E-6	5.86E-6
HCT-15	0.342	2.148	2.074	2.031	1.853	0.117	0.109	96	94	84	-66	-68	1.68E-6	3.62E-6	7.82E-6
HT29	0.313	1.696	1.743	1.768	1.719	0.097	0.044	103	105	102	-69	-86	2.01E-6	3.94E-6	7.74E-6
KM12	0.561	2.346	2.390	2.474	2.314	0.115	0.092	102	107	98	-80	-84	1.87E-6	3.57E-6	6.82E-6
SW-620	0.383	2.401	2.414	2.362	2.389	0.429	0.133	101	98	99	2	-65	3.23E-6	1.08E-5	5.92E-5
CNS Cancer															
SF-268	0.722	2.137	2.143	2.162	2.021	0.818	0.101	100	102	92	7	-86	3.10E-6	1.18E-5	4.09E-5
SF-295	0.992	3.054	2.989	2.999	2.964	0.336	0.180	97	97	96	-66	-82	1.91E-6	3.90E-6	7.95E-6
SF-539	1.032	2.715	2.694	2.559	2.518	0.016	0.020	99	91	88	-98	-98	1.60E-6	2.97E-6	5.50E-6
SNB-19	0.857	2.083	2.047	2.043	2.048	0.929	0.140	97	97	97	6	-84	3.29E-6	1.16E-5	4.20E-5
SNB-75	0.860	1.667	1.551	1.511	1.530	0.292	0.003	86	81	83	-66	-100	1.67E-6	3.60E-6	7.80E-6
U251	0.895	2.642	2.603	2.576	2.535	0.115	0.025	98	96	94	-87	-97	1.75E-6	3.30E-6	6.23E-6
Melanoma															
LOX IMVI	0.493	2.814	2.766	2.710	2.666	0.003	0.009	98	96	94	-99	-98	1.68E-6	3.06E-6	5.55E-6
MALME-3M	0.818	1.649	1.716	1.684	1.617	0.377	0.143	108	104	96	-54	-83	2.03E-6	4.37E-6	9.41E-6
M14	0.549	1.863	1.911	1.938	1.867	0.109	0.045	104	106	100	-80	-92	1.90E-6	3.60E-6	6.81E-6
MDA-MB-435	0.672	2.853	2.846	2.773	2.715	0.401	0.205	100	96	94	-40	-70	2.12E-6	5.00E-6	2.14E-5
SK-MEL-2	1.330	2.674	2.742	2.801	2.811	0.363	0.196	105	109	110	-73	-85	2.13E-6	4.00E-6	7.51E-6
SK-MEL-28	0.712	2.027	2.033	1.934	1.935	0.019	.	100	93	93	-97	-100	1.68E-6	3.08E-6	5.64E-6
SK-MEL-5	0.909	3.089	3.036	3.029	2.974	0.003	0.018	98	97	95	-100	-98	1.70E-6	3.07E-6	5.55E-6
UACC-257	1.080	2.204	2.129	2.145	2.141	0.940	0.310	93	95	94	-13	-71	2.59E-6	7.57E-6	4.31E-5
UACC-62	0.911	2.456	2.468	2.478	2.383	0.202	0.071	101	101	95	-78	-92	1.83E-6	3.55E-6	6.90E-6
Ovarian Cancer															
IGROV1	0.583	2.143	2.287	2.307	2.111	0.436	0.215	109	111	98	-25	-63	2.45E-6	6.23E-6	4.48E-5
OVCAR-4	0.702	1.437	1.401	1.359	1.265	0.028	0.007	95	89	77	-96	-99	1.42E-6	2.78E-6	5.41E-6
OVCAR-5	0.595	1.693	1.660	1.594	1.591	0.082	0.086	97	91	91	-86	-86	1.70E-6	3.26E-6	6.24E-6
OVCAR-8	0.504	2.368	2.324	2.320	2.297	0.413	0.136	98	97	96	-18	-73	2.54E-6	6.95E-6	3.81E-5
NCI/ADR-RES	0.763	2.486	2.462	2.412	2.316	0.541	0.134	99	96	90	-29	-82	2.17E-6	5.70E-6	2.46E-5
SK-OV-3	0.770	1.945	1.932	2.000	1.963	0.567	0.022	99	105	102	-26	-97	2.53E-6	6.21E-6	2.15E-5
Renal Cancer															
786-0	0.899	2.707	2.725	2.851	2.717	0.234	0.029	101	108	101	-74	-97	1.95E-6	3.77E-6	7.29E-6
A498	1.565	2.186	2.043	1.998	1.962	0.285	0.053	77	70	64	-82	-97	1.25E-6	2.74E-6	6.05E-6
ACHN	0.515	1.945	1.922	1.845	1.789	0.029	0.002	98	93	89	-94	-100	1.63E-6	3.06E-6	5.72E-6
CAKI-1	1.002	3.017	2.968	3.029	2.977	0.901	0.222	98	101	98	-10	-78	2.78E-6	8.06E-6	3.88E-5
RXF 393	1.073	1.906	1.861	1.834	1.803	0.114	0.106	95	91	88	-89	-90	1.63E-6	3.13E-6	5.99E-6
SN12C	0.977	2.800	2.718	2.787	2.678	0.155	0.117	96	99	93	-84	-88	1.75E-6	3.35E-6	6.42E-6
TK-10	0.712	1.568	1.572	1.593	1.672	0.653	0.033	100	103	112	-8	-95	3.28E-6	8.54E-6	3.01E-5
UO-31	0.881	2.410	2.285	2.283	2.116	0.265	0.046	92	92	81	-70	-95	1.60E-6	3.43E-6	7.38E-6
Prostate Cancer															
PC-3	0.519	2.211	2.128	2.076	1.968	0.346	0.024	95	92	86	-33	-95	1.99E-6	5.24E-6	1.85E-5
DU-145	0.415	1.624	1.742	1.763	1.664	0.341	0.002	110	111	103	-18	-100	2.75E-6	7.11E-6	2.47E-5
Breast Cancer															
MCF7	0.726	2.833	2.709	2.832	2.859	0.409	0.067	94	100	101	-44	-91	2.26E-6	5.00E-6	1.36E-5
MDA-MB-231/ATCC	0.847	2.098	2.150	2.127	2.013	0.119	0.039	104	102	93	-86	-95	1.74E-6	3.31E-6	6.30E-6
HS 578T	1.662	2.626	2.547	2.528	2.536	1.235	0.977	92	90	91	-26	-41	2.23E-6	6.01E-6	> 1.00E-4
BT-549	1.004	2.026	1.934	2.008	1.945	0.100	0.103	91	98	92	-90	-90	1.70E-6	3.20E-6	6.03E-6
T-47D	0.572	1.258	1.298	1.360	1.309	0.419	0.082	106	115	107	-27	-86	2.68E-6	6.32E-6	2.48E-5
MDA-MB-468	0.821	1.401	1.353	1.323	1.326	0.636	0.239	92	86	87	-23	-71	2.18E-6	6.23E-6	3.70E-5

Table A.11. In-Vitro Testing Results (GI_{50}, TGI, and LC_{50}) of Compound **3.40.**

National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

NSC : 772961 / 1	Experiment ID : 1307NS20	Test Type : 08	Units : Molar
Report Date : November 05, 2013	Test Date : July 02, 2013	QNS :	MC :
COMI : ST-18 (127022)	Stain Reagent : SRB Dual-Pass Related	SSPL : 0YWM	

			Log10 Concentration											
	Time		Mean Optical Densities					Percent Growth						
Panel/Cell Line	Zero	Ctrl	-8.0	-7.0	-6.0	-5.0	-4.0	-8.0	-7.0	-6.0	-5.0	-4.0	GI50	TGI	LC50
Leukemia															
CCRF-CEM	0.264	1.173	1.164	1.122	1.042	0.351	0.367	99	94	86	10	11	2.94E-6	> 1.00E-4	> 1.00E-4
HL-60(TB)	1.348	3.361	3.329	3.382	3.330	1.452	0.911	98	101	98	5	-32	3.31E-6	1.37E-5	> 1.00E-4
K-562	0.194	1.506	1.554	1.554	1.510	0.275	0.200	104	104	100	6	.	3.42E-6	> 1.00E-4	> 1.00E-4
MOLT-4	0.729	2.907	2.936	2.854	2.709	1.019	0.765	101	98	91	13	2	3.37E-6	> 1.00E-4	> 1.00E-4
RPMI-8226	0.748	2.224	2.289	2.255	2.134	0.746	0.511	104	102	94	.	-32	2.93E-6	9.93E-6	> 1.00E-4
SR	0.292	0.960	0.957	0.939	0.937	0.392	0.291	100	97	97	15	.	3.72E-6	9.50E-5	> 1.00E-4
Non-Small Cell Lung Cancer															
A549/ATCC	0.343	1.731	1.770	1.741	1.617	0.482	0.092	103	101	92	10	-73	3.24E-6	1.32E-5	5.26E-5
HOP-62	0.402	1.299	1.306	1.257	1.331	0.706	0.091	101	95	104	34	-77	5.87E-6	2.02E-5	5.68E-5
HOP-92	1.215	1.762	1.741	1.666	1.610	1.065	0.314	96	82	72	-12	-74	1.83E-6	7.14E-6	4.06E-5
NCI-H226	0.592	1.698	1.612	1.582	1.584	0.659	0.037	92	90	90	6	-94	2.98E-6	1.15E-5	3.64E-5
NCI-H23	0.923	2.768	2.737	2.711	2.558	1.199	0.245	98	97	89	15	-74	3.34E-6	1.48E-5	5.42E-5
NCI-H322M	0.774	2.042	2.083	1.949	1.913	1.062	0.485	103	93	90	23	-37	3.92E-6	2.39E-5	> 1.00E-4
NCI-H460	0.330	2.880	2.998	2.956	2.857	0.188	0.055	105	103	99	-43	-83	2.21E-6	4.97E-6	1.48E-5
Colon Cancer															
COLO 205	0.392	1.805	1.810	1.862	1.945	0.006	0.104	100	104	110	-98	-74	1.94E-6	3.37E-6	5.85E-6
HCC-2998	1.001	3.068	3.096	3.127	3.161	1.366	0.252	101	103	105	18	-75	4.24E-6	1.55E-5	5.39E-5
HCT-116	0.219	1.970	2.013	1.987	1.867	0.239	0.129	102	101	94	1	-41	2.98E-6	1.06E-5	> 1.00E-4
HCT-15	0.271	1.762	1.682	1.694	1.642	0.286	0.154	95	95	92	1	-43	2.89E-6	1.05E-5	> 1.00E-4
HT29	0.223	1.086	1.108	1.095	1.084	0.163	0.090	103	101	100	-27	-60	2.47E-6	6.11E-6	5.05E-5
KM12	0.671	2.719	2.676	2.660	2.581	0.269	0.138	98	97	93	-60	-79	1.92E-6	4.06E-6	8.62E-6
SW-620	0.397	2.502	2.442	2.396	2.317	0.686	0.175	97	95	91	14	-56	3.40E-6	1.57E-5	8.22E-5
CNS Cancer															
SF-268	0.487	1.737	1.707	1.659	1.644	0.704	0.254	98	94	93	17	-48	3.68E-6	1.84E-5	> 1.00E-4
SF-295	0.897	2.721	2.591	2.552	2.562	0.667	0.132	93	91	91	-26	-85	2.25E-6	6.03E-6	2.56E-5
SF-539	0.826	2.321	2.327	2.238	2.099	0.634	0.102	100	94	85	-23	-88	2.11E-6	6.10E-6	2.60E-5
SNB-19	1.039	2.468	2.364	2.346	2.247	1.452	1.200	93	91	85	29	11	4.17E-6	> 1.00E-4	> 1.00E-4
SNB-75	0.860	1.676	1.582	1.552	1.435	0.916	0.326	89	85	70	7	-62	2.10E-6	1.26E-5	6.67E-5
U251	0.634	2.377	2.356	2.342	2.228	0.930	0.164	99	98	91	17	-74	3.60E-6	1.54E-5	5.43E-5
Melanoma															
MALME-3M	0.499	1.159	1.144	1.108	1.100	0.452	0.063	98	92	91	-9	-87	2.56E-6	8.06E-6	3.31E-5
M14	0.465	1.942	1.878	1.847	1.941	0.482	0.243	96	94	100	1	-48	3.20E-6	1.05E-5	> 1.00E-4
MDA-MB-435	0.437	1.959	1.972	1.970	1.882	0.313	0.033	101	101	95	-28	-92	2.31E-6	5.89E-6	2.18E-5
SK-MEL-2	1.008	2.243	2.251	2.169	2.215	0.899	0.144	101	94	98	-11	-86	2.75E-6	7.94E-6	3.33E-5
SK-MEL-28	0.614	1.892	1.939	1.860	1.823	0.443	0.155	104	97	95	-28	-75	2.31E-6	5.92E-6	2.96E-5
SK-MEL-5	0.567	2.811	2.806	2.795	2.715	0.080	-0.012	100	99	96	-86	-100	1.78E-6	3.36E-6	6.34E-6
UACC-257	0.681	1.483	1.483	1.488	1.389	0.796	0.382	100	101	88	14	-44	3.30E-6	1.76E-5	> 1.00E-4
UACC-62	0.795	2.508	2.464	2.421	2.251	0.327	0.193	97	95	85	-59	-76	1.75E-6	3.90E-6	8.68E-6
Ovarian Cancer															
IGROV1	0.661	2.238	2.247	2.233	2.236	0.954	0.261	101	100	100	19	-61	4.10E-6	1.72E-5	7.36E-5
OVCAR-3	0.750	1.918	1.887	1.908	1.889	0.635	0.057	97	99	97	-15	-92	2.63E-6	7.30E-6	2.81E-5
OVCAR-4	0.517	0.892	0.863	0.841	0.787	0.506	-0.018	92	86	72	-2	-100	1.98E-6	9.36E-6	3.08E-5
OVCAR-5	0.517	1.699	1.691	1.675	1.616	0.661	0.088	99	98	93	12	-83	3.40E-6	1.34E-5	4.50E-5
OVCAR-8	0.432	1.857	1.880	1.819	1.771	0.516	0.099	102	97	94	6	-77	3.16E-6	1.18E-5	4.70E-5
NCI/ADR-RES	1.003	3.013	3.047	3.041	2.869	0.903	0.215	102	101	93	-10	-79	2.61E-6	8.00E-6	3.83E-5
SK-OV-3	0.779	2.083	2.192	2.210	2.297	1.162	0.600	108	110	116	29	-23	5.79E-6	3.64E-5	> 1.00E-4
Renal Cancer															
786-0	0.762	2.598	2.471	2.481	2.587	0.722	0.333	93	94	99	-5	-56	2.96E-6	8.90E-6	7.52E-5
A498	1.469	2.209	2.105	2.034	2.077	0.675	0.126	86	76	82	-54	-91	1.72E-6	4.01E-6	9.33E-6
ACHN	0.397	1.782	1.814	1.805	1.686	0.416	0.156	102	102	93	1	-61	2.95E-6	1.05E-5	6.70E-5
CAKI-1	0.438	2.164	2.120	2.111	2.143	0.458	0.141	97	97	99	1	-68	3.16E-6	1.04E-5	5.50E-5
RXF 393	0.868	1.563	1.493	1.482	1.462	0.445	0.097	90	88	85	-49	-89	1.84E-6	4.33E-6	1.08E-5
SN12C	0.737	2.401	2.357	2.312	2.274	0.334	0.226	97	95	92	-55	-69	1.94E-6	4.25E-6	9.28E-6
TK-10	0.571	1.368	1.360	1.325	1.385	0.625	0.249	99	95	102	7	-56	3.52E-6	1.28E-5	7.92E-5
UO-31	0.718	2.347	2.125	2.070	1.977	0.529	0.062	86	83	77	-26	-91	1.83E-6	5.57E-6	2.31E-5
Prostate Cancer															
PC-3	0.555	1.749	1.687	1.652	1.510	0.695	0.348	95	92	80	12	-37	2.75E-6	1.73E-5	> 1.00E-4
DU-145	0.348	1.541	1.557	1.521	1.555	0.566	0.151	101	98	101	18	-57	4.15E-6	1.75E-5	8.16E-5
Breast Cancer															
MCF7	0.486	2.368	2.526	2.593	2.529	0.474	0.316	108	112	109	-3	-35	3.36E-6	9.48E-6	> 1.00E-4
MDA-MB-231/ATCC	0.611	1.331	1.316	1.273	1.197	0.366	0.244	98	92	81	-40	-60	1.81E-6	4.68E-6	3.13E-5
HS 578T	1.495	2.405	2.322	2.368	2.258	1.281	0.754	91	96	84	-14	-50	2.21E-6	7.14E-6	> 1.00E-4
BT-549	0.888	1.892	1.857	1.833	1.889	0.826	0.168	97	94	100	-7	-81	2.92E-6	8.59E-6	3.80E-5
T-47D	0.405	0.951	0.995	0.965	0.968	0.477	0.206	108	103	103	13	-49	3.90E-6	1.63E-5	> 1.00E-4
MDA-MB-468	0.721	1.323	1.296	1.324	1.304	0.800	0.300	95	100	97	13	-58	3.62E-6	1.52E-5	7.62E-5

Table A.12. In-Vitro Testing Results (GI_{50}, TGI, and LC_{50}) of Compound **3.45.**

National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

NSC : 772962 / 1	Experiment ID : 1307NS20	Test Type : 08	Units : Molar
Report Date : November 29, 2013	Test Date : July 02, 2013	QNS :	MC :
COMI : ST-19 (127023)	Stain Reagent : SRB Dual-Pass Related	SSPL : 0YWM	

			Log10 Concentration												
	Time		Mean Optical Densities					Percent Growth							
Panel/Cell Line	Zero	Ctrl	-8.0	-7.0	-6.0	-5.0	-4.0	-8.0	-7.0	-6.0	-5.0	-4.0	GI50	TGI	LC50
Leukemia															
CCRF-CEM	0.264	1.194	1.166	1.170	1.043	0.431	0.390	97	97	84	18	14	3.25E-6	> 1.00E-4	> 1.00E-4
HL-60(TB)	1.348	3.384	3.389	3.466	3.322	1.227	1.085	100	104	97	-9	-20	2.78E-6	8.23E-6	> 1.00E-4
K-562	0.194	1.697	1.830	1.787	1.148	0.281	0.270	109	106	63	6	5	1.71E-6	> 1.00E-4	> 1.00E-4
MOLT-4	0.729	3.012	3.055	3.056	2.789	0.941	0.842	102	102	90	9	5	3.14E-6	> 1.00E-4	> 1.00E-4
RPMI-8226	0.748	2.309	2.299	2.288	2.068	0.672	0.639	99	99	85	-10	-15	2.32E-6	7.80E-6	> 1.00E-4
SR	0.292	1.027	1.070	1.066	0.937	0.315	0.397	106	105	88	3	14	2.79E-6	> 1.00E-4	> 1.00E-4
Non-Small Cell Lung Cancer															
A549/ATCC	0.343	1.786	1.735	1.697	1.532	0.364	0.181	96	94	82	1	-47	2.51E-6	1.07E-5	> 1.00E-4
HOP-62	0.402	1.302	1.316	1.323	1.345	0.522	0.080	102	102	105	13	-80	3.97E-6	1.39E-5	4.75E-5
HOP-92	1.215	1.767	1.714	1.658	1.584	1.108	0.495	90	80	67	-9	-59	1.67E-6	7.64E-6	6.54E-5
NCI-H226	0.592	1.782	1.792	1.732	1.689	0.642	0.096	101	96	92	4	-84	3.02E-6	1.12E-5	4.13E-5
NCI-H23	0.923	2.838	2.775	2.675	2.515	1.179	0.685	97	91	83	13	-26	2.99E-6	2.20E-5	> 1.00E-4
NCI-H322M	0.774	2.008	2.005	1.984	1.917	1.043	0.533	100	98	93	22	-31	4.00E-6	2.58E-5	> 1.00E-4
NCI-H460	0.330	2.918	2.992	2.957	2.731	0.169	0.180	103	101	93	-49	-46	2.00E-6	4.52E-6	> 1.00E-4
Colon Cancer															
COLO 205	0.392	1.874	1.942	1.997	1.956	0.192	0.234	105	108	106	-51	-40	2.26E-6	4.72E-6	.
HCC-2998	1.001	3.157	3.147	3.074	2.984	1.177	0.586	100	96	92	8	-42	3.17E-6	1.46E-5	> 1.00E-4
HCT-116	0.219	1.962	2.010	2.103	1.845	0.157	0.194	103	108	93	-28	-11	2.27E-6	5.85E-6	> 1.00E-4
HCT-15	0.271	1.785	1.695	1.714	1.013	0.260	0.236	94	95	49	-4	-13	9.51E-7	8.38E-6	> 1.00E-4
HT29	0.223	1.144	1.163	1.255	1.225	0.136	0.131	102	112	109	-39	-41	2.50E-6	5.45E-6	> 1.00E-4
KM12	0.671	2.821	2.868	2.875	2.360	0.284	0.351	102	102	79	-58	-48	1.62E-6	3.77E-6	.
SW-620	0.397	2.531	2.479	2.380	2.209	0.496	0.290	98	93	85	5	-27	2.72E-6	1.40E-5	> 1.00E-4
CNS Cancer															
SF-268	0.487	1.745	1.758	1.781	1.613	0.582	0.316	101	103	89	8	-35	3.03E-6	1.50E-5	> 1.00E-4
SF-295	0.897	2.740	2.619	2.602	1.842	0.291	0.093	93	93	51	-68	-90	1.02E-6	2.70E-6	7.11E-6
SF-539	0.826	2.303	2.192	2.170	2.156	0.366	0.327	92	91	90	-56	-60	1.88E-6	4.14E-6	9.13E-6
SNB-19	1.039	2.459	2.352	2.308	2.185	1.371	1.231	92	89	81	23	14	3.43E-6	> 1.00E-4	> 1.00E-4
SNB-75	0.860	1.695	1.520	1.500	1.416	0.848	0.438	79	77	67	-1	-49	1.75E-6	9.52E-6	> 1.00E-4
U251	0.634	2.435	2.405	2.314	2.306	0.657	0.207	98	93	93	1	-67	2.94E-6	1.04E-5	5.57E-5
Melanoma															
MALME-3M	0.499	1.128	1.115	1.130	1.001	0.366	0.118	98	100	80	-27	-76	1.90E-6	5.61E-6	2.94E-5
M14	0.465	1.947	2.019	2.003	1.997	0.606	0.340	105	104	103	10	-27	3.70E-6	1.83E-5	> 1.00E-4
MDA-MB-435	0.437	2.057	1.985	1.908	1.380	0.101	0.046	96	91	58	-77	-90	1.15E-6	2.69E-6	6.31E-6
SK-MEL-2	1.008	2.280	2.371	2.373	2.300	0.647	0.252	107	107	102	-36	-75	2.37E-6	5.48E-6	2.30E-5
SK-MEL-28	0.614	1.976	1.932	1.880	1.844	0.449	0.201	97	93	90	-27	-67	2.21E-6	5.89E-6	3.73E-5
SK-MEL-5	0.567	2.901	2.960	2.872	2.505	0.020	0.098	103	99	83	-96	-83	1.53E-6	2.90E-6	5.51E-6
UACC-257	0.681	1.492	1.431	1.398	1.349	0.693	0.521	92	88	82	1	-24	2.51E-6	1.15E-5	> 1.00E-4
UACC-62	0.795	2.467	2.325	2.296	2.087	0.271	0.251	92	90	77	-66	-68	1.55E-6	3.46E-6	7.74E-6
Ovarian Cancer															
IGROV1	0.661	2.173	2.250	2.333	2.114	0.820	0.401	105	111	96	10	-39	3.45E-6	1.62E-5	> 1.00E-4
OVCAR-3	0.750	1.920	1.978	2.007	1.837	0.572	0.234	105	107	93	-24	-69	2.33E-6	6.26E-6	3.83E-5
OVCAR-4	0.517	0.931	0.872	0.862	0.854	0.563	0.163	86	83	81	11	-68	2.80E-6	1.38E-5	5.86E-5
OVCAR-5	0.517	1.777	1.701	1.704	1.637	0.638	0.152	94	94	89	10	-71	3.09E-6	1.32E-5	5.54E-5
OVCAR-8	0.432	1.908	1.846	1.834	1.823	0.563	0.279	96	95	94	9	-35	3.30E-6	1.59E-5	> 1.00E-4
NCI/ADR-RES	1.003	3.112	3.127	3.104	2.883	0.999	0.941	101	100	89	.	-6	2.74E-6	9.90E-6	> 1.00E-4
SK-OV-3	0.779	2.076	2.146	2.263	2.098	0.948	0.764	105	114	102	13	-2	3.83E-6	7.43E-5	> 1.00E-4
Renal Cancer															
786-0	0.762	2.615	2.658	2.696	2.641	0.755	0.461	102	104	101	-1	-40	3.18E-6	9.78E-6	> 1.00E-4
A498	1.469	2.223	2.099	2.039	2.100	0.657	0.134	84	76	84	-55	-91	1.75E-6	4.00E-6	9.16E-6
ACHN	0.397	1.839	1.851	1.816	1.733	0.483	0.262	101	98	93	6	-34	3.10E-6	1.41E-5	> 1.00E-4
CAKI-1	0.438	2.210	2.224	2.141	1.818	0.388	0.382	101	96	78	-12	-13	2.05E-6	7.43E-6	> 1.00E-4
RXF 393	0.868	1.553	1.526	1.560	1.524	0.396	0.211	96	101	96	-54	-76	2.02E-6	4.34E-6	9.35E-6
SN12C	0.737	2.450	2.317	2.249	2.172	0.203	0.224	92	88	84	-73	-70	1.64E-6	3.43E-6	7.18E-6
TK-10	0.571	1.417	1.448	1.481	1.432	0.567	0.242	104	107	102	-1	-58	3.20E-6	9.82E-6	7.32E-5
UO-31	0.718	2.366	2.172	2.148	1.984	0.640	0.130	88	87	77	-11	-82	2.02E-6	7.51E-6	3.55E-5
Prostate Cancer															
PC-3	0.555	1.808	1.697	1.648	1.477	0.684	0.607	91	87	74	10	4	2.36E-6	> 1.00E-4	> 1.00E-4
DU-145	0.348	1.596	1.674	1.679	1.516	0.324	0.157	106	107	94	-7	-55	2.71E-6	8.51E-6	7.86E-5
Breast Cancer															
MCF7	0.486	2.310	2.390	2.511	2.023	0.514	0.526	104	111	84	2	2	2.59E-6	> 1.00E-4	> 1.00E-4
MDA-MB-231/ATCC	0.611	1.318	1.310	1.268	1.180	0.340	0.259	99	93	81	-44	-58	1.75E-6	4.41E-6	2.63E-5
HS 578T	1.495	2.619	2.575	2.622	2.513	1.514	1.061	96	100	91	2	-29	2.86E-6	1.13E-5	> 1.00E-4
BT-549	0.888	1.947	1.999	2.011	1.946	0.684	0.448	105	106	100	-23	-50	2.55E-6	6.50E-6	> 1.00E-4
T-47D	0.405	0.964	0.983	1.023	0.943	0.464	0.359	103	111	96	11	-11	3.47E-6	3.01E-5	> 1.00E-4
MDA-MB-468	0.721	1.332	1.296	1.315	1.175	0.714	0.436	94	97	74	-1	-40	2.10E-6	9.71E-6	> 1.00E-4

Table A.13. In-Vitro Testing Results (GI_{50}, TGI, and LC_{50}) of Compound **3.46.**

National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

NSC : 772959 / 1	Experiment ID : 1306NS17	Test Type : 08	Units : Molar
Report Date : January 30, 2014	Test Date : June 24, 2013	QNS :	MC :
COMI : ST-16 (126750)	Stain Reagent : SRB Dual-Pass Related	SSPL : 0YWM	

		Log10 Concentration													
	Time	Mean Optical Densities						Percent Growth							
Panel/Cell Line	Zero	Ctrl	-8.0	-7.0	-6.0	-5.0	-4.0	-8.0	-7.0	-6.0	-5.0	-4.0	GI50	TGI	LC50
Leukemia															
CCRF-CEM	0.443	2.210	2.197	2.156	1.972	0.741	0.599	99	97	87	17	9	3.34E-6	> 1.00E-4	> 1.00E-4
HL-60(TB)	0.773	2.502	2.487	2.347	2.552	1.404	0.639	99	91	103	36	-17	6.26E-6	4.76E-5	> 1.00E-4
K-562	0.197	1.522	1.380	1.381	1.434	0.373	0.279	89	89	93	13	6	3.48E-6	> 1.00E-4	> 1.00E-4
MOLT-4	0.729	2.621	2.585	2.525	2.486	0.983	0.818	98	95	93	13	5	3.47E-6	> 1.00E-4	> 1.00E-4
RPMI-8226	0.654	2.201	2.225	2.173	1.918	0.690	0.393	102	98	82	2	-40	2.51E-6	1.14E-5	> 1.00E-4
SR	0.281	0.995	0.986	0.948	1.083	0.358	0.275	99	93	112	11	-2	4.10E-6	6.64E-5	> 1.00E-4
Non-Small Cell Lung Cancer															
A549/ATCC	0.375	1.630	1.584	1.592	1.508	0.522	0.389	96	97	90	12	1	3.26E-6	> 1.00E-4	> 1.00E-4
HOP-62	0.655	1.874	1.871	1.860	1.932	1.275	0.832	100	99	105	51	14	1.05E-5	> 1.00E-4	> 1.00E-4
HOP-92	1.382	1.857	1.808	1.754	1.734	1.368	0.679	90	78	74	-1	-51	2.09E-6	9.68E-6	9.59E-5
NCI-H226	0.693	1.853	1.800	1.757	1.569	0.973	0.281	95	92	75	24	-60	3.13E-6	1.94E-5	7.69E-5
NCI-H23	0.309	0.942	0.916	0.886	0.929	0.371	0.212	96	91	98	10	-31	3.49E-6	1.72E-5	> 1.00E-4
NCI-H322M	0.854	2.203	2.212	2.104	2.136	1.528	1.169	101	93	95	50	23	9.99E-6	> 1.00E-4	> 1.00E-4
NCI-H460	0.342	2.963	3.028	3.042	2.943	0.334	0.078	102	103	99	-2	-77	3.05E-6	9.48E-6	4.33E-5
NCI-H522	0.685	1.522	1.507	1.529	1.563	0.833	0.528	98	101	105	18	-23	4.26E-6	2.72E-5	> 1.00E-4
Colon Cancer															
COLO 205	0.675	2.696	2.631	2.613	2.746	0.058	-0.014	97	96	102	-91	-100	1.86E-6	3.38E-6	6.11E-6
HCC-2998	0.751	2.832	2.797	2.824	2.716	1.278	0.860	98	100	94	25	5	4.39E-6	> 1.00E-4	> 1.00E-4
HCT-116	0.164	1.554	1.501	1.403	1.379	0.247	-0.042	96	89	87	6	-100	2.88E-6	1.14E-5	3.37E-5
HCT-15	0.281	1.666	1.589	1.581	1.551	0.355	0.227	94	94	92	5	-19	3.04E-6	1.65E-5	> 1.00E-4
HT29	0.186	1.152	1.168	1.172	1.235	0.178	0.012	102	102	109	-4	-94	3.30E-6	9.16E-6	3.24E-5
KM12	0.566	2.385	2.290	2.328	2.371	0.831	0.337	95	97	99	15	-41	3.81E-6	1.84E-5	> 1.00E-4
SW-620	0.386	2.419	2.414	2.374	2.367	1.071	0.531	100	98	97	34	7	5.54E-6	> 1.00E-4	> 1.00E-4
CNS Cancer															
SF-268	0.747	2.009	1.920	1.896	1.861	1.049	0.793	93	91	88	24	4	3.93E-6	> 1.00E-4	> 1.00E-4
SF-295	0.994	2.855	2.727	2.710	2.734	1.066	0.718	93	92	93	4	-28	3.06E-6	1.32E-5	> 1.00E-4
SF-539	0.773	2.260	2.283	2.226	2.125	1.099	0.073	102	98	91	22	-91	3.91E-6	1.57E-5	4.36E-5
SNB-19	0.895	2.226	2.061	2.111	2.013	1.360	1.072	88	91	84	35	13	4.92E-6	> 1.00E-4	> 1.00E-4
SNB-75	0.795	1.548	1.476	1.428	1.343	1.028	0.710	90	84	73	31	-11	3.50E-6	5.52E-5	> 1.00E-4
U251	0.508	2.011	1.859	1.893	1.926	0.819	0.409	90	92	94	21	-20	4.00E-6	3.26E-5	> 1.00E-4
Melanoma															
LOX IMVI	0.613	3.111	3.003	2.958	3.052	1.956	0.074	96	94	98	54	-88	1.06E-5	2.39E-5	5.39E-5
MALME-3M	0.910	1.849	1.874	1.774	1.789	0.934	0.701	103	92	94	3	-23	3.01E-6	1.26E-5	> 1.00E-4
M14	0.447	1.729	1.706	1.632	1.711	0.555	0.144	98	92	99	8	-68	3.46E-6	1.29E-5	5.84E-5
MDA-MB-435	0.559	2.397	2.381	2.377	2.331	0.645	0.298	99	99	96	5	-47	3.21E-6	1.23E-5	> 1.00E-4
SK-MEL-2	0.936	2.066	2.034	2.050	2.117	1.052	0.323	97	99	104	10	-65	3.78E-6	1.36E-5	6.24E-5
SK-MEL-28	0.406	1.310	1.292	1.296	1.287	0.709	0.358	98	98	97	34	-12	5.52E-6	5.46E-5	> 1.00E-4
SK-MEL-5	0.850	2.985	2.931	2.900	2.796	0.117	0.120	97	96	91	-86	-86	1.71E-6	3.26E-6	6.24E-6
UACC-257	0.777	1.590	1.519	1.493	1.493	1.001	0.574	91	88	88	28	-26	4.26E-6	3.26E-5	> 1.00E-4
UACC-62	0.883	2.575	2.505	2.529	2.344	1.359	0.305	96	97	86	28	-65	4.20E-6	2.00E-5	6.84E-5
Ovarian Cancer															
IGROV1	0.786	2.344	2.366	2.381	2.438	1.378	0.969	101	102	106	38	12	6.66E-6	> 1.00E-4	> 1.00E-4
OVCAR-3	0.744	1.718	1.694	1.699	1.682	0.817	0.259	98	98	96	7	-65	3.32E-6	1.27E-5	6.18E-5
OVCAR-4	0.855	1.496	1.472	1.440	1.346	0.904	0.688	96	91	77	8	-20	2.43E-6	1.90E-5	> 1.00E-4
OVCAR-5	0.598	1.623	1.640	1.606	1.587	0.884	0.179	102	98	97	28	-70	4.76E-6	1.92E-5	6.23E-5
OVCAR-8	0.451	1.881	1.851	1.889	1.802	0.855	0.549	98	101	94	28	7	4.69E-6	> 1.00E-4	> 1.00E-4
NCI/ADR-RES	0.712	2.279	2.300	2.253	2.217	0.990	0.781	101	98	96	18	4	3.87E-6	> 1.00E-4	> 1.00E-4
SK-OV-3	0.875	2.041	2.071	2.150	2.144	1.320	0.985	103	109	109	38	9	6.79E-6	> 1.00E-4	> 1.00E-4
Renal Cancer															
786-0	0.684	2.448	2.389	2.272	2.372	0.987	0.468	97	90	96	17	-32	3.82E-6	2.25E-5	> 1.00E-4
A498	1.633	2.138	1.957	1.958	1.996	1.585	0.641	64	64	72	-3	-61	1.96E-6	9.13E-6	6.51E-5
ACHN	0.313	1.468	1.465	1.514	1.377	0.350	0.290	100	104	92	3	-7	2.98E-6	2.01E-5	> 1.00E-4
CAKI-1	0.706	2.567	2.580	2.527	2.425	0.997	0.711	101	98	92	16	.	3.57E-6	> 1.00E-4	> 1.00E-4
RXF 393	0.973	1.594	1.604	1.583	1.542	0.882	0.460	102	98	92	-9	-53	2.58E-6	8.08E-6	8.65E-5
SN12C	0.576	2.000	1.987	2.085	1.834	0.592	0.144	99	106	88	1	-75	2.75E-6	1.03E-5	4.68E-5
TK-10	0.698	1.608	1.578	1.536	1.606	0.904	0.645	97	92	100	23	-8	4.42E-6	5.59E-5	> 1.00E-4
UO-31	0.888	2.423	2.268	2.255	2.272	1.154	1.013	90	89	90	17	8	3.56E-6	> 1.00E-4	> 1.00E-4
Prostate Cancer															
PC-3	0.530	1.907	1.955	1.878	1.760	0.683	0.504	103	98	89	11	-5	3.18E-6	4.89E-5	> 1.00E-4
DU-145	0.493	1.716	1.704	1.654	1.725	0.965	0.541	99	95	101	39	4	6.55E-6	> 1.00E-4	> 1.00E-4
Breast Cancer															
MCF7	0.452	2.247	2.413	2.475	2.429	0.473	0.350	109	113	110	1	-23	3.56E-6	1.12E-5	> 1.00E-4
MDA-MB-231/ATCC	0.567	1.307	1.310	1.293	1.303	0.749	0.230	100	98	99	25	-59	4.58E-6	1.96E-5	7.72E-5
HS 578T	1.118	2.268	2.236	2.221	2.177	1.619	1.203	97	96	92	44	7	7.36E-6	> 1.00E-4	> 1.00E-4
BT-549	0.854	1.750	1.725	1.656	1.666	0.901	0.256	97	90	91	5	-70	2.99E-6	1.17E-5	5.42E-5
T-47D	0.771	1.808	1.831	1.822	1.790	0.864	0.740	102	101	98	9	-4	3.47E-6	4.90E-5	> 1.00E-4
MDA-MB-468	0.950	1.610	1.591	1.564	1.513	1.020	0.880	97	93	85	11	-7	2.96E-6	3.88E-5	> 1.00E-4

Table A.14. In-Vitro Testing Results (GI_{50}, TGI, and LC_{50}) of Compound **3.48.**

Appendix B – Supplementary Figures and Tables for Chapter 4

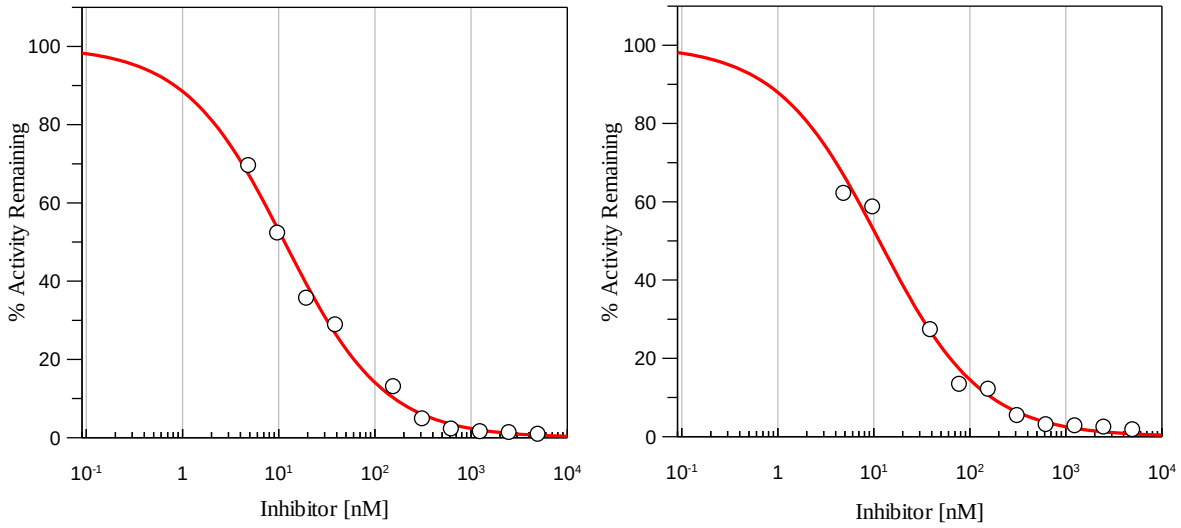

Figure B.1. **(a)** IC_{50} plot for **4.09** (left). **(b)** IC_{50} plot for **4.10** (right).

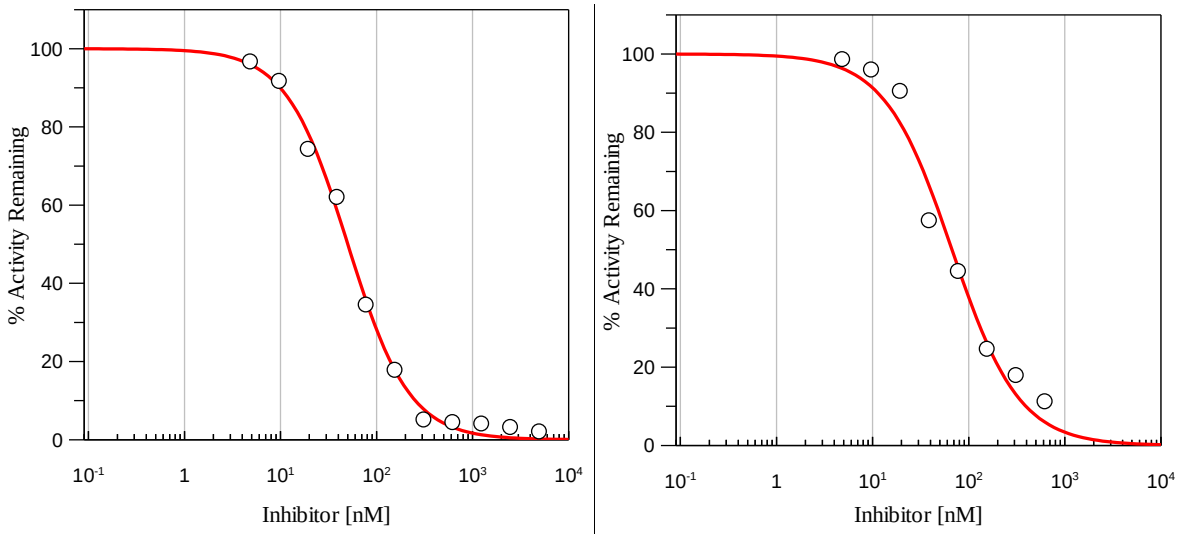

Figure B.2. **(a)** IC_{50} plot for **4.13** (left). **(b)** IC_{50} plot for **4.14** (right).

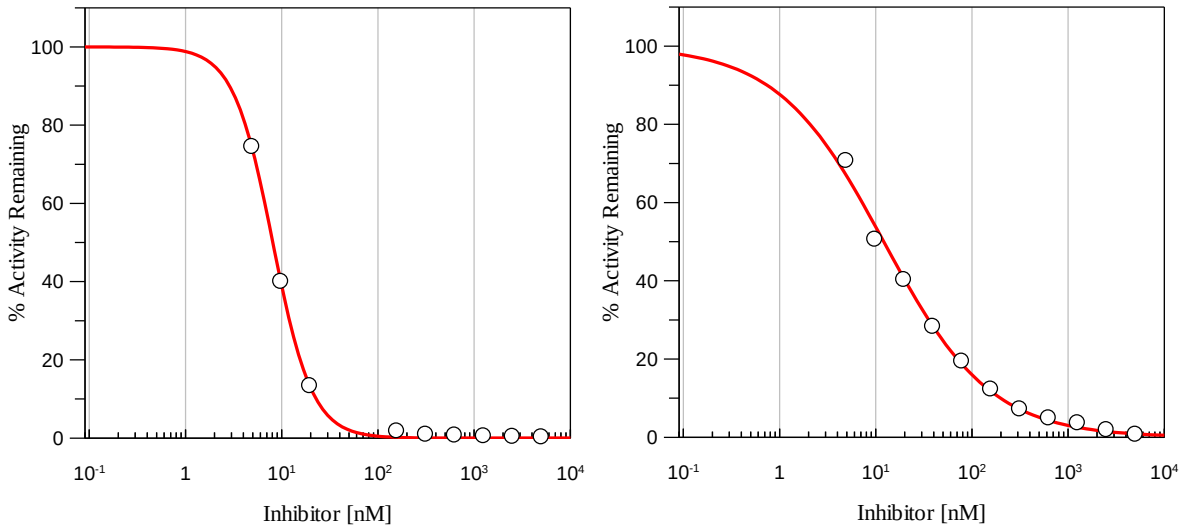

Figure B.3a. IC_{50} plot for **4.11** using 5 nM (left) and 10 nM (right) STS.

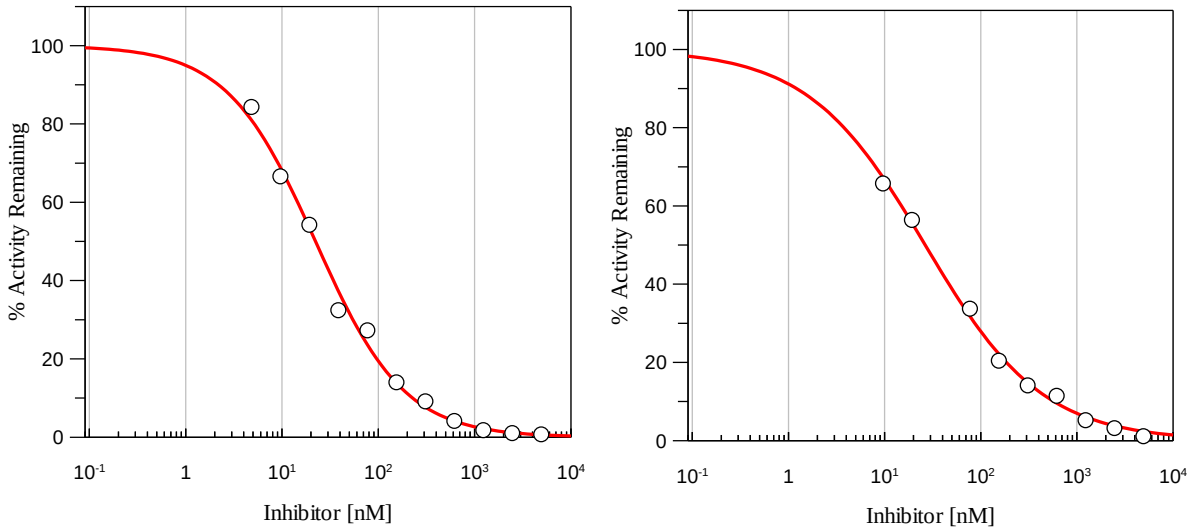

Figure B.3b. IC_{50} plot for **4.11** using 20 nM (left) and 40 nM (right) STS.

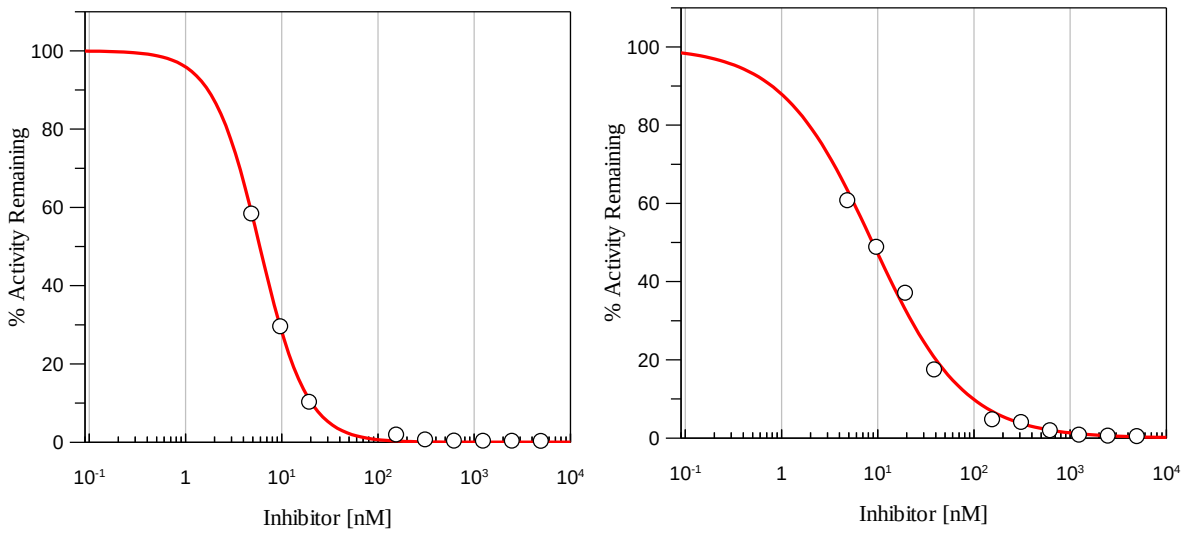

Figure B.4a. IC_{50} plot for **4.12** using 5 nM (left) and 10 nM (right) STS.

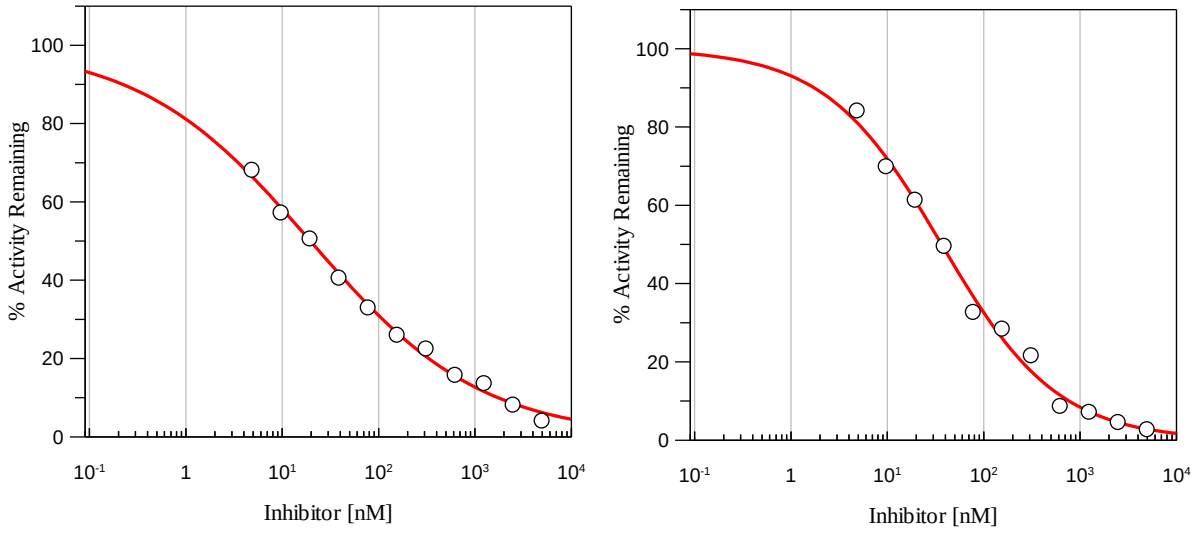

Figure B.4b. IC_{50} plot for **4.12** using 20 nM (left) and 40 nM (right) STS.

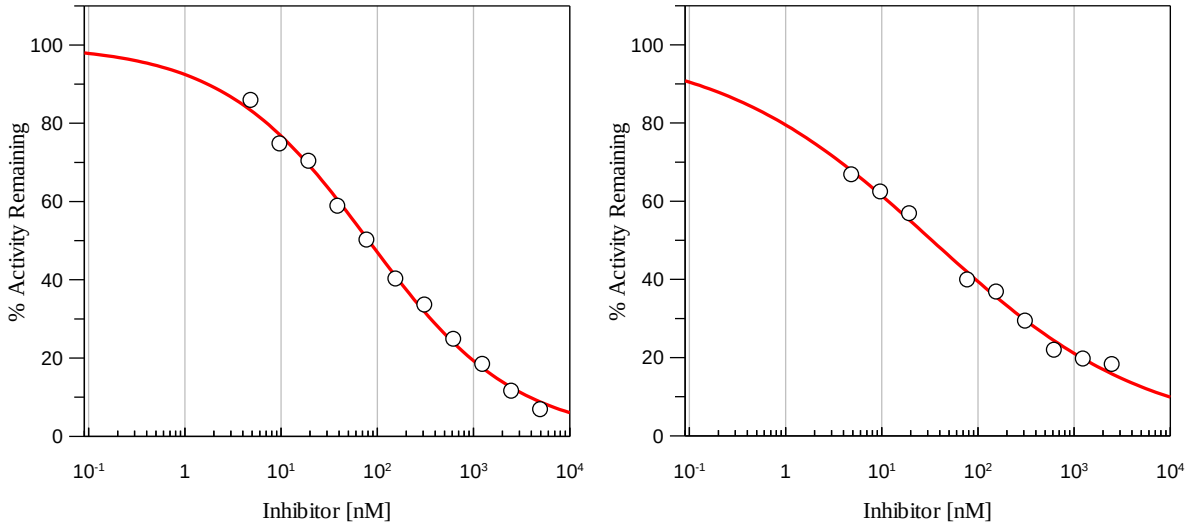

Figure B.5. **(a)** IC_{50} plot for **4.15** (left). **(b)** IC_{50} plot for **4.16** (right).

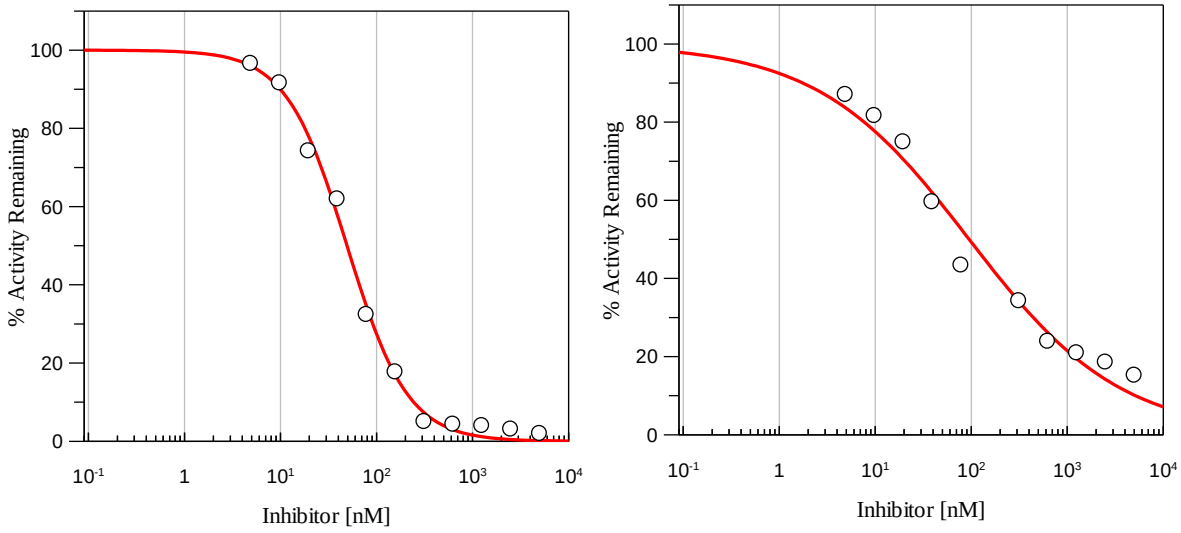

Figure B.6. **(a)** IC_{50} plot for **4.27** (left). **(b)** IC_{50} plot for **4.28** (right).

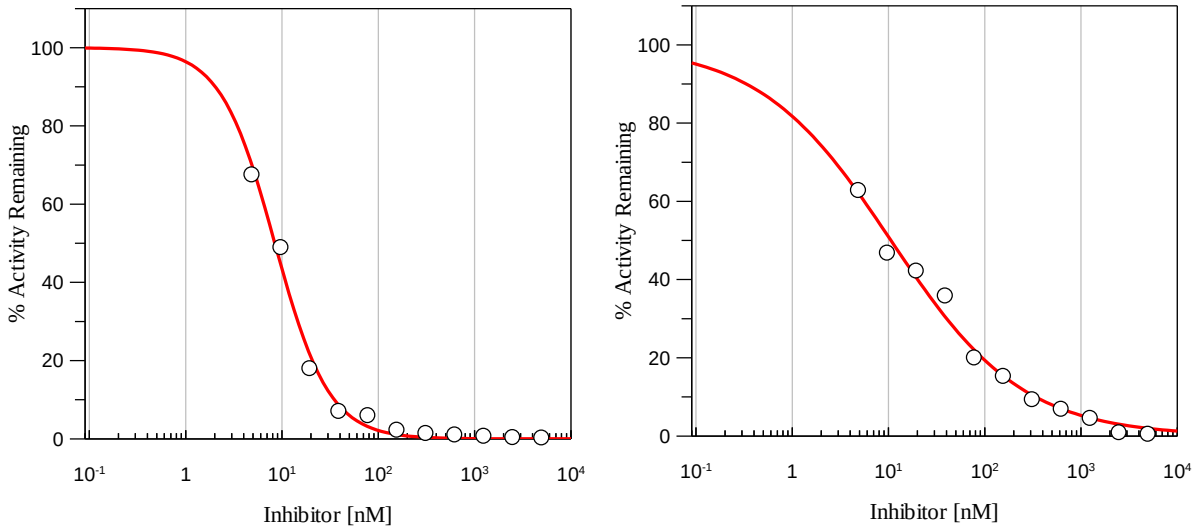

Figure B.7a. IC_{50} plot for **4.49** using 5 nM (left) and 10 nM (right) STS.

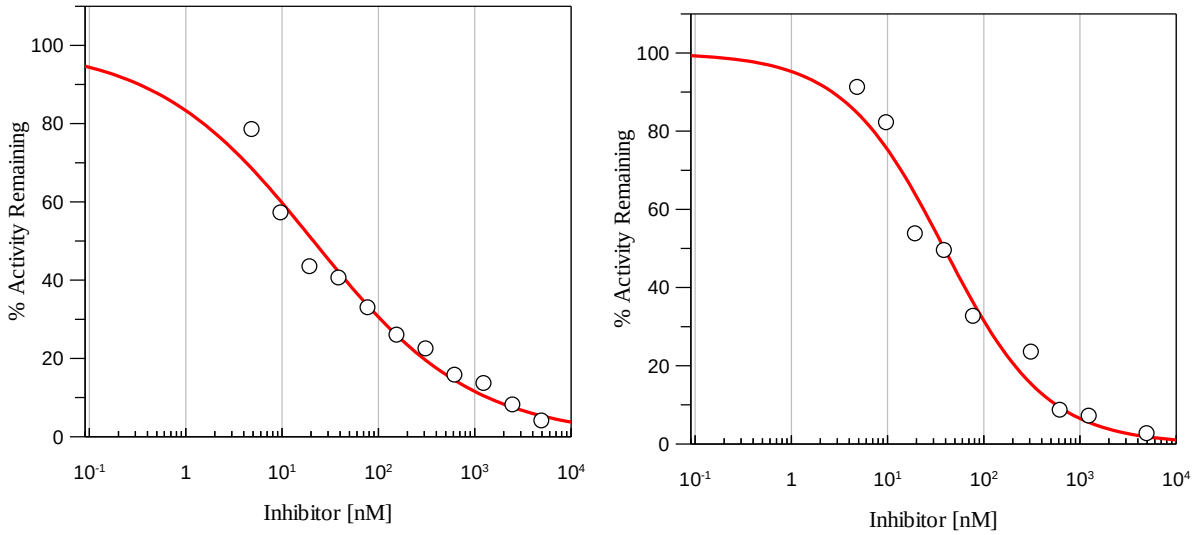

Figure B.7b. IC_{50} plot for **4.49** using 20 nM (left) and 40 nM (right) STS.

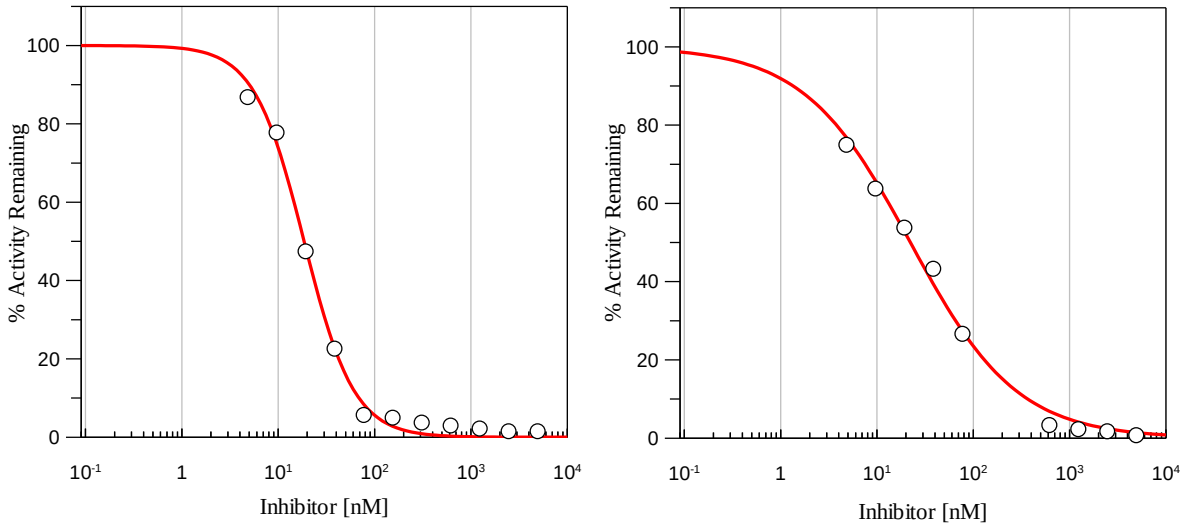

Figure B.8. **(a)** IC_{50} plot for **4.51** (left). **(b)** IC_{50} plot for **4.52** (right).

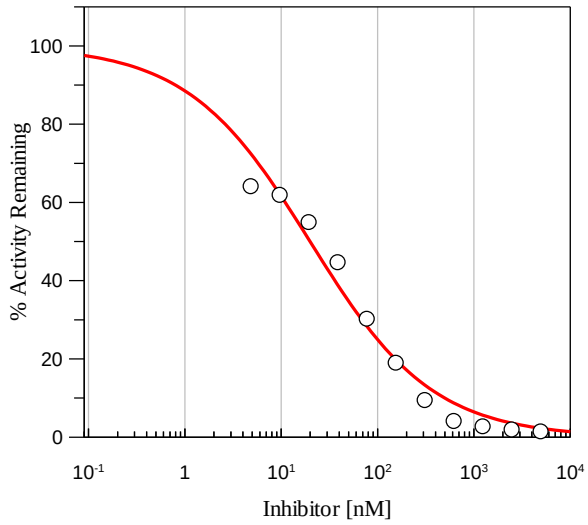

Figure B.9. IC_{50} plot for **4.53**.

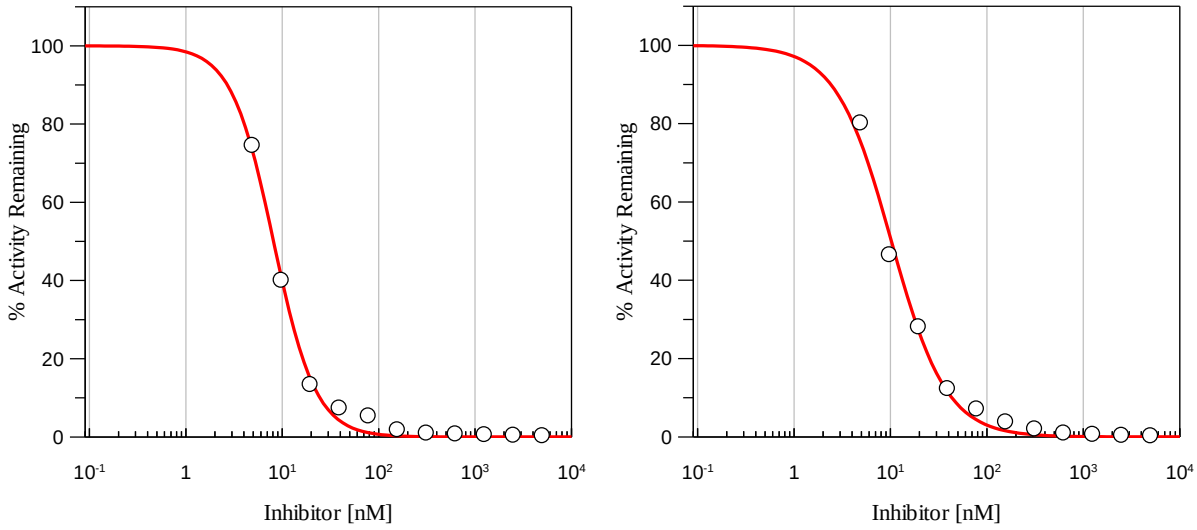

Figure B.10a. IC_{50} plot for **4.54** using 5 nM (left) and 10 nM (right) STS.

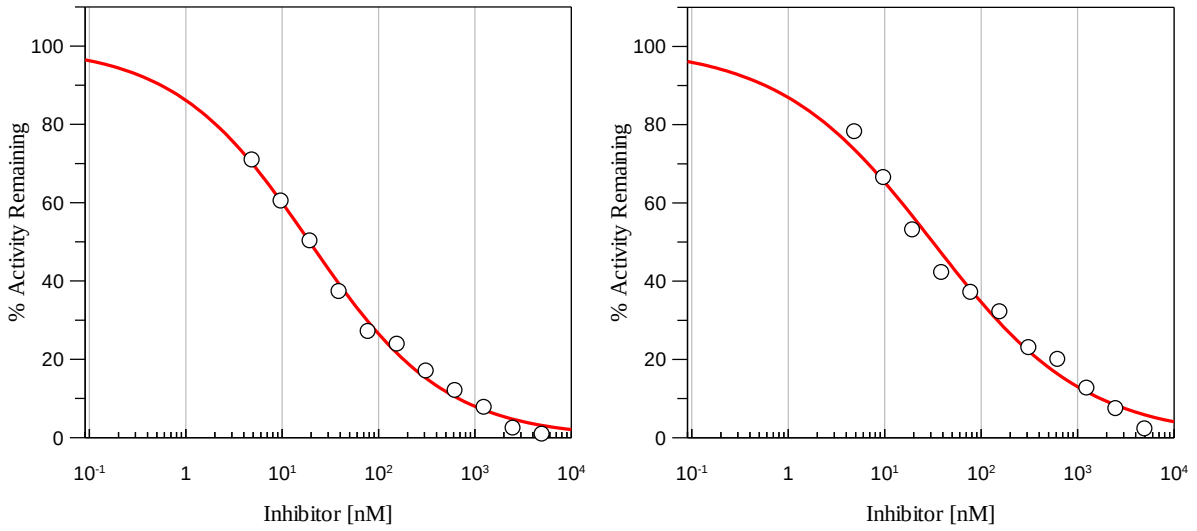

Figure B.10b. IC_{50} plot for **4.54** using 20 nM (left) and 40 nM (right) STS.

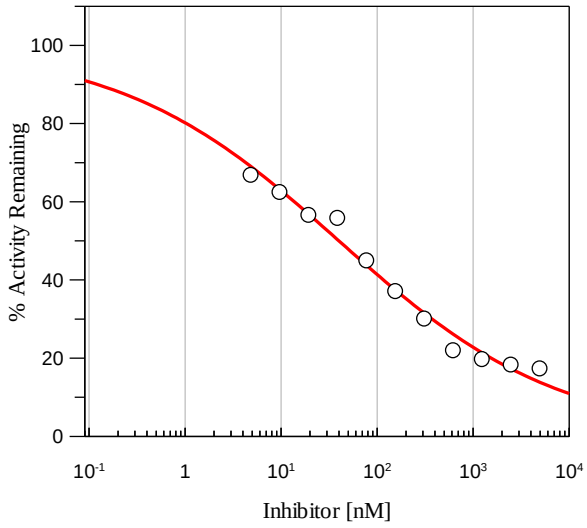

Figure B.11. IC_{50} plot for **4.2.**

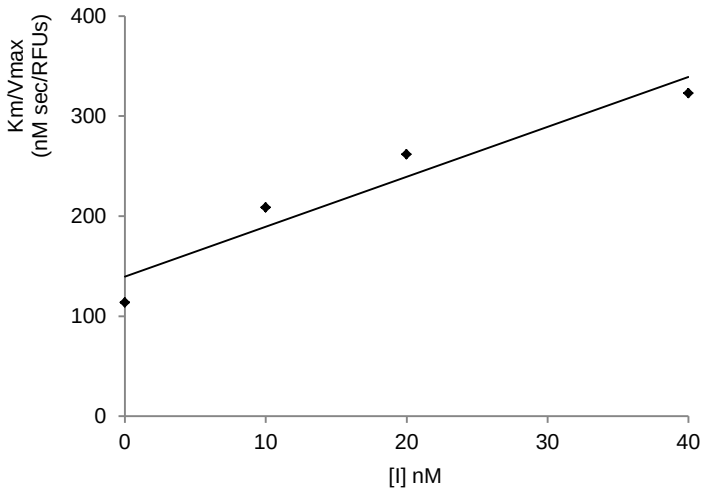

Figure B.12. Replot of the data from **Figure B.11** to determine K_i of **4.2**.

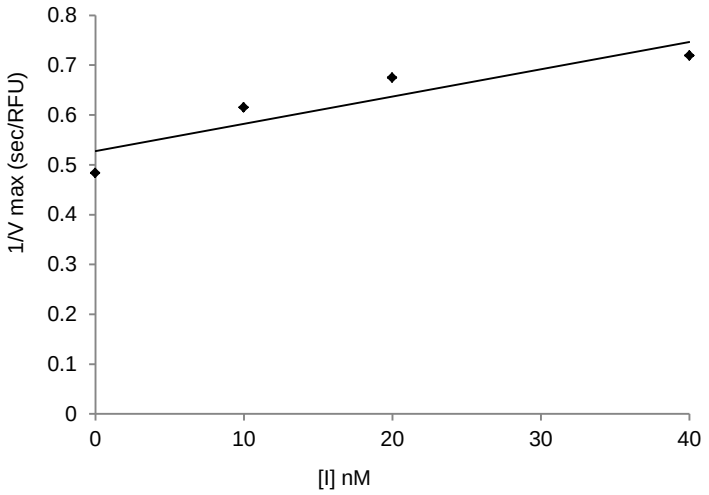

Figure B.13. Replot of the data from **Figure B.11** to determine αK_i of **4.2**.

National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

NSC : 772971 / 1	Experiment ID : 1306NS06	Test Type : 08	Units : Molar
Report Date : September 11, 2013	Test Date : June 10, 2013	QNS :	MC :
COMI : ST-31 (127061)	Stain Reagent : SRB Dual-Pass Related	SSPL : 0YWM	

			Log10 Concentration												
	Time		Mean Optical Densities					Percent Growth							
Panel/Cell Line	Zero	Ctrl	-8.0	-7.0	-6.0	-5.0	-4.0	-8.0	-7.0	-6.0	-5.0	-4.0	GI50	TGI	LC50
Leukemia															
CCRF-CEM	0.522	2.383	2.367	2.241	2.100	0.473	0.325	99	92	85	-9	-38	2.34E-6	7.95E-6	> 1.00E-4
K-562	0.205	1.509	1.448	1.413	1.289	0.153	0.113	95	93	83	-25	-45	2.02E-6	5.84E-6	> 1.00E-4
MOLT-4	0.676	2.368	2.355	2.272	2.110	0.512	0.338	99	94	85	-24	-50	2.08E-6	5.99E-6	> 1.00E-4
RPMI-8226	0.972	2.634	2.656	2.770	2.509	0.643	0.441	101	108	93	-34	-55	2.17E-6	5.40E-6	5.99E-5
SR	0.447	1.779	1.588	1.596	1.477	0.232	0.205	86	86	77	-48	-54	1.65E-6	4.14E-6	2.06E-5
Non-Small Cell Lung Cancer															
A549/ATCC	0.461	2.272	2.228	2.239	1.917	0.359	0.053	98	98	80	-22	-89	1.98E-6	6.07E-6	2.62E-5
HOP-62	0.508	1.515	1.462	1.453	1.468	0.119	0.021	95	94	95	-77	-96	1.83E-6	3.58E-6	7.00E-6
HOP-92	1.215	1.704	1.673	1.637	1.492	0.260	0.162	94	86	57	-79	-87	1.12E-6	2.62E-6	6.14E-6
NCI-H226	0.667	1.776	1.703	1.612	1.535	0.286	0.186	93	85	78	-57	-72	1.62E-6	3.78E-6	8.85E-6
NCI-H23	0.829	2.132	2.032	2.025	1.977	0.367	0.122	92	92	88	-56	-85	1.84E-6	4.10E-6	9.12E-6
NCI-H322M	0.920	2.235	2.114	2.152	2.147	0.972	0.080	91	94	93	4	-91	3.05E-6	1.10E-5	3.68E-5
NCI-H460	0.306	2.892	2.968	2.938	2.762	0.172	0.051	103	102	95	-44	-83	2.11E-6	4.83E-6	1.42E-5
NCI-H522	1.054	2.491	2.300	2.339	2.221	0.329	0.194	87	89	81	-69	-82	1.61E-6	3.48E-6	7.49E-6
Colon Cancer															
COLO 205	0.575	2.463	2.415	2.448	2.457	0.559	0.065	97	99	100	-3	-89	3.05E-6	9.39E-6	3.54E-5
HCC-2998	1.298	3.215	3.119	3.126	3.273	0.422	0.134	95	95	103	-67	-90	2.05E-6	4.02E-6	7.90E-6
HCT-116	0.284	2.390	2.316	2.415	2.130	0.009	0.011	96	101	88	-97	-96	1.60E-6	2.99E-6	5.57E-6
HCT-15	0.415	2.367	2.291	2.305	2.100	0.127	0.067	96	97	86	-70	-84	1.71E-6	3.58E-6	7.49E-6
HT29	0.300	1.615	1.651	1.632	1.732	0.053	0.050	103	101	109	-83	-83	2.03E-6	3.71E-6	6.76E-6
KM12	0.530	2.406	2.335	2.293	2.233	0.129	0.084	96	94	91	-76	-84	1.76E-6	3.51E-6	7.00E-6
SW-620	0.340	2.249	2.181	2.124	2.096	0.193	0.103	96	93	92	-43	-70	2.04E-6	4.78E-6	1.78E-5
CNS Cancer															
SF-268	0.659	1.976	1.900	1.883	1.771	0.582	0.121	94	93	84	-12	-82	2.28E-6	7.55E-6	3.52E-5
SF-295	0.646	2.713	2.663	2.676	2.567	0.683	0.203	98	98	93	2	-69	2.96E-6	1.06E-5	5.44E-5
SF-539	0.866	2.392	2.435	2.338	2.304	0.142	0.048	103	96	94	-84	-94	1.77E-6	3.39E-6	6.47E-6
SNB-19	0.617	1.900	1.797	1.804	1.723	0.681	0.030	92	93	86	5	-95	2.79E-6	1.12E-5	3.54E-5
SNB-75	0.823	1.553	1.414	1.392	1.283	0.658	0.085	81	78	63	-20	-90	1.43E-6	5.73E-6	2.69E-5
U251	0.710	2.460	2.393	2.380	2.140	0.119	0.026	96	95	82	-83	-96	1.56E-6	3.13E-6	6.28E-6
Melanoma															
LOX IMVI	0.427	2.377	2.298	2.216	2.228	0.011	0.029	96	92	92	-97	-93	1.67E-6	3.07E-6	5.62E-6
MALME-3M	0.900	1.739	1.644	1.607	1.609	0.623	0.253	89	84	84	-31	-72	1.99E-6	5.41E-6	2.93E-5
M14	0.553	1.958	1.918	1.987	1.896	0.189	0.076	97	102	96	-66	-86	1.92E-6	3.91E-6	7.98E-6
MDA-MB-435	0.555	2.437	2.400	2.529	2.204	0.173	0.200	98	105	88	-69	-64	1.74E-6	3.63E-6	7.58E-6
SK-MEL-2	1.219	2.486	2.438	2.545	2.533	0.448	0.113	96	105	104	-63	-91	2.10E-6	4.18E-6	8.33E-6
SK-MEL-28	0.546	1.740	1.789	1.828	1.815	0.097	0.056	104	107	106	-82	-90	1.99E-6	3.66E-6	6.74E-6
SK-MEL-5	0.889	3.022	3.026	2.841	2.850	0.123	0.013	100	92	92	-86	-99	1.72E-6	3.28E-6	6.26E-6
UACC-257	0.913	1.957	1.896	1.889	1.786	0.546	0.051	94	93	84	-40	-94	1.87E-6	4.74E-6	1.52E-5
UACC-62	0.900	2.488	2.402	2.350	2.016	0.109	0.014	95	91	70	-88	-99	1.34E-6	2.78E-6	5.76E-6
Ovarian Cancer															
IGROV1	0.561	1.951	1.996	1.969	1.918	0.292	0.109	103	101	98	-48	-81	2.12E-6	4.68E-6	1.15E-5
OVCAR-4	0.576	1.079	1.062	1.050	1.001	0.550	0.084	97	94	84	-5	-86	2.44E-6	8.88E-6	3.64E-5
OVCAR-5	0.640	1.537	1.517	1.473	1.461	0.199	0.027	98	93	92	-69	-96	1.81E-6	3.72E-6	7.62E-6
OVCAR-8	0.631	2.587	2.543	2.526	2.334	0.583	0.191	98	97	87	-8	-70	2.46E-6	8.30E-6	4.80E-5
NCI/ADR-RES	0.676	2.044	2.016	2.008	1.882	0.385	0.113	98	97	88	-43	-83	1.95E-6	4.70E-6	1.49E-5
SK-OV-3	0.781	1.898	1.875	1.937	1.871	0.664	-0.008	98	103	98	-15	-100	2.65E-6	7.36E-6	2.58E-5
Renal Cancer															
786-0	0.818	2.651	2.629	2.597	2.602	0.368	0.011	99	97	97	-55	-99	2.04E-6	4.35E-6	9.26E-6
A498	1.633	2.170	2.084	1.998	1.918	0.900	0.055	84	68	53	-45	-97	1.07E-6	3.48E-6	1.25E-5
ACHN	0.511	1.908	1.967	1.904	1.683	0.424	0.015	104	100	84	-17	-97	2.17E-6	6.78E-6	2.58E-5
CAKI-1	0.698	2.805	2.792	2.821	2.521	0.543	0.152	99	101	87	-22	-78	2.17E-6	6.24E-6	3.13E-5
SN12C	0.669	2.257	2.225	2.156	2.028	0.077	0.049	98	94	86	-88	-93	1.60E-6	3.10E-6	6.01E-6
TK-10	0.800	1.581	1.493	1.555	1.604	0.667	0.041	89	97	103	-17	-95	2.77E-6	7.26E-6	2.67E-5
UO-31	0.702	2.125	1.897	1.894	1.801	0.168	0.118	84	84	77	-76	-83	1.50E-6	3.19E-6	6.76E-6
Prostate Cancer															
PC-3	0.871	2.669	2.686	2.622	2.281	0.421	0.057	101	97	78	-52	-93	1.65E-6	4.00E-6	9.70E-6
DU-145	0.413	1.436	1.448	1.418	1.424	0.342	0.028	101	98	99	-17	-93	2.63E-6	7.11E-6	2.70E-5
Breast Cancer															
MCF7	0.591	2.838	2.724	2.730	2.632	0.271	0.123	95	95	91	-54	-79	1.91E-6	4.23E-6	9.35E-6
MDA-MB-231/ATCC	0.721	1.869	1.827	1.782	1.630	0.070	0.045	96	92	79	-90	-94	1.49E-6	2.93E-6	5.78E-6
HS 578T	1.409	2.506	2.426	2.436	2.331	1.143	0.664	93	94	84	-19	-53	2.14E-6	6.56E-6	8.21E-5
BT-549	0.902	1.943	1.878	1.859	1.780	0.081	0.024	94	92	84	-91	-97	1.57E-6	3.03E-6	5.83E-6
T-47D	0.635	1.502	1.476	1.434	1.429	0.540	0.083	97	92	91	-15	-87	2.45E-6	7.22E-6	3.06E-5
MDA-MB-468	0.909	2.118	2.059	1.943	1.957	0.711	0.240	95	86	87	-22	-74	2.18E-6	6.29E-6	3.50E-5

Table B.1. In-Vitro Testing Results (GI_{50}, TGI, and LC_{50}) of **4.9.**

National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

NSC : 772970 / 1	Experiment ID : 1307NS20	Test Type : 08	Units : Molar
Report Date : November 05, 2013	Test Date : July 02, 2013	QNS :	MC :
COMI : ST-30 (127058)	Stain Reagent : SRB Dual-Pass Related	SSPL : 0YWM	

			Log10 Concentration											
	Time		Mean Optical Densities					Percent Growth						
Panel/Cell Line	Zero	Ctrl	-8.0	-7.0	-6.0	-5.0	-4.0	-8.0	-7.0	-6.0	-5.0	-4.0	GI50	TGI	LC50
Leukemia															
CCRF-CEM	0.264	1.188	1.196	1.156	1.062	0.267	0.209	101	97	86	.	-21	2.65E-6	1.04E-5	> 1.00E-4
HL-60(TB)	1.348	3.431	3.403	3.390	3.375	0.980	0.672	99	98	97	-27	-50	2.40E-6	6.04E-6	9.81E-5
K-562	0.194	1.900	1.923	1.897	1.857	0.209	0.138	101	100	97	1	-29	3.10E-6	1.07E-5	> 1.00E-4
MOLT-4	0.729	2.983	2.905	2.951	2.815	0.795	0.585	97	99	93	3	-20	2.98E-6	1.34E-5	> 1.00E-4
RPMI-8226	0.748	2.248	2.283	2.285	2.207	0.563	0.498	102	103	97	-25	-33	2.44E-6	6.27E-6	> 1.00E-4
SR	0.292	1.010	0.989	0.999	1.015	0.284	0.237	97	98	101	-3	-19	3.09E-6	9.41E-6	> 1.00E-4
Non-Small Cell Lung Cancer															
A549/ATCC	0.343	1.626	1.613	1.641	1.559	0.326	0.071	99	101	95	-5	-79	2.81E-6	8.89E-6	4.02E-5
HOP-62	0.402	1.339	1.350	1.341	1.354	0.067	0.083	101	100	102	-83	-79	1.90E-6	3.54E-6	6.60E-6
HOP-92	1.215	1.772	1.735	1.692	1.592	0.866	0.238	93	86	68	-29	-80	1.53E-6	5.03E-6	2.58E-5
NCI-H226	0.592	1.727	1.685	1.710	1.720	0.549	0.154	96	99	99	-7	-74	2.90E-6	8.53E-6	4.36E-5
NCI-H23	0.923	2.857	2.798	2.796	2.611	0.585	0.286	97	97	87	-37	-69	2.00E-6	5.06E-6	2.59E-5
NCI-H322M	0.774	1.978	1.883	1.928	1.933	0.859	0.025	92	96	96	7	-97	3.30E-6	1.17E-5	3.54E-5
NCI-H460	0.330	2.933	2.925	2.937	2.783	0.077	0.022	100	100	94	-77	-93	1.81E-6	3.56E-6	6.98E-6
Colon Cancer															
COLO 205	0.392	1.967	1.955	1.965	2.018	0.327	0.063	99	100	103	-17	-84	2.78E-6	7.26E-6	3.13E-5
HCC-2998	1.001	3.131	3.192	3.148	3.243	0.294	0.216	103	101	105	-71	-78	2.06E-6	3.97E-6	7.63E-6
HCT-116	0.219	2.059	2.036	1.943	1.911	0.096	0.107	99	94	92	-56	-51	1.92E-6	4.17E-6	9.06E-6
HCT-15	0.271	1.812	1.626	1.674	1.580	0.031	0.056	88	91	85	-89	-79	1.59E-6	3.09E-6	5.99E-6
HT29	0.223	1.166	1.149	1.174	1.138	0.075	0.065	98	101	97	-67	-71	1.94E-6	3.92E-6	7.92E-6
KM12	0.671	2.801	2.717	2.720	2.633	0.271	0.080	96	96	92	-60	-88	1.89E-6	4.05E-6	8.64E-6
SW-620	0.397	2.534	2.373	2.370	2.316	0.388	0.047	92	92	90	-2	-88	2.70E-6	9.42E-6	3.58E-5
CNS Cancer															
SF-268	0.487	1.782	1.749	1.739	1.709	0.502	0.092	97	97	94	1	-81	2.99E-6	1.03E-5	4.18E-5
SF-295	0.897	2.752	2.496	2.540	2.589	0.067	0.080	86	89	91	-93	-91	1.68E-6	3.13E-6	5.86E-6
SF-539	0.826	2.313	2.335	2.157	2.014	0.195	0.118	101	89	80	-76	-86	1.55E-6	3.24E-6	6.77E-6
SNB-19	1.039	2.507	2.361	2.375	2.382	1.272	0.102	90	91	91	16	-90	3.53E-6	1.41E-5	4.18E-5
SNB-75	0.860	1.706	1.557	1.555	1.371	0.175	0.172	82	82	60	-80	-80	1.19E-6	2.70E-6	6.14E-6
U251	0.634	2.377	2.350	2.306	2.193	0.303	0.044	98	96	89	-52	-93	1.90E-6	4.28E-6	9.64E-6
Melanoma															
MALME-3M	0.499	1.124	1.120	1.062	1.078	0.330	0.058	99	90	93	-34	-88	2.17E-6	5.40E-6	1.97E-5
M14	0.465	1.959	1.917	1.857	1.844	0.195	0.142	97	93	92	-58	-70	1.91E-6	4.11E-6	8.82E-6
MDA-MB-435	0.437	2.097	2.040	2.041	1.790	0.043	0.062	97	97	81	-90	-86	1.53E-6	2.98E-6	5.83E-6
SK-MEL-2	1.008	2.219	2.203	2.213	2.244	0.623	0.154	99	100	102	-38	-85	2.35E-6	5.34E-6	1.79E-5
SK-MEL-28	0.614	1.958	1.947	1.940	1.892	0.164	0.149	99	99	95	-73	-76	1.85E-6	3.67E-6	7.27E-6
SK-MEL-5	0.567	2.828	2.773	2.712	2.626	0.034	0.063	98	95	91	-94	-89	1.67E-6	3.11E-6	5.78E-6
UACC-257	0.681	1.491	1.433	1.442	1.431	0.414	0.131	93	94	93	-39	-81	2.10E-6	5.04E-6	1.82E-5
UACC-62	0.795	2.489	2.440	2.421	2.123	0.320	0.084	97	96	78	-60	-89	1.60E-6	3.69E-6	8.49E-6
Ovarian Cancer															
IGROV1	0.661	2.223	2.217	2.191	2.300	0.640	0.097	100	98	105	-3	-85	3.22E-6	9.35E-6	3.71E-5
OVCAR-3	0.750	1.941	1.991	1.987	1.975	0.494	0.032	104	104	103	-34	-96	2.43E-6	5.63E-6	1.81E-5
OVCAR-4	0.517	0.918	0.877	0.861	0.828	0.063	0.079	90	86	78	-88	-85	1.47E-6	2.94E-6	5.90E-6
OVCAR-5	0.517	1.763	1.792	1.784	1.691	0.322	0.106	102	102	94	-38	-80	2.16E-6	5.17E-6	1.96E-5
OVCAR-8	0.432	1.899	1.912	1.873	1.781	0.460	0.148	101	98	92	2	-66	2.92E-6	1.07E-5	5.85E-5
NCI/ADR-RES	1.003	3.103	3.134	3.152	2.949	0.935	0.362	101	102	93	-7	-64	2.69E-6	8.55E-6	5.70E-5
SK-OV-3	0.779	1.973	1.967	2.028	2.060	0.662	0.044	99	105	107	-15	-94	2.94E-6	7.53E-6	2.76E-5
Renal Cancer															
786-0	0.762	2.615	2.492	2.475	2.593	0.655	0.124	93	92	99	-14	-84	2.70E-6	7.50E-6	3.28E-5
A498	1.469	2.251	2.201	2.073	2.131	1.095	0.009	94	77	85	-25	-99	2.06E-6	5.87E-6	2.15E-5
ACHN	0.397	1.836	1.880	1.853	1.715	0.362	0.028	103	101	92	-9	-93	2.59E-6	8.17E-6	3.08E-5
CAKI-1	0.438	2.252	2.292	2.308	2.278	0.278	0.037	102	103	101	-37	-92	2.36E-6	5.43E-6	1.75E-5
RXF 393	0.868	1.578	1.555	1.544	1.570	0.352	0.267	97	95	99	-59	-69	2.03E-6	4.21E-6	8.72E-6
SN12C	0.737	2.462	2.418	2.382	2.250	0.206	0.147	97	95	88	-72	-80	1.72E-6	3.54E-6	7.27E-6
TK-10	0.571	1.451	1.426	1.410	1.432	0.544	0.054	97	95	98	-5	-91	2.92E-6	8.98E-6	3.36E-5
UO-31	0.718	2.269	2.083	2.087	2.135	0.500	0.020	88	88	91	-30	-97	2.19E-6	5.63E-6	1.97E-5
Prostate Cancer															
PC-3	0.555	1.760	1.688	1.660	1.541	0.599	0.134	94	92	82	4	-76	2.55E-6	1.11E-5	4.72E-5
DU-145	0.348	1.604	1.632	1.605	1.615	0.466	0.027	102	100	101	9	-92	3.60E-6	1.24E-5	3.84E-5
Breast Cancer															
MCF7	0.486	2.312	2.051	2.117	2.083	0.196	0.084	86	89	87	-60	-83	1.80E-6	3.93E-6	8.60E-6
MDA-MB-231/ATCC	0.611	1.383	1.390	1.315	1.295	0.275	0.156	101	91	89	-55	-75	1.86E-6	4.14E-6	9.23E-6
HS 578T	1.495	2.350	2.317	2.295	2.270	1.109	0.770	96	94	91	-26	-49	2.23E-6	6.00E-6	> 1.00E-4
BT-549	0.888	1.947	1.885	1.883	1.894	0.312	0.254	94	94	95	-65	-71	1.91E-6	3.93E-6	8.07E-6
T-47D	0.405	1.006	0.955	0.944	0.928	0.168	0.088	91	90	87	-59	-78	1.80E-6	3.96E-6	8.72E-6
MDA-MB-468	0.721	1.357	1.330	1.339	1.349	0.630	0.199	96	97	99	-13	-72	2.74E-6	7.69E-6	4.21E-5

Table B.2. In-Vitro Testing Results (GI_{50}, TGI, and LC_{50}) of **4.10.**

National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

NSC : 772965 / 1	Experiment ID : 1306NS09	Test Type : 08	Units : Molar
Report Date : January 30, 2014	Test Date : June 17, 2013	QNS :	MC :
COMI : ST-25 (127030)	Stain Reagent : SRB Dual-Pass Related	SSPL : 0YWM	

			Log10 Concentration											
	Time		Mean Optical Densities					Percent Growth						
Panel/Cell Line	Zero	Ctrl	-8.0	-7.0	-6.0	-5.0	-4.0	-8.0	-7.0	-6.0	-5.0	-4.0	GI50	TGI	LC50
Leukemia															
CCRF-CEM	0.434	2.058	1.963	2.075	1.980	0.420	0.288	94	101	95	-3	-34	2.88E-6	9.25E-6	> 1.00E-4
K-562	0.223	1.609	1.578	1.640	1.550	0.302	0.141	98	102	96	6	-37	3.22E-6	1.36E-5	> 1.00E-4
MOLT-4	0.780	2.747	2.656	2.570	2.512	0.766	0.537	95	91	88	-2	-31	2.65E-6	9.55E-6	> 1.00E-4
RPMI-8226	1.046	2.933	2.832	2.939	2.819	0.896	0.602	95	100	94	-14	-42	2.55E-6	7.37E-6	> 1.00E-4
SR	0.596	2.126	2.042	1.980	2.010	0.496	0.368	95	90	92	-17	-38	2.45E-6	7.02E-6	> 1.00E-4
Non-Small Cell Lung Cancer															
A549/ATCC	0.458	2.293	2.311	2.268	2.124	0.468	0.101	101	99	91	1	-78	2.83E-6	1.02E-5	4.40E-5
HOP-62	0.667	1.760	1.549	1.700	1.787	0.585	0.012	81	95	102	-12	-98	2.87E-6	7.81E-6	2.74E-5
HOP-92	1.173	1.769	1.737	1.704	1.713	0.771	0.138	95	89	91	-34	-88	2.11E-6	5.31E-6	1.95E-5
NCI-H226	0.682	1.781	1.657	1.749	1.622	0.066	0.070	89	97	86	-90	-90	1.59E-6	3.06E-6	5.89E-6
NCI-H23	0.924	2.607	2.481	2.451	2.403	0.892	0.213	92	91	88	-4	-77	2.60E-6	9.15E-6	4.30E-5
NCI-H322M	0.984	2.330	2.220	2.241	2.302	1.340	0.096	92	93	98	26	-90	4.68E-6	1.69E-5	4.52E-5
NCI-H460	0.305	2.873	2.955	2.986	2.882	0.161	0.060	103	104	100	-47	-80	2.19E-6	4.79E-6	1.21E-5
NCI-H522	0.876	2.003	1.843	1.869	1.816	0.581	0.234	86	88	83	-34	-73	1.93E-6	5.15E-6	2.58E-5
Colon Cancer															
COLO 205	0.569	2.407	2.309	2.389	2.440	0.028	-0.001	95	99	102	-95	-100	1.83E-6	3.29E-6	5.90E-6
HCC-2998	1.045	2.986	3.050	3.177	3.153	1.295	0.026	103	110	109	13	-98	4.09E-6	1.31E-5	3.71E-5
HCT-116	0.288	2.145	2.124	2.121	2.074	0.038	0.048	99	99	96	-87	-84	1.79E-6	3.35E-6	6.29E-6
HCT-15	0.342	2.056	2.050	2.022	1.921	0.090	0.094	100	98	92	-74	-73	1.79E-6	3.59E-6	7.19E-6
HT29	0.313	1.634	1.680	1.666	1.679	0.146	0.063	103	102	103	-53	-80	2.19E-6	4.57E-6	9.52E-6
KM12	0.561	2.249	2.245	2.285	2.348	0.170	0.068	100	102	106	-70	-88	2.08E-6	4.01E-6	7.72E-6
SW-620	0.383	2.406	2.356	2.406	2.358	0.531	0.127	98	100	98	7	-67	3.37E-6	1.25E-5	5.91E-5
CNS Cancer															
SF-268	0.722	2.074	1.995	2.060	1.994	0.796	0.122	94	99	94	5	-83	3.14E-6	1.15E-5	4.22E-5
SF-295	0.992	2.963	2.943	2.915	2.892	0.796	0.154	99	98	96	-20	-85	2.51E-6	6.76E-6	2.93E-5
SF-539	1.032	2.699	2.680	2.711	2.659	0.117	0.021	99	101	98	-89	-98	1.80E-6	3.34E-6	6.20E-6
SNB-19	0.857	2.148	2.014	2.038	2.063	1.032	0.146	90	91	93	14	-83	3.49E-6	1.38E-5	4.55E-5
SNB-75	0.860	1.628	1.489	1.546	1.492	0.668	0.106	82	89	82	-22	-88	2.03E-6	6.11E-6	2.65E-5
U251	0.895	2.713	2.669	2.583	2.491	0.180	0.045	98	93	88	-80	-95	1.68E-6	3.34E-6	6.63E-6
Melanoma															
LOX IMVI	0.493	2.826	2.695	2.767	2.681	0.017	0.125	94	97	94	-97	-75	1.70E-6	3.11E-6	5.69E-6
MALME-3M	0.818	1.588	1.555	1.546	1.599	0.586	0.239	96	94	101	-28	-71	2.49E-6	6.04E-6	3.24E-5
M14	0.549	1.858	1.787	1.810	1.850	0.234	0.051	95	96	99	-57	-91	2.07E-6	4.31E-6	8.97E-6
MDA-MB-435	0.672	2.695	2.733	2.660	2.599	0.482	0.267	102	98	95	-28	-60	2.32E-6	5.90E-6	4.78E-5
SK-MEL-2	1.330	2.693	2.728	2.761	2.718	0.920	0.123	103	105	102	-31	-91	2.46E-6	5.86E-6	2.09E-5
SK-MEL-28	0.712	2.002	2.008	2.066	1.957	0.100	0.084	100	105	96	-86	-88	1.80E-6	3.38E-6	6.35E-6
SK-MEL-5	0.909	3.079	3.059	3.074	2.881	-0.002	0.094	99	100	91	-100	-90	1.64E-6	2.99E-6	5.47E-6
UACC-257	1.080	2.203	2.232	2.203	2.115	1.038	0.316	103	100	92	-4	-71	2.75E-6	9.11E-6	4.89E-5
UACC-62	0.911	2.445	2.356	2.347	2.311	0.285	0.071	94	94	91	-69	-92	1.81E-6	3.72E-6	7.64E-6
Ovarian Cancer															
IGROV1	0.583	2.057	2.068	2.024	2.196	0.631	0.095	101	98	109	3	-84	3.63E-6	1.09E-5	4.09E-5
OVCAR-4	0.702	1.420	1.375	1.412	1.298	0.700	0.179	94	99	83	.	-75	2.49E-6	9.92E-6	4.67E-5
OVCAR-5	0.595	1.679	1.647	1.666	1.661	0.135	0.173	97	99	98	-77	-71	1.88E-6	3.63E-6	6.99E-6
OVCAR-8	0.504	2.342	2.304	2.342	2.273	0.466	0.230	98	100	96	-8	-54	2.79E-6	8.46E-6	8.03E-5
NCI/ADR-RES	0.763	2.501	2.538	2.524	2.335	0.639	0.208	102	101	90	-16	-73	2.39E-6	7.03E-6	3.95E-5
SK-OV-3	0.770	1.949	1.934	1.980	1.950	0.714	0.001	99	103	100	-7	-100	2.93E-6	8.54E-6	2.89E-5
Renal Cancer															
786-0	0.899	2.671	2.695	2.620	2.667	0.674	0.050	101	97	100	-25	-94	2.50E-6	6.30E-6	2.29E-5
A498	1.565	2.205	2.186	2.100	2.068	0.251	0.069	97	84	79	-84	-96	1.50E-6	3.04E-6	6.18E-6
ACHN	0.515	1.905	1.923	1.963	1.861	0.219	-0.003	101	104	97	-58	-100	2.01E-6	4.24E-6	8.93E-6
CAKI-1	1.002	2.921	2.752	2.928	2.798	0.952	0.308	91	100	94	-5	-69	2.77E-6	8.89E-6	5.01E-5
RXF 393	1.073	1.878	1.805	1.844	1.716	0.352	0.300	91	96	80	-67	-72	1.60E-6	3.49E-6	7.64E-6
SN12C	0.977	2.830	2.678	2.701	2.712	0.239	0.247	92	93	94	-76	-75	1.81E-6	3.57E-6	7.06E-6
TK-10	0.712	1.561	1.556	1.501	1.584	0.794	0.052	99	93	103	10	-93	3.69E-6	1.24E-5	3.83E-5
UO-31	0.881	2.364	1.885	2.165	2.112	0.475	0.041	68	87	83	-46	-95	1.80E-6	4.40E-6	1.20E-5
Prostate Cancer															
PC-3	0.519	2.315	2.145	2.193	2.068	1.021	0.065	91	93	86	28	-87	4.19E-6	1.75E-5	4.74E-5
DU-145	0.415	1.572	1.603	1.586	1.568	0.809	-0.005	103	101	100	34	-100	5.72E-6	1.80E-5	4.24E-5
Breast Cancer															
MCF7	0.726	2.837	2.715	2.723	2.787	0.515	0.176	94	95	98	-29	-76	2.38E-6	5.90E-6	2.81E-5
MDA-MB-231/ATCC	0.847	2.024	2.039	1.998	1.929	0.389	0.111	101	98	92	-54	-87	1.94E-6	4.26E-6	9.37E-6
HS 578T	1.662	2.617	2.583	2.611	2.616	1.395	0.873	96	99	100	-16	-48	2.69E-6	7.27E-6	> 1.00E-4
BT-549	1.004	1.980	1.978	1.944	2.013	0.376	0.196	100	96	103	-63	-80	2.10E-6	4.20E-6	8.40E-6
T-47D	0.572	1.256	1.166	1.196	1.195	0.571	-0.002	87	91	91	.	-100	2.82E-6	9.96E-6	3.16E-5
MDA-MB-468	0.821	1.354	1.283	1.293	1.280	0.583	0.083	87	89	86	-29	-90	2.05E-6	5.59E-6	2.21E-5

Table B.3. In-Vitro Testing Results (GI_{50}, TGI, and LC_{50}) of Compound **4.53.**

Appendix C – Supplementary X-ray Crystallographic Data for Compound 3.33

Crystal data

$C_{25}H_{28}F_3NO_3S$	$F(000)$ = 1008
M_r = 479.54	*Density* = 1.392 g cm^{-3}
Monoclinic, $P2_1$	Mo $K\alpha$ radiation, λ = 0.71073 Å
a = 9.611 (3) Å	Cell parameters from 1582 reflections
b = 13.461 (4) Å	θ = 1.5–30°
c = 17.786 (6) Å	μ = 0.19 mm^{-1}
β = 96.239 (18)°	T = 200 K
V = 2287.5 (12) Å^3	Plate, colourless
Z = 4	0.25 × 0.08 × 0.02 mm

Data collection

Bruker Kappa APEX II diffractometer	6503 reflections with $I > 2\sigma(I)$
Radiation source: fine-focus sealed tube with graphite monochromator	R_{int} = 0.067
ω and phi scan	θ_{max} = 26.0°, θ_{min} = 3.0°
Absorption correction: empirical (using intensity measurements) SADABS	h = −11 11
T_{min} = 0.953, T_{max} = 0.996	k = −16 16
25349 measured reflections	l = −21 21
8534 independent reflections	

Refinement

Refinement on F^2	Hydrogen site location: inferred from neighbouring sites
Least-squares matrix: full	H-atom parameters constrained
$R[F^2 > 2\sigma(F^2)]$ = 0.080	$w = 1/[\sigma^2(F_o^2) + (0.0019P)^2 + 5.0121P]$ where $P = (F_o^2 + 2F_c^2)/3$

$wR(F^2) = 0.177$	$(\Delta/\sigma)_{max} < 0.001$
S = 1.20	$\Delta\rho_{max} = 0.61$ e Å^{-3}
8534 reflections	$\Delta\rho_{min} = -0.38$ e Å^{-3}
609 parameters	Extinction correction: ?, $Fc^*=kFc[1+0.001xFc^2\lambda^3/\sin(2\theta)]^{-1/4}$
19 restraints	Extinction coefficient: ?
0 constraints	Absolute structure: Flack H D (1983), Acta Cryst. A39, 876-881
Primary atom site location: structure-invariant direct methods	Flack parameter: −0.06 (14)

Fractional atomic coordinates and isotropic or equivalent isotropic displacement parameters (Å^2)

	x	*y*	*z*	U_{iso}*/U_{eq}	Occ. (<1)
S1A	0.06736 (17)	−0.03120 (13)	0.69284 (9)	0.0363 (4)	
N1A	0.1541 (6)	0.0612 (4)	0.7280 (3)	0.0369 (13)	
H1AA	0.2311	0.0771	0.7082	0.044*	
O1A	−0.0460 (5)	−0.0493 (4)	0.7378 (3)	0.0449 (12)	
O2A	0.1602 (5)	−0.1106 (4)	0.6805 (3)	0.0487 (13)	
O3A	0.0256 (7)	0.8482 (4)	0.8772 (3)	0.0648 (17)	
H3AA	0.0503	0.8799	0.8402	0.097*	
C1A	0.1154 (8)	0.1223 (5)	0.7912 (4)	0.0378 (17)	
H1AC	0.0135	0.1119	0.7952	0.045*	
C2A	0.1957 (11)	0.0950 (6)	0.8672 (4)	0.062 (2)	
H2AB	0.2905	0.0703	0.8603	0.075*	
H2AC	0.1454	0.0431	0.8929	0.075*	
C3A	0.2044 (10)	0.1937 (5)	0.9139 (4)	0.060 (2)	
H3AB	0.1646	0.1845	0.9624	0.072*	
H3AC	0.3027	0.2160	0.9245	0.072*	
C4A	0.1207 (8)	0.2671 (5)	0.8652 (4)	0.0392 (17)	

H4AA	0.0209	0.2518	0.8716	0.047*
C5A	0.1400 (7)	0.2346 (5)	0.7824 (4)	0.0362 (16)
C6A	0.1357 (8)	0.3759 (5)	0.8782 (3)	0.0361 (16)
H6AA	0.2330	0.3956	0.8694	0.043*
C7A	0.0332 (7)	0.4320 (4)	0.8216 (3)	0.0316 (15)
H7AA	−0.0623	0.4101	0.8319	0.038*
C8A	0.0476 (8)	0.4010 (5)	0.7390 (4)	0.0443 (18)
H8AA	−0.0271	0.4336	0.7050	0.053*
H8AB	0.1388	0.4245	0.7249	0.053*
C9A	0.0376 (8)	0.2886 (5)	0.7276 (4)	0.0421 (17)
H9AA	−0.0584	0.2662	0.7343	0.050*
H9AB	0.0561	0.2722	0.6754	0.050*
C10A	0.1111 (11)	0.4080 (5)	0.9584 (4)	0.057 (2)
H10A	0.0127	0.3945	0.9668	0.068*
H10B	0.1724	0.3690	0.9958	0.068*
C11A	0.1418 (12)	0.5178 (6)	0.9699 (4)	0.064 (3)
H11A	0.1059	0.5392	1.0174	0.076*
H11B	0.2445	0.5273	0.9762	0.076*
C12A	0.0804 (8)	0.5832 (5)	0.9074 (4)	0.0431 (18)
C13A	0.0378 (7)	0.5434 (5)	0.8359 (4)	0.0351 (16)
C14A	0.0761 (9)	0.6865 (5)	0.9194 (4)	0.050 (2)
H14A	0.1079	0.7129	0.9677	0.060*
C15A	0.0266 (8)	0.7494 (5)	0.8621 (4)	0.0435 (18)
C16A	−0.0162 (8)	0.7116 (5)	0.7910 (4)	0.0478 (19)
H16A	−0.0472	0.7555	0.7508	0.057*
C17A	−0.0140 (8)	0.6104 (5)	0.7783 (4)	0.0417 (17)
H17A	−0.0480	0.5853	0.7299	0.050*
C18A	0.2879 (8)	0.2518 (6)	0.7636 (4)	0.0470 (19)

H18A	0.3046	0.3232	0.7591	0.071*		
H18B	0.3008	0.2190	0.7157	0.071*		
H18C	0.3543	0.2242	0.8040	0.071*		
C19A	−0.0136 (7)	0.0040 (5)	0.6025 (4)	0.0333 (15)		
C20A	−0.1121 (7)	0.0758 (5)	0.5972 (4)	0.0349 (15)		
H20A	−0.1399	0.1061	0.6415	0.042*		
C21A	−0.1725 (8)	0.1049 (6)	0.5256 (4)	0.0467 (18)		
C22A	−0.1319 (10)	0.0602 (7)	0.4624 (5)	0.065 (2)		
H22A	−0.1724	0.0804	0.4136	0.078*		
C23A	−0.0315 (11)	−0.0145 (8)	0.4692 (5)	0.079 (3)		
H23A	−0.0058	−0.0463	0.4251	0.095*		
C24A	0.0307 (8)	−0.0430 (6)	0.5387 (4)	0.0546 (19)		
H24A	0.1013	−0.0927	0.5437	0.066*		
C25A	−0.2771 (10)	0.1876 (7)	0.5223 (6)	0.068 (3)		
F1AA	−0.302 (2)	0.231 (2)	0.4529 (9)	0.111 (4)	0.449	(13)
F1AB	−0.3762 (18)	0.1735 (15)	0.4589 (8)	0.111 (4)	0.551	(13)
F2AA	−0.2279 (9)	0.2722 (6)	0.5519 (7)	0.095 (3)	0.686	(11)
F2AB	−0.234 (2)	0.2510 (13)	0.4784 (19)	0.095 (3)	0.314	(11)
F3AA	−0.3697 (8)	0.1777 (6)	0.5709 (5)	0.094 (3)	0.780	(10)
F3AB	−0.405 (3)	0.152 (2)	0.503 (2)	0.094 (3)	0.220	(10)
S1B	0.47973 (16)	0.46528 (12)	0.32239 (9)	0.0346 (4)		
N1B	0.5455 (6)	0.3803 (4)	0.2771 (3)	0.0412 (14)		
H1BA	0.6354	0.3689	0.2886	0.049*		
O1B	0.3480 (5)	0.4922 (3)	0.2837 (3)	0.0417 (12)		
O2B	0.5842 (5)	0.5393 (3)	0.3412 (3)	0.0495 (13)		
O3B	0.3301 (7)	−0.4081 (3)	0.1431 (3)	0.0649 (17)		
H3BA	0.3344	−0.4403	0.1838	0.097*		
C1B	0.4726 (7)	0.3178 (5)	0.2178 (4)	0.0358 (16)		

H1BC	0.3701	0.3307	0.2175	0.043*
C2B	0.5121 (10)	0.3438 (5)	0.1388 (4)	0.056 (2)
H2BB	0.6088	0.3701	0.1421	0.067*
H2BC	0.4473	0.3944	0.1144	0.067*
C3B	0.5001 (10)	0.2452 (5)	0.0932 (4)	0.052 (2)
H3BB	0.5933	0.2208	0.0828	0.062*
H3BC	0.4400	0.2533	0.0447	0.062*
C4B	0.4318 (7)	0.1740 (4)	0.1475 (4)	0.0336 (15)
H4BA	0.3303	0.1919	0.1436	0.040*
C5B	0.4946 (7)	0.2058 (5)	0.2262 (4)	0.0313 (15)
C6B	0.4383 (8)	0.0631 (4)	0.1345 (3)	0.0316 (15)
H6BA	0.5388	0.0424	0.1402	0.038*
C7B	0.3648 (8)	0.0096 (4)	0.1936 (4)	0.0327 (15)
H7BA	0.2650	0.0317	0.1847	0.039*
C8B	0.4143 (8)	0.0412 (5)	0.2741 (3)	0.0373 (16)
H8BA	0.5104	0.0160	0.2880	0.045*
H8BB	0.3528	0.0108	0.3089	0.045*
C9B	0.4136 (7)	0.1549 (5)	0.2842 (4)	0.0338 (15)
H9BA	0.3158	0.1792	0.2785	0.041*
H9BB	0.4562	0.1721	0.3357	0.041*
C10B	0.3714 (10)	0.0304 (5)	0.0559 (4)	0.049 (2)
H10C	0.2701	0.0459	0.0509	0.059*
H10D	0.4142	0.0681	0.0165	0.059*
C11B	0.3910 (10)	−0.0786 (5)	0.0437 (4)	0.054 (2)
H11C	0.4877	−0.0900	0.0313	0.065*
H11D	0.3263	−0.0999	−0.0005	0.065*
C12B	0.3661 (8)	−0.1420 (5)	0.1104 (4)	0.0409 (17)
C13B	0.3607 (7)	−0.1022 (5)	0.1823 (4)	0.0316 (15)

C14B	0.3575 (9)	−0.2449 (5)	0.0989 (4)	0.0471 (19)
H14B	0.3648	−0.2721	0.0502	0.057*
C15B	0.3384 (8)	−0.3063 (5)	0.1591 (4)	0.0402 (17)
C16B	0.3346 (7)	−0.2693 (5)	0.2304 (4)	0.0374 (16)
H16B	0.3254	−0.3126	0.2718	0.045*
C17B	0.3442 (7)	−0.1677 (5)	0.2412 (4)	0.0375 (16)
H17B	0.3395	−0.1418	0.2905	0.045*
C18B	0.6504 (7)	0.1833 (5)	0.2404 (4)	0.0414 (17)
H18D	0.6642	0.1114	0.2460	0.062*
H18E	0.6899	0.2168	0.2868	0.062*
H18F	0.6973	0.2072	0.1977	0.062*
C19B	0.4367 (7)	0.4156 (4)	0.4089 (3)	0.0293 (14)
C20B	0.3302 (7)	0.3474 (4)	0.4075 (4)	0.0339 (15)
H20B	0.2764	0.3314	0.3611	0.041*
C21B	0.3011 (8)	0.3016 (5)	0.4748 (4)	0.0406 (17)
C22B	0.3764 (10)	0.3293 (7)	0.5421 (5)	0.064 (3)
H22B	0.3563	0.2995	0.5881	0.077*
C23B	0.4812 (9)	0.4002 (7)	0.5430 (4)	0.059 (2)
H23B	0.5308	0.4195	0.5898	0.070*
C24B	0.5154 (9)	0.4445 (5)	0.4752 (4)	0.051 (2)
H24B	0.5888	0.4917	0.4751	0.061*
C25B	0.1927 (10)	0.2240 (6)	0.4725 (5)	0.056 (2)
F1B	0.1313 (8)	0.2187 (6)	0.5344 (4)	0.138 (3)
F2B	0.2460 (7)	0.1342 (4)	0.4644 (5)	0.120 (3)
F3B	0.0921 (6)	0.2318 (4)	0.4170 (4)	0.098 (2)

Geometric parameters (Å, °)

S1A—O2A	1.425 (5)	F1AB—F3AB	0.90 (3)

S1A—O1A	1.441 (5)	F1AB—F2AB	1.72 (3)
S1A—N1A	1.587 (6)	S1B—O1B	1.421 (5)
S1A—C19A	1.772 (7)	S1B—O2B	1.428 (5)
N1A—C1A	1.473 (8)	S1B—N1B	1.571 (6)
N1A—H1AA	0.8800	S1B—C19B	1.768 (6)
O3A—C15A	1.358 (8)	N1B—C1B	1.466 (8)
O3A—H3AA	0.8400	N1B—H1BA	0.8800
C1A—C2A	1.528 (10)	O3B—C15B	1.400 (8)
C1A—C5A	1.540 (9)	O3B—H3BA	0.8400
C1A—H1AC	1.0000	C1B—C5B	1.528 (9)
C2A—C3A	1.564 (10)	C1B—C2B	1.534 (9)
C2A—H2AB	0.9900	C1B—H1BC	1.0000
C2A—H2AC	0.9900	C2B—C3B	1.554 (9)
C3A—C4A	1.489 (9)	C2B—H2BB	0.9900
C3A—H3AB	0.9900	C2B—H2BC	0.9900
C3A—H3AC	0.9900	C3B—C4B	1.555 (9)
C4A—C6A	1.488 (9)	C3B—H3BB	0.9900
C4A—C5A	1.567 (9)	C3B—H3BC	0.9900
C4A—H4AA	1.0000	C4B—C6B	1.513 (8)
C5A—C9A	1.495 (9)	C4B—C5B	1.524 (9)
C5A—C18A	1.513 (10)	C4B—H4BA	1.0000
C6A—C7A	1.530 (8)	C5B—C9B	1.521 (8)
C6A—C10A	1.534 (9)	C5B—C18B	1.521 (9)
C6A—H6AA	1.0000	C6B—C7B	1.510 (9)
C7A—C13A	1.520 (9)	C6B—C10B	1.539 (9)
C7A—C8A	1.548 (9)	C6B—H6BA	1.0000
C7A—H7AA	1.0000	C7B—C13B	1.518 (8)
C8A—C9A	1.528 (10)	C7B—C8B	1.520 (9)

C8A—H8AA	0.9900	C7B—H7BA	1.0000
C8A—H8AB	0.9900	C8B—C9B	1.541 (9)
C9A—H9AA	0.9900	C8B—H8BA	0.9900
C9A—H9AB	0.9900	C8B—H8BB	0.9900
C10A—C11A	1.516 (10)	C9B—H9BA	0.9900
C10A—H10A	0.9900	C9B—H9BB	0.9900
C10A—H10B	0.9900	C10B—C11B	1.498 (9)
C11A—C12A	1.488 (10)	C10B—H10C	0.9900
C11A—H11A	0.9900	C10B—H10D	0.9900
C11A—H11B	0.9900	C11B—C12B	1.502 (10)
C12A—C13A	1.400 (9)	C11B—H11C	0.9900
C12A—C14A	1.408 (10)	C11B—H11D	0.9900
C13A—C17A	1.414 (9)	C12B—C13B	1.393 (9)
C14A—C15A	1.369 (10)	C12B—C14B	1.400 (9)
C14A—H14A	0.9500	C13B—C17B	1.392 (8)
C15A—C16A	1.383 (10)	C14B—C15B	1.381 (9)
C16A—C17A	1.382 (10)	C14B—H14B	0.9500
C16A—H16A	0.9500	C15B—C16B	1.366 (9)
C17A—H17A	0.9500	C16B—C17B	1.383 (9)
C18A—H18A	0.9800	C16B—H16B	0.9500
C18A—H18B	0.9800	C17B—H17B	0.9500
C18A—H18C	0.9800	C18B—H18D	0.9800
C19A—C20A	1.349 (9)	C18B—H18E	0.9800
C19A—C24A	1.404 (9)	C18B—H18F	0.9800
C20A—C21A	1.397 (9)	C19B—C20B	1.372 (9)
C20A—H20A	0.9500	C19B—C24B	1.385 (9)
C21A—C22A	1.370 (11)	C20B—C21B	1.402 (9)
C21A—C25A	1.496 (12)	C20B—H20B	0.9500

C22A—C23A	1.391 (13)	C21B—C22B	1.381 (11)
C22A—H22A	0.9500	C21B—C25B	1.473 (11)
C23A—C24A	1.368 (11)	C22B—C23B	1.387 (12)
C23A—H23A	0.9500	C22B—H22B	0.9500
C24A—H24A	0.9500	C23B—C24B	1.415 (10)
C25A—F2AB	1.26 (3)	C23B—H23B	0.9500
C25A—F3AA	1.313 (11)	C24B—H24B	0.9500
C25A—F2AA	1.322 (13)	C25B—F1B	1.305 (9)
C25A—F3AB	1.33 (4)	C25B—F3B	1.310 (10)
C25A—F1AA	1.364 (19)	C25B—F2B	1.326 (10)
C25A—F1AB	1.407 (16)		
O2A—S1A—O1A	118.9 (3)	F3AA—C25A—C21A	113.5 (7)
O2A—S1A—N1A	109.7 (3)	F2AA—C25A—C21A	114.6 (8)
O1A—S1A—N1A	108.1 (3)	F3AB—C25A—C21A	109.8 (14)
O2A—S1A—C19A	106.4 (3)	F1AA—C25A—C21A	114.0 (11)
O1A—S1A—C19A	105.4 (3)	F1AB—C25A—C21A	109.0 (11)
N1A—S1A—C19A	107.9 (3)	F3AB—F1AB—C25A	66 (3)
C1A—N1A—S1A	125.0 (5)	F3AB—F1AB—F2AB	110 (4)
C1A—N1A—H1AA	117.5	C25A—F1AB—F2AB	46.0 (11)
S1A—N1A—H1AA	117.5	C25A—F2AB—F1AB	53.6 (10)
C15A—O3A—H3AA	109.5	F1AB—F3AB—C25A	75 (3)
N1A—C1A—C2A	113.3 (6)	O1B—S1B—O2B	120.2 (3)
N1A—C1A—C5A	114.6 (5)	O1B—S1B—N1B	108.9 (3)
C2A—C1A—C5A	104.9 (6)	O2B—S1B—N1B	108.4 (3)
N1A—C1A—H1AC	107.9	O1B—S1B—C19B	103.8 (3)
C2A—C1A—H1AC	107.9	O2B—S1B—C19B	106.6 (3)
C5A—C1A—H1AC	107.9	N1B—S1B—C19B	108.4 (3)
C1A—C2A—C3A	104.9 (6)	C1B—N1B—S1B	126.8 (5)

C1A—C2A—H2AB	110.8	C1B—N1B—H1BA	116.6
C3A—C2A—H2AB	110.8	S1B—N1B—H1BA	116.6
C1A—C2A—H2AC	110.8	C15B—O3B—H3BA	109.5
C3A—C2A—H2AC	110.8	N1B—C1B—C5B	116.5 (6)
H2AB—C2A—H2AC	108.8	N1B—C1B—C2B	112.5 (5)
C4A—C3A—C2A	105.1 (6)	C5B—C1B—C2B	105.6 (5)
C4A—C3A—H3AB	110.7	N1B—C1B—H1BC	107.3
C2A—C3A—H3AB	110.7	C5B—C1B—H1BC	107.3
C4A—C3A—H3AC	110.7	C2B—C1B—H1BC	107.3
C2A—C3A—H3AC	110.7	C1B—C2B—C3B	105.8 (5)
H3AB—C3A—H3AC	108.8	C1B—C2B—H2BB	110.6
C6A—C4A—C3A	121.7 (6)	C3B—C2B—H2BB	110.6
C6A—C4A—C5A	113.6 (5)	C1B—C2B—H2BC	110.6
C3A—C4A—C5A	104.5 (6)	C3B—C2B—H2BC	110.6
C6A—C4A—H4AA	105.2	H2BB—C2B—H2BC	108.7
C3A—C4A—H4AA	105.2	C2B—C3B—C4B	102.4 (5)
C5A—C4A—H4AA	105.2	C2B—C3B—H3BB	111.3
C9A—C5A—C18A	110.5 (6)	C4B—C3B—H3BB	111.3
C9A—C5A—C1A	116.4 (6)	C2B—C3B—H3BC	111.3
C18A—C5A—C1A	109.3 (6)	C4B—C3B—H3BC	111.3
C9A—C5A—C4A	109.9 (5)	H3BB—C3B—H3BC	109.2
C18A—C5A—C4A	112.0 (6)	C6B—C4B—C5B	113.5 (5)
C1A—C5A—C4A	98.2 (5)	C6B—C4B—C3B	119.1 (6)
C4A—C6A—C7A	109.9 (6)	C5B—C4B—C3B	104.2 (5)
C4A—C6A—C10A	113.6 (6)	C6B—C4B—H4BA	106.4
C7A—C6A—C10A	108.6 (6)	C5B—C4B—H4BA	106.4
C4A—C6A—H6AA	108.2	C3B—C4B—H4BA	106.4
C7A—C6A—H6AA	108.2	C9B—C5B—C18B	111.4 (6)

C10A—C6A—H6AA	108.2	C9B—C5B—C4B	108.4 (5)
C13A—C7A—C6A	111.8 (6)	C18B—C5B—C4B	112.4 (5)
C13A—C7A—C8A	114.9 (5)	C9B—C5B—C1B	115.8 (5)
C6A—C7A—C8A	111.9 (5)	C18B—C5B—C1B	109.6 (5)
C13A—C7A—H7AA	105.8	C4B—C5B—C1B	98.7 (5)
C6A—C7A—H7AA	105.8	C7B—C6B—C4B	109.5 (5)
C8A—C7A—H7AA	105.8	C7B—C6B—C10B	108.4 (6)
C9A—C8A—C7A	112.5 (6)	C4B—C6B—C10B	113.6 (5)
C9A—C8A—H8AA	109.1	C7B—C6B—H6BA	108.4
C7A—C8A—H8AA	109.1	C4B—C6B—H6BA	108.4
C9A—C8A—H8AB	109.1	C10B—C6B—H6BA	108.4
C7A—C8A—H8AB	109.1	C6B—C7B—C13B	112.9 (6)
H8AA—C8A—H8AB	107.8	C6B—C7B—C8B	113.6 (5)
C5A—C9A—C8A	111.6 (6)	C13B—C7B—C8B	113.8 (5)
C5A—C9A—H9AA	109.3	C6B—C7B—H7BA	105.2
C8A—C9A—H9AA	109.3	C13B—C7B—H7BA	105.2
C5A—C9A—H9AB	109.3	C8B—C7B—H7BA	105.2
C8A—C9A—H9AB	109.3	C7B—C8B—C9B	112.5 (5)
H9AA—C9A—H9AB	108.0	C7B—C8B—H8BA	109.1
C11A—C10A—C6A	110.6 (7)	C9B—C8B—H8BA	109.1
C11A—C10A—H10A	109.5	C7B—C8B—H8BB	109.1
C6A—C10A—H10A	109.5	C9B—C8B—H8BB	109.1
C11A—C10A—H10B	109.5	H8BA—C8B—H8BB	107.8
C6A—C10A—H10B	109.5	C5B—C9B—C8B	111.0 (5)
H10A—C10A—H10B	108.1	C5B—C9B—H9BA	109.4
C12A—C11A—C10A	114.9 (7)	C8B—C9B—H9BA	109.4
C12A—C11A—H11A	108.5	C5B—C9B—H9BB	109.4
C10A—C11A—H11A	108.5	C8B—C9B—H9BB	109.4

C12A—C11A—H11B	108.5	H9BA—C9B—H9BB	108.0
C10A—C11A—H11B	108.5	C11B—C10B—C6B	111.4 (6)
H11A—C11A—H11B	107.5	C11B—C10B—H10C	109.3
C13A—C12A—C14A	120.3 (6)	C6B—C10B—H10C	109.3
C13A—C12A—C11A	120.4 (6)	C11B—C10B—H10D	109.3
C14A—C12A—C11A	119.2 (7)	C6B—C10B—H10D	109.3
C12A—C13A—C17A	117.4 (6)	H10C—C10B—H10D	108.0
C12A—C13A—C7A	121.9 (6)	C10B—C11B—C12B	114.2 (7)
C17A—C13A—C7A	120.4 (6)	C10B—C11B—H11C	108.7
C15A—C14A—C12A	120.8 (7)	C12B—C11B—H11C	108.7
C15A—C14A—H14A	119.6	C10B—C11B—H11D	108.7
C12A—C14A—H14A	119.6	C12B—C11B—H11D	108.7
O3A—C15A—C14A	117.9 (7)	H11C—C11B—H11D	107.6
O3A—C15A—C16A	122.2 (6)	C13B—C12B—C14B	120.5 (6)
C14A—C15A—C16A	119.8 (6)	C13B—C12B—C11B	122.1 (6)
C17A—C16A—C15A	120.2 (7)	C14B—C12B—C11B	117.3 (6)
C17A—C16A—H16A	119.9	C17B—C13B—C12B	117.7 (6)
C15A—C16A—H16A	119.9	C17B—C13B—C7B	122.2 (6)
C16A—C17A—C13A	121.4 (7)	C12B—C13B—C7B	120.0 (6)
C16A—C17A—H17A	119.3	C15B—C14B—C12B	119.4 (7)
C13A—C17A—H17A	119.3	C15B—C14B—H14B	120.3
C5A—C18A—H18A	109.5	C12B—C14B—H14B	120.3
C5A—C18A—H18B	109.5	C16B—C15B—C14B	121.3 (6)
H18A—C18A—H18B	109.5	C16B—C15B—O3B	122.6 (6)
C5A—C18A—H18C	109.5	C14B—C15B—O3B	116.0 (6)
H18A—C18A—H18C	109.5	C15B—C16B—C17B	118.8 (6)
H18B—C18A—H18C	109.5	C15B—C16B—H16B	120.6
C20A—C19A—C24A	122.6 (7)	C17B—C16B—H16B	120.6

C20A—C19A—S1A	119.4 (5)	C16B—C17B—C13B	122.3 (6)
C24A—C19A—S1A	118.0 (6)	C16B—C17B—H17B	118.9
C19A—C20A—C21A	118.9 (6)	C13B—C17B—H17B	118.9
C19A—C20A—H20A	120.6	C5B—C18B—H18D	109.5
C21A—C20A—H20A	120.6	C5B—C18B—H18E	109.5
C22A—C21A—C20A	119.9 (7)	H18D—C18B—H18E	109.5
C22A—C21A—C25A	123.0 (8)	C5B—C18B—H18F	109.5
C20A—C21A—C25A	117.1 (7)	H18D—C18B—H18F	109.5
C21A—C22A—C23A	120.2 (8)	H18E—C18B—H18F	109.5
C21A—C22A—H22A	119.9	C20B—C19B—C24B	122.7 (6)
C23A—C22A—H22A	119.9	C20B—C19B—S1B	118.8 (5)
C24A—C23A—C22A	120.8 (8)	C24B—C19B—S1B	118.4 (5)
C24A—C23A—H23A	119.6	C19B—C20B—C21B	119.7 (6)
C22A—C23A—H23A	119.6	C19B—C20B—H20B	120.1
C23A—C24A—C19A	117.6 (8)	C21B—C20B—H20B	120.1
C23A—C24A—H24A	121.2	C22B—C21B—C20B	119.1 (7)
C19A—C24A—H24A	121.2	C22B—C21B—C25B	121.2 (7)
F2AB—C25A—F3AA	140.3 (13)	C20B—C21B—C25B	119.6 (7)
F2AB—C25A—F2AA	62.2 (13)	C21B—C22B—C23B	120.5 (7)
F3AA—C25A—F2AA	93.6 (10)	C21B—C22B—H22B	119.8
F2AB—C25A—F3AB	116.0 (19)	C23B—C22B—H22B	119.8
F3AA—C25A—F3AB	57.6 (16)	C22B—C23B—C24B	121.1 (7)
F2AA—C25A—F3AB	134.2 (16)	C22B—C23B—H23B	119.5
F2AB—C25A—F1AA	35.0 (12)	C24B—C23B—H23B	119.5
F3AA—C25A—F1AA	125.2 (11)	C19B—C24B—C23B	116.8 (7)
F2AA—C25A—F1AA	90.4 (14)	C19B—C24B—H24B	121.6
F3AB—C25A—F1AA	81.7 (17)	C23B—C24B—H24B	121.6
F2AB—C25A—F1AB	80.4 (14)	F1B—C25B—F3B	106.0 (8)

F3AA—C25A—F1AB	93.8 (10)	F1B—C25B—F2B	104.9 (8)
F2AA—C25A—F1AB	128.1 (11)	F3B—C25B—F2B	104.7 (8)
F3AB—C25A—F1AB	38.3 (14)	F1B—C25B—C21B	113.5 (7)
F1AA—C25A—F1AB	45.5 (10)	F3B—C25B—C21B	115.2 (7)
F2AB—C25A—C21A	105.4 (12)	F2B—C25B—C21B	111.6 (7)

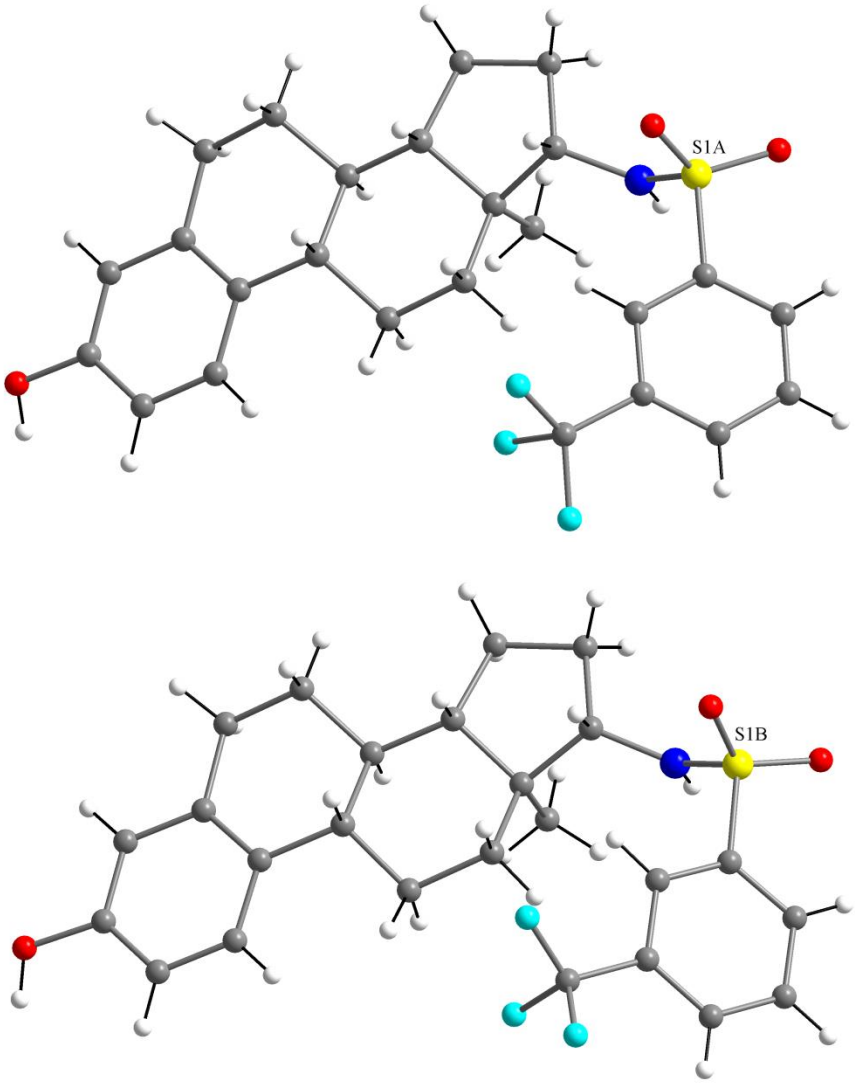

Fig. B.14. Two crystallographic-independent molecules **A** and **B**.