Newly Developed Piperidinyl Sulfamides as Tyrosyl-DNA Phosphodiesterase 1 (Tdp 1) Inhibitors, and Study of Anticancer Activity of Piperidinyl Sulfamides Derivatives and Seven-Membered Cyclic Sulfamide Analogs Using the National Cancer Institute 60 Human Cancer Cell Line (NCI 60) Screen

Jung Ho Jun

Submitted to the Department of Chemistry and the Faculty of the Graduate School of the University of Kansas in partial fulfillment of the requirements of the degree of Doctor of Philosophy

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Abstract

Jung Ho Jun, Ph. D

Department of Chemistry, October 2013

University of Kansas

Sulfur containing compounds have become increasingly important in the development of biological agents for pharmaceutical and industrial use. Cyclic sulfamides, in particular, have been found to be useful as cancer, HIV protease inhibitors and other therapeutic treatments. As the need for new and improved inhibitors is warranted by the serious cancer disease, the search for new synthetic pathways to access novel sulfamides is ongoing. To this end, the work discussed herein focuses on the synthesis of newly developed sulfamides utilizing the reductive amination and Mitsunobu reaction to generate novel chiral amino ester containing sulfamide compounds. These compounds are being screened for their biological activities as Tyrosyl-DNA phosphodiesterase 1 **(**Tdp 1) inhibitors and anti-cancer drugs. Initially, reductive amination, CSI coupling, and Mitsunobu reaction were employed to generate piperidinyl sulfamides, and these compounds were screened for Tdp1 inhibition. These compounds were submitted to Dr. Pomier's group at NIH to carry out the gel study to select active compounds. We also checked the binding effect through the protein docking study. In addition, these sulfamide compounds were screened from NCI 60-cancer cell lines to check the bioactivity and *in vitro* cytotoxicity evaluation. To understand anti-cancer activity of cyclic sulfamides, symmetric and unsymmetric seven-membered sulfamides compounds were tested in 60 cancer cell line from the National Cancer Institute. These compounds were made when I studied for the Master degree at the University of Kansas. RCM was employed to generate symmetric seven-membered cyclic sulfamides similar in structure to known active HIV protease inhibitor DMP 323.

Functionalization of these compounds employing “robust *S*-linchpins” in conjunction with RCM yields an array of new *S*-heterocycles. Further work in sulfamides employed a combination of RCM with different coupling routes to generate unsymmetric seven-membered cyclic sulfamides with varied substitution in their P1/P1' and P2/P2' periphery in attempts to broaden the scope of this chemistry and to generate new biologically active compounds.

To my friend and wife
To my love

JungRim Moon

My soul mate and intimate prayer

To my family with love

My father and mother
My daughter, ***Talia Jun***
My sister’s family

Acknowledgements

First of all, I would like to thank the Lord for guiding my way, and my eternal friend, lovely wife and soul mate, *Jungrim Moon*. As I always mentioned, you are a precious gift from God. You always give me lots of ideas of how to endure torrential life and go straight toward our promised future with God. You have shown your love and support to me every single day and every moment. I sincerely appreciate your prayer that you always hope my life goes well. I love you and will love you forever.

My daughter, Talia. You are the greatest gift and an angel for me and your mom from the Lord. I still remember the first day when your mom gave birth to you. I enjoyed holding you in my arm as my first and only daughter. You were so cute and pretty!!! When you held my finger with your hand for the first time, I cried with joy and grace. The very first day of your walking, your mommy and daddy were so excited and proud of you. Now you are already the first grade student. Talia, I pray to God that you always enjoy your life and journey, and be in his graceful guidance.

My family, father, mother, my younger sister's family, and my father-in-law family. All of you are the Lord's greatest gift to me. I am entirely blessed to have you always praying on my behalf. Dad, you are my hero and master of my adventurous sailing. Whenever I needed your wisdom and experience, you always constantly encouraged and supported me. Dad, I will never forget memories of us climbing the mountains together every New Year's Day. Mom, thank you for always supporting my efforts and for being my best friend. You have been a major part of my life for so long. I will pray for your health and pleasure. My younger sister, Jungrim Jun, you are my lovely friend and I hope you are always happy with your son, Jinho. Without your thoughtful consideration of my life, it would have been impossible to achieve this goal. Jinho, you will be remembered always as my cute nephew and I want you to grow like Joseph with BIG DREAM! My mother-in-law and father-in-law, thank you for your continuous prayer for my family. My wife's sister family, Misuk and Jaesung. I won't forget all of the memories that we had last summer. It was really great time with you.

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Many thanks to all of the other professors who have contributed to my intellectual and personal development: Dr. Robert Carlson, Dr. Helena, Malinakova, Dr. David Benson, Dr. Jeffrey Aube, Dr. Andrew Borovik, and Dr. Brian Blagg. I thank you all.

I want to thank all of Hanson group members who are in my memory. Don, Matt, Joel, Joe, Andy, Poon, Rusty, Shubashish, Mianji, Punitha, Alan, Steve, Maria, Josh, Thiwanka, and so on. I really miss the fun we had and lots of memories with you guys!! I do not think I will ever have an experience as enjoyable as I had working with you guys. I hope to see you all at Paul's 60th Birthday Party!!!! (It will come very soon).

Vineet, my previous colleague at SAIC-Frederick. I enjoyed every moment last five years with you as LSC group member. I have many memories with you from setting our lab to the renovation and more. I hope we keep in touch and I will be watching your career from afar. You are my good friend!

Many current Hanson group members. Even if we never work together in a lab, I feel like we've been worked altogether for a long time. Naeem, Pradip, Qin, Saqib, Joanna, Jana, Soma, Rambabu, Salim, Kyu Ok, Susanthi, Moon Young, and more. Thank you for listening my Skype- presentation at group meeting and reading my thesis. Kyu Ok, I will continuously watch your next steps as a young chemist. Naeem, I appreciate your time and effort to set up seminar schedules for me. Moon Young, I appreciate your technical support for each time. Guys! Remember this. One day you will move on leaving DOOMSDAY behind forever and ever. The day will come, the sun will rise, and we'll be fine!

Finally, I would like to thank Dr. Changkiu Lee and Dr. Insook Han-Lee, my former advisors and organic chemistry professors at the Kangwon National University in Korea. Your enthusiasm for organic chemistry encouraged many students including me to step up to higher field and to study abroad to explore new and advanced academic area. You introduced me a new life view to plan and help to educate next generation. I am forever grateful for your commitment to me. I hope you continue to harvest a greater research result every year.

Table of Contents

Thesis Explanation:

This thesis is separated into three major parts and is set up to be easily perused by the interested reader.

Chapter 1 consists of an introduction of biologically active sulfamides and synthetic approaches to sulfamides.

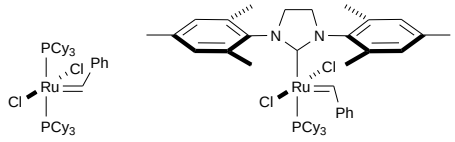
Chapter 2 contains the synthesis of newly developed piperidinyl sulfamides as Tyrosyl-DNA phosphodiesterase 1 (Tdp 1) Inhibitors.

Chapter 3 is the study of anticancer activity of piperidinyl sulfamides derivatives using the National Cancer Institute 60 human cancer cell line (NCI 60) screening.

Chapter 4 is discussion about the anticancer activity of seven-membered cyclic sulfamide analogs using the Nnational Cancer Institute 60 human cancer cell line (NCI 60) Screening.

Chapter 5 is the experimental section consisting of an explanation of all synthetic methods and selected ^{1}H and ^{13}C NMR spectra for new compounds that have been synthesized. In spectral data for pertinent new compounds is reported. This section also contains the results of one and five dose experimental data from NCI 60 cell line.

For the purpose of simplicity, Grubbs 1st generation catalyst and 2nd generation catalyst have been designated **I** and **II**, and refer to the structures listed below:



Grubbs 1st Generation **I**

Grubbs 2nd Generation **II**

Abbreviations:

AIDS	Acquired Immune Deficiency Syndrome
Ala	Alanine
Bn	Benzyl
Boc	*tert*-butoxy carbonyl
***n*-BuLi**	*n*-butyl lithium
CH_2Cl_2	methylene chloride
DCM	methylene chloride
Cs_2CO_3	cesium carbonate
CSI	chlorosulfonyl isocyanate
Cat-I	phenyl methylene bis(tricyclohexylphosphine) ruthenium dichloride
Cat-II	tricyclohexylphosphine [1,3-bis(2,4,6-trimethylphenyl)-4,5-dihydroimidazole-2-ylidene][benzylidine]-ruthenium(IV) dichloride
DEAD	diethylazodicarboxylate
DIAD	diisopropylazodicarboxylate
DMSO	dimethyl sulfoxide
DMF	dimethyl formamide
EDC	*N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide
FAB-MS	Fast Atom Bombardment-Mass Spectrometry
HIV	Human Immunodeficiency Virus
HRMS	High Resolution Mass Spectrometry
Hz	Hertz
K_2CO_3	potassium carbonate
LAH	lithium aluminum hydride
$LiAlH_4$	lithium aluminum hydride
***m*-CPBA**	*meta*-chloro perbenzoic acid
MeCN	acetonitrile

MHz	Megahertz
NOE	nuclear overhauser enhancement
NMO	4-methylmorpholine *N*-oxide
NMR	Nuclear Magnetic Resonance
$PhCH_3$	toluene
PhCl	chlorobenzene
PhH	benzene
ppm	parts per million
PR	protease
RCM	ring-closing metathesis
^{t}Bu	*tert*-butyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	Thin layer chromatography
TMSI	1-(trimethylsily)imidazole

CHAPTER 1

Introduction:

Biologically Active Sulfamides and Synthetic Approaches to Sulfamides

1.1. Introduction

Over the past decade, the sulfamide ($R_2NSO_2NR_2$) functionality has found extensive use in medicinal chemistry for the development of novel small molecule therapeutic agents and high affinity protein ligands.[1,2] The synthesis of the first sulfamide was reported in 1892 by Traube, who prepared it from sulfuryl chloride and gaseous ammonia.[3]

The utility of sulfamides can be attributed to the ability to variably substitute with up to four different substituents, which are distributed on the two nitrogen atoms, thus offering diversity. Moreover, the sulfamide functional group can also act as a useful biosteric replacement for sulfonamide, sulfamate, urea, carbamate, ketoamide, ester, and amide functionalities when incorporated into putative pharmaceutical agents, as it has the potential to construct several electrostatic interactions with protein and other targets.[4]

Notably, numerous compounds have been reported as marketed and investigational drugs in which the free or substituted sulfamide moiety plays a key role in dictating potent biological activity (Figure 1.1). Doripenem (**1.1**), structurally related to penicillin, is an ultra-broad spectrum injectable antibiotic that was recently approved by the Food and Drug Administration (FDA) for the treatment of complicated intra-abdominal infections and complicated urinary tract infections.[5] It is currently on market by Johnson & Johnson and is a beta-lactam that belongs to the subgroup of carbapenems. Initially, it was launched in 2005 by the Shionogi Company of Japan under the brand name Finibax. Quinagolide (Norprolac, **1.2**), is a selective, dopamine receptor agonist that is used for the treatment of elevated levels of peptide hormone prolactin.[6] JNJ-26990990 (**1.3**), a primary sulfamide used for the treatment of epileptic seizures is reported to have entered phase II clinical trials as a broad-spectrum anticonvulsant drug.[7] Macitentan (**1.4**), has currently entered phase III human clinical trials for pulmonary arterial hypertension. Famotidine (**1.5**), is a histamine-2 (H2) blocker, which is now on the market for the treatment of ulcers in the stomach.

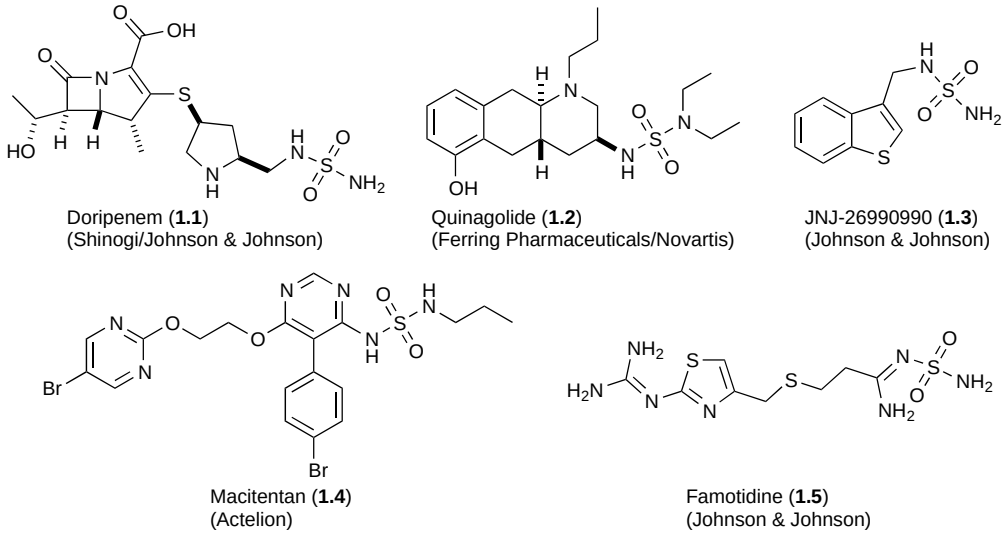
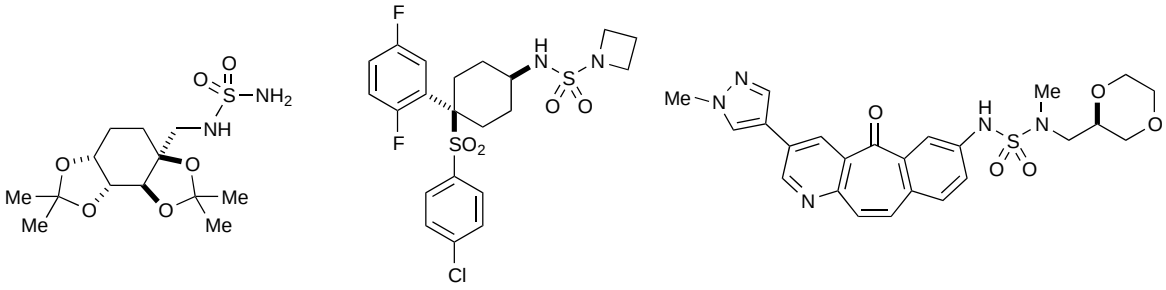


Figure 1.1. *Examples of sulfamide-containing drugs*.

Interestingly, sulfamides have also seen widespread utilization in early, and late-stage, drug discovery efforts in several therapeutic areas, such as glaucoma, cancer, obesity, epilepsy and other neurological disorders.[8] In particular, the search for therapeutics for the treatment of cancer remains an ongoing endeavor with a World Health Organization (WHO)[9] survey revealing that approximately 7.6 million people worldwide died (around 13% of all deaths) from cancer in 2008.

In this regard, several acyclic sulfamides have emerged as potential cancer drugs and are shown in Figure 1.2. Compound **1.8** has undergone clinical investigation at Merck as an orally dosed c-Met (Mesenchymal epithelial transition factor, tyrosine kinase receptor) inhibitor which inhibits the expression of hepatocyte growth factor (HGF, scatter factor).[10,1a] Aberrant activation of c-Met can increase the tumorigenicity and metastatic potential of tumor cells, so it is hypothesized that the inhibition of c-Met could suppress tumor aggressiveness and decrease the lethal disruptions to embryogenesis.[11] Recently, kinesin spindle protein (KSP) has been the focus of intense interest as a novel biological target for anticancer therapy by GlaxoSmithKline.[12] Further, biphenyl sulfamide **1.9** was found to exhibit potent inhibitory activity against kinesin spindle protein (KSP) with *in vitro* anti-proliferative activity against

human cells with mutant KSP (HCT116 D130V).[11] Sulfamide compound **1.10** was rationally designed and tested for the steroid inhibition of glucose 6-phosphate dehydrogenase (G6PD) in HEK293T cells, with good activity, and was retained with this sulfamide compound.[13] Moreover, a series of sulfamidocyclopropanecarboxylates **1.11** were discovered as potent, highly selective and orally bioavailable aggrecanase inhibitors in 2011.[14] Aggrecanases are considered as possible drug targets for the treatment of osteoarthritis, a degenerative joint disease. While other potent MMP compounds bear a hydroxamate zinc-binding group that tend to lack metabolic stability,[15] and inhibit other MMPs such as MMP-3, MMP-9, and MMP-13 in broad range selectivity panel, **1.11** has a carboxylate zinc-binding group which has good oral bioavailability and was identified as highly selective aggrecanase-2 inhibitor. It is widely admitted that a diversity of unacceptable hostile events, such as musculoskeletal disorder, that have been



1.6, Carbonic anhydrase-II (CA II) Inhibitor
Ki = 25–650 mM
(Johnson & Johnson)

1.7, g-Secretase inhibitor
IC_{50} = 0.15 ± 0.07 nM
(Merck)

1.8, MK-2461, c-Met inhibitor
c-Met IC_{50} = 2 nM
(Merck)

1.9, Kinesin spindle protein (KSP) inhibitor
HCT116D130V IC_{50} = 5.4 nM
(GlaxoSmithKline)

1.10, Glucose 6-Phosphate Dehydrogenase
HEK293T IC_{50} = 9.2 mM
(University of Manchester)

1.11, Aggrecanase Inhibitor
Agg2 IC_{50} = 8.4 nM
(Japan Tobacco Inc)

Figure 1.2. *Biologically active acyclic sulfamide compounds in early- and late-stage drug-discovery.*

clinically perceived with the use of broad spectrum MMP inhibitors arose from a lack of selectivity, and hence the identification of highly selective MMP inhibitors is greatly desired.

Cyclic sulfamides are an important class of compounds and can be found in a number of pharmaceutically useful compounds. Notably, cyclic sulfamides have been reported to be general templates suitable for the design of inhibitors against a variety of biological targets including HIV, serine proteases, γ-secretase as shown in Figure 1.3. Cyclic sulfamide compound **1.11** was developed by Merck as a γ-secretase inhibitor[16] as alternative motifs to the acyclic sulfonamide derivatives reported in 2005 for inhibiting γ-secretase.[17] Compound **1.12** is a potent and orally-bioavailable Factor Xa inhibitor.[18] Factor Xa (FXa) is a serine protease that plays a critical role in the sequence of blood coagulation cascade by catalyzing the proteolytic conversion of prothrombin to active thrombin. Compound **1.13** was discovered as a potent inhibitor of Norwalk virus for viral gastroenteritis, and displayed enhanced binding, increased aqueous solubility, and better bioavailability.[19] Fused cyclic sulfamide compound **1.14**

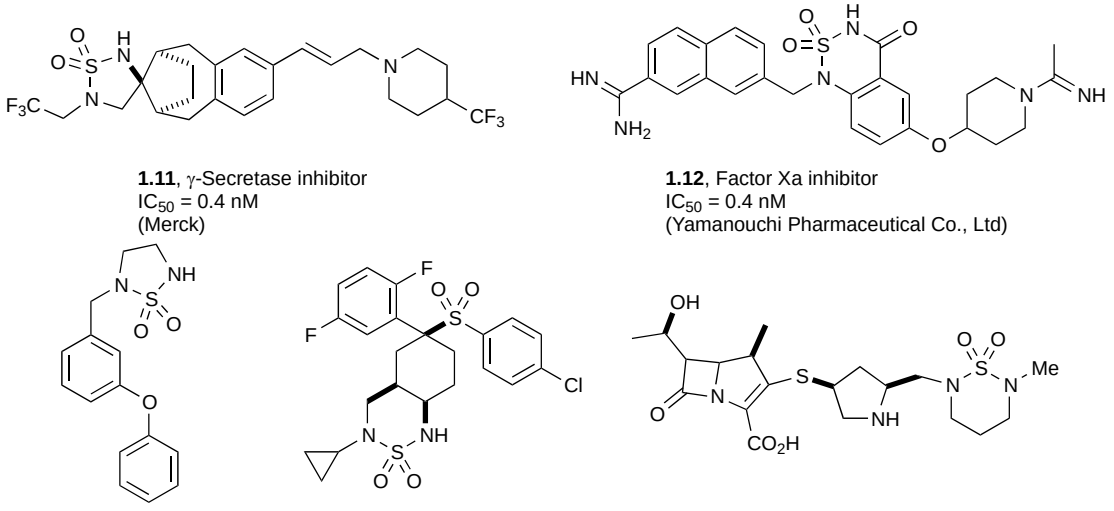


Figure 1.3. *Representative examples of cyclic sulfamide compounds in clinical discovery.*

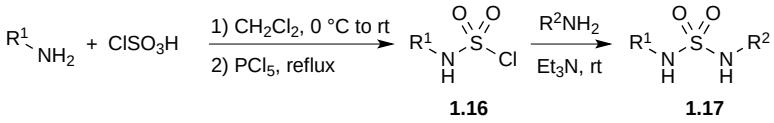
represents yet another example of γ-secretase inhibitors containing the sulfamide moiety and was developed by Merck for the treatment of Alzheimer's disease (AD).[20] The Korean Institute of Science and Technology (KIST) has reported the development of carbapenem compounds

comprising a pendant cyclic sulfamide such as in **1.15**, which was found to exhibit potent antibacterial activity.[21]

1.2. Methods for generation of acyclic sulfamides

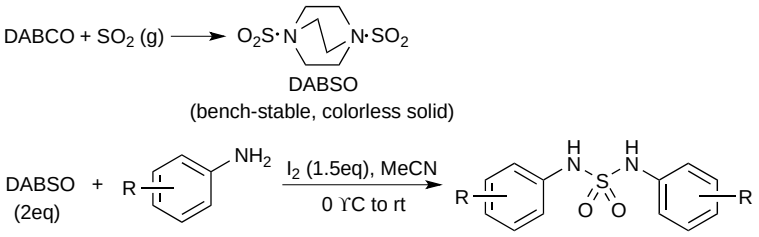
The significance of the sulfamide functional group is increasingly growing in bioactive small molecule, medicinal and supramolecular chemistry, yet surprisingly few selective synthetic methods are available for its elaboration.[22,23] In this section, several selected general as well as efficient procedures are introduced for the generation of acyclic symmetric and asymmetric sulfamides.

Leschinsky and co-workers have reported the construction of acyclic, non-symmetric substituted sulfamides as shown in Scheme 1.1. Thus, sequential treatment of primary or secondary amines with chlorosulfonic acid and PCl_5 provides the substituted chlorosulfonamides **1.16**.[24] Treatment of the chlorosulfonamide with a second amine furnishes the desired di-, tri- or tetra-substituted sulfamides **1.17**.[25]



Scheme 1.1.

Application of DABCO-*bis*(sulfur dioxide) [DABSO] as a convenient source of sulfur dioxide was reported for the preparation of sulfonamides and sulfamides (Scheme 1.2).[26] DABSO was conveniently prepared from the direct combination of DABCO and SO_2 in quantitative yield, and was reported to be a bench-stable solid reagent. Treatment of two equivalents of DABSO with anilines and iodine allowed for the preparation of *N,N'*-diarylsulfamide derivatives, in moderate yields.[27]



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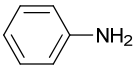
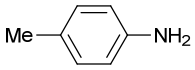
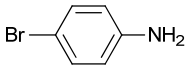
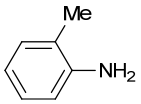
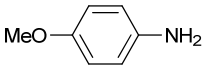
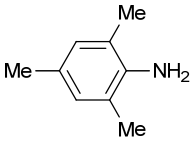
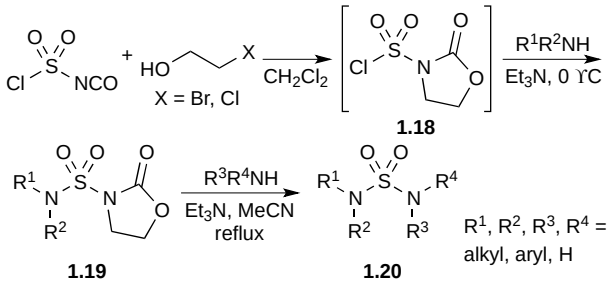
1		63%	4		65%
2		73%	5		51%
3		60%	6		52%

Scheme 1.2. *Preparation of symmetric sulfamide using DABSO.*

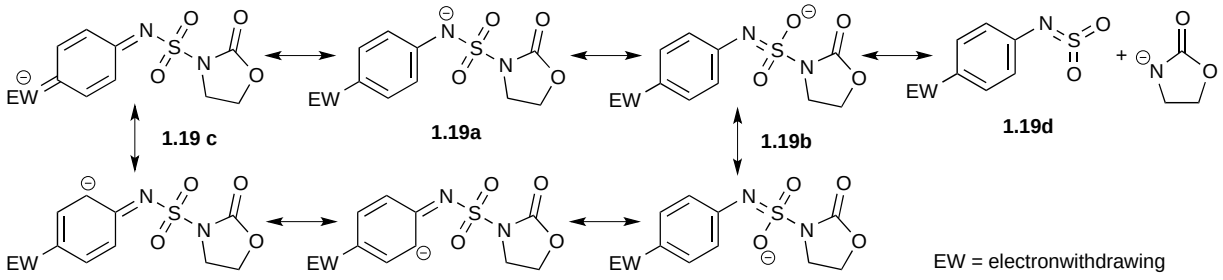
Nonhazardous sulfamide derivatives such as **1.19** have also been reported for the synthesis of non-symmetric sulfamide **1.20** (Scheme 1.3).[28] Chlorosulfonylisocyanate (CSI) was treated with 2-bromo or 2-chloroethanol to furnish the *N*-sulfamoyloxazolidinoes **1.18**. Addition of primary amine to the *in situ* generated chlorosulfonyloxazolidinone **1.18**, in the presence of Et_3N, afforded asymmetric intermediate **1.19** via the intermolecular S_N2 displacement of the halide. A second addition of primary amine to the oxazolidinone **1.19**, with base in CH_3CN, afforded a variety of sulfamides **1.20** in good yields as listed in the Table within Scheme 1.3. It is noteworthy that the first amine addition has to be a primary amine, *vide infra*.



entry	R_1	R_2	R_3	R_4	Yield (%)
1	*p*-MePh	H	*i*-Pr	H	74
2	*p*-$MeSO_2$Ph	H	*i*-Pr	H	85
3	*p*-MeSPh	H	*i*-Pr	H	62
4	*p*-ClPh	H	*i*-Pr	H	84
5	Ph	H	*i*-Pr	H	87
6	*p*-$MeSO_2$Ph	H	*p*-MePh	H	68
7	*i*-Pr	H	*t*-amyl	H	73

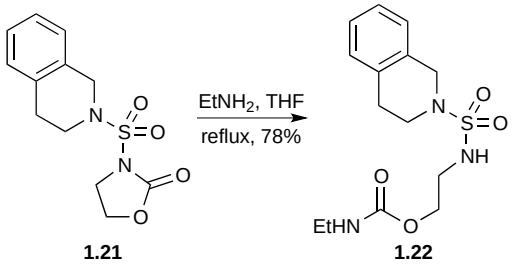
Scheme 1.3. *Preparation of ozaxolidinone and non-symmetric sulfamide.*

The postulated intermediate for the *trans*-sulfamoylation reaction is likely to involve the *N*-sulfamoylamine species **1.19d** (Scheme 1.4). This intermediate is formed via the deprotonation of the *N*-sulfamoyloxazolidinones **1.19** to generate species **1.19a**, which is stabilized through either mesomeric forms **1.19b** or **1.19c** depending on the substituents present. Presumably, only the form **1.19b** will lead to the formation of the sulfamide species via **1.19d**.



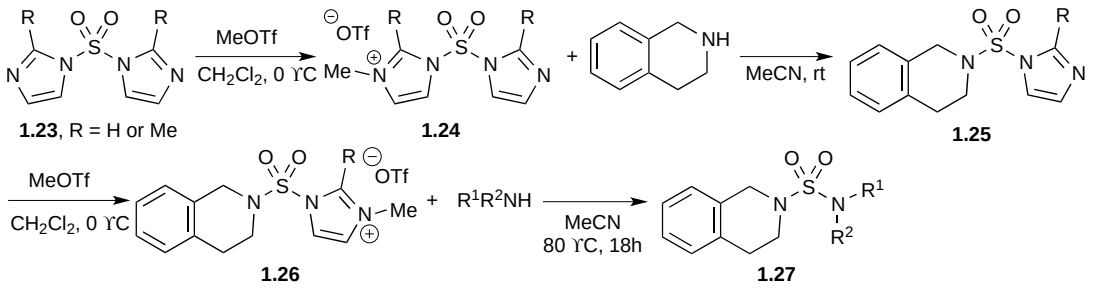
Scheme 1.4. *Resonance effect involved in the formation of **1.19d**.*

In 2003, Burns and coworkers studied "primary amine effects" of the aforementioned *trans*-sulfamoylation reaction[29] and reported that when a secondary cyclic amine such as 1,2,3,4-tetrahydroisoquinalone was attached to a sulfamide, the oxazolidin-2-one group was not displaced by a primary amine, but rather resulted in ring-opening of the oxazolidinone ring to furnish sulfamide **1.22** (Scheme 1.5).



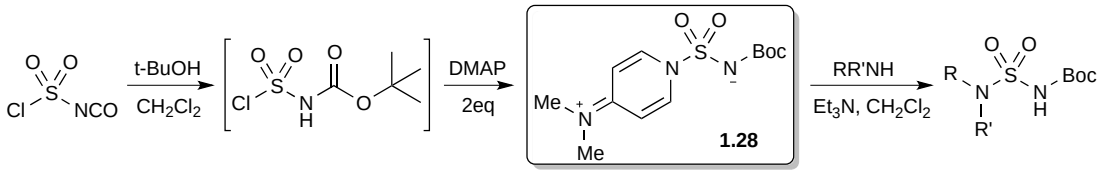
Scheme 1.5.

Burns and coworkers further investigated alternatives to the oxazolidin-2-one moiety for the preparation of non-symmetric sulfamides.[29] Immidazolium salts are known to be a superior leaving groups for the synthesis of sulfamides.[30] In this regard, *N,N'*-sulfuryldiimidazole **1.23** was prepared by reacting an excess of imidazole with sulfuryl chloride.[31] The *N,N'*-sulfuryldiimidazoles were then allowed to undergo sequential and selective monoalkylation, followed by subsequent displacement for the preparation of a variety of sulfonylureas, including both sterically-crowded and electronically-deactivated amines. Thus, alkylation of **1.23** was carried out utilizing MeOTf in CH_2Cl_2 resulting in salt **1.24**. Precipitation and filtration of the imidazolium group of salt **1.24**, and treatment with an amine generated the corresponding imidazoylsulfonylurea **1.25**. A second addition of MeOTf in CH_2Cl_2 generated salt **1.26**, which was heated to 80 °C in CH_3CN in the presence of a primary or secondary amine, to convert the triflate salt **1.26** to the desired sulfamide product **1.27** in moderate to good yield (Scheme 1.6).



Scheme 1.6. *Immidazolium salts for the synthesis of a non-symmetric sulfamides.*

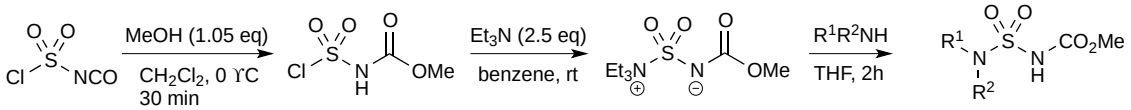
In 2001, Montero and coworkers reported the introduction of a new method for the synthesis of non-symmetric sulfamides utilizing Burgess-type reagents (Scheme 1.7**)**. [32] Treatment of chlorosulfonyl isocyanate (CSI) with *tert*-BuOH in CH_2Cl_2 afforded *N*-(*tert*-butoxycarbonyl)-*N*-[4-(dimethylazaniumylidene)-1,4-dihydropyridin-1-ylsulfonyl]-azanide **1.28** as a colorless crystal, which was non-moisture sensitive and stable at ambient temperature in good yield and which exists in the zwitterionic form similar to the Burgess reagent.[33]

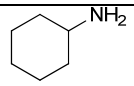
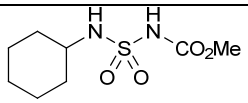
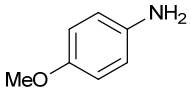
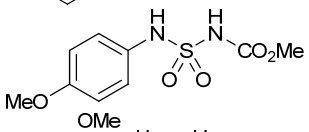
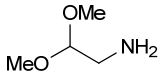
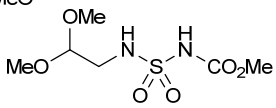
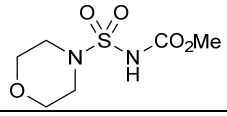


Scheme 1.7. *Sulfamoylating agent: N-(tert-Butoxycarbonyl)-N-[4-(dimethylazaniumylidene)-1,4-dihydropyridin-1-ylsulfonyl] azanide.*

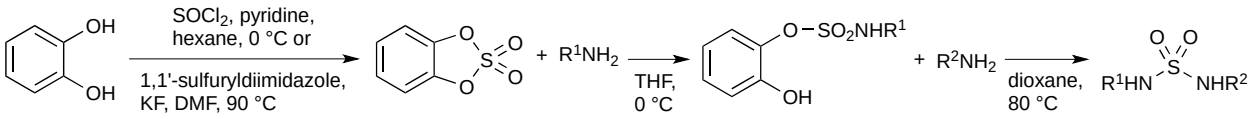
Similarly, K. C. Nicolaou and coworkers explored the synthesis of non-symmetric, linear sulfamides from primary and secondary amines (Scheme 1.8).[34] A Burgess reagent could be generated appropriately by the treatment of chlorosulfonyl isocyanate with an alcohol of interest and exposing to Et_3N at 0 ºC. Reactions of the Burgess reagent with starting amines furnished several linear sulfamides in high yields. This Burgess reagent provides a mild alternative, avoiding direct use of toxic and corrosive agents which contain traces of acid, such as HCl, making them incapable of associating with acid-sensitive functionality (Entry 3).[35]

Scheme 1.8. *Synthesis of linear sulfamides.*



Entry	Amine	Product	Yield (%)
1			83
2			97
3			98
4			87

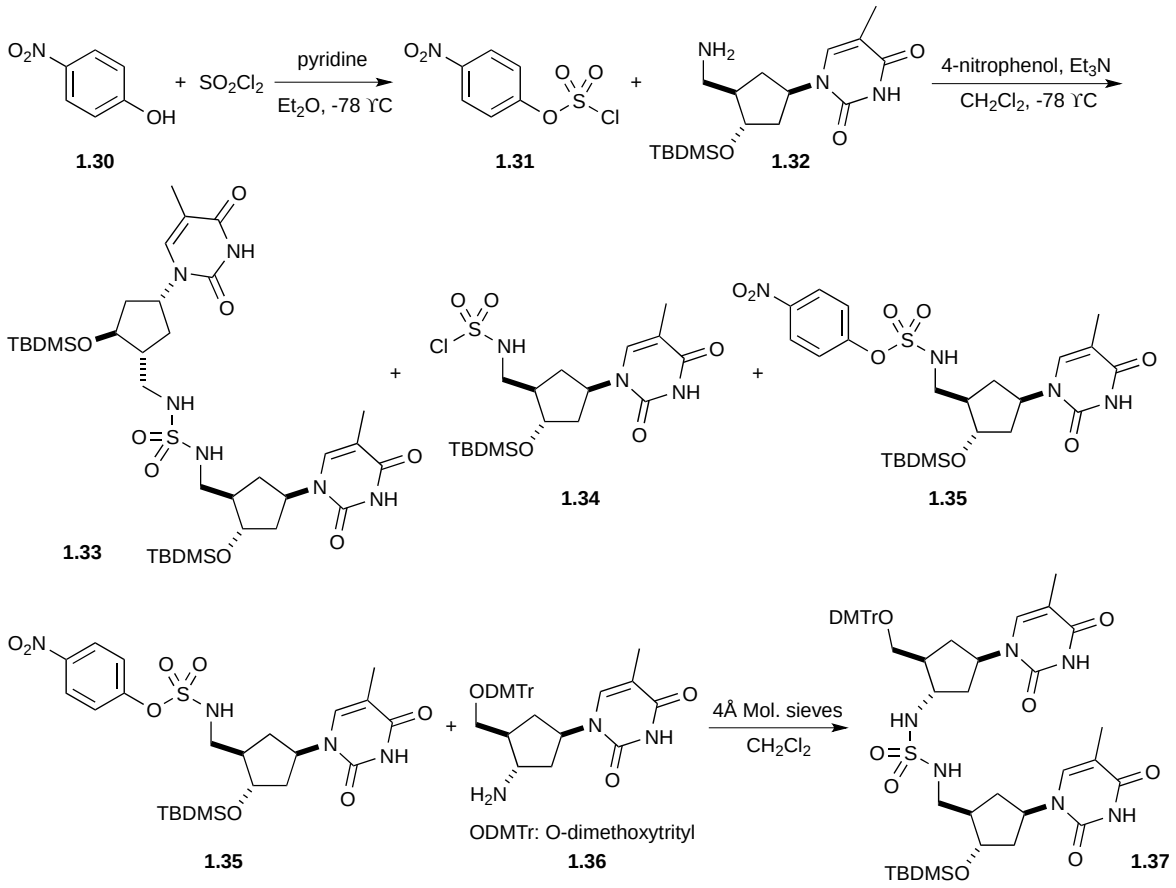
Catechol-derived cyclic sulfates **1.29** have been established as a useful intermediate in the synthesis of sulfamide compounds.[36] Until the mid-1990s, the procedures for the preparation of catechol sulfates suffered from low yield and lack of general applicability.[37] In 1994, the Tickner group reported a high yielding and efficient synthesis of catechol cyclic sulfate (Scheme 1.9),[38] which is readily prepared by reacting the catechol component with 1,1'-sulfurylimidazole in the presence of KF in DMF at 85–90 °C. There are several advantages to this method. Firstly, the use of 1,1'-sulfurylimidazole avoids the competing ring chlorination which often occurs when sulfuryl chloride is employed. Secondly, since this reaction is carried out under neutral conditions the potential oxidation of the starting catechol is circumvented. Potassium fluoride serves as an effective non-nucleophilic base which is tolerated by most functionalities.



catechol — catechol sulfate (**1.29**)

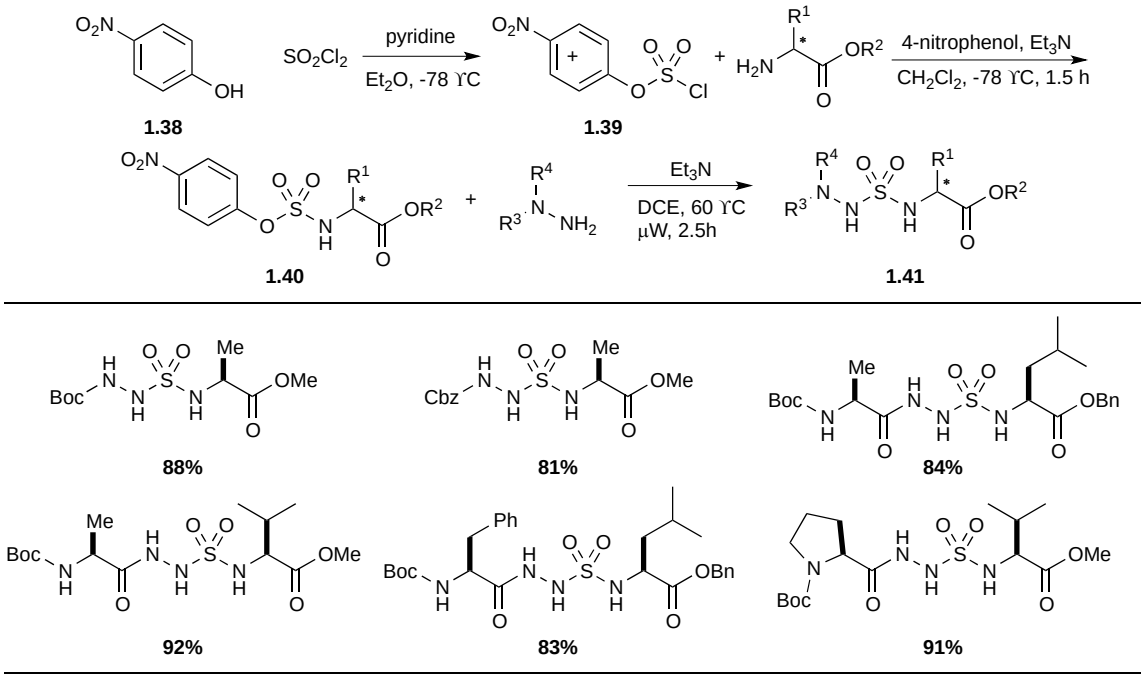
Scheme 1.9. *Synthesis of catechol sulfate and asymmetric linear sulfamides.*

Fettes and co-workers have reported the preparation of 4-nitrophenyl chlorosulfates for the preparation of non-symmetric sulfamides (Scheme 1.10).[39] Addition of sulfuryl chloride to a solution of 4-nitrophenol **1.30** and pyridine in Et_2O at -78 °C for 4 h afforded 4-nitrophenyl chlorosulfate **1.31** in 83% overall yield as a stable crystalline solid. **1.31** was then reacted with amine **1.32** at room temperature or -78 °C to afford the symmetrical sulfamide **1.33** as the major product and 4-nitrophenyl sulfamate **1.35** as a minor product, with none of the sulfamoyl chloride **1.34** being isolated. The mechanism of nucleophilic substitution reaction of **1.31** includes nucleophilic attack at sulfur with either S-Cl or S-OAr bond scission with the S-OAr bond cleavage being the major reaction pathway.[40] The authors note that if the major pathway is the S-OAr bond cleavage, the more active sulfamoyl chloride **1.34** is generated *in situ* and reacts with the amine **1.32** to give the unwanted dimerized compound **1.33**. Thus the less active 4-nitrophenyl sulfamate **1.35** is probably derived via the S-Cl bond cleavage reaction pathway. To avoid the generation of unwanted symmetrical dimer **1.33**, an excess of 4-nitrophenol and Et_3N were added and 4-nitrophenyl sulfamoyl chloride **1.34** could be prepared in 68% yield. 4-nitrophenyl sulfamide **1.35** was then treated with secondary amine **1.36** in CH_2Cl_2 to afford asymmetric sulfamide **1.37** in 83% yield.



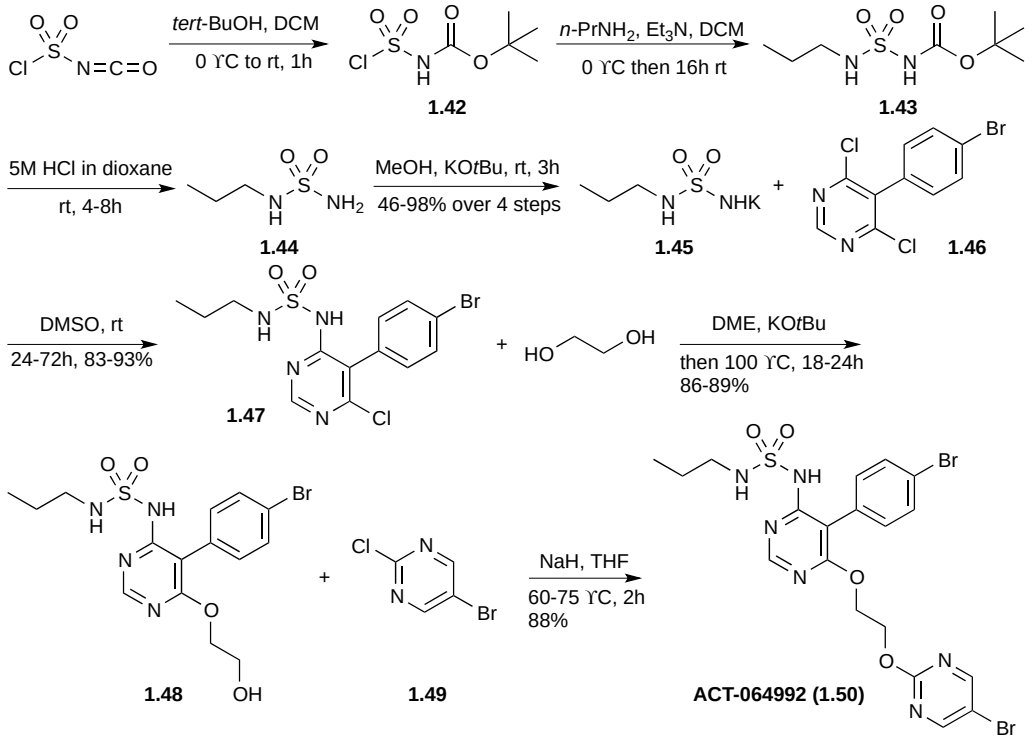
Scheme 1.10.

Recently, the Lubell group has reported an effective method to generate *N*-aminosulfamide using *p*-nitrophenylsulfamidate esters (Scheme 1.11).[41] This method entailed the reaction of 4-nitrophenol **1.38**, sulfuryl chloride, and pyridine in Et_2O at -78 °C[39,42] to afford the desired product as a crystalline and relatively stable solid that can be stored for several month under an inert gas. It must be noted that in order to prepare sulfamate **1.40**, 2 equiv. of 4-nitrophenol was required to avoid the formation of symmetric sulfamide as mentioned before. It is also noteworthy that microwave irradiation improved the formation of *N*-aminosulfamides **1.41** to more than 80% yield as compared to 36% yield with conventional heating at reflux for 24 h.



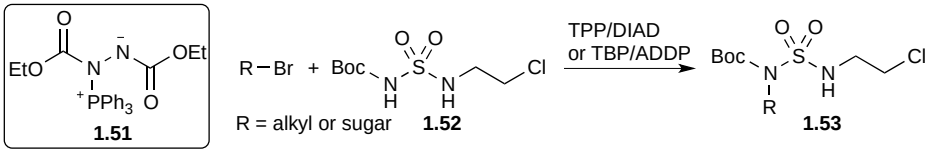
Scheme 1.11. *Synthesis of p-nitrophenylsulfamidate esters and N-aminosulfamides.*

The Bolli group reported a new method to generate a sulfamide compound ACT-064992, (macitentan, **1.50**) as an orally active, potent dual endothelin receptor antagonist for regulating blood pressure (Scheme 1.12).[43] The procedure starts by reacting chlorosulfonyl isocyanate (CSI) with *t*-BuOH to provide the Boc-protected amino-sulfonyl chloride **1.42**, which was subsequently added to *n*-propylamine to furnish **1.43**. Boc-deprotection using HCl in CH_2Cl_2 solution afforded **1.44**. Generation of potassium salt **1.45** and addition of pyrimidine **1.46** allowed the preparation of the desired sulfamide **1.47** via a nucleophilic aromatic substitution (S_NAr). Introduction of an ethylene glycol side-chain via a second S_NAr reaction furnished the corresponding alcohol **1.48**. Attachment of 2-chloropyrimidine **1.49** afforded the final product **1.50** in 88 % yield.



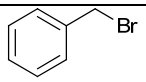
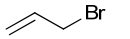
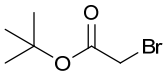
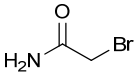
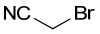
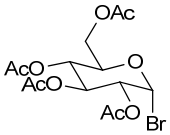
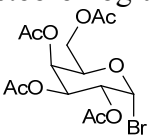
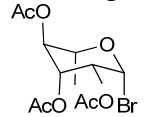
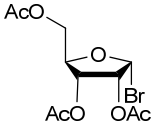
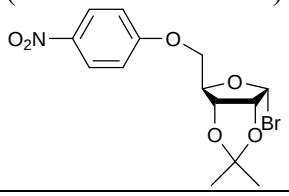
Scheme 1.12. *Synthesis of sulfamide potassium salt and general route for the preparation of ACT-064992 (**1.50**).*

In 2001, Montero reported an efficient *N*-alkylation method for the generation of unsymmetric sulfamide using an alkyl bromide and a Mitsunobu betaine (Scheme 1.13).[44] In this reaction, the Mitsunobu betaine intermediate **1.51** is produced *in situ* from PPh_3 and an azodicarboxylate, which performs the role of a base to deprotonate the sulfamoyl carbamate NH. This reaction was employed in alkylation as well as glycosylation reactions utilizing two redox couples; (a) triphenylphosphine (TPP) and diisopropylazodicarboxylate (DIAD) and (b) tri-*n*-butylphosphine (TBP) and 1,1'-(azodicarbonyl)-dipiperidine (ADDP), and the results are highlighted in Table 1.1.



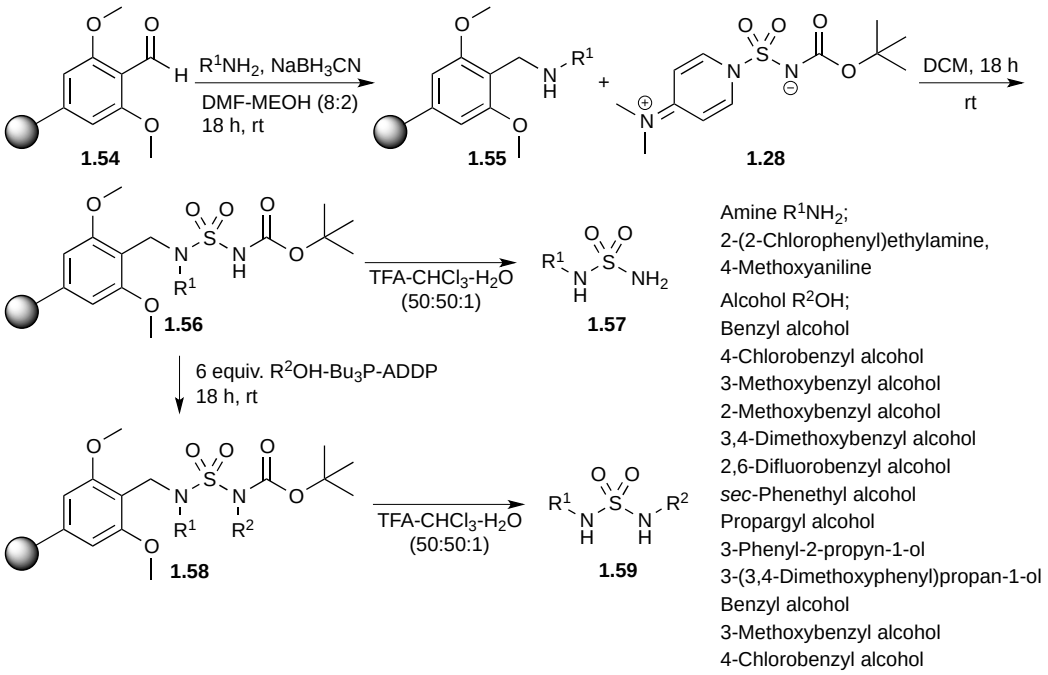
Scheme 1.13. *N-alkylation method reported by Montero.*[44]

Table 1.1. *Reaction of **1.52** with alkyl bromide under Mitsunobu conditions using two different redox couples.*

entry	R-Br	TPP/DIAD	TBP/ADDP
1		37%	86%
2		41%	88%
3		37%	60%
4		20%	45%
5		32%	56%
6	 (α-acetobromoglucose)	35% β-anomer	60% β-anomer
7	 (α-acetobromogalactose)	23% β-anomer	44% β-anomer
8	 (α-acetobromorhamnose)	10% β-anomer	45% β-anomer
9	 (α-acetobromoribose)	21% β-anomer	42% β-anomer
10		20% β-anomer	40% β-anomer

The Vidal group reported the generation of mono- and di-substituted acyclic sulfamides using solid-support resins and sulfamoylating agent **1.28** (Scheme 1.14).[45] Montero and co-workers have described the preparation of a sulfamoylating agent **1.28** and its reactivity with various amines (*vide infra*).[32] Vidal and coworkers employed the Burgess Type reagent **1.28**

with polystyrene (PS)-supported benzylamine amine **1.55** to prepare Boc-substituted sulfamide **1.56** by the reaction of excess of sulfamoylating agent **1.28** (3 equiv.) and PS-benzyl amine **1.55** in DMF-CH_2Cl_2 at room temperature. Utilization of TFA allowed simultaneous deprotection and cleavage from the resin to provide sulfamide **1.57**. On the other hand, Mitsunobu alkylation of **1.56** and subsequent cleavage in TFA-$CHCl_3$-H_2O (50:50:1) afforded the non-symmetric sulfamide **1.59**.



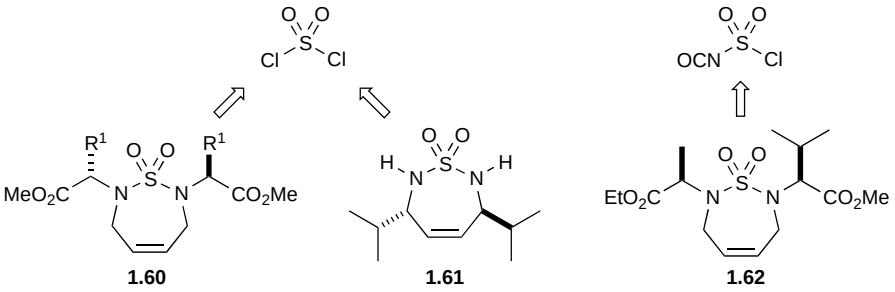
Scheme 1.14. *Synthesis of acyclic sulfamides using solid-support resins and **1.28**.*

1.3 Methods to generate cyclic sulfamide

Exploratory studies related to the design and synthesis of functionalized cyclic sulfamides have been achieved for the invention of pharmaceutical compounds such as HIV protease inhibitors, virus inhibitor, and diabetes treatment.[46] In this section, various methods for the generation of cyclic sulfamides are summarized.

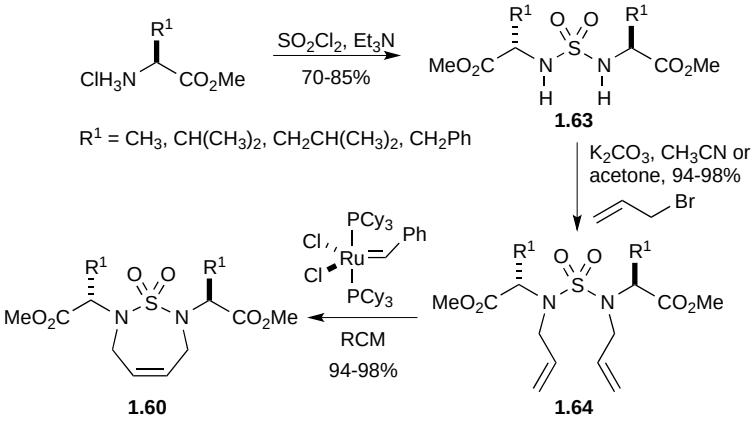
In 2000, Hanson and coworkers reported new methods employing ring-closing metathesis (RCM) to generate C_2-symmetric sulfamides **1.60** and **1.61** and the asymmetric cyclic sulfamide

1.62 starting from from sulfuryl chloride (SO_2Cl_2) and chlorosulfonyl isocyanate ($OCNSO_2Cl$) (Scheme 1.15).[47]



Scheme 1.15.

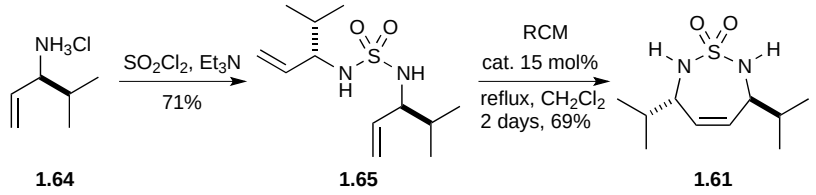
The synthetic route to the amino acid-derived C_2-symmetric sulfamides is outlined in Scheme 1.16. Condensation of amino ester with sulfuryl chloride generates sulfamide **1.63** in 70–85% yield. Diallylation to the sulfamide, and RCM using the first-generation Grubbs catalyst (**G-I**),[48] subsequently afforded the C_2-symmetric cyclic sulfamide **1.60** in excellent yields.



Scheme 1.16. *Synthesis of C_2-symmetric cyclic sulfamide.*

Next a route to afford the substituted, C_2-symmetric sulfamide **1.61** is described in Scheme 1.17. In this method, amine **1.64** obtained from amino ester via Swern oxidation and Wittig reaction, was coupled with sulfuryl chloride to furnish sulfamide **1.65** in 71% yield.

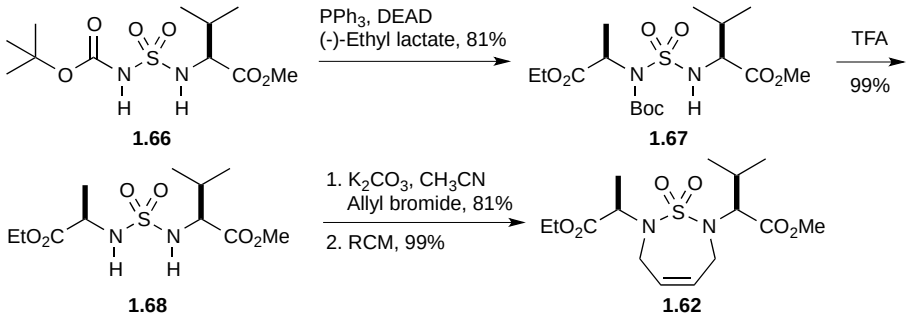
Subsequent RCM using 15 mol% of the **G-I** catalyst generated the desired sulfamide **1.61** in 69% yield.



1.64 **1.65** **1.61**

Scheme 1.17. *Synthesis of C_2-symmetric cyclic sulfamide.*

The Hanson group also developed a strategy to the unsymmetric cyclic sulfamide utilizing CSI chemistry as outlined in Scheme 1.18. Starting substrate **1.66** was obtained by reacting chlorosulfonyl isocyanate (CSI), *t*-BuOH and an amino ester, and was subsequently utilized in a regioselective Mitsunobu reaction and deprotection to afford unsymmetric intermediate **1.68**. Subsequent diallylation and RCM produced the unsymmetric cyclic sulfamide **1.62** in excellent yield.

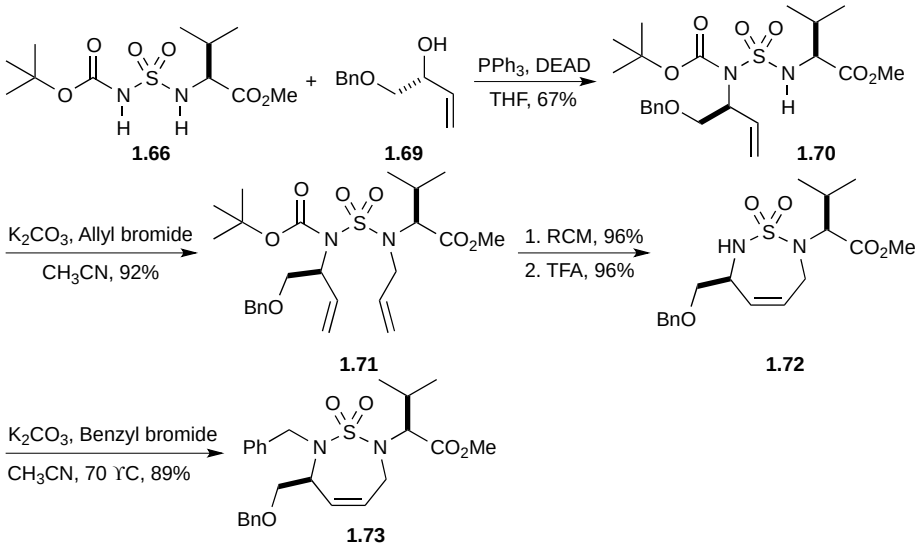


1.66 **1.67** **1.68** **1.62**

Scheme 1.18. *Synthesis of unsymmetric cyclic sulfamide.*

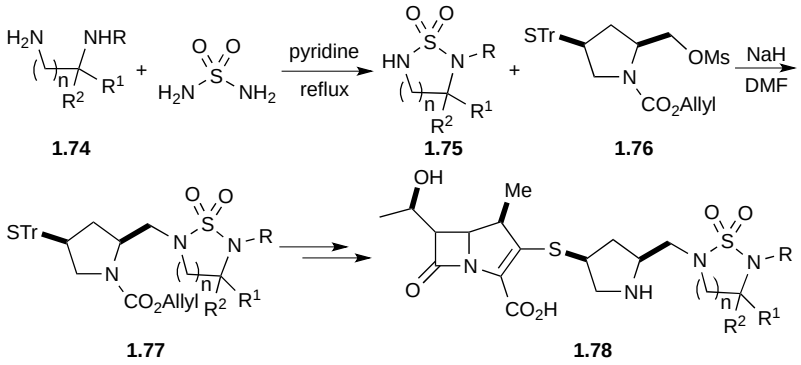
In 2003, Hanson and coworkers reported the synthesis of tri- and tetra-substituted non-symmetric cyclic sulfamide compounds (Scheme 1.19).[49] In this strategy they employed the Mitsunobu reaction to install a stereogenic center using the chiral, non-racemic secondary allyl alcohol **1.69** to produce sulfamide **1.70** in good yield. Allylation followed by RCM using the

second-generation Grubbs catalyst (**G-II**),[48] and Boc-deprotection produced cyclic sulfamide **1.72**. Benzylation afforded the desired trisubstituted cyclic sulfamide **1.73** in excellent yield.



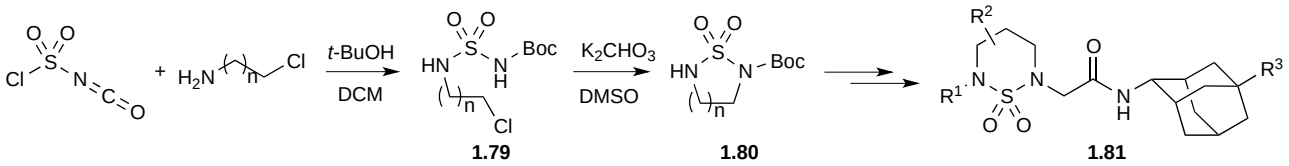
Scheme 1.19. *Synthesis of tri- and tetra-substituted non-symmetric cyclic sulfamide compounds.*

In 2009, Oh and co-workers reported the preparation of substituted cyclic sulfamides **1.75** via the condensation of the corresponding diamines **1.74** with sulfamide in refluxing pyridine (Scheme 1.20).[21, 50] Their method was then employed for the preparation of 1β-methylcarbapenems **1.78** which possess excellent *in vitro* antibacterial activity.



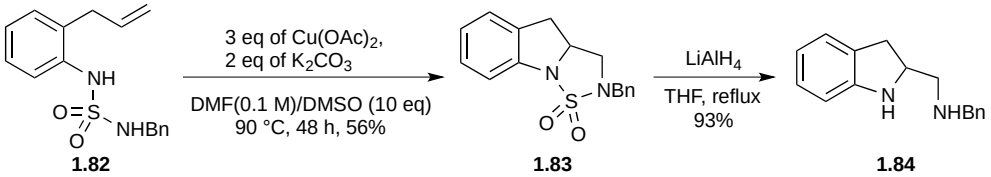
Scheme 1.20. *Synthesis of a substituted cyclic sulfamide.*

Ahn and co-workers have described a component coupling reaction for the synthesis cyclic sulfamide **1.81** as shown in Scheme 1.21.[46b] Intermediate **1.79** was synthesized via sequential addition of *t*-BuOH and the corresponding mustards to chlorosulfonyl isocyanate (CSI) in CH_2Cl_2 at 0 °C. The *N*-Boc cyclic sulfamide **1.80** was then obtained simply by the treatment of the intermediate **1.79** with K_2CO_3 in DMSO.



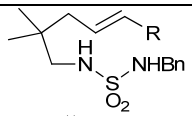
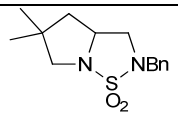
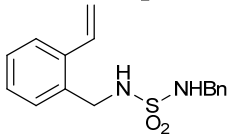
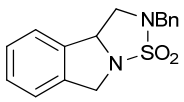
Scheme 1.21. *Synthesis of cyclic sulfamide from chlorosulfonyl isocyanate (CSI).*

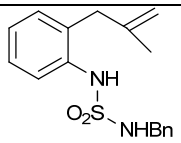
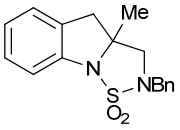
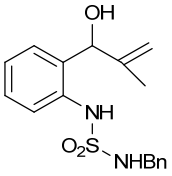
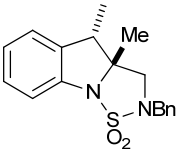
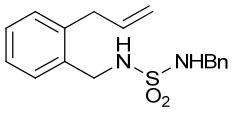
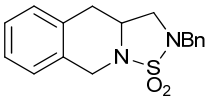
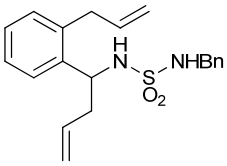
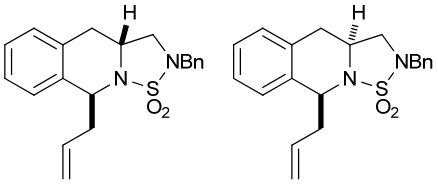
In 2005, Chemler and coworkers reported $Cu(OAc)_2$ as an excellent promoter for the intramolecular diamination of inactivated olefins which have the sulfamide moiety (Scheme 1.22).[51] Acyclic sulfamide **1.82** was treated with $Cu(OAc)_2$ (1.2 eq) in the presence of K_2CO_3 as a base at high temperature (90 °C) to provide the desired cyclic sulfamide **1.83** in up to 92%. Free diamine **1.84** can be furnished by the reduction of the sulfamide **1.83** with $LiAlH_4$ in THF under refluxing conditions in 93% yield.



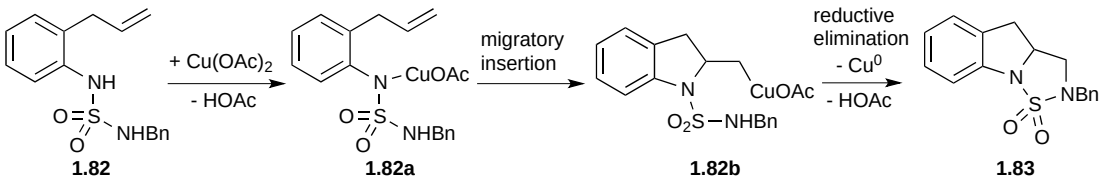
Scheme 1.22. *Intramolecular diamination of olefins.*

Table 1.2.

entry	substrate	product	Yield (%)
1			73
2			56

3			57
4			71
		Selectivity = > 20 : 1	
5			83
6			34
		Selectivity = 1.2 : 1	

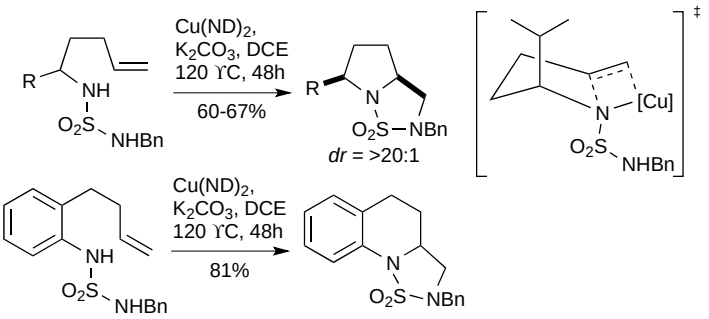
The proposed mechanism suggests that intramolecular diamination is likely initiated by the engagement of $Cu(OAc)_2$ to sulfamide nitrogen to deliver intermediate **1.82a** (Scheme 1.23). Migratory insertion allows formation of the new sp^3 N-C bond to furnish the intermediate **1.82b**. The organocopper species **1.82b** is then suggested to undergo ligand exchange with the remaining nitrogen, followed by reductive elimination to afford cyclic sulfamide **1.83**.



Scheme 1.23. *Mechanism of diamination*

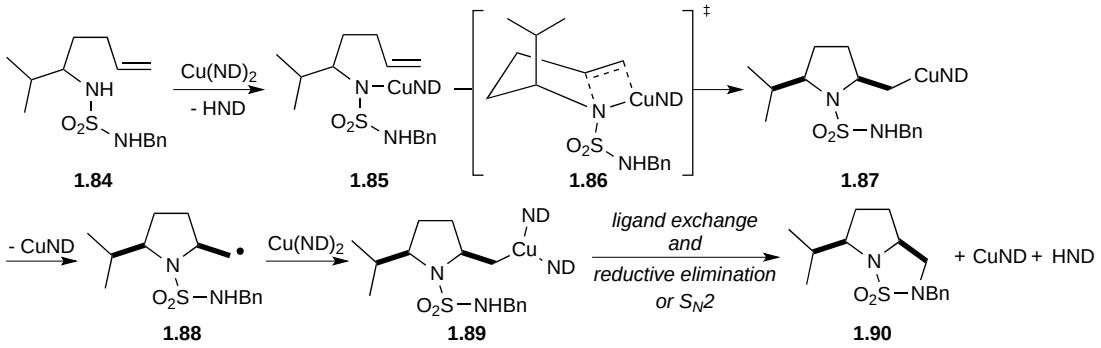
In 2007, the Chemler group expanded the intramolecular diamination using copper (II) carboxylate for the synthesis of cyclic sulfamides (Scheme 1.24).[52] The organic soluble copper (II) neodecanoate [$Cu(ND)_2$] allowed for shorter reaction times (90 °C, 24 h) alongside more general organic solvents (DCE, toluene) under the refluxing conditions. A notable development

in this regard was the use of microwave heating (120 °C for 20 min) to further reduce reaction times.



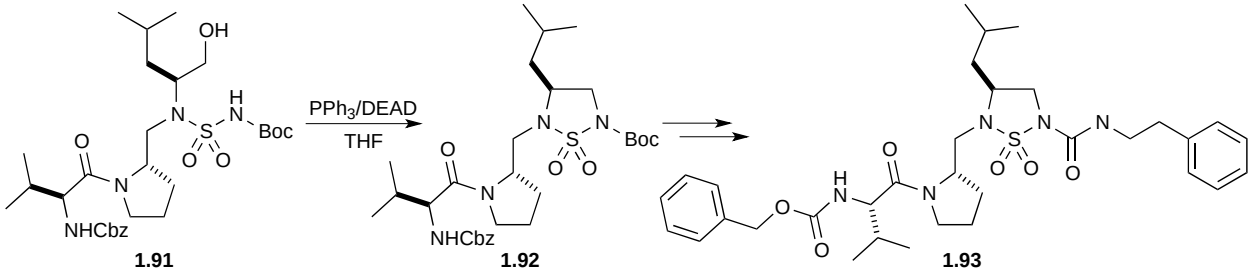
Scheme 1.24. Intramolecular diamination

The reaction mechanism for the copper (II) carboxylate-promoted intramolecular diamination is proposed in Scheme 1.25. Ligand exchange of **1.84** with $Cu(ND)_2$ generates the N–Cu bond, followed by *syn*-aminocupration via transition state **1.86**, to stereoselectively generate *cis*-pyrrolidine **1.87**. The organocopper (II) intermediate **1.87** generates primary radical **1.88** via C–N bond homolysis. Since it is necessary to lose another electron from the substrate, copper needs to be involved in the second C–N bond forming process. The resulting intermediate **1.89** then undergoes ligand exchange and reductive elimination or S_N2 to afford the cyclized unsymmetric sulfamide compound **1.90**.



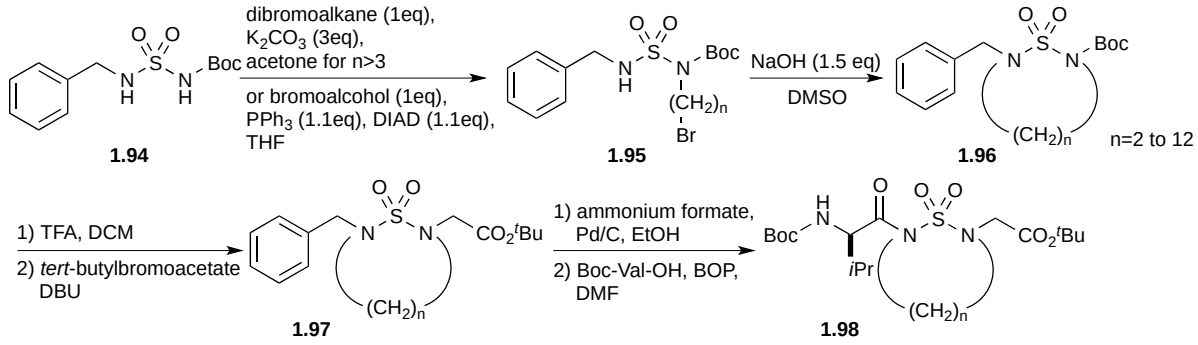
Scheme 1.25. *Proposed diamination mechanism.*

In 2004, the Groutas group explored the synthesis of cyclic sulfamides for the generation of potential inhibitors of human leukocyte elastase (HLE) (Scheme 1.26).[53] Primary alcohol **1.91** was formed via the reduction of the corresponding amino ester and subsequently utilized in an intramolecular Mitsunobu reaction to furnish a cyclized sulfamide **1.93**.



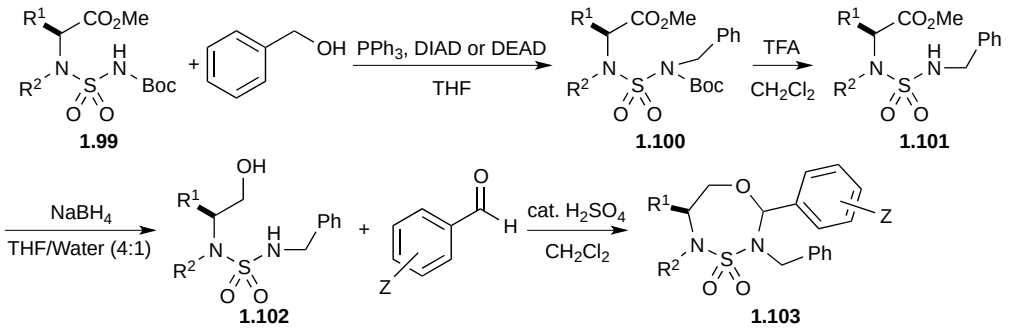
Scheme 1.26. *Synthesis of the clinical potential HLE inhibitor **1.93**.*

In 2003, the Montero group reported a two-step protocol starting from *N*-benzyl-*N*'-*tert*-buthoxycarbonylsulfamide **1.94** to generate cyclic sulfamides **1.96** (Scheme 1.27).[54] Thus, regioselective *N*-alkylation of **1.94** was carried out using dibromoalkanes in the presence of K_2CO_3 in acetone to obtain **1.95** in moderate to good yield for n>3. Alternatively, bromo-alcohols were utilized in a Mitsunobu alkylation reaction for the preparation of **1.95**. Subjection of **1.95** to basic conditions under reflux furnished a variety of cyclic sulfamides **1.96**. Boc-deprotection and the coupling with *t*-BuOH bromoacetate in the presence of DBU gave **1.97**. Hydrogenation and peptidic coupling using BOP with *N*-Boc-protected valine, generated sulfamide **1.98** in good yield.



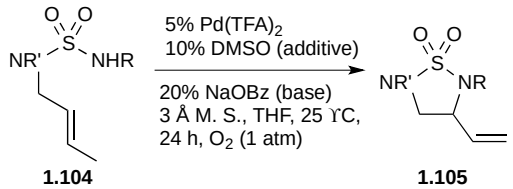
Scheme 1.27. *General synthesis of n-membered cyclic sulfamide.*

A new heterocyclic class of cyclic sulfamides, 1,4,3,5-oxathiadiazepane-4,4-dioxanes were reported in 2012 as potential analogs of anti-HIV compounds (Scheme 1.28).[55] The key reaction for the preparation of the cyclic sulfamide **1.103** was the condensation of hydroxysulfamide **1.102** with aldehydes.[56] Sulfamoylation of amino acid methyl ester generated compound **1.99** which was then allowed to undergo a Mitsunobu alkylation with benzyl alcohol to afford **1.100**. Subsequent deprotection of the Boc group using TFA, followed by reduction of the ester using $NaBH_4$, furnished **1.102**. Hydroxysulfamide **1.102** was subjected to a cyclodehydration reaction by the treatment of a variety of substituted aromatic aldehydes in CH_2Cl_2 to afford cyclic sulfamide **1.103**.



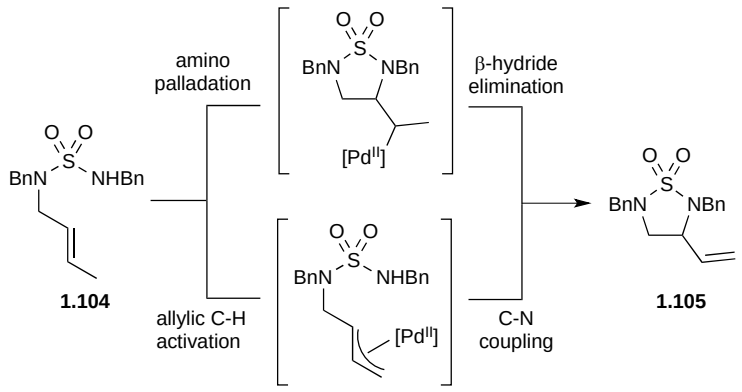
Scheme 1.28. *Synthesis of substituted amino alcohol sulfamides.*

In 2010, Stahl and co-workers reported the utilization of Pd-catalyzed hydroamination of allylic sulfamides **1.104** for the synthesis of cyclic sulfamides **1.105** as shown in Scheme 1.29.[57] Treatment of the allyl sulfamide with $Pd(TFA)_2$ in the presence of sodium benzoate, catalytic DMSO and molecular oxygen allowed for an oxidative cyclization of allylic sulfamide to generate the desired cyclic sulfamide.



Scheme 1.29. *Aerobic oxidative cyclization of sulfamide.*

There are two different possible mechanisms to explain this oxidative cyclization reaction (Scheme 1.30): (1) aminopalladation of the alkene followed by the β–hydride elimination or (2) formation of a π-allyl-palladium (II) intermediate via allylic C-H activation followed by the C-N coupling.



Scheme 1.30. *Possible mechanism for the palladium-catalyzed oxidative cyclization reaction.*

Ligand **1.106** and **1.107** which are known to facilitate allylic C-H activation were tested but only low yields of sulfamide products were observed (Figure 1.4). A further study to distinguish these two mechanisms was carried out, whereby the homoallyl amine derivative **1.108** was synthesized. Cyclization of this substrate would provide evidence in favor of an allylic C–H activation pathway. However, treating this substrate **1.108** under the optimized cyclization reaction conditions resulted in complete recovery of starting material after 24 hrs. Thus, it can be concluded that cyclization via allylic C–H activation does not occur.

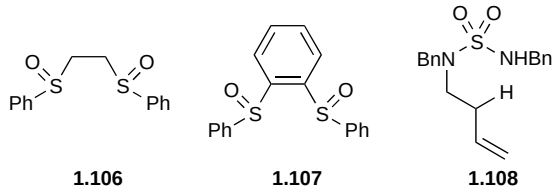
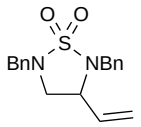
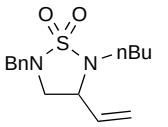
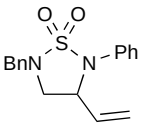
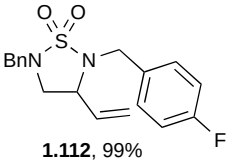
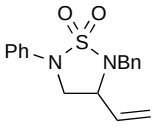
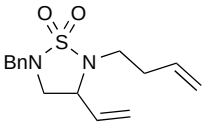
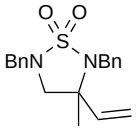
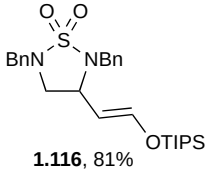
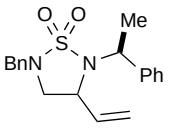
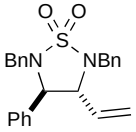
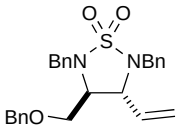
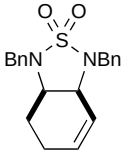


Figure 1.4. *Ligands and homoallyl amine derivative.*

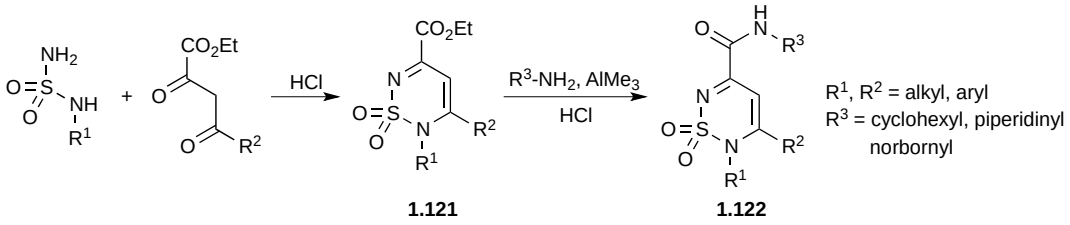
Employment of the optimized reaction conditions with a variety of sulfamides afforded an array of cyclic sulfamides in good to excellent yields (Table 1.3). Substrates bearing both

aliphatic or aryl *N*-substituents were found to undergo cyclization efficiently (Table 1.3, **1.109–1.114**). Quaternary C–N bond formation (**1.115**) stemming from the use of a tri-substituted alkene was found to occur in quantitative yield while employment of a silyloxy allyl amine furnished the corresponding silyloxy vinyl ether **1.116**. Allylic substituents larger than a methyl group delivered diastereomeric product in good to high yield (**1.118–1.120**, diastereomeric ratios > 29:1).

Table 1.3. *Aerobic oxidative cyclization of sulfamide.*

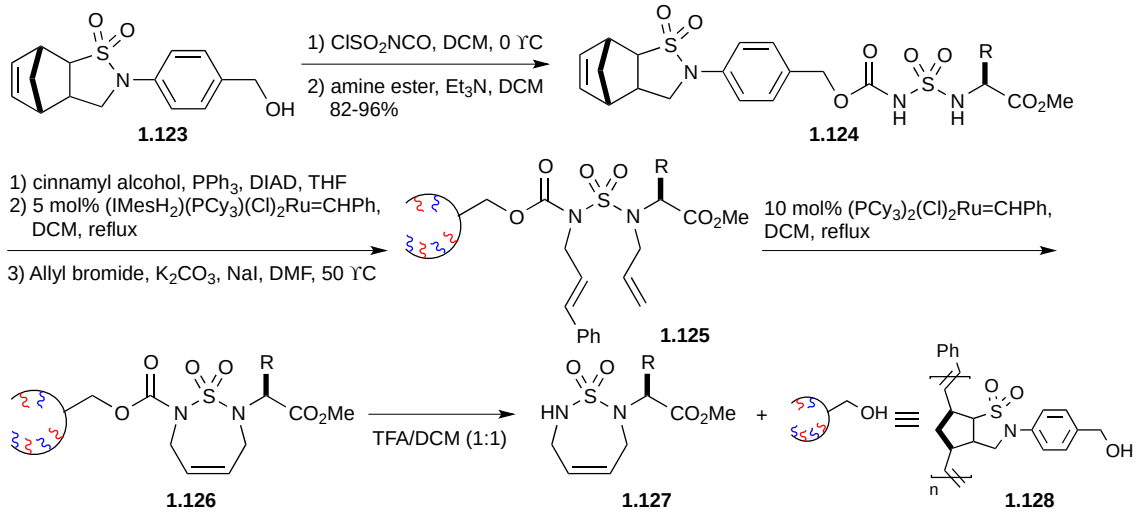
 **1.109**, 99%	 **1.110**, 99%	 **1.111**, 97%	 **1.112**, 99%
 **1.113**, 92%	 **1.114**, 99%	 **1.115**, 99%	 **1.116**, 81%
 **1.117**, 90% (d.r.= 6:1)	 **1.118**, 95% (d.r.> 30:1)	 **1.119**, 98% (d.r.> 29:1)	 **1.120**, 73% (d.r.> 30:1)

In 2007, the Girón group reported a new carboxamide series of 1,1-dioxo-1,2-dihydro-$1^{\lambda 6}$-1,2,6-thiadiazine derivatives that have a cannabinoid (psychotropic constituent)-like molecular structure (Scheme 1.31).[58] The general synthetic route for the formation of substituted 1,2,6-thiadiazine 5-carboxamides employs a cyclocondensation with mono-substituted sulfamide and 2,4-dioxocarboxylic acid ethyl ester under the acidic conditions to furnish **1.121**, and subsequent amination with an exogenous amine to afford **1.122** in high yield.



Scheme 1.31. *Synthesis of 1,1-dioxo-1,2,6-thiadiazine compounds.*

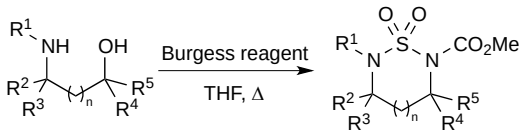
In 2003, Hanson and coworkers reported a result of the synthesis of a variety of amino acid derived unsymmetric cyclic sulfamide compounds utilizing ring-opening metathesis (ROM) polymerization-derived oligomers as soluble supports (Scheme 1.32).[59] In this method, norbornenyl-tagged sulfonamide **1.123**[60] was allowed to undergo sequential reactions with chlorosulfonyl isocyanate and amino acid methyl esters to afford the norbornenyl-tagged sulfamoyl carbamate **1.124**, which was polymerized with 5 mol% of the **G-II** catalyst to generate the soluble oligomer. Dissolving the oligomer in DMF, followed by bis-allylation (allyl bromide, NaI, K_2CO_3) furnished diene **1.125**. Upon precipitation with water, oligomer **1.125** was treated with 10 mol% of the **G-I** catalyst to afford the cyclized compound **1.126**. Cleavage by the TFA: CH_2Cl_2 (1:1) furnished unsymmetric cyclic sulfamide **1.127**. Overall, this method represents a chromatography-free method for the preparation of cyclic sulfamides using a soluble support.

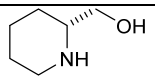
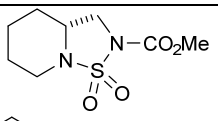
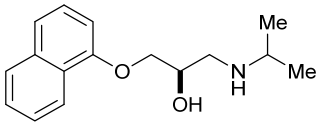
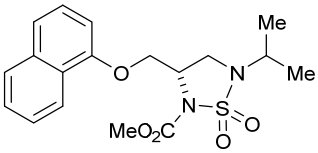
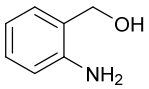
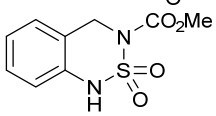
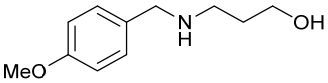
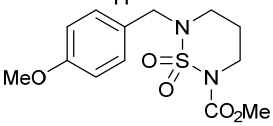
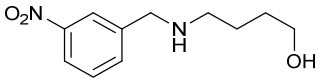
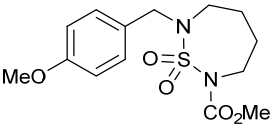
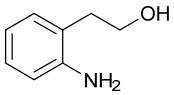
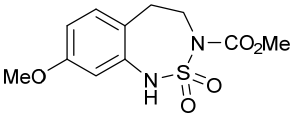
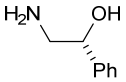
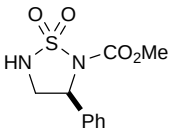
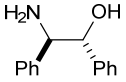
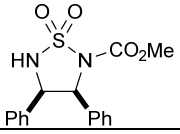


Scheme 1.32. *Synthesis of cyclic sulfamide using ring opening metathesis (ROM) oligomers*

In 2002, K. C. Nicolaou and coworkers explored the synthesis of nonsymmetric cyclic sulfamides from amino alcohols (Table 1.4).[34] In this regard, a Burgess reagent-facilitated cyclic sulfamide synthesis was reported employing primary and secondary amino alcohols under optimized condition. Initially, the reaction mixtures (1.0 equiv. of amino alcohol and 2.5 equiv. of the Burgess reagent) were heated for specified hours in THF to yield cyclic sulfamides

Table 1.4. *Synthesis of non-symmetric cyclic sulfamides from amino alcohols.*

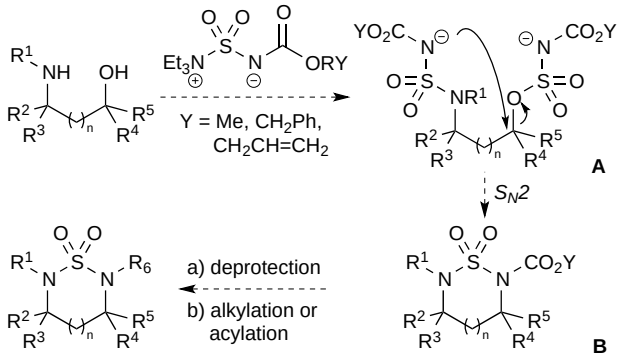


Entry	Starting amine	Product	Yield (%)
1			77[a]
2			89[b]
3			45[c]
4			93[a]
5			81[a]
6			90[a]
7			90[d]
8			76[d]

[a] THF, reflux, 2 h; [b] THF, reflux, 21 h; [c] THF, reflux, 8 h; [d] 0 ºC, 1 h, then 25 ºC, 5 h

(entries 1, 2, and 3). Non-benzylic alcohols were employed to explore a series of six-, seven-, and eight-membered ring analogues (entries 4, 5, and 6) under optimized reaction condition (THF, refluxing 2 h). Utilizing primary aliphatic amines, in conjunction with secondary benzylic alcohols, the reaction mixture was commenced at 0 ºC and allowed to warm to 25 ºC for 5 h to produce compounds in high yields (entries 7 and 8).

The proposed mechanistic conversion of amino alcohols into non-symmetric cyclic sulfamides using a Burgess-type reagent is shown in Scheme 1.33. Thus, treatment of excess amounts of the Burgess reagent with an amino alcohol leads to a mono-protected, nonsymmetric cyclic sulfamide **B** through the S_N2 reaction of the proposed intermediate **A**. Potentially, deprotection of the carbamate **B** and substitution provides an array of pharmaceutically useful sulfamides such as high-affinity protein ligands[61] and inhibitors of enzymes including HIV proteases.[62]



Scheme 1.33. *Proposed conversion of amino alcohols into cyclic sulfamides.*

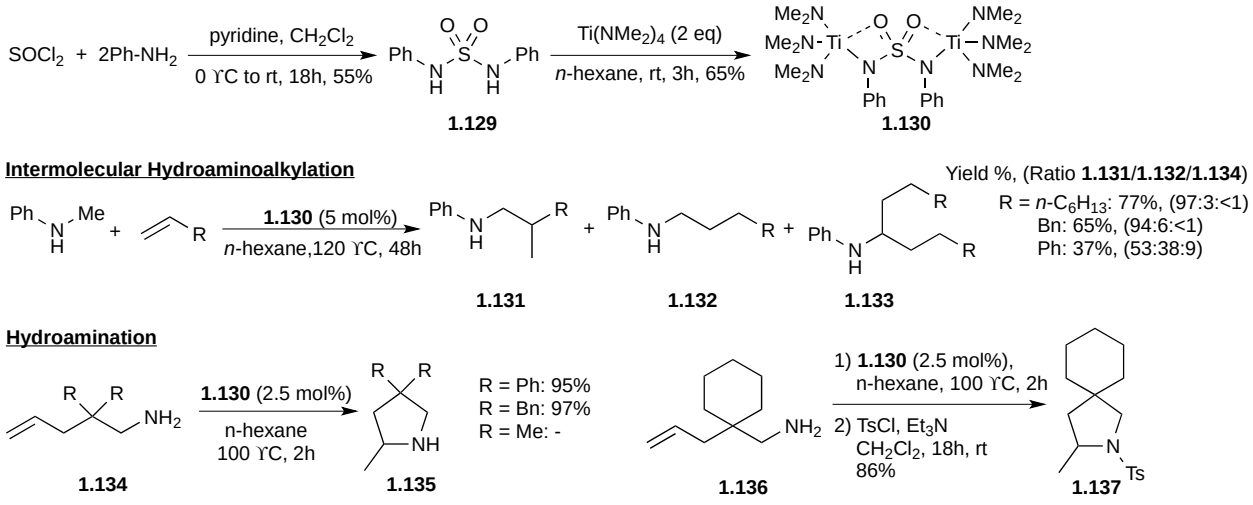
1.4. Sulfamide catalysts

Recently, asymmetric organocatalysis has emerged as a "third pillar" of enantioselective catalysts, together with biocatalysis and metal catalysis.[63,64] Although the potential of proline-catalyzed asymmetric intramolecular aldol reactions have been shown by Hajos and Wiechert in the 1970s,[65] the pioneering discovery of L-proline-catalyzed direct intermolecular asymmetric

aldol reactions by Barbas et al. opened a new gate of asymmetric organocatalysis.[66,67] Since this seminal discovery, organocatalysis has accumulated the attention of the synthetic community. In this section, various roles of sulfamide catalysts for several types of reactions are highlighted.

1.4.1. Hydroaminoalkylation and hydroamination

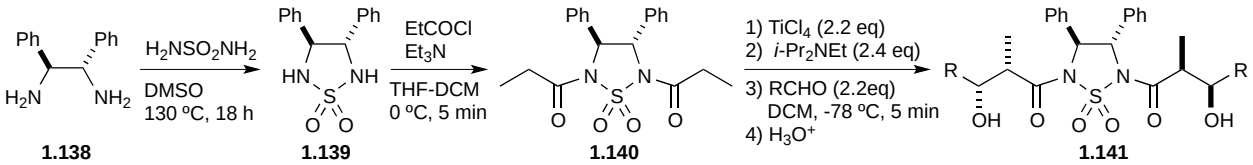
In 2012, Doyle group reported hydroaminoalkylation and hydroamination reactions using titanium complexes with sulfamide ligands as precatalysts (Scheme 1.34).[68] Diphenylsulfamide **1.129** was prepared from SO_2Cl_2 and aniline in the presence of pyridine. Subsequently, sulfamide **1.129** was reacted with two equivalents of $Ti(NMe_2)_4$ at room temperature to furnish the dinuclear titanium complex **1.130** in 65% yield. This sulfamide-titanium complex was used for catalyzing the hydroamination of olefins. Thus, hydroaminoalkylation of 1-octene, allylbenzene and styrene with *N*-methylaniline was carried out in the presence of 5 mol% of complex **1.130** at 120 ºC for 48 hours in *n*-hexane to afford the desired product in moderate to good yield. Catalyst **1.130** was also found to be useful for the intramolecular hydroamination of several aminoalkenes **1.134** and **1.136** under mild conditions employing 2.5 mol% of catalyst loading to produce cyclized amines **1.135** and **1.137**. To date, mechanisms for these reactions are yet to be reported.



Scheme 1.34. *Hydroaminoalkylation and hydroamination reactions using titanium complexes.*

1.4.2. Aldol reaction

A Ti-enolate-derived diastereoselective aldol reaction using a cyclic sulfamide chiral auxiliary for the preparation of *syn*-aldol products was reported in 1992 by Ahn and co-workers.[69] Chiral sulfamide auxiliary **1.140** was synthesized through the coupling reaction of propionyl chloride and cyclic sulfamide **1.139** which was obtained from the reaction of (1*S*,*2S*)-diphenyl-1,2-diaminoethane (**1.138**) and sulfamide. The titanium enolate of **1.140** was generated by the treatment of **1.140** with $TiCl_4$ in the presence of DIEA in CH_2Cl_2 at -78 °C. This enolate was treated with aldehyde at -78 °C for 5 minutes to afford the *syn*-aldol product in high yield (89–93%) (Table 1.5).



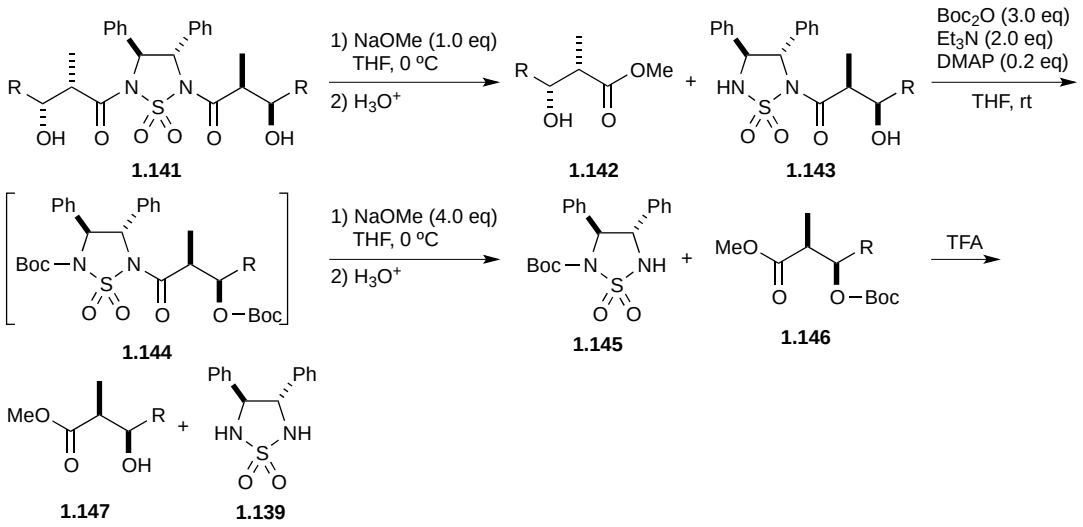
1.138 **1.139** **1.140** **1.141**

Scheme 1.35. *Synthesis of chiral sulfamide auxiliary **1.140** and aldol reaction.*

Table 1.5. *Asymmetric aldol reactions of titanium enolate of **1.140**.*

Entry	R in RCHO	Stereoselectivity	Yield (%)
1	Ph	>96:4	91
2	Me	>97:3	90
3	*i*-Pr	>97:3	93
4	(*trans*)-MeCH=CH	>95:5	89

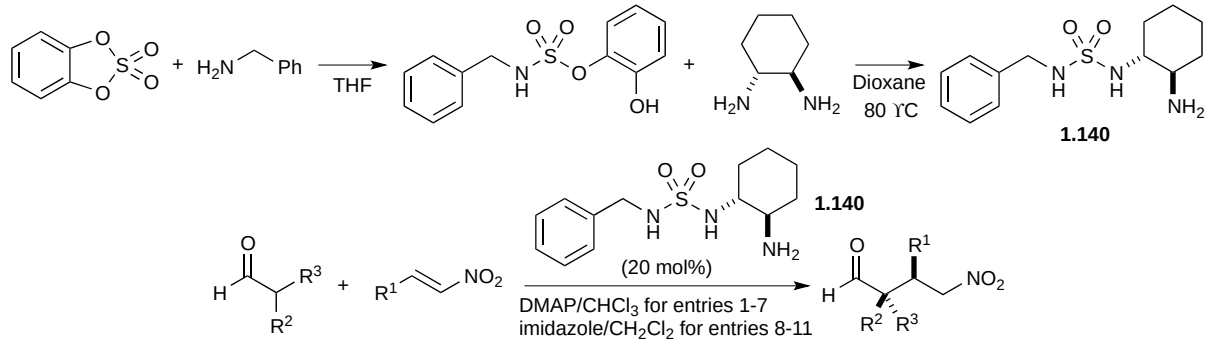
Each of the absolute stereochemistries of the *syn* products were identified by comparing the optical rotation of **1.142** with reported data.[70] The conversion of dialdol **1.141** into **1.142** and recovery of the sulfamide chiral auxiliary **1.139** are described in Scheme 1.36. Treatment of **1.141** with NaOMe generated aldol product **1.142** and sulfamide **1.143** in quantitative yield. Sulfamide **1.143** was, however, found to resist a second cleavage. In order to circumvent this issue, Boc-protection of **1.143** and treatment with NaOMe produced **1.145** and **1.146**. Subsequent treatment of reaction mixture with TFA furnished **1.147** and sulfamide chiral auxiliary **1.139**.



Scheme 1.36.

1.4.3. Conjugate addition

Asymmetric conjugate addition of ketone or aldehyde to nitro-olefins is a very useful reaction to prepare chiral *γ*–amino acids. In 2009, Chan and coworkers reported that chiral bifunctional sulfamides were highly efficient organocatalysts in the conjugate addition of aldehydes to *trans*-*β*–aryl-nitroethenes in the presence of base additives (Scheme 1.37).[71] In this regard, the chiral cyclohexanediamine unit exerted high enantiofacial selectivity in this reaction. Sulfamide catalyst **1.140** was prepared via stepwise reaction of the corresponding amines and (*1R,2R*)-cyclohexane-1,2-diamine with catechol sulfate.[72]

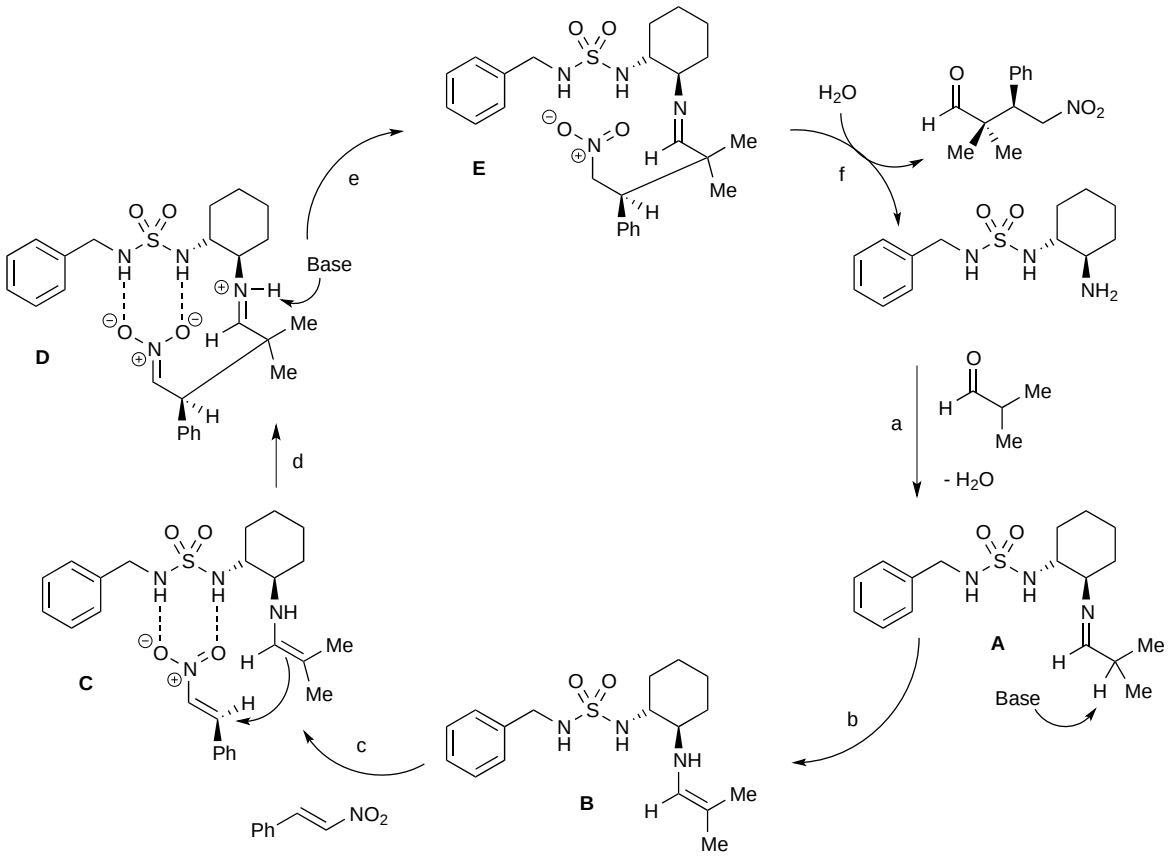


Scheme 1.37. *Conjugate addition of aldehydes to β–aryl-nitroethenes catalyzed by chiral bifunctional sulfamide.*

Entry	R_1	R_2	R_3	T (h)	Yield (%)	ee (%)
1	Ph	CH_3	CH_3	3	83	99
2	4-MeO-Ph	CH_3	CH_3	2	79	99
3	4-Cl-Ph	CH_3	CH_3	3	79	98
4	4-NO_2-Ph	CH_3	CH_3	3	74	99
5	PhCH=CH	CH_3	CH_3	24	53	98
6	2-furanyl	CH_3	CH_3	6	94	98
7	2-thiophenyl	CH_3	CH_3	24	99	99
8	Ph	$CH_2(CH_2)_2CH_2$		23	41	91
9	Ph	H	CH_3	23	96 (2/1)	78/70
10	Ph	H	CH_2CH_3	24	72 (2/1)	91/93
11	Ph	H	Ph	21	90 (2/1)	82/80

Table for Scheme 1.37. *Conjugate addition of aldehydes to β–aryl-nitroethenes catalyzed by chiral bifunctional sulfamide.*

A catalytic mechanism of conjugate addition of aldehyde to nitro ethene using a chiral sulfamide catalyst is proposed in Scheme 1.38. Initially, the catalyst and isobutyraldehyde generate the imine intermediate **A**. Tautomerization of **A** is promoted by the base additive (DMAP or imidazole) and generates enamine **B**. As is shown in Scheme 1.37, two hydrogen bonds are postulated to form between the nitro group of nitrostyrene and the sulfamide (intermediate **C**) to attenuate the electrophilic nature of nitrostyrene. Attack of the enamine to the *re*-face of the nitrostyrene double bond provides intermediate **D**. Consequent proton transfer and hydrolysis affords the desired chiral aldehyde product and regenerates the chiral sulfamide catalyst for an ensuing cycle.

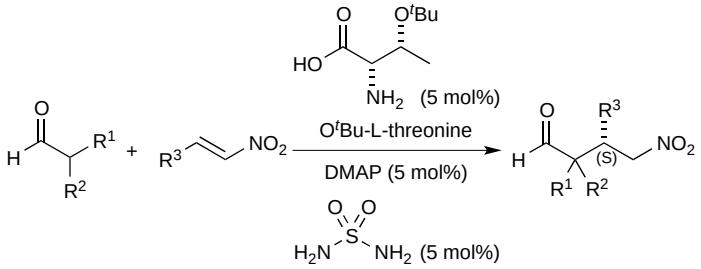


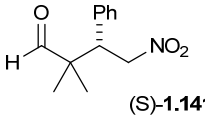
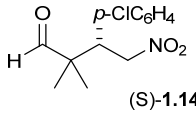
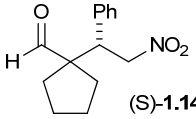
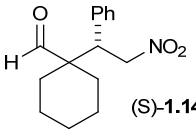
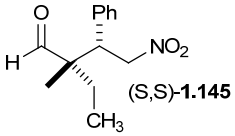
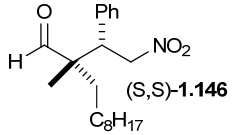
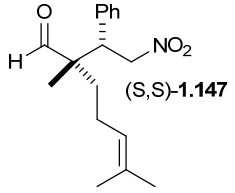
Scheme 1.38. *Proposed catalytic mechanism.*

1.4.4. Michael addition

In 2011, the Nugent group reported a Michael addition using a sulfamide as a catalyst/hydrogen bond donor to generate enantioenriched quaternary carbon containing compounds (Table 1.6). [73] In this regard, reaction of isobutyraldehyde, cyclopentanecarboxaldehyde, and cyclohexanecarboxaldehyde with 2-substituted-nitroethanes in the presence of O^{t}Bu-L-threonine, DMAP and sulfamide as an H-bond donor afforded **1.141**–**1.144** in high yield and high *ee*. Addition of aldehyde with nonequivalent *α,α*‘-substituents to *β*-nitrostyrene provided the Michael products **1.145**–**1.147** containing a stereogenic quaternary carbons in good yield and excellent *ee*, albeit with moderate diastereomeric ratios.

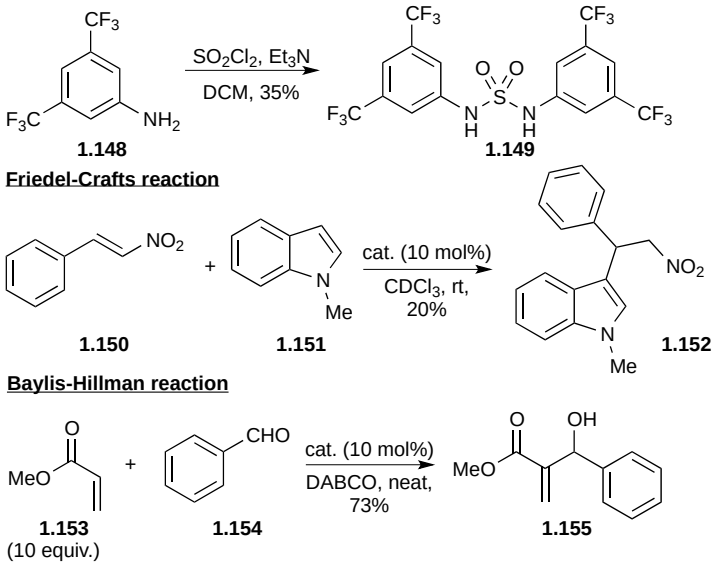
Table 1.6. *Various aldehyde additions to 2-substituted-nitroethenes.*



Entry	Product	T (h)	Yield (%)	*dr*	*ee* (%)
1	 (S)-**1.141**	7	97	-	98
2	 (S)-**1.142**	24	98	-	96
3	 (S)-**1.143**	7	89	-	97
4	 (S)-**1.144**	48	88	-	91
5	 (S,S)-**1.145**	12	84	70:30	97
6	 (S,S)-**1.146**	12	71	78:28	91
7	 (S,S)-**1.147**	12	70	77:23	98

In 2009, Shea and coworkers reported a new type of H-bond catalysis using a sulfamide catalyst (Scheme 1.39).[74] The sulfamide catalyst **1.149** can be readily synthesized from the

reaction of aniline **1.148** and SO_2Cl_2 in CH_2Cl_2 in 35% yield. The Friedel-Craft reaction between *β*–nitrostyrene **1.150** and *N*-methyl indole **1.151** was carried out with 10 mol% of sulfamide **1.149** to provide 3-alkylated indole compound **1.152** in 20% yield. The Baylis-Hillman reaction was performed between methyl acrylate **1.153** and benzaldehyde **1.154** in the presence of the sulfamide catalyst **1.149** and DABCO as a co-catalyst to furnish the vinyl ketone **1.155** in 73% of yield.



Scheme 1.39. Fridel-Crafts reaction and Baylis-Hillman reaction using sulfamide catalysis.

1.4.5. Mitsunobu-like coupling

Sulfamides have been reported for the preparation of a Mitsunobu-type betaine for coupling between alcohols and carboxylic acids and imides (Figure 1.5).[75] In 1994, Castro and coworkers isolated an unprecedented adduct **1.165** between triphenylphosphine and 3,3-dimethyl-1,2,5-thiadiazolidine 1,1-dioxide **1.159**, which was synthesized from diamine **1.161** and sulfamide ($SO_2(NH_2)_2$. Interestingly, the initial study involved the preparation of **1.160** using **1.156** or **1.157** with **1.158** or **1.159** for the identification of molecules for the treatment of migraine headaches (Figure 1.5).[76]

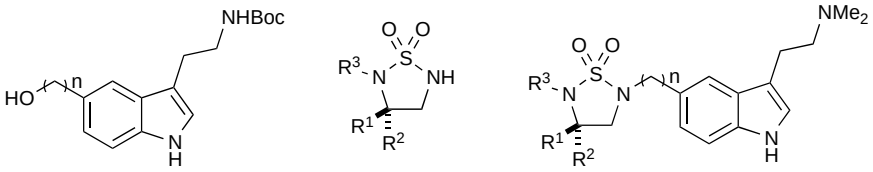


Figure 1.5.

Under the standard Mitsunobu conditions, alcohol **1.156** and **1.157** failed to furnish the expected alkylated product when reacted with **1.159** (R^1=R^2= Me, R^3= H), but afforded a white solid was identified as the betaine **1.165** (Figure 1.6). It is important to note that when only one proton on the cyclic sulfamide (R^1=R^2= H, R^3= Me) is available, the betaine cannot be generated. On the other hand, using acidic component HX with a lower pKa than that of betaine **1.165** allows for the generation of ion pair **1.166**. Reaction with alcohol would then furnish the oxyphosphonium salt **1.167** and cyclic sulfamide **1.159**. Subsequent S_N2 displacement with the nucleophilic component afforded the corresponding product **1.168** with inversion of chiral center. Summary of the reactions with various alcohols and carboxylic acids and imides are given in Table 1.7.

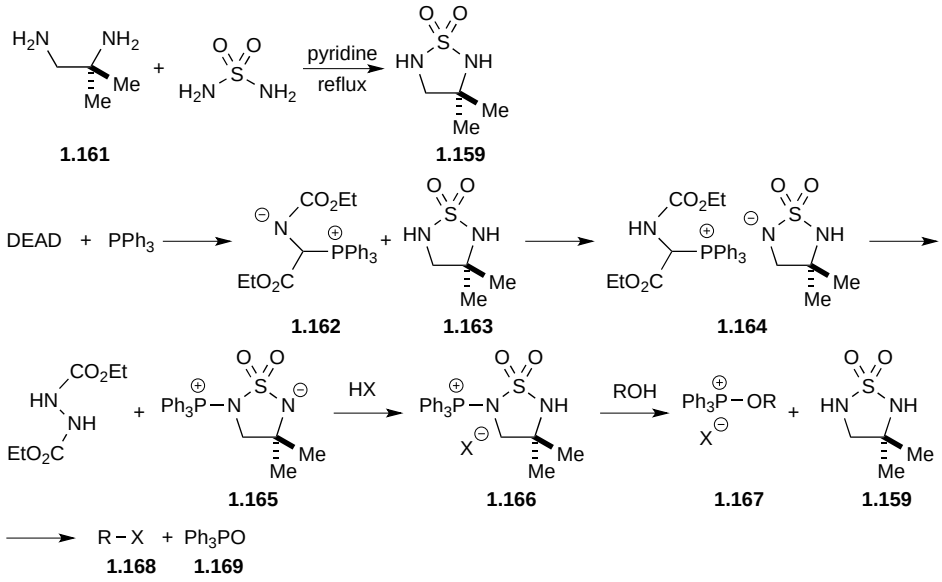
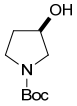
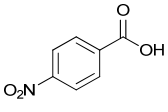
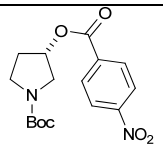
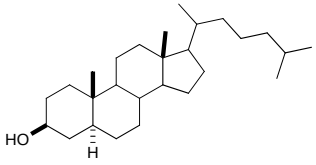
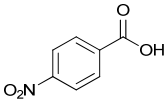
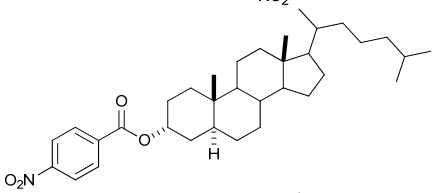
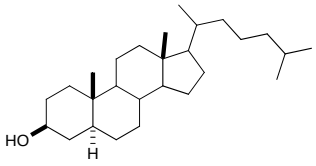
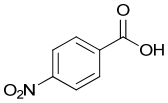
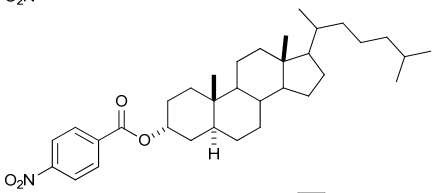
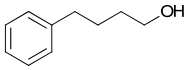
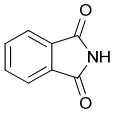
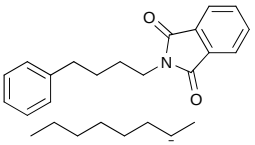
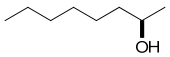
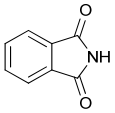
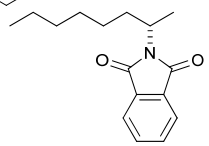


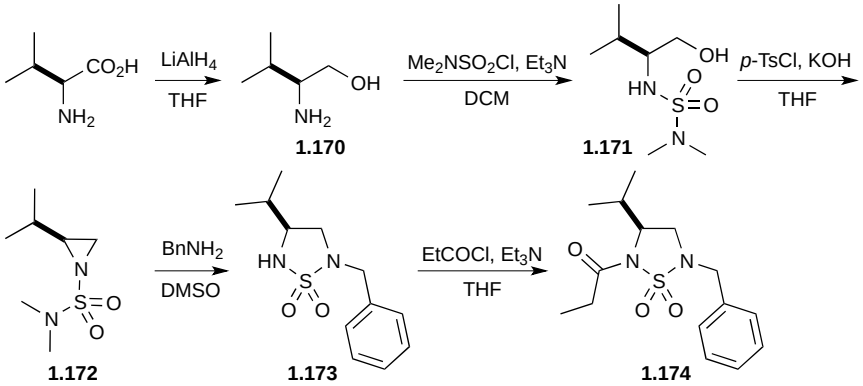
Figure 1.6. *Mechanism of coupling reaction through a Mitsunobu-like process.*

Table 1.7. *Utilization of betaine **1.165** in a Mitsunobu-like process.*

entry	substrate	HX	conditions	product	Yield (%)
1			CH_2Cl_2, rt, 5 h		89
2			CH_2Cl_2, rt, 64 h		53
3			Toluene, rt, 17 h		95
4			CH_2Cl_2, rt, 2.5 h		98
5			CH_2Cl_2, rt, 65 h		80

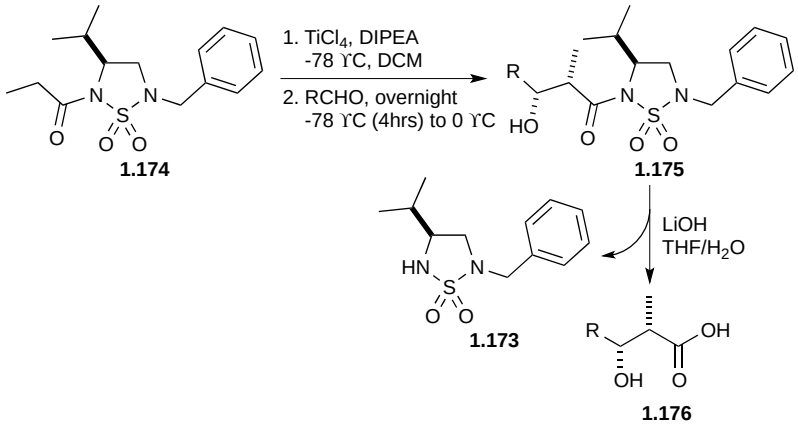
1.4.6. Utilization of cyclic sulfamide as chiral auxiliaries

Cyclic sulfamides have been employed as chiral auxiliaries for the production of chiral, non-racemic molecules. In 2010, Dewynter and coworkers reported the application of an acyclic sulfamide as a chiral auxiliary for facilitating asymmetric aldol and alkylation reactions.[77] Thus, *N*-propionyl sulfamide **1.174** was efficiently synthesized in five steps and shown in Scheme 1.40.



Scheme 1.40. *Preparation of cyclosulfamide **1.173** as a chiral auxiliary.*

With this sulfamide in hand, the authors were able to accomplish a number of diasteroselective aldol reactions using chiral auxiliary **1.174** as represented in Scheme 1.41. Reaction with 1.2 equiv. of $TiCl_4$ and **1.174** in CH_2Cl_2 at -78 °C for 30 min followed by the addition of *N*-diisopropyl ethylamine generated the titanium enolate of **1.174**. The enolate was then reacted with aldehyde at the same temperature for 4 hours, warmed to 0 °C, and stirred overnight to afford the aldol product as a single diastereoisomeric, *syn*-aldol product **1.175** (dr >99:1). In this regard, a variety of aldehydes were used and the results are summarized in Table 1.7. The aldol products were subsequently hydrolyzed with 3 equiv. of LiOH monohydrate in THF/H_2O (1:1) at 0 °C to afford the corresponding carboxylic acids **1.176**, as well as the chiral auxiliary **1.173**, without any loss of the stereochemical integrity (Table 1.8).



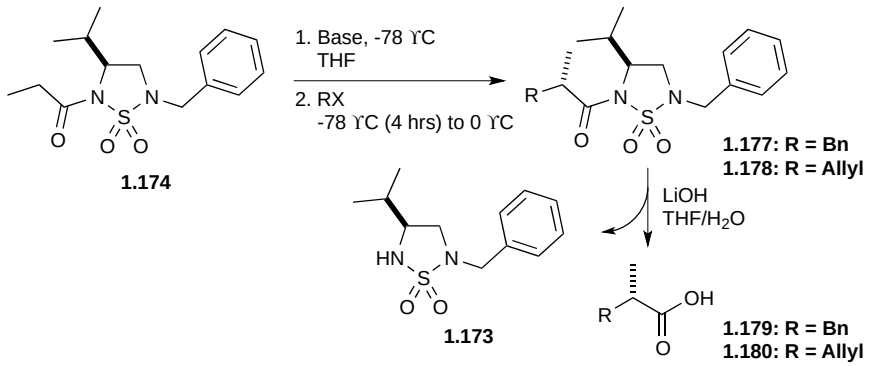
Scheme 1.41. *Diastereoselective aldol reactions.*

Table 1.8.

Entry	aldehyde	dr	% Yield	Product	Yield of recovery **1.173**	% Yield	Product
1	*i*Pr-CHO	>99:1	93%	**1.175a**	96%	95%	**1.176a**
2	Ph-CHO	>99:1	88%	**1.175b**	98%	96%	**1.176b**
3	*n*Pr-CHO	>99:1	92%	**1.175c**	95%	94%	**1.176c**
4	*c*Hex-CHO	>99:1	87%	**1.175d**	93%	93%	**1.176d**

Chiral sulfamide **1.174** was also reported in asymmetric alkylation reactions. Treatment of **1.174** with NaHMDS or LiHMDS and addition of benzyl bromide or allyl bromide allows for

generation of **1.177** and **1.178** (Scheme 1.42 and Table 1.9). Removal of the chiral auxiliary **1.173** using lithium hydroxide afford only one diastereomer in each case.



Scheme 1.42. *Stereocontrolled alkylation*.

Table 1.9.

Entry	R-X	Base	dr	Yield	Product	Yield of recovery **1.173**	Yield	Product
1	Bn-Br	NaHMDS	>99:1	30%	**1.177**	96%	95%	**1.179**
2	Bn-Br	LiHMDS	>99:1	58%	**1.177**	97%	91%	**1.179**
3	Allyl-Br	NaHMDS	>99:1	48%	**1.178**	98%	94%	**1.180**
4	Allyl-Br	LiHMDS	>99:1	60%	**1.178**	97%	92%	**1.180**
5	Allyl-I	LiHMDS	>99:1	78%	**1.178**	98%	96%	**1.180**

1.5. Conclusions

In conclusion, sulfamide compounds have been reported possessing a variety of biological activities for the treatment of life threating illnesses such as AIDS and cancers. Despite the indisputable utility of sulfamide compounds, existing routes for their construction were lacking in the literature. With this essential need, synthetic approaches for acyclic and cyclic sulfamide compounds were summarized in this chapter. Many methods from typical procedures to advanced innovative approaches for the synthesis of sulfamides have been developed by scientists featuring symmetric and non-symmetric sulfamides to serve as high affinity protein ligands and pharmaceutically useful agents. There are several investigational and commercially available drugs containing sulfamide moiety in order to treat different types of

diseases. In this regard, generation of new molecular structures and chemical methodologies to discover new pharmacophores are important to survey more pharmaceutically active sulfamide-containing compounds. Several technological advances will undoubtedly enable the formation of new, structurally unique sulfamides exhibiting biological potential. Furthermore, advances in sulfamide organocatalysts will expand a pathway to develop and produce a powerful arsenal for both drug and reagent development.

1.6. References

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CHAPTER 2

Newly Developed Piperidinyl Sulfamides as

Tyrosyl-DNA phosphodiesterase 1 (Tdp 1) Inhibitors

2.1. Abstract

Tyrosyl-DNA phosphodiesterase 1 (Tdp1) is an enzyme that catalyzes the hydrolysis of 3'-phosphotyrosyl bonds.[1] Such linkages form *in vivo* following the DNA processing activity of topoisomerase I (Top1). For this reason, Tdp1 has been implicated in the repair of irreversible Top1-DNA covalent complexes, which can be generated by either exogenous or endogenous factors. Tdp1 has been regarded as a potential therapeutic co-target of Top1 in that it seemingly counteracts the effects of Top1 inhibitors, such as camptothecin and indenoisoquinolines and its clinically used derivatives. Thus, by reducing the repair of Top1-DNA lesions, Tdp1 inhibitors have the potential to augment the anticancer activity of Top1 inhibitors. There are no known specific pharmacological inhibitors of Tdp1. In our attempts to design new chemical scaffolds for anti-cancer activity against various protein targets, we have recently synthesized a host of piperidinyl-based sulfamides. Some of these compounds have shown activity in screening for Tdp 1 inhibition activity in biochemical assays against recombinant Tdp1. Using molecular modeling and homology studies, a small library of compounds has been synthesized and tested further.

2.2. Introduction

Most people and living organisms on planet earth are exposed to substances that are known to damage DNA, which is caused by UV light, radiation (including x-rays and gamma rays), plastics, cigarette smoke, pesticides, micronutrient deficiency, hydrolysis or thermal disruption, etc. While rare, mistakes also occur during DNA replication, namely, when a cell copies its DNA in preparation for cell division. Ultimately, damaged DNA can be prompted to a tumor cell by proliferating through continuous cell division. Many anticancer drugs used for

chemotherapy generate their anti-cancer activity by damaging DNA in rapidly replicating tumor cells, and this poses a significant risk of generating a new cancer, such as leukemia. Therefore, there is a high demand to develop new inhibitors that may help to repair DNA or oppose the unwanted action of these anticancer agents. Topoisomerase I (Top1) inhibitors, such as Camptothecins,[2] are chemotherapeutic agents which prevent the replication of single strand DNA molecules, ultimately leading to cell death (Figure 2.1). The natural product camptothecin (**2.1**) was first isolated from the bark of the Chinese tree, *Camptotheca acuminata*, by the National Cancer Institute (NCI).[3] The water-soluble derivatives of Camptothecin–Topotecan (**2.2**) and Irinotecan (**2.3**) were developed successfully and approved by the US Food and Drug Administration (FDA): Topotecan for ovarian and lung cancers and Irinotecan for colorectal cancer.[4]

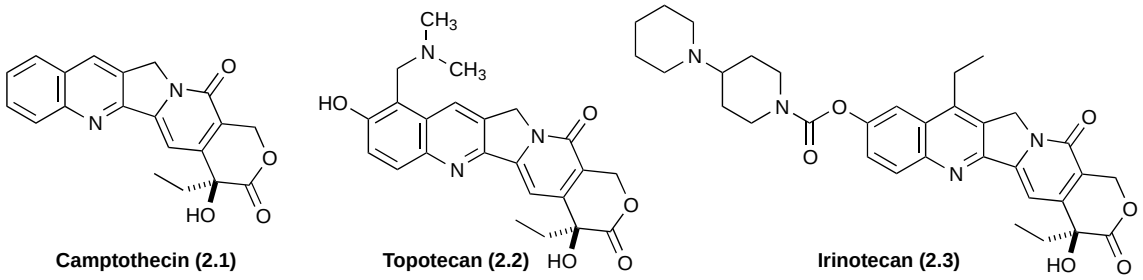


Figure 2.1. *Structures of Top1 inhibitors*.

2.3. Topoisomerase I (Top1) and Tyrosyl-DNA phosphodiesterase 1 (Tdp1)

Nuclear DNA (nDNA) is approximately a 2 meter-long polymer that is located in a cell nuclear volume of 10^{-17} m^3. Because it is highly compacted, cellular DNA must have many curved DNA domains/loops and points of contact between these domains (Figure 2.2).[5]

Furthermore, DNA metabolism needs the two strands of the double helix to be separated to serve as templates for transcription, replication, recombination and repair and this fundamental processes commences during the cell cycle to maintain its own integrity and generate genetic diversity. Due to the size and mass of replication and transcription complexes, the rigid complexes do not rotate easily around the DNA helix. This limitation of free rotation of the flanking DNA domain generates supercoiled DNA, which needs to be relaxed by topoisomerases. TOP1 relieves DNA torsional stress and relaxes supercoiled DNA by nicking the DNA and rotating the broken strand around the TOP1-bound DNA strand. The yellow circle in Figure 2.2 **A** shows the covalently linked catalytic tyrosine of TOP1. Figure 2.2 **B** is an expanded version of DNA-relaxation by a TOP1 cleavage complex (TOP1cc). The first step is a trans-esterification reaction catalyzed by the TOP1 whereby the catalytic tyrosine (Y) is linked to the 3'-DNA end (nicking step) (Figure 2.2 **B**, left). A nucleophilic attack by the tyrosine residue of TOP1 on the phosphate moiety of the substrate releases tyrosine and forms a new covalent enzyme-DNA complex, TOP1cc (Figure 2.3).[6] In the second step, the torsion strain from DNA supercoiling allows the controlled rotation of the 5' end of the nicked DNA strand around the intact strand (Figure 2.2 **B**, middle). Once the DNA is relaxed, the nucleophilic attack of the tyrosyl-DNA-phosphodiester bond by the free 5'-hydroxyl end of the nicked DNA is required to religate (bind back) with the corresponding 3' end of DNA, which is called the closing step of the nicking-closing reaction (DNA religation, Figure 2.2 **B**, right and Figure 2.3). TOP1ccs are generally ephemeral that they are not detectable because the closing step is much faster than the nicking step. It is crucial that any misalignment of the 5'-hydroxyl-DNA end with the scissile tyrosyl-DNA-phosphodiester bond leads to an accumulation of TOP1cc, and it will end up as DNA modification[7] or result in apoptosis.[8]

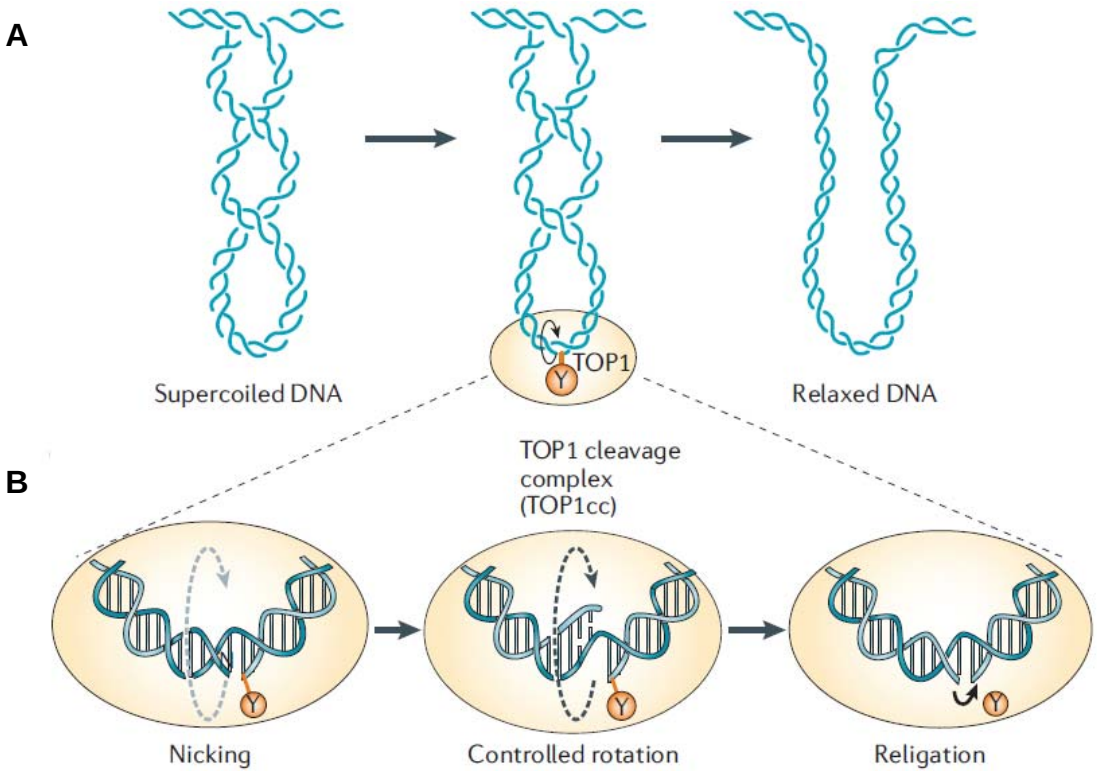


Figure 2.2. *Relaxation of DNA supercoiling by TOP1-mediated DNAcleavage complexes*.[5] (This figure was copied from 'Pommier, Y., Topoisomerase I inhibitors: camptothecins and beyond. *Nature Reviews Cancer* **2006,** *6*, 789–802)

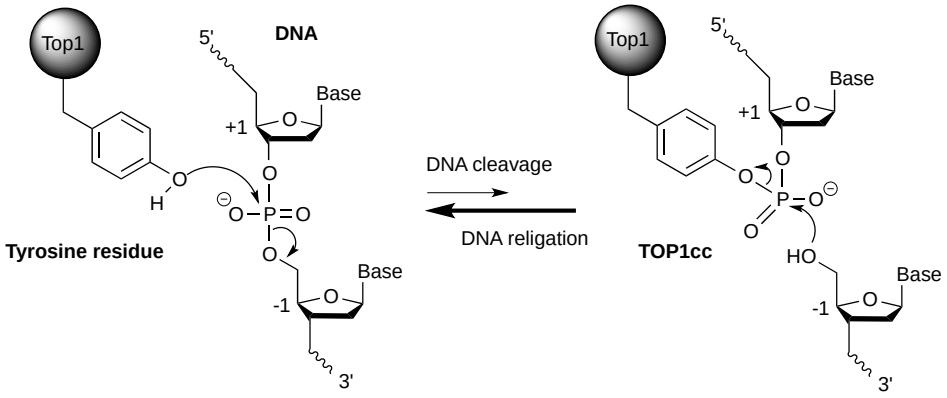


Figure 2.3. *Trans-esterification catalyzed by Top1*.

Camptothecin, a Top1 inhibitor, targets Top1 and novel Top1 inhibitors are in development as anticancer agents that prevent the religation of DNA after cleavage during replication (Figure 2.4).[9] Mechanistically and undesirably, Top1 inhibitors selectively bind to the TOP1-DNA interface and damage DNA by trapping covalent complexes between the Top1 catalytic tyrosine and the 3′-end of the broken DNA.[10]

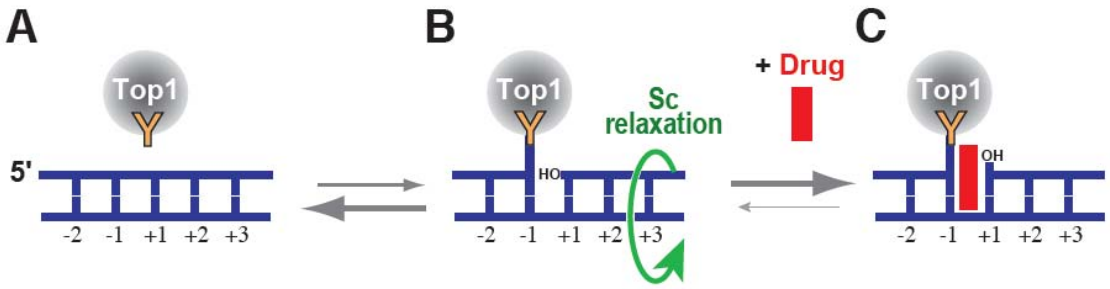


Figure 2.4. *Mechanism for each of Top1 and inhibitor with DNA* (This figure was copied from "Pommier, Y. et al., *Progress in Nucleic Acid Research and Molecular Biology, Vol 81*, Moldave, K., Ed. 2006; pp 179–229.")

Tyrosyl-DNA phosphodiesterase 1 (Tdp1) is a recently discovered DNA repair enzyme that catalyzes the cleavage (hydrolysis) of phosphodiester bond between the Top1 catalytic tyrosine residue and a DNA 3'-phosphate as shown Figure 2.5.[1, 11, 12, 13] When the 5'-hydroxyl end of the broken DNA is too far to carry out the nucleophilic addition resulting in DNA religation, then Tdp1 hydrolyzes the intermediate tyrosyl-phosphodiester bond using a water molecule.[7c, 14] Tdp1 repairs topoisomerase I (Top1)-DNA covalent complexes by this mechanism and Tdp1 has the potential to enhance the negative activity of Top 1 inhibitors in cancer cell as mentioned before.[15] The PNKP (Polynucleotide kinase 3'-phosphatase) enzyme

can then hydrolyze the damaged DNA by either removing 3'-phosphates from, or by phosphorylating 5'-hydroxyl groups on the broken DNA backbone. This is now a substrate for DNA polymerase, which is an enzyme that assists in DNA replication, by adding free nucleotides to the 3' end of a newly forming strand, and ligase which helps the combining of DNA strands together by catalyzing the formation of a phosphodiester bond. As discussed above, Tdp1 counteracts the action of Top1 inhibitors and possibly decrease their effectiveness in reducing cancer cells. Tdp1 repairs DNA lesions and chemotherapeutic-mediated DNA damage, such as the DNA breaks prompted by top1 inhibitors. Thus, Tdp1 is a potentially rational anticancer target whose inhibition should improve the activity of cancer chemotherapeutics.

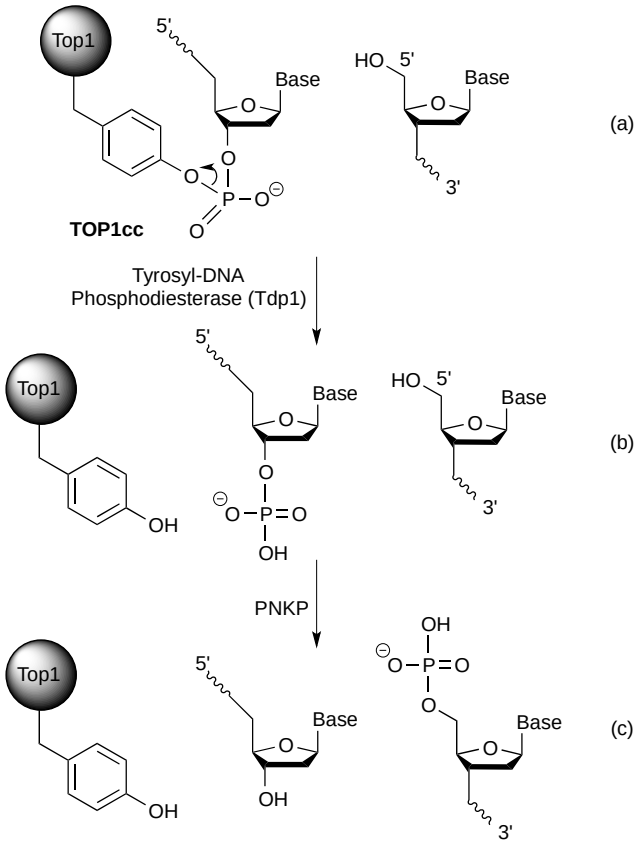


Figure 2.5. *Action of Tdp1 and PNKP.*

Tdp1 inhibitors have become a major area of drug research for anti-cancer treatment.[5,16] A recent study on a steroid-linked benzenesulfonate (NSC 88915) and other derivatives reported that both the steroid and phenylsulfonyl ester moieties of NSC 88915 are required for Tdp1 inhibition (Figure 2.6).[17] In particular, the *p*-Br-substituted benzenesulfonate NSC 88915 showed the best result among the derivatives, its IC_{50} value was 7.7 ± 0.8 μM.

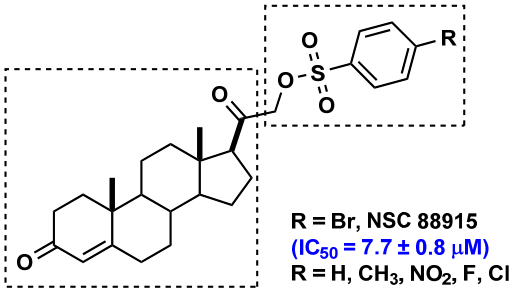


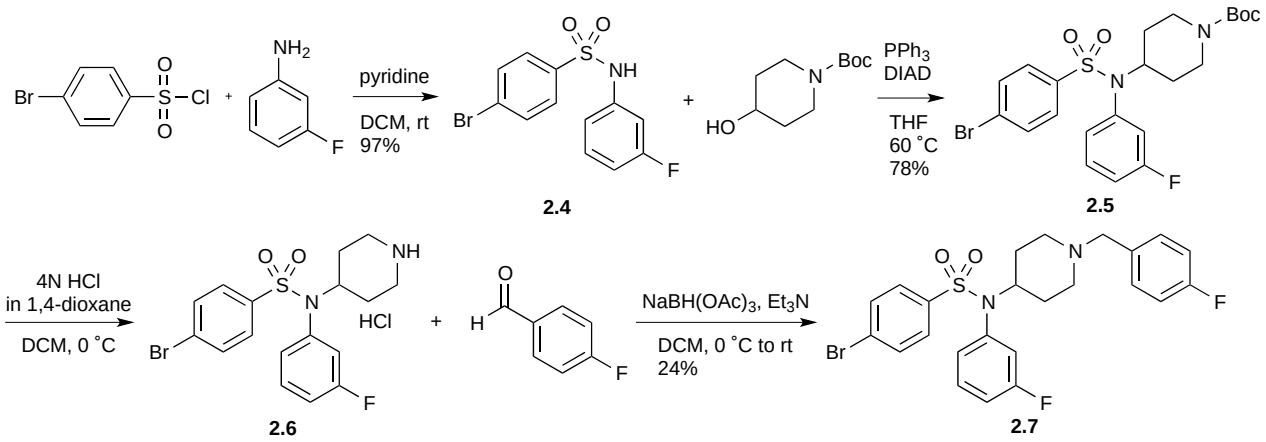
Figure 2.6. *Structure of NSC88915 and other derivatives.*

Since only a limited number of weak Tdp1 inhibitors have been reported,[18] we commenced the investigation by designing and developing new desired compounds from a sulfamide moiety. The structural features considered for initial scaffold design were based on various literature reports on compounds tested as inhibitor of Aggrecanase-1,[19] TACE [20] or KSP (Kinesin Spindle Protein).[21] Herein, we report the study of a small library of sulfamide compounds, designed, synthesized and tested for Tdp1 inhibitory activity

2.4. Chemistry

The initial study was the synthesis of the sulfonamide compound **2.7**, which has a *p*-bromo phenyl ring similar to NSC88915 (Scheme 2.1). The synthesis route for the sulfonamide **2.7** was started from the reaction of *p*-bromo benzenesulfonyl chloride with *m*-fluoroaniline

under basic conditions. After the generation of sulfonamide intermediate **2.4**, a Mitsunobu reaction was carried out to generate the piperidine-containing secondary amine **2.5**. Reductive amination after deprotection of Boc group, using HCl, was successfully accomplished to generate sulfonamide compound **2.7**.



Scheme 2.1. *Preparation of sulfonamide* ***2.7***.

In order to observe the effect of an amino ester moiety on DNA binding or H-bonding, we changed the phenyl ring to an amino ester. The new scaffold design is composed of three fragments as outlined in Figure 2.7, namely, a western subunit (hydrophilic amino ester), central subunit (sulfamide with phenyl ring), and eastern subunit (benzyl piperidine). Modification of the structure of the inhibitor was mainly focused on these three fragments.

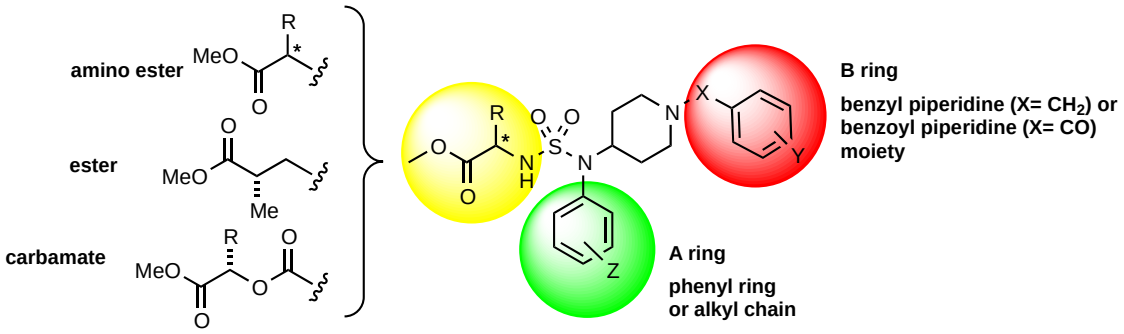
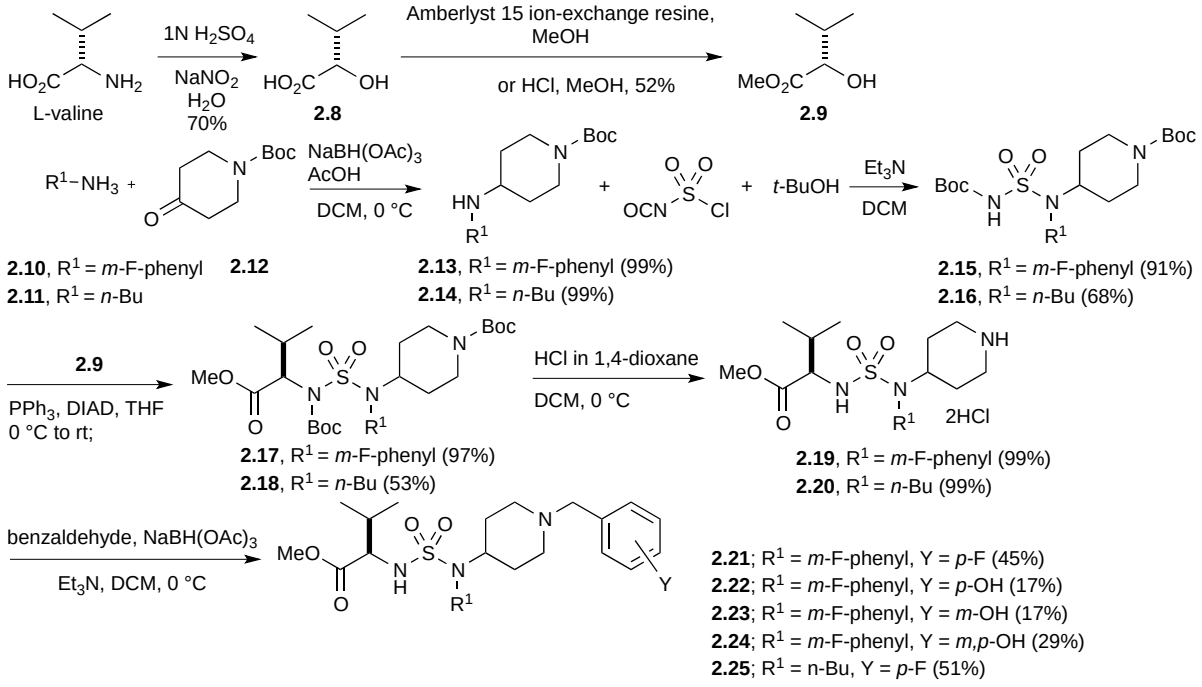


Figure 2.7. *Scaffold design for Tdp 1 analogues.*

Piperidinyl sulfamides derivatives **2.21–2.25** were prepared starting from the reductive amination reaction of *p*-fluoroaniline **2.10** or *n*-butylamine **2.11** and *N*-Boc-4-piperidinone **2.12** to give **2.13** and **2.14** (Scheme 2.2).[22] The secondary amines **2.13** and **2.14** were coupled with chlorosulfonyl isocyanate (CSI) and *t*-BuOH in the presence of Et_3N as a base to the corresponding Boc-protected sulfamide moieties **2.15** and **2.16**.[23] The subsequent Mitsunobu reaction was carried out with the α-hydroxyl amino ester **2.9** to generate the amino ester-linked sulfamides **2.17** and **2.18**.[24] In this regard, L-valine was converted to the α-hydroxyl carboxylic acid **2.8** using the Van Slyke[25] reaction which maintains the chiral integrity using H_2SO_4 and $NaNO_2$ in water at 0 °C overnight with vigorous generation of nitrogen gas being observed.[26] Amino ester **2.9** was obtained from amino acid **2.8** via esterification using MeOH/acid.[27,28] Deprotection of the Boc group furnished the secondary amines **2.19** and **2.20**, and reductive amination afforded the piperidinyl sulfamides **2.21**–**2.25**.[29]



Scheme 2.2. *Preparation of compounds **2.21–2.25**.*

2.4.1. Initial Gel Study

Results of the initial gel study of sulfamide compounds **2.21**, **2.25**, and other intermediates are shown in Figure 2.8. Key points of Figure 2.8, include: (**A**) Sulfamide intermediates and final sulfamide compounds; (**B**) Tdp1 biochemical assays. Single-stranded 14Y (14-mer strand) was used as substrates and ^{32}P-Radiolabeling (*) was at the 5' terminus of the strand. Tdp1 catalyzes the hydrolysis of the 3'-phosphotyrosine bond using water molecule and converts 14Y to an oligonucleotide with 3'-phosphate 14P; (**C**) Gel illustrates Tdp1 inhibition by sulfamides with single strand 14Y. 3'-Phosphate oligonucleotide product (14P) was developed faster than the corresponding tyrosyl oligonucleotide substrate (14Y). Reactions were performed with sulfamides in concentration 0.01, 0.1, 1.0, 10, and 100 μM, and (**D**) Cleavage inhibition analysis of the gel shown in panel **C** was calculated as percentage. Gel study for compounds LSC-JHJ-I-55-1 and LSC-JHJ-I-64-1 was carried out together but they are intermediates for other projects. Compound **2.21** showed the best result among the tested compounds with a measured IC_{50} value of 23.7 μM.

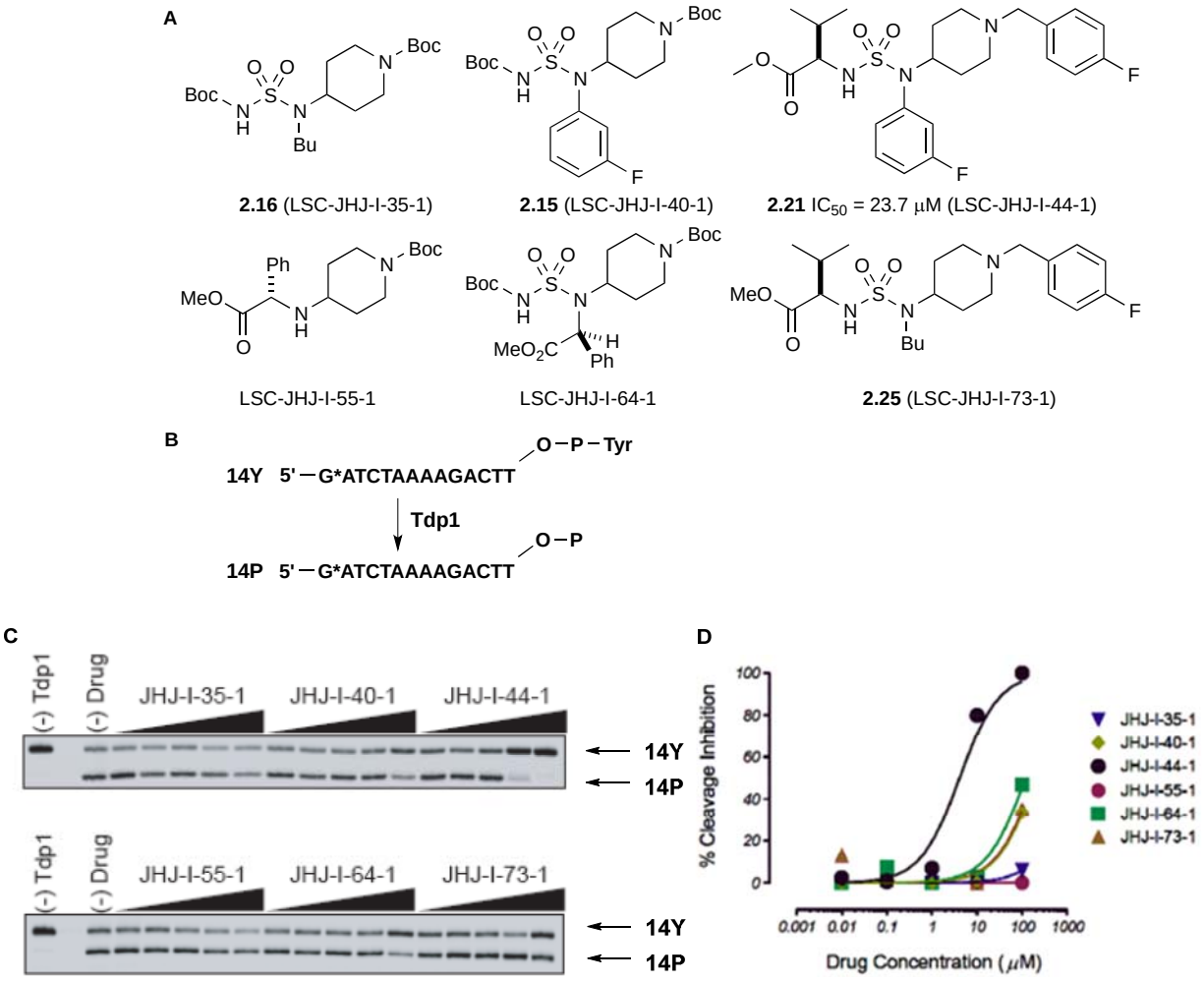
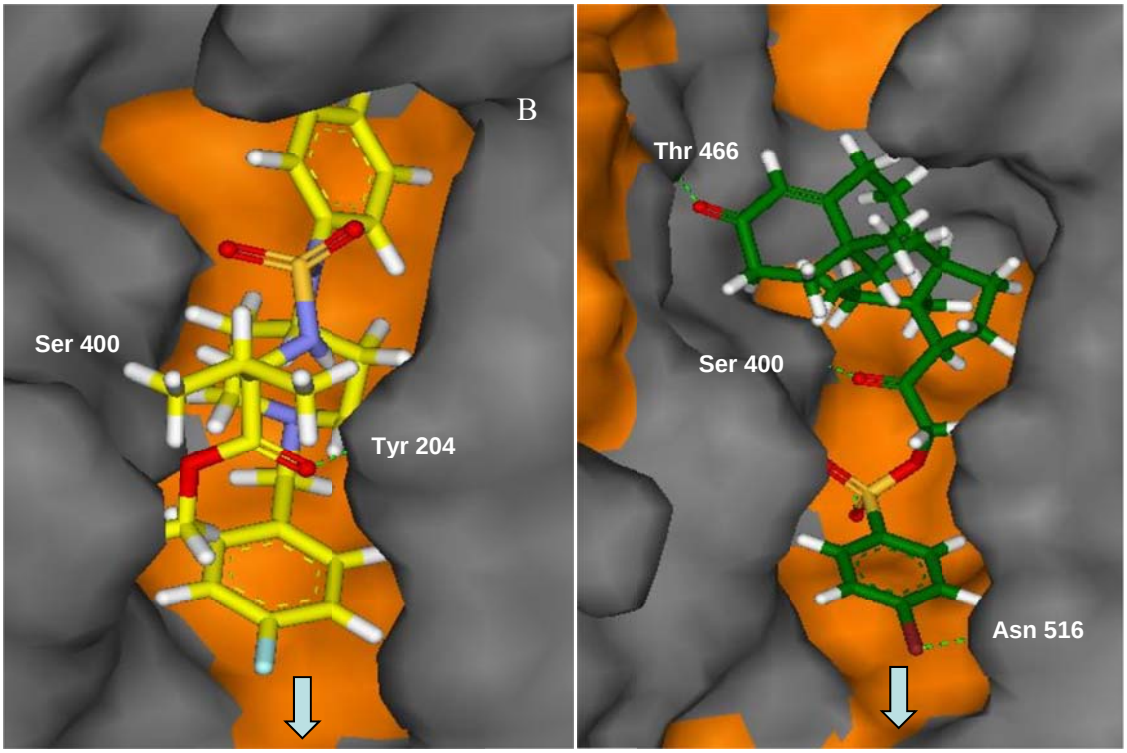


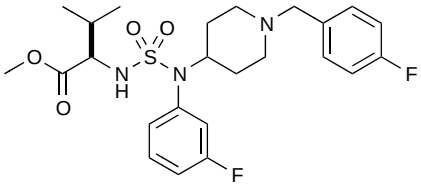
Figure 2.8. *Inhibition of Tdp1 activity by sulfamides and intermediates (Initial Gel Study).*

2.4.2. Protein Docking Study

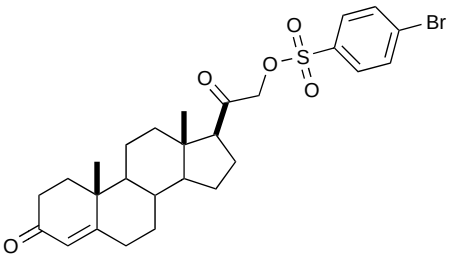
Dr. Iwona Weidlich carried out molecular modeling of piperidinyl sulfamide derivatives (Figure 2.9). The arrow indicates the direction to the Tdp1 active site. Hydrophobic and hydrophilic surface areas of the protein are colored in grey and orange, respectively. The coloring of atoms is as follows: carbon – yellow (**A**), green (**B**); nitrogen – blue; oxygen – red; sulfur - orange. Ligands are displayed in stick representations, while all hydrogen atoms have been shown. Hydrogen bonds are represented by green dotted lines. Both inhibitors bind in the

same binding pocket and form hydrogen bond with Serine 400. In newly synthesized compound **2.21**, the benzyl piperidine moiety is oriented towards Tdp1 active site and the hydrophobic amino ester moiety forms a hydrogen bond with Tyrosine 204.





Piperidinyl sulfamide derivative (**2.21**)

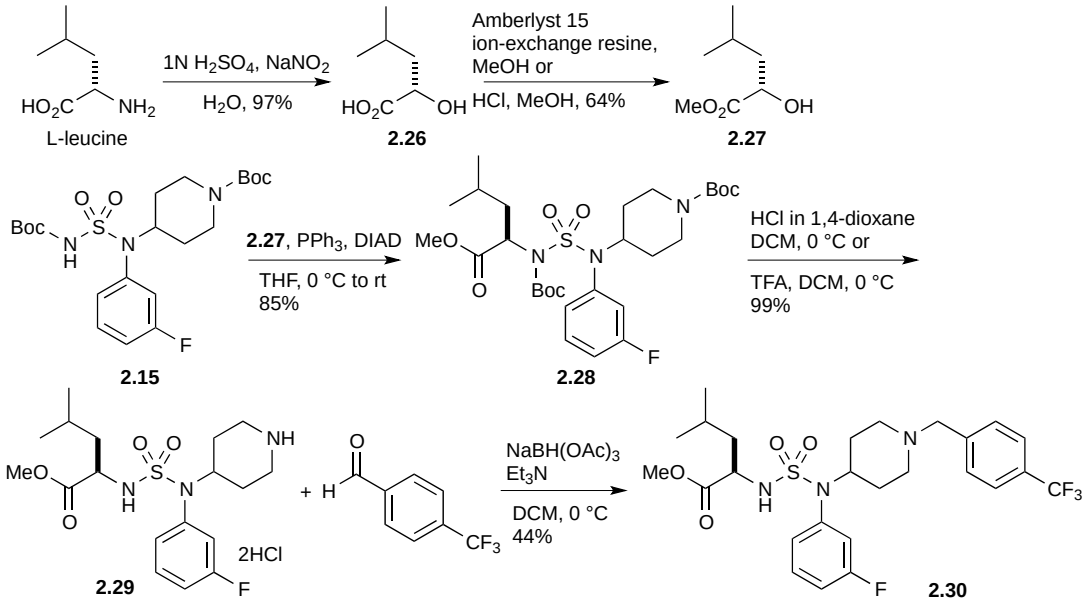


Phenyl sulfonyl ester derivative (**NSC 88915**)

Figure 2.9. *Comparison of the best docking poses of active Compound* ***2.21*** *(JHJ-I-44-1,* ***A)*** *and reported earlier phenyl sulfonyl ester derivative (NSC 88915,* ***B)***[30] *docked into the active site of Tdp1 (H263, K265, H493, and K495).*

2.4.3. Synthesis of Piperidinyl Sulfamides

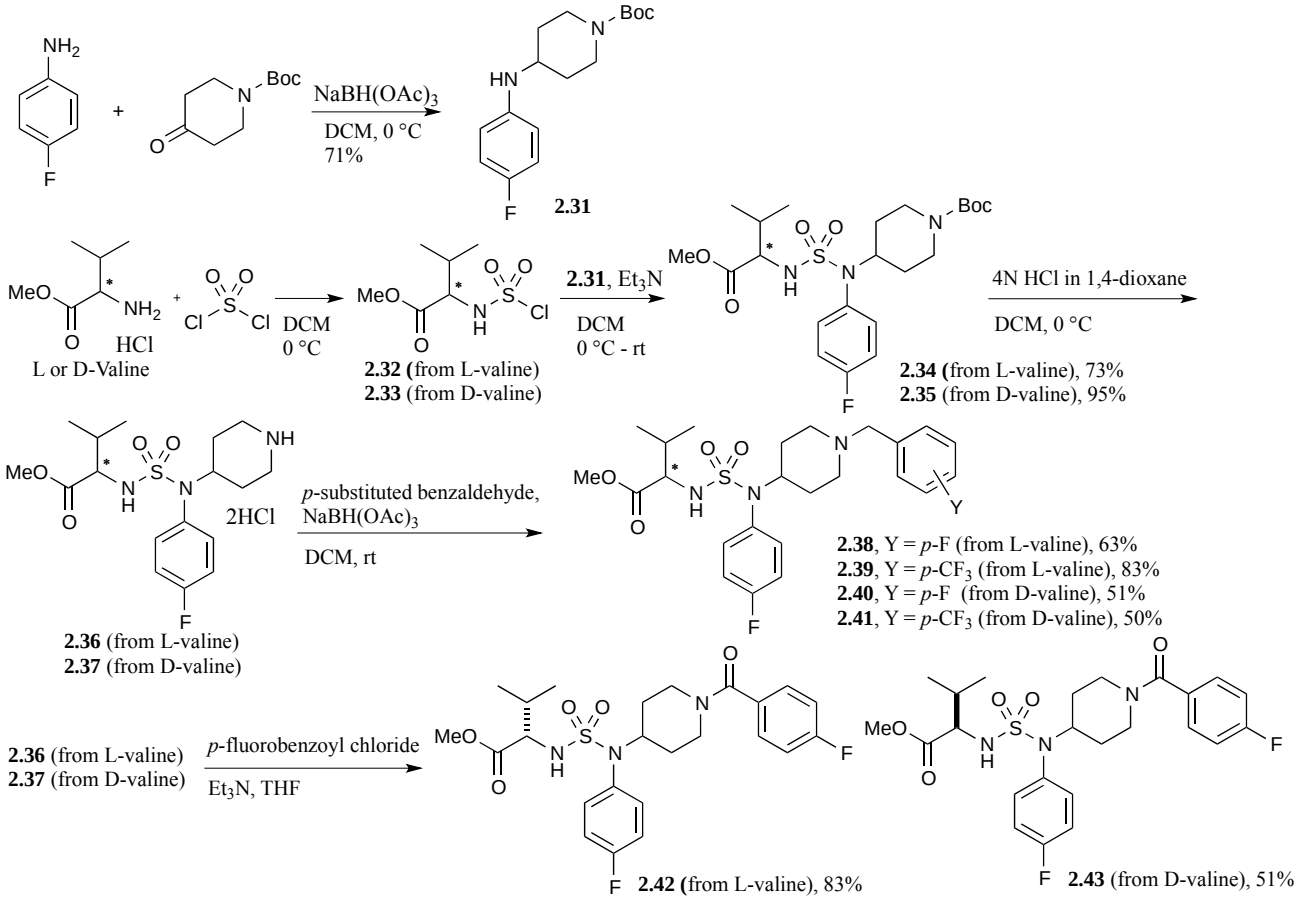
Since we obtained the promising results of the gel study and protein docking study, we continued to make an analogue of the piperidinyl sulfamide. The same synthetic route was utilized for the synthesis of sulfamide compound **2.30** where L-leucine was used as the starting substrate as outlined in Scheme 2.3. After the conversion of L-leucine to α-hydroxy ester **2.27** through **2.26** via esterification, it was used for the Mitsunobu reaction with **2.15** to furnish intermediate **2.28**. In an analogous manner to the aforementioned L-valine-derived analogues **2.21**–**2.25**, de-protection and reductive amination with *p*-substituted benzaldehyde generated compound **2.30**.



Scheme 2.3. *Preparation of compound **2.30**.*

An alternative route to obtain piperidinyl sulfamides is described below (Scheme 2.4). Sulfuryl chloride was coupled with L- or D-valine methyl ester hydrochlorides to generate

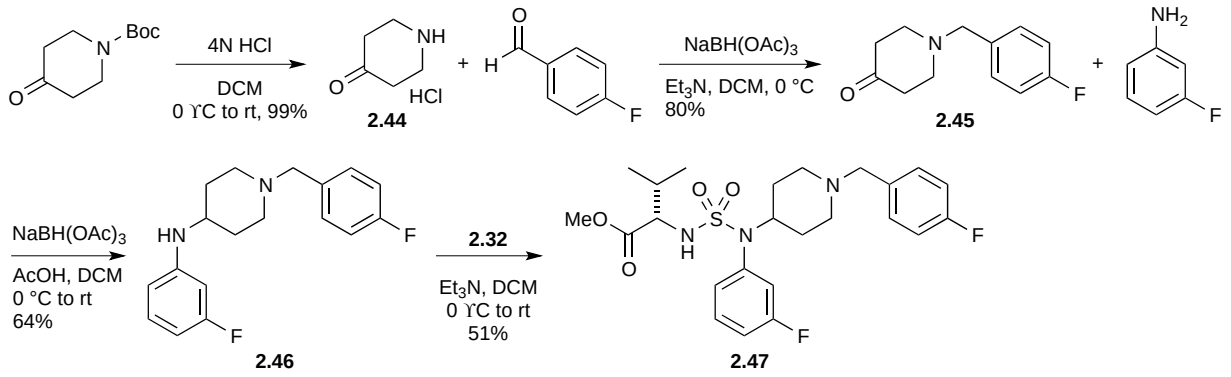
sulfamoyl chlorides **2.32** and **2.33** at a low temperature.[31] The sulfamoyl chloride **2.32** and **2.33** then reacted with *p*-F-phenyl piperidinyl amine **2.31** to furnish sulfamides **2.34** and **2.35**. The secondary piperidinyl amine **2.36** and **2.37** were prepared using HCl (4N in 1,4-dioxane), and reductive amination afforded sulfamides **2.38**, **2.39**, **2.40** and **2.41**. Acylation also proceeded from compound **2.36** and **2.37** to generate compound **2.42,**[32] and compound **2.43** was synthesized from D-valine.



Scheme 2.4. *Synthetic route for the preparation of compounds **2.38–2.43**.*

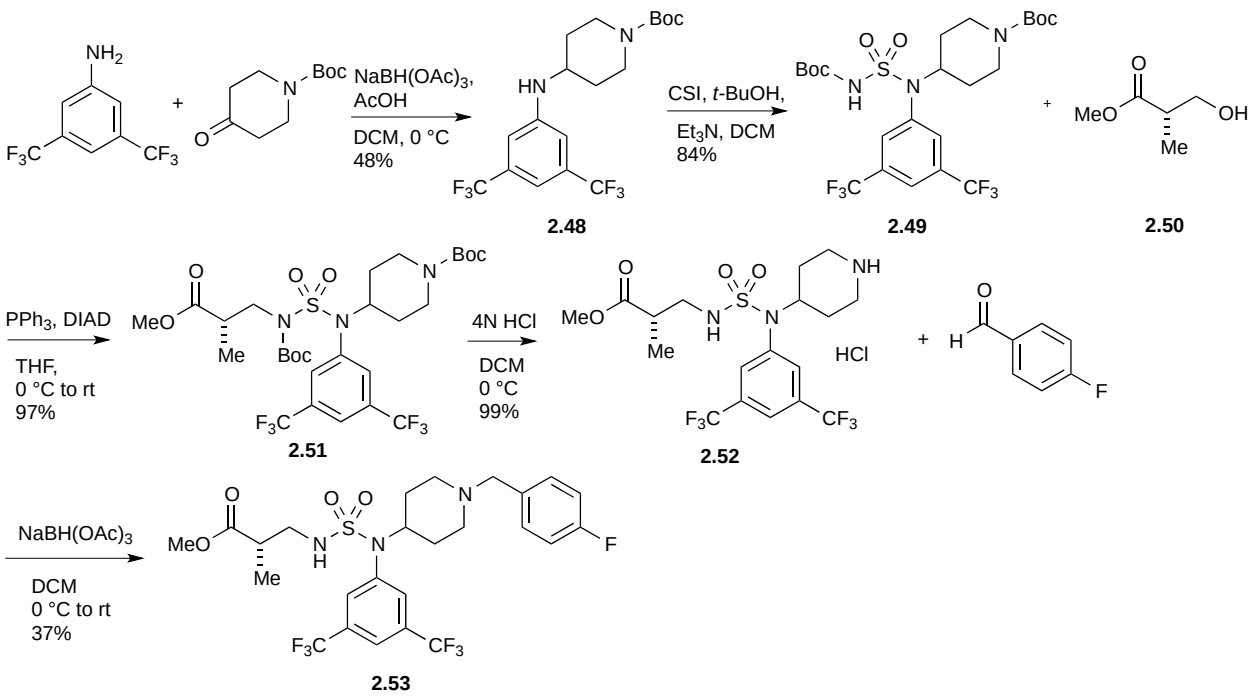
Synthesis of **2.47** was accomplished through the route described below (Scheme 2.5). *N*-Boc-1-piperidone was deprotected using HCl (4N in 1,4-dioxane) to generate free-base **2.44**,

which was reacted with benzaldehyde under reductive amination conditions to afford compound **2.45** in 80% yield. Reductive amination reaction with *m*-fluoroaniline at 0 °C to room temperature, generated amine **2.46** which was coupled with **2.32** under basic condition to complete the synthesis of sulfamide compound **2.47** in 51%.



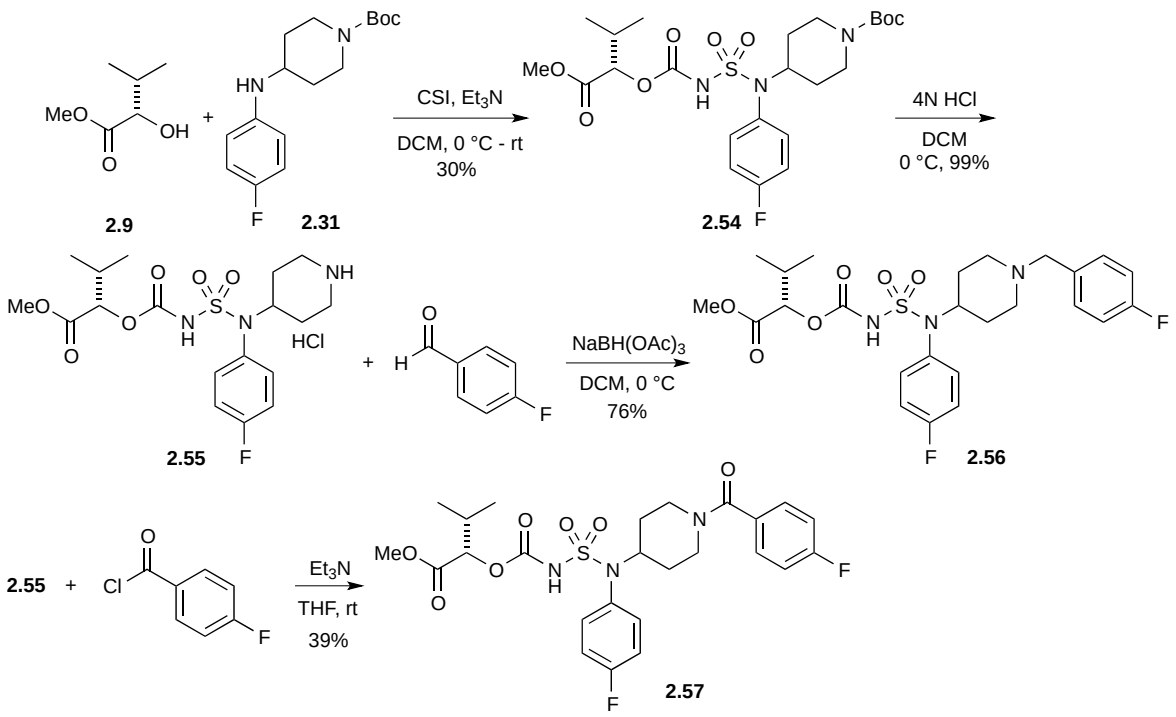
Scheme 2.5. *Synthesis of sulfamide compound **2.47** derived from L-valine.*

Secondary amine **2.48** was prepared by the reductive amination of 3,5-bis-trifluoromethyl aniline and *N*-Boc-4-piperidinone (Scheme 2.6). The secondary amine **2.48** was coupled with chlorosulfonyl isocyanate (CSI) and *t*-BuOH in the presence of Et_3N as a base to generate the corresponding Boc-protected sulfamide moiety **2.49**. To diversify the amino ester moiety of the compound, methyl (*S*)-(+)-3-hydroxy-2-methyl propionate **2.50** was used for the Mitsunobu reaction with intermediate **2.49** to afford the corresponding compound **2.51**. Compound **2.51**, when treated with 4N HCl solution in 1,4-dioxane at 0 °C, yielded **2.52**. Intermediate **2.52** was reacted with *p*-fluorobenzaldehyde to furnish compound **2.53** through reductive amination, which was carried out at 0 °C and warmed to room temperature.



Scheme 2.6. *Preparation of compound **2.53**.*

An alternative route to the modified amino ester part is through the use of chlorosulfonyl isocyanate (CSI) chemistry (Scheme 2.7). A solution of CSI and α-hydroxyl ester **2.9**, generated from L-valine via diazotization and esterification, was cannulated to a solution of secondary amine **2.31** in CH_2Cl_2 at 0 °C to obtain a carbamate compound **2.54** in moderate yield. Generation of secondary amine **2.55** via Boc-deprotection of **2.54** and reductive amination with benzaldehyde, afforded the sulfur-containing carbamate compound **2.56**. The coupling reaction with **2.55** and *p*-fluorobenzoyl chloride generated compound **2.57** containing a carbonyl group.



Scheme 2.7. *Preparation of compound **2.56** and **2.57**.*

2.5. Biology

2.5.1. Expression and Purification of Tdp1.

Wild-type and mutant (H493R) human Tdp1 enzymes were expressed in *E. coli* BL21 (DE3) cells and purified as described earlier,[33] following the described method in reference (*Nucleic Acids Res*. **2007**, *35*, 4474–4484). Human Tdp1 expressing plasmid pHN1910 (a gift from Dr. Howard Nash, Laboratory of Molecular Biology, National Institute of Mental Health, National Institutes of Health) was constructed using vector pET-15b (Novagen, Madison, WI, USA) with full-length human Tdp1 and an additional His-tag sequence of MGSSHHHHHHSSGLVPRGSHMLEDP in its N terminus. The His-tagged human Tdp1 was purified from Novagen BL21 cells using chelating sepharose^{TM} fast flow column (Amersham Biosciences, Piscataway, NJ, USA) according to the company's protocol. The collected

fractions were assayed immediately for Tdp1 activity. Fractions that showed Tdp1 activity were pooled and dialyzed in 20 % glycerol, 50 mM Tris-HCl, pH 8.0, 100 mM NaCl, 10 mM β-mercaptoethanol and 2 mM EDTA. Dialyzed samples were aliquoted and stored at -80 °C. Tdp1 concentration was determined using the Bradford protein assay (Bio-Rad Laboratories, Hercules, CA, USA). Tdp1 purity was determined as a single ~70kDa band representing over 95% of the detectable proteins stained by Coomassie after SDS–polyacrylamide gel electrophoresis (SDS-PAGE).

2.5.2. Tdp1 Gel-Based Assay

A 1 nM 5'-^{32}P-labeled DNA substrate was incubated with 0.1 nM recombinant Tdp1 in the absence or presence of inhibitor for 20 min at 25 °C in a reaction buffer containing 50 μM Tris-HCl (pH 7.5), 80 mM KCl, 2 mM EDTA, and 40 μg/mL bovine serum albumin (BSA). Reactions were terminated by the addition of two volumes of gel loading buffer (96% (v/v) formamide, 10 μM EDTA, 1% (w/v) xylene cyanol, and 1% (w/v) bromphenol blue). The samples were subsequently heated to 95 °C for 5 min and subjected to 18% sequencing gel electrophoresis. A concentration of 100 nM was used when employing the SCAN1 mutant H493R. In addition, H493R reactions were divided in half. One-half of the reaction was run on a sequencing gel, while the other half was analyzed by 4-20% SDS-PAGE electrophoresis. Imaging and quantification were performed using the Typhoon 8600 and ImageQuant software (Molecular Dynamics), respectively.

The results of the final gel study with 17 compounds including compound **2.21** are shown in Figure 2.10. While the compound **2.21** is showing Tdp1 inhibition identical to our previous observations, the compound **2.47** is inactive against Tdp1. This experiment is showing very

interesting result. Compound **2.21** has (*R*)-configuration on the amino ester moiety, whereas compound **2.47** has (*S*)-configuration. From this observation, it could be concluded that the chirality of the compound contributes to the affinity of inhibitor for the active site of the Tdp1 enzyme.

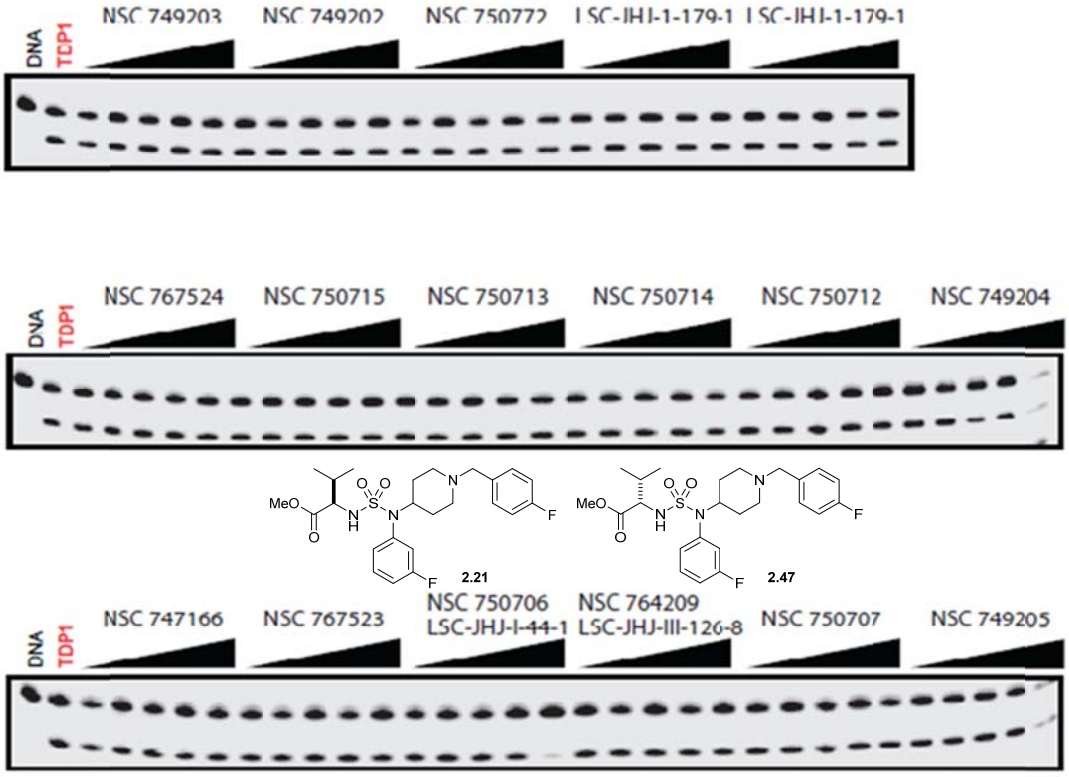


Figure 2.10. *Gel study with final compounds.*

2.6. Molecular Modeling of Piperidinyl Sulfamide Derivatives

2.6.1. Preparation of ligand structures

The piperidinyl sulfamide derivatives described in this paper were drawn in the program ChemBioDraw Ultra 12.0.[34] Additional molecular construction and modeling of the derivatives were performed using the building tools available in MACROMODEL 2011 (Schrödinger

Inc.).[35] The ligands were minimized using the OPLS-2005 force field. The preparation procedure in GLIDE requires the preparation of the structures in the appropriate ionization state. We used 2D to 3D conversion program LigPrep[36] to generate accurate energy minimized molecular structures, expands tautomeric and ionization states, ring conformations, and stereoisomers to produce broad chemical and structural diversity of ligand libraries for further computational analyses.

2.6.2. Molecular Docking

To investigate the binding mode of the piperidinyl sulfamide derivatives to Tdp1 at the molecular level, we performed docking analysis using the high-resolution structure of Tdp1, co-crystallized with a peptide-vanadate-DNA substrate mimic (PDB accession code 1NOP). After construction of a molecular model from 1NOP (published earlier)[30] the prepared ligands were docked into the substrate binding pocket of Tdp1 using the program GLIDE (Schrödinger)[37] with the Extra Precision mode. A set of Grid files was generated with residues H263, K265, H493 and K495 at the center of the binding box defining the space through which the center of the docked ligand is allowed to move. The size of the cube box was set to 16 Å edge in length in order to explore a large region of the protein. To conduct a more precise analysis of docked poses of the ligands, we mapped the output docking poses to the pharmacophores of the lead compounds[30] using absolute positioning in program MOE.[38]

2.7. Conclusion

Overall, the routes described in this chapter are applicable to the synthesis of sulfamides related to a promising Tdp1 inhibitor. We identified piperidinyl sulfamide derivative **2.21**,

which has exhibited inhibitory activity against Tdp1 at low μM concentrations. The inhibitory activity was confirmed using a gel-based assay. Through the analysis of concentration versus percentage inhibition curves, we estimated the IC_{50} value for **2.21** as 23.7 μM (Figure 2.7). To investigate the binding mode of piperidinyl sulfamide derivatives to Tdp1 at the molecular level, we studied docking analysis. From a stereoview representation of **2.21** (Figure 2.10), we found that the benzyl piperidine moiety is oriented towards the Tdp1 active site and hydrophobic amino ester moiety forms a hydrogen bond with Tyrosine 204. We are currently investigating more compounds with varying pharmacophores that might be active against Tdp1.

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CHAPTER 3

Study of Anticancer Activity of Piperidinyl Sulfamides Derivatives

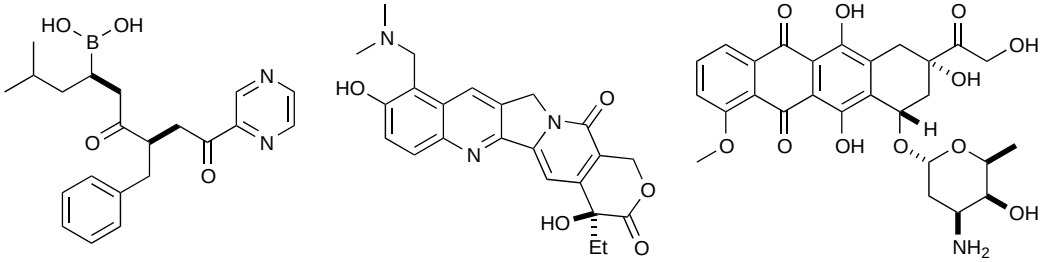
Using the USA National Cancer Institute 60 Human Cancer Cell Line (NCI 60) Screen

3.1. Introduction

Cancer is not one disease, but rather many diseases in which abnormal cells divide without control and are able to occupy other tissues. Cancer cells can spread to other parts of the human body through the blood and lymph systems.[1] Damaged or mutated DNA affects normal cell growth and division, while the immortal cells become a mass of tissue called tumor. In spite of enormous developments in the field of medical research area, which have resulted in higher cure rates for a number of malignancies, cancer is the second ranked leading cause of death after heart disorders in developing, as well as, advanced countries.[2] Although major advances have been made in the chemotherapeutic treatment of some patients, high obligation to the demanding task of discovering new anti-cancer drugs remains crucially important. As a major pioneering cancer research center, the US National Cancer Institute (NCI) has played a significant role in leading the discovery and development of cancer treatment. Since 1955, NCI has provided screening support to cancer researchers globally. In the late 1980s, 60 anticancer drug screens were developed with the aim of changing the emphasis of drug discovery from murine neoplasms (household rats and mice tumors) to human solid tumors as an *in vitro* drug-discovery tool.[3] Since then, it was available to identify the clinical activity of the compounds for the human adult tumor, such as lung, colon, breast, and prostate cancers.

The compounds shown in Figure 3.1 are examples identified by the NCI 60 cell line screen. The first boronic acid compound (NSC 681239, Bortezomib, **3.1**) is the first therapeutic proteasome inhibitor, which was synthesized in 1995 at Myogenics Topotecan (NSC 609699, **3.2**),[4] and is a TOP1 inhibitor to treat ovarian cancer and lung cancer, as well as other types of

cancers.[5] Doxoruicin (NSC 123127, **3.3**) is microbial product for breast cancer, bladder cancer, and stomach cancer.[6]



NSC 681239 (Bortezomib, **3.1**) NSC 609699 (Topotecan, **3.2**) NSC 123127 (Doxorubicin, **3.3**)

Figure 3.1. *Examples of compounds identified by NCI 60 cell line*.

The discovery and development of potential anticancer drugs by NCI are based on a series of sequential screening and detailed testing steps to identify new, effective lead compounds and to eliminate inactive and/or highly toxic materials from further consideration. With this concern, Tdp1 related compounds were submitted and screened against the NCI-60 cell line, and the results will be discussed.

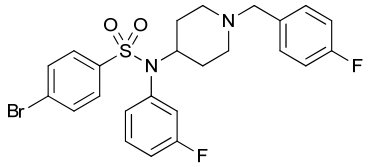
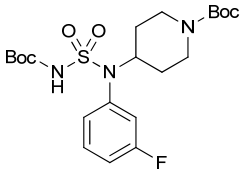
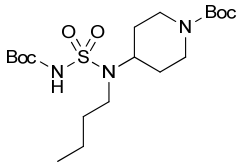
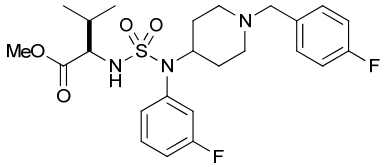
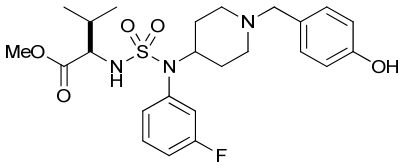
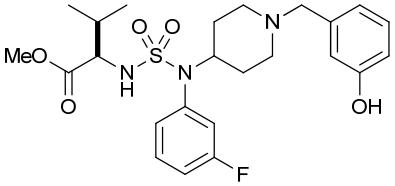
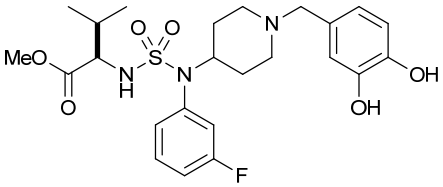
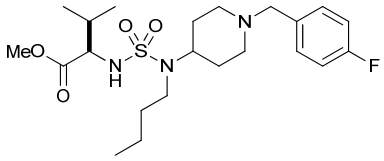
3.2. NCI 60 Cell Line Screening

Pharmacological evaluation of anticancer activity was carried out on selected compounds by the developmental therapeutic program of *Frederick National Laboratory for Cancer Research*, Frederick, Maryland. All selected compounds for *in vitro* cancer screening have been given a unique NSC (National Service Center) number. Compounds in Table 3.1 were submitted to the NCI-60 cell line screen and evaluated for their *in vitro* anticancer activity at a single dose (1×10^{-5}M or 10 μM) against the full NCI-60 cell line panels (Table 3.1). Some sulfamide intermediates were also submitted to compare the NCI-60 cell line results with final compound

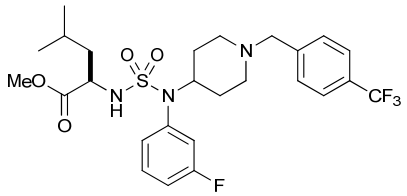
results. Details of the methodologies for the NCI-60 cell line screening are described at http://dtp.nci.nih.gov/branches/btb/ivclsp.html.[3, 7] Briefly, the panel is organized into nine subpanels representing diverse histologies: leukemia, melanoma, and cancers of lung, colon, kidney, ovary, breast, prostate, and central nervous system. The human tumor cells are grown in supplemented RPMI 1640 medium containing 5% fetal bovine serum and 2 mL glutamine for 24 h. The cells are inoculated into 96-well microtiter plates in 100 μL at plating densities ranging from 5,000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates are incubated at 37 °C, 5 % CO_2, 95 % air and 100 % relative humidity for 24 h prior to addition of experimental drugs. The submitted compounds **2.7-2.57** in Table 3.1 were dissolved in DMSO and incubated with cells at five concentrations with 10-fold dilutions, the highest being 10^{-4} M and the others being 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8} M. The assay is terminated by the addition of cold trichloroacetic acid, and the cells are fixed and stained with sulforhodamine B. Bound stain is solubilized, and the absorbance is read on an automated plate reader. The cytostatic parameter that is 50% growth inhibition (GI_{50}, concentrations required to inhibit the growth by 50%) was calculated from time zero, control growth, and the five concentration level absorbance. The cytotoxic parameter that is inhibitory concentrations (LC_{50}, lethal concentration, standard measure of the toxicity of the medium that kills half of the sample population in a specified period, lower number means more toxic) represents the average of two independent experiments. *In vitro* screening is a two-stage process starting with the evaluation of all compounds against the NCI-60 human tumor cell lines with a single dose of 10.0 μM, which is done by following the same protocol as for five-dose screening. The output from the single dose screen was reported as a mean graph (given in the Supplementary data section with general interpretation) of the percentage growth of the treated

cells. Results of each test agents are reported as percentage growth of the treated cell when compared with untreated control cells. The value numbers from the single dose screen were analyzed by the COMPARE program with only the compounds that showed more than 60% of growth inhibition in at least 8 tumor cell lines selected for further testing, while the others were assumed as inactive.

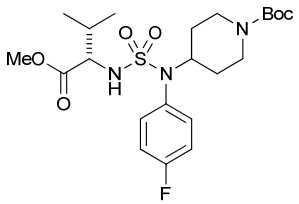
Table 3.1. *List of compounds screened for NCI 60 cell lines.*

NSC No.	Structure	NSC No.	Structure
750772 (**2.7**)		750710 (**2.15**)	
750711 (**2.16**)		750706 (**2.21**)	
747166 (**2.22**)		767523 (**2.23**)	
767524 (**2.24**)		750707 (**2.25**)	

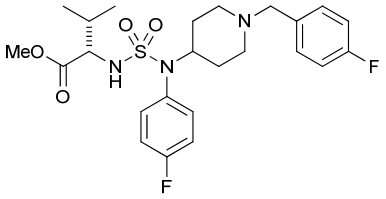
749205 (**2.30**)



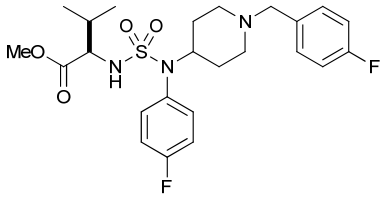
749201 (**2.34**)



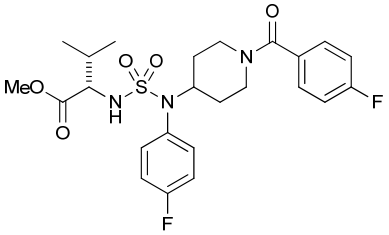
750715 (**2.38**)



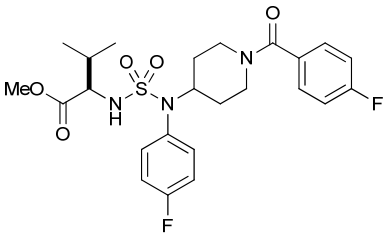
750713 (**2.40**)



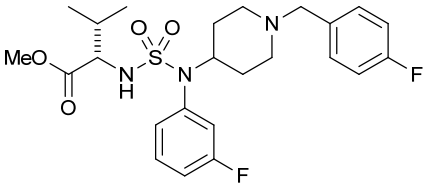
750714 (**2.42**)



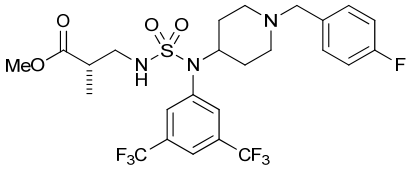
750712 (**2.43**)



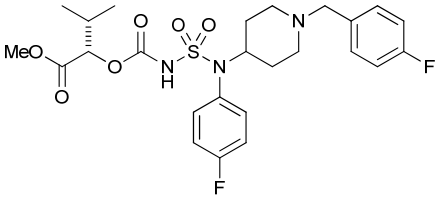
764209 (**2.47**)



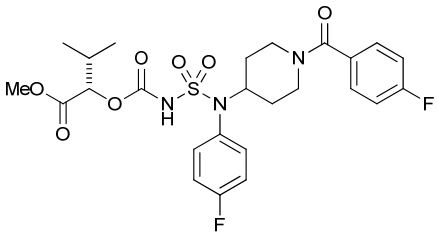
749204 (**2.53**)



749203 (**2.56**)



749202 (**2.57**)



3.2.1. *In vitro* anticancer activity

The one-dose data for the aforementioned screen is reported as a mean graph of the percent growth of treated cells and will be similar in appearance to the mean graphs from the 5-dose assay. The number reported for the one-dose assay is growth relative to the no-drug control, and relative to the time zero number of cells. This allows detection of both growth inhibition

(values between 0 and 100) and lethality (values less than 0). This is the same as for the 5-dose assay, described on http://dtp.nci.nih.gov/branches/btb/ivclsp.html. For example, a value of 100 means no growth inhibition. A value of 40 would mean 60% growth inhibition. A value of 0 means no net growth over the course of the experiment. A value of -40 would mean 40% lethality. A value of -100 means all cells are dead. Information from the one-dose mean graph is available for COMPARE analysis (http://dtp.nci.nih.gov/docs/compare/compare.html). The primary, one-dose screening data showed that **NSC 749204** (**2.53**) was active, while other compounds were determined as inactive. Table 3.2 is the summary of one-dose experiments for each compound. Even if it was not selectively considered using a 60% of growth inhibition as criterion, many compounds were moderately sensitive on the non-small cell lung cancer (HOP-92) and leukemia (HL-60(TB) cell lines. Compound **2.7** (NSC 750772) showed 35.19% growth inhibition against the RPMI-8226 cell line (Leukemia), compound **2.15** (NSC 750710), 67.08% against the HOP-92 cell line (Non-small cell lung cancer), compound **2.16** (NSC 750711), 78.38% against the HL-60(TB) cell line (Leukemia), compound **2.21** (NSC 750706), 43.68% against the HL-60(TB) cell line (Leukemia), compound **2.22** (NSC 747166), 12.34% against the HOP-62 cell line (Non-small cell lung cancer), compound **2.23** (NSC 767523), 67.24% against the UO-31 cell line (Renal cancer), compound **2.24** (NSC 767524), 33.30% against the CCRF-CEM cell line (Leukemia), compound **2.25** (NSC 750707), 69.67% against the UO-31 cell line (Renal cancer), compound **2.30** (NSC 749205), 39.58% against the HOP-92 cell line (Non-small cell lung cancer), compound **2.34** (NSC 749201), 71.49% against the HOP-92 cell line (Non-small cell lung cancer), compound **2.38** (NSC 750715), 76.86% against the CCRF-CEM cell line (Leukemia), compound **2.40** (NSC 750713), 39.99% against the HL-60(TB) cell line (Leukemia), compound **2.42** (NSC 750714), 43.07% against the HL-60(TB) cell line (Leukemia), compound

2.43 (NSC 750712), 62.35% against the A498 cell line (Renal cancer), compound **2.47** (NSC 764209), 87.42% against the SNB-75 cell line (CNS cancer), compound **2.53** (NSC 749204), 19.49% against the HT29 cell line (Colon cancer), compound **2.56** (NSC 749203), 41.46% against the MOLT-4 cell line (Leukemia), and compound **2.57** (NSC 749202), 83.92% against the NCI-H322M cell line (Non-small cell lung cancer). A compound that reduced the growth of a cell line to 32% or less (negative number indicate kills), is considered *in vitro* active.[8,9] The output from the NCI 60-cell lines single dose screen of **NSC 749204** was reported as a mean graph (Figure 3.2).

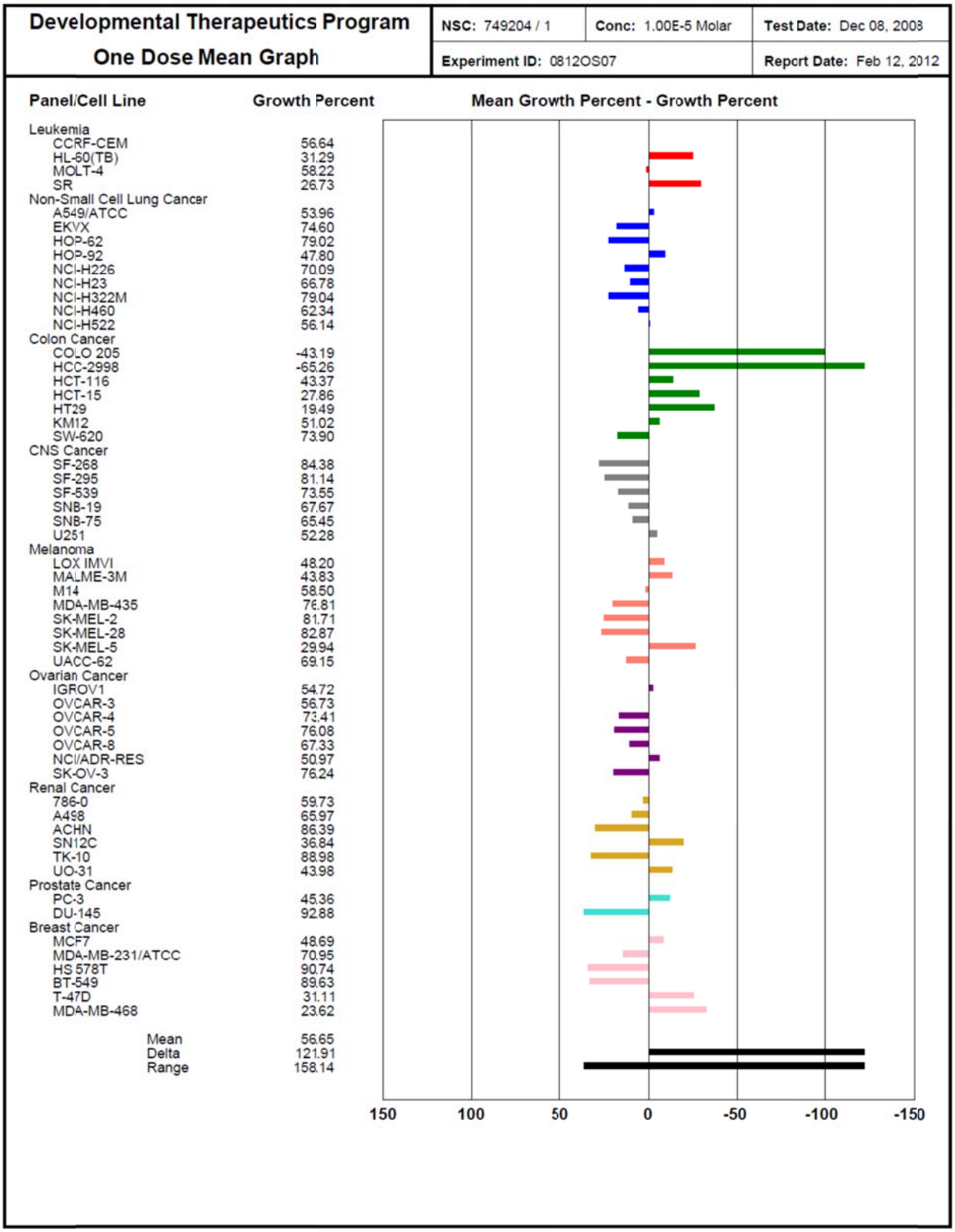
Table 3.2. *Anti-cancer screening data of compounds*.

Comp. No. (NSC No.)	60 cell line assay in one dose at 10^{-5} concentration						
	Range of growth percentage	Most sensitive cell line	Growth % of most sensitive cell line	Mean	Delta	range	activity[a]
2.7 (750772)	35.19 to 112.96	Leukemia (RPMI-8226)	35.19	82.41	47.22	77.77	inactive
2.15 (750710)	67.08 to 134.43	Non-small cell lung cancer (HOP-92)	67.08	100.62	33.54	67.35	inactive
2.16 (750711)	78.38 to 132.86	Leukemia (HL-60(TB))	78.38	103.06	24.68	54.48	inactive
2.21 (750706)	43.68 to 115.29	Leukemia (HL-60(TB))	43.68	81.90	38.22	71.61	inactive
2.22 (747166)	12.34 to 208.78	Non-small cell lung cancer (HOP-62)	12.34	100.63	88.29	196.44	**active**
2.23 (767523)	67.24 to 117.68	Renal Cancer (UO-31)	67.24	97.34	30.10	50.44	inactive
2.24 (767524)	33.20 to 109.93	Leukemia (CCRF-CEM)	33.20	84.99	51.79	76.73	inactive
2.25 (750707)	69.67 to 118.35	Renal cancer (UO-31)	69.67	98.62	28.95	48.68	inactive
2.30 (749205)	39.58 to 107.51	Non-small cell lung cancer (HOP-92)	39.58	78.32	38.74	67.93	inactive
2.34	71.49 to 130.53	Non-small cell lung cancer	71.49	97.81	26.32	59.04	inactive

(749201)		(HOP-92)					
2.38 (750715)	76.86 to 124.74	Leukemia (CCRF-CEM)	76.86	98.79	21.93	47.88	inactive
2.40 (750713)	39.99 to 131.35	Leukemia (HL-60(TB))	39.99	96.65	56.66	91.36	inactive
2.42 (750714)	43.07 to 131.06	Leukemia (HL-60(TB))	43.07	99.49	56.42	87.99	inactive
2.43 (750712)	62.35 to 131.82	Renal cancer (A498)	62.35	100.64	38.29	69.47	inactive
2.47 (764209)	87.42 to 126.22	CNS (SNB-75)	87.42	105.30	17.88	38.80	inactive
2.53 (749204)	19.49 to 92.88	Colon cancer (HT29)	19.49	56.65	121.91	158.14	**active**
2.56 (749203)	41.46 to 116.72	Leukemia (MOLT-4)	41.46	98.11	56.65	84.64	inactive
2.57 (749202)	83.92 to 127.35	Non-small cell lung cancer (NCI-H322M)	83.92	99.52	15.60	43.43	inactive

[a] Compounds active of that particular cell lines, which shown growth inhibition ≤ 32% cell growth reduction following 48 h incubation with test compounds.

Figure 3.2. *Selected NCI60-cell lines screening data for one dose study of* **2.53** *(NSC 749204).*



3.2.2. Five-dose assay

When the result of growth inhibition is satisfied to more than 60% over 8 cell lines, the compound is selected for the five-dose assay. To explain the data, the activity of a one-test compound on three non-small-cell lung cancer cell lines is shown in Figure 3.3.[3] The response parameters GI_{50} (50% growth inhibition) and LC_{50} (50% lethal concentration) are extracted from concentration-response curves by linear interpolation. TGI (total growth inhibition, concentration at which the total growth inhibition is 100%) is indicated as the x-axis intercept. Five-dose assays are carried out with 10-fold dilutions at five different concentrations (0.01, 0.1, 1, 10 and 100 μM). Thus, for EKVX cell line, GI_{50} = 0.12, the TGI = 0.84 and the LC_{50} of effect is not reached.

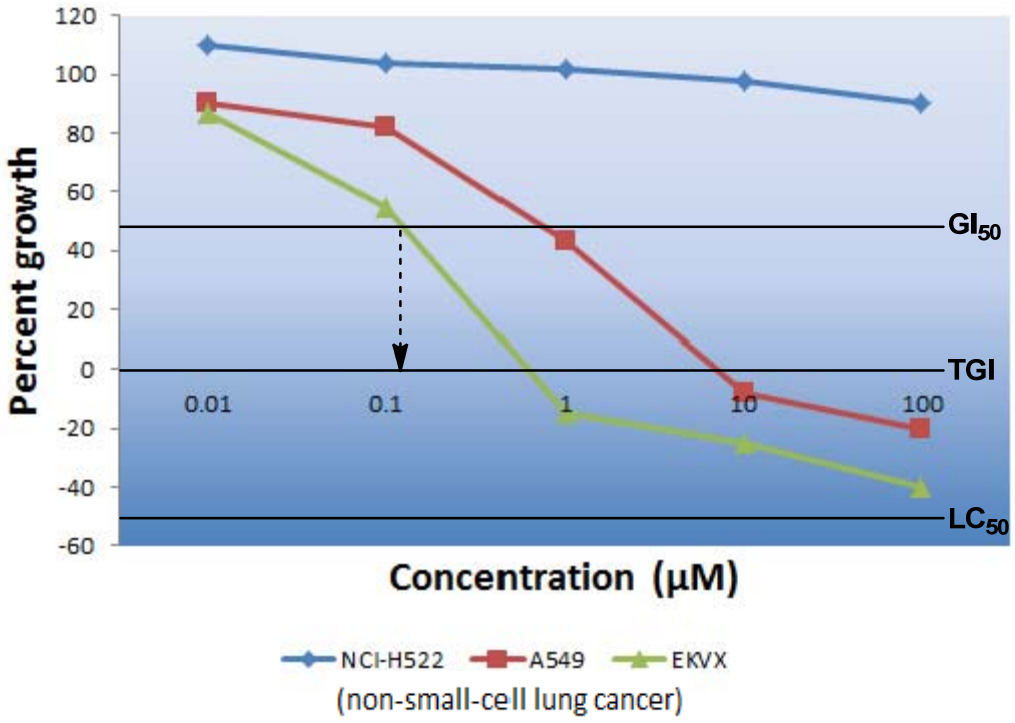


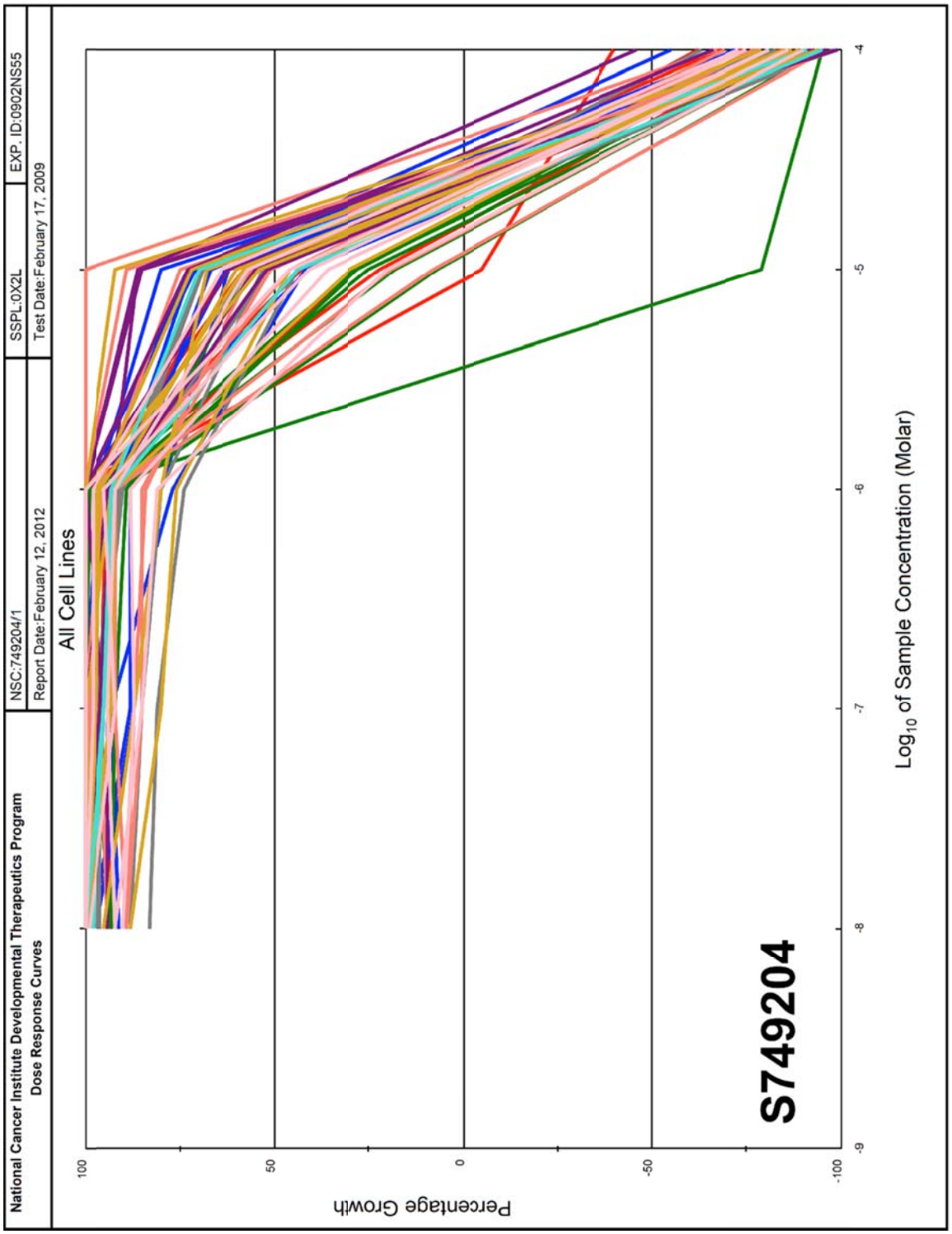
Figure 3.3. *Activity of a one-test compound on three non-small-cell lung cancer cell lines. This graph was depicted with a hypothetical number to explain GI_{50}, TGI, and LC_{50} value.* (This Figure was copied from 'Shoemaker, R. H., The NCI60 human tumor cell line anticancer drug screen. *Nat. Rev. Cancer* **2006**, *6*, 813–823').

The complete *in-vitro* anti-cancer data collected on the 60 subpanel cell lines for the most active compound, **2.53** (NSC 749204), is highlighted in Tables 3.3 and 3.4. Secondary screening was carried out on this active compound in order to determine its cytostatic and cytotoxic activities. Compound **2.53** (NSC 749204) satisfied 60% of growth inhibition as a criterion over 8 cell lines and was further selected for the NCI full panel five-dose assay at 10-fold dilutions using five different concentrations (0.01, 0.1, 1, 10 and 100 μM). The result of compound **2.53** for five-dose screening is given with three response parameters (GI_{50}, TGI and LC_{50}) for each cell line from $\log_{10}$ of sample concentration (molar) vs. percentage growth inhibition curves of nine cancer diseases (Figures 3.4 and 3.5). NCI renamed the IC_{50} value, the concentration that causes 50% growth inhibition, the GI_{50} value (growth inhibitory activity) to emphasize the correction for the cell count at time zero. Namely, GI_{50} is the concentration of test compound where $100 \times (T-T_0) / (C-T_0) = 50$. T is the optical density of the test well after a 48-h period of exposure to test drug, T_0 is the optical density at time zero, and C is the controlled optical density. The GI_{50} value (growth inhibitory activity) corresponds to the concentration of the compound causing 50% decrease in net cell growth, namely it is the growth inhibitory power of the testing compound. The TGI value (cytostatic activity, the inhibition of cell growth and multiplication) is the concentration of the compound resulting in total growth inhibition. The LC_{50} value, signifies cytotoxic activity (the quality of being toxic to cells), and is the concentration of the compound causing a net 50% loss of initial cells at the end of the incubation period of 48 h. Furthermore, a mean graph midpoint (MID) is calculated giving an averaged activity parameter over all cell lines.

Compound **2.53** (NSC 749204) shows moderate to good anticancer activity against many tested cell lines responding nine different panels with GI_{50} values between 1.88 and 21.0 μM.

Regarding sensitivity against some individual cell lines, the compound showed good activity against colon cancer COLO 205 and HCC-2998 cell lines with GI_{50} value 1.88 and 3.01 μM, respectively. Generally, obtained data shows a good sensitivity profile towards colon cancer (least for COLO 205 cell line, GI_{50} = 1.88 μM and maximum for SW-620 cell line, GI_{50} = 11.1 μM). The compound also shows the sensitivity toward leukemia (least for SR cell line, GI_{50} = 3.18 μM and maximum for CCRF-CEM cell line, GI_{50} = 12.8 μM) and the breast cancer subpanel (least for MDA-MB-468 cell line, GI_{50} = 3.25 μM and maximum for MDA-MB-231/ATCC cell line, GI_{50} = 12.7 μM). Compound **2.53** also exhibited sensitivity toward some of cell lines of the melanoma cancer cell panel such as LOX IMVI (GI_{50} = 8.10 μM), MALME-3M (GI_{50} = 6.89 μM), M14 (GI_{50} = 3.75 μM), and SK-MEL-5 (GI_{50} = 3.19 μM). All remaining subpanel cell lines showed maximum sensitive toward tested compounds with not more than 21 μM of GI_{50} concentrations.

Figure 3.4. *Nine panel dose response curves of compound* ***2.53*** *(NSC 749204).*



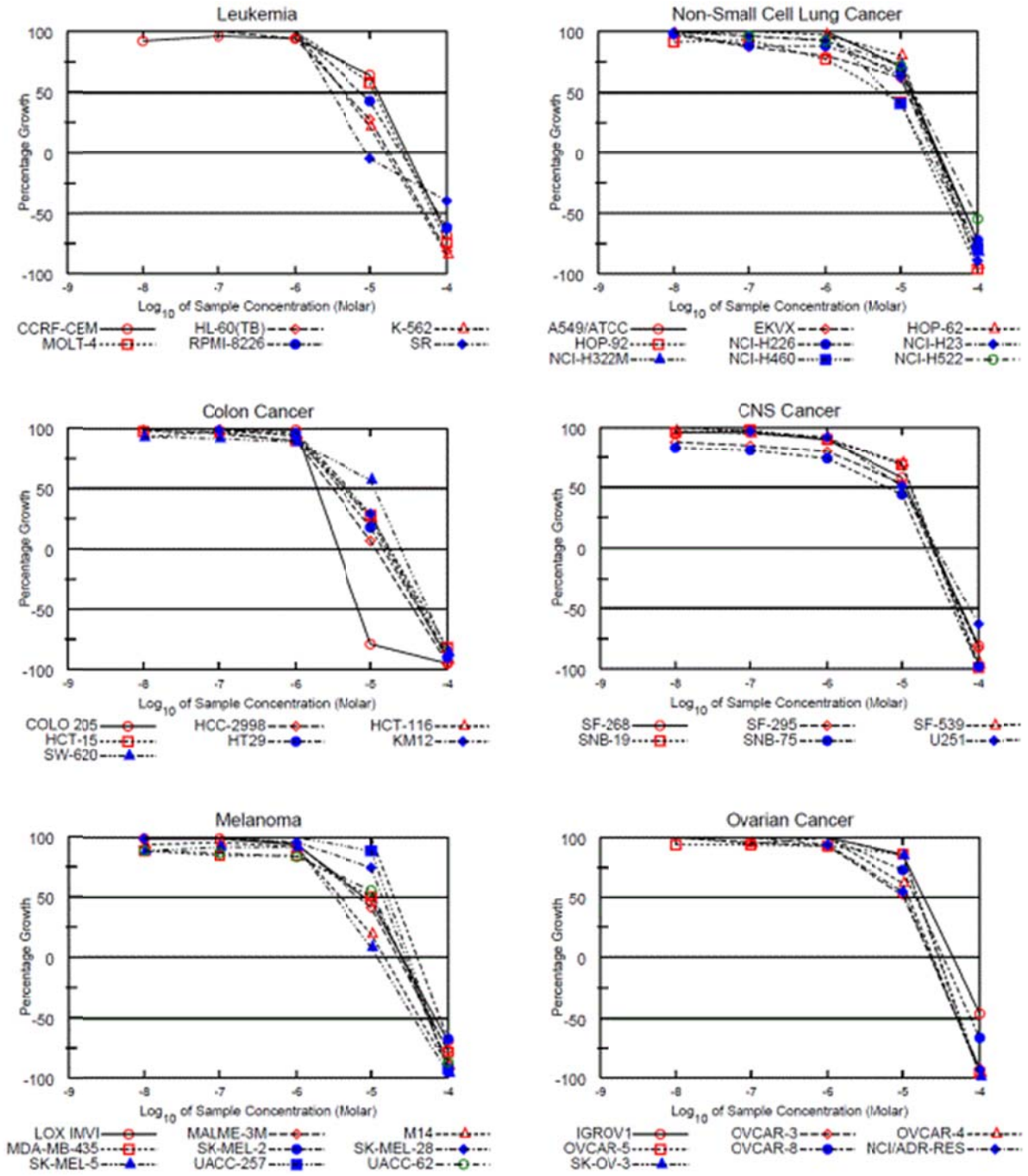


Figure 3.5. *Dose response (% growth verses sample concentration at NCI fixed protocol, μM) obtained from the NCI's in vitro disease-oriented human tumor cells line of compound* ***2.53*** *(NSC 749204) on nine cancer panels.*

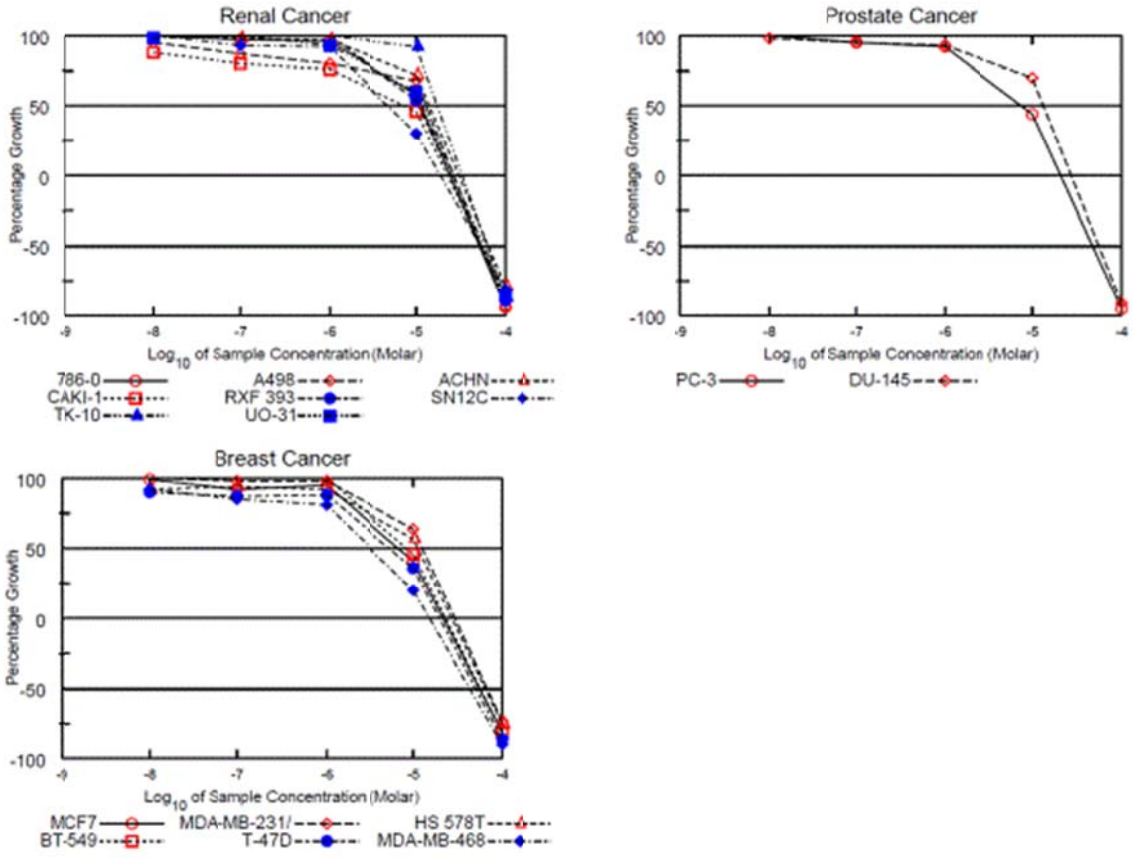


Figure 3.5. *Dose response (% growth verses sample concentration at NCI fixed protocol, μM) obtained from the NCI's in vitro disease-oriented human tumor cells line of compound* ***2.53*** *(NSC 749204) on nine cancer panels. (Continued)*

Table 3.3. *Result of the five-dose assay for compound* ***2.53*** *(NSC 749204).*

National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

NSC : 749204 / 1	Experiment ID : 0902NS55	Test Type : 08	Units : Molar
Report Date : February 12, 2012	Test Date : February 17, 2009	QNS :	MC :
COMI : LSC-JHJ-I-150-1 (81538)	Stain Reagent : SRB Dual-Pass Related	SSPL : 0X2L	

			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	0.568	1.796	1.699	1.753	1727	1.354	0.185	92	96	94	64	-68	1.28E-5	3.07E-5	7.36E-5
HL-60(TB)	0.660	1.253	1.323	1.276	1218	0.829	0.129	112	104	94	28	-81	4.69E-6	1.82E-5	5.25E-5
K-562	0.229	1.171	1.239	1.284	1232	0.433	0.037	107	112	107	22	-84	4.63E-6	1.60E-5	4.78E-5
MOLT-4	0.524	1.392	1.440	1.526	1673	1.026	0.135	105	115	132	58	-74	1.15E-5	2.74E-5	6.54E-5
RPMI-8226	0.559	1.672	1.712	1.761	1714	1.033	0.214	104	108	104	43	-62	7.57E-6	2.56E-5	7.72E-5
SR	0.208	0.518	0.526	0.581	0598	0.199	0.125	102	120	126	-5	-40	3.81E-6	9.22E-6	> 1.00E-4
Non-Small Cell Lung Cancer															
A549/ATCC	0.242	1.163	1.207	1.228	1178	0.895	0.065	105	107	102	71	-73	1.40E-5	3.10E-5	6.91E-5
EKVX	0.628	1.445	1.421	1.342	1282	1.133	0.176	97	87	80	62	-72	1.22E-5	2.89E-5	6.84E-5
HOP-62	0.512	1.153	1.138	1.178	1132	1.027	0.034	98	104	97	80	-93	1.50E-5	2.90E-5	5.62E-5
HOP-92	0.836	1.267	1.229	1.236	1167	1.015	0.033	91	93	77	42	-96	5.76E-6	2.00E-5	4.62E-5
NCI-H226	0.727	1.463	1.449	1.373	1377	1.218	0.201	98	88	88	67	-72	1.32E-5	3.02E-5	6.91E-5
NCI-H23	0.532	1.764	1.753	1.714	1678	1.307	0.058	99	96	93	63	-89	1.22E-5	2.59E-5	5.53E-5
NCI-H322M	0.646	1.480	1.559	1.519	1493	1.246	0.118	110	105	102	72	-82	1.39E-5	2.94E-5	6.21E-5
NCI-H460	0.206	2.115	2.211	2.143	2112	0.997	0.044	105	101	100	41	-79	7.13E-6	2.21E-5	5.77E-5
NCI-H522	0.310	1.859	1.908	1.804	1739	1.424	0.141	103	96	92	72	-55	1.49E-5	3.70E-5	9.18E-5
Colon Cancer															
COLO 205	0.275	0.897	0.959	0.943	0890	0.057	0.015	110	107	99	-79	-95	1.88E-6	3.59E-6	6.85E-6
HCC-2998	0.744	2.842	2.828	2.755	2624	0.889	0.039	99	96	90	7	-95	3.01E-6	1.17E-5	3.62E-5
HCT-116	0.199	1.484	1.399	1.452	1416	0.525	0.016	93	98	95	25	-92	4.41E-6	1.64E-5	4.37E-5
HCT-15	0.448	2.365	2.325	2.304	2181	0.981	0.079	98	97	90	28	-82	4.42E-6	1.79E-5	5.08E-5
HT29	0.137	0.967	0.976	0.980	0918	0.285	0.014	101	102	94	18	-90	3.78E-6	1.46E-5	4.25E-5
KM12	0.205	1.064	1.094	1.053	1037	0.456	0.023	103	99	97	29	-89	4.92E-6	1.76E-5	4.68E-5
SW-620	0.223	1.279	1.205	1.191	1168	0.820	0.031	93	92	89	57	-86	1.11E-5	2.49E-5	5.58E-5
CNS Cancer															
SF-268	0.406	1.430	1.389	1.394	1325	1.004	0.079	96	96	90	58	-81	1.15E-5	2.63E-5	6.02E-5
SF-295	0.773	1.903	1.768	1.735	1679	1.370	0.133	88	85	80	53	-83	1.05E-5	2.45E-5	5.72E-5
SF-539	0.529	1.740	1.703	1.679	1636	1.375	0.023	97	95	91	70	-96	1.32E-5	2.84E-5	5.29E-5
SNB-19	0.666	1.646	1.607	1.623	1548	1.345	0.009	96	98	90	69	-99	1.30E-5	2.58E-5	5.13E-5
SNB-75	0.612	1.170	1.073	1.064	1025	0.857	0.012	83	81	74	44	-98	6.24E-6	2.04E-5	4.58E-5
U251	0.177	1.047	1.061	1.022	0980	0.620	0.065	102	97	92	51	-63	1.02E-5	2.79E-5	7.65E-5
Melanoma															
LOX IMVI	0.231	1.611	1.593	1.594	1531	0.860	0.072	99	99	94	46	-69	8.10E-6	2.50E-5	6.84E-5
MALME-3M	0.783	1.449	1.451	1.448	1415	1.058	0.179	100	100	95	41	-77	6.89E-6	2.23E-5	5.90E-5
M14	0.315	1.245	1.188	1.206	1167	0.494	0.031	94	96	92	19	-90	3.75E-6	1.50E-5	4.28E-5
MDA-MB-435	0.429	1.744	1.593	1.542	1547	1.098	0.093	89	85	85	51	-78	1.02E-5	2.48E-5	6.04E-5
SK-MEL-2	0.369	0.827	0.888	0.896	0917	0.854	0.122	113	115	119	106	-67	2.10E-5	4.10E-5	7.98E-5
SK-MEL-28	0.504	1.412	1.401	1.421	1380	1.182	0.035	99	101	96	75	-93	1.40E-5	2.78E-5	5.53E-5
SK-MEL-5	0.358	1.647	1.511	1.547	1550	0.462	0.014	89	92	92	8	-96	3.19E-6	1.20E-5	3.61E-5
UACC-257	0.837	1.584	1.648	1.650	1596	1.501	0.060	109	109	102	89	-93	1.64E-5	3.08E-5	5.81E-5
UACC-62	0.522	2.012	1.848	1.816	1777	1.353	0.069	89	87	84	56	-87	1.10E-5	2.46E-5	5.51E-5
Ovarian Cancer															
IGROV1	0.198	1.371	1.640	1.682	1549	1.208	0.107	123	126	115	86	-46	1.88E-5	4.49E-5	> 1.00E-4
OVCAR-3	0.405	1.242	1.243	1.209	1187	0.843	0.032	100	96	93	52	-92	1.04E-5	2.30E-5	5.11E-5
OVCAR-4	0.459	1.436	1.442	1.392	1435	1.067	0.017	101	95	100	62	-96	1.19E-5	2.47E-5	5.10E-5
OVCAR-5	0.383	0.946	0.914	0.910	0909	0.870	0.014	94	94	93	86	-96	1.58E-5	2.97E-5	5.58E-5
OVCAR-8	0.228	0.929	0.937	0.968	0954	0.738	0.078	101	106	104	73	-66	1.46E-5	3.34E-5	7.67E-5
NCI/ADR-RES	0.344	1.259	1.255	1.263	1203	0.843	0.023	100	100	94	55	-93	1.07E-5	2.34E-5	5.09E-5
SK-OV-3	0.450	1.126	1.182	1.144	1133	1.025	0.006	108	103	101	85	-99	1.55E-5	2.90E-5	5.43E-5
Renal Cancer															
786-0	0.947	2.397	2.418	2.354	2344	1.784	0.055	101	97	96	58	-94	1.12E-5	2.40E-5	5.11E-5
A498	0.846	1.509	1.475	1.422	1380	1.294	0.051	95	87	80	68	-94	1.29E-5	2.62E-5	5.34E-5
ACHN	0.340	1.337	1.342	1.320	1304	1.058	0.073	101	98	97	72	-79	1.40E-5	3.00E-5	6.45E-5
CAKI-1	0.722	1.845	1.712	1.621	1580	1.235	0.104	88	80	76	46	-86	7.23E-6	2.23E-5	5.36E-5
RXF 393	0.685	1.284	1.346	1.358	1318	1.006	0.075	110	112	106	54	-89	1.06E-5	2.37E-5	5.32E-5
SN12C	0.335	1.231	1.228	1.168	1158	0.600	0.063	100	93	92	30	-81	4.69E-6	1.85E-5	5.23E-5
TK-10	0.877	1.376	1.388	1.414	1385	1.337	0.114	102	108	102	92	-87	1.72E-5	3.27E-5	6.21E-5
UO-31	0.259	1.269	1.251	1.292	1203	0.866	0.040	98	102	93	60	-85	1.17E-5	2.60E-5	5.76E-5
Prostate Cancer															
PC-3	0.349	1.125	1.140	1.090	1061	0.694	0.017	102	95	92	44	-95	7.61E-6	2.08E-5	4.75E-5
DU-145	0.337	1.401	1.379	1.346	1323	1.086	0.032	98	95	93	70	-91	1.34E-5	2.74E-5	5.60E-5
Breast Cancer															
MCF7	0.295	1.592	1.575	1.487	1524	0.833	0.058	99	92	95	41	-81	6.91E-6	2.19E-5	5.62E-5
MDA-MB-231/ATCC	0.326	0.927	0.930	0.918	0913	0.711	0.087	100	98	98	64	-73	1.27E-5	2.93E-5	6.77E-5
HS 578T	0.630	1.152	1.110	1.120	1109	0.927	0.161	92	94	92	57	-75	1.13E-5	2.71E-5	6.51E-5
BT-549	0.995	1.486	1.518	1.533	1487	1.220	0.186	106	109	100	46	-81	8.34E-6	2.29E-5	5.67E-5
T-47D	0.740	1.538	1.458	1.436	1443	1.026	0.102	90	87	88	36	-86	5.34E-6	1.96E-5	5.04E-5
MDA-MB-468	0.456	1.207	1.147	1.097	1067	0.608	0.046	92	85	81	20	-90	3.25E-6	1.53E-5	4.34E-5

Table 3.4. *Anti-tumor activity ($GI_{50}/\mu M$)[a], TGI[b] and toxicity ($LC_{50}/\mu M$)[c] data of* ***2.53*** *(NSC 749204) selected for 5 dose studies for the NCI60-cell lines screen.*

NSC 749204							
Panel/cell line	GI_{50}	TGI	LC_{50}	Panel/cell line	GI_{50}	TGI	LC_{50}
Leukemia				*Melanoma*			
CCRF-CEM	12.8	30.7	73.6	LOX IMVI	8.10	25.0	68.4
HL-60(TB)	4.69	18.2	52.5	MALME-3M	6.89	22.3	59.0
K-562	4.63	16.0	47.8	M14	3.75	15.0	42.8
MOLT-4	11.5	27.4	65.4	MDA-MB-435	10.2	24.8	60.4
RPMI-8226	7.57	25.6	77.2	SK-MEL-2	21.0	41.0	79.8
SR	3.81	9.22	>100	SK-MEL-28	14.0	27.8	55.3
				SK-MEL-5	3.19	12.0	36.1
Non-small cell lung cancer				UACC-257	16.4	30.8	58.1
A549/ATCC	14.0	31.0	69.1	UACC-62	11.0	24.6	55.1
EKVX	12.2	28.9	68.4				
HOP-62	15.0	29.0	56.2	*Ovarian cancer*			
HOP-92	5.76	20.0	46.2	IGROV1	18.8	44.9	>100
NCI-H226	13.2	30.2	69.1	OVCAR-3	10.4	23.0	51.1
NCI-H23	12.2	25.9	55.3	OVCAR-4	11.9	24.7	51.0
NCI-H322M	13.9	29.4	62.1	OVCAR-5	15.8	29.7	55.8
NCI-H460	7.13	22.1	57.7	OVCAR-8	14.6	33.4	76.7
NCI-H522	14.9	37.0	91.8	NCI/ADR-RES	10.7	23.4	50.9
				SK-OV-3	15.5	29.0	54.3
Colon cancer							
COLO 205	1.88	3.59	6.85	*Renal cancer*			
HCC-2998	3.01	11.7	36.2	786-0	11.2	24.0	51.1
HCT-116	4.41	16.4	43.7	A498	12.9	26.2	53.4
HCT-15	4.42	17.9	50.8	ACHN	14.0	30.0	64.5
HT29	3.78	14.6	42.5	CAKI-1	7.23	22.3	53.6
KM12	4.92	17.6	46.8	RXF 393	10.6	23.7	53.2
SW-620	11.1	24.9	55.8	SN12C	4.69	18.5	52.3
				TK-10	17.2	32.7	62.1
CNS cancer				UO-31	11.7	26.0	57.6
SF-268	11.5	26.3	60.2				

SF-295	10.5	24.5	57.2	*Prostate cancer*			
SF-539	13.2	26.4	52.9	PC-3	7.61	20.8	47.5
SNB-19	13.0	25.8	51.3	DU-145	13.4	27.4	56.0
SNB-75	6.24	20.4	45.8				
U251	11.1	27.9	76.5	*Breast cancer*			
				MCF7	6.91	21.9	56.2
				MDA-MB-231/ATCC	12.7	29.3	67.7
				HS 578T	11.3	27.1	65.1
				BT-549	8.34	22.9	56.7
				T-47D	5.34	19.6	50.4
				MDA-MB-468	3.25	15.3	43.4

[a] GI_{50}: 50% growth inhibition, concentration of drug resulting in a 50% reduction in net protein increase compared with control cells.
[b] TGI: total cell growth inhibition
[c] LC_{50}: lethal concentration, concentration of drug lethal to 50% of cells.

The criterion for selectivity of a compound depends on the ratio obtained by dividing the full panel MID (the average sensitivity of all cell lines towards the test agent) by their individual subpanel MID (the average sensitivity of all cell lines of a particular subpanel towards the test agent). Ratios between 3 and 6 refer to moderate selectivity; ratios greater than 6 indicate high selectivity towards the corresponding cell line, while compounds not meeting either of these criteria were rated non-selective.[10] Following this criterion, compound **2.53** (NSC 749204) was found to be mildly selective toward the colon cancer panel. In addition, compound **2.53** was also found to demonstrate mild to no-selectivity in both the leukemia and breast cancer subpanels.

Table 3.5. *Calculated value of GI50 of the cell lines: full cell line panel, MG-MID and selectivity ratio of the compound* ***2.53*** *(NSC 749204).*

Panel	Cell line	GI_{50} (10^{-6} M)			
		Concentration per cell line	Subpanel concentration	Subpanel MID	Selectivity ratio
Leukemia	CCRF-CEM	12.8			
	HL-60(TB)	4.69			
	K-562	4.63	45	7.500	1.340
	MOLT-4	11.5			
	RPMI-8226	7.57			
	SR	3.81			
Non-small cell	A549/ATCC	14.0			
lung cancer	EKVX	12.2			
	HOP-62	15.0			
	HOP-92	5.76			
	NCI-H226	13.2	108.29	12.032	0.835
	NCI-H23	12.2			
	NCI-H322M	13.9			
	NCI-H460	7.13			
	NCI-H522	14.9			
Colon cancer	COLO 205	1.88			
	HCC-2998	3.01			
	HCT-116	4.41			
	HCT-15	4.42	33.52	4.789	2.098
	HT29	3.78			
	KM12	4.92			
	SW-620	11.1			
CNS cancer	SF-268	11.5			
	SF-295	10.5			
	SF-539	13.2	65.54	10.923	0.920
	SNB-19	13.0			
	SNB-75	6.24			
	U251	11.1			
Melanoma	LOX IMVI	8.10			
	MALME-3M	6.89	94.53	10.503	0.957
	M14	3.75			

	MDA-MB-435	10.2			
	SK-MEL-2	21.0			
	SK-MEL-28	14.0			
	SK-MEL-5	3.19			
	UACC-257	16.4			
	UACC-62	11.0			
Ovarian cancer	IGROV1	18.8			
	OVCAR-3	10.4			
	OVCAR-4	11.9			
	OVCAR-5	15.8	97.7	13.957	0.720
	OVCAR-8	14.6			
	NCI/ADR-RES	10.7			
	SK-OV-3	15.5			
Renal cancer	786-0	11.2			
	A498	12.9			
	ACHN	14.0			
	CAKI-1	7.23	89.52	11.190	0.898
	RXF 393	10.6			
	SN12C	4.69			
	TK-10	17.2			
	UO-31	11.7			
Prostate cancer	PC-3	7.61	21.01	10.505	0.957
	DU-145	13.4			
Breast cancer	MCF7	6.91			
	MDA-MB-231/ATCC	12.7			
	HS 578T	11.3	47.84	7.973	1.260
	BT-549	8.34			
	T-47D	5.34			
	MDA-MB-468	3.25			

The log molar concentration of the resulted screening of compound **2.53** (NSC 749204) shown for each of the parameters; for log GI_{50} ranged from -5.73 to -4.68, for log TGI ranged from -5.45 to -4.39, for log LC_{50} ranged from -5.16 to -4.00 (Table 3.6). A mean graph midpoint

(MG-MID) calculated for each of the parameters; log GI_{50} (-5.05), log TGI (-4.64), and log LC_{50} (-4.26).

Table 3.6. *Values of the log molar concentration of response parameter ($log_{10}GI_{50}$, $log_{10}TGI$ and $log_{10}LC_{50}$) of the compound **2.53** (NSC 749204).*

Cancer disease	Used cell lines	$log_{10}GI_{50}$	$log_{10}TGI$	$log_{10}LC_{50}$
Leukemia	CCRF-CEM	-4.89	-4.51	-4.13
	HL-60(TB)	-5.33	-4.74	-4.28
	K-562	-5.33	-4.80	-4.32
	MOLT-4	-4.94	-4.56	-4.18
	RPMI-8226	-5.12	-4.59	-4.11
	SR	-5.42	-5.04	-4.00
Non-small cell lung cancer	A549/ATCC	-4.86	-4.51	-4.16
	EKVX	-4.91	-4.54	-4.16
	HOP-62	-4.83	-4.54	-4.25
	HOP-92	-5.24	-4.70	-4.33
	NCI-H226	-4.88	-4.52	-4.16
	NCI-H23	-4.92	-4.59	-4.26
	NCI-H322M	-4.86	-4.53	-4.21
	NCI-H460	-5.15	-4.65	-4.24
	NCI-H522	-4.83	-4.43	-4.04
Colon cancer	COLO 205	-5.73	-5.45	-5.16
	HCC-2998	-5.52	-4.93	-4.44
	HCT-116	-5.36	-4.78	-4.36
	HCT-15	-5.35	-4.75	-4.29
	HT29	-5.42	-4.84	-4.37
	KM12	-5.31	-4.75	-4.33
	SW-620	-4.95	-4.60	-4.25
CNS cancer	SF-268	-4.94	-4.58	-4.22
	SF-295	-4.98	-4.61	-4.24
	SF-539	-4.88	-4.58	-4.28

	SNB-19	-4.89	-4.59	-4.29
	SNB-75	-5.20	-4.69	-4.34
	U251	-4.99	-4.55	-4.12
Melanoma	LOX IMVI	-5.09	-4.60	-4.16
	MALME-3M	-5.16	-4.65	-4.23
	M14	-5.43	-4.82	-4.37
	MDA-MB-435	-4.99	-4.61	-4.22
	SK-MEL-2	-4.68	-4.39	-4.10
	SK-MEL-28	-4.85	-4.56	-4.26
	SK-MEL-5	-5.50	-4.92	-4.44
	UACC-257	-4.79	-4.51	-4.24
	UACC-62	-4.96	-4.61	-4.26
Ovarian cancer	IGROV1	-4.73	-4.35	-4.00
	OVCAR-3	-4.98	-4.64	-4.29
	OVCAR-4	-4.92	-4.61	-4.29
	OVCAR-5	-4.80	-4.53	-4.25
	OVCAR-8	-4.84	-4.48	-4.12
	NCI/ADR-RES	-4.97	-4.63	-4.29
	SK-OV-3	-4.81	-4.54	-4.27
Renal cancer	786-0	-4.95	-4.62	-4.29
	A498	-4.89	-4.58	-4.27
	ACHN	-4.85	-4.52	-4.19
	CAKI-1	-5.14	-4.65	-4.27
	RXF 393	-4.98	-4.62	-4.27
	SN12C	-5.33	-4.73	-4.28
	TK-10	-4.76	-4.49	-4.21
	UO-31	-4.93	-4.59	-4.24
Prostate cancer	PC-3	-5.12	-4.68	-4.32
	DU-145	-4.87	-4.56	-4.25
Breast cancer	MCF7	-5.16	-4.66	-4.25
	MDA-MB-231/ATCC	-4.90	-4.53	-4.17
	HS 578T	-4.95	-4.57	-4.19
	BT-549	-5.08	-4.64	-4.25

	T-47D	-5.27	-4.71	-4.30
	MDA-MB-468	-5.49	-4.82	-4.36
MID		-5.05	-4.64	-4.26
Delta		0.68	0.81	0.9
Range		1.05	1.1	1.16

3.3. Conclusion

Compounds synthesized for the study of Tdp1 inhibition were screened in the NCI-60 cancer cell line assay to identify their anti-cancer activity. Among the selected compounds for screening, compound **2.53** (NSC 749204) was selected for five-dose experiments and showed moderate-to-good anticancer activity against many tested cell lines responding nine different panels with GI_{50} values between 1.88 and 21.0 μM. Compound **2.53** (NSC 749204) was found to be mildly selective in the colon cancer panel, as well as mildly-to-non-selective in the leukemia and breast cancer subpanel.

3.4. References

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CHAPTER 4

Study of Anticancer Activity of Seven-membered Cyclic Sulfamide Analogs

Using the USA National Cancer Institute 60 Human Cancer Cell Line (NCI 60) Screen

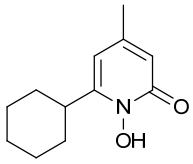
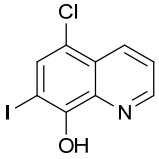
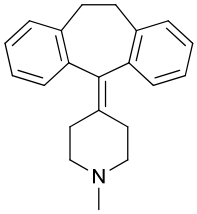
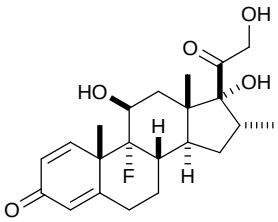
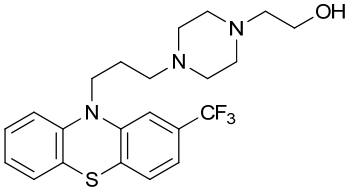
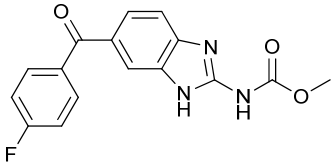
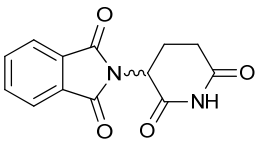
4.1. Introduction

Among the various human diseases, cancer, human immunodeficiency virus infection / acquired immunodeficiency syndrome (HIV/AIDS), and hepatitis C virus (HCV) are among the most devastating diseases in contemporary human history. Accordingly, development and discovery of novel potent, significantly selective, and less toxic antitumor, antiviral drugs is one of the main hurdles to overcome health problems. Manytimes, AIDS patients have accompany cancers and other lethal diseases because the immune system is so weakened by the HIV in a human body.

Drug repositioning (drug repurposing, reprofiling and indication switch) has gained attention from drug discovery.[1] Development of a new pharmaceutical product requires at least 10 to 15 years and costs between $500 million and $2 billion.[2] Thus, the identification and characterization of new pharmacological activities through screening from existing therapeutic drugs is an effective method to accelerate the translation of discoveries in short time and to save the development cost. It also opens new applications of the subsequent target identification and validation.

There are several examples of newly rescued drugs from old drugs **4.1**–**4.7** shown in Table 4.1.[3] These drugs are newly indicated for cancers that affect blood, bone marrow, and lymph nodes. With successful results from old drugs to new treatments, scientists are becoming more and more interested in drug repositioning.[4]

Table 4.1. *Developed treatments for hematological malignancies from old drugs.*

Drugs	Original indications	New indications	Notes
 Ciclopirox (**4.1**)	Fungal infection	Leukemia	Preclinical[5]
 Clioquinol (**4.2**)	Fungal and protozoal infection	Leukemia and Myeloma	Phase I[6]
 Cyproheptadine (**4.3**)	Antihistamine	Leukemia, Mantle cell lymphoma, and myeloma	Preclinical[7]
 Dexamethasone (**4.4**)	Inflammatory and autoimmune conditions	Multiple myeloma	Clinical, FDA approved[8]
 Fluphenazine (**4.5**)	Mental and emotional disorders	Multiple myeloma and other plasma cell neoplasm	Phase II[9]
 Flubendazole (**4.6**)	Antihelmintics	Leukemia, myeloma	Preclinical[10]
 Thalidomide (**4.7**)	Morning sickness	Multiple myeloma	Clinical, FDA approved[11]

Recently, focused studies of the effective inhibitions of selective cancer cells by HIV protease inhibitors have surfaced in the literature (Figure 4.1).[12] Nelfinavir (**4.8**) is an HIV protease inhibitor that is recently being evaluated in an oncology clinical trial as a potential candidate of cancer therapeutic treatment.[13] Liu reported that Nelfinavir (**4.8**) selectively inhibits the growth of HER2-positive breast cancer cells *in vitro*.[12a] Although breast cancer is one of the leading causes of cancer death, only few treatment options are available, and development of new drug targets is still in need. In 2012, Dennis and coworkers reported that Nelfinavir (**4.8**) and bortezomib (**4.9**) are able to induce endoplasmic reticulum (ER) stress, whereas the combination enhances ATF3 and CHOP expression to cause cell death.[12b] Betulinic acid (**4.10**) is a natural product possessing biological activities such as including anti-cancer, anti-malarial, anti-inflammatory and anti-HIV properties.[12c,d] Cobicistat (**4.11**), a potential inhibitor of cytochrome P450 3A enzymes, has been developed as a pharmacoenhancer (booster) for coformulation with HIV drugs.[12e] Tenofovir alafenamide fumarate (TAF), or GS 7340, (**4.12**) is under development by Gilead Sciences for use in the treatment of HIV infection. Cobicistat (**4.11**) is a substrate of breast cancer resistance protein (BCRP), and experimental data shown that Cobicistat (**4.11**) has a competitive mode of inhibition with coadministrated agent **4.12** during intestinal absorption to inhibit breast cancer resistance protein (BCRP).

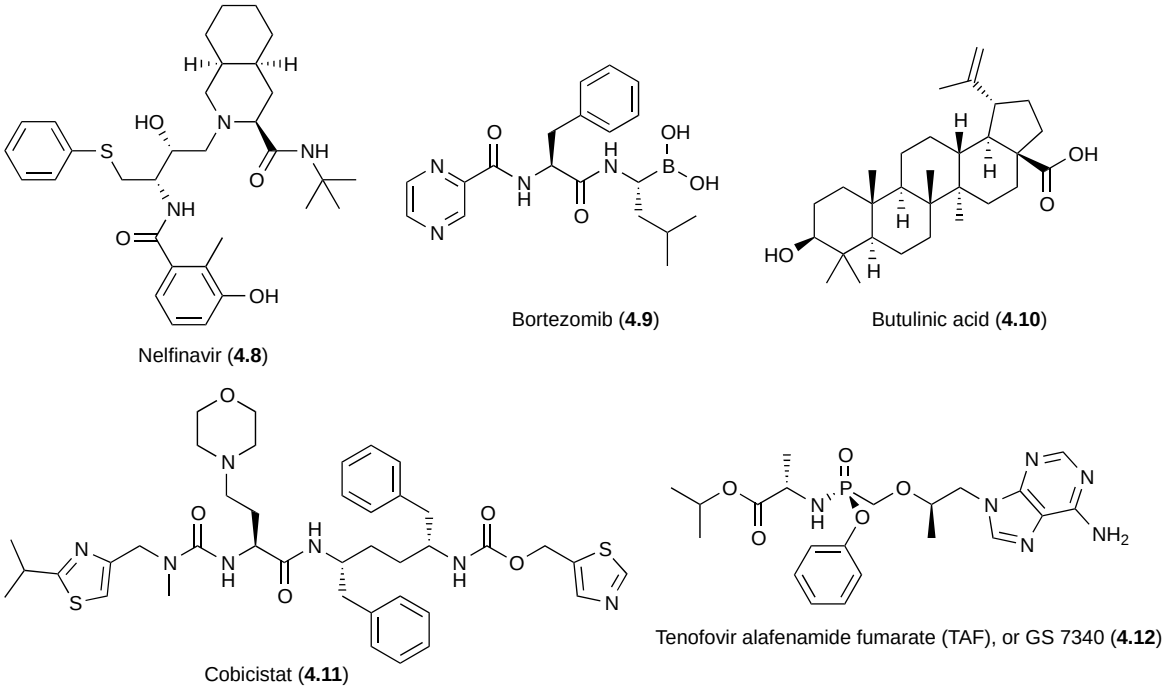


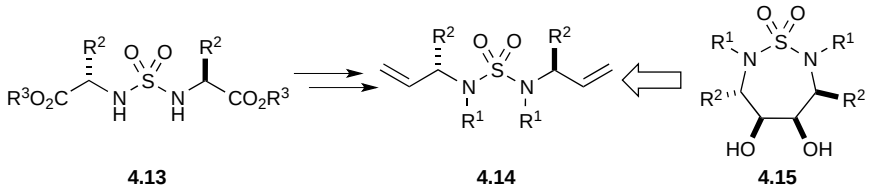
Figure 4.1. *Active compounds possessing biological activity on cancer cell.*

In this regard, we previously synthesized and reported an array of sulfur-based potential HIV-PR inhibitors (DMP 323 analogs in Jung Ho Jun Master Thesis) that we now have submitted to the NCI-60 cancer cell line screen and herein report the summarized results in order to discuss possible opportunities in an oncology study.

4.2. Summary of the synthesis of cyclic sulfamide compounds

Cyclic urea-based compounds have demonstrated antiviral activity and there are prominent examples of highly potent HIV protease inhibitors developed by pharmaceutical industry.[14,15,16] Previous studies have elucidated the effect of substituents, absolute and relative stereochemistry, hydrophobicity etc., on the hydrogen bonding and catalytic aspartate interactions with enzyme, and thereby, overall inhibitor potency.[17] It is well known that modification with sulfamide functional group provides an attractive and versatile opportunity for the selective and potent modulation of protein function.[18] These observations inspired us to

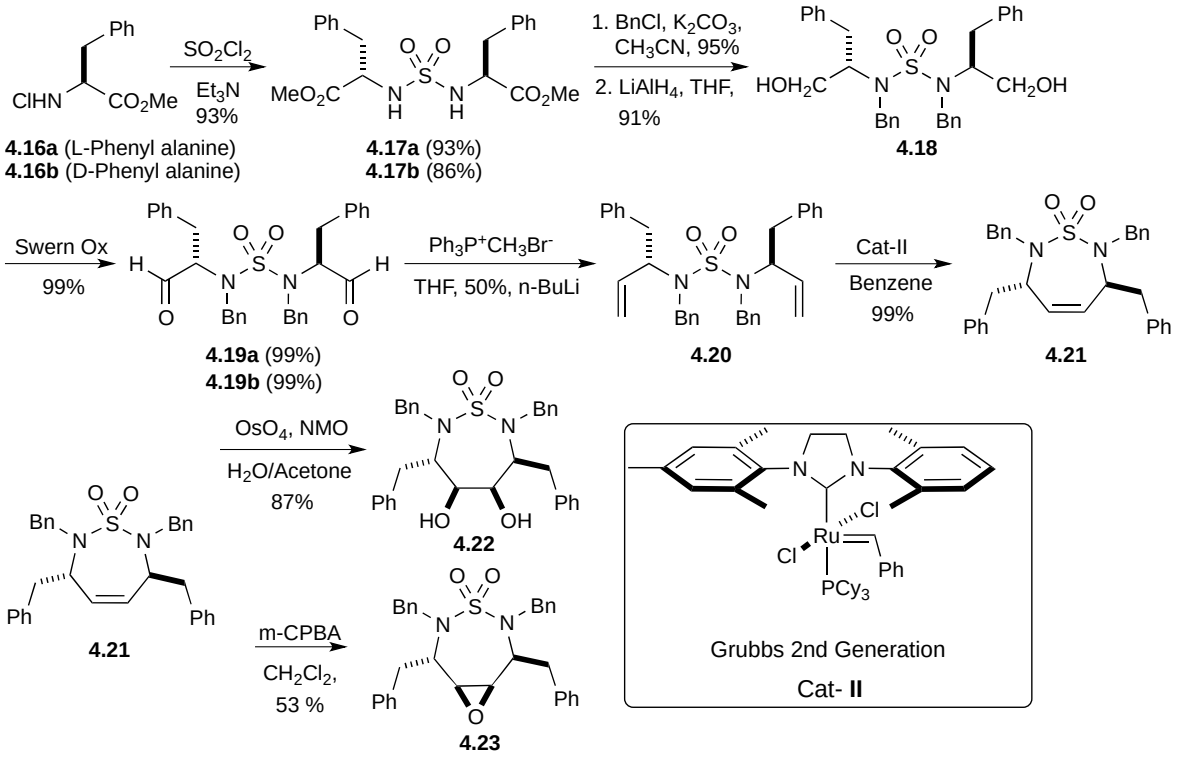
explore the potential of cyclic sulfamide analogs of ureas, for anti-cancer activity. Since it is already published in my Master thesis and paper,[19] the methodologies in synthesizing cyclic sulfamide compounds utilizing ring-closing metathesis (RCM) are only summarized in this section. Synthesis of cyclic sulfamide **4.15**, which has alkyl substituents at the P1/P1' positions, was accomplished from **4.13** (Scheme 4.1). Alkylation of C_2-cymmetric sulfamide **4.13**, followed by the conversion of the ester groups to terminal olefins, RCM, and subsequent dihydroxylation generated cyclic sulfamide **4.15**.



Scheme 4.1. *Symmetric Sulfamides from SO_2Cl_2: Ester as Latent Olefin.*

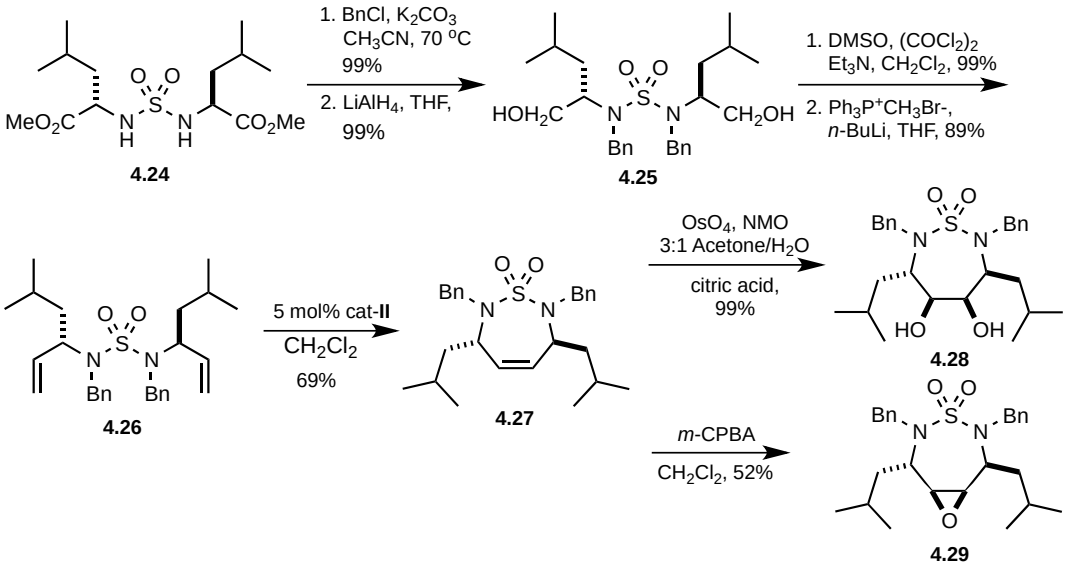
The initial synthesis of amino ester derived C_2-symmetric sulfamides **4.22** and further synthesis is described in Scheme 4.2. Condensation of a slight excess of phenylalanine•HCl with SO_2Cl_2 in CH_2Cl_2 at 0 ºC furnished sulfamides **4.17** in 93% yield. Benzylation using benzyl bromide and reduction by the addition of $LiAlH_4$ cautiously into a reaction mixture in THF at low temperature (0 ºC) allowed primary alcohol **4.18** in 91%. Swern oxidation and following Wittig reaction[20] using *n*-butyl lithium provided terminal olefin **4.20** in moderate yield. Addition of the **G-II** catalyst in 3–6 mol% in refluxing benzene was found to be highly efficient for the metathesis of these substrates to provide cyclic C_2-symmetric sulfamides **4.21** in quantitative yield. This pathway represents the first important example of a C_2-symmetric sulfamide that has functionality occupying the P1/P1' and P2/P2' positions. Each reaction of dihydroxylation and epoxidation from **4.21** yielded diol **4.22** and the 7-membered epoxy sulfamide **4.23**.

Scheme 4.2



With the desire for a more effective route to C_2-symmetric sulfamides with bulky endocyclic substituents occupying the P1/P1' positions, an improved synthetic pathway was explored. This route employed a two-directional chain synthesis[21] on the leucine-derived, C_2-symmetric sulfamide **4.24** (Scheme 4.3). Dialkylation of **4.24** with benzyl bromide under standard conditions (K_2CO_3, CH_3CN, 70 ºC) and $LiAlH_4$ reduction gave the corresponding *bis*-benzylated sulfamide **4.25** in 99% yield. Next, a two-step protocol was used to convert the diol moieties to sulfamide diene **4.26**. Swern oxidation in 99% followed by *bis*-Wittig olefination (PPh_3CH_2Br, *n*-BuLi, THF) yielded **4.26** in 89%. With use of 5 mol% of the **G-II** catalyst, C_2-symmetric sulfamide **4.27** was furnished in moderate yield. Dihydroxylation proceeded smoothly to produce sulfamide diol **4.28** in 99% yield. Alternatively, treatment with *m*-CPBA yielded epoxy sulfamide **4.29** in an un-optimized yield of 52%.

Scheme 4.3



Attempt at the installation of an α-hydroxyl amine at the P2/P2' positions is shown in Scheme 4.4. Several efforts were studied to open the epoxide ring using sodium azide with various conditions (Table 4.2).[22] The first reaction condition using sodium azide and epoxide **4.29** in DMF and H_2O (7:1) did not afford the ring-opened product. The second reaction condition utilized cerium chloride and NaN_3 in CH_3CN and H_2O (9:1), yet also failed to yield the desired products. Finally, the reaction condition using NH_4Cl with NaN_3 in DMF and H_2O (7:1) furnished desired product **4.30** and **4.31** in less than 30% yield (ratio 1.3:1)[23]. These two α-hydroxyl azides, **4.30** and **4.31**, could be distinguished by NOE analysis (Figure 4.2). The relationship between H1 and H3 of **4.30** is *cis,* since no NOE was seen between H2 and either H1 or H3.

Investigations using the Staudinger reaction will be explored in the future. Under the simple reaction condition (PPh_3 and H_2O), sulfamides **4.30** and **4.31** should be able to be converted to α-hydroxyl amines **4.32** and **4.33**. Since the α-hydroxyl amines have a higher

probability to engage in hydrogen bonds, the degree of coordination between these α-hydroxyl amines and aspartate residues present in the active site of HIV-PR could potentially be enhanced in comparison with the corresponding diol compound. These efforts will be reported in due course.

Scheme 4.4. *Ring opening reaction using NaN_3.*

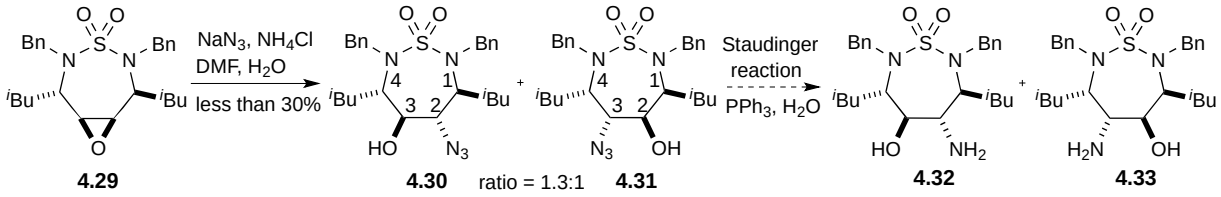
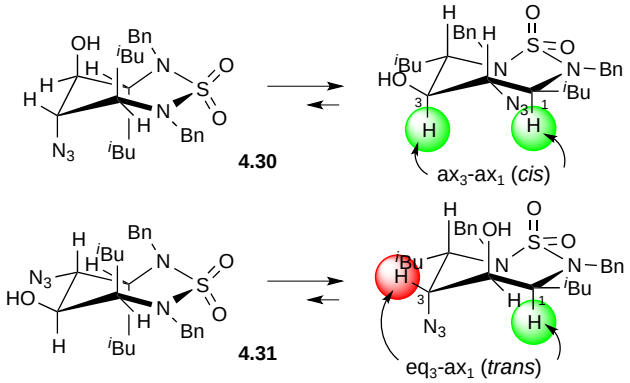


Table 4.2. *Result of ring opening reaction using various conditions.*

Condition	Solvent	Yield
NaN_3	DMF:H_2O = 7:1	None
$CeCl_3 \cdot 7H_2O/NaN_3$	CH_3CN:H_2O = 9:1	None
NH_4Cl/NaN_3	DMF:H_2O = 7:1	30%

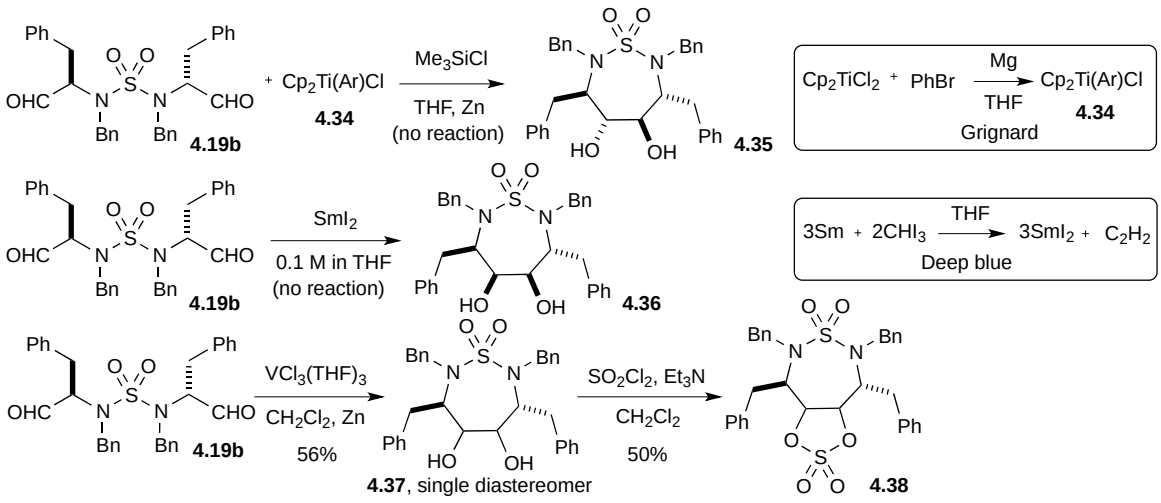
Figure 4.2



An attractive route to diol **4.37** is outlined in Scheme 4.5 and utilizes the pinacol coupling reaction that converts internal or external aldehydes to *cis* or *trans* diols using various catalysts.

The first trial to generate a *trans*-diol using titanium cyclopentadiene catalyst was performed.[24] The reaction of aldehyde **4.19b**, titanium catalyst **4.34**, and TMSCl in the presence of catalytic amount of Zn powder in THF did not furnish the desired trans-diol, **4.35**. Secondly, a widely known method to furnish *cis*-diol using SmI_2 was applied to the pinacol coupling reaction, but the desired product *cis*-diol **4.38** was not generated.[25] Fortunately, by using a protocol reported by Pederson and coworkers,[26] pinacol coupling reaction of the aldehyde **4.19b** with a vanadium (II) reagent, $[V_2Cl_3(THF)_6]_2[Zn_2Cl_6]$ generated diol **4.37** as a single diastereomer in 56% yield. We next embarked upon studies to elucidate the stereochemistry of diol **4.37**.

Scheme 4.5



There are three possible stereochemical outcomes of the pinacol coupling, namely two *trans*-diastereomers **4.37a**, **4.37b**, and the *cis*-diol **4.37c** (Figure 4.3). ^{1}H NMR NOE studies allowed us to assign the product as the *cis*-diol **4.37c**.

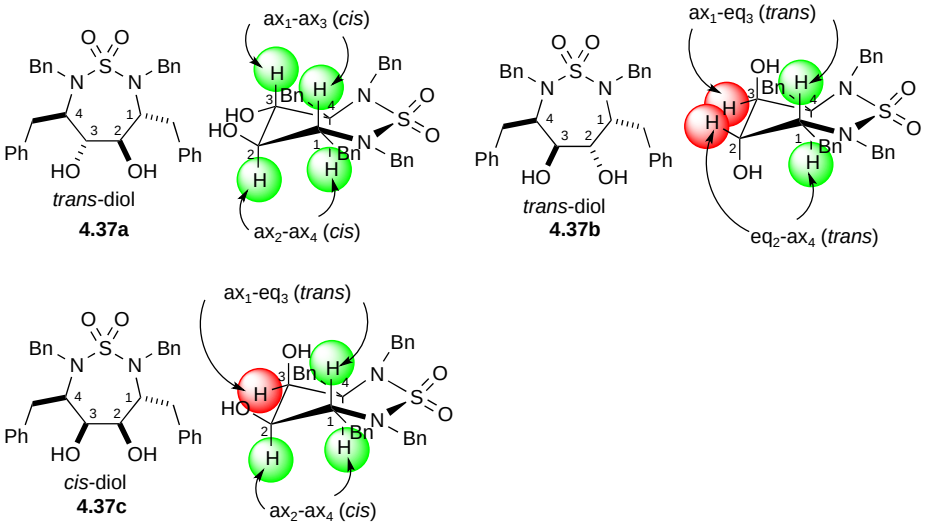
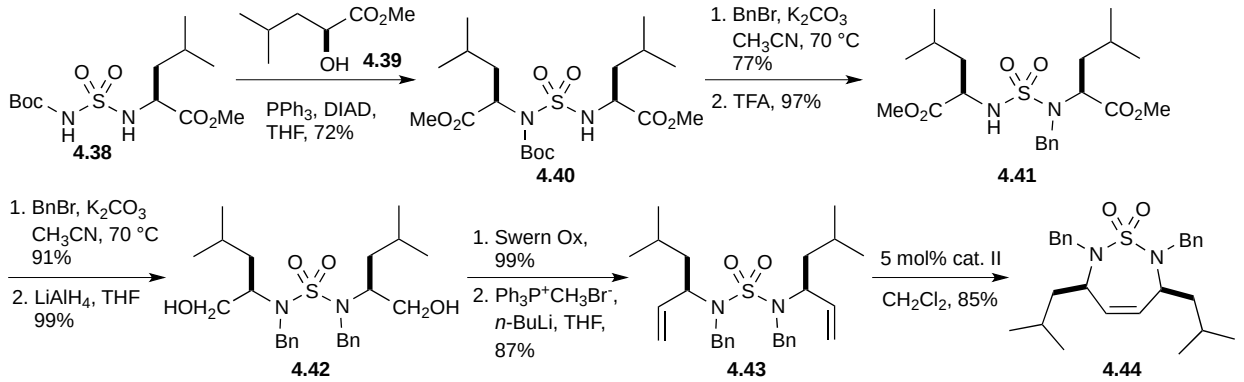


Figure 4.3

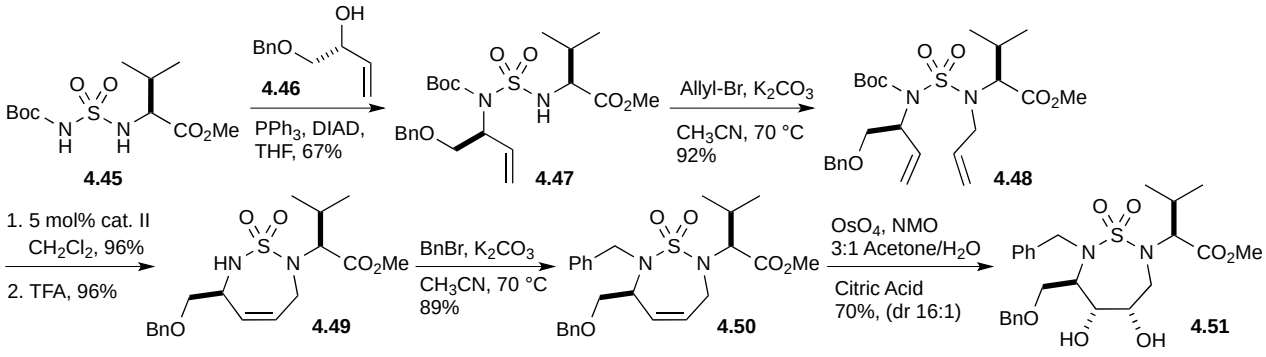
An approach to synthesize *meso* compound **4.44** utilizing CSI and chiral amino acid was developed (Scheme 4.6). Mitsunobu reaction of α-hydroxy ester **4.39**, which was generated by the reaction of *α*-hydroxy amino acid and amberlyst-15 ion exchange resin in MeOH, and the unsymmetric sulfamide **4.38**,[27] provided the *N*-Boc protected sulfamide **4.40** in 72% yield. Benzylation and deprotection of Boc group furnished **4.41**, and further benzylation and $LiAlH_4$ reduction produced *meso* sulfamide **4.42**. Swern oxidation followed by Wittig reaction generated terminal olefin **4.43**, and RCM using the **G-II** catalyst (5 mol%) furnished cyclized *meso* sulfamide **4.44** in good yields.

Scheme 4.6



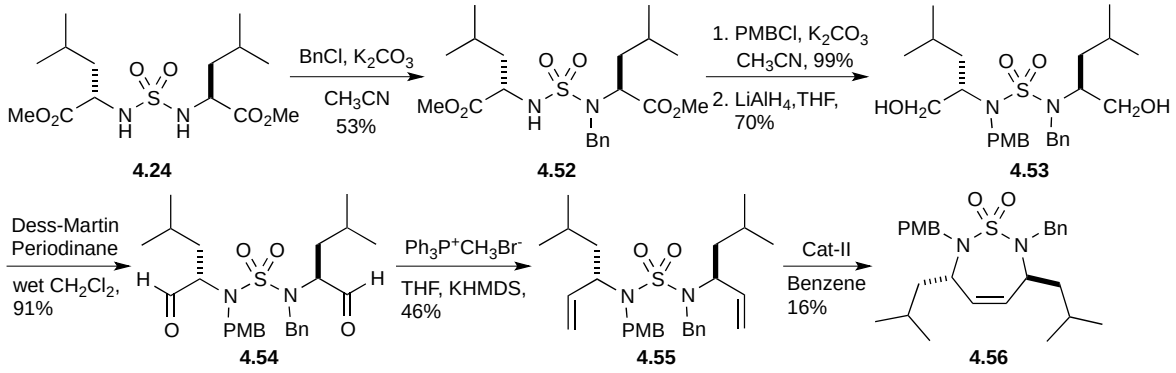
The newest route to unsymmetric sulfamides is represented in Scheme 4.6. This approach involves the use of the Mitsunobu reaction of sulfamoyl carbamates to apply a stereogenic center occupying the P1 position in a tri-differentiated sulfamide.[19] Mitsunobu reaction of sulfamide **4.45** with readily prepared chiral nonracemic secondary allylic alcohol **4.46** provided sulfamide **4.47** in 67% yield. Allylation afforded sulfamide diene **4.48** in 92%. RCM (96%), Boc-deprotection (96%) and benzylation (89%) gave the desired cyclic sulfamide **4.50** in excellent yield. Finally, dihydroxylation gave sulfamide diol **4.51** in good yield (70%) and with high diastereoselectivity (*dr*=16:1).

Scheme 4.7



Another new approach to unsymmetric sulfamides is represented in Scheme 4.8.[19] This strategy involves mono benzylation followed by *p*-methoxy benzylation on the nitrogen to generate diverse substituents occupying the P2 and P2' positions in a tri-differentiated sulfamide. Mono benzylation of sulfamide **4.24** with benzyl bromide allowed sulfamide **4.52** in 53% yield with dibenzylated sulfamide as a byproduct. *p*-Methoxy benzylation of **4.52** on the rest of nitrogen and reduction of ester gave diol **4.53**. Dess-Martin oxidation of **4.53** in wet CH_2Cl_2 organic solvent generated unstable dialdehyde **4.54**.[28] Wittig reaction using KHMDS to produce terminal olefin **4.55**, and RCM in refluxing benzene generated desired cyclic sulfamide **4.56**.

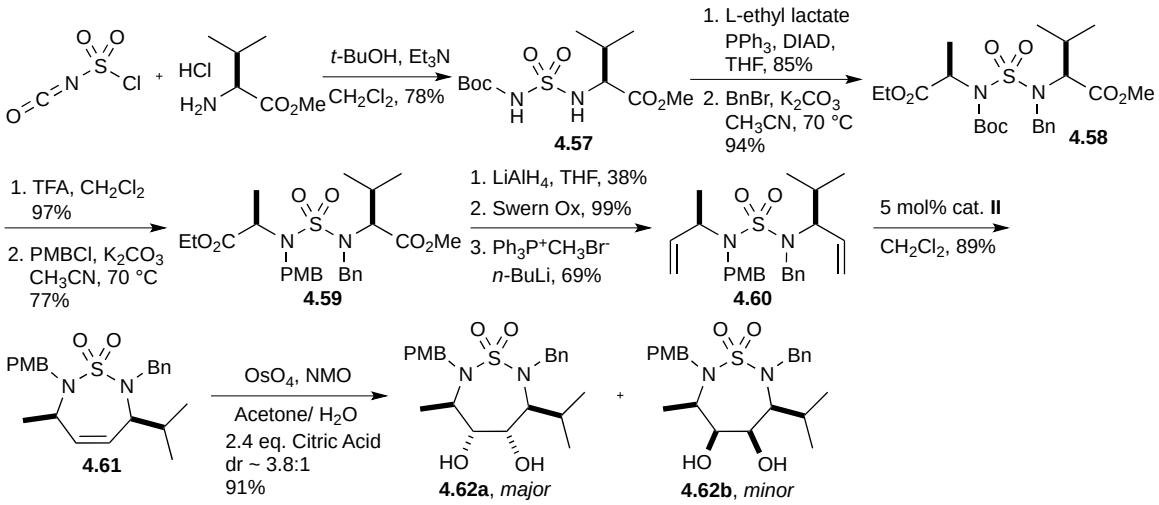
Scheme 4.8



The methods outlined below were exploited further in the synthesis of an unsymmetric sulfamide bearing tetra-differentiated P1/P1'/P2/P2' regions (Scheme 4.9).[19] Mitsunobu reaction of sulfamide **4.57**[29] and L-ethyl lactate, and benzylation of sulfamoyl carbamate furnished unsymmetric sulfamide **4.58** in 94% yield. Boc-deprotection and protection of the remaining sulfamide nitrogen with *p*-methoxybenzyl chloride gave 77% of sulfamide **4.59**. $LiAlH_4$ reduction (38%), Swern oxidation (99%), and Wittig olefination (69%) gave the metathesis precursor **4.60**. Ring-closing metathesis with 5 mol % of the **G-II** catalyst afforded 69% of

unsymmetric sulfamide **4.61**. Conversion of the cyclic internal olefin to the corresponding diol via *cis*-dihydroxylation was the final step toward analogues of DMP 323. Dihydroxylation furnished the sulfamide diol **4.62** in 91% yield, albeit with modest diastereoselectivity (*dr*=3.9:1).

Scheme 4.9



4.3. Anticancer drug discovery NCI-60 cell line screening at National Cancer Institute (NCI)

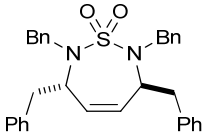
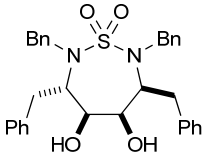
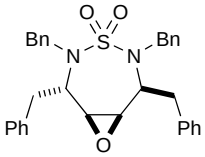
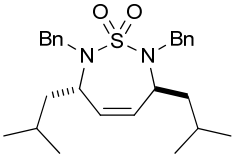
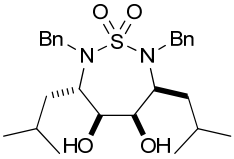
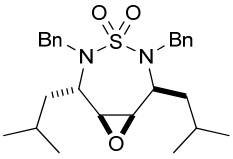
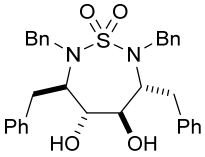
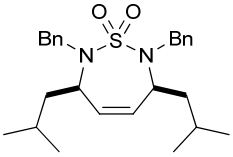
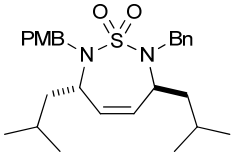
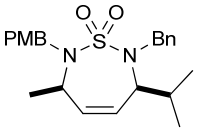
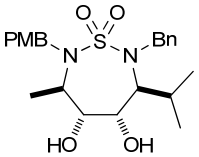
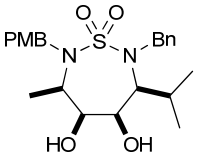
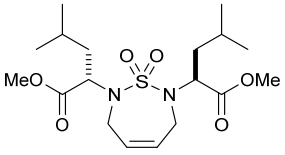
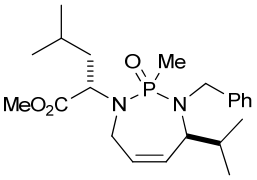
4.3.1. One-dose assay

Pharmacological evaluation of the anticancer activity was carried out on the selected compounds by developmental therapeutic program of the *National Cancer Institute (NCI),* Frederick, Maryland. All the selected 29 compounds for *in vitro* cancer screening have been given a unique NSC (National Service Center) number. The compounds **4.21–4.78** in Table 4.3 were submitted to NCI-60 cell line screening. Compounds **4.63–4.78** were prepared by our group members and synthetic methods and supplemental data can be found in cited references.[30,19b] Cyclic sulfamide, urea, and phosphorus containing compounds **4.63–4.78** were

screened to obtain structure activity relationships (SAR). Details of the methodologies for NCI-60 cell line screening are described at http://dtp.nci.nih.gov/branches/btb/ivclsp.html.[31] Briefly, the panel is organized into nine subpanels representing diverse histologies: leukemia, melanoma, and cancers of lung, colon, kidney, ovary, breast, prostate, and central nervous system. The human tumor cells are grown in supplemented RPMI 1640 medium containing 5% fetal bovine serum and 2 mL glutamine for 24 h. The cells are inoculated into 96-well microtiter plates in 100 μL at plating densities ranging from 5,000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the micro-titer plates are incubated at 37° C, 5 % CO_2, 95 % air and 100 % relative humidity for 24 h prior to addition of experimental drugs. The selected compounds **4.21–4.78** were dissolved in DMSO and incubated with cells at five concentrations with 10-fold dilutions, the highest being 10^{-4} M and the others being 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8} M. The assay is terminated by addition of cold trichloroacetic acid, and the cells are fixed and stained with sulforhodamine B. Bound stain was solubilized, and the absorbance read on an automated plate reader. The cytostatic parameter that is 50% growth inhibition (GI_{50}, concentrations required to inhibit growth by 50%) was calculated from time zero, control growth, and the five concentration level absorbance. The cytotoxic parameter that is, inhibitory concentrations (LC_{50}, lethal concentration, standard measure of the toxicity of the medium that kills half of the sample population in a specified period, lower number means more toxic) represent the average of two independent experiments. The *in vitro* screening is a two-stage process started with the evaluation of all the compounds against the NCI-60 human tumor cell lines with a single dose of 10.0 μM, which is done by following same protocol as for five-dose screening. The output from the single dose screen was reported as a mean graph (given in the Supplementary data with general interpretation). Only the compounds, which showed more than

60% of growth inhibition in at least 8 tumor cell lines, were selected for further testing and the others were assumed as inactive.

Table 4.3. *List of compounds screened for NCI 60-cell lines.*

Compd No. NSC No.	Structure	Activity	Compd No. NSC No.	Structure	Activity
4.21 NSC 764190		Active	**4.22** NSC 751486		Active
4.23 NSC 751478		Active	**4.27** NSC 751468		
4.28 NSC 751469			**4.29** NSC 751470		
4.37C NSC 764189		Active	**4.44** NSC 751477		
4.56 NSC 751472			**4.61** NSC 751473		
4.62a NSC 751479			**4.62b** NSC 751483		
4.63 NSC 751467			**4.64** NSC 764191		

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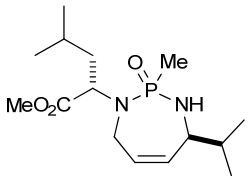

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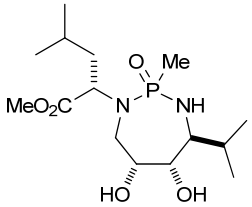

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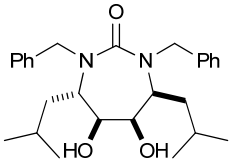

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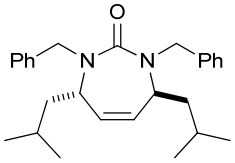

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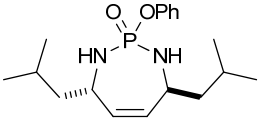

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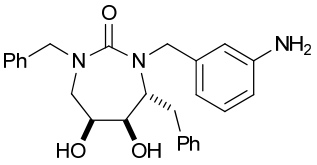
NSC 764197



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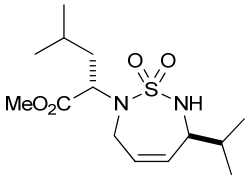
NSC 767525



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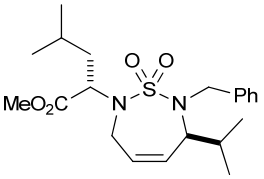

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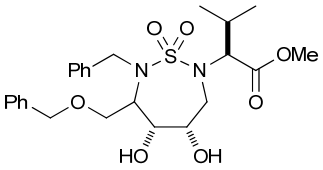

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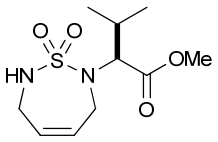

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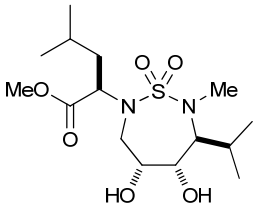
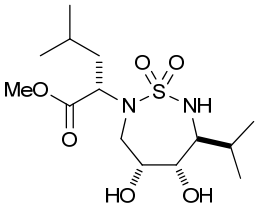
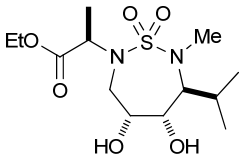
NSC 767529



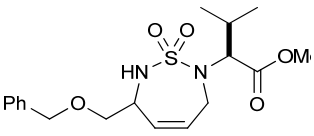
4.76
NSC 767530



4.77
NSC 767531



4.78
NSC 767532



The one-dose data is reported as a mean graph of the percent growth of treated cells and is similar in appearance to mean graphs from the 5-dose assay. The number reported for the one-dose assay is growth relative to the no-drug control, and relative to the time zero number of cells.

This allows detection of both growth inhibition (values between 0 and 100) and lethality (values less than 0). This is the same as for the 5-dose assay, described on http://dtp.nci.nih.gov/branches/btb/ivclsp.html. For example, a value of 100 means no growth inhibition. A value of 40 would mean 60% growth inhibition. A value of 0 means no net growth over the course of the experiment. A value of -40 would mean 40% lethality. A value of -100 means all cells are dead. Information from the One-dose mean graph is available for COMPARE analysis (http://dtp.nci.nih.gov/docs/compare/compare.html). This primary one-dose screening showed that compounds (**4.21**, **4.22**, **4.23**, and **4.37c**) were active, while other compounds are determined as inactive. Table 4.4 is the summary of one-dose experiment for each compound. Even if it was not selectively considered 60% of growth inhibition as criterion, many compounds are moderately sensitive on the breast cancer (MDA-MB-468), renal cancer (UO-31 and CAKI-1) and leukemia (MOLT-4 and SR).

Compound **4.21** (NSC 764190) showed 11.87% growth inhibition against the SR cell line (Leukemia), compound **4.22** (NSC 751486), 0% against the UO-31 cell line (Renal cancer), compound **4.23** (NSC 751478), 6.52% against MDA-MB-468 the cell line (Breast cancer), compound **4.27** (NSC 751468), 39.65% against MDA-MB-468 the cell line (Breast cancer), compound **4.28** (NSC 751469), 46.61% against HCT-116 the cell line (Colon cancer), compound **4.29** (NSC 751470), 35.50% against MDA-MB-468 the cell line (Breast cancer), **4.37c** (NSC 751489), 18.03% against MDA-MB-468 the cell line (Breast cancer), compound **4.44** (NSC 751477), 41.68% against the MDA-MB-468 cell line (Breast cancer), compound **4.56** (NSC 751472), 44.15% against the MDA-MB-468 cell line (Breast cancer), compound **4.61** (NSC 751473), 81.62% against the HT29 cell line (Colon cancer), compound **4.62a** (NSC 751479), 67.93% against the SNB-75 cell line (CNS cancer), compound **4.62b** (NSC 751483), 75.56%

against the UO-31 cell line (Renal cancer), Compound **4.63** (NSC 751467), 69.49% against the SNB-19 cell line (CNS cancer), compound **4.64** (NSC 751491), 71.87% against the Leukemia MOLT-4 cell line (Leukemia), compound **4.65** (NSC 764192), 78.05% against the SNB-75 cell line (CNS cancer), compound **4.66** (NSC 764193), 87.73% against the UO-31 cell line (Renal cancer), compound **4.67** (NSC 764194), 43.31% against the CAKI-1 cell line (Renal cancer), compound **4.68** (NSC 764195), 57.52% against the HCT-116 cell line (Colon cancer), compound **4.69** (NSC 764196), 73.57% against the CAKI-1 cell line (Renal cancer), compound **4.70** (NSC 764197), 82.35% against the UO-31 cell line (Renal cancer), compound **4.71** (NSC 767525), 79.70% against the NCI-H522 cell line (Non-small cell lung cancer), compound **4.72** (NSC 767526), 34.17% against the MOLT-4 cell line (Leukemia), compound **4.73** (NSC 767527), 39.80% against the MOLT-4 cell line (Leukemia), compound **4.74** (NSC 767528), 82.41% against the SR cell line (Leukemia), compound **4.75** (NSC 767529), 76.18% against the SNB-75 cell line (CNS cancer), compound **4.76** (NSC 767530), 80.47% against the SR cell line (Leukemia), compound **4.77** (NSC 767531), 64.25% against the UO-31 cell line (Renal cancer), and compound **4.78** (NSC 767532), 64.80% against the UO-31 cell line (Renal cancer) (Table 4.4). The compounds which reduced the growth of the cell lines to 32% or less (negative number indicate kills) are considered *in vitro* active.[32,33] The output from the NCI-60 cell lines single dose screen of **NSC 764190** was reported as a mean graph (Figure 4.4).

Table 4.4. *Anti-cancer screening data of compounds.*

NSC No.	60-cell line assay in one-dose at 10^{-5} concentration					
	Range of growth percentage	Most sensitive cell line	Growth % of most sensitive cell line	Mean	Delta	range
751467	69.49 to 127.06	CNS cancer (SNB-19)	69.49	100.68	31.19	57.57
751468	39.65 to 140.80	Breast cancer (MDA-MB-468)	39.65	85.77	46.12	101.15
751469	46.61 to 113.74	Colon cancer (HCT-116)	46.61	81.18	34.57	67.13
751470	35.50 to 123.55	Breast cancer (MDA-MB-468)	35.50	79.44	43.94	88.05
751472	44.15 to 132.68	Breast cancer (MDA-MB-468)	44.15	81.64	37.49	88.53
751473	81.62 to 126.01	Colon cancer (HT29)	81.62	102.22	20.60	44.39
751477	41.68 to 110.24	Breast cancer (MDA-MB-468)	41.68	76.40	35.15	68.99
751478	6.52 to 114.43	Breast cancer (MDA-MB-468)	6.52	46.84	40.32	107.91
751479	67.93 to 150.32	CNS cancer (SNB-75)	67.93	100.64	32.71	82.39
751483	75.56 to 125.47	Renal cancer (UO-31)	75.56	99.69	24.13	49.91
751486	-45.75 to 60.35	Renal cancer (UO-31)	0	-0.81[a]	44.94	106.10
751489	18.03 to 102.81	Breast cancer (MDA-MB-468)	18.03	65.12	47.09	91.32
764190	11.87 to 99.65	Leukemia (SR)	11.87	55.00	43.13	87.78
764191	71.87 to 116.41	Leukemia (MOLT-4)	71.87	96.44	24.57	44.54
764192	78.05 to 120.37	CNS cancer (SNB-75)	78.05	103.32	25.27	42.32
764193	87.73 to 125.96	Renal cancer (UO-31)	87.73	104.45	16.72	38.23
764194	43.31 to 107.76	Renal cancer (CAKI-1)	43.31	82.97	39.66	64.45
764195	57.52 to 122.20	Colon cancer (HCT-116)	57.52	85.01	38.72	75.91
764196	73.57 to 127.74	Renal cancer (CAKI-1)	73.57	101.26	27.69	54.17
764197	82.35 to 129.96	Renal cancer (UO-31)	82.35	99.55	17.20	47.61
767525	79.70 to 119.76	Non-small cell lung cancer (NCI-H522)	79.70	101.95	22.25	40.06
767526	34.17 to 115.22	Leukemia (MOLT-4)	34.17	80.46	46.29	81.05
767527	39.80 to 110.48	Leukemia (MOLT-4)	39.80	85.89	46.09	70.68
767528	82.41 to 119.28	Leukemia (SR)	82.41	102.75	20.34	36.87
767529	76.18 to 122.73	CNS cancer (SNB-75)	76.18	101.04	30.17	51.86
767530	80.47 to 187.29	Leukemia (SR)	80.47	104.72	24.25	106.82
767531	64.25 to 119.03	Renal cancer (UO-31)	64.25	100.51	36.26	55.75
767532	64.80 to 119.30	Renal cancer (UO-31)	64.80	99.27	34.47	54.50

[a] Negative indicates the cell kill

Figure 4.4. *Selected NCI60-cell lines screening data for one-dose study of* ***4.21 (NSC 764190)***.

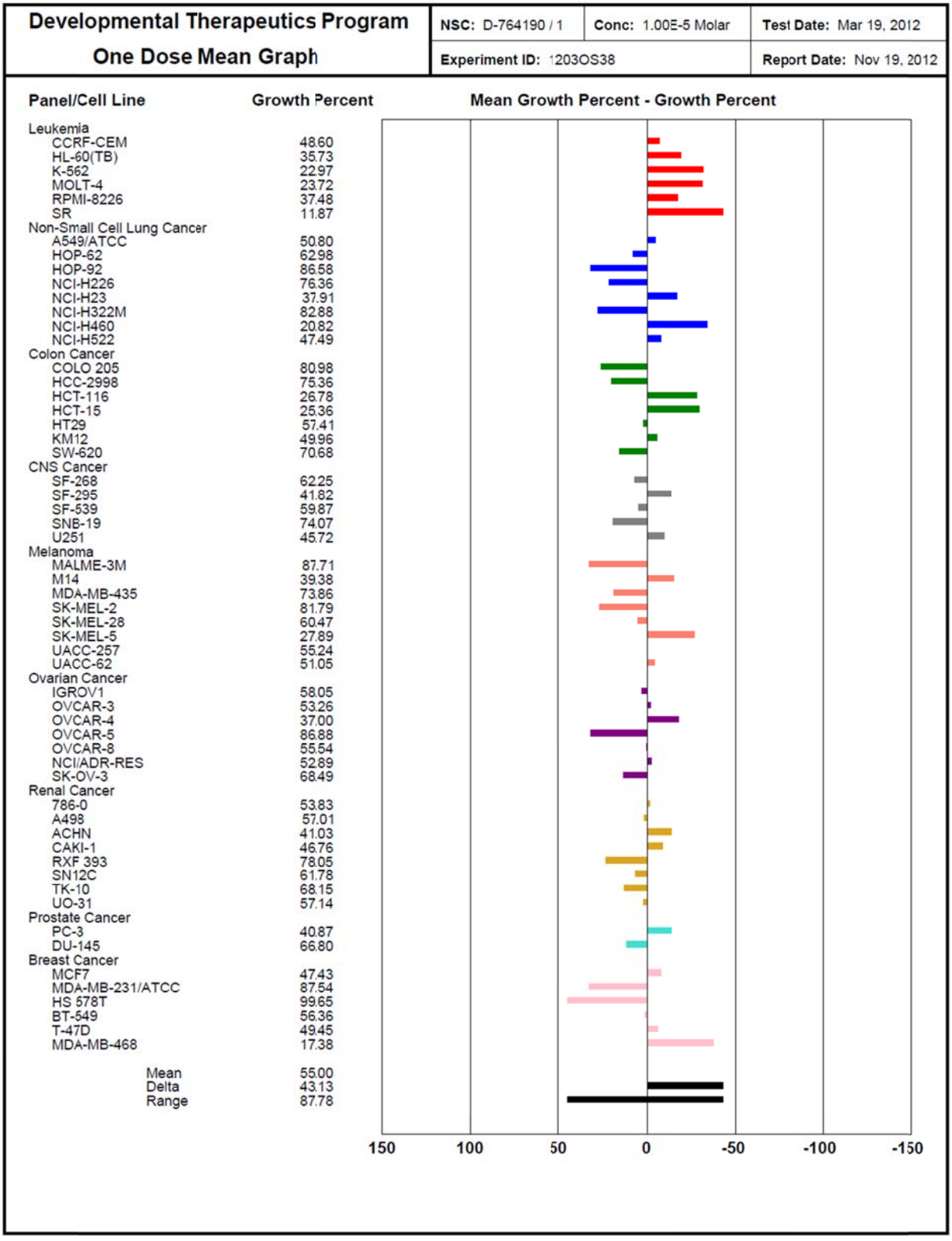


Table 4.5 represents the growth percent inhibition (100 - growth percent) of compounds that inhibited more than 50% of growth inhibition for one-dose studies from the NCI60-cell lines screen. Generally, compounds were selectively sensitive on the leukemia, colon cancer, prostate cancer and breast cancer cell. Especially, almost every compound in Table 4.5 showed strong inhibition of the breast cancer cell line (MDA-MB-468).

Table 4.5. *Growth percent inhibition of compounds inhibited more than 50% for one-dose studies for the NCI 60-cell lines screen.*

Panel/cell line	**NSC 751468**	**NSC 751470**	**NSC 751472**	**NSC 751477**	**NSC 751478**	**NSC 751486**	**NSC 764189**	**NSC 764190**	**NSC 767527**
Leukemia									
CCRF-CEM					70.51	92.67		51.40	
HL-60(TB)					54.91			64.27	
K-562					76.65	96.88	58.11	77.03	
MOLT-4					64.56	94.35	73.65	76.28	61.20
RPMI-8226					72.12		62.31	62.52	
SR					70.44	95.55	58.23	88.13	
Colon cancer									
HCT-116		51.37	50.59		82.98		68.99	73.22	
HCT-15					73.92	96.84		74.64	
HT29	55.97				53.32	93.05			
KM12					59.78			50.04	
SW-620						90.78			
Prostate cancer									
PC-3				52.03	57.96	95.11	60.25	59.13	
V66666DU-145					65.74				
Breast cancer									
MCF7							66.13	52.57	
MDA-MB-231/ATCC					75.79		58.41		
HS 578T						80.33			
BT-549						84.33			
T-47D					67.51	92.13		50.55	
MDA-MB-468	60.35	64.5	55.85	58.32	93.48		81.97	82.62	

4.3.2. Five-dose assay

The log mean values for GI_{50} and LC_{50} in NCI-60 cell lines for compounds **4.21**, **4.22**, **4.23**, and **4.37c** are provided in Table 4.6 along with the log delta value (the maximum sensitivity in excess of the mean) and the log range (the maximum difference between the least sensitive and the most sensitive cell lines). These parameters provided insights into selectivity and potency of anti-tumor agents. Large values of the delta and range indicate high selectivity for some histological cancers over others. The lower median log GI_{50} values of compounds **4.22**, **4.23** and **4.37c** show that these three compounds are active, followed by **4.21**. The high median log LC_{50} value of **4.21**, along with the low delta and range value, indicates the complete absence of cytotoxicity against all cell lines.

Table 4.6. *Cytostatic (GI_{50}) and cytotoxic (LC_{50}) parameters for **4.21 (NSC 764190)**, **4.22 (NSC 751486)**,**4.23 (NSC 751478)**, and **4.37c (NSC 764189)**.*

Compound	GI_{50}			LC_{50}		
	Median	Delta	Range	Median	Delta	Range
4.21 (**NSC 764190**)	-4.94	1.63	2.57	-4.0	0	0.0
4.22 (**NSC 751486**)	-5.49	0.58	0.85	-4.8	0.18	0.28
4.23 (**NSC 751478**)	-5.31	1.25	1.44	-5.12	0	0.0
4.37c (**NSC 764189**)	-5.30	0	0.0	-5.3	0	0.0

The complete *in-vitro* anti-cancer data collected on NCI-60 subpanel cell lines for the four most active compounds informed are shown in Table 4.7. Secondary screening was carried out on these active compounds (**4.21**, **4.22**, **4.23**, and **4.37c**) in order to determine their cytostatic and cytotoxic activities. Generally, cyclic sulfamides possessing benzyl group substituted at the 3- and 6-positions have antitumor activities in several cancer cells. Cyclic sulfamides **4.27**, **4.28**, **4.29**, **4.44**, **4.56**, **4.61**, **4.62a** and **4.62b** which have alkyl substituents at the 3- and 6-positions do not have noticeable sensitivities toward the 60 tumor cell screening line. To compare as *in vitro* SAR data, unsymmetric phosphorus-containing analogues of DMD 232 **4.64–4.66**, **4.69**, cyclic

ureas **4.67**, **4.68**, **4.70**, and di- or tri-substituted unsymmetric cyclic sulfamides **4.71–4.78** were submitted to 60-cell lines additionally. One-dose experimental results show that these compounds did not possess enough biological availability to warrant additional five-dose screening. The result of compound **4.21** for five-dose screening is given by three response parameters (GI_{50}, TGI and LC_{50}) for each cell line from $\log_{10}$ of sample concentration (molar) vs percentage growth inhibition curves in nine cancer diseases (Figure 4.5).

Table 4.7. *Anti-tumor activity (GI_{50}/μM)[a], TGI[b] and toxicity (LC_{50}/μM)[c] data of compounds selected for 5 dose studies for the NCI60-cell lines screen.*

Panel/cell line	**4.21 (NSC 764190)**			**4.22 (NSC 751486)**			**4.23 (NSC 751478)**			**4.37c (NSC 764189)**		
	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}
Leukemia												
CCRF-CEM	3.85	>100	>100	5.96	>20	>20	>7.5	>7.5	>7.5	>5.0	>5.0	>5.0
HL-60(TB)	3.43	>100	>100	2.97	8.72	>20	4.75	>7.5	>7.5	>5.0	>5.0	>5.0
K-562	2.00	>100	>100	3.05	9.38	>20	3.34	>7.5	>7.5	>5.0	>5.0	>5.0
MOLT-4	2.15	>100	>100	2.86	1.02	>20	4.12	>7.5	>7.5	>5.0	>5.0	>5.0
RPMI-8226	0.859	>100	>100	4.49	>20	>20	4.92	>7.5	>7.5	>5.0	>5.0	>5.0
SR	1.66	>100	>100	nd	nd	nd	nd	nd	nd	nd	nd	nd
Non-small cell lung cancer												
A549/ATCC	7.67	>100	>100	2.99	7.62	19.4	3.78	>7.5	>7.5	>5.0	>5.0	>5.0
EKVX	nd	>100	>100	3.43	9.86	>20	6.55	>7.5	>7.5	>5.0	>5.0	>5.0
HOP-62	>100	>100	>100	3.71	7.34	14.5	>7.5	>7.5	>7.5	>5.0	>5.0	>5.0
HOP-92	4.40	>100	>100	3.15	6.68	14.1	>7.5	>7.5	>7.5	>5.0	>5.0	>5.0
NCI-H226	4.35	>100	>100	3.04	6.17	12.5	>7.5	>7.5	>7.5	>5.0	>5.0	>5.0
NCI-H23	1.66	>100	>100	3.11	6.71	14.5	3.47	>7.5	>7.5	>5.0	>5.0	>5.0
NCI-H322M	>100	>100	>100	4.48	11.8	>20	>7.5	>7.5	>7.5	>5.0	>5.0	>5.0
NCI-H460	2.75	>100	>100	3.41	7.91	18.3	2.53	>7.5	>7.5	>5.0	>5.0	>5.0
NCI-H522	5.46	>100	>100	0.847	4.79	14.5	1.83	>7.5	>7.5	>5.0	>5.0	>5.0
Colon cancer												
COLO 205	>100	>100	>100	4.26	10.5	>20	>7.5	>7.5	>7.5	>5.0	>5.0	>5.0
HCC-2998	>100	>100	>100	3.41	6.20	11.3	>7.5	>7.5	>7.5	>5.0	>5.0	>5.0
HCT-116	0.535	>100	>100	2.43	5.05	10.5	0.844	>7.5	>7.5	>5.0	>5.0	>5.0
HCT-15	3.51	>100	>100	4.37	1.82	>20	3.92	>7.5	>7.5	>5.0	>5.0	>5.0
HT29	42.6	>100	>100	2.63	5.64	12.1	>7.5	>7.5	>7.5	>5.0	>5.0	>5.0
KM12	9.92	>100	>100	2.94	5.84	11.6	7.19	>7.5	>7.5	>5.0	>5.0	>5.0
SW-620	nd	>100	>100	3.18	7.12	15.9	>7.5	>7.5	>7.5	>5.0	>5.0	>5.0
CNS cancer												
SF-268	7.85	>100	>100	3.44	8.03	18.8	6.08	>7.5	>7.5	>5.0	>5.0	>5.0
SF-295	0.667	>100	>100	2.62	5.68	12.3	1.80	>7.5	>7.5	>5.0	>5.0	>5.0
SF-539	>100	>100	>100	3.37	6.76	13.6	>7.5	>7.5	>7.5	>5.0	>5.0	>5.0
SNB-19	>100	>100	>100	4.69	>20	>20	>7.5	>7.5	>7.5	>5.0	>5.0	>5.0
SNB-75	>100	>100	>100	2.31	7.28	>20	>7.5	>7.5	>7.5	>5.0	>5.0	>5.0

U251	1.11	>100	>100	3.07	6.47	13.6	1.84	>7.5	>7.5	>5.0	>5.0	>5.0
Melanoma												
LOX IMVI	1.40	>100	>100	3.28	7.09	15.3	5.38	>7.5	>7.5	>5.0	>5.0	>5.0
MALME-3M	>100	>100	>100	2.75	6.37	14.7	>7.5	>7.5	>7.5	>5.0	>5.0	>5.0
M14	nd	>100	>100	3.04	6.07	12.1	>7.5	>7.5	>7.5	>5.0	>5.0	>5.0
MDA-MB-435	>100	>100	>100	3.33	7.10	15.1	>7.5	>7.5	>7.5	>5.0	>5.0	>5.0
SK-MEL-2	>100	>100	>100	2.80	5.76	11.8	>7.5	>7.5	>7.5	>5.0	>5.0	>5.0
SK-MEL-28	>100	>100	>100	3.35	6.90	14.2	1.79	>7.5	>7.5	>5.0	>5.0	>5.0
SK-MEL-5	1.14	>100	>100	3.05	5.80	11.1	2.89	>7.5	>7.5	>5.0	>5.0	>5.0
UACC-257	2.23	>100	>100	2.89	5.93	12.2	6.75	>7.5	>7.5	>5.0	>5.0	>5.0
UACC-62	2.66	>100	>100	2.86	6.40	14.3	nd	nd	nd	>5.0	>5.0	>5.0
Ovarian cancer												
IGROV1	>100	>100	>100	3.57	7.24	14.7	5.06	>7.5	>7.5	>5.0	>5.0	>5.0
OVCAR-3	2.34	>100	>100	2.90	6.07	12.7	4.03	>7.5	>7.5	>5.0	>5.0	>5.0
OVCAR-4	0.483	>100	>100	2.72	7.26	19.4	>7.5	>7.5	>7.5	>5.0	>5.0	>5.0
OVCAR-5	>100	>100	>100	3.57	9.10	>20	>7.5	>7.5	>7.5	>5.0	>5.0	>5.0
OVCAR-8	>100	>100	>100	4.20	11.2	>20	>7.5	>7.5	>7.5	>5.0	>5.0	>5.0
NCI/ADR-RES	7.03	>100	>100	3.40	8.58	>20	5.23	>7.5	>7.5	>5.0	>5.0	>5.0
SK-OV-3	>100	>100	>100	4.15	10.6	>20	>7.5	>7.5	>7.5	>5.0	>5.0	>5.0
Renal cancer												
786-0	>100	>100	>100	3.24	6.86	14.5	5.12	>7.5	>7.5	>5.0	>5.0	>5.0
A498	1.77	>100	>100	2.45	5.18	10.9	>7.5	>7.5	>7.5	>5.0	>5.0	>5.0
ACHN	2.10	>100	>100	6.01	>20	>20	5.88	>7.5	>7.5	>5.0	>5.0	>5.0
CAKI-1	2.83	>100	>100	3.65	7.33	14.7	0.949	>7.5	>7.5	>5.0	>5.0	>5.0
RXF 393	28.0	>100	>100	2.60	5.24	10.5	>7.5	>7.5	>7.5	>5.0	>5.0	>5.0
SN12C	9.42	>100	>100	3.26	7.68	18.1	>7.5	>7.5	>7.5	>5.0	>5.0	>5.0
TK-10	8.26	>100	>100	3.77	8.38	18.6	3.90	>7.5	>7.5	>5.0	>5.0	>5.0
UO-31	>100	>100	>100	2.73	5.96	13.1	>7.5	>7.5	>7.5	>5.0	>5.0	>5.0
Prostate cancer												
PC-3	0.580	>100	>100	3.48	10.4	>20	3.06	>7.5	>7.5	>5.0	>5.0	>5.0
DU-145	>100	>100	>100	3.21	7.55	17.8	>7.5	>7.5	>7.5	>5.0	>5.0	>5.0
Breast cancer												
MCF7	>100	>100	>100	4.77	13.6	>20	4.27	>7.5	>7.5	>5.0	>5.0	>5.0
MDA-MB-231/ATCC	>100	>100	>100	2.82	6.39	14.5	>7.5	>7.5	>7.5	>5.0	>5.0	>5.0
HS 578T	>100	>100	>100	3.27	8.38	>20	>7.5	>7.5	>7.5	>5.0	>5.0	>5.0
BT-549	>100	>100	>100	2.65	5.53	11.6	4.09	>7.5	>7.5	>5.0	>5.0	>5.0
T-47D	>100	>100	>100	3.95	10.2	>20	6.81	>7.5	>7.5	>5.0	>5.0	>5.0
MDA-MB-468	0.267	60.1	>100	2.98	7.17	17.3	0.274	>7.5	>7.5	>5.0	>5.0	>5.0

nd: not determined.

[a] GI_{50}: 50% growth inhibition, concentration of drug resulting in a 50% reduction in net protein increase compared with control cells.

[b] TGI: total cell growth inhibition

[c] LC_{50}: lethal concentration, concentration of drug lethal to 50% of cells.

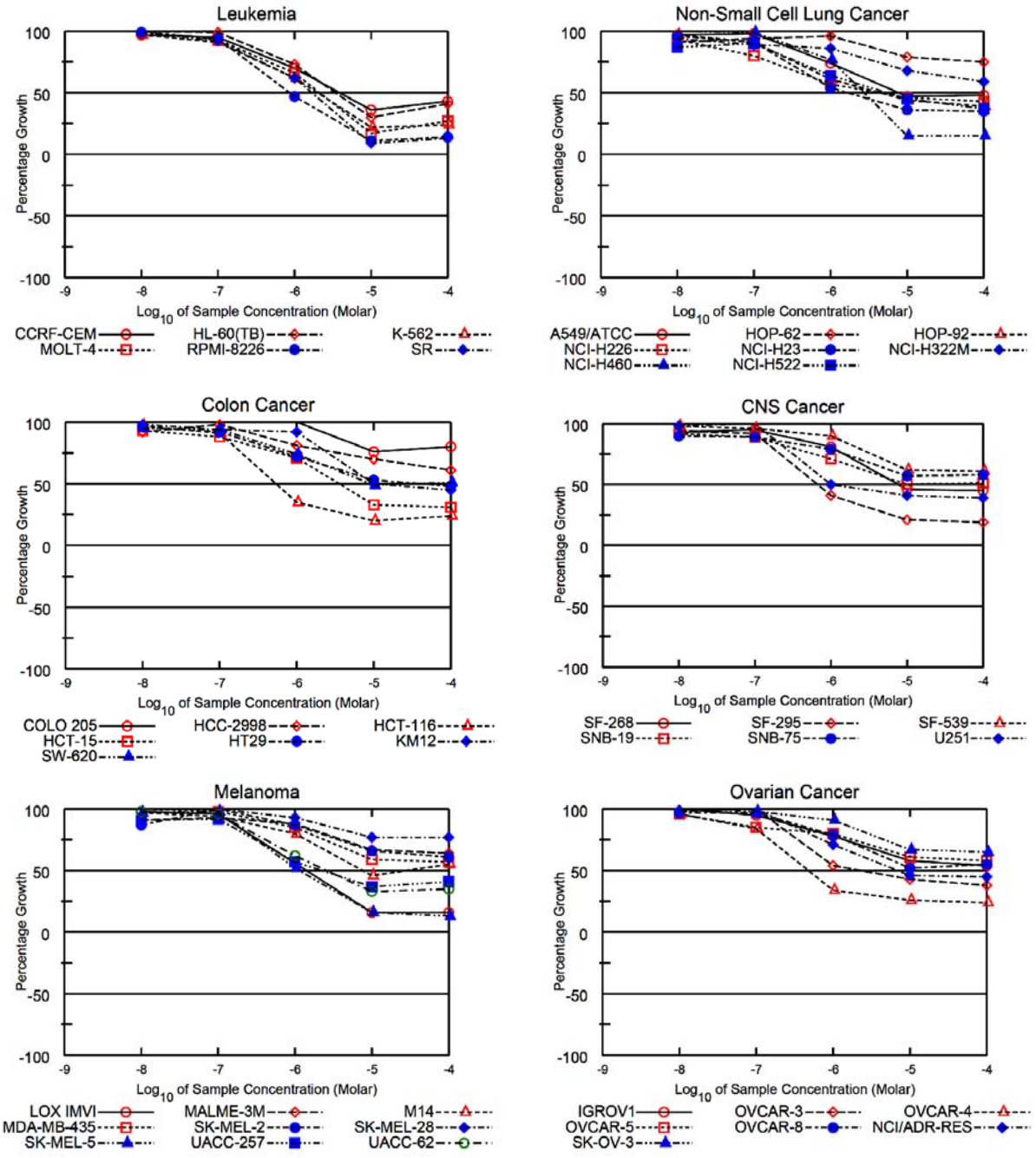


Figure 4.5. *Dose response curves (% growth verses samples concentration at NCI fixed protocol, μM) obtained from the NCI in vitro disease-oriented human cancer cell line of compounds* **4.21** *(NSC 764190) in nine cancer diseases.*

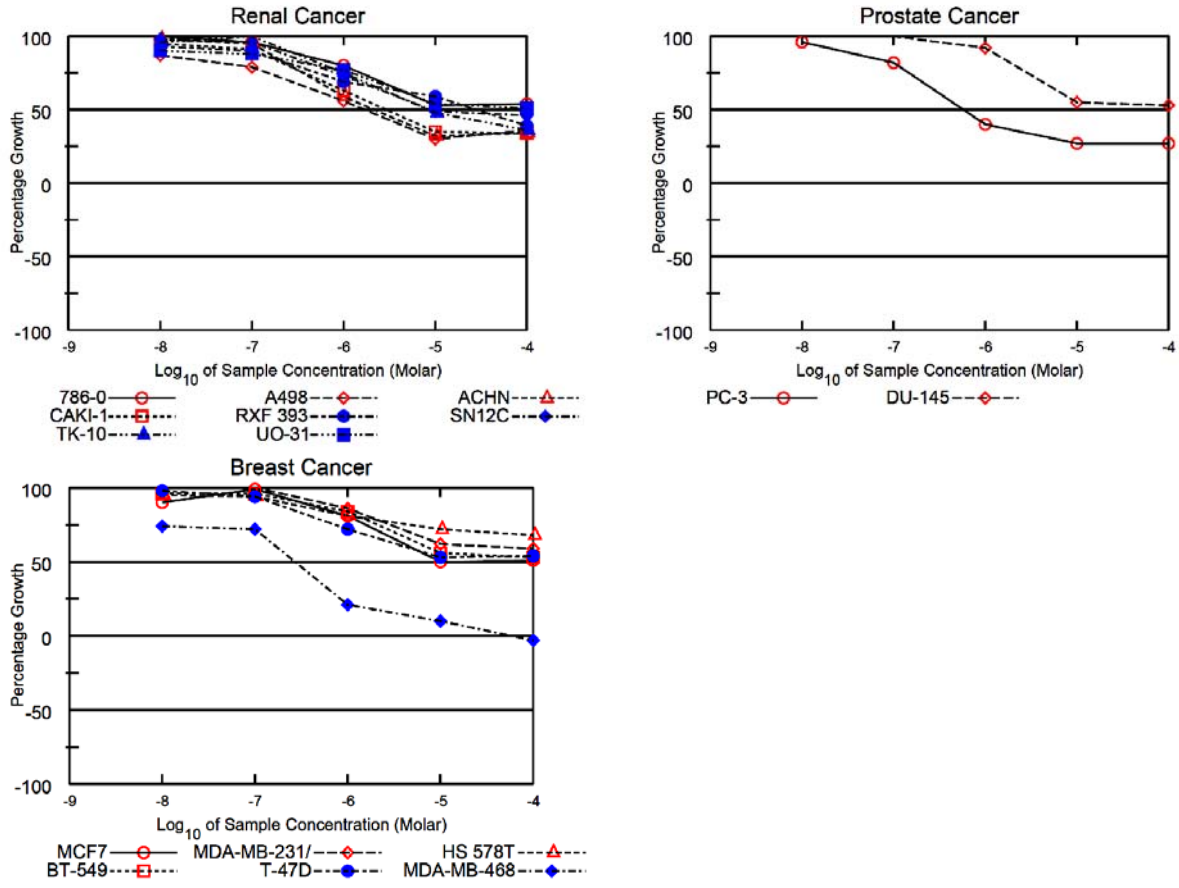


Figure 4.5. *Dose response curves (% growth verses samples concentration at NCI fixed protocol, μM) obtained from the NCI in vitro disease-oriented human cancer cell line of compounds* ***4.21*** *(NSC 764190) in nine cancer diseases (continued).*

The criterion for selectivity of a compound depends on the ratio obtained by dividing the full panel MID (the average sensitivity of all cell lines towards the test agent) by their individual subpanel MID (the average sensitivity of all cell lines of a particular subpanel towards the test agent). The ratios between 3 and 6 refer to moderate selectivity; ratios greater than 6 indicate high selectivity towards the corresponding cell line, while compounds not meeting either of there criteria are rated non-selective.[34] Since it is difficult for the calculation of GI_{50} in cases which have values of >7.5 μM, they are excluded from the calculation. Following this criterion, compound **4.23** (**NSC 751478**) was found to be mildly selective toward every cancer panel.

Table 4.8. *Calculated value of GI50 of the cell lines: full cell line panel, MG-MID and selectivity ratio of compound* ***4.23*** *(**NSC 751478**).*

Panel	Cell line	GI_{50} (10^{-6} M)			
		Concentration per cell line	Subpanel concentration	Subpanel MID	Selectivity ratio
Leukemia	CCRF-CEM	>7.5			
	HL-60(TB)	4.75			
	K-562	3.34	17.13	4.282	0.922
	MOLT-4	4.12			
	RPMI-8226	4.92			
	SR	nd			
Non-small cell lung cancer	A549/ATCC	3.78			
	EKVX	6.55			
	HOP-62	>7.5			
	HOP-92	>7.5			
	NCI-H226	>7.5	18.16	3.632	1.088
	NCI-H23	3.47			
	NCI-H322M	>7.5			
	NCI-H460	2.53			
	NCI-H522	1.83			
Colon cancer	COLO 205	>7.5			
	HCC-2998	>7.5			
	HCT-116	0.844			
	HCT-15	3.92	11.954	3.984	0.991
	HT29	>7.5			
	KM12	7.19			
	SW-620	>7.5			
CNS cancer	SF-268	6.08			
	SF-295	1.80			
	SF-539	>7.5	9.72	3.24	1.219
	SNB-19	>7.5			
	SNB-75	>7.5			
	U251	1.84			
Melanoma	LOX IMVI	5.38			
	MALME-3M	>7.5			
	M14	>7.5			
	MDA-MB-435	>7.5			
	SK-MEL-2	>7.5	16.81	4.203	0.940
	SK-MEL-28	1.79			
	SK-MEL-5	2.89			
	UACC-257	6.75			
	UACC-62	nd			
Ovarian cancer	IGROV1	5.06			
	OVCAR-3	4.03			
	OVCAR-4	>7.5			
	OVCAR-5	>7.5	14.32	4.773	0.828
	OVCAR-8	>7.5			
	NCI/ADR-RES	5.23			
	SK-OV-3	>7.5			
Renal cancer	786-0	5.12			
	A498	>7.5			
	ACHN	5.88	15.849	3.962	0.997
	CAKI-1	0.949			
	RXF 393	>7.5			

	SN12C	>7.5			
	TK-10	3.90			
	UO-31	>7.5			
Prostate cancer	PC-3	3.06	3.06	3.06	1.291
	DU-145	>7.5			
Breast cancer	MCF7	4.27			
	MDA-MB-231/ATCC	>7.5			
	HS 578T	>7.5	15.444	3.861	1.023
	BT-549	4.09			
	T-47D	6.81			
	MDA-MB-468	0.274			

nd: not determined

Based on the discussion on the criterion of selectivity, compound **4.22** (**NSC 751486**) was found to be mild selective in the colon cancer panel. It was also found to be mildly selective in every cancer panel.

Table 4.9. *Calculated value of GI50 of the cell lines: full cell line panel, MG-MID and selectivity ratio of compound **4.22** (**NSC 751486**).*

Panel	Cell line	GI_{50} (10^{-6} M)			
		Concentration per cell line	Subpanel concentration	Subpanel MID	Selectivity ratio
Leukemia	CCRF-CEM	5.96			
	HL-60(TB)	2.97			
	K-562	3.05	19.33	3.866	0.863
	MOLT-4	2.86			
	RPMI-8226	4.49			
	SR	nd			
Non-small cell	A549/ATCC	2.99			
lung cancer	EKVX	3.43			
	HOP-62	3.71			
	HOP-92	3.15			
	NCI-H226	3.04	28.167	3.130	1.066
	NCI-H23	3.11			
	NCI-H322M	4.48			
	NCI-H460	3.41			
	NCI-H522	0.847			
Colon cancer	COLO 205	4.26			
	HCC-2998	3.41			
	HCT-116	2.43			
	HCT-15	4.37	23.22	3.317	1.006
	HT29	2.63			
	KM12	2.94			
	SW-620	3.18			
CNS cancer	SF-268	3.44	19.50	3.25	1.027
	SF-295	2.62			

	SF-539	3.37			
	SNB-19	4.69			
	SNB-75	2.31			
	U251	3.07			
Melanoma	LOX IMVI	3.28			
	MALME-3M	2.75			
	M14	3.04			
	MDA-MB-435	3.33			
	SK-MEL-2	2.80	27.35	3.039	1.098
	SK-MEL-28	3.35			
	SK-MEL-5	3.05			
	UACC-257	2.89			
	UACC-62	2.86			
Ovarian cancer	IGROV1	3.57			
	OVCAR-3	2.90			
	OVCAR-4	2.72			
	OVCAR-5	3.57	24.51	3.501	0.953
	OVCAR-8	4.20			
	NCI/ADR-RES	3.40			
	SK-OV-3	4.15			
Renal cancer	786-0	3.24			
	A498	2.45			
	ACHN	6.01			
	CAKI-1	3.65	27.71	3.464	0.964
	RXF 393	2.60			
	SN12C	3.26			
	TK-10	3.77			
	UO-31	2.73			
Prostate cancer	PC-3	3.48	6.69	3.345	0.998
	DU-145	3.21			
Breast cancer	MCF7	4.77			
	MDA-MB-231/ATCC	2.82			
	HS 578T	3.27	20.44	3.407	0.980
	BT-549	2.65			
	T-47D	3.95			
	MDA-MB-468	2.98			

nd: not determined

The next table, Table 4.10, contains the calculated values of the selectivity ratio of compound **4.21** (**NSC 764190**). Cases with values over 100 of the GI_{50} value were excluded from the calculation. Following the selectivity criterion, compound **4.21** (**NSC 764190**) was found to be mildly selective toward the leukemia (selectivity ratio = 2.238) and melanoma (selectivity ratio = 2.801) cancer panels. Even though it was chosen in only one cell line from each of the prostate cancer and breast cancer panels for the calculation of the selectivity ratio,

compound **4.21** (**NSC 764190**) was indicated to be highly selective toward these two cancer panels.

Table 4.10. *Calculated value of GI50 of the cell lines: full cell line panel, MG-MID and selectivity ratio of the compound* ***4.21*** *(**NSC 764190**).*

Panel	Cell line	GI_{50} (10^{-6} M)			
		Concentration per cell line	Subpanel concentration	Subpanel MID	Selectivity ratio
Leukemia	CCRF-CEM	3.85			
	HL-60(TB)	3.43			
	K-562	2.00	13.949	2.325	2.238
	MOLT-4	2.15			
	RPMI-8226	0.859			
	SR	1.66			
Non-small cell lung cancer	A549/ATCC	7.67			
	EKVX	nd			
	HOP-62	>100			
	HOP-92	4.40			
	NCI-H226	4.35	26.29	4.382	1.188
	NCI-H23	1.66			
	NCI-H322M	>100			
	NCI-H460	2.75			
	NCI-H522	5.46			
Colon cancer	COLO 205	>100			
	HCC-2998	>100			
	HCT-116	0.535			
	HCT-15	3.51	56.565	14.141	0.368
	HT29	42.6			
	KM12	9.92			
	SW-620	nd			
CNS cancer	SF-268	7.85			
	SF-295	0.667			
	SF-539	>100	9.627	3.209	1.622
	SNB-19	>100			
	SNB-75	>100			
	U251	1.11			
Melanoma	LOX IMVI	1.40			
	MALME-3M	>100			
	M14	nd			
	MDA-MB-435	>100			
	SK-MEL-2	>100	7.43	1.858	2.801
	SK-MEL-28	>100			
	SK-MEL-5	1.14			
	UACC-257	2.23			
	UACC-62	2.66			
Ovarian cancer	IGROV1	>100			
	OVCAR-3	2.34			
	OVCAR-4	0.483	9.853	3.284	1.585
	OVCAR-5	>100			
	OVCAR-8	>100			

	NCI/ADR-RES	7.03			
	SK-OV-3	>100			
Renal cancer	786-0	>100			
	A498	1.77			
	ACHN	2.10			
	CAKI-1	2.83	52.38	8.73	0.596
	RXF 393	28.0			
	SN12C	9.42			
	TK-10	8.26			
	UO-31	>100			
Prostate cancer	PC-3	0.580	0.58	0.58	8.972
	DU-145	>100			
Breast cancer	MCF7	>100			
	MDA-MB-231/ATCC	>100			
	HS 578T	>100	0.267	0.267	19.491
	BT-549	>100			
	T-47D	>100			
	MDA-MB-468	0.267			

nd: not determined

The log molar concentration of the resulted screening of compound **4.23** (NSC 751478) shown for each of the parameters; for log GI_{50} ranged from -6.56 to -5.12, for log TGI ranged -5.12 only, for log LC_{50} ranged -5.12 only (Table 4.11). A mean graph midpoint (MG-MID) calculated for each of the parameters; log GI_{50} (-5.31), log TGI (-5.12), and log LC_{50} (-5.12).

Table 4.11. *Values of the log molar concentration of response parameter ($log_{10}GI_{50}$, $log_{10}TGI$ and $log_{10}LC_{50}$) of the* ***4.23*** *(NSC 751478).*

Cancer disease	Used cell lines	$log_{10}GI_{50}$	$log_{10}TGI$	$log_{10}LC_{50}$
Leukemia	CCRF-CEM	> -5.12	> -5.12	> -5.12
	HL-60(TB)	-5.32	> -5.12	> -5.12
	K-562	-5.48	> -5.12	> -5.12
	MOLT-4	-5.38	> -5.12	> -5.12
	RPMI-8226	-5.31	> -5.12	> -5.12
Non-small cell lung cancer	A549/ATCC	-5.42	> -5.12	> -5.12
	EKVX	-5.18	> -5.12	> -5.12
	HOP-62	> -5.12	> -5.12	> -5.12
	HOP-92	> -5.12	> -5.12	> -5.12
	NCI-H226	> -5.12	> -5.12	> -5.12
	NCI-H23	-5.46	> -5.12	> -5.12
	NCI-H322M	> -5.12	> -5.12	> -5.12
	NCI-H460	-5.60	> -5.12	> -5.12
	NCI-H522	-5.74	> -5.12	> -5.12

Colon cancer	COLO 205	> -5.12	> -5.12	> -5.12
	HCC-2998	> -5.12	> -5.12	> -5.12
	HCT-116	-6.07	> -5.12	> -5.12
	HCT-15	-5.41	> -5.12	> -5.12
	HT29	> -5.12	> -5.12	> -5.12
	KM12	-5.14	> -5.12	> -5.12
	SW-620	> -5.12	> -5.12	> -5.12
CNS cancer	SF-268	-5.22	> -5.12	> -5.12
	SF-295	-5.75	> -5.12	> -5.12
	SF-539	> -5.12	> -5.12	> -5.12
	SNB-19	> -5.12	> -5.12	> -5.12
	SNB-75	> -5.12	> -5.12	> -5.12
	U251	-5.73	> -5.12	> -5.12
Melanoma	LOX IMVI	-5.27	> -5.12	> -5.12
	MALME-3M	> -5.12	> -5.12	> -5.12
	M14	> -5.12	> -5.12	> -5.12
	MDA-MB-435	> -5.12	> -5.12	> -5.12
	SK-MEL-2	> -5.12	> -5.12	> -5.12
	SK-MEL-28	> -5.12	> -5.12	> -5.12
	SK-MEL-5	-5.75	> -5.12	> -5.12
	UACC-257	-5.54	> -5.12	> -5.12
	UACC-62	-5.17	> -5.12	> -5.12
Ovarian cancer	IGROV1	-5.30	> -5.12	> -5.12
	OVCAR-3	-5.39	> -5.12	> -5.12
	OVCAR-4	> -5.12	> -5.12	> -5.12
	OVCAR-5	> -5.12	> -5.12	> -5.12
	OVCAR-8	> -5.12	> -5.12	> -5.12
	NCI/ADR-RES	-5.28	> -5.12	> -5.12
	SK-OV-3	> -5.12	> -5.12	> -5.12
Renal cancer	786-0	-5.29	> -5.12	> -5.12
	A498	> -5.12	> -5.12	> -5.12
	ACHN	-5.23	> -5.12	> -5.12
	CAKI-1	-6.02	> -5.12	> -5.12
	RXF 393	> -5.12	> -5.12	> -5.12
	SN12C	> -5.12	> -5.12	> -5.12
	TK-10	-5.41	> -5.12	> -5.12
	UO-31	> -5.12	> -5.12	> -5.12
Prostate cancer	PC-3	-5.51	> -5.12	> -5.12
	DU-145	> -5.12	> -5.12	> -5.12
Breast cancer	MCF7	-5.37	> -5.12	> -5.12
	MDA-MB-231/ATCC	> -5.12	> -5.12	> -5.12
	HS 578T	> -5.12	> -5.12	> -5.12
	BT-549	-5.39	> -5.12	> -5.12
	T-47D	-5.17	> -5.12	> -5.12
	MDA-MB-468	-6.56	> -5.12	> -5.12
MID		-5.31	-5.12	-5.12
Delta		1.25	0	0
Range		1.44	0	0

The log molar concentration of the resulted screening of compound **4.22** (NSC 751486) shown for each of the parameters; for log GI_{50} ranged from -6.07 to -5.22, for log TGI ranged from -5.32 to -4.70, for log LC_{50} ranged from -4.98 to -4.70 (Table 4.12). A mean graph midpoint (MG-MID) calculated for each of the parameters; log GI_{50} (-5.49), log TGI (-5.10), and log LC_{50} (-4.80).

Table 4.12. *Values of the log molar concentration of response parameter ($log_{10}GI_{50}$, $log_{10}TGI$ and $log_{10}LC_{50}$) of the* ***4.22*** *(NSC 751486).*

Cancer disease	Used cell lines	$log_{10}GI_{50}$	$log_{10}TGI$	$log_{10}LC_{50}$
Leukemia	CCRF-CEM	-5.22	> -4.70	> -4.70
	HL-60(TB)	-5.53	-5.06	> -4.70
	K-562	-5.52	-5.03	> -4.70
	MOLT-4	-5.54	-4.99	> -4.70
	RPMI-8226	-5.35	> -4.70	> -4.70
Non-small cell lung cancer	A549/ATCC	-5.52	-5.12	-4.71
	EKVX	-5.47	-5.01	> -4.70
	HOP-62	-5.43	-5.13	-4.84
	HOP-92	-5.50	-5.18	-4.85
	NCI-H226	-5.52	-5.21	-4.90
	NCI-H23	-5.51	-5.17	-4.84
	NCI-H322M	-5.35	-4.93	> -4.70
	NCI-H460	-5.47	-5.10	-4.74
	NCI-H522	-6.07	-5.32	-4.84
Colon cancer	COLO 205	-5.37	-4.98	> -4.70
	HCC-2998	-5.47	-5.21	-4.95
	HCT-116	-5.61	-5.30	-4.98
	HCT-15	-5.36	-4.74	> -4.70
	HT29	-5.58	-5.25	-4.92
	KM12	-5.53	-5.23	-4.94
	SW-620	-5.50	-5.15	-4.80
CNS cancer	SF-268	-5.46	-5.10	-4.73
	SF-295	-5.58	-5.25	-4.91
	SF-539	-5.47	-5.17	-4.87
	SNB-19	-5.33	> -4.7	> -4.70
	SNB-75	-5.64	-5.14	> -4.70
	U251	-5.51	-5.19	-4.87
Melanoma	LOX IMVI	-5.48	-5.15	-4.81
	MALME-3M	-5.56	-5.20	-4.83
	M14	-5.52	-5.22	-4.92
	MDA-MB-435	-5.48	-5.15	-4.82
	SK-MEL-2	-5.55	-5.24	-4.93
	SK-MEL-28	-5.47	-5.16	-4.85
	SK-MEL-5	-5.52	-5.24	-4.96
	UACC-257	-5.54	-5.23	-4.91

	UACC-62	-5.54	-5.19	-4.84
Ovarian cancer	IGROV1	-5.45	-5.14	-4.83
	OVCAR-3	-5.54	-5.22	-4.90
	OVCAR-4	-5.57	-5.14	-4.71
	OVCAR-5	-5.45	-5.04	> -4.70
	OVCAR-8	-5.38	-4.95	> -4.70
	NCI/ADR-RES	-5.47	-5.07	> -4.70
	SK-OV-3	-5.38	-4.98	> -4.70
Renal cancer	786-0	-5.49	-5.16	-4.84
	A498	-5.61	-5.29	-4.96
	ACHN	-5.22	> -4.70	> -4.70
	CAKI-1	-5.44	-5.13	-4.83
	RXF 393	-5.58	-5.28	-4.98
	SN12C	-5.49	-5.11	-4.74
	TK-10	-5.42	-5.08	-4.73
	UO-31	-5.56	-5.22	-4.88
Prostate cancer	PC-3	-5.46	-4.98	> -4.70
	DU-145	-5.49	-5.12	-4.75
Breast cancer	MCF7	-5.32	-4.87	> -4.70
	MDA-MB-231/ATCC	-5.55	-5.19	-4.84
	HS 578T	-5.49	-5.08	> -4.70
	BT-549	-5.58	-5.26	-4.94
	T-47D	-5.40	-4.99	> -4.70
	MDA-MB-468	-5.53	-5.14	-4.76
MID		-5.49	-5.10	-4.80
Delta		0.58	0.22	0.18
Range		0.85	0.22	0.28

The log molar concentration of the resulted screening of compound **4.37c** (NSC 764189) shown for each of the parameters; for log GI_{50} ranged - 5.30 only, for log TGI ranged - 5.30 only, for log LC_{50} ranged - 5.30 only. A mean graph midpoint (MG-MID) calculated for each of the parameters; log GI_{50} (-5.30), log TGI (-5.30), and log LC_{50} (-5.30) (refer to the Supplementary data in chapter 5**)**.

The log molar concentration of the resulted screening of compound **4.21** (NSC 764190) shown for each of the parameters; for log GI_{50} ranged from -6.57 to -4.00, for log TGI ranged from -4.22 to -4.00, for log LC_{50} ranged -4.00 only (Table 4.13). A mean graph midpoint (MG-MID) calculated for each of the parameters; log GI_{50} (-4.94), log TGI (-4.0), and log LC_{50} (-4.0).

Table 4.13. *Values of the log molar concentration of response parameter ($log_{10}GI_{50}$, $log_{10}TGI$ and $log_{10}LC_{50}$) of the* ***4.21*** *(NSC 764190).*

Cancer disease	Used cell lines	$log_{10}GI_{50}$	$log_{10}TGI$	$log_{10}LC_{50}$
Leukemia	CCRF-CEM	-5.41	> -4.00	> -4.00
	HL-60(TB)	-5.46	> -4.00	> -4.00
	K-562	-5.70	> -4.00	> -4.00
	MOLT-4	-5.67	> -4.00	> -4.00
	RPMI-8226	-6.07	> -4.00	> -4.00
	SR	-5.78	> -4.00	> -4.00
Non-small cell lung cancer	A549/ATCC	-5.12	> -4.00	> -4.00
	HOP-62	> -4.00	> -4.00	> -4.00
	HOP-92	-5.36	> -4.00	> -4.00
	NCI-H226	-5.36	> -4.00	> -4.00
	NCI-H23	-5.78	> -4.00	> -4.00
	NCI-H322M	> -4.00	> -4.00	> -4.00
	NCI-H460	-5.56	> -4.00	> -4.00
	NCI-H522	-5.26	> -4.00	> -4.00
Colon cancer	COLO 205	> -4.00	> -4.00	> -4.00
	HCC-2998	> -4.00	> -4.00	> -4.00
	HCT-116	-6.27	> -4.00	> -4.00
	HCT-15	-5.45	> -4.00	> -4.00
	HT29	-4.37	> -4.00	> -4.00
	KM12	-5.00	> -4.00	> -4.00
	SW-620	nd	> -4.00	> -4.00
CNS cancer	SF-268	-5.11	> -4.00	> -4.00
	SF-295	-6.18	> -4.00	> -4.00
	SF-539	> -4.00	> -4.00	> -4.00
	SNB-19	> -4.00	> -4.00	> -4.00
	SNB-75	> -4.00	> -4.00	> -4.00
	U251	-5.96	> -4.00	> -4.00
Melanoma	LOX IMVI	-5.85	> -4.00	> -4.00
	MALME-3M	> -4.00	> -4.00	> -4.00
	M14	nd	> -4.00	> -4.00
	MDA-MB-435	> -4.00	> -4.00	> -4.00
	SK-MEL-2	> -4.00	> -4.00	> -4.00
	SK-MEL-28	> -4.00	> -4.00	> -4.00
	SK-MEL-5	-5.94	> -4.00	> -4.00
	UACC-257	-5.65	> -4.00	> -4.00
	UACC-62	-5.57	> -4.00	> -4.00
Ovarian cancer	IGROV1	> -4.00	> -4.00	> -4.00
	OVCAR-3	-5.63	> -4.00	> -4.00
	OVCAR-4	-6.32	> -4.00	> -4.00
	OVCAR-5	> -4.00	> -4.00	> -4.00
	OVCAR-8	> -4.00	> -4.00	> -4.00
	NCI/ADR-RES	-5.15	> -4.00	> -4.00
	SK-OV-3	> -4.00	> -4.00	> -4.00
Renal cancer	786-0	> -4.00	> -4.00	> -4.00
	A498	-5.75	> -4.00	> -4.00
	ACHN	-5.68	> -4.00	> -4.00
	CAKI-1	-5.55	> -4.00	> -4.00

	RXF 393	-4.55	> -4.00	> -4.00
	SN12C	-5.03	> -4.00	> -4.00
	TK-10	-5.08	> -4.00	> -4.00
	UO-31	> -4.00	> -4.00	> -4.00
Prostate cancer	PC-3	-6.24	> -4.00	> -4.00
	DU-145	> -4.00	> -4.00	> -4.00
Breast cancer	MCF7	> -4.00	> -4.00	> -4.00
	MDA-MB-231/ATCC	> -4.00	> -4.00	> -4.00
	HS 578T	> -4.00	> -4.00	> -4.00
	BT-549	> -4.00	> -4.00	> -4.00
	T-47D	> -4.00	> -4.00	> -4.00
	MDA-MB-468	-6.57	> -4.22	> -4.00
MID		-4.94	-4.0	-4.0
Delta		1.63	0.22	0
Range		2.57	0.22	0.0

4.4. Conclusion

In conclusion, an analog of DMP 323 from a variety of synthesized symmetric, and unsymmetric cyclic sulfamides and additional urea and phosphorus-containing compounds were screened in NCI 60 cancer cell line to identify biologically active compounds. With the concept of drug repositioning, we studied a new opportunity of application of these compounds which are known HIV protease inhibitors to potential agents for the treatment of cancer. The two-stage process of *in vitro* screening was carried out: wherein 29 compounds were selected for one-dose study and 4 compounds selected for a five-dose study for *in vitro* cytotoxicity evaluation. Generally, compounds were selectively sensitive on the leukemia, colon cancer, prostate cancer and breast cancer cell. Notably, almost every compound demonstrated strong inhibition against breast cancer (MDA-MB-468). The primary one-dose study revealed that compounds **4.21**, **4.22**, **4.23**, and **4.37c** possessed high activity against different cancer types. Four such compounds were further tested in the five-dose experiment. To understand the mechanism of the observed cytotoxicity, investigations with a representative compound are underway to learn about the effect for apoptosis, migration, anchorage independent growth and cellular senescence.

4.5. References

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CHAPTER 5

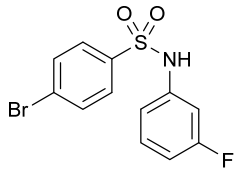
Experimental data

5.1. General Methods

All air and moisture sensitive reactions were carried out in flame- or oven dried glassware under argon or nitrogen using standard gas tight syringes, cannulas, and septa. Methylene chloride (CH_2Cl_2), tetrahydrofuran (THF), diethyl ether (Et_2O), and toluene were purified by passage through a Solv-Tek solvent purification system employing activated Al_2O_3, or used them immediately after purchasing from Sigma-Aldrich as anhydrous solvent grade. Triethylamine (Et_3N) was stored over KOH. Sodium triacetoxyborohydride (97%) was purchased from Sigma-Aldrich and was not further purified. All amino acids and amines were purchased from Sigma-Aldrich. Thin layer chromatography was performed on silica gel $60F_{254}$ plates (EM-5717, Merck). Visualization of TLC spots were effected using $KMnO_4$ stain or UV lamp (254 nm). Flash column chromatography was performed with Teledyne ISCO CombiFlash companion using various sizes of Teledyne columns or Grace® Flash Cartridges. Deuterochloroform ($CDCl_3$) with and without TMS (0.03% (v/v)) was purchased from Sigma-Aldrich and stored in desiccator at room temperature. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ (unless otherwise noted) on either Varian-400 MHz spectrometer operating at 400MHz and 100 MHz, respectively.

5.2. Experimental Procedure and data: Chapter 2

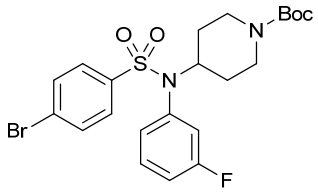
4-Bromo-*N*-(3-fluorophenyl)benzenesulfonamide (**2.4**)



To a solution of 3-fluoroaniline (1.0 mL, 10.39 mmol) and pyridine (1.3 mL, 16.07 mmol) in CH_2Cl_2 (20 mL) was added a solution of 4-bromobenzene sulfonyl chloride (2.65 g, 10.44 mmol) in CH_2Cl_2 (30 mL) at room temperature. The color of solution was changed to light orange. The reaction mixture was stirred overnight. The reaction mixture was evaporated, and purified by the ISCO-Flash column chromatography in 0% to 40% of EtOAc in hexane to get white solid as a product **2.4** (3.31 g, 97 %).

Analytical data for **2.4:** R_f = 0.83 (Sol. EtOAc:Hexane = 1/1); FTIR (neat) 3240, 2383, 2368, 1612, 1601, 1573, 1483, 1468, 1399, 1389, 1334, 1265, 1154, 1130, 1088, 1067, 1009, 962, 913, 823, 762, 742, 682, 630 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.70–7.65 (m, 2H), 7.62–7.57 (m, 2H), 7.25 (s, 1H), 7.20 (ddd, *J* = 8.2, 8.2, 6.3 Hz, 1H), 6.91 (ddd, *J* = 10.0, 2.3, 2.3 Hz, 1H), 6.85–6.80 (m, 2H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 163.18 (d, *J* = 247.2 Hz), 137.81, 137.74 (d, *J* = 6.4 Hz), 132.68, 130.88 (d, *J* = 9.3 Hz), 128.84, 128.67, 116.69 (d, *J* = 3.1 Hz), 112.61 (d, *J* = 21.2 Hz), 108.64 (d, *J* = 25.3 Hz); HRMS $(M+Na)^+$ calcd for $C_{12}H_9BrFNNaO_2S^+$ (M+Na) required 351.9419, found 351.9410.

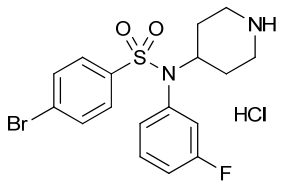
***tert*-Butyl 4-(4-bromo-*N*-(3-fluorophenyl)phenylsulfonamido)piperidine-1-carboxylate (2.5)**



To a solution of **2.4** (0.52 g, 1.56 mmol) and DIAD (0.92 mL, 4.67 mmol) in THF (10 mL) was added a solution of *tert*-butyl-4-hydroxy-1-piperidinecarboxylate (0.38 g, 1.88 mmol) and PPh_3 (1.23 g, 4.70 mmol) in THF (10 mL) at room temperature and heated to 60 ºC for overnight. The reaction mixture was evaporated, and purified by the ISCO-Flash column chromatography in 0% to 40% of EtOAc in hexane to get white solid as a product **2.5** (0.63 g, 78 %).

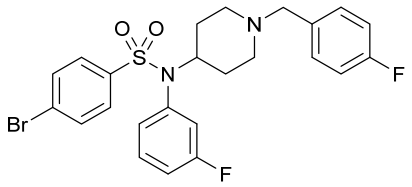
Analytical data for **2.5**: R_f = 0.68 (Sol. EtOAc:Hexane = 1/1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66–6.55 (m, 4H), 7.31 (ddd, *J* = 8.1, 6.4, 6.4 Hz, 1H), 7.11 (dddd, *J* = 8.3, 8.3, 2.5, 0.8 Hz, 1H), 6.83–6.72 (m, 2H), 4.29 (tt, *J* = 12.1, 3.8 Hz, 1H), 4.12 (m, 2H), 2.74 (t, *J* = 12.9 Hz, 2H), 1.76 (d, *J* = 12.2 Hz, 2H), 1.37 (s, 9H), 1.27–1.26 (m, 2H).

4-Bromo-*N*-(3-fluorophenyl)-*N*-(piperidin-4-yl)benzenesulfonamide hydrochloride (**2.6**)



To a solution of **2.5** (0.63g, 1.22 mmol) in CH_2Cl_2 (20 mL) was added 4N HCl in 1,4-dioxane (20 mL,) at 0 °C and stirred overnight. A reaction mixture was evaporated to remove solvent, and then the mixture was dried under reduced vacuum to furnish a white solid as a product **2.6**. It used without further purification.

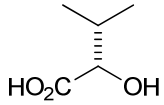
4-Bromo-*N*-(1-(4-fluorobenzyl)piperidin-4-yl)-*N*-(3-fluorophenyl)benzenesulfonamide (2.7)



To a solution of **2.6** (0.34 g, 0.76 mmol), 4-fluorobenzaldehyde (0.09 mL, 0.84 mmol) and Et_3N (0.1 mL, 0.72 mmol) in CH_2Cl_2 (10 mL) was added $NaBH(OAc)_3$ (0.52 g, 2.44 mmol) at 0 °C and stirred for overnight. The reaction mixture was extracted with CH_2Cl_2 (100 mL X 3) and then the combined organic layer was dried over $MgSO_4$, filtered, evaporated, and purified by the ISCO-Flash column chromatography in 0% to 40% of EtOAc in hexane to get white oil as a product **2.7** (0.10 g, 24 %).

Analytical data for **2.7**: R_f = 0.81 (Sol. EtOAc:Hexane = 1/1); FTIR (neat) 3241, 3098, 2986, 2383, 2309, 1796, 1770, 1733, 1611, 1600, 1573, 1482, 1467, 1400, 1389, 1334, 1264, 1154, 1129, 1087, 1067, 1009, 962, 912, 823, 762, 741, 682, 621 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.64–7.55 (m, 2H), 7.57 (d, *J* = 8.6 Hz, 2H), 7.29 (m, 1H), 7.23–7.13 (m, 2H), 7.09 (ddd, *J* = 8.2, 2.4, 2.4 Hz, 1H), 6.95 (dd, *J* = 8.5, 8.5 Hz, 2H), 6.79–6.73 (m, 2H), 4.22–4.08 (m, 1H), 3.39 (s, 2H), 2.92–2.74 (m, 2H), 2.14–1.94 (m, 2H), 1.78-1.65 (m, 2H), 1.52–1.33 (m, 2H); ^{13}C NMR (101 MHz, Chloroform) δ 162.58 (d, *J* = 248.9 Hz), 162.58 (d, *J* = 248.9 Hz), 140.20, 136.63 (d, *J* = 8.9 Hz), 132.43, 130.08 (d, *J* = 9.2 Hz), 128.94, 128.84, 128.34 (d, *J* = 2.7 Hz), 127.76, 119.82 (d, *J* = 21.4 Hz), 116.47 (d, *J* = 20.5 Hz), 115.52 (d, *J* = 21.4 Hz), 115.21 (d, *J* = 21.1 Hz), 62.00, 57.99, 52.89, 31.77; HRMS $(M+H)^+$ calcd for $C_{24}H_{24}BrF_2N_2O_2S^+$ (M+H) required 521.0710, found 521.0846.

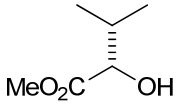
(*S*)-2-hydroxy-3-methylbutanoic acid (**2.8**)



To a solution of L-valine (5.00 g, 42.72 mmol) in 1N H_2SO_4 (100 mL) was added slowly a solution of $NaNO_2$ (6.02 g, 87.25 mmol) at 0 °C and stirred overnight. The reaction mixture was extracted with diethyl ether (100 mL X 4) and concentrated by azeotropic distillation with toluene to provide yellow oil. A yellow oil was dried under reduced vacuum to furnish a crystal as white needles **2.8** (3.50 g, 70%).

Analytical data for **2.8**: FTIR (neat) 3428, 2968, 2936, 2879, 1716, 1645, 1211, 1136, 1027, 727, 616 cm^{-1}; ^{1}H NMR (CD_3CN, 400 MHz) δ 4.03 (d, 1H), 2.06 (m, 1H), 0.99 (d, J = 8.0 Hz, 3H), 0.87 (d, J = 8.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 175.6, 117.7, 74.7, 31.9, 18.4.

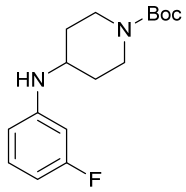
(*S*)-methyl 2-hydroxy-3-methylbutanoate (**2.9**)



To a solution of **2.8** (3.49 g, 29.54 mmol) in methanol (50 mL) was added amberlyst-15 ion exchange resin at room temperature and stirred for overnight. The reaction mixture was filtered and evaporated to remove solvent to give yellow oil. The yellow oil was purified by the ISCO-Flash column chromatography in 0% to 40% of EtOAc in hexane to get yellow oil as a product **2.9** (2.03 g, 52%).

Analytical data for **2.9**: R_f = 0.71 (Sol. EtOAc:Hexane = 1/1, checked by $KMnO_4$ stain solution); FTIR (neat) 2958, 2922, 2851, 1743, 1672, 1428, 1621, 1428, 1276, 1175, 1147, 936, 860, 756 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 4.02 (dd, *J* = 6.0, 3.6 Hz, 1H), 3.76 (s, 3H), 2.80 (d, *J* = 6.0 Hz, 1H), 2.04 (dqq, *J* = 6.9, 6.9, 3.4 Hz, 1H), 0.99 (d, *J* = 6.9 Hz, 3H), 0.84 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 175.5, 75.3, 52.4, 32.3, 18.7, 16.2.

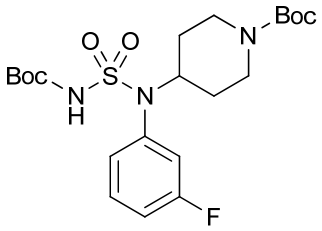
***tert*-Butyl 4-(3-fluorophenylamino)piperidine-1-carboxylate** (**2.13**)



To a solution of 1-Boc-4-piperidone (7.92 g, 39.77 mmol) and 3-fluoroaniline (4.0 mL, 41.61 mmol) in CH_2Cl_2 (100 mL) was added $NaBH(OAc)_3$ (26.05 g, 122.90 mmol) at 0 °C and stirred for 2 hrs. To a reaction mixture was added glacial acetic acid (5.0 mL, 87.34 mmol) at 0 °C and stirred at ambient temperature overnight. The reaction mixture was washed with saturated aqueous $NaHCO_3$ solution. The mixture was extracted with CH_2Cl_2 (200 mL X 3) and then the combined organic layer was dried over $MgSO_4$, filtered, evaporated, and purified by the ISCO-Flash column chromatography in 0% to 40% of EtOAc in hexane to get white solid as a product **2.13** (11.21 g, 99%).

Analytical data for **2.13**: Rf = 0.88 (Sol. EtOAc:Hexane = 1/1); FTIR (neat) 3353, 3010, 2168, 2141, 1666, 1621, 1494, 1421, 1236, 1160, 936, 756, 687, 609 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.08 (ddd, *J* = 8.1, 6.8, 6.8 Hz, 1H), 6.40–6.38 (m, 1H), 6.36–6.32 (m, 1H), 6.28 (ddd, *J* = 4.45, 2.3, 2.3 Hz, 1H), 4.18-3.93 (m, 2H), 3.66 (s, 1H), 3.43-3.32 (m, 1H), 2.92 (t, *J* = 12.0 Hz, 2H), 2.09–1.94 (td, *J* = 7.4, 2.7 Hz, 2H), 1.47 (s, 9H), 1.39–1.26 (m, 2H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 164.29 (d, *J* = 242.7 Hz), 154.87, 148.69 (d, *J* = 11.0 Hz), 130.53 (d, *J* = 10.2 Hz), 109.18 (d, *J* = 2.2 Hz), 103.90 (d, *J* = 21.6 Hz), 99.84 (d, *J* = 25.4 Hz), 79.79, 76.82, 50.25, 32.35, 28.56; HRMS $(M+Na)^+$ calcd for $C_{16}H_{23}FN_2NaO_2^+$ (M+Na) required 317.1636, found 317.1635.

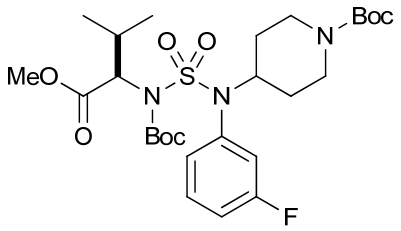
tert-Butyl 4-((_N_-(tert-butoxycarbonyl)sulfamoyl)(3-fluorophenyl)amino)piperidine-1-carboxylate (2.15)



To a solution of chlorosulfonyl isocyanate (1.5 mL, 17.23 mmol) in CH_2Cl_2 (20 mL) was added to a solution of *tert*-butyl alcohol (1.65 mL, 17.25 mmol) in CH_2Cl_2 (20 mL) at 0 °C. This solution was cannulated to a solution of **2.13** (3.46 g, 11.75 mmol) and Et_3N (3.0 mL, 21.52 mmol) in CH_2Cl_2 (30 mL) at 0 °C. After that, the reaction mixture was stirred at ambient temperature for overnight. The reaction mixture was extracted with CH_2Cl_2 (100 mL X 4) and dried over $MgSO_4$, filtered, evaporated, and purified by the ISCO-Flash column chromatography in 0% to 40% of EtOAc in hexane to get white solid as a product **2.15** (6.20 g, 91%).

Analytical data for **2.15**: R_f = 0.80 (Sol. EtOAc:Hexane = 1/1); FTIR (neat) 3745, 3712, 3067, 2977, 2944, 2863, 2357, 2325, 1735, 1659, 1436, 1366, 1353, 1243, 1168, 1135, 1056, 979, 827, 725, 694, 616 cm^{-1}; ^1H NMR (400 MHz, Chloroform-*d*) δ 7.38 (ddd, *J* = 8.2, 6.4, 6.4 Hz, 1H), 7.15 (dddd, *J* = 8.3, 8.3, 2.5, 0.9 Hz, 1H), 7.07–7.01 (m, 2H), 6.95 (ddd, *J* = 4.4, 2.4, 2.4 Hz, 1H), 4.41 (tt, *J* = 12.1, 3.8 Hz, 1H), 4.20–4.05 (m, 2H), 2.78 (t, *J* = 11.9 Hz, 2H), 2.03–1.94 (m, 2H), 1.52 (s, 9H), 1.38 (s, 9H), 1.27 (dtd, *J* = 15.5, 4.0 Hz, 2H); ^{13}C NMR (101 MHz, $cdcl_3$) δ 162.70 (d, *J* = 249.0 Hz), 154.56, 149.52, 135.99 (d, *J* = 9.7 Hz), 130.38 (d, *J* = 9.1 Hz), 128.11 (d, *J* = 3.5 Hz), 119.48 (d, *J* = 21.7 Hz), 116.89 (d, *J* = 20.9 Hz). 83.87, 79.91, 59.35, 59.35, 31.81, 28.49, 28.17.

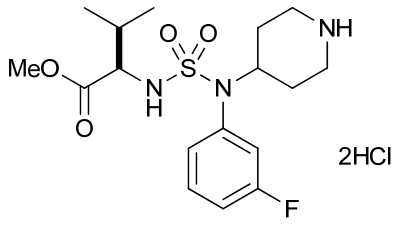
(*R*)-*tert*-Butyl 4-((*N*-(tert-butoxycarbonyl)-*N*-(1-methoxy-3-methyl-1-oxobutan-2-yl) sulfamoyl)(3-fluorophenyl)amino)piperidine-1-carboxylate (2.17)



To a solution of **2.13** (1.01 g, 2.14 mmol) and DIAD (3.0 mL, 6.59 mmol) in THF (20 mL) was added a solution of **2.9** (0.34 g, 2.60 mmol) and PPh_3 (1.69 g, 6.44 mmol) in THF (20 mL) at room temperature and then the reaction mixture was heated at 65 °C for overnight. The reaction mixture was extracted with CH_2Cl_2 (150 mL X 3) and the combined organic layer was dried over $MgSO_4$, filtered, evaporated, and purified by the ISCO-Flash column chromatography in 0% to 40% of EtOAc in hexane to get white oil as a product **2.17** (1.22 g, 97%).

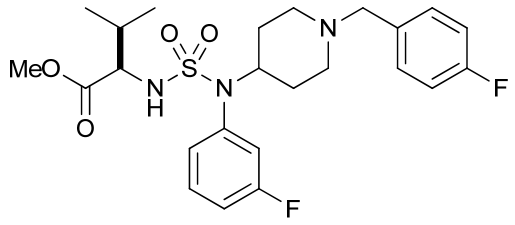
Analytical data for **2.17**: R_f = 0.86 (Sol. EtOAc:Hexane = 1/1); $[\alpha]^{25}_D$ = + 1.188 (*c* = 1.094, $CHCl_3$); FTIR (neat) 3333, 2991, 2978, 2924, 2853, 1666, 1611, 1588, 1524, 1476, 1430, 1366, 1342, 1311, 1233, 1187, 1167, 1139, 998, 980, 936, 862, 821, 756, 627 cm^{-1}; ^1H NMR (400 MHz, Chloroform-*d*) δ 7.36 (ddd, *J* = 8.2, 6.5, 6.5 Hz, 1H), 7.14 (ddd, *J* = 10.8, 10.8, 2.5 Hz, 1H), 7.11–7.07 (m, 1H), 6.99 (ddd, *J* = 9.6, 2.2, 2.2 Hz, 1H), 4.47 (tt, *J* = 12.0, 3.7 Hz, 1H), 4.30 (d, *J* = 8.8 Hz, 1H), 4.12 (q, *J* = 7.2 Hz, 2H), 3.59 (s, 3H), 2.80 (t, *J* = 12.2 Hz, 2H), 2.42 (ddt, *J* = 13.5, 8.7, 6.8 Hz, 1H), 2.03–1.94 (m, 2H), 1.50 (s, 9H), 1.38 (s, 9H), 1.26 (ddd, *J* = 8.7, 3.9, 2.2 Hz, 2H), 1.07 (d, *J* = 6.4 Hz, 3H), 0.92 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (101 MHz, cdcl$_3$) δ 170.18, 162.34 (d, *J* = 247.6 Hz), 154.59, 150.64, 135.87 (d, *J* = 10.1 Hz), 129.73 (d, *J* = 9.1 Hz), 129.01 (d, *J* = 3.2 Hz), 120.27 (d, *J* = 21.8 Hz), 116.50 (d, *J* = 21.0 Hz), 84.59, 79.83, 60.60, 59.43, 59.27, 52.07, 28.49, 28.30, 28.09, 22.28, 19.50.

(*R*)-Methyl 2-(*N*-(3-fluorophenyl)-*N*-(piperidin-4-yl)sulfamoylamino)-3-methylbutanoate dihydrochloride (2.19)



To a solution of **2.17** (0.39 g, 0.66 mmol) in CH_2Cl_2 (20 mL) was added 4N HCl in 1,4-dioxane (5 mL) at 0 °C and stirred overnight. A reaction mixture was evaporated to remove solvent, and then the mixture was dried under reduced vacuum to furnish a white solid as a product **2.19**. It used without further purification.

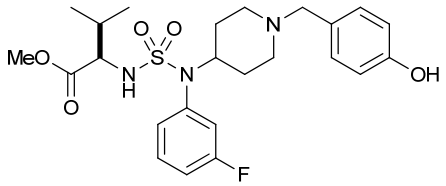
(*R*)-Methyl 2-(*N*-(1-(4-fluorobenzyl)piperidin-4-yl)-*N*-(3-fluorophenyl)sulfamoylamino)-3-methyl butanoate (2.21)



To a solution of **2.19** (0.13 g, 0.34 mmol) and 4-fluorobenzaldehyde (0.045 mL, 0.42 mmol) in CH_2Cl_2 (20 mL) was added $NaBH(OAc)_3$ (0.22 g, 1.03 mmol) at 0 °C and stirred for 2 hrs. To a solution was added glacial acetic acid (0.04 mL, 0.70 mmol) at 0 °C and stirred overnight. The reaction mixture was extracted with CH_2Cl_2 (150 mL X 3) and EtOAc (200 mL) and then the combined organic layer was dried over $MgSO_4$, filtered, evaporated, and purified by the ISCO-Flash column chromatography in 0% to 60% of EtOAc in hexane to get white oil as a product **2.21** (0.0742 g, 45%).

Analytical data for **2.21**: R_f = 0.89 (Sol. EtOAc:Hexane = 1/1); $[\alpha]^{25}_D$ = + 23.661 (*c* = 0.224, CH_2Cl_2); FTIR (neat) 3316, 2925, 2854, 2383, 23236, 1589, 1512, 1495, 1338, 1226, 1151, 938, 828, 760, 685 cm^{-1}; ^1H NMR (400 MHz, Chloroform-*d*) δ 7.32 (ddd, *J* = 16.3, 16.3, 8.2 Hz, 1H), 7.21–7.15 (m, 2H), 7.10–7.02 (m, 2H), 6.99–6.95 (m, 2H), 6.94–6.93 (m, 1H), 5.02 (d, *J* = 9.4 Hz, 1H), 3.89 (tt, *J* = 12.1, 3.8 Hz, 1H), 3.77 (dd, *J* = 9.4, 5.0 Hz, 1H), 3.73 (s, 3H), 3.42 (s, 2H), 2.90–2.87 (m, 2H), 2.11–2.02 (m, 2H), 2.02–1.98 (m, 1H), 1.97–1.90 (m, 1H), 1.83 (dt, *J* = 12.3, 3.1 Hz, 1H), 1.47 (dddd, *J* = 19.6, 12.2, 7.6, 4.0 Hz, 2H), 0.94 (d, *J* = 6.8 Hz, 3H), 0.88 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.47, 164.6 (d, *J* = 248.5 Hz), 164.2 (d, *J* = 247.5 Hz), 140.0 (d, *J* = 11.1 Hz), 135.34, 132.86, 132.44 (d, *J* = 84.8 Hz), 129.65, 121.08 (d, *J* = 21.2), 117.91 (d, *J* = 21.2), 117.20 (d, *J* = 22.2 Hz), 63.88, 63.62, 60.26, 54.84, 54.78, 54.52, 34.07, 33.42, 33.15, 20.89, 19.83; HRMS $(M+H)^+$ calcd for $C_{24}H_{32}F_2N_3O_4S^+$ (M+H) required 496.2081, found 496.2090.

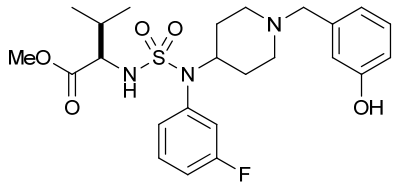
(*R*)-Methyl 2-(*N*-(3-fluorophenyl)-*N*-(1-(4-hydroxybenzyl)piperidin-4-yl)sulfamoyl-amino)-3-methylbutanoate (2.22)



A solution of **2.19** (0.38 g, 0.98 mmol), 4-hydroxybenzaldehyde (0.15 g, 1.11 mmol) in CH_2Cl_2 (20 mL) was treated with $NaBH(OAc)_3$ (0.65 g, 3.05 mmol) at room temperature and stirred for 2 hrs. To a reaction mixture was added glacial acetic acid (0.11 mL, 1.92 mmol) at 0 ºC and stirred overnight. A reaction mixture was quenched with aqueous $NaHCO_3$ solution and extracted with CH_2Cl_2 (150 mL X 2) and then the combined organic layer was dried over $MgSO_4$, filtered, evaporated, and purified by the ISCO-Flash column chromatography in 0% to 40% of EtOAc in hexane to get white solid as a product **2.22** (0.08 g, 17%).

Analytical data for **2.22**: R_f = 0.29 (Sol. EtOAc:Hexane = 1/1); $[\alpha]^{25}_D$ = + 27.471 (*c* = 0.597, CH_2Cl_2); FTIR (neat) 3711, 2925, 2855, 2369, 1731, 1610, 1593, 1516, 1366, 1248, 1144, 1131, 1059, 1033, 1010, 831, 777, 692 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.26 (ddd, *J* = 14.7, 8.0, 0 Hz, 1H), 7.05 (d, *J* = 8.3 Hz, 2H), 7.02(m, 2H), 6.89 (m, 1H), 6.62 (d, *J* = 8.3 Hz, 2H), 4.97 (d, *J* = 9.4 Hz, 1H), 3.87 (t, *J* = 11.9 Hz, 1H), 3.74 (dd, *J* = 9.3, 5.0 Hz, 1H), 3.71 (s, 3H), 3.36 (s, 2H), 2.90 (d, *J* = 10.9 Hz, 2H), 2.10–1.96 (m, 3H), 1.87 (ddd, J = 43.9, 12.2, 0 Hz, 2H), 1.47 (m, 2H), 0.92 (d, *J* = 6.8 Hz, 3H), 0.86 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.33, 162.37 (d, *J* = 249.5 Hz), 155.14, 137.62, 130.69, 130.28 (d, *J* = 83.8 Hz), 129.82 (d, *J* = 9.1 Hz), 127.42 (d, *J* = 3.0 Hz), 118.85 (d, *J* = 22.2 Hz), 115.52 (d, *J* = 48.5 Hz), 115.17, 62.03, 61.41, 58.04, 52.67, 52.61, 52.42, 31.92, 31.09, 30.82, 18.74, 17.66; HRMS $(M+H)^+$ calcd for $C_{24}H_{33}FN_3O_5S^+$ (M+H) required 494.2125, found 494.2160.

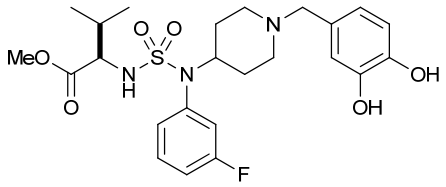
(*R*)-Methyl 2-(*N*-(3-fluorophenyl)-*N*-(1-(3-hydroxybenzyl)piperidin-4-yl)sulfamoyl-amino)-3-methylbutanoate (2.23)



A solution of **2.19** (0.18 g, 0.45 mmol), 3-hydroxybenzaldehyde (0.06 g, 0.45 mmol) in CH_2Cl_2 (10 mL) was treated with $NaBH(OAc)_3$ (0.29 g, 1.36 mmol) at room temperature and stirred for 2 hrs. To a reaction mixture was added glacial acetic acid (0.05 mL, 0.87 mmol) at 0 ºC and stirred overnight. A reaction mixture was quenched with aqueous $NaHCO_3$ solution and extracted with CH_2Cl_2 (100 mL X 2) and then the combined organic layer was dried over $MgSO_4$, filtered, evaporated, and purified by the ISCO-Flash column chromatography in 0% to 40% of EtOAc in hexane to get white solid as a product **2.23** (38.1 mg, 17%).

Analytical data for **2.23**: R_f = 0.42 (Sol. EtOAc:Hexane = 1/1); $[\alpha]^{25}_D$ = + 1.538 (*c* = 0.130, CH_2Cl_2); FTIR (neat) 3274, 2964, 2874, 2845, 2299, 2256, 1738, 1591, 1486, 1455, 1339, 1264, 1207, 1161, 1140, 1054, 982, 911, 888, 858, 777, 730, 692, 649, 605 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.28 (ddd, *J* = 14.7, 8.0, 0 Hz, 1H) 7.11 (dd, *J* = 7.8, 7.8 Hz, 1H), 7.03 (m, 2H), 6.91 (d, *J* = 9.5 Hz, 1H), 6.76–6.63 (m, 3H), 5.00 (d, *J* = 9.4 Hz, 1H), 3.87 (t, *J* = 12.0 Hz, 1H), 3.76 (dd, *J* = 8.2, 3.3 Hz, 1H), 3.71 (s, 3H), 3.38 (s, 2H), 2.90 (d, *J* = 11.0 Hz, 2H), 2.06 (t, *J* = 12.2 Hz, 2H), 1.99 (m, 1H), 1.85 (ddd, *J* = 44.2, 12.4, 0 Hz, 2H), 1.49 (ddd, *J* = 20.8, 11.6, 8.8 Hz, 2H), 0.92 (d, *J* = 6.8 Hz, 3H), 0.86 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.33, 161.15, 155.89, 137.42, 129.86 (d, *J* = 9.1 Hz), 129.39, 127.42 (d, *J* = 3.0 Hz), 121.31, 118.85 (d, *J* = 22.2 Hz), 116.01, 115.96 (d, *J* = 11.1 Hz), 115.69, 114.48, 62.34, 61.43, 57.97, 52.84, 52.78, 52.43, 31.92, 31.11, 30.84, 18.75, 17.67; HRMS $(M+H)^+$ calcd for $C_{24}H_{33}FN_3O_5S^+$ (M+H) required 494.2125, found 494.3777.

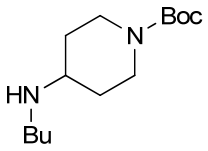
(*R*)-Methyl 2-((*N*-(1-(3,4-dihydroxybenzyl)piperidin-4-yl)-*N*-(3-fluorophenyl)sulfamoyl)-amino)-3-methylbutanoate (2.24)



A solution of **2.19** (0.16 g, 0.41 mmol), 3,4-dihydroxybenzaldehyde (0.06 g, 0.45 mmol) in CH_2Cl_2 (10 mL) was treated with $NaBH(OAc)_3$ (0.29 g, 1.36 mmol) at room temperature and stirred for 2 hrs. To a reaction mixture was added glacial acetic acid (0.05 mL, 0.87 mmol) at 0 ºC and stirred overnight. A reaction mixture was quenched with aqueous $NaHCO_3$ solution and extracted with CH_2Cl_2 (100 mL X 2) and then the combined organic layer was dried over $MgSO_4$, filtered, evaporated, and purified by the ISCO-Flash column chromatography in 0% to 40% of EtOAc in hexane to get white solid as a product **2.24** (0.06 g, 28.5%).

Analytical data for **2.24**: R_f = 0.07 (Sol. EtOAc:Hexane = 1/1); $[\alpha]^{25}_D$ = + 2.033 (*c* = 0.246, CH_2Cl_2); FTIR (neat) 3283, 2964, 2875, 2360, 2257, 1737, 1667, 1607, 1592, 1486, 1445, 1339, 1266, 1206, 1161, 1139, 1055, 982, 912, 787, 731, 693, 650, 604 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.23 (ddd, J = 15.5, 7.9, 0 Hz, 1H), 6.98 (t, *J* = 7.5 Hz, 1H), 6.91 (d, *J* = 7.8 Hz, 1H), 6.84 (d, *J* = 9.3 Hz, 1H), 6.62–6.47 (m, 3H), 5.50 (bs, 3H), 3.87 (t, *J* = 11.5 Hz, 1H), 3.71 (s, 1H), 3.69 (s, 3H), 3.37 (s, 2H), 2.95 (d, *J* = 10.2 Hz, 2H), 2.13 (t, *J* = 11.5 Hz, 2H), 1.98 (dq, *J* = 11.6, 6.8 Hz, 1H), 1.84 (ddd, *J* = 47.1, 11.4, 0 Hz, 2H), 1.53–1.43 (m, 2H), 0.89 (d, *J* = 6.8 Hz, 3H), 0.84 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.45, 162.29 (d, *J* = 249.5 Hz), 144.70, 144.27, 137.49 (d, *J* = 9.1 Hz), 129.94 (d, *J* = 9.1 Hz), 127.12, 122.11, 118.67, 118.45, 116.91,115.89 (d, *J* = 20.2 Hz), 114.93, 61.63, 61.52, 57.04, 52.44, 52.12, 51.99, 31.83, 30.04, 29.72, 18.76, 17.69; HRMS $(M+H)^+$ calcd for $C_{24}H_{33}FN_3O_6S^+$ (M+H) required 510.2074, found 510.2185.

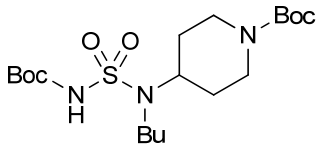
***tert*-Butyl 4-(butylamino)piperidine-1-carboxylate (2.14)**



To a solution of 1-Boc-4-piperidone (6.28 g, 31.50 mmol) and butylamine (3.10 mL, 31.24 mmol) in CH_2Cl_2 (200 mL) was added $NaBH(OAc)_3$ at 0 °C and stirred for 2 hrs. To a reaction mixture was added glacial acetic acid (3.6 mL, 62.89 mmol) at 0 °C and stirred at ambient temperature overnight. The reaction mixture was washed with saturated aqueous $NaHCO_3$ solution. The mixture was extracted with CH_2Cl_2 (150 mL X 3) and then the combined organic layer was dried over $MgSO_4$, filtered, and evaporated to a product **2.14** as a colorless oil with spectral data identical to those previously reported.[1] It used without further purification.

Analytical data for **2.14**: FTIR (neat) 3711, 2960, 2933, 2862, 2383, 2368, 1683, 1422, 1365, 1274, 1239, 1159, 865, 732, 649 cm^{-1}; HRMS $(M+H)^+$ calcd for $C_{14}H_{29}N_2O_2^+$ (M+H) required 257.2224, found 257.2230.

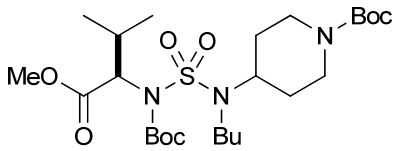
***tert*-Butyl 4-((*N*-(tert-butoxycarbonyl)sulfamoyl)(butyl)amino)piperidine-1-carboxylate (2.16)**



A solution of the *tert*-butyl alcohol (1.8 mL, 18.82 mmol) in CH_2Cl_2 (20 mL) was treated with a solution of CSI (1.65 mL, 18.96 mmol) in CH_2Cl_2 (20 mL) at 0 °C. This mixture was cannulated to a solution of **2.14** and Et_3N (3.3 mL, 23.68 mmol) in CH_2Cl_2 (20 mL). The reaction mixture was stirred at ambient temperature for overnight. The reaction mixture was extracted with CH_2Cl_2 (150 mL X 3) and the combined organic layer was dried over $MgSO_4$, filtered, evaporated, and purified by the ISCO-Flash column chromatography in 0% to 40% of EtOAc in hexane to get white oil as a product **2.16** (4.66 g, 68%).

Analytical data for **2.16**: R_f = 0.69 (Sol. EtOAc:Hexane = 1/1); FTIR (neat) 3259, 2974, 2935, 2873, 1734, 1668, 1436, 1366, 1244, 1136, 1022, 916, 869, 829, 771, 729, 604 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.49 (s, 1H), 4.27–4.24 (m, 1H), 4.13 (ddd, *J* = 14.3, 7.1, 2.9 Hz, 2H), 3.91–3.83 (m, 1H), 3.27 (t, *J* = 8.0 Hz, 2H), 2.76 (t, *J* = 11.6 Hz, 2H), 1.81 (d, *J* = 11.5 Hz, 2H), 1.64–1.53 (m, 2H), 1.48 (d, *J* = 2.6 Hz, 9H), 1.47 (d, *J* = 2.7 Hz, 9H), 1.31 (dd, *J* = 8.9, 6.2 Hz, 2H), 1.26 (m, 2H), 0.91 (td, *J* = 7.2, 2.6 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.43, 154.81, 150.21, 149.40, 84.57, 83.48, 80.08, 57.17, 45.20, 33.44, 28.63, 28.27, 28.09, 20.29, 13.98; HRMS $(M+H)^+$ calcd for $C_{19}H_{37}N_3NaO_6S^+$ (M+H) required 458.2295, found 458.2294.

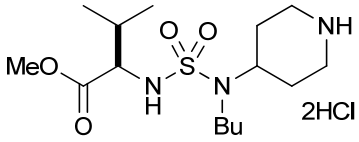
(*R*)-*tert*-Butyl 4-((*N*-(tert-butoxycarbonyl)-*N*-(1-methoxy-3-methyl-1-oxobutan-2-yl)-sulfamoyl) (butyl)amino)piperidine-1-carboxylate (2.18)



To a solution of **2.16** (0.10 g, 0.73 mmol), **2.9** (0.29 g, 0.66 mmol), and PPh_3 (1.96 g, 7.45 mmol) in THF (5 mL) was added DEAD (40% wt. in toluene, 32 mL, 1.28 mmol) at 0 ºC and stirred 10 min. The reaction mixture was heated at 60 ºC. The reaction mixture was quenched with 1M aqueous HCl solution and extracted with CH_2Cl_2 (100 mL X 3) and then the combined organic layer was dried over $MgSO_4$, filtered, evaporated, and purified by the ISCO-Flash column chromatography in 0% to 20% of EtOAc in hexane to get white oil as a product **2.18** (0.19 g, 53%).

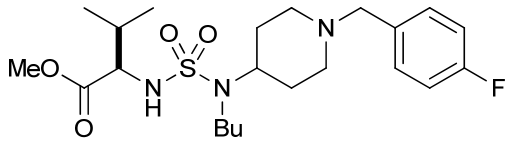
Analytical data for **2.18**: R_f = 0.8 (Sol. EtOAc:Hexane = 1/1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.46 (dd, *J* = 8.9, 2.6 Hz, 1H), 4.26–4.15 (m, 2H), 3.87 (td, *J* = 12.0, 3.1 Hz, 1H), 3.67 (s, 3H), 3.51–3.43 (m, 1H), 3.21–3.05 (m, 1H), 2.70 (m, 2H), 2.43 (m, 1H), 1.81 (d, *J* = 11.9 Hz, 1H), 1.67 (d, *J* = 12.7 Hz, 1H), 1.62–1.48 (m, 2H), 1.40 (d, *J* = 3.2 Hz, 9H), 1.38 (d, *J* = 2.7 Hz, 9H), 1.29–1.16 (m, 4H), 1.14–1.06 (m, 6H), 0.93 (dd, *J* = 7.0, 2.5 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.61, 154.63, 150.73, 83.96, 79.73, 66.84, 56.12, 52.14, 45.59, 33.70, 31.87, 29.11, 28.42, 28.18, 27.97, 22.37, 20.38, 20.12, 19.59, 14.48, 13.80.

(*R*)-Methyl 2-(*N*-butyl-*N*-(piperidin-4-yl)sulfamoylamino)-3-methylbutanoate 2HCl (**2.20**)



To a solution of **2.18** (0.19 g, 0.35 mmol) in CH_2Cl_2 (10 mL) was added 4N HCl in 1,4-dioxane (2.4 mL) at 0 °C and stirred overnight. A reaction mixture was evaporated to remove solvent, and then the mixture was dried under reduced vacuum to furnish a white solid as a product **2.20**. It used without further purification.

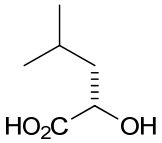
(*R*)-Methyl 2-(*N*-butyl-*N*-(1-(4-fluorobenzyl)piperidin-4-yl)sulfamoylamino)-3-methyl-butanoate (2.25)



A solution of **2.20** (0.1481 g, 0.3507 mmol) and *p*-fluorobenzaldehyde (0.04 mL, 0.38 mmol) in CH_2Cl_2 (20 mL) was treated with Et_3N (0.15 mL, 1.08 mmol) at 0 ºC and stirred for 10 minutes. $NaBH(OAc)_3$ (0.23 g, 1.07 mmol) was added to a reaction mixture at 0 ºC and stirred at ambient temperature overnight. The reaction mixture was extracted with CH_2Cl_2 (100 mL X 3) and then the combined organic layer was dried over $MgSO_4$, filtered, evaporated, and purified by the ISCO-Flash column chromatography in 0% to 40% of EtOAc in hexane to get colorless oil as a product **2.25** (0.08 g, 51%).

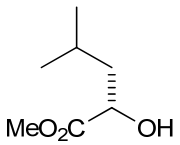
Analytical data for **2.25**: R_f = 0.28 (Sol. EtOAc:Hexane = 1/1); $[\alpha]^{25}_D$ = + 7.209 (*c* = 0.652, CH_2Cl_2); FTIR (neat) 3295, 2958, 2874, 2801, 2342, 2296, 1740, 1508, 1468, 1325, 1276, 1166, 1153, 1133, 1015, 995, 924, 861, 828, 770, 629 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.30–7.24 (m, 2H), 7.01 (m, 2H), 4.84, (d, *J* = 9.9 Hz, 1H), 3.77 (s, 3H), 3.70 (dd, *J* = 10.0, 5.2 Hz, 1H), 3.53–3.47 (m, 1H), 3.46 (s, 2H), 3.10 (dd, *J* = 10.3, 5.0 Hz, 2H), 2.92 (d, *J* = 9.8 Hz, 2H), 2.10–1.95 (m, 3H), 1.78 (dt, *J* = 13.5, 3.0 Hz, 2H), 1.72 (dt, *J* = 8.1, 4.0 Hz, 2H), 1.58 (dddd, *J* = 15.8, 11.2, 7.8, 5.2 Hz, 2H), 1.37–1.20 (m, 2H), 1.01 (d, *J* = 6.7 Hz, 3H), 0.93 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.12, 162.23 (d, *J* = 246.4 Hz), 134.13, 130.78 (d, *J* = 8.1 Hz), 115.25 (d, *J* = 22.2 Hz), 77.61, 77.29, 76.97, 62.28, 61.22, 56.80, 53.36, 53.31, 52.56, 44.36, 33.95, 31.89, 31.09, 30.77, 20.40, 19.23, 18.02, 14.04; HRMS $(M+H)^+$ calcd for $C_{22}H_{37}FN_3O_4S^+$ (M+H) required 458.2489, found 458.2465.

(*S*)-2-Hydroxy-4-methylpentanoic acid (2.26)



To a solution of L-leucine (10.10 g, 77.02 mmol) in 1N H_2SO_4 (100 mL) was added dropwise a solution of $NaNO_2$ (12.41 g, 179.86 mmol) at 0 °C and stirred overnight. The reaction mixture was extracted with diethyl ether (200 mL X 3) and concentrated by azeotropic distillation with toluene to provide yellow solid. A yellow solid was dried under reduced vacuum to furnish a crystal as white needles **2.26** (9.92 g, 97%).

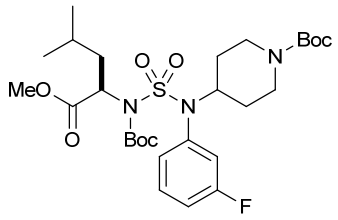
(*S*)-Methyl 2-hydroxy-4-methylpentanoate (2.27)



To a solution of **2.26** (9.20 g, 69.61 mmol) in methanol (100 mL) was added amberlyst-15 ion exchange resin at room temperature and stirred for overnight. The reaction mixture was filtered and evaporated to remove solvent to give yellow oil. The yellow oil was purified by the ISCO-Flash column chromatography in 0% to 40% of EtOAc in hexane to get yellow oil as a product **2.27** (6.56 g, 64%) R_f = 0.71 (Sol. EtOAc:Hexane = 1/1, checked by $KMnO_4$ stain solution).

Analytical data for **2.27**: ^{1}H NMR (CD_3CN, 400 MHz) δ ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.21–4.12 (m, 1H), 3.72 (s, 3H), 2.86 (s, 1H), 1.83 (dt, *J* = 13.5, 6.8 Hz, 1H), 1.56–1.46 (m, 2H), 0.89 (dd, *J* = 6.7, 4.1 Hz, 6H); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 175.5, 75.3, 52.4, 32.3, 18.7, 16.2.

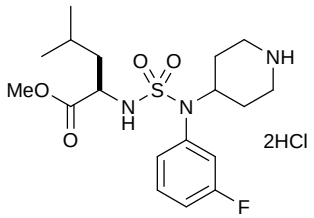
(*R*)-*tert*-Butyl 4-((*N*-(*tert*-butoxycarbonyl)-*N*-(1-methoxy-4-methyl-1-oxopentan-2-yl)-sulfamoyl)(3-fluorophenyl)amino)piperidine-1-carboxylate (2.28)



To a solution of **2.15** (1.04 g, 2.20 mmol) and DIAD (2.9 mL, 6.66 mmol) in THF (20 mL) was added a solution of **2.27** (0.36 g, 2.45 mmol) and PPh_3 (1.73 g, 6.583 mmol) in THF (20 mL) at 0 ºC and then the reaction mixture was heated at 60 °C for overnight. The reaction mixture was extracted with CH_2Cl_2 (150 mL X 3) and the combined organic layer was dried over $MgSO_4$, filtered, evaporated, and purified by the ISCO-Flash column chromatography in 0% to 20% of EtOAc in hexane to get white oil as a product **2.28** (1.12 g, 85%).

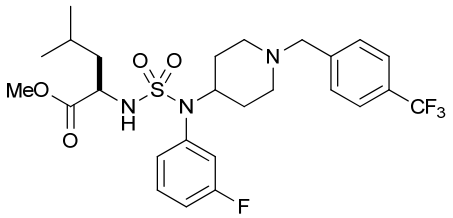
Analytical data for **2.28**: R_f = 0.81 (Sol. EtOAc:Hexane = 1/1); $[\alpha]^{25}_D$ = + 25.000 (*c* = 0.168, CH_2Cl_2); FTIR (neat) 2957, 2870, 1732, 1693, 1592, 1486, 1425, 1365, 1275, 1238, 1146, 979, 884, 865, 845, 772, 718, 694, 615 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.37 (ddd, *J* = 16.2, 16.2, 8.2 Hz, 1H), 7.14 (dddd, *J* = 8.3, 8.3, 2.5, 0.8 Hz, 1H), 7.06 (dd, *J* = 7.9, 0.8 Hz, 1H), 6.97 (ddd, *J* = 9.5, 2.3, 2.3 Hz, 1H), 4.66 (t, *J* = 6.6 Hz, 1H), 4.54–4.40 (m, 1H), 4.14–4.10 (m, 2H), 3.63 (s, 2H), 2.82–2.76 (m, 2H), 2.17 (s, 2H), 2.00 (d, *J* = 11.4 Hz, 3H), 1.51 (s, 9H), 1.39 (s, 9H), 1.31–1.19 (m, 3H), 0.81 (d, *J* = 6.3 Hz, 3H), 0.76 (d, *J* = 6.3 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.97, 162.54 (d, *J* = 248.5 Hz), 154.66, 150.69, 135.85 (d, *J* = 10.1 Hz), 129.98 (d, *J* = 9.1 Hz), 128.95, 120.22 (d, *J* = 22.2 Hz), 116.69 (d, *J* = 21.2 Hz), 84.64, 79.90, 59.59, 52.45, 40.44, 31.80, 31.15, 28.56, 28.24, 25.02, 23.05, 22.87, 22.13, 14.34; HRMS $(M+Na)^+$ calcd for $C_{28}H_{44}FN_3NaO_8S^+$ (M+Na) required Exact Mass: 624.2725, found 624.2726.

(*R*)-Methyl 2-(*N*-(3-fluorophenyl)-*N*-(piperidin-4-yl)sulfamoylamino)-4-methylpentanoate 2HCl (2.29)



To a solution of **2.28** (1.12 g, 1.87 mmol) in CH_2Cl_2 (40 mL) was added 4N HCl in 1,4-dioxane (4 mL) at 0 °C and stirred overnight. A reaction mixture was evaporated to remove solvent, and then the mixture was dried under reduced vacuum to furnish a white solid as a product **2.29**. It used without further purification.

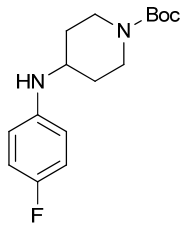
(*R*)-Methyl 2-(*N*-(3-fluorophenyl)-*N*-(1-(4-(trifluoromethyl)benzyl)piperidin-4-yl)sulfamoyl-amino)-4-methylpentanoate (**2.30**)



A solution of **2.29** (0.07 g, 0.15 mmol), 4-trifluoromethylbenzaldehyde (0.03 g, 0.17 mmol) in CH_2Cl_2 (3 mL) was treated with Et_3N (0.06 mL, 0.43 mmol) and $NaBH(OAc)_3$ (0.10 g, 0.47 mmol) at room temperature and stirred for overnight. A reaction mixture was quenched with aqueous $NaHCO_3$ solution and extracted with CH_2Cl_2 (50 mL X 3) and then the combined organic layer was dried over $MgSO_4$, filtered, evaporated, and purified by the ISCO-Flash column chromatography in 0% to 30% of EtOAc in hexane to get colorless syrup as a product **2.30** (0.04 g, 44%).

Analytical data for **2.30**: R_f = 0.61 (Sol. EtOAc:Hexane = 1/1); $[\alpha]^{25}_D$ = + 6.320 (*c* = 0.269, CH_2Cl_2); FTIR (neat) 3280, 2956, 2872, 2383, 2368, 2342, 1744, 1606, 1592, 1485, 1435, 1324, 1160, 1120, 1064, 1018, 981, 822, 787, 692, 644 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.49 (d, *J* = 8.1 Hz, 2H), 7.32 (d, *J* = 7.5 Hz, 2H), 7.30–7.21 (m, 1H), 7.09–7.01 (m, 2H), 6.94 (dt, *J* = 9.6, 2.3 Hz, 1H), 4.92 (d, *J* = 9.5 Hz, 1H), 3.98–3.82 (m, 2H), 3.78–3.64 (m, 3H), 3.61 (ddd, *J* = 9.3, 6.6, 4.3 Hz, 1H), 3.45 (s, 2H), 2.88–2.80 (m, 2H), 2.06 (td, *J* = 11.8, 2.6 Hz, 2H), 1.94–1.78 (m, 2H), 1.66 (dq, *J* = 13.3, 6.6 Hz, 1H), 1.53–1.36 (m, 2H), 1.27–1.18 (m, 1H), 0.87 (d, *J* = 6.5 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.53, 162.66 (d, *J* = 249.5 Hz), 142.39, 138.06 (d, *J* = 10.1 Hz), 130.07 (d, *J* = 9.1 Hz), 129.42, 129.35 (d, *J* = 5.0 Hz), 127.68 (d, *J* = 3.0 Hz), 125.39 (d, *J* = 3.0 Hz), 125.31 (d, *J* = 4.0 Hz), 119.11 (d, *J* = 21.2 Hz), 116.02 (d, *J* = 21.2 Hz), 62.28, 58.29, 55.09, 53.63, 53.13, 52.73, 42.99, 31.64, 31.41, 24.55, 22.74, 22.23; HRMS $(M+H)^+$ calcd for $C_{26}H_{34}F_4N_3O_4S^+$ (M+H) required 560.2206, found 560.2542.

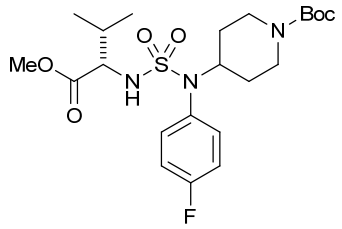
***tert*-Butyl 4-(4-fluorophenylamino)piperidine-1-carboxylate (2.31)**



To a solution of 1-Boc-4-piperidone (5.28 g, 26.49 mmol) and *p*-fluoroaniline (2.54 mL, 26.45 mmol) in CH_2Cl_2 (60 mL) was added $NaBH(OAc)_3$ (9.73 g, 45.90 mmol) at 0 °C and stirred for 2 hrs. To a reaction mixture was added glacial acetic acid (3 mL, 52.41 mmol) at 0 °C and stirred at ambient temperature overnight. The reaction mixture was washed with saturated aqueous $NaHCO_3$ solution. The mixture was extracted with CH_2Cl_2 (200 mL X 3) and then the combined organic layer was dried over $MgSO_4$, filtered, evaporated, and purified by the ISCO-Flash column chromatography in 0% to 40% of EtOAc in hexane to get white solid as a product **2.31** (5.51 g, 71%).

Analytical data for **2.31**: R_f = 0.75 (Sol. EtOAc:Hexane = 1/1); FTIR (neat) 3355, 2982, 2945, 2923, 2847, 1670, 1612, 1530, 1505, 1419, 1366, 1316, 1233, 1139, 1097, 973, 858, 821, 773, 645 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 6.88–6.80 (m, 2H), 6.69–6.59 (m, 2H), 4.85 (s, 1H), 4.01 (ddd, J = 13.6, 3.4, 3.4 Hz, 2H), 3.38 (tt, *J* = 10.2, 3.9 Hz, 1H), 2.97-2.91 (m, 2H), 1.96 (dd, *J* = 13.3, 3.3 Hz, 2H), 1.45 (s, 9H), 1.29 (dddd, *J* = 13.0, 11.4, 10.2, 4.2 Hz, 2H); ^{13}C NMR (101 MHz, CD_3OD) δ 156.83, 154.79 (d, *J* = 57.6 Hz), 143.83, 114.88 d, *J* = 22.2 Hz), 114.50 (d, *J* = 7.1 Hz), 79.58, 50.34, 31.63, 27.25; HRMS $(M+H)^+$ calcd for $C_{16}H_{23}FN_2NaO_2^+$ (M+H) required 317.1336, found 317.1640.

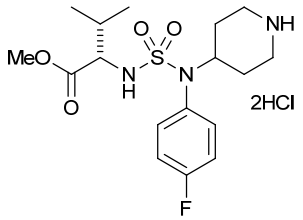
(*S*)-*tert*-Butyl 4-((4-fluorophenyl)(*N*-(1-methoxy-3-methyl-1-oxobutan-2-yl)sulfamoyl) amino)-piperidine-1-carboxylate (2.34)



To a solution of sulfuryl chloride (0.30 mL, 3.70 mmol) in CH_2Cl_2 (20 mL) was added L-valine methyl ester (0.62 g, 3.70 mmol) in CH_2Cl_2 (20 mL) slowly at 0 ºC and stirred for 30 minutes to obtain **2.32**. A solution of **2.31** (0.31 g, 1.06 mmol) and Et_3N (1.3 mL, 9.33 mmol) in CH_2Cl_2 (20 mL) was treated with a solution of **2.32** at 0 ºC and stirred for overnight. The reaction mixture was evaporated and purified by the ISCO-Flash column chromatography in 0% to 30% of EtOAc in hexane to get colorless syrup as a product **2.34** (0.38 g, 73%).

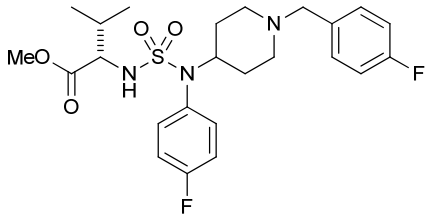
Analytical data for **2.34**: R_f = 0.71 (Sol. EtOAc:Hexane = 1/1); $[\alpha]^{25}_D$ = - 4.52 (*c* = 0.310, CH_2Cl_2); FTIR (neat) 3274, 2969, 2935, 2871, 2383, 2324, 1740, 1682, 15061427, 1365, 1335, 1275, 1263, 1250, 1210, 1164, 1135, 1092, 1056, 1092, 1056, 955, 935, 869, 822, 737, 703, 642, 612 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.17 (dd, *J* = 8.2, 5.1 Hz, 2H), 7.02 (dd, *J* = 8.8, 8.8 Hz, 2H), 5.08 (d, *J* = 9.4 Hz, 1H), 4.14–4.05 (m, 2H), 3.98 (tt, *J* = 11.9, 3.1 Hz, 1H), 3.74 (dd, *J* = 9.4, 5.1 Hz, 1H), 3.71 (s, 3H), 2.71 (dd, *J* = 11.8, 11.8 Hz, 2H), 2.00 (qd, *J* = 19.0, 6.3 Hz, 1H), 1.91 (d, *J* = 12.4 Hz, 1H), 1.81 (d, *J* = 12.3 Hz, 1H), 1.36 (s, 9H), 1.29–1.14 (m, 2H), 0.91 (d, *J* = 6.8 Hz, 3H), 0.86 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.56, 162.66 (d, *J* = 249.5 Hz), 154.60, 133.60 (d, *J* = 9.1 Hz), 132.19 (d, *J* = 3.0 Hz), 116.14 (d, *J* = 23.2 Hz), 79.93, 61.73, 58.11, 53.66, 52.59, 43.37, 32.11, 31.75, 31.45, 28.52, 18.98, 17.96; HRMS $(M+Na)^+$ calcd for $C_{22}H_{34}FN_3NaO_6S^+$ (M+Na) required 510.2045, found 510.2040.

(*S*)-Methyl 2-(*N*-(4-fluorophenyl)-*N*-(piperidin-4-yl)sulfamoylamino)-3-methylbutanoate 2HCl (**2.36**)



To a solution of **2.34** (0.38 g, 0.78 mmol) in CH_2Cl_2 (40 mL) was added 4N HCl in 1,4-dioxane (4 mL) at 0 °C and stirred overnight. A reaction mixture was evaporated to remove solvent, and then the mixture was dried under reduced vacuum to furnish a white solid as a product **2.36**. It used without further purification.

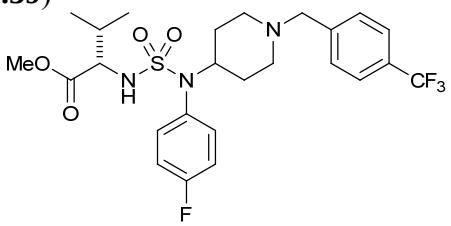
(*S*)-Methyl 2-(*N*-(1-(4-fluorobenzyl)piperidin-4-yl)-*N*-(4-fluorophenyl)sulfamoylamino)-3-methylbutanoate (**2.38**)



To a solution of **2.36** (0.05 g, 0.12 mmol) and 4-fluorobenzaldehyde (14 μL, 0.13 mmol) in CH_2Cl_2 (10 mL) was treated with Et_3N (18 μL, 0.13 mmol) and $NaBH(OAc)_3$ (0.08 g, 0.37 mmol) at 0 °C and stirred overnight. The reaction mixture was evaporated, and purified by the ISCO-Flash column chromatography in 0% to 60% of EtOAc in hexane to get colorless solid oil as a product **2.38** (0.04 g, 63%).

Analytical data for **2.38**: R_f = 0.43 (Sol. EtOAc:Hexane = 1/1); $[\alpha]^{25}_D$ = - 5.031 (*c* = 0.318, CH_2Cl_2); FTIR (neat) 3280, 2958, 2927, 2854, 2383, 2368, 2185, 1738, 1603, 1505, 1454, 1337, 1208, 1161, 1133, 1057, 960, 871, 823, 740, 622 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.22–7.15 (m, 4H), 7.03 (t, 2H), 6.94 (t, *J* = 8.6 Hz, 2H), 5.30 (s, 1H), 4.93 (d, *J* = 9.4 Hz, 1H), 3.87 (tt, *J* = 12.0, 4.0 Hz, 1H), 3.76 (dd, *J* = 9.5, 5.0 Hz, 1H), 3.73 (s, 3H), 3.39 (s, 2H), 2.86 (d, *J* = 10.9 Hz, 2H), 2.09–1.98 (m, 3H), 1.95–1.88 (m, 1H), 1.80 (dq, *J* = 8.5, 2.6 Hz, 1H), 1.84–1.78 (m, 1H), 1.41 (dddd, *J* = 15.6, 12.2, 6.1, 3.1 Hz, 2H), 0.93 (d, *J* = 6.8 Hz, 3H), 0.88 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.37, 162.41 (d, *J* = 250.5 Hz), 162.11 (d, *J* = 246.4 Hz), 133.42, 133.34, 131.96 (d, *J* = 4.4 Hz), 130.80 (d, *J* = 8.1 Hz), 115. 87 (d, *J* = 22.2 Hz), 115.11 (d, *J* = 21.2 Hz), 61.56, 61.42, 57.67, 52.57, 52.52, 52.40, 31.91, 31.09, 30.78, 18.76, 17.68; HRMS $(M+H)^+$ calcd for $C_{24}H_{32}F_2N_3O_4S^+$ (M+H) required 496.2076, found 496.2074.

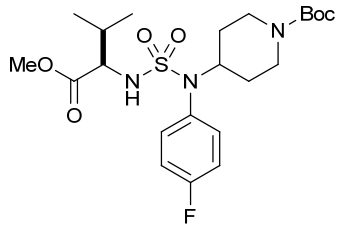
(*S*)-Methyl 2-(*N*-(4-fluorophenyl)-*N*-(1-(4-(trifluoromethyl)benzyl)piperidin-4-yl)sulfamoyl-amino)-3-methylbutanoate (**2.39**)



To a solution of **2.36** (0.05 g, 0.12 mmol) and 4-trifluorobenzaldehyde (18 μL, 0.13 mmol) in CH_2Cl_2 (10 mL) was treated with Et_3N (18 μL, 0.13 mmol) and $NaBH(OAc)_3$ (0.08 g, 0.37 mmol) at 0 °C and stirred overnight. The reaction mixture was evaporated, and purified by the ISCO-Flash column chromatography in 0% to 60% of EtOAc in hexane to get colorless solid oil as a product **2.39** (0.06 g, 83%).

Analytical data for **2.39**: R_f = 0.70 (Sol. EtOAc:Hexane = 1/1); $[\alpha]^{25}_D$ = - 3.786 (*c* = 0.449, CH_2Cl_2); FTIR (neat) 3295, 2956, 2924, 2853, 2342, 2300, 1739, 1619, 1602, 1506, 1454, 1421, 1323, 1262, 1209, 1161, 1121, 1064, 1018, 959, 931, 871, 822, 740, 621 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.51 (d, *J* = 8.1 Hz, 2H), 7.34 (d, *J* = 8.0 Hz, 2H), 7.16–7.11 (m, 2H), 7.01–6.95 (m, 2H), 4.96 (d, *J* = 9.4 Hz, 1H), 3.82 (tt, *J* = 12.1, 3.7 Hz, 1H), 3.69 (dd, *J* = 9.4, 5.0 Hz, 1H), 3.66 (s, 3H), 3.41 (s, 2H), 2.79 (d, *J* = 11.0 Hz, 2H), 2.02 (t, *J* = 11.2 Hz, 2H), 1.95 (dq, *J* = 12.9, 7.1 Hz, 1H), 1.88–1.83 (m, 1H), 1.77–1.72 (m, 1H), 1.36 (ddd, *J* = 12.3, 8.8, 3.8 Hz, 2H), 0.87 (d, *J* = 6.8 Hz, 3H), 0.81 (d, *J* = 6.3 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.48, 162.51 (d, *J* = 250.5 Hz), 142.31, 133.52 (d, *J* = 8.1 Hz), 132.19 (d, *J* = 4.0 Hz), 129.22, 125.26 (d, *J* = 4.4 Hz), 125.19 (d, *J* = 3.0 Hz), 115.93 (d, *J* = 22.2 Hz), 62.17, 61.54, 57.96, 53.05, 52.99, 52.46, 32.01, 31.52, 31.21, 18.84, 17.79; HRMS $(M+H)^+$ calcd for $C_{25}H_{32}F_4N_3O_4S^+$ (M+H) required 546.2044, found 546.2049.

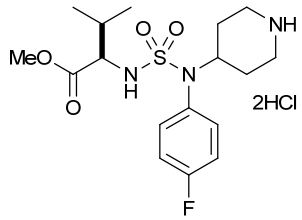
(*R*)-*tert*-Butyl 4-((4-fluorophenyl)(*N*-(1-methoxy-3-methyl-1-oxobutan-2-yl)sulfamoyl) amino)piperidine-1-carboxylate (**2.35**)



To a solution of sulfuryl chloride (0.48 mL, 5.92 mmol) in CH_2Cl_2 (40 mL) was added D-valine methyl ester (1.00 g, 5.97 mmol) in CH_2Cl_2 (20 mL) slowly at 0 ºC and stirred for 4 hrs to furnish **2.33**. To a solution of **2.27** (0.95 g, 3.22 mmol) and Et_3N (2.50 mL, 17.94 mmol) in CH_2Cl_2 (20 mL) was added **2.33** solution at 0 ºC and stirred for overnight. The reaction mixture was evaporated and purified by the ISCO-Flash column chromatography in 0% to 30% of EtOAc in hexane to get colorless syrup as a product **2.35** (1.50 g, 95%).

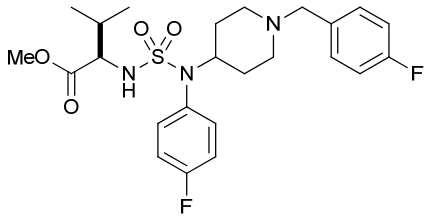
Analytical data for **2.35**: R_f = 0.68 (Sol. EtOAc:Hexane = 1/1); $[\alpha]^{25}_D$ = - 1.199 (*c* = 0.584, CH_2Cl_2); FTIR (neat) 3333, 2991, 2924, 2853, 1666, 1611, 1588, 1524, 1448, 1430, 1366, 1342, 1249, 1233, 1167, 1139, 1099, 1076, 998, 936, 821, 756, 627 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.19–7.14 (m, 2H), 7.07–6.96 (m, 2H), 4.95 (dd, *J* = 9.0, 4.8 Hz, 1H), 4.15–4.05 (m, 2H), 3.97 (tt, *J* = 7.4, 5.0 Hz, 2H), 3.76–3.71 (m, 4H), 2.70 (t, *J* = 11.5 Hz, 2H), 1.86 (m, 2H), 1.34 (s, 9H), 1.27–1.16 (m, 2H), 0.91 (t, *J* = 6.4 Hz, 3H), 0.86 (t, *J* = 5.7 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.29, 162.44 (d, *J* = 250.5 Hz), 154.37, 133.34 (d, *J* = 9.1 Hz), 131.90, 115.92 (d, *J* = 22.2 Hz), 79.72, 61.47, 61.12, 57.93, 52.39, 43.14, 31.92, 31.52, 31.23, 28.30, 18.74, 17.68.

(*R*)-Methyl 2-(*N*-(4-fluorophenyl)-*N*-(piperidin-4-yl)sulfamoylamino)-3-methylbutanoate 2HCl (**2.37**)



To a solution of **2.35** (1.50 g, 3.08 mmol) in CH_2Cl_2 (60 mL) was added 4N HCl in 1,4-dioxane (6 mL) at 0 °C and stirred overnight. A reaction mixture was evaporated to remove solvent, and then the mixture was dried under reduced vacuum to furnish a white solid as a product **2.37**. It used without further purification.

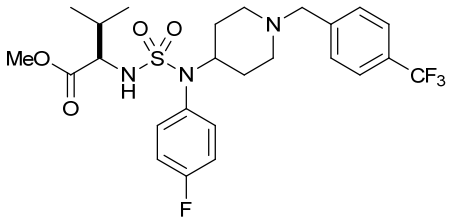
(*R*)-Methyl 2-(*N*-(1-(4-fluorobenzyl)piperidin-4-yl)-*N*-(4-fluorophenyl)sulfamoylamino)-3-methylbutanoate (**2.40**)



To a solution of **2.37** (0.11 g, 0.26 mmol), 4-fluorobenzaldehyde (0.04 g, 0.32 mmol) and Et_3N (0.04 mL, 0.29 mmol) in CH_2Cl_2 (18 mL) was treated with $NaBH(OAc)_3$ (0.17 g, 0.78 mmol) at 0 °C and stirred overnight. The reaction mixture was evaporated, and purified by the ISCO-Flash column chromatography in 0% to 30% of EtOAc in hexane to get colorless syrup as a product **2.40** (0.07 g, 51%).

Analytical data for **2.40**: R_f = 0.42 (Sol. EtOAc:Hexane = 1/1); $[\alpha]^{25}_D$ = + 3.767 (*c* = 0.584, CH_2Cl_2); FTIR (neat) 3293, 2956, 2933 2383, 2325, 2299, 1738, 1603, 1505, 1468, 1453, 1435, 1337, 1259, 1208, 1162, 1132, 1058, 960, 931, 823, 740, 619 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.21–7.16 (m, 4H), 7.05–7.01 (m, 2H), 6.97–6.92 (m, 2H), 4.98 (d, *J* = 9.4 Hz, 1H), 3.91–3.82 (m, 1H), 3.75 (ddd, *J* = 9.8, 5.2, 1.8 Hz, 1H), 3.72 (s, 3H), 3.40 (s, 2H), 2.87 (d, 2H), 2.08–1.96 (m, 2H), 1.94–1.87 (m, 2H), 1.80 (d, *J* = 12.4 Hz, 1H), 1.48–1.36 (m, 2H), 0.92 (dd, *J* = 6.8, 1.7 Hz, 3H), 0.87 (dd, *J* = 6.9, 1.7 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.63, 162.64 (d, *J* = 250.5 Hz), 162.26 (*J* = d, 246.4 Hz), 133.68 (d, *J* = 8.7 Hz), 133.52, 132.32 (d, *J* = 3.3 Hz), 130.88 (d, *J* = 7.9 Hz), 116.05 (d, *J* = 22.6 Hz), 115.25 (d, *J* = 21.2 Hz), 61.99, 61.68, 58.12, 52.95, 52.90, 52.61, 32.16, 31.53, 31.23, 18.99, 17.94; HRMS $(M+H)^+$ calcd for $C_{24}H_{32}F_2N_3O_4S^+$ (M+H) required 496.2081, found 496.2543.

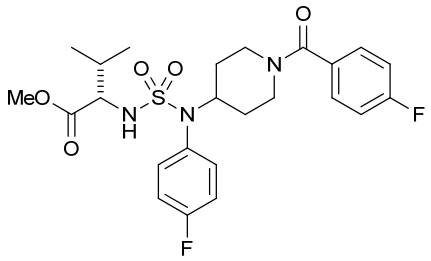
(*R*)-Methyl 2-(*N*-(4-fluorophenyl)-*N*-(1-(4-(trifluoromethyl)benzyl)piperidin-4-yl)sulfamoyl -amino)-3-methylbutanoate (**2.41**)



To a solution of **2.37** (0.12 g, 0.29 mmol) and 4-trifluorobenzaldehyde (0.06 g, 0.32 mmol) in CH_2Cl_2 (18 mL) was treated with Et_3N (0.04 mL, 0.29 mmol) and $NaBH(OAc)_3$ (0.18 g, 0.87 mmol) at 0 °C and stirred overnight. The reaction mixture was evaporated, and purified by the ISCO-FlashColumn chromatography in 0% to 30% of EtOAc in hexane to get colorless syrup as a product **2.41** (0.0779 g, 50%).

Analytical data for **2.41**: R_f = 0.67 (Sol. EtOAc:Hexane = 1/1); $[\alpha]^{25}_D$ = + 2.734 (*c* = 0.695, CH_2Cl_2); FTIR (neat) 3242, 2957, 2804, 2326, 2299, 1739, 1614, 1601, 1574, 1505, 1469, 1324, 1263, 1209, 1161, 1126, 1066, 1018, 961, 871, 822, 740, 622 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.52 (d, *J* = 8.3 Hz, 2H), 7.35 (d, *J* = 8.2 Hz, 2H),7.22–7.19 (m, 2H), 7.04 (dd, *J* = 8.4, 8.4 Hz, 2H), 4.92 (d, *J* = 9.4 Hz, 1H), 3.89 (tt, *J* = 12.0, 3.8 Hz, 1H), 3.77 (dd, *J* = 9.4, 5.1 Hz, 1H), 3.73 (d, *J* = 0.5 Hz, 3H), 3.48 (s, 2H), 2.86 (d, *J* = 11.5 Hz, 2H), 2.10 (ddd, *J* = 11.8, 11.8, 1.8 Hz, 2H), 2.02 (qd, *J* = 11.4, 4.7 Hz, 1H), 1.94–1.79 (m, 2H), 1.44 (dddd, *J* = 34.9, 18.7, 14.7, 10.8 Hz, 2H), 0.95 (d, 6.8 Hz, 3H), 0.89 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.48, 162.51 (d, *J* = 248.9 Hz),, 142.31, 133.52 (d, *J* = 8.7 Hz), 132.19 (d, *J* = 3.3 Hz), 129.22, 125.26 (d, *J* = 3.7 Hz), 125.18 (d, *J* = 3.8 Hz), 115.93 (d, *J* = 22.6 Hz), .62.17, 61.54, 57.96, 53.05, 52.99, 52.46, 52.45, 32.01, 31.52, 31.21, 18.84, 17.79; HRMS $(M+H)^+$ calcd for $C_{25}H_{32}F_4N_3O_4S^+$ (M+H) required 546.2049, found 546.2187.

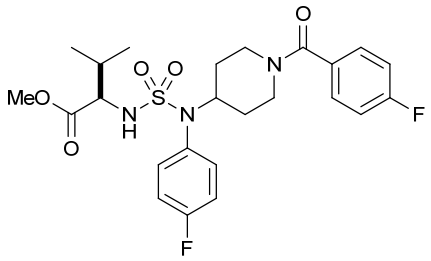
(*S*)-Methyl 2-((*N*-(1-(4-fluorobenzoyl)piperidin-4-yl)-*N*-(4-fluorophenyl)sulfamoyl)amino)-3-methylbutanoate (**2.42**)



A solution of **2.36** (0.09 g, 0.22 mmol), Et_3N (0.09 mL, 0.65 mmol) and 4-fluorobenzoyl chloride (0.04 g, 0.24 mmol) in THF (10 mL) was stirred at room temperature for overnight. The reaction mixture was evaporated, and purified by the ISCO-Flash column chromatography in 0% to 50% of EtOAc in hexane to get colorless syrup as a product **2.42** (0.09 g, 83%).

Analytical data for **2.42**: R_f = 0.35 (Sol. EtOAc:Hexane = 1/1); $[\alpha]^{25}_D$ = - 1.232 (*c* = 0.0594, CH_2Cl_2); FTIR (neat) 3334, 2937, 2863, 2383, 2368, 1737, 1672, 1614, 1505, 1431, 1366, 1339, 1233, 1167, 1138, 1058, 1014, 961, 936, 845, 822, 759, 737, 663, 617 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.24 (dd, J = 8.4, 5.4 Hz, 2H), 7.12 (dd, J = 8.8, 5.0 Hz, 2H), 7.01 (d, *J* = 8.5 Hz, 2H), 6.97 (d, *J* = 8.6 Hz, 2H), 5.08 (d, *J* = 9.4 Hz, 1H), 4.06 (ddt, *J* = 11.0, 7.2, 3.7 Hz, 1H), 3.69 (dd, *J* = 9.5, 5.1 Hz, 1H), 3.66 (s, 3H), 3.00 (m, 1H), 2.74 (m, 1H), 1.95 (dt, *J* = 13.6, 5.1 Hz, 2H), 1.84 (m, 2H), 1.36–1.25 (m, 2H), 1.18 (t, *J* = 6.9 Hz, 1H), 0.86 (d, *J* = 6.8 Hz, 3H), 0.81 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.35, 169.28, 163.37 (d, *J* = 251.5 Hz), 162.51 (d, *J* = 250.5 Hz), 133.61 (d, *J* = 9.1 Hz), 131.78 (d, *J* = 3.0 Hz), 131.43 (d, *J* = 4.0 Hz), 129.24 (d, *J* = 8.1 Hz), 116.12 (d, *J* = 23.2 Hz), 115.52 (d, *J* = 22.2 Hz), 61.50, 57.58, 52.43, 31.86, 18.77, 17.68; HRMS $(M+H)^+$ calcd for $C_{24}H_{30}F_2N_3O_5S^+$ (M+H) required 510.1874, found 510.3023.

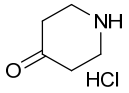
(*R*)-Methyl 2-((*N*-(1-(4-fluorobenzoyl)piperidin-4-yl)-*N*-(4-fluorophenyl)sulfamoyl)amino)-3-methylbutanoate (**2.43**)



A solution of **2.37** (0.11 g, 0.27 mmol), Et_3N (0.11 mL, 0.79 mmol) and 4-fluorobenzoyl chloride (0.03 mL, 0.25 mmol) in THF (10 mL) was stirred at room temperature for overnight. The reaction mixture was evaporated, and purified by the ISCO-Flash column chromatography in 0% to 50% of EtOAc in hexane to get colorless syrup as a product **2.43** (0.07 g, 51%).

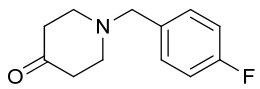
Analytical data for **2.43**: R_f = 0.33 (Sol. EtOAc:Hexane = 1/1); $[\alpha]^{25}_D$ = + 1.146 (*c* = 0.584, CH_2Cl_2); FTIR (neat) 3261, 2961, 2927, 2856, 2366, 1739, 1622, 1604, 1505, 1435, 1336, 1155, 1138, 1062, 955, 935, 846, 760, 739, 704, 641, 615cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (dd, *J* = 8.6, 5.4 Hz, 2H), 7.17 (dd, *J* = 8.8, 4.9 Hz, 2H), 7.04 (q, *J* = 8.8 Hz, 4H), 5.02 (d, *J* = 9.4 Hz, 1H), 4.72 (s, 1H), 4.11 (tt, *J* = 11.9, 2.3 Hz, 1H), 3.78–3.73 (dd, *J* = 9.4, 5.0 Hz, 1H), 3.71 (s, 3H), 3.20–2.93 (bm, 2H), 2.92–2.63 (bm, 2H), 2.00 (ddd, *J* = 8.8, 8.8, 6.8 Hz, 2H), 1.95–1.76 (bm, 2H), 0.92 (d, *J* = 6.8 Hz, 3H), 0.86 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.33, 169.30, 163.38 (d, *J* = 251.5 Hz), 162.52 (d, *J* = 250.5 Hz), 133.29 (d, *J* = 9.1 Hz), 131.73 (d, *J* = 3.0 Hz), 131.39 (d, *J* = 3.0 Hz), 129.23 (d, *J* = 9.1 Hz), 116.22 (d, *J* = 22.2 Hz), 115.53 (d, *J* = 22.2 Hz), 61.48, 57.62, 52.45, 31.88, 29.67, 18.76, 17.64; HRMS $(M+H)^+$ calcd for $C_{24}H_{30}F_2N_3O_5S^+$ (M+H) required 510.1874, found 510.3173.

Piperidin-4-one hydrochloride (**2.44**)



4N HCl (30 mL) in 1,4-dioxane was added to a solution of 4-Boc-1-piperidone (5.18 g, 26.00 mmol) at 0 °C and stirred overnight at room temperature. The mixture was evaporated to get white solid as a product **2.44**. It was used for next reaction without further purification.

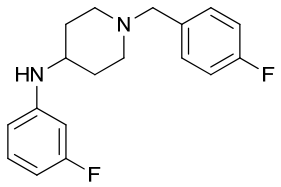
1-(4-Fluorobenzyl)piperidin-4-one (**2.45**)



$NaBH(OAc)_3$ (16.67 g, 78.77 mmol) was added to a solution of 4-fluorobenzaldehyde (3.1 mL, 28.90 mmol) and **2.44** (3.53 g, 26.00 mmol) in CH_2Cl_2 (100 mL) at 0 °C and stirred at room temperature for overnight. The mixture was quenched with $NaHCO_3$ and extracted with CH_2Cl_2 (200 mL X 3) and the combined organic layer was dried over $MgSO_4$. The mixture was filtered, concentrated, and purified by the ISCO-Flash column chromatography in 0% to 40% of EtOAc in hexane to furnish colorless syrup as a product **2.45** (4.29 g, 80%).

Analytical data for **2.45**: R_f = 0.29 (Sol. EtOAc:Hexane = 1/1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 (dd, *J* = 8.4, 5.5 Hz, 2H), 6.95 (dd, *J* = 8.7, 8.7, 2H), 3.51 (s, 2H), 2.66 (t, *J* = 5.9 Hz, 4H), 2.37 (t, *J* = 5.9 Hz, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 209.16, 162.26 (d, *J* = 246.4 Hz), 134.0 (d, *J* = 3.0 Hz), 130.62 (d, *J* = 8.1 Hz), 115.35 (d, *J* = 21.2 Hz), 61.22, 52.92, 41.33.

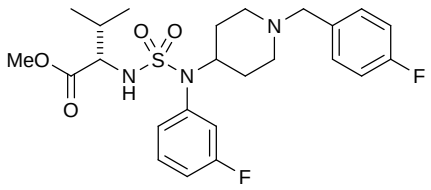
1-(4-Fluorobenzyl)-*N*-(3-fluorophenyl)piperidin-4-amine (**2.46**)



$NaBH(OAc)_3$ (13.45 g, 63.44 mmol) was added to a solution of **2.45** (4.29 g, 20.72 mmol) and *p*-fluoroaniline (2.60 mL, 27.05 mmol) in CH_2Cl_2 (50 mL) at 0 °C and stirred for 2 hrs. Acetic acid (2.40 mL, 41.93 mmol) was treated at 0 °C and stirred for overnight at room temperature. The mixture was transferred to a separatory funnel using CH_2Cl_2 (100 mL) and distilled water (50 mL). The mixture was extracted with CH_2Cl_2 (100 mL X 3) and EtOAc (100 mL) and the combined organic layer was dried over $MgSO_4$. The mixture was filtered, concentrated, and purified by the ISCO-Flash column chromatography in 0% to 40% of EtOAc in hexane to furnish white solid as a product **2.46** (4.03 g, 64%).

Analytical data for **2.46**: R_f = 0.19 (Sol. EtOAc:Hexane = 1/1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (dd, *J* = 8.7, 5.6 Hz, 2H), 7.07 (ddd, *J* = 8.1, 8.1, 4.6 Hz, 1H), 7.04–6.98 (dd, *J* = 8.7, 8.7 Hz, 2H), 6.35 (ddd, *J* = 10.6, 8.2, 2.4 Hz, 2H), 6.27 (ddd, *J* = 11.8, 2.3, 2.3 Hz, 1H), 3.66 (d, *J* = 7.8 Hz, 1H), 3.48 (s, 2H), 3.22–3.29 (m, 1H), 2.82 (d, *J* = 12.0 Hz, 2H), 2.13 (ddd, *J* = 11.4, 11.4, 2.9 Hz, 2H), 2.05–2.00 (m, 2H), 1.47 (dddd, *J* = 10.8, 10.8, 10.8, 3.7 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.53 (d, *J* = 223.2 Hz), 162.11 (d, *J* = 225.2 Hz), 149.14 (d, *J* = 10.1 Hz), 134.37 (d, *J* = 3.0 Hz), 130.73 (d, *J* = 8.1 Hz), 130.55 (d, *J* = 10.1 Hz), 115.24 (d, *J* = 21.2 Hz), 109.26 ((d, *J* =2.0 Hz), 103.67 (d, *J* = 21.2 Hz), 99.81 (d, *J* = 25.3 Hz), 62.50, 52.41, 50.19, 32.62.

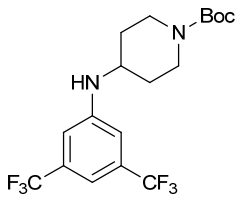
(*S*)-Methyl 2-((*N*-(1-(4-fluorobenzyl)piperidin-4-yl)-*N*-(3-fluorophenyl)sulfamoyl)amino)-3-methylbutanoate (**2.47**)



A mixture of L-valine methylester HCl (0.06 g, 0.37 mmol) in CH_2Cl_2 (20 mL) was added slowly to a solution of sulfuryl chloride (0.03 mL, 0.37 mmol) at 0 °C and stirred for 4 hrs. A solution of **2.46** (0.07 g, 0.24 mmol) and Et_3N (0.15 mL, 1.07 mmol) in CH_2Cl_2 (20 mL) was added to a reaction mixture at 0 °C and stirred at room temperature for overnight. The mixture was evaporated and purified by the ISCO-Flash column chromatography in 0% to 50% of EtOAc in hexane to get colorless syrup as product **2.47** (0.06 g, 51%).

Analytical data for **2.47**: R_f = 0.33 (Sol. EtOAc:Hexane = 1/1); $[\alpha]^{25}_D$ = - 4.872 (*c* = 0.390, CH_2Cl_2); FTIR (neat) 3284, 2960, 2801, 2762, 2256, 1738, 1669, 1607, 1592, 1508, 1485, 1468, 1339, 1264, 1220, 1162, 1139, 1055, 981, 908, 861, 828, 728, 691, 646 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.30 (ddd, *J* = 6.9, 6.9, 6.9 Hz, 1H), 7.16 (dd, *J* = 7.5, 7.5 Hz, 2H), 7.06 (ddd, *J* = 8.3, 1.2, 1.2 Hz, 1H), 7.06–7.01 (m, 1H), 6.94 (dd, *J* = 7.5, 7.5 Hz, 2H), 5.00 (d, *J* = 9.3 Hz, 1H), 3.88 (ddd, *J* = 11.9, 8.7, 3.2 Hz, 1H), 3.76 (ddd, *J* = 9.4, 2.0, 1.1 Hz, 1H), 3.73 (d, *J* = 1.2 Hz, 3H), 3.40 (s, 2H), 2.87 (d, *J* = 9.5 Hz, 2H), 2.10–1.97 (m, 3H), 1.93 (d, *J* = 12.2 = Hz, 1H), 1.82 (d, *J* = 12.3 Hz, 1H) 1.50–1.38 (m, 2H), 0.94 (d, *J* = 6.8 Hz, 3H), 0.88 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.30, 162.41 (d, *J* = 249.5 Hz), 161.97 (d, *J* = 245.4 Hz), 137.86, 137.77, 130.54 (d, *J* = 8.1 Hz), 129.84 (d, *J* = 9.1 Hz), 127.51 (d, *J* = 4.0 Hz), 118.93 (d, *J* = 22.2 Hz), 115.77 (d, *J* = 21.2 Hz), 115.00 (d, *J* = 21.2 Hz), 61.88, 61.42, 58.21, 52.85, 52.79, 52.40, 31.93, 31.43, 31.16, 18.75, 17.66; HRMS $(M+H)^+$ calcd for $C_{24}H_{32}F_2N_3O_4S^+$ (M+H) required 496.2082, found 496.2135.

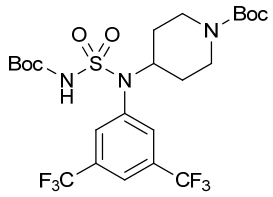
***tert*-Butyl 4-(3,5-bis(trifluoromethyl)phenylamino)piperidine-1-carboxylate** (**2.48**)



To a solution of 1-Boc-4-piperidone (5.16g, 25.89 mmol) and 3,5-bis(trifluoromethyl)aniline (4.0 mL, 25.80 mmol) in CH_2Cl_2 (100 mL) was added $NaBH(OAc)_3$ (16.30 g, 76.92 mmol) at 0 °C and stirred for 2 hrs. To a reaction mixture was added glacial acetic acid (3 mL, 52.41 mmol) at 0 °C and stirred at ambient temperature overnight. The reaction mixture was washed with saturated aqueous $NaHCO_3$ solution. The mixture was extracted with CH_2Cl_2 (100 mL X 3) and then the combined organic layer was dried over $MgSO_4$, filtered, evaporated, and purified by the ISCO-Flash column chromatography in 0% to 40% of EtOAc in hexane to get white solid as a product **2.48** (5.1053 g, 48%).

Analytical data for **2.48**: R_f = 0.37 (Sol. EtOAc:Hexane = 1/1); FTIR (neat) 3337, 2941, 2859, 1671, 1615, 1588, 1477, 1432, 1405, 1367, 1275, 1237, 1167, 1116, 1088, 979, 927, 858, 822, 731, 683, 624 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.08 (s, 1H), 6.91 (s, 2H), 4.44 (d, *J* = 7.1 Hz, 1H), 4.13–4.00 (m, 2H), 3.52–3.40 (m, 1H), 3.47 (s, 1H), 2.95 (t, *J* = 11.9 Hz, 2H), 2.00 (d, *J* = 15.4 Hz, 2H), 1.45 (s, 9H), 1.36 (td, *J* = 14.3, 4.4 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.42, 151.39 (d, *J* = 712.8 Hz), 132.64 (q, *J* = 32.6 Hz), 123.78 (q, *J* = 272.6 Hz), 111.04 (dd, *J* = 223.7, 3.3 Hz), 80.02, 49.95, 32.03, 31.74, 28.49.

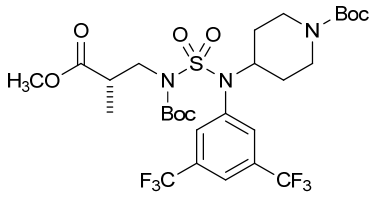
***tert*-Butyl 4-((3,5-bis(trifluoromethyl)phenyl)(*N*-(tert-butoxycarbonyl)sulfamoyl)amino) piperidine-1-carboxylate** (**2.49**)



To a solution of chlorosulfonyl isocyanate (1.0 mL, 11.49 mmol) in CH_2Cl_2 (60 mL) was added to a solution of *tert*-butyl alcohol (1.1 mL, 11.50 mmol) in CH_2Cl_2 (20 mL) at 0 °C. This solution was cannulated to a solution of **2.48** (3.98 g, 9.65 mmol) and Et_3N (2.0 mL, 14.35 mmol) in CH_2Cl_2 (40 mL) at 0 °C. After that, the reaction mixture was stirred at ambient temperature for overnight. The reaction mixture was extracted with CH_2Cl_2 (100 mL X 2) and dried over $MgSO_4$, filtered, evaporated, and purified by the ISCO-Flash column chromatography in 0% to 40% of EtOAc in hexane to get white solid as a product **2.49** (4.78 g, 84%).

Analytical data for **2.49**: R_f = 0.83 (Sol. EtOAc:Hexane = 1/1); FTIR (neat) 2981, 2934, 2866, 2383, 2369, 2325, 1736, 1666, 1436, 1366, 1276, 1247, 1131, 1075, 979, 917, 834, 770, 734, 705, 674, 610 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.94 (s, 1H), 7.67 (s, 2H), 7.57 (s, 1H), 4.42 (tt, *J* = 12.1, 3.8 Hz, 1H), 4.19–4.04 (m, 2H), 2.78 (bt, *J* = 11.9 Hz, 2H), 2.02–1.98 (m, 2H), 1.48 (s, 9H), 1.33 (s, 9H), 1.15 (dddd, *J* = 12.5, 12.3, 12.3, 4.5 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.47, 152.13 (d, *J* = 500.4 Hz), 136.73, 133.12 (q, *J* = 34.1 Hz), 132.75, 123.59, 122.78 (q, *J* = 273.0 Hz), 84.27, 80.23, 59.58, 53.61, 31.85, 28.44, 28.07.

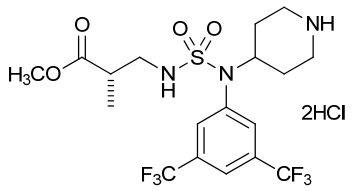
(*S*)-*tert*-Butyl 4-((3,5-bis(trifluoromethyl)phenyl)(*N*-(tert-butoxycarbonyl)-*N*-(3-methoxy-2-methyl-3-oxopropyl)sulfamoyl)amino)piperidine-1-carboxylate (**2.51**)



To a solution of **2.49** (0.51 g, 0.86 mmol) and DIAD (0.35 g, 1.73 mmol) in THF (30 mL) was added a solution of methyl (s)-(+)-3-hydroxy-2-methylpropionate **2.50** (0.10 mL, 0.86 mmol) and PPh_3 (0.45 g, 1.72 mmol) in THF (30 mL) at room temperature and stirred at room temperature for overnight. The reaction mixture was extracted with CH_2Cl_2 (100 mL X 3) and the combined organic layer was dried over $MgSO_4$, filtered, evaporated, and purified by the ISCO-Flash column chromatography in 0% to 30% of EtOAc in hexane to get colorless oil as a product **2.51** (1.22 g, 97%).

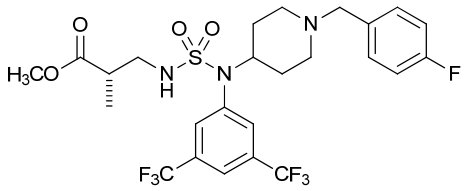
Analytical data for **2.51**: R_f = 0.34 (Sol. EtOAc:Hexane = 1/1); $[\alpha]^{25}_D$ = - 0.992 (*c* = 0.504, CH_2Cl_2); FTIR (neat) 2985, 2943, 2882, 2383, 2368, 1672, 1623, 1556, 1478, 1433, 1405, 1368, 1274, 1238, 1166, 1116, 1088, 1068, 1001, 979, 944, 927, 855, 773, 705, 682, 646 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.95 (s, 1H), 7.62 (s, 2H), 4.45 (m, 1H), 4.13 (s, 2H), 3.63 (dd, *J* = 12.0, 8.0 Hz, 1H), 3.61 (s, 3H), 3.40 (dd, *J* = 12.0, 8.0 Hz, 1H), 2.80 (m, 2H), 2.58 (m, 1H), 2.01 (d, *J* = 8.0 Hz, 2H), 1.55 (s, 9H), 1.37 (s, 9H), 1.13 (dt, *J* = 12.9, 7.7 Hz, 2H), 1.02 (d, *J* = 7.2 Hz, 3H); HRMS $(M+Na)^+$ calcd for $C_{28}H_{39}F_6N_3NaO_8S^+$ (M+Na) required 714.2254, found 714.2257.

(*S*)-Methyl 3-(*N*-(3,5-bis(trifluoromethyl)phenyl)-*N*-(piperidin-4-yl)sulfamoylamino)-2-methylpropanoate HCl (**2.52**)



To a solution of **2.51** (0.30 g, 0.44 mmol) in CH_2Cl_2 (20 mL) was added 4N HCl in 1,4-dioxane (6 mL) at 0 °C and stirred overnight. A reaction mixture was evaporated to remove solvent, and then the mixture was dried under reduced vacuum to furnish a white solid as a product **2.52**. It used without further purification.

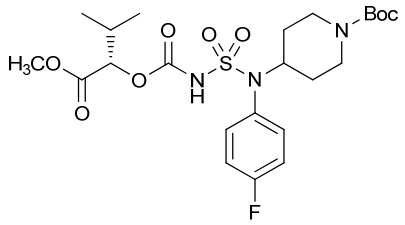
(*S*)-Methyl 3-(*N*-(3,5-bis(trifluoromethyl)phenyl)-*N*-(1-(4-fluorobenzyl)piperidin-4-yl)-sulfamoylamino)-2-methylpropanoate (**2.53**)



To a solution of **2.52** (0.12 g, 0.23 mmol), 4-fluorobenzaldehyde (0.03 g, 0.26 mmol) and Et_3N (0.1 mL, 0.72 mmol) in CH_2Cl_2 (10 mL) was added $NaBH(OAc)_3$ (0.15 g, 0.73 mmol) at 0 °C and stirred for overnight. The reaction mixture was extracted with CH_2Cl_2 (100 mL X 2) and then the combined organic layer was dried over $MgSO_4$, filtered, evaporated, and purified by the ISCO-Flash column chromatography in 0% to 40% of EtOAc in hexane to get white oil as a product **2.53** (0.05 g, 37%).

Analytical data for **2.53:** R_f = 0.45 (Sol. EtOAc:Hexane = 1/1); $[\alpha]^{25}_D$ = + 1.714 (*c* = 0.175, $CHCl_3$); FTIR (neat) 3317, 2945, 2815, 2349, 1727, 1620, 1520, 1475, 1436, 1397, 1372, 1275, 1221, 1169, 1124, 1087, 1035, 943, 858, 844, 769, 702, 682, 662 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.30–7.22 (m, 2H), 7.08 (s, 1H), 7.02–6.95 (m, 2H), 6.87 (s, 2H), 5.35 (s, 1H), 4.29–4.23 (m, 1H), 4.02 (d, *J* = 7.8 Hz, 1H), 3.70 (s, 1H), 3.65–3.60 (m, 1H), 3.48 (s, 3H), 3.38–3.29 (m, 1H), 3.27 (d, *J* = 6.4 Hz, 1H), 2.86–2.77 (m, 2H), 2.16 (td, *J* = 11.4, 2.7 Hz, 2H), 2.05–1.96 (m, 2H), 1.49 (dtd, *J* = 13.6, 10.4, 3.6 Hz, 2H), 1.22 (d, *J* = 7.3 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.62, 162.22 (d, *J* = 244.9 Hz), 147.88, 134.07 (d, *J* = 3.2 Hz), 132.64 (q, *J* = 32.6 Hz), 130.75 (d, *J* = 7.9 Hz), 123.77 (q, *J* = 272.6 Hz), 115.24 (d, *J* = 21.2 Hz), 112.18 (d, *J* = 3.0 Hz), 109.99 (p, *J* = 3.9 Hz), 71.59, 69.62, 69.13, 62.41, 52.32, 52.12, 46.12, 39.43, 32.26, 14.98.

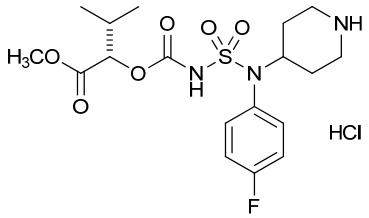
(*S*)-*tert*-Butyl 4-((4-fluorophenyl)(*N*-((1-methoxy-3-methyl-1-oxobutan-2-yloxy)carbonyl) sulfamoyl)amino)piperidine-1-carboxylate (**2.54**)



To a solution of **2.31** (0.93 g, 3.17 mmol) and Et_3N (0.66 mL, 4.74 mmol) in CH_2Cl_2 (20 mL) was cannulated a solution of chlorosulfonyl isocyanate (0.33 mL, 3.79 mmol) and (*S*)-methyl 2-hydroxy-3-methylbutanoate **2.9** (0.50 g, 3.81 mmol) at 0 °C and stirred for overnight. The reaction mixture was extracted with CH_2Cl_2 (100 mL X 2) and then the combined organic layer was dried over $MgSO_4$, filtered, evaporated, and purified by the ISCO-Flash column chromatography in 0% to 50% of EtOAc in hexane to get white solid as a product **2.54** (0.50 g, 30 %).

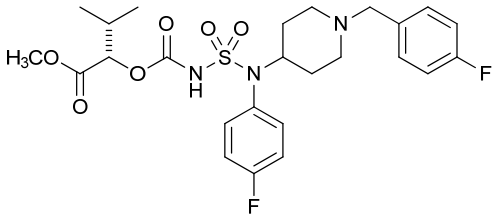
Analytical data for **2.54**: R_f = 0.70 (Sol. EtOAc:Hexane = 1/1); $[\alpha]^{25}_D$ = + 33.939 (*c* = 0.165, CH_2Cl_2); FTIR (neat) 3077, 2972, 2936, 2878, 1743, 1667, 1602, 1506, 1452, 1435, 1366, 1265, 1234, 1212, 1168, 1133, 1093, 1064, 954, 938, 873, 738, 641 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.09 (s, 1H), 7.25 (dd, *J* = 9.0, 4.9 Hz, 2H), 7.07 (dd, *J* = 5.2, 5.2 Hz, 2H), 4.85 (d, *J* = 4.3 Hz, 1H), 4.33 (tt, *J* = 11.9, 3.6 Hz, 1H), 4.10–3.96 (m, 2H), 3.75 (s, 3H), 2.79–2.59 (m, 2H), 2.28–2.15 (m, 1H), 1.90 (t, *J* = 11.5 Hz, 2H), 1.32 (s, 9H), 1.25–1.15 (m 2H), 1.08 (dddd, *J* = 12.4, 12.4 12.3, 4.2 Hz, 1H), 0.96 (d, *J* = 6.9 Hz, 3H), 0.89 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.96, 164.36, 161.88, 152.74 (d, *J* = 378.8 Hz), 134.29 (d, *J* = 8.9 Hz), 130.12 (d, *J* = 3.2 Hz), 116.45 (d, *J* = 22.7 Hz), 79.97, 78.59, 59.33, 53.64, 52.54, 30.18, 28.49, 18.82, 17.21.

(*S*)-Methyl 2-(*N*-(4-fluorophenyl)-*N*-(piperidin-4-yl)sulfamoylcarbamoyloxy)-3-methyl-butanoate hydrochloride (**2.55**)



To a solution of **2.54** (0.5 g, 0.94 mmol) in CH_2Cl_2 (20 mL) was added 4N HCl in 1,4-dioxane (6 mL) at 0 °C and stirred overnight. A reaction mixture was evaporated to remove solvent, and then the mixture was dried under reduced vacuum to furnish a white solid as a product **2.55**. It used without further purification.

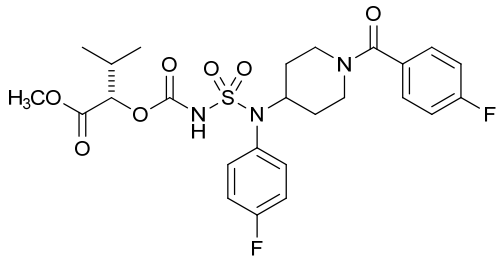
(*S*)-Methyl 2-(*N*-(1-(4-fluorobenzyl)piperidin-4-yl)-*N*-(4-fluorophenyl)sulfamoyl carbamoyloxy)-3-methylbutanoate (**2.56**)



To a solution of **2.55** (0.09 g, 0.19 mmol), 4-fluorobenzaldehyde (0.03 mL, 0.22 mmol) and Et_3N (0.08 mL, 0.22 mmol) in CH_2Cl_2 (10 mL) was added $NaBH(OAc)_3$ (0.14 g, 0.64 mmol) at 0 °C and stirred for overnight. The reaction mixture was extracted with CH_2Cl_2 (100 mL X 2) and then the combined organic layer was dried over $MgSO_4$, filtered, evaporated, and purified by the ISCO-Flash column chromatography in 0% to 40% of EtOAc in hexane to get white oil as a product **2.56** (0.0786 g, 76 %).

Analytical data for **2.56**: R_f = 0.24 (Sol. EtOAc:Hexane = 1/1); $[\alpha]^{25}_D$ = + 16. 467 (*c* = 0.753, CH_2Cl_2); FTIR (neat) 2956, 2925, 2852, 2383, 2357, 1750, 1661, 1627, 1604, 1505, 1463, 1264, 1226, 1209, 1089, 918, 874, 838, 791, 739, 626 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.29–7.23 (m, 2H), 7.15 (dd, *J* = 8.6, 5.5 Hz, 2H), 7.04 (dd, *J* = 8.9, 8.9, 2H), 6.96 (dd, *J* = 8.7, 8.7, 2H), 4.84 (d, *J* = 4.6 Hz, 1H), 4.23 (tt, *J* = 12.0, 3.9 Hz, 1H), 3.75 (s, 3H), 3.49 (d, *J* = 13.2 Hz, 1H), 3.41 (d, *J* = 13.2 Hz, 1H), 3.05–2.91 (m, 3H), 2.28–2.08 (m, 3H), 2.02–1.90 (m, 2H), 1.63–1.38 (m, 2H), 0.93 (t, *J* = 6.5 Hz, 3H), 0.86 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.35, 162.76 (d, *J* = 249.2 Hz), δ 162.53 (d, *J* = 246.7 Hz), 152.77, 134.20 (d, *J* = 8.7 Hz), 131.54 (d, *J* = 8.1 Hz), 131.20, 130.62, 116.10 (d, *J* = 22.6 Hz), 115.43 (d, *J* = 21.4 Hz), 77.88, 60.65, 57.95, 52.22, 52.01, 30.85, 30.37, 30.19, 29.78, 18.68, 17.40; HRMS $(M+H)^+$ calcd for $C_{25}H_{32}F_2N_3O_6S^+$ (M+H) required 540.1980, found 540.2224.

(*S*)-Methyl 2-(*N*-(1-(4-fluorobenzoyl)piperidin-4-yl)-*N*-(4-fluorophenyl)sulfamoyl carbamoyloxy)-3-methylbutanoate (**2.57**)



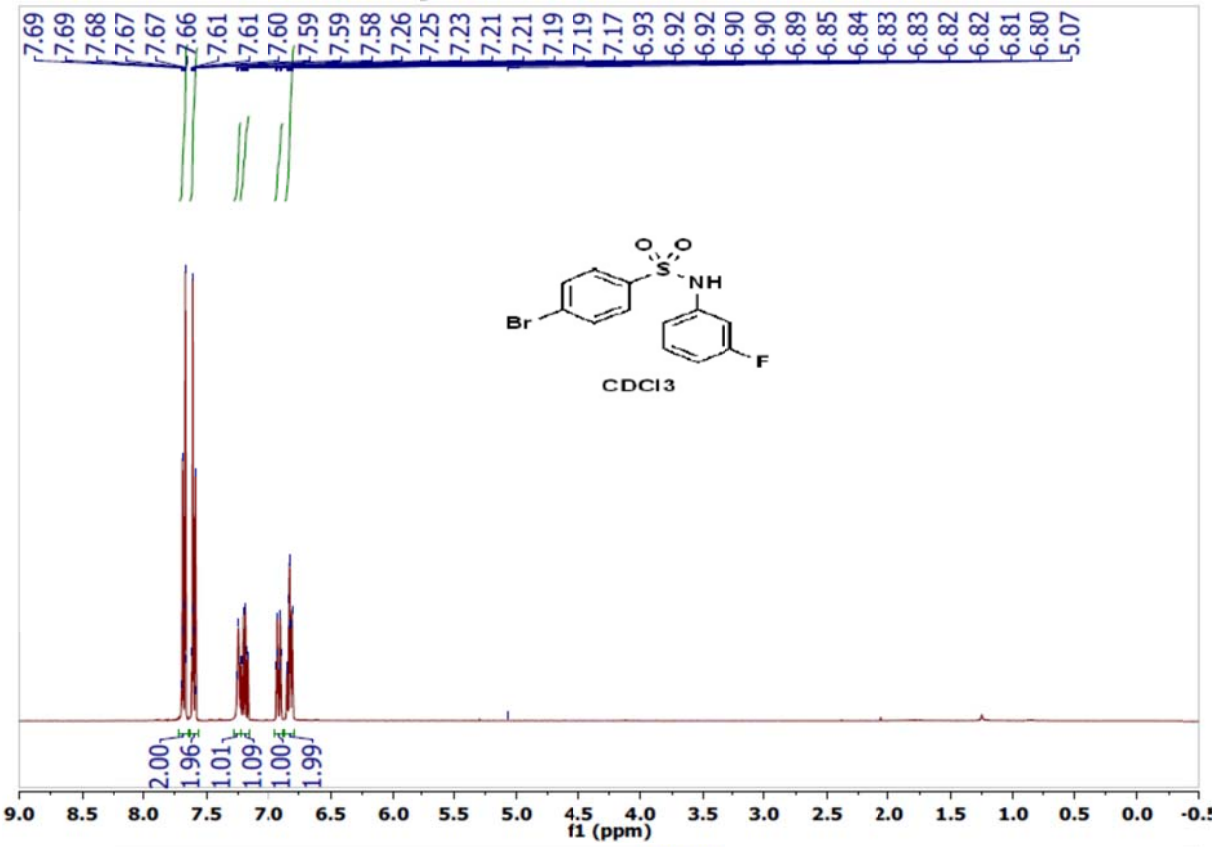
To a solution of **2.55** (0.10 g, 0.21 mmol) and Et_3N (0.09 mL, 0.65 mmol) in THF (8 mL) was added 4-fluorobenzoyl chloride (0.03 g, 0.22 mmol) at room temperature and stirred for overnight. The reaction mixture was evaporated, and purified by the ISCO-Flash column chromatography in 0% to 40% of EtOAc in hexane to get white solid as a product **2.57** (0.0458 g, 39 %).

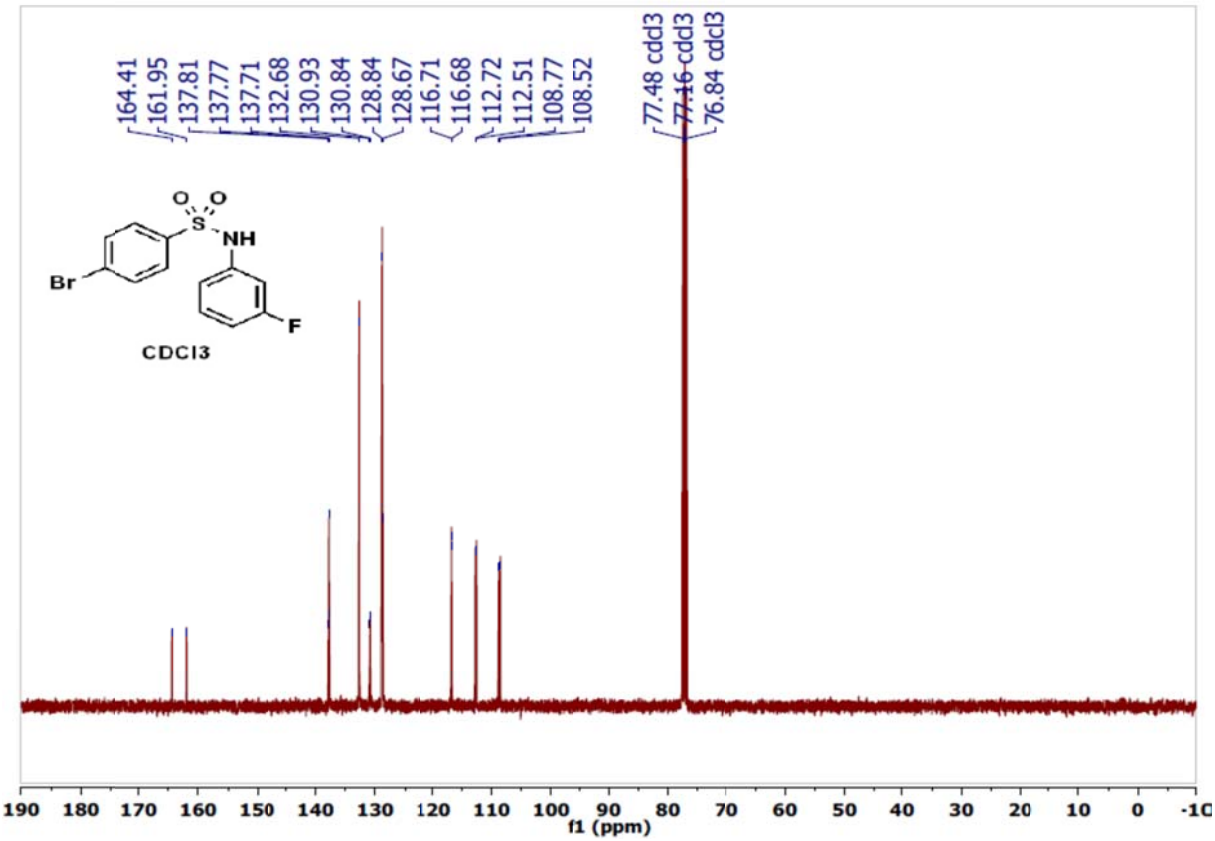
Analytical data for **2.57:** R_f = 0.30 (Sol. EtOAc:Hexane = 1/1); $[\alpha]^{25}_D$ = + 25.410 (*c* = 0.488, CH_2Cl_2); FTIR (neat) 2964, 2933, 2878, 1741, 1604, 1506, 1449, 1370, 1281, 1213, 1170, 1151, 1067, 1013, 954, 938, 846, 760, 739, 615 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.27–7.21 (m, 4H), 7.09 (dd, *J* = 8.42, 8.42 Hz, 2H), 7.00 (dd, *J* = 8.6, 8.6 Hz, 2H), 4.85 (d, *J* = 4.2 Hz, 1H), 4.69 (s, 1H), 4.48 (tt, *J* = 12.1, 3.9 Hz, 1H), 3.75 (s, 3H), 2.92 (bd, *J* = 4.8, 2H), 2.23 (ttd, *J* = 6.9, 6.9, 4.2 Hz, 1H), 2.14–1.86 (m, 2H), 1.45–1.00 (m, 4H), 0.97 (d, *J* = 6.9 Hz, 3H), 0.89 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.70, 169.30, 163.36 (d, *J* = 249.9 Hz), 163.00 (d, *J* = 250.7 Hz), 150.64, 133.99 (d, *J* = 9.0 Hz), 131.35 (d, *J* = 3.2 Hz), 129.67 (d, *J* = 3.2 Hz), 129.25 (d, *J* = 8.6 Hz), 116.45 (d, *J* = 22.6 Hz), 115.46 (d, *J* = 21.7 Hz), 78.43, 58.83, 52.36, 50.92, 29.93, 29.65, 18.63, 16.95; HRMS $(M+H)^+$ calcd for $C_{25}H_{30}F_2N_3O_7S^+$ (M+H) required 554.1772, found 554.1864.

5.3. Appendix A

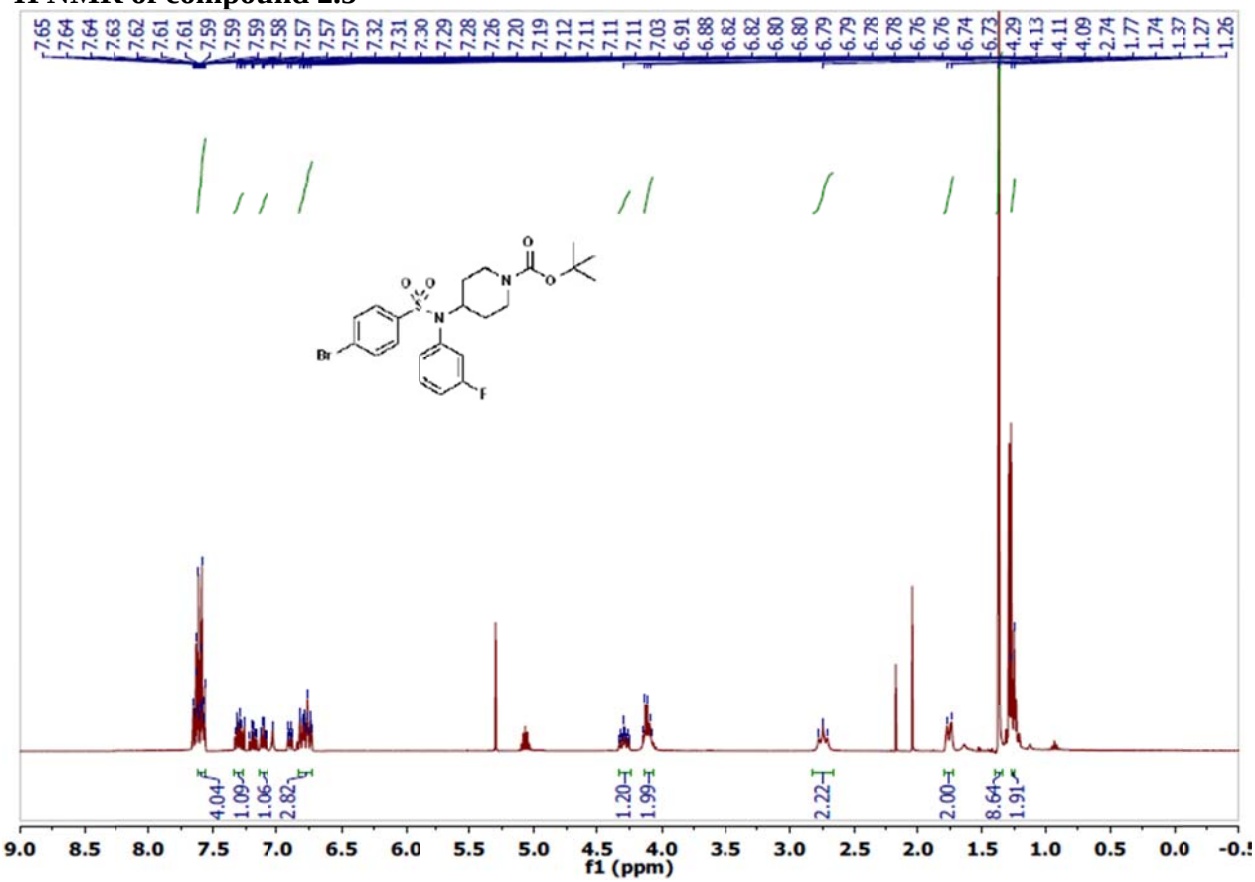
Selected ^{1}H and ^{13}C NMR's

^{1}H NMR and ^{13}C NMR of compound 2.4

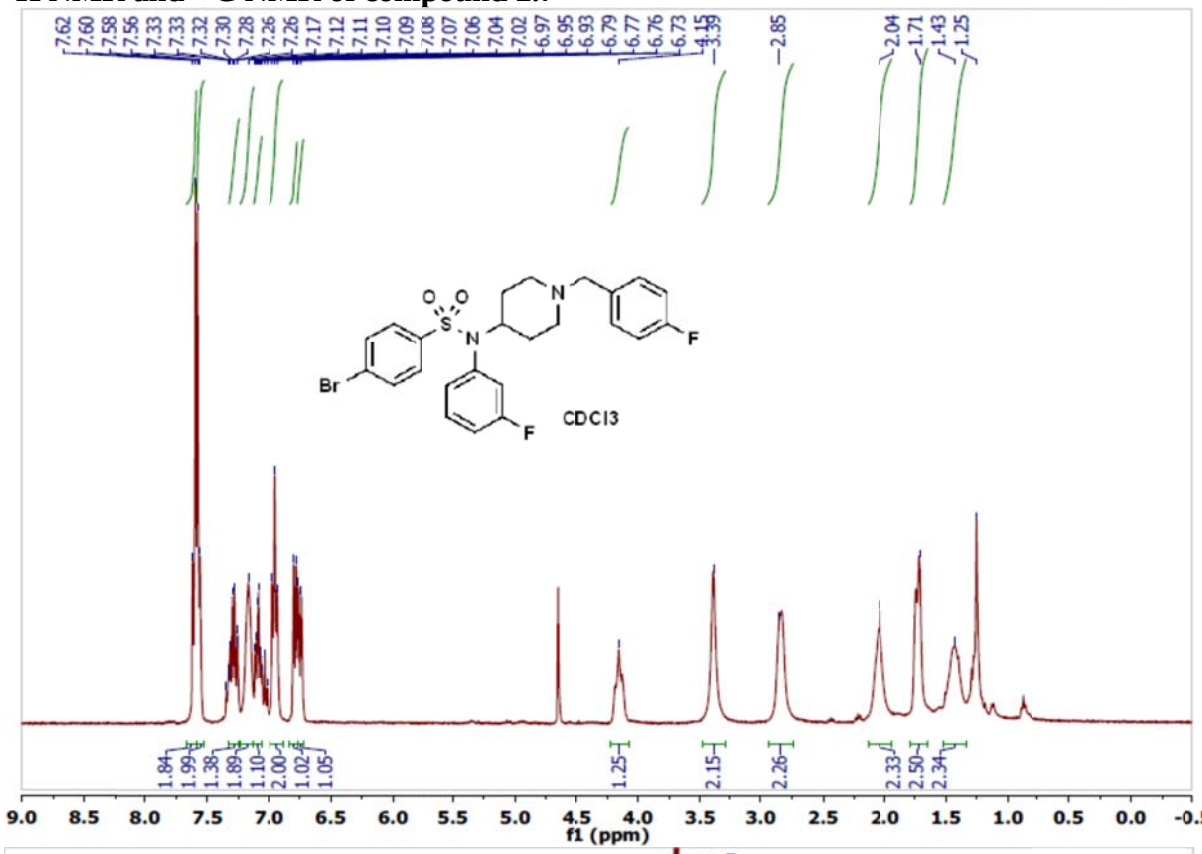


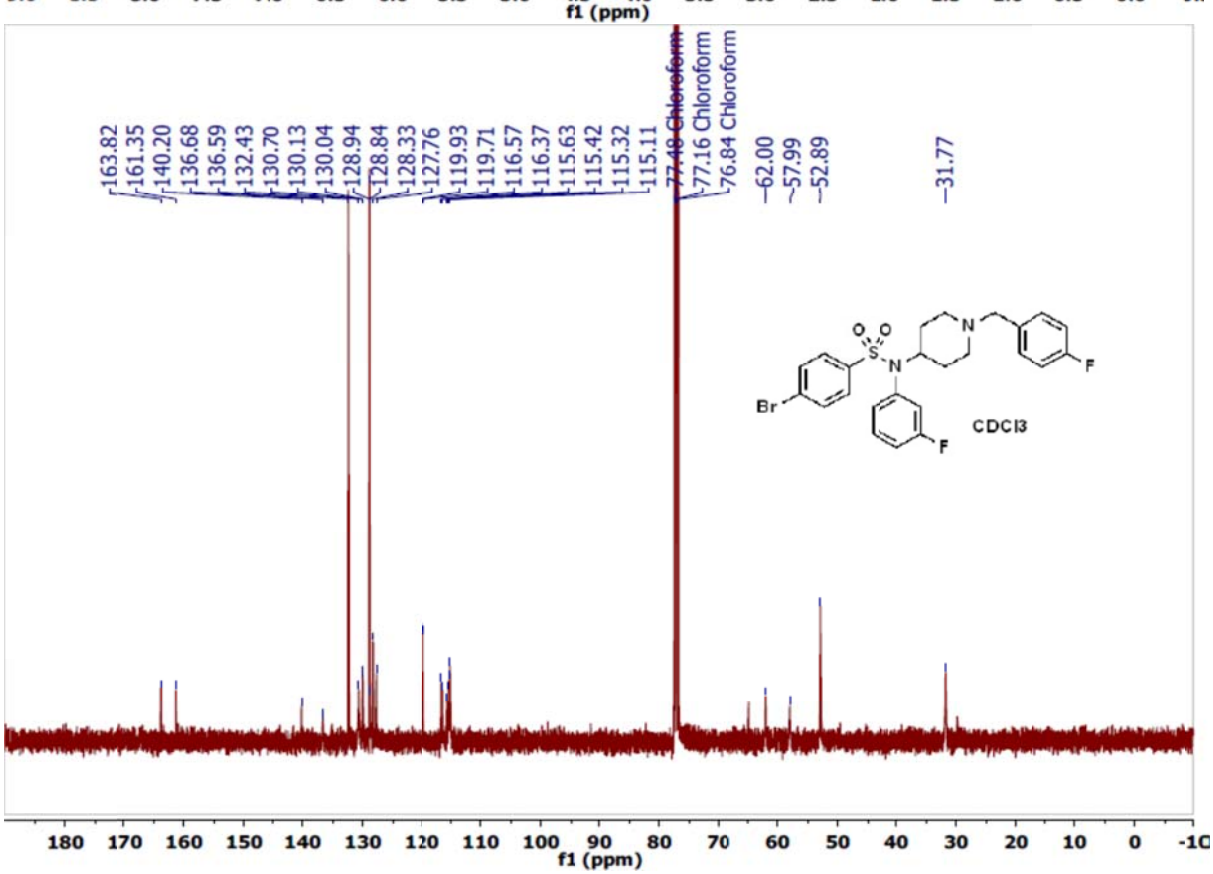


^{1}H NMR of compound 2.5

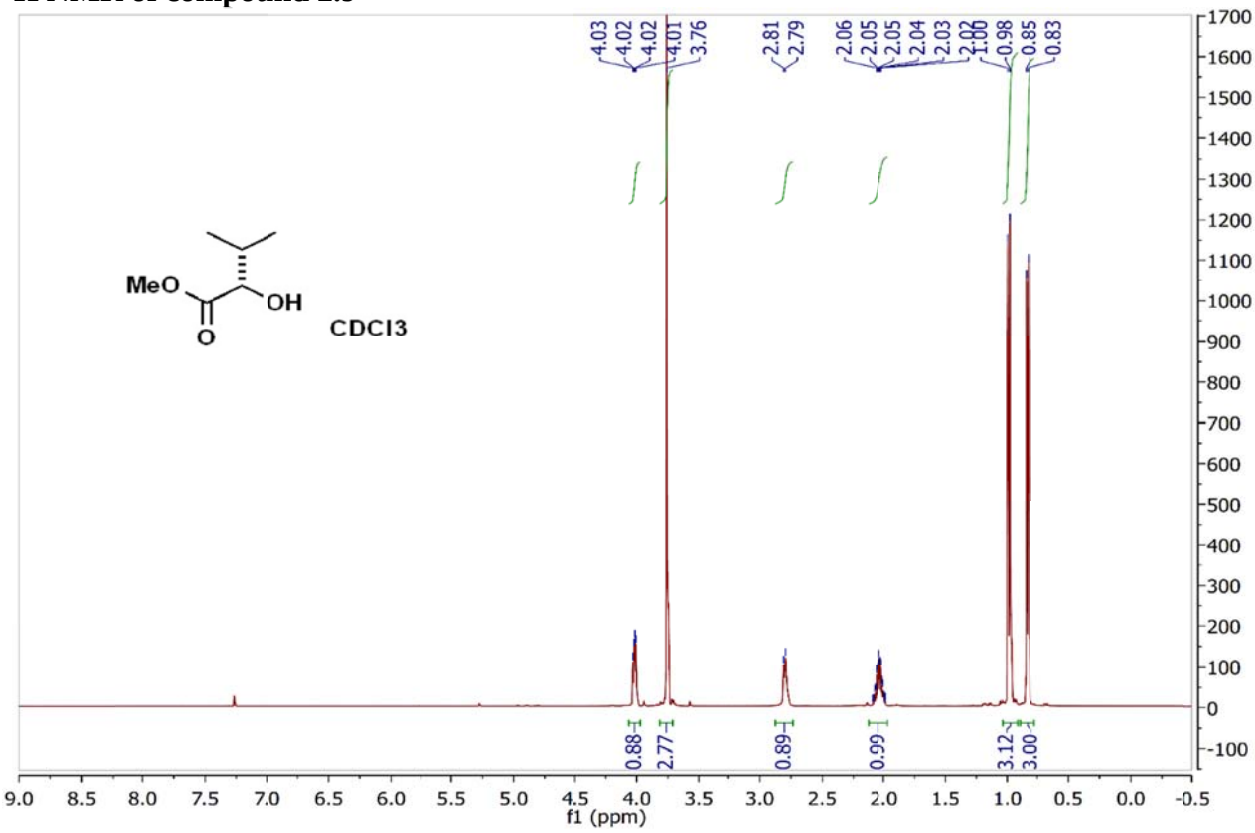


^{1}H NMR and ^{13}C NMR of compound 2.7

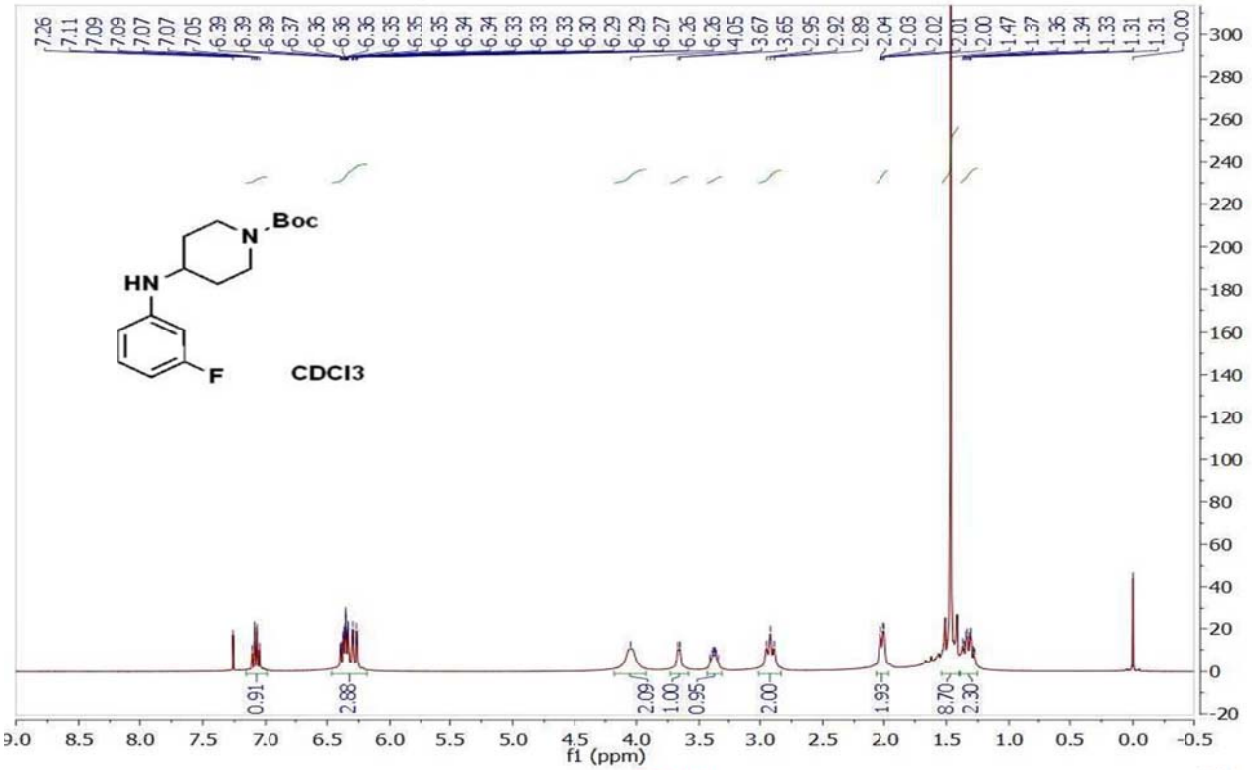


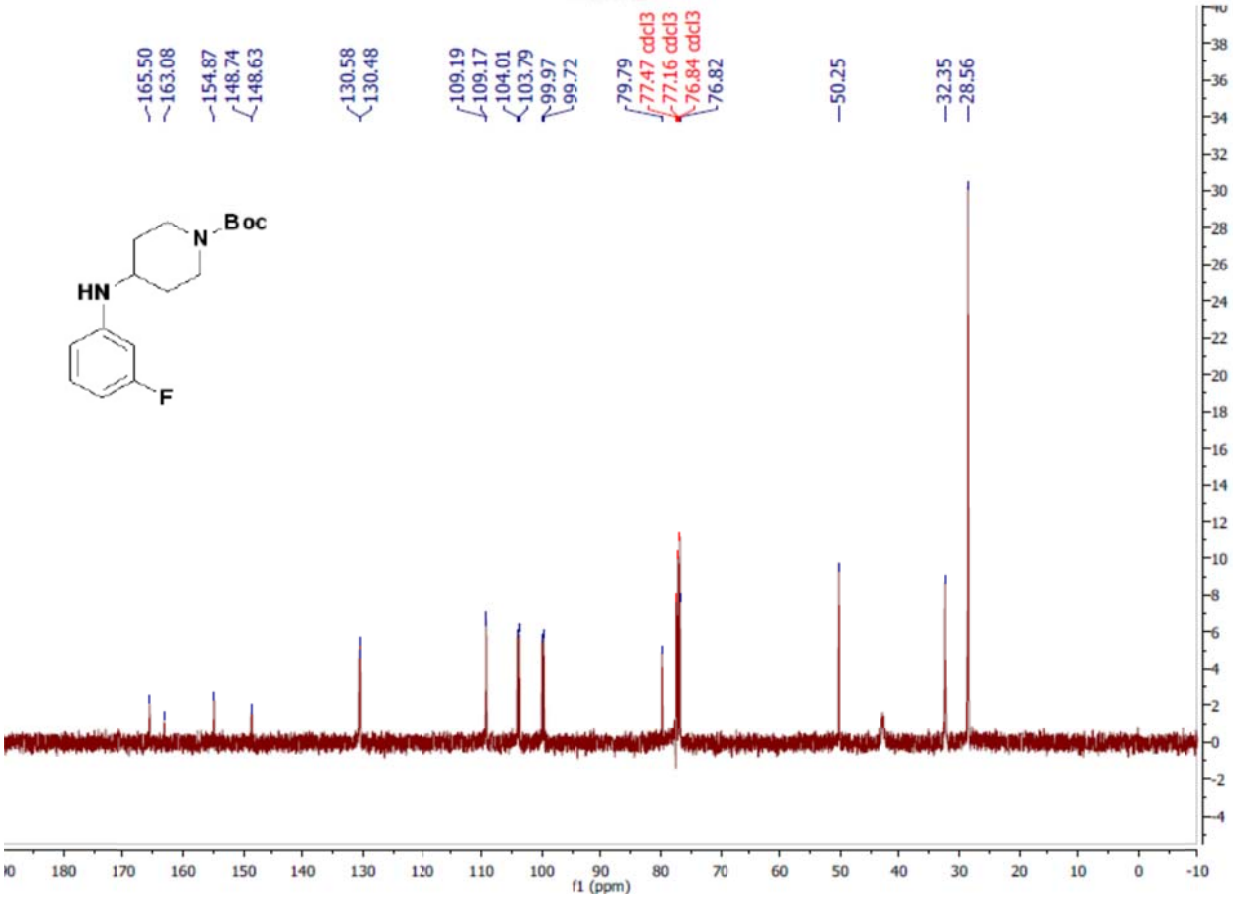


^{1}H NMR of compound 2.9

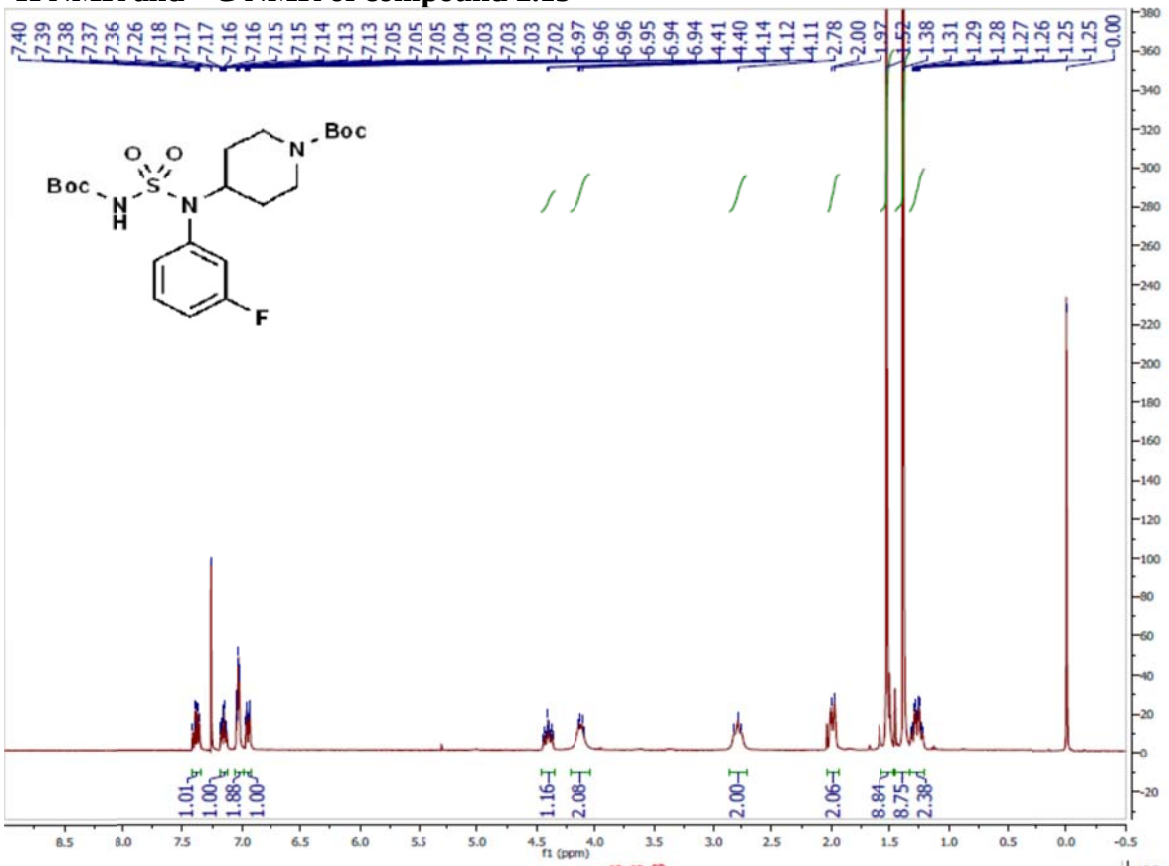


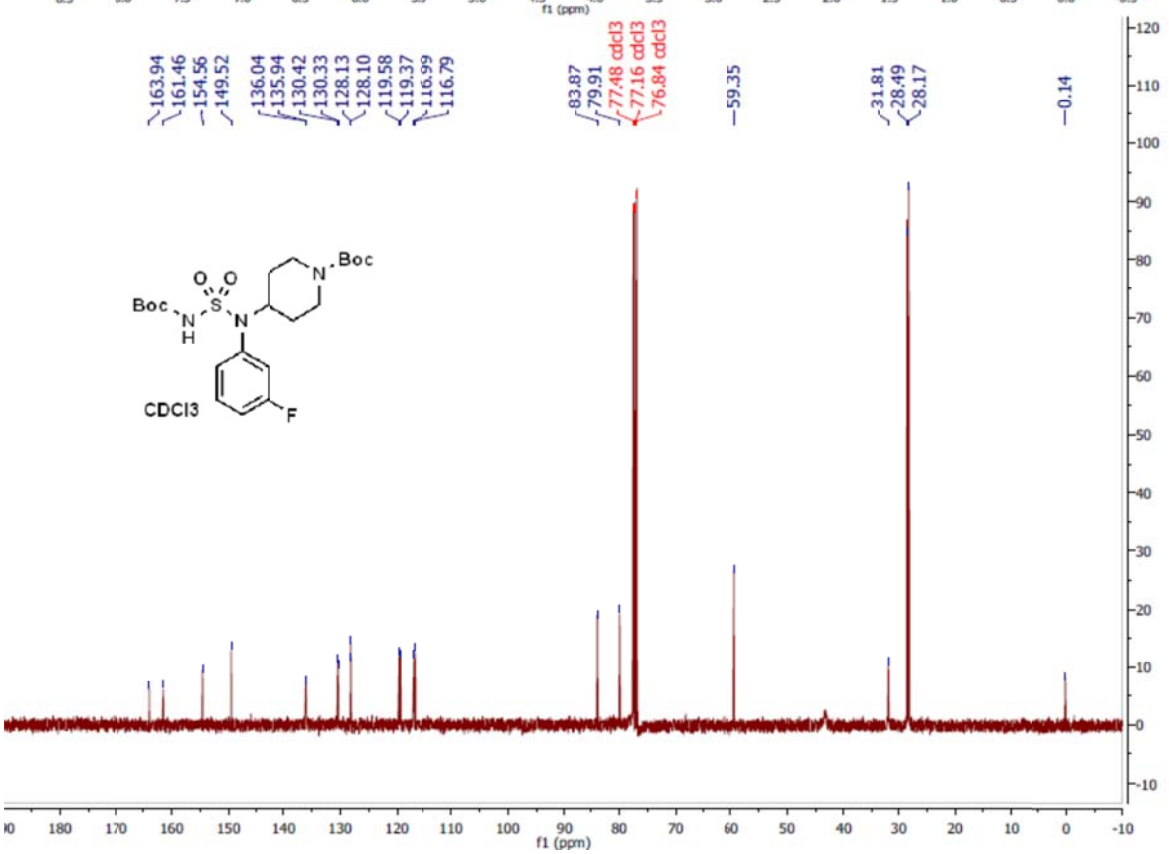
^{1}H NMR and ^{13}C NMR of compound 2.13



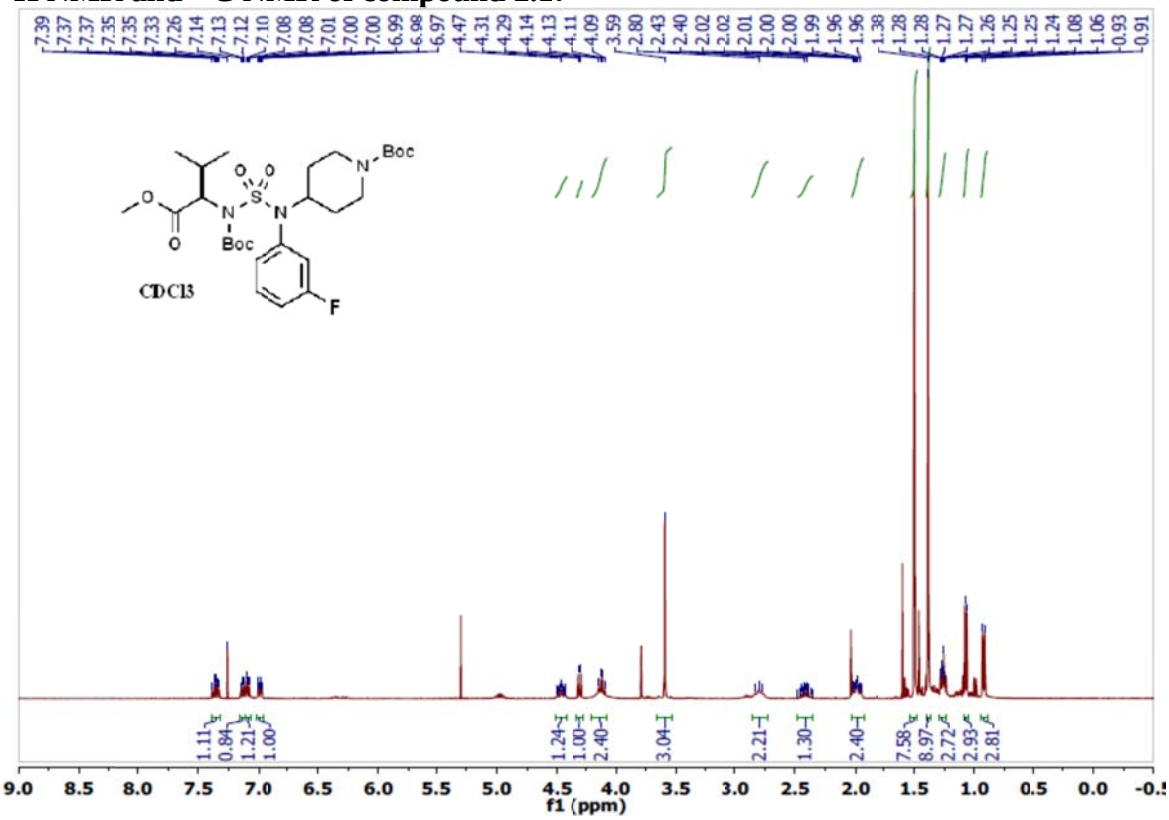


^{1}H NMR and ^{13}C NMR of compound 2.15

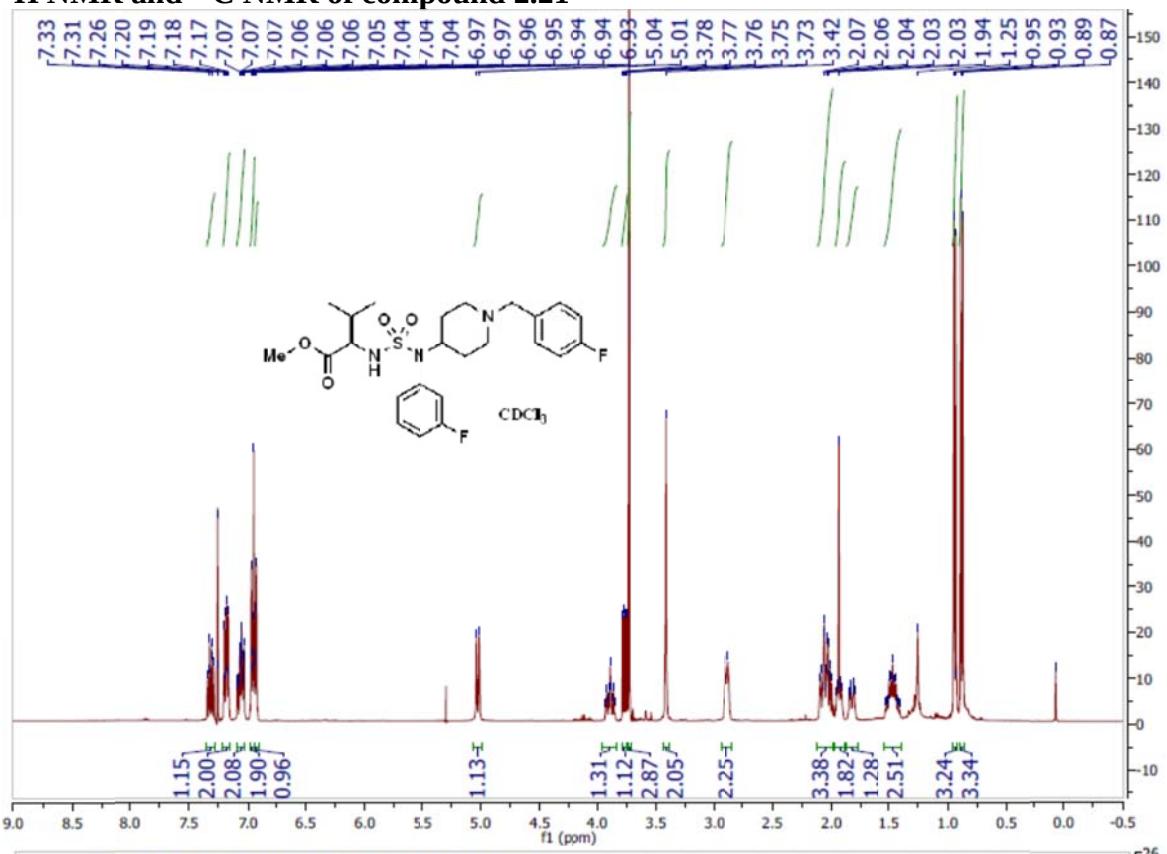


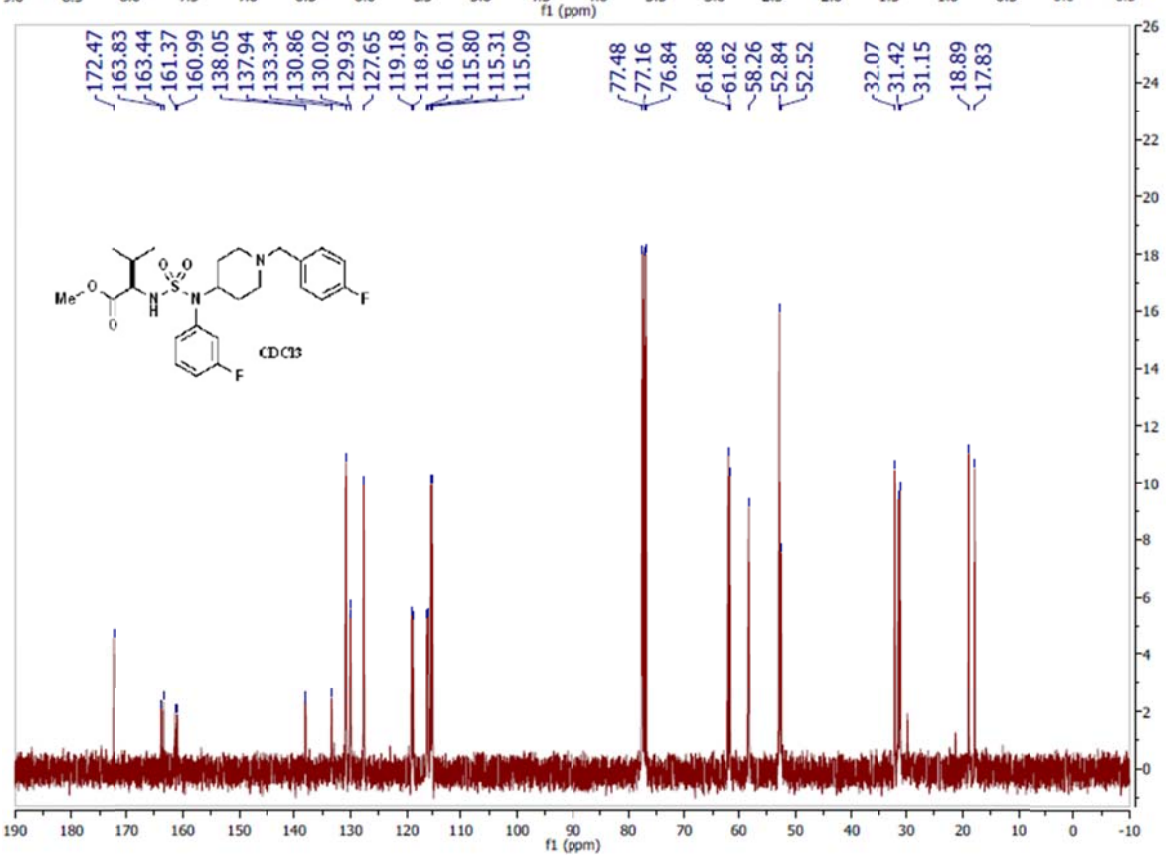


^{1}H NMR and ^{13}C NMR of compound 2.17

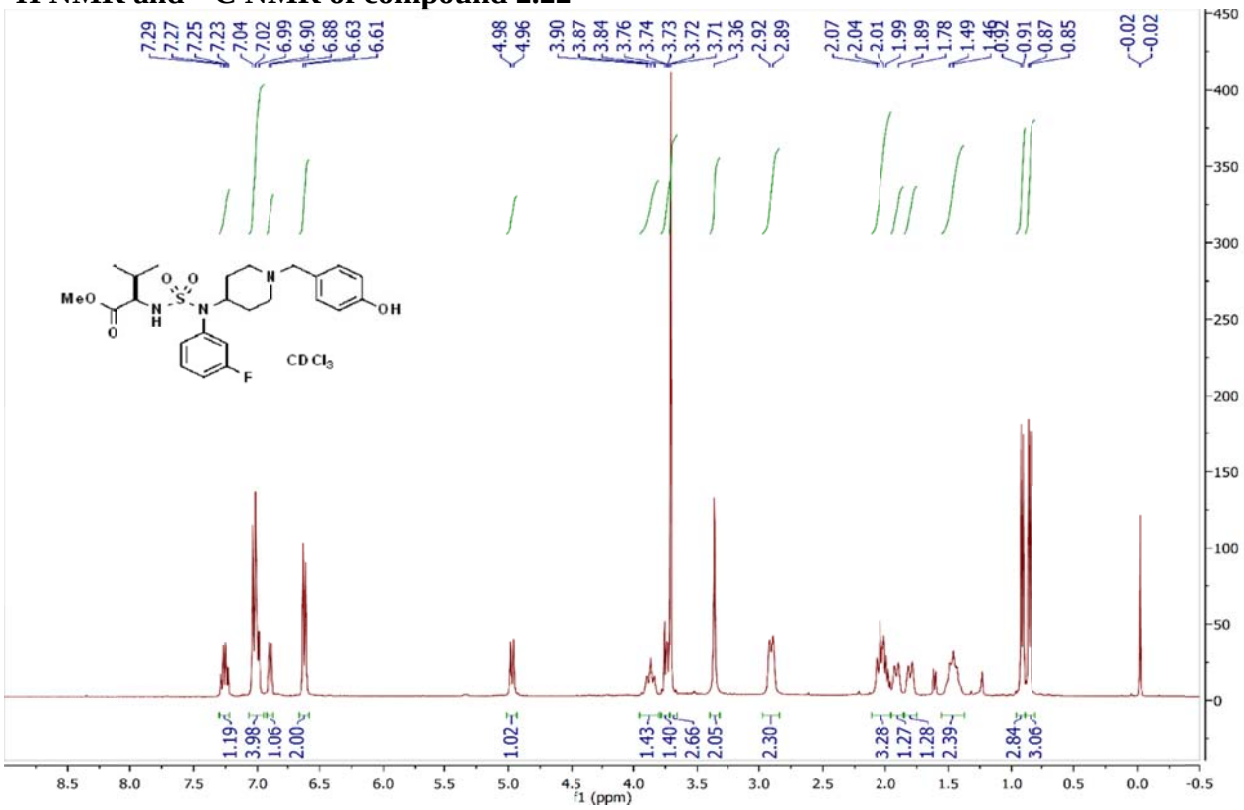


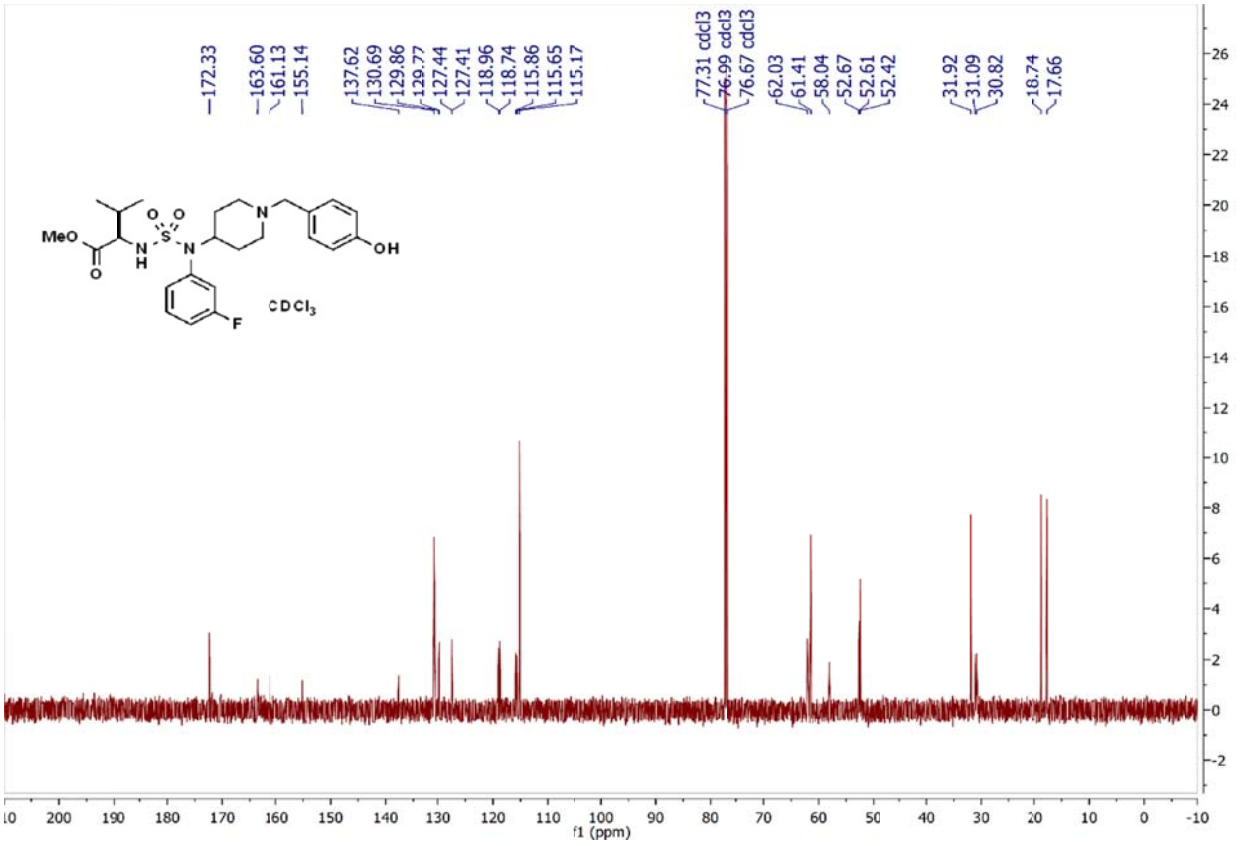
^{1}H NMR and ^{13}C NMR of compound 2.21



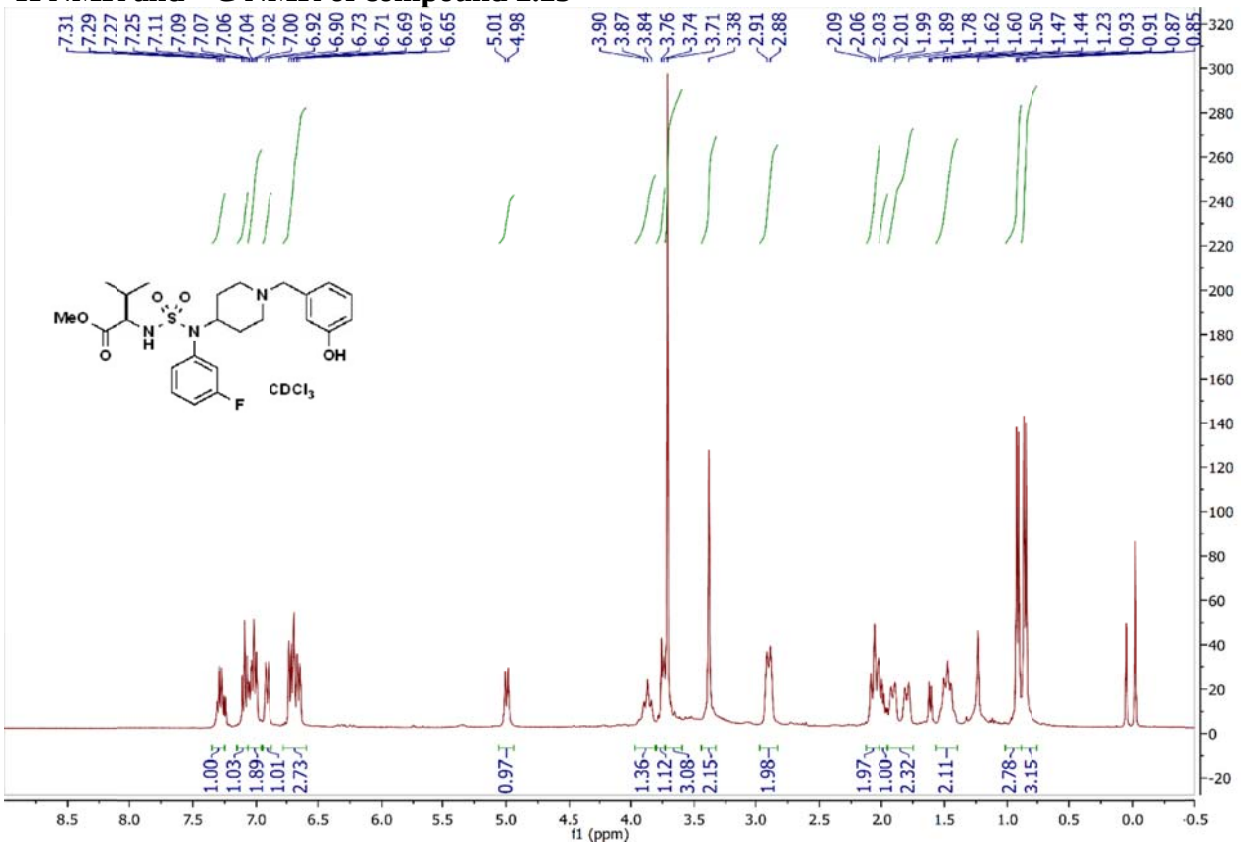


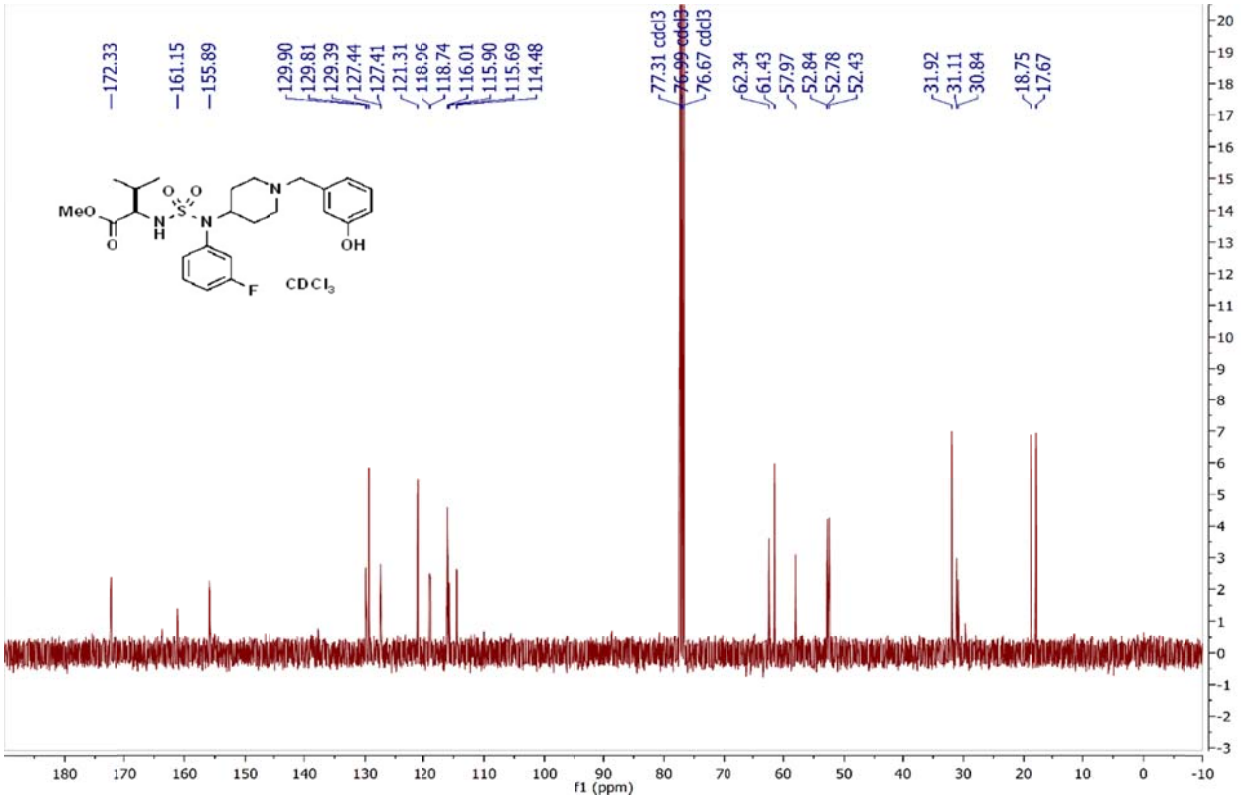
^{1}H NMR and ^{13}C NMR of compound 2.22



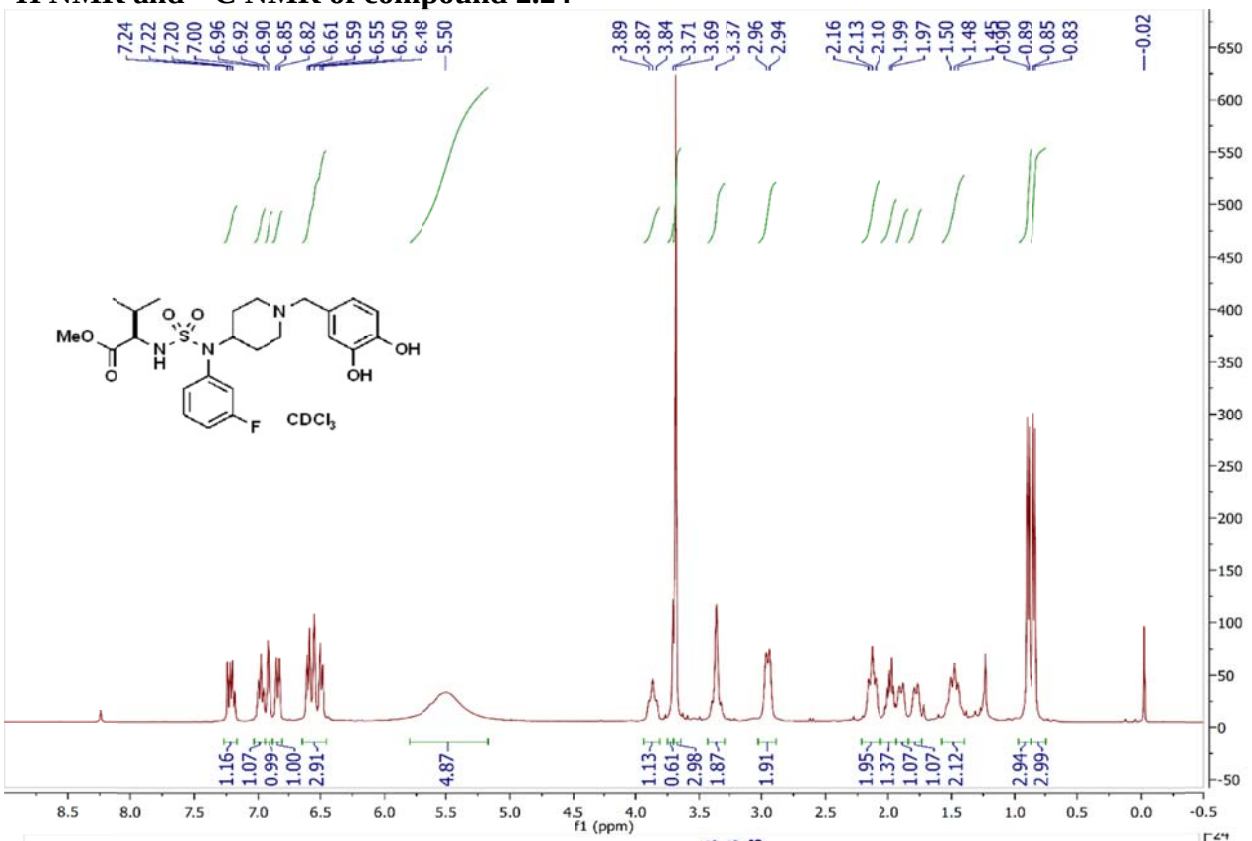


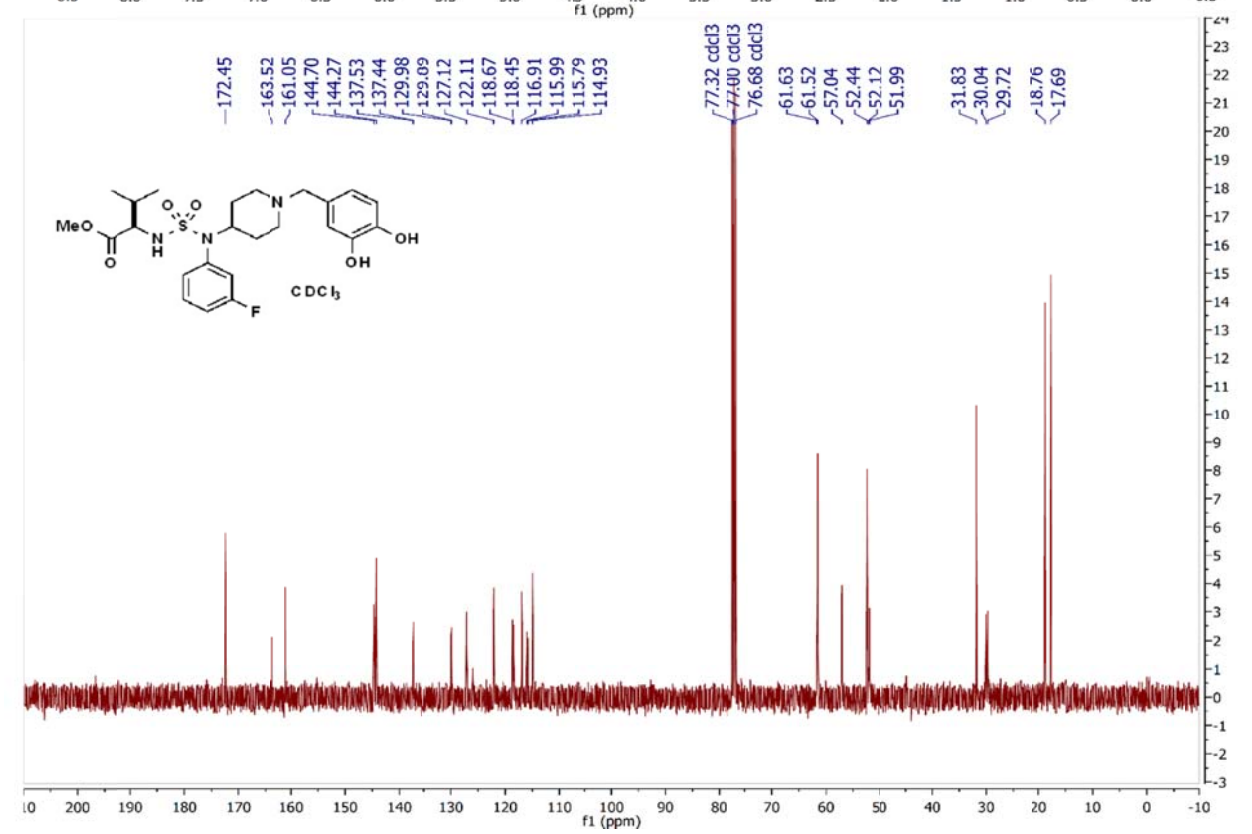
^{1}H NMR and ^{13}C NMR of compound 2.23



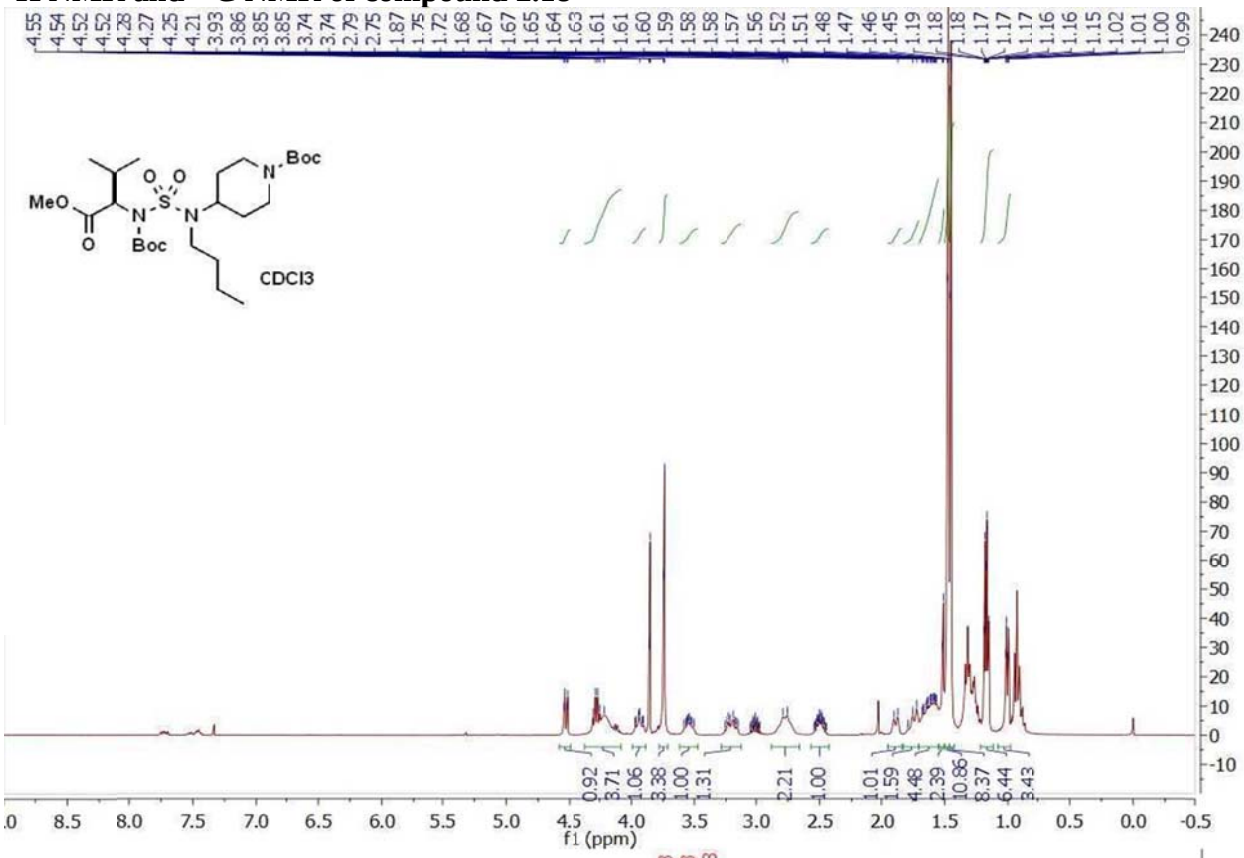


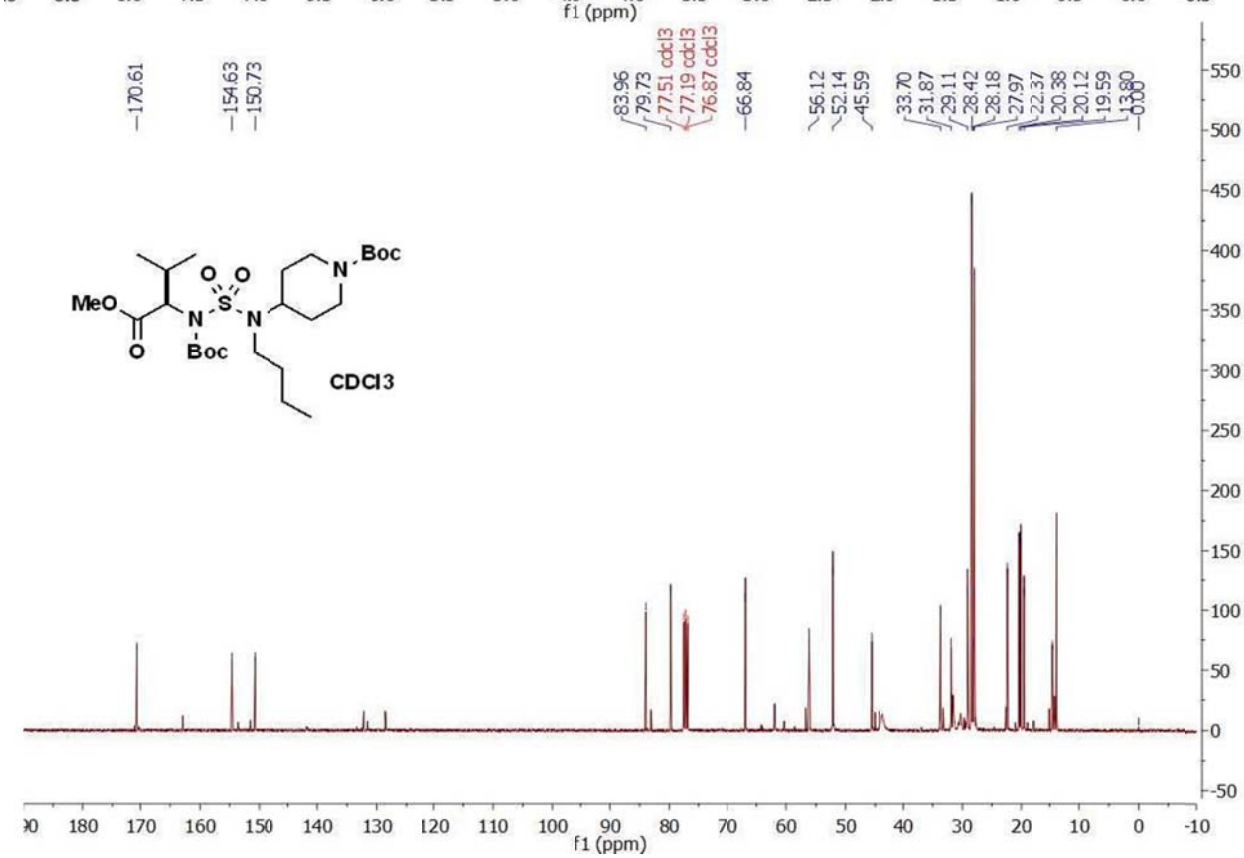
^{1}H NMR and ^{13}C NMR of compound 2.24



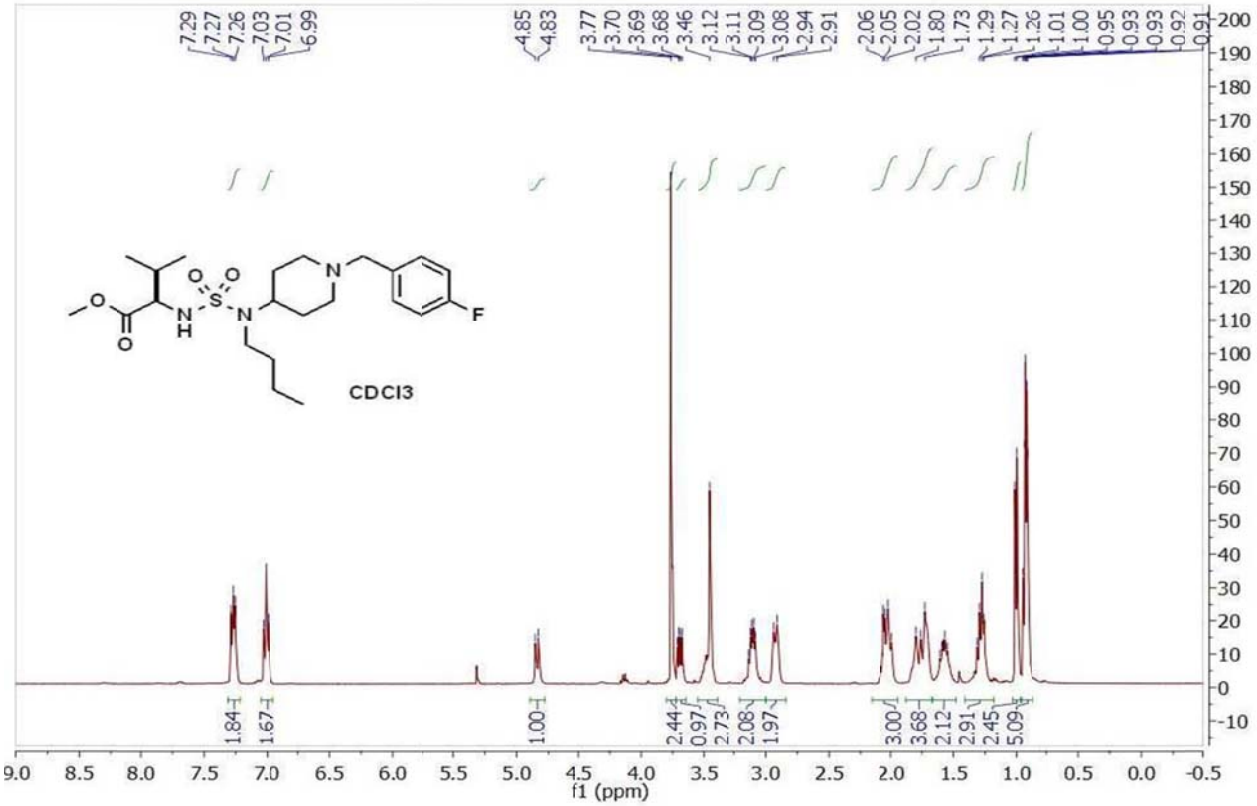


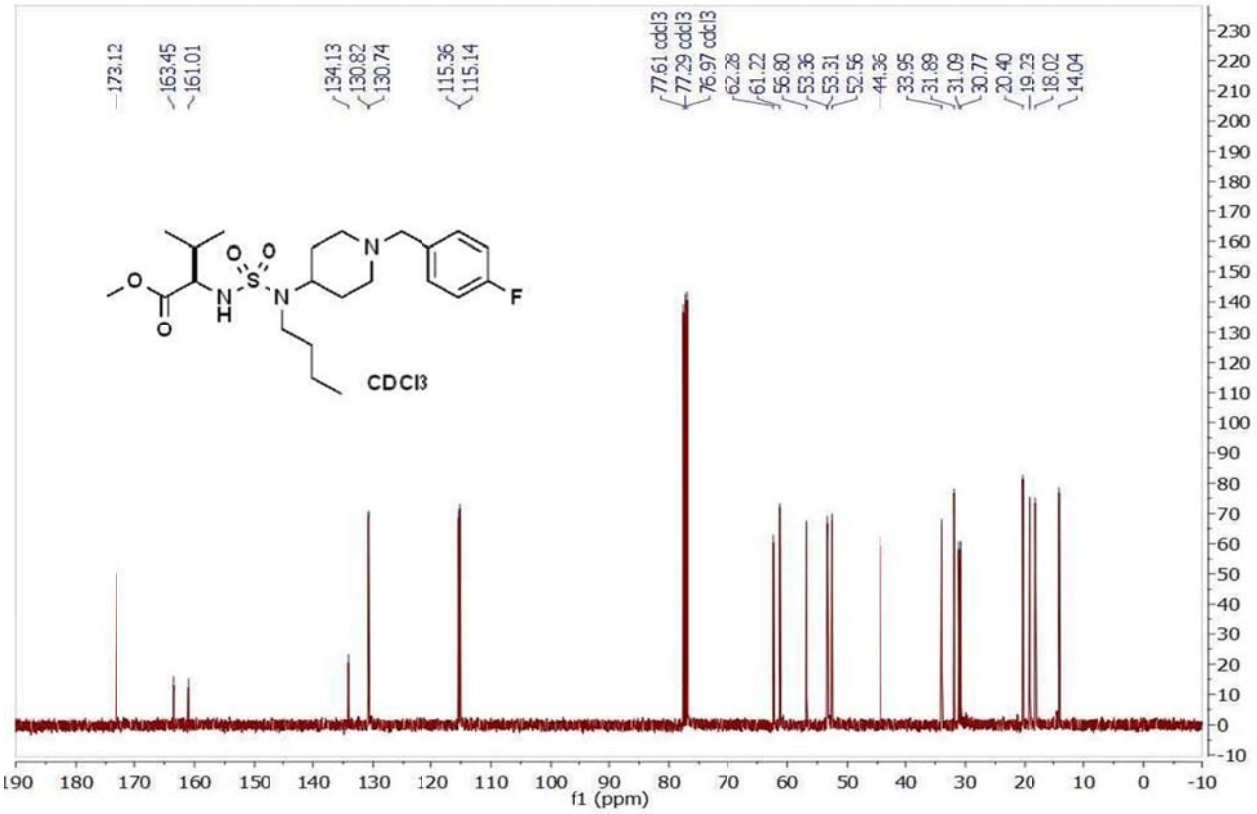
^{1}H NMR and ^{13}C NMR of compound 2.18



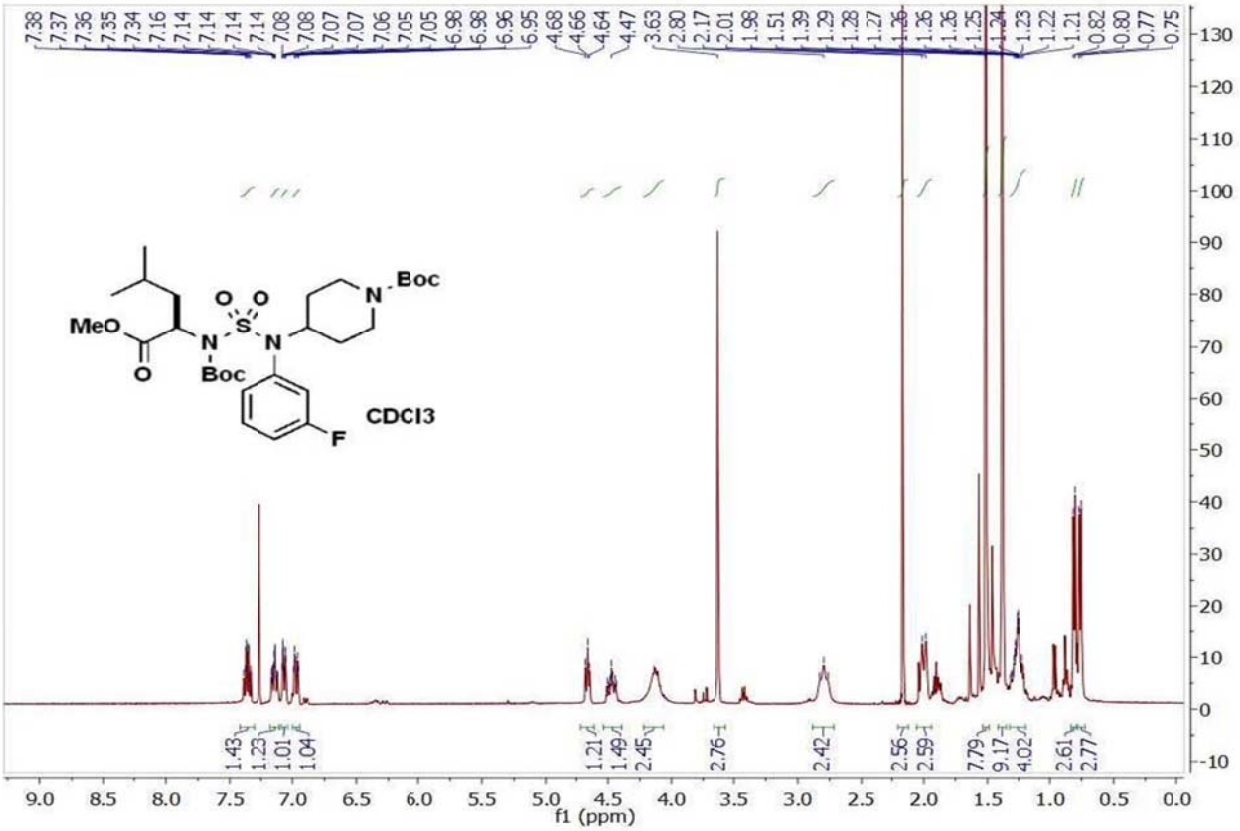


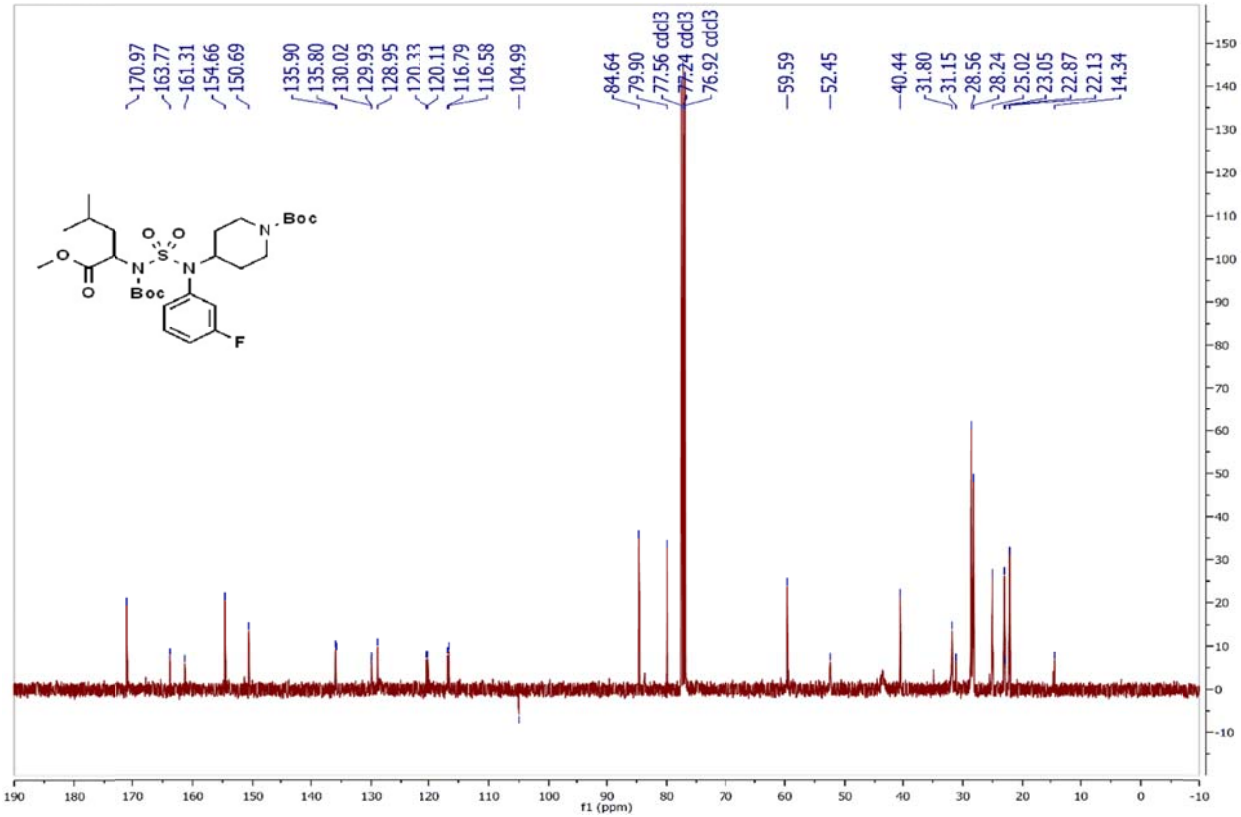
^{1}H NMR and ^{13}C NMR of compound 2.25



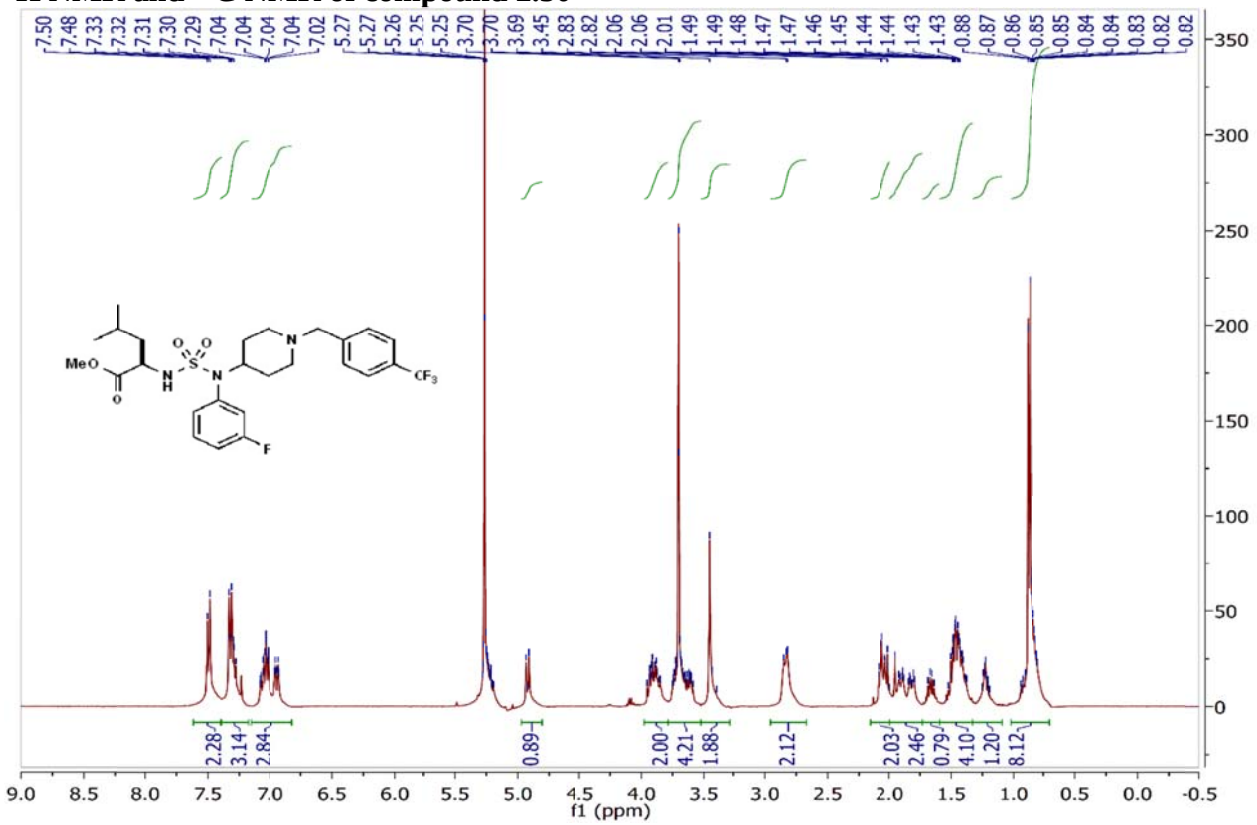


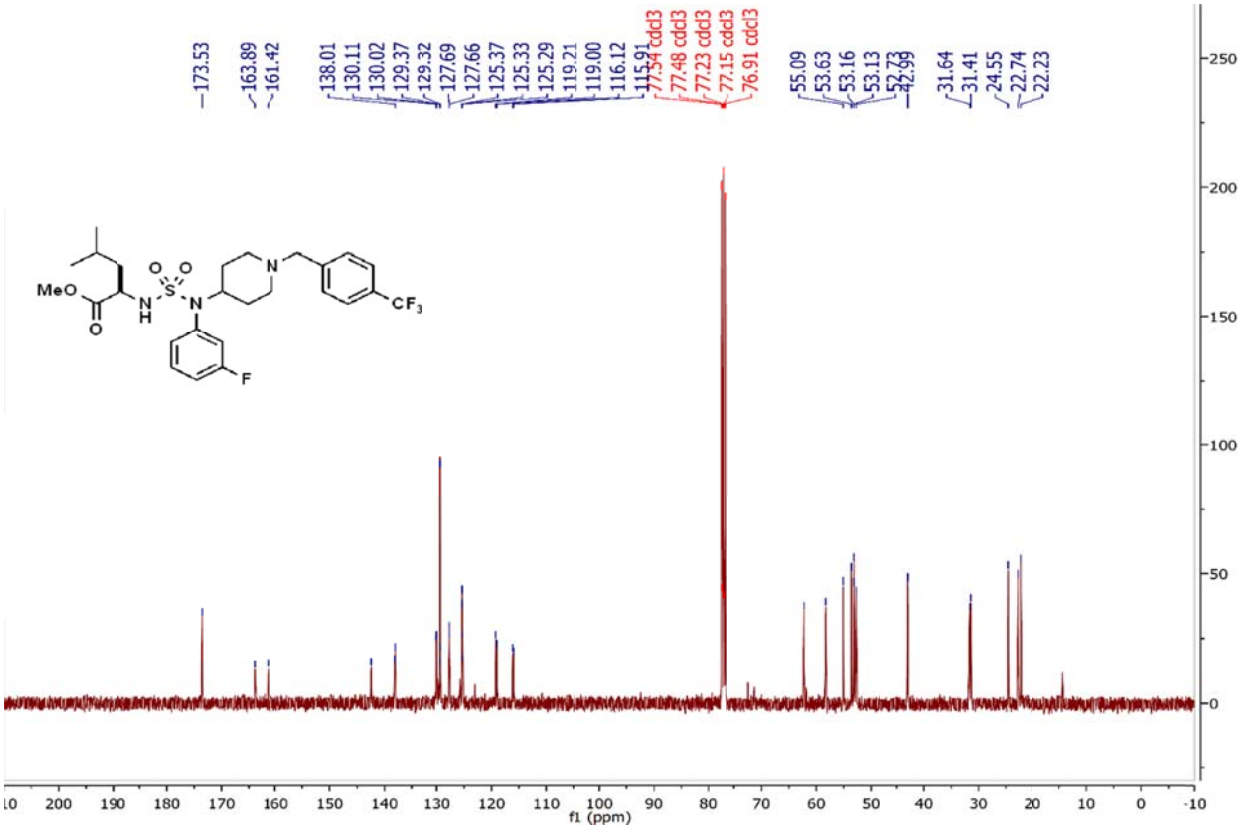
^{1}H NMR and ^{13}C NMR of compound 2.28



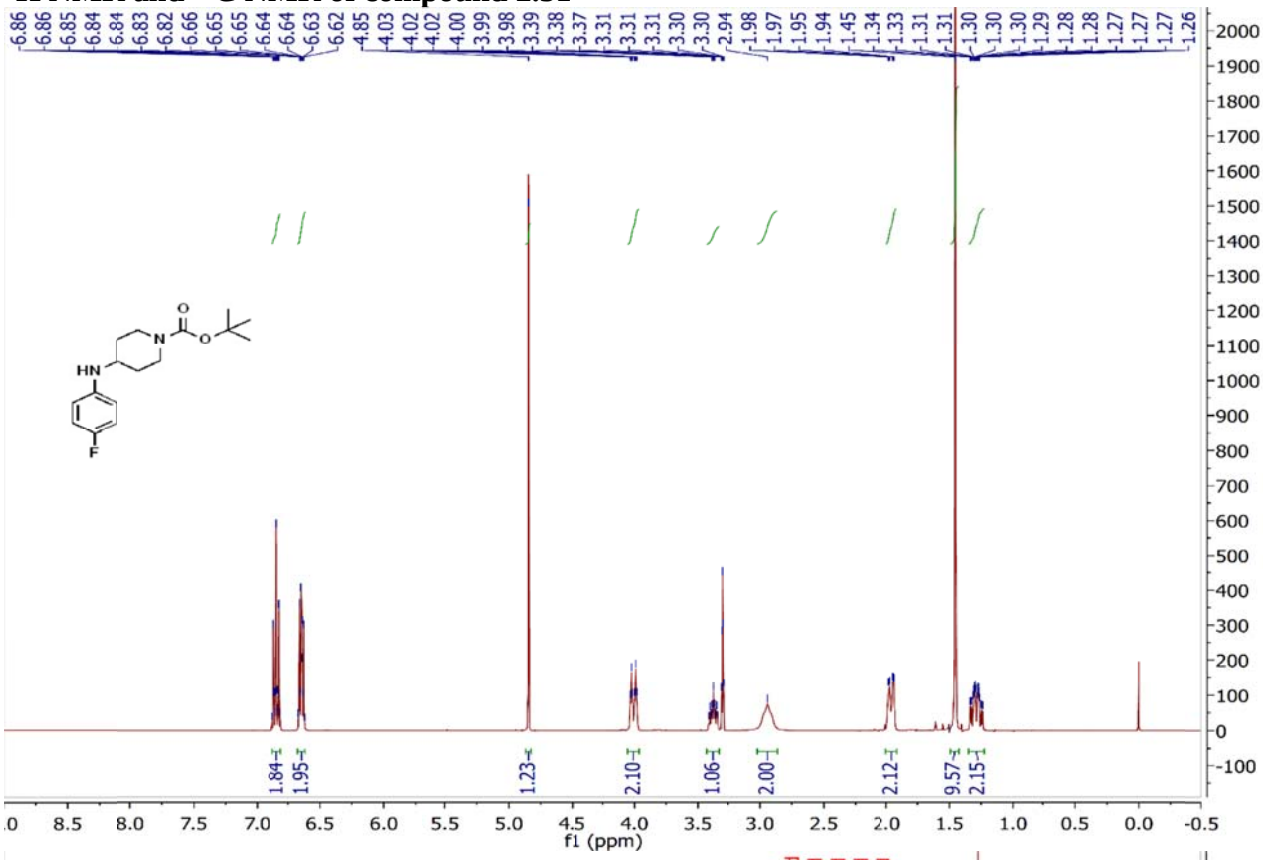


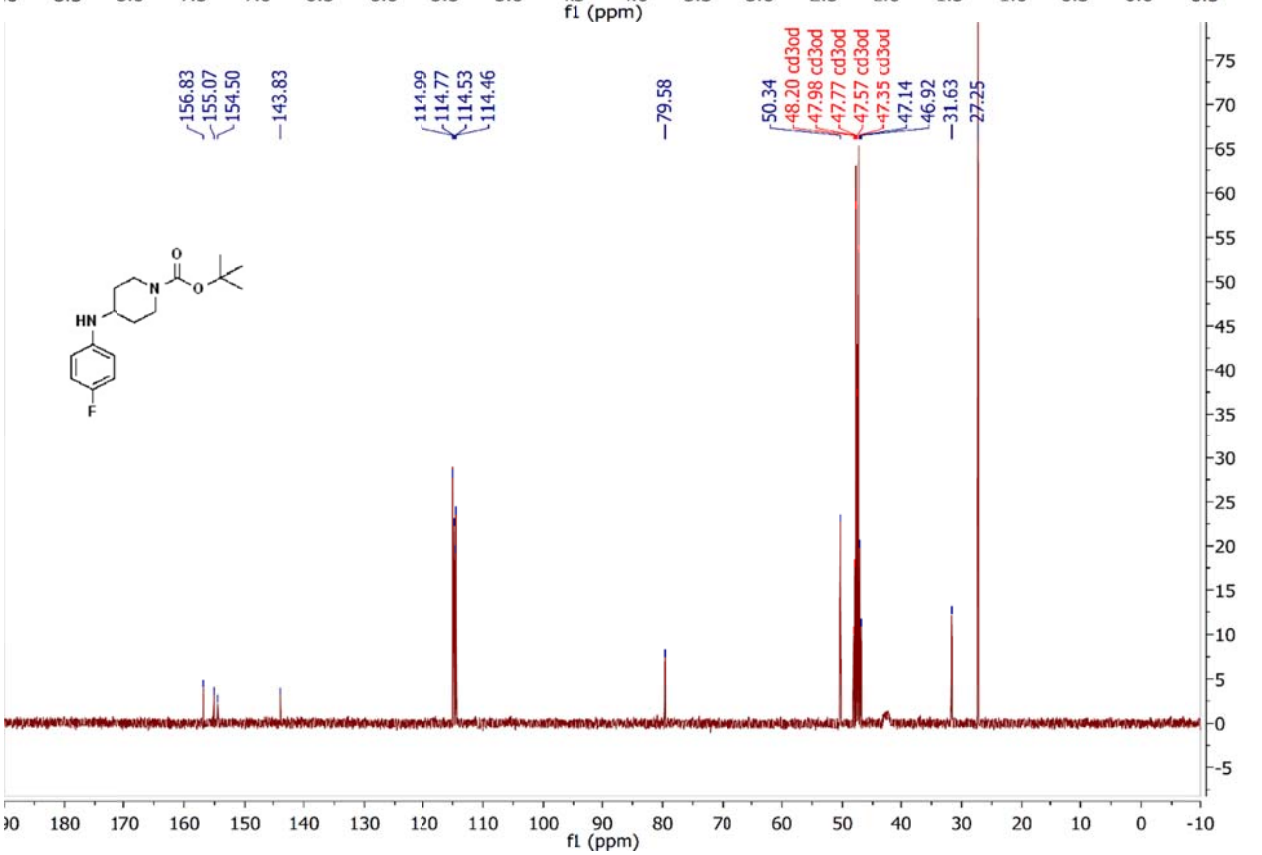
^{1}H NMR and ^{13}C NMR of compound 2.30



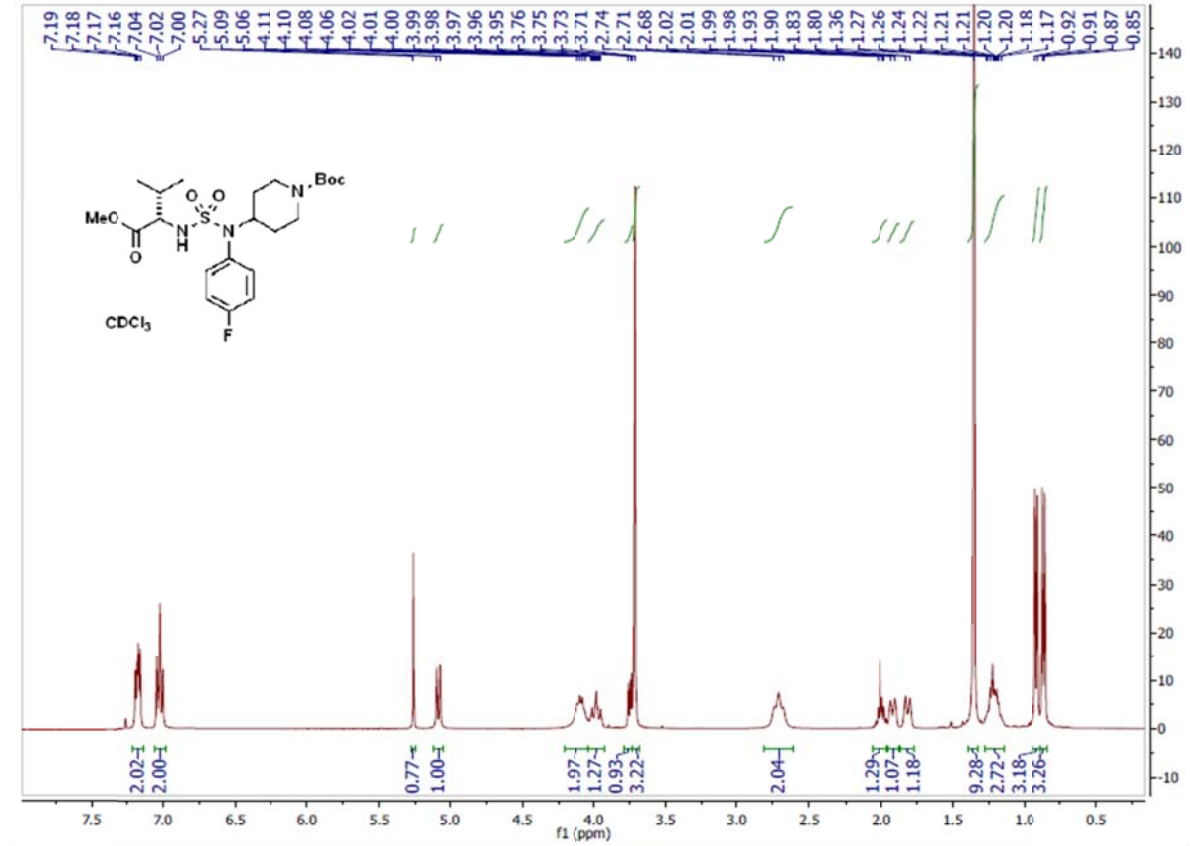


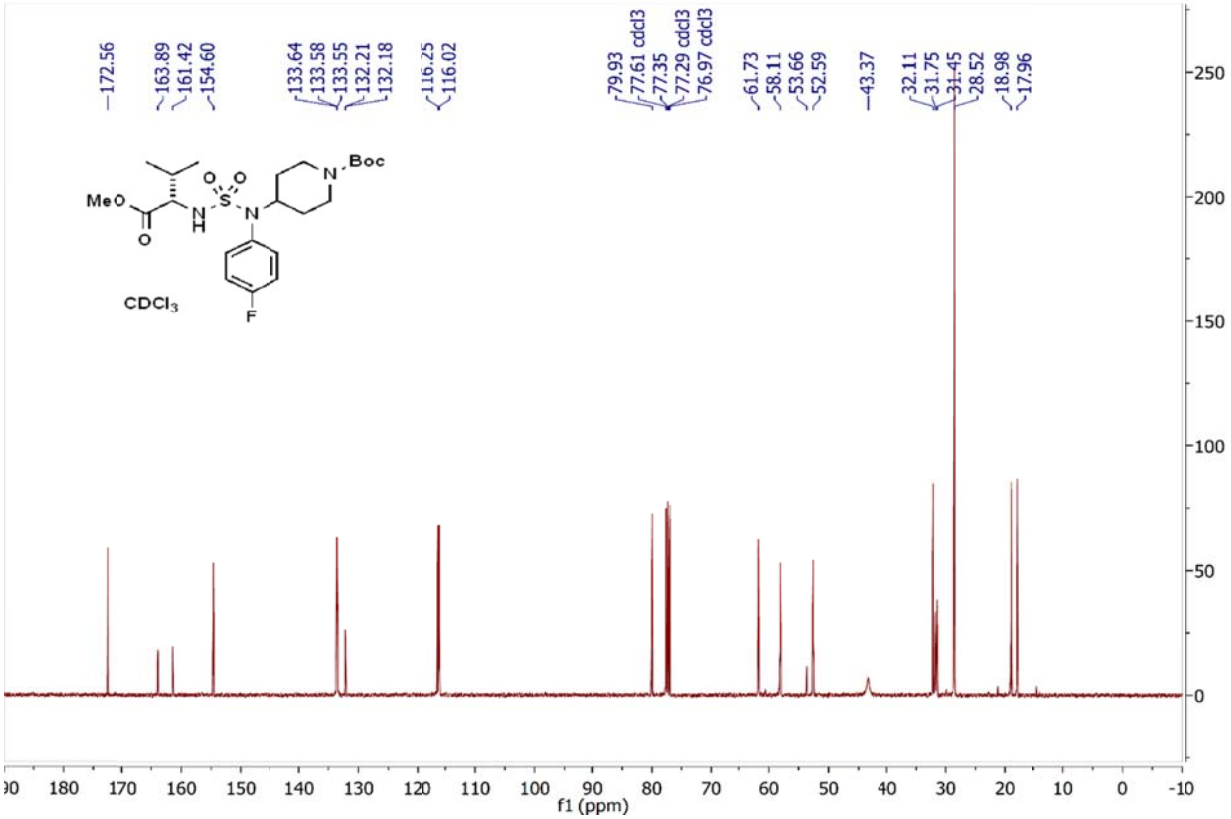
^{1}H NMR and ^{13}C NMR of compound 2.31



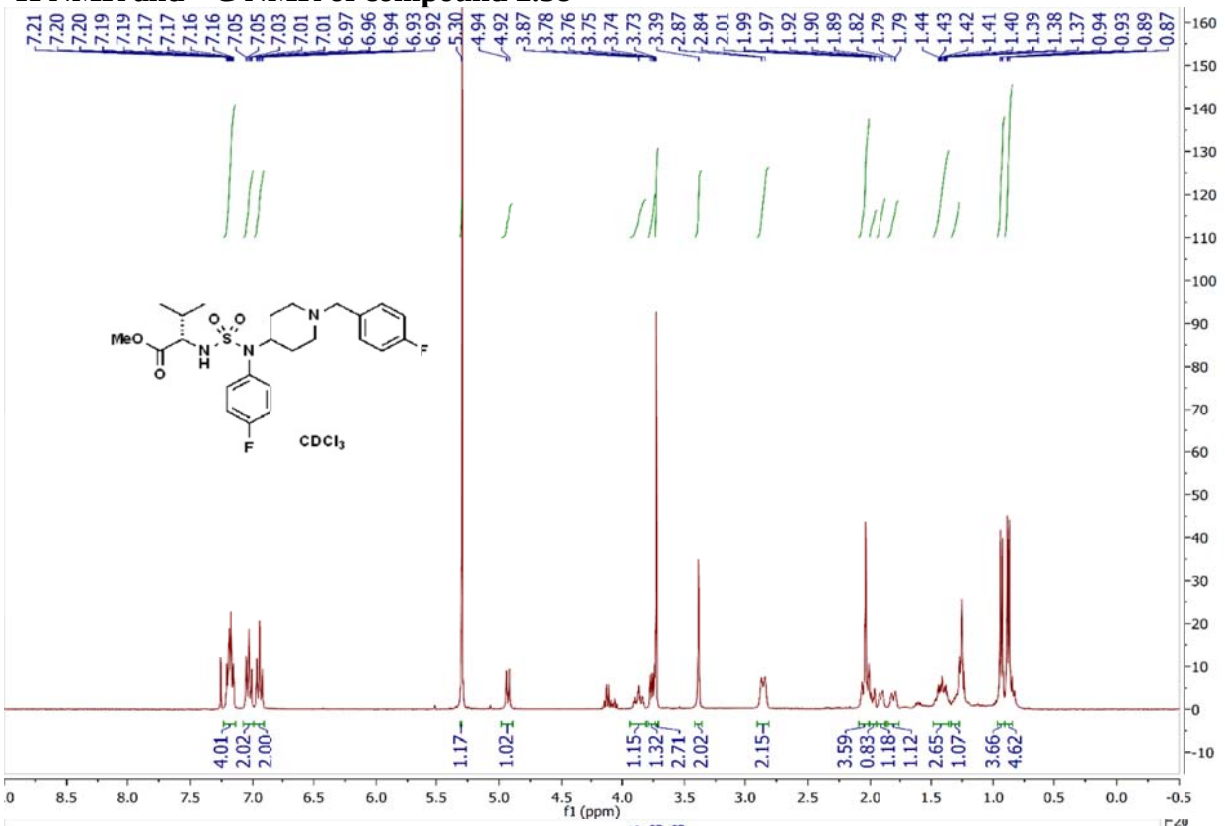


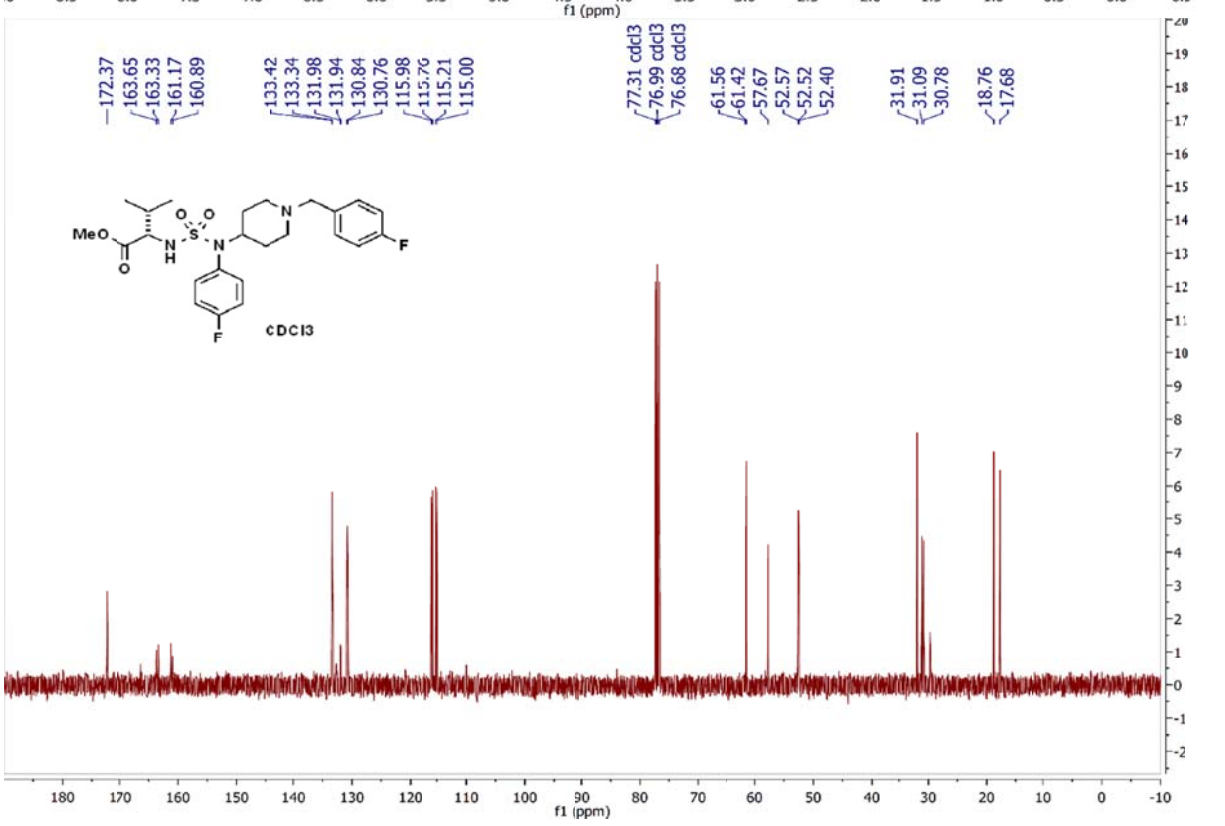
^{1}H NMR and ^{13}C NMR of compound 2.34



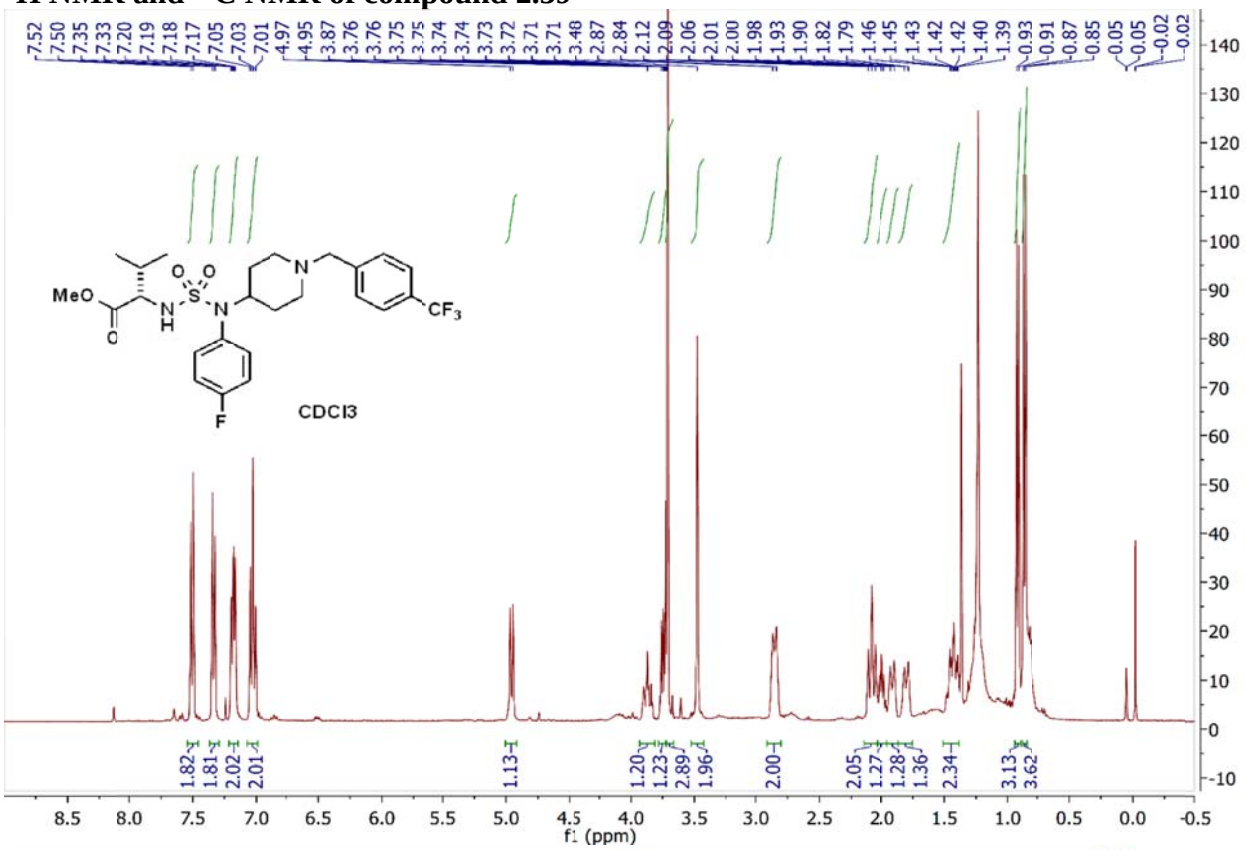


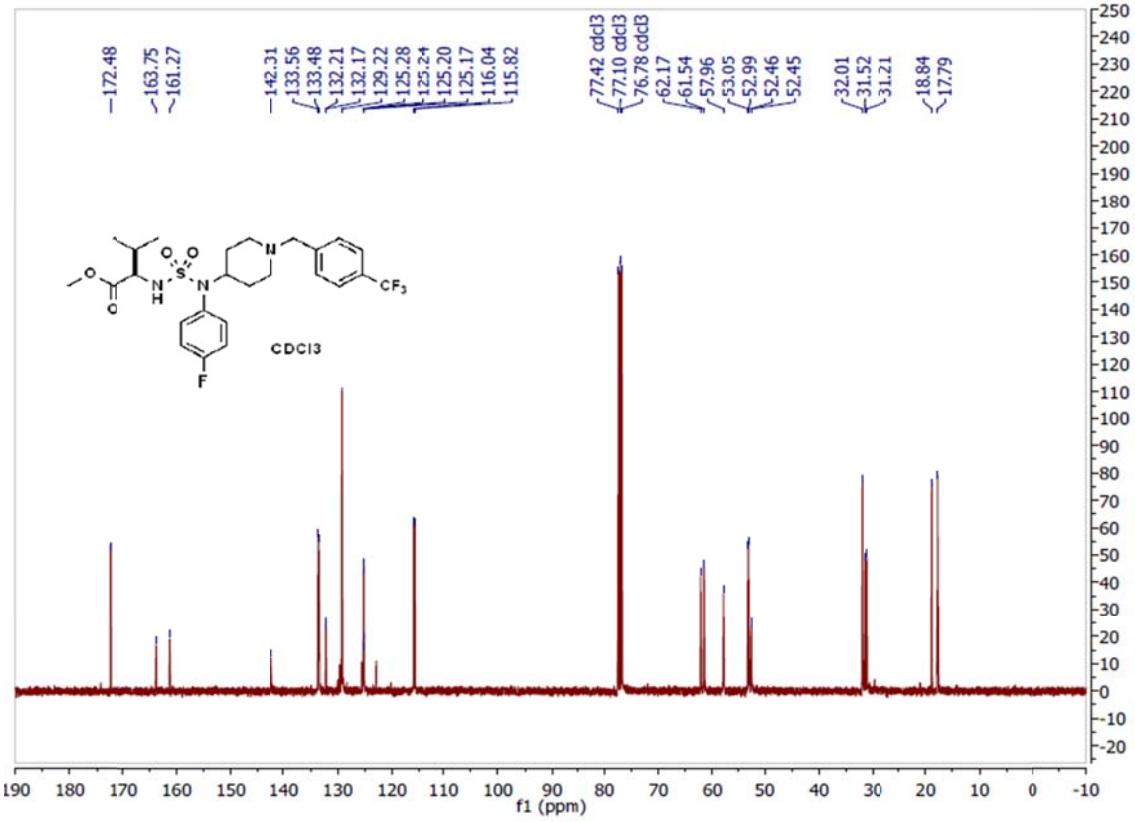
^{1}H NMR and ^{13}C NMR of compound 2.38



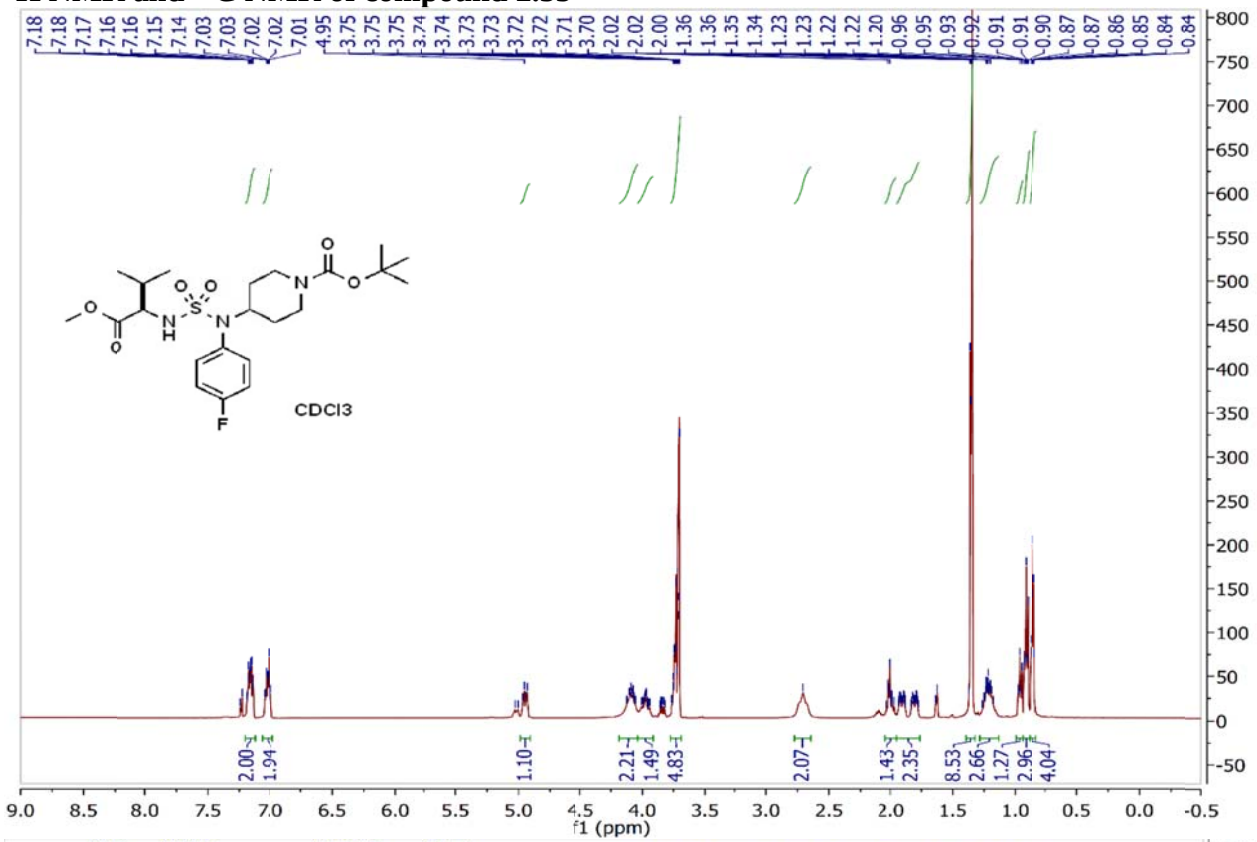


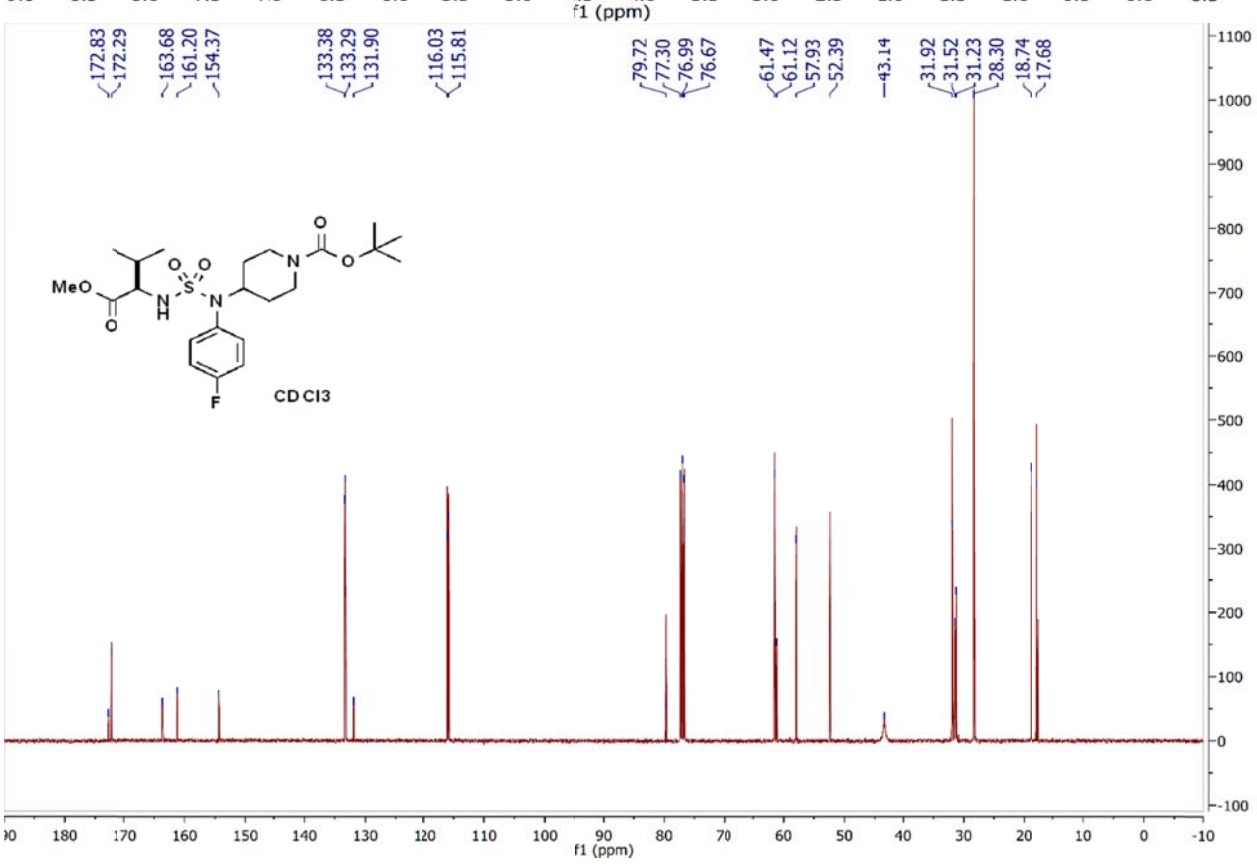
^{1}H NMR and ^{13}C NMR of compound 2.39



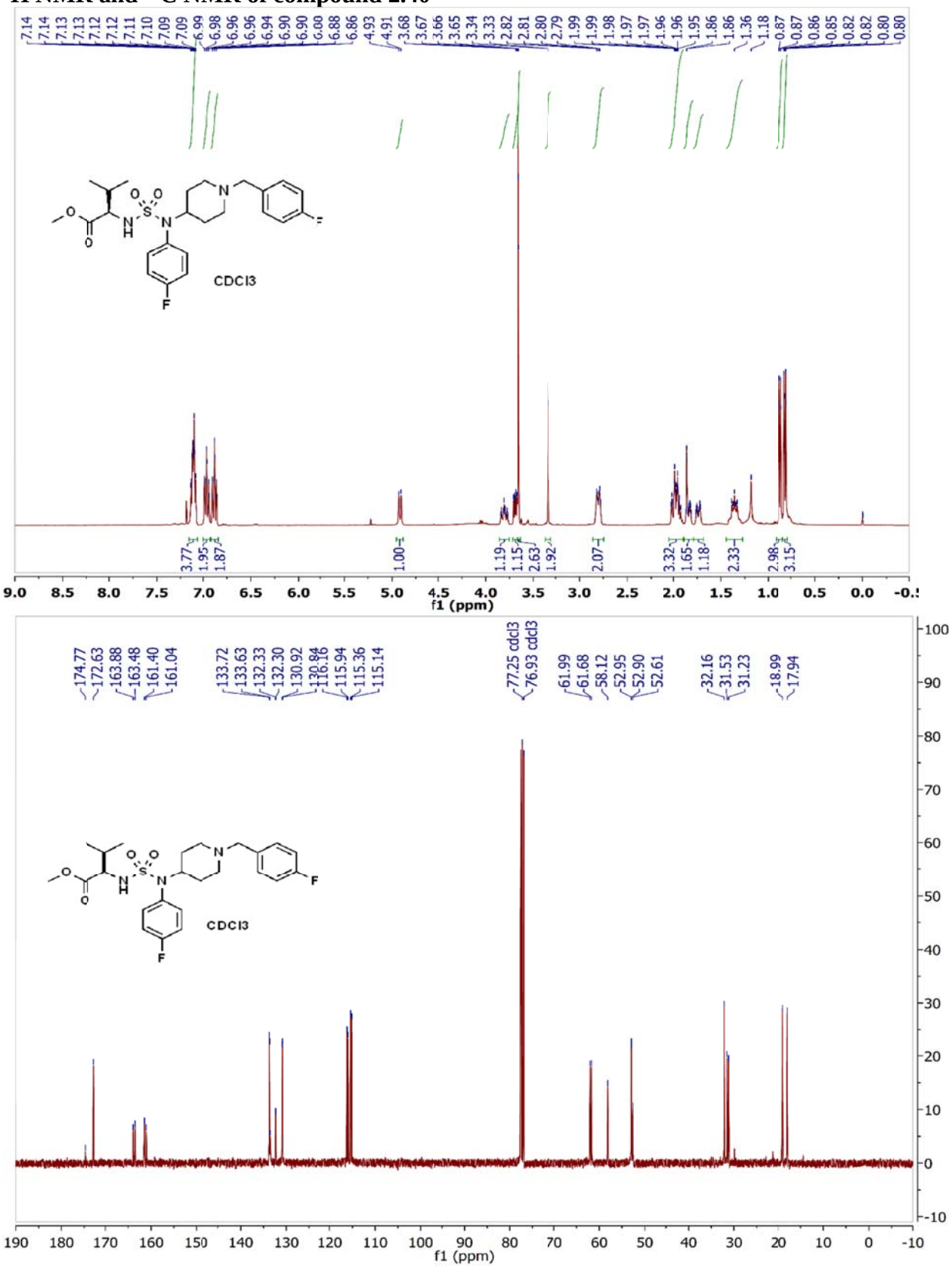


^{1}H NMR and ^{13}C NMR of compound 2.35

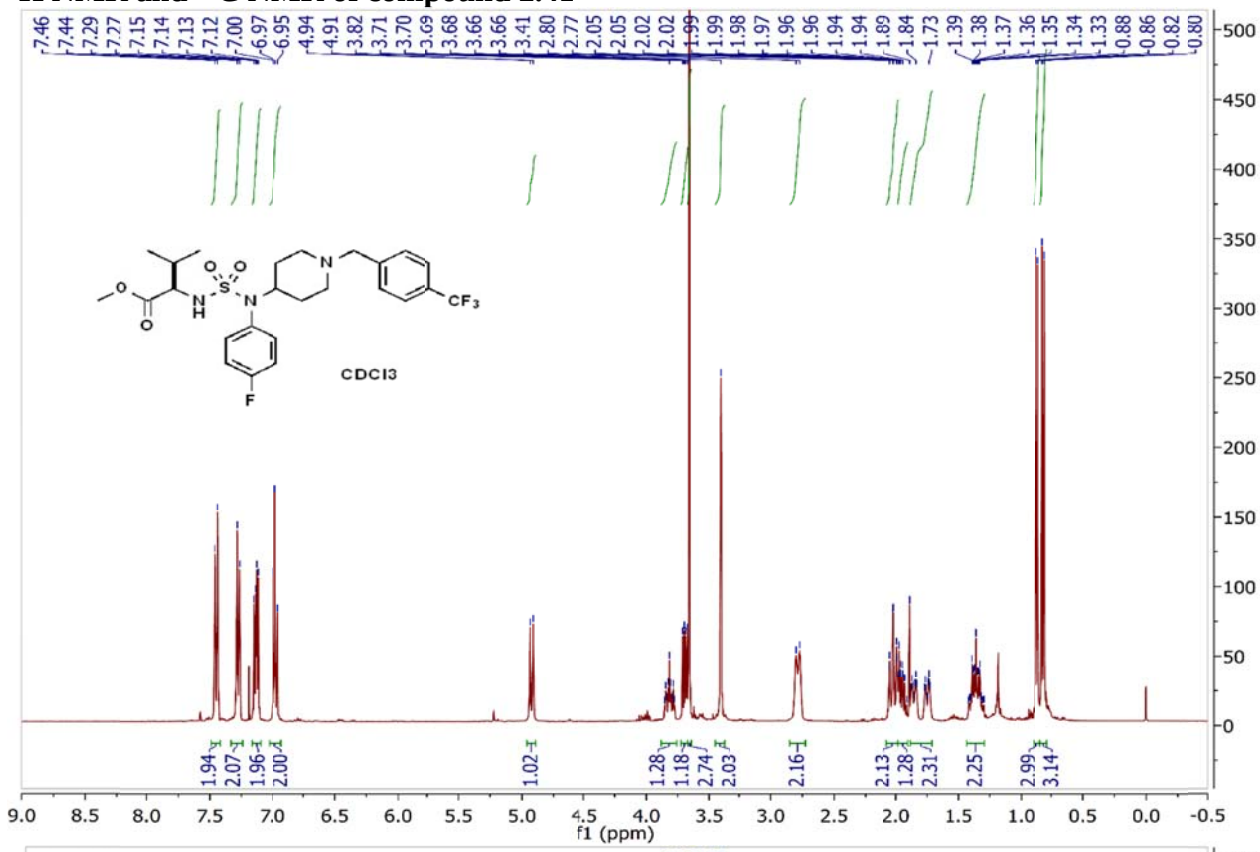


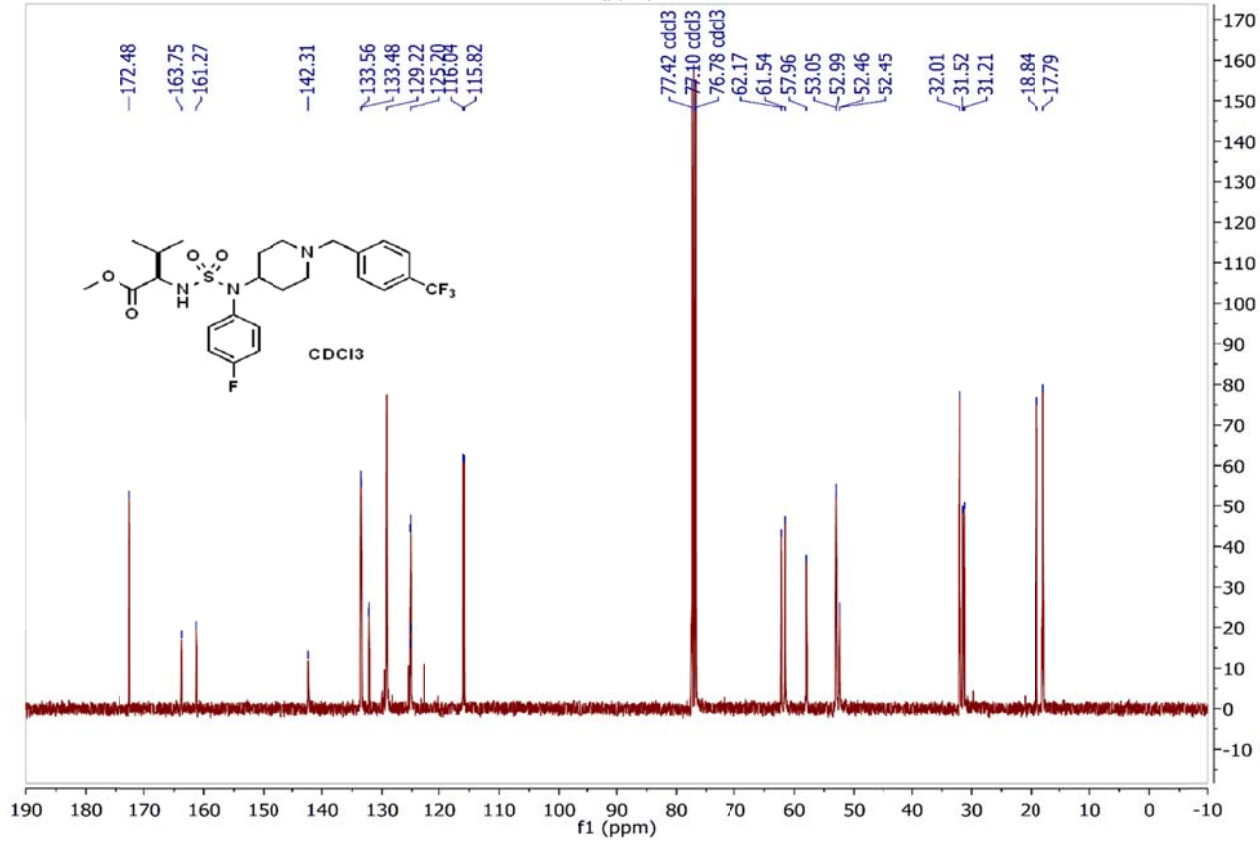


^{1}H NMR and ^{13}C NMR of compound 2.40

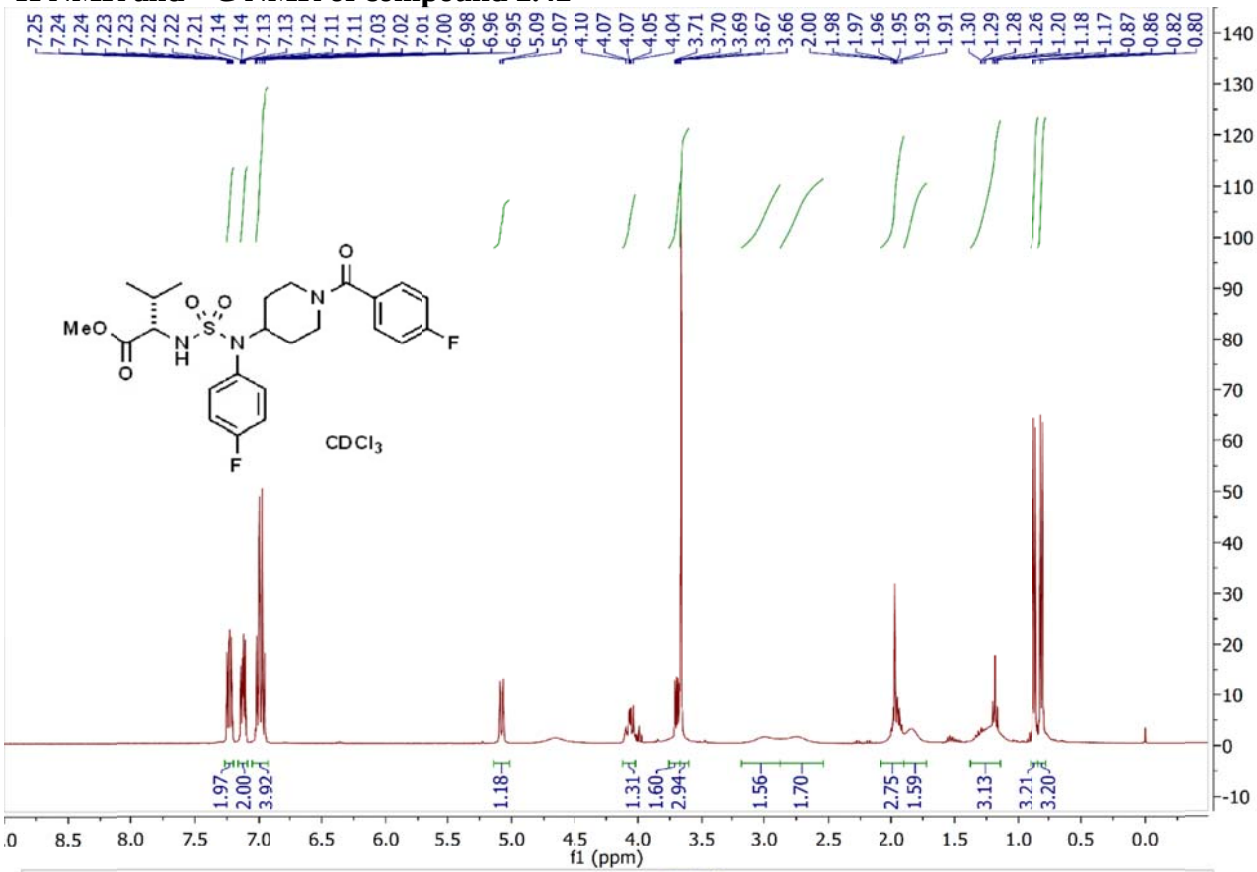


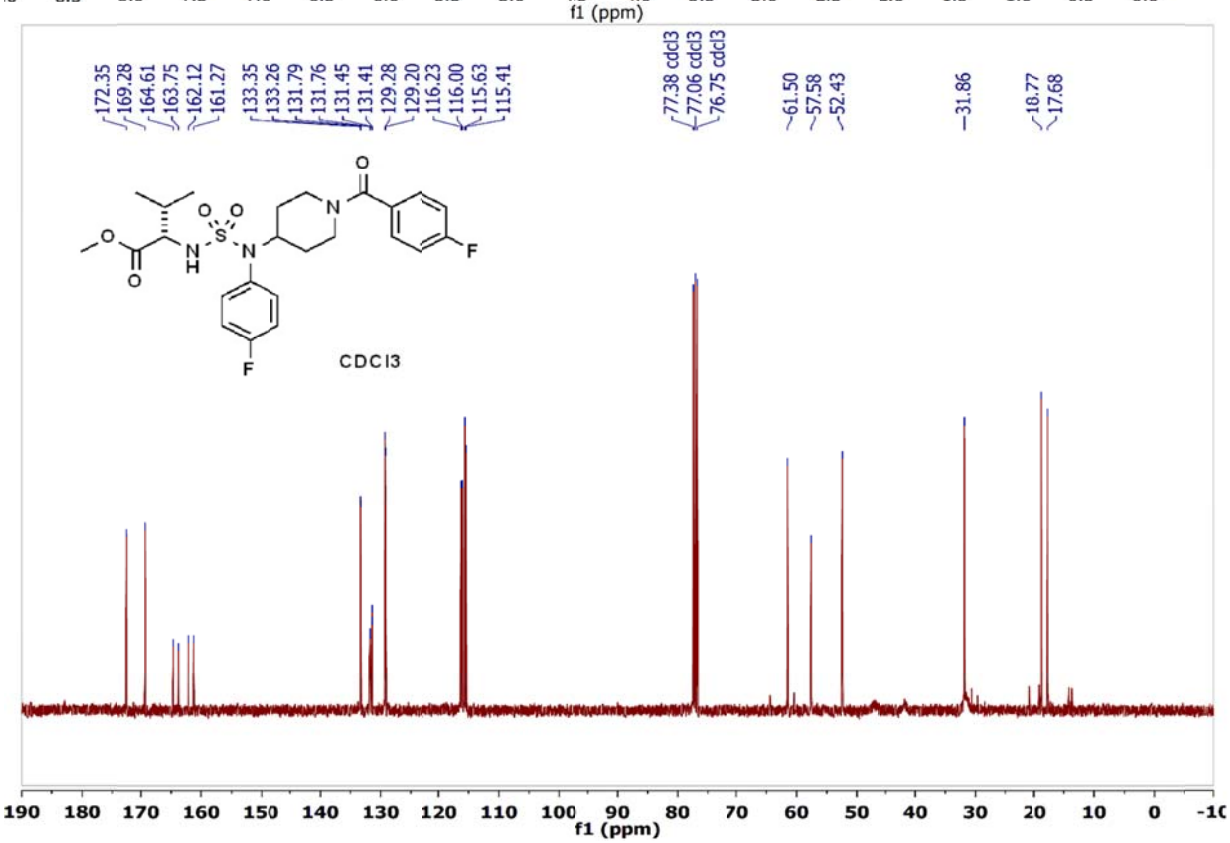
^{1}H NMR and ^{13}C NMR of compound 2.41



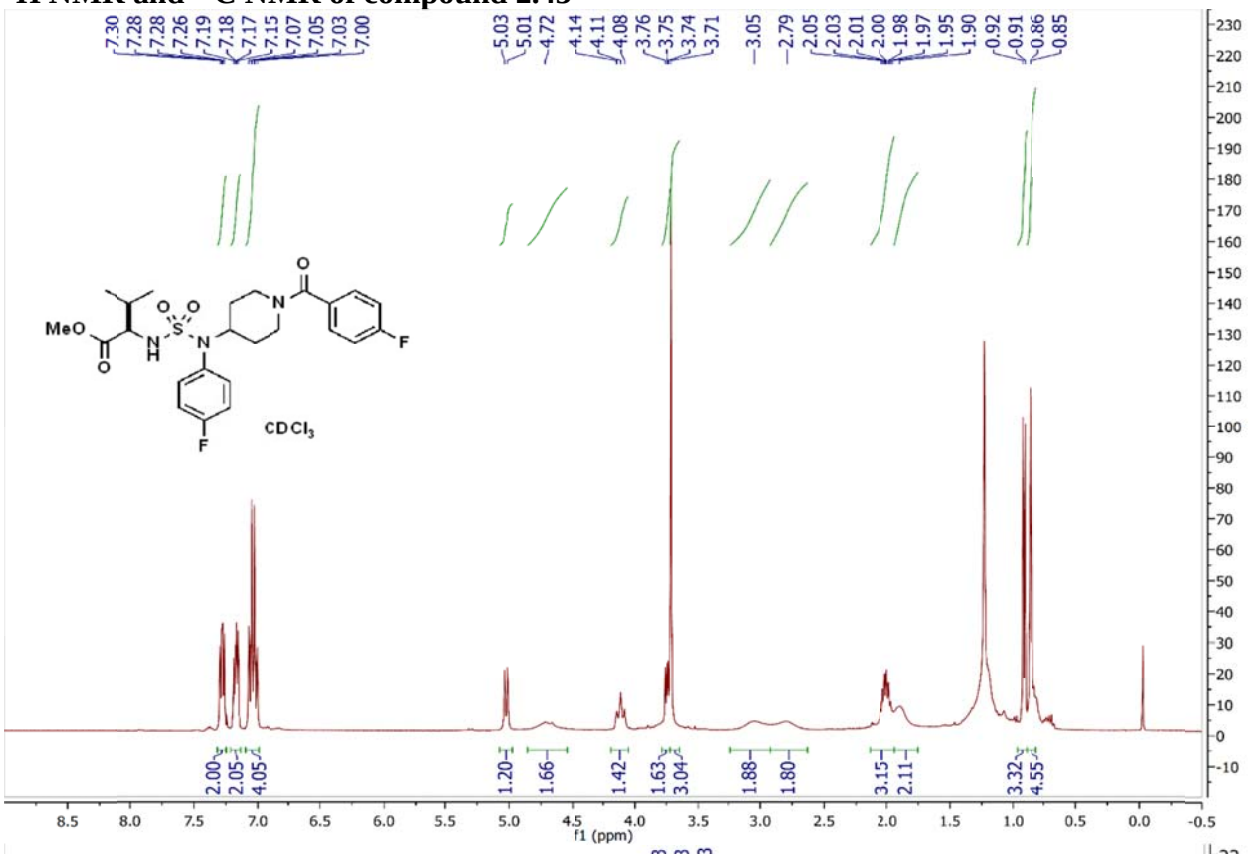


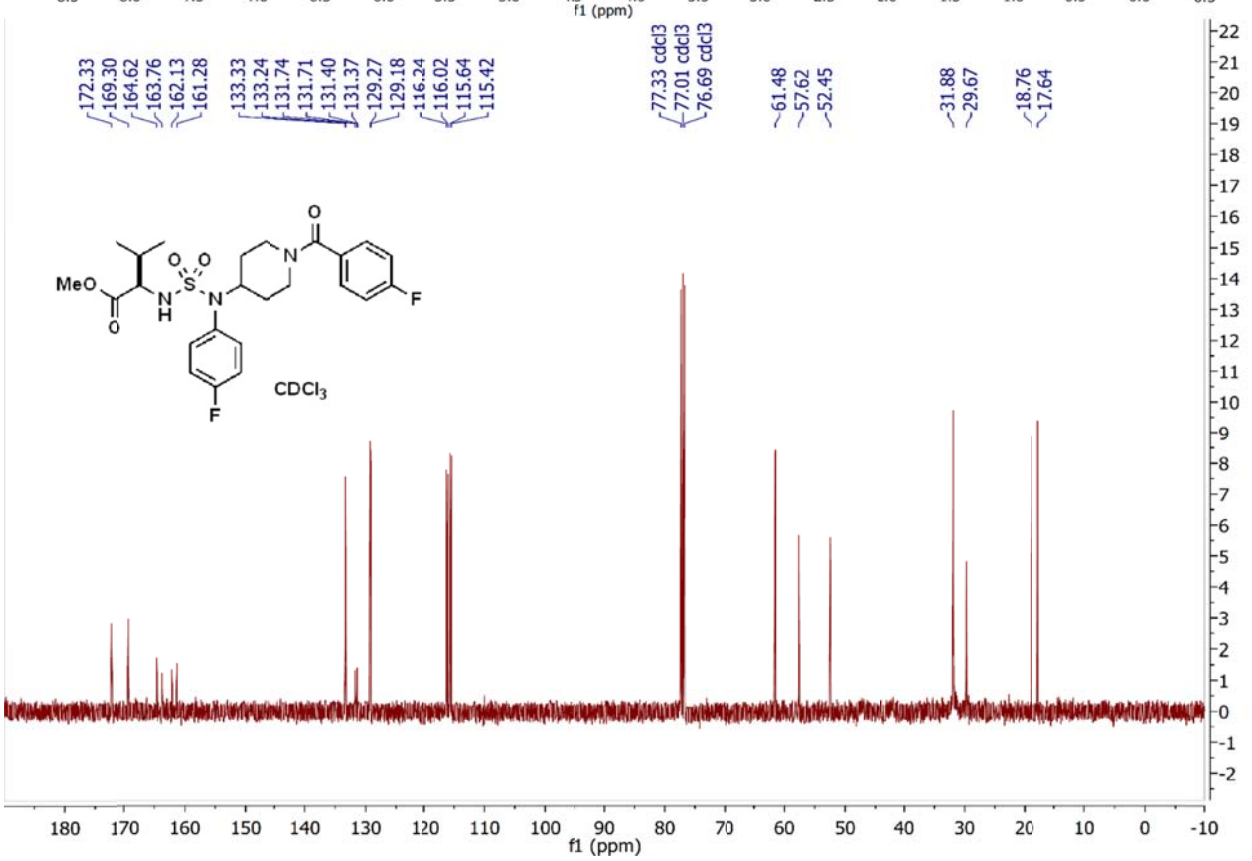
^{1}H NMR and ^{13}C NMR of compound 2.42



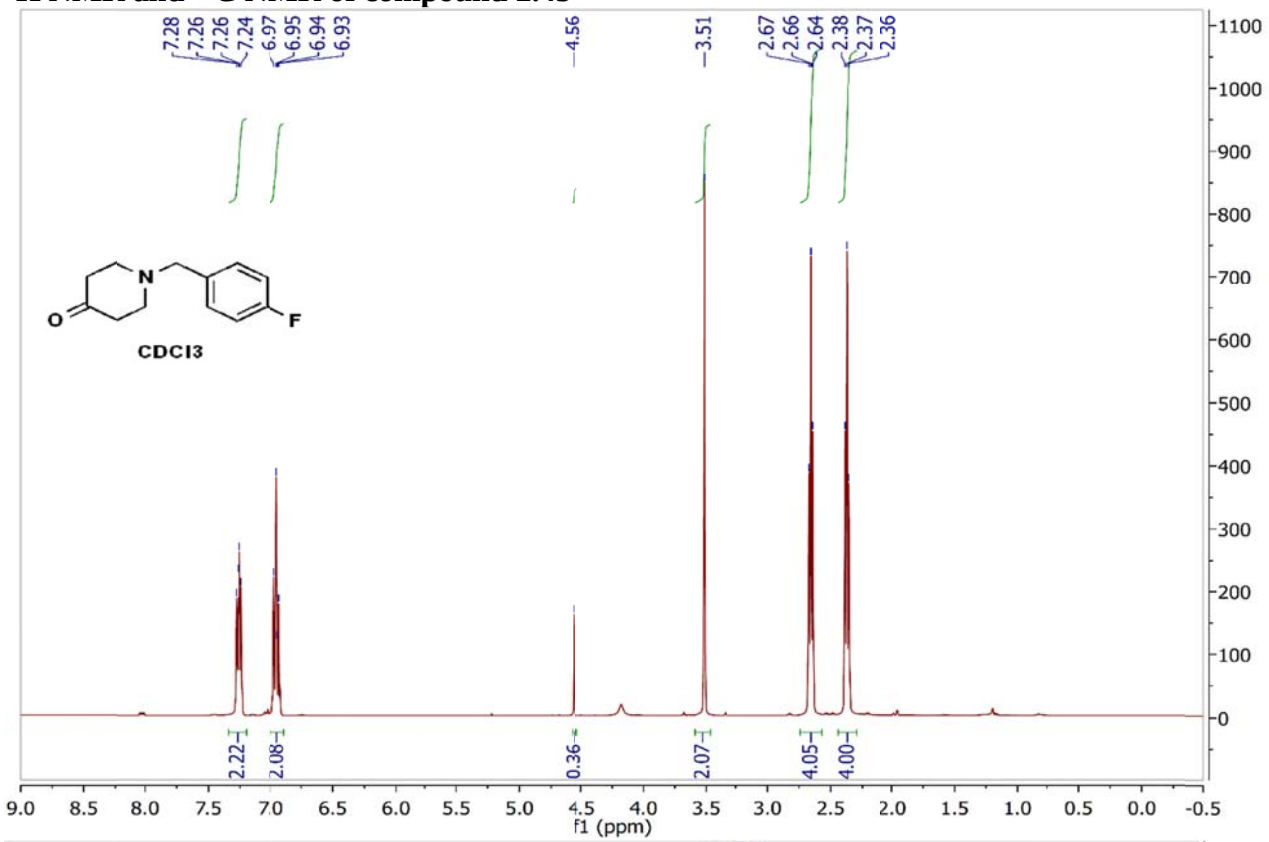


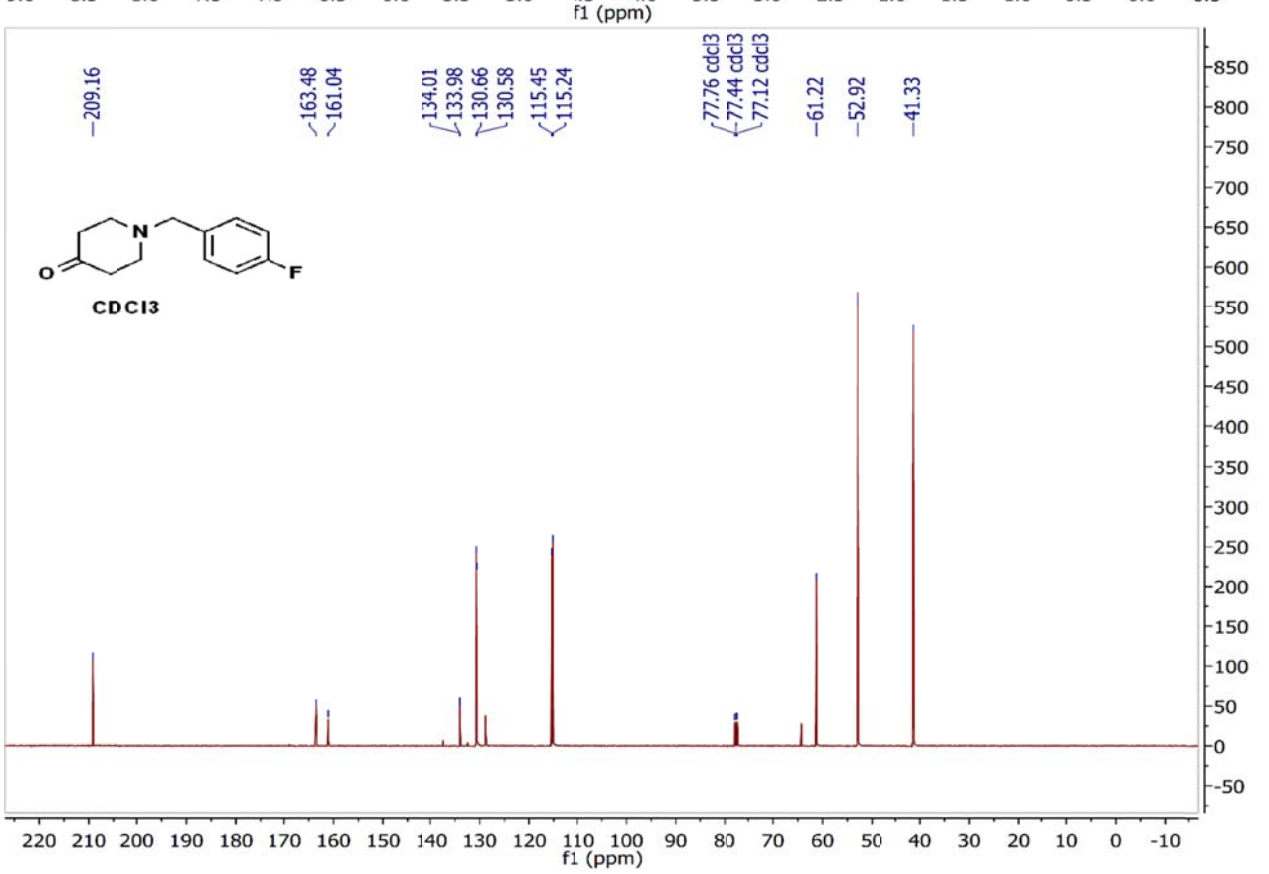
^{1}H NMR and ^{13}C NMR of compound 2.43



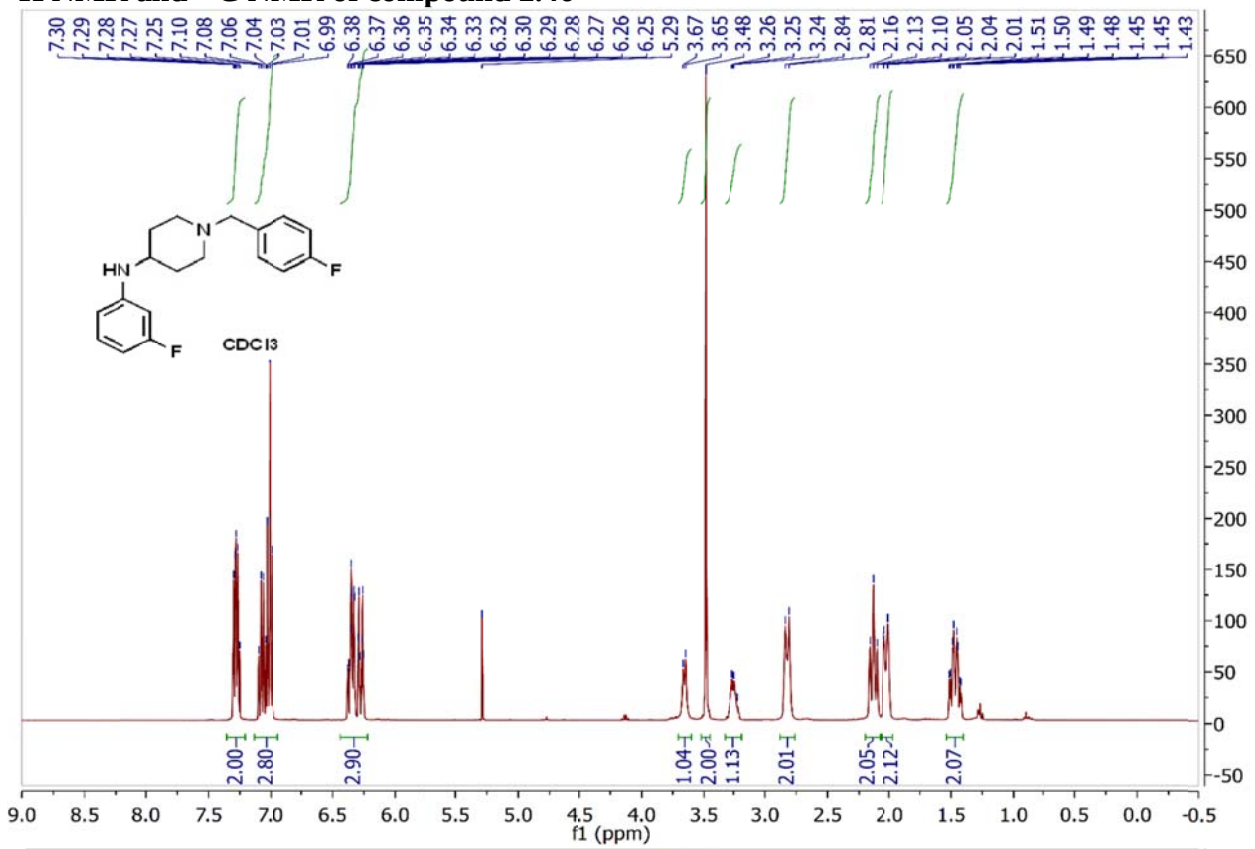


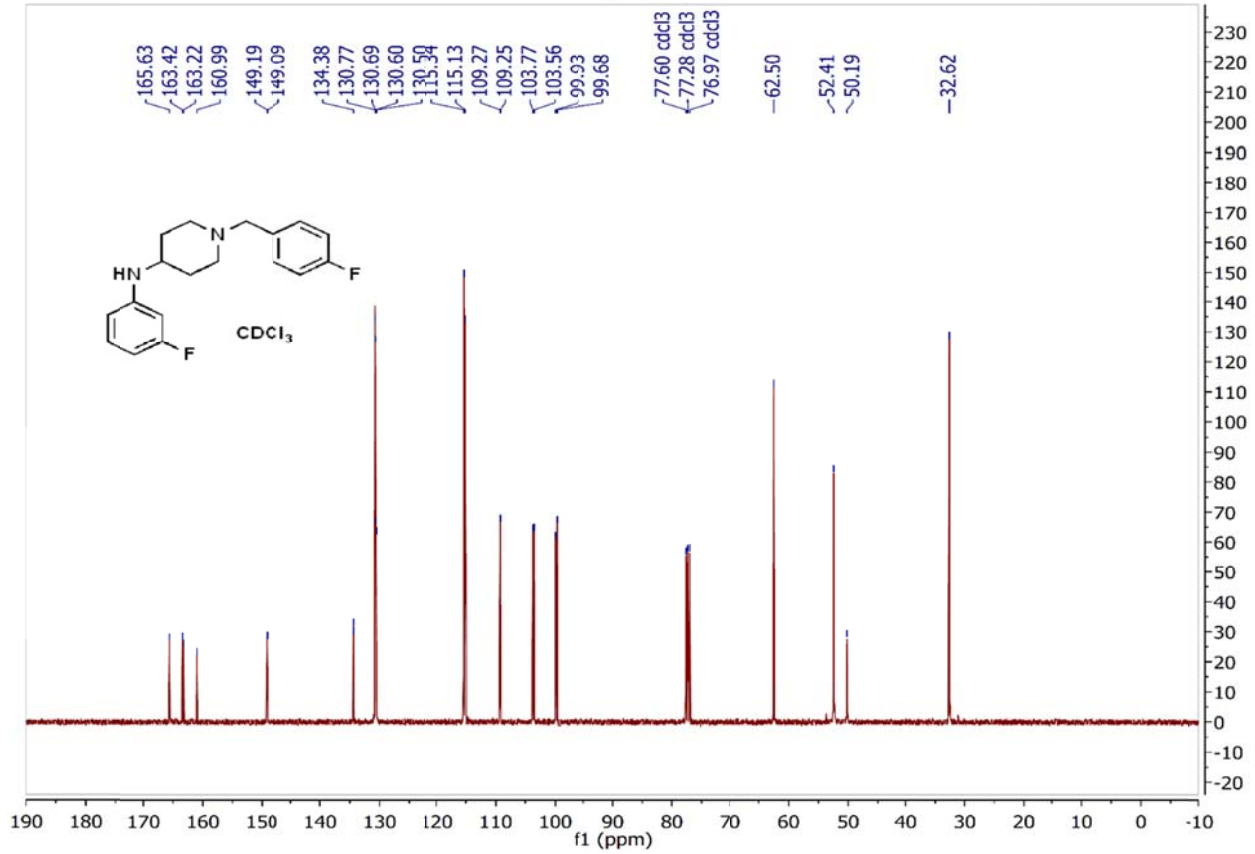
^{1}H NMR and ^{13}C NMR of compound 2.45



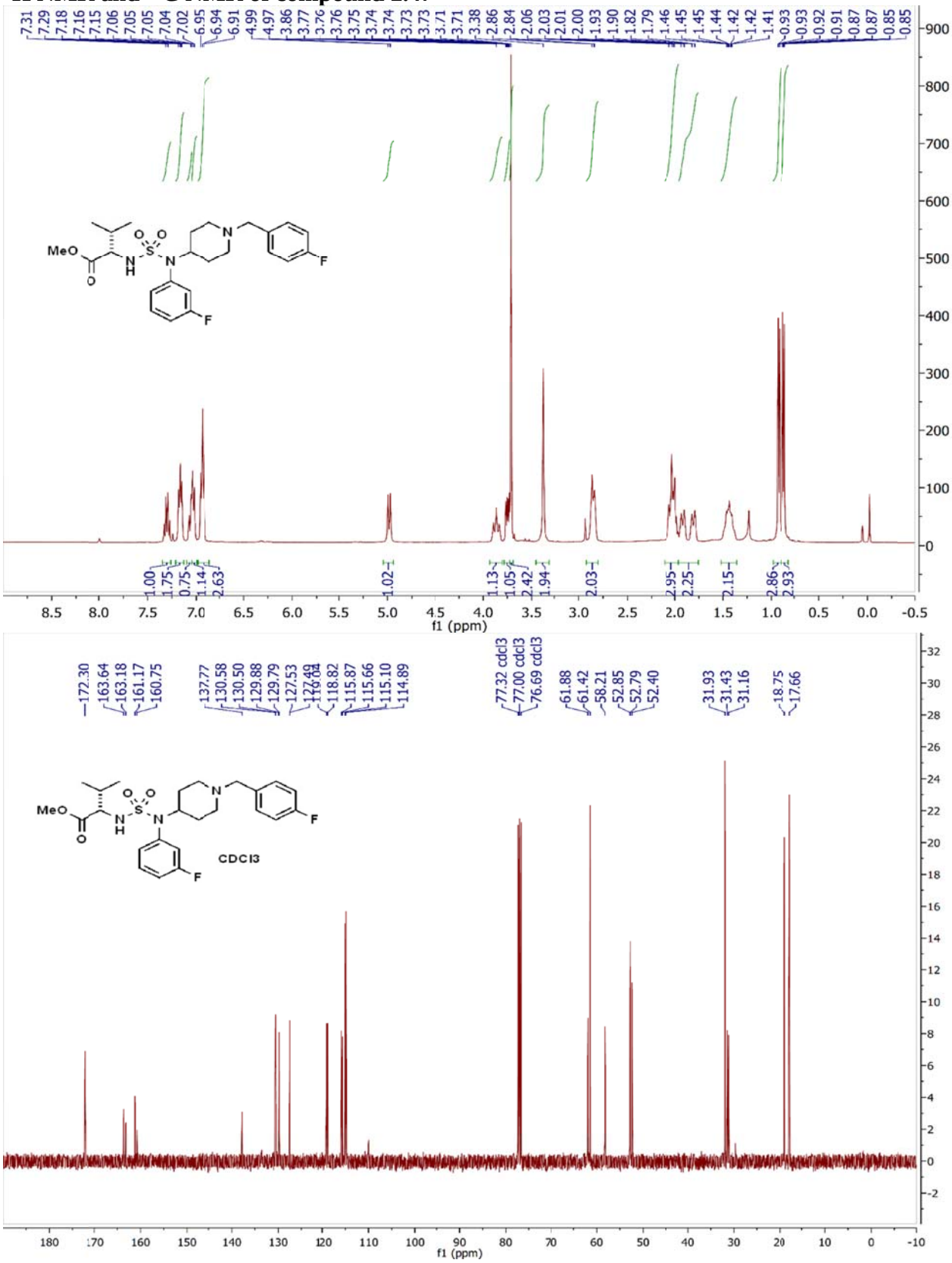


^{1}H NMR and ^{13}C NMR of compound 2.46

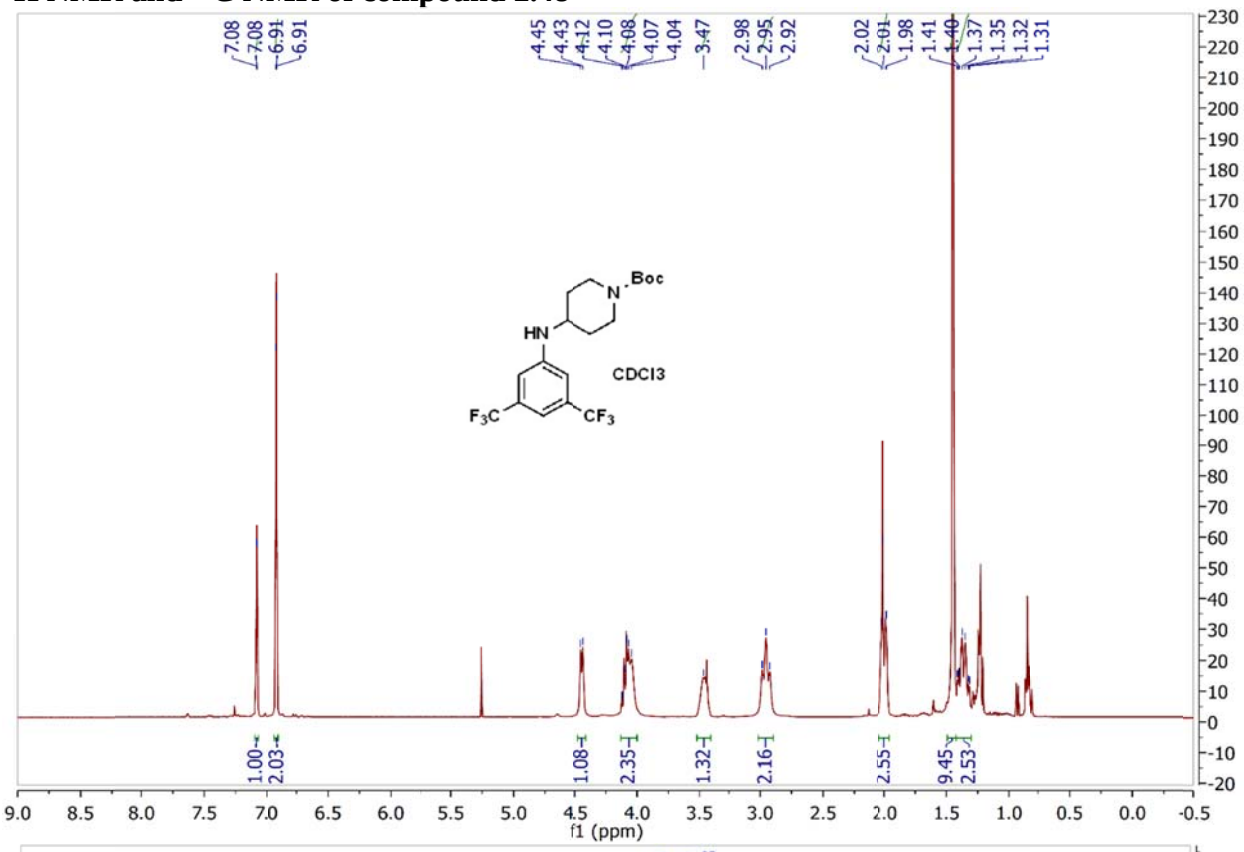


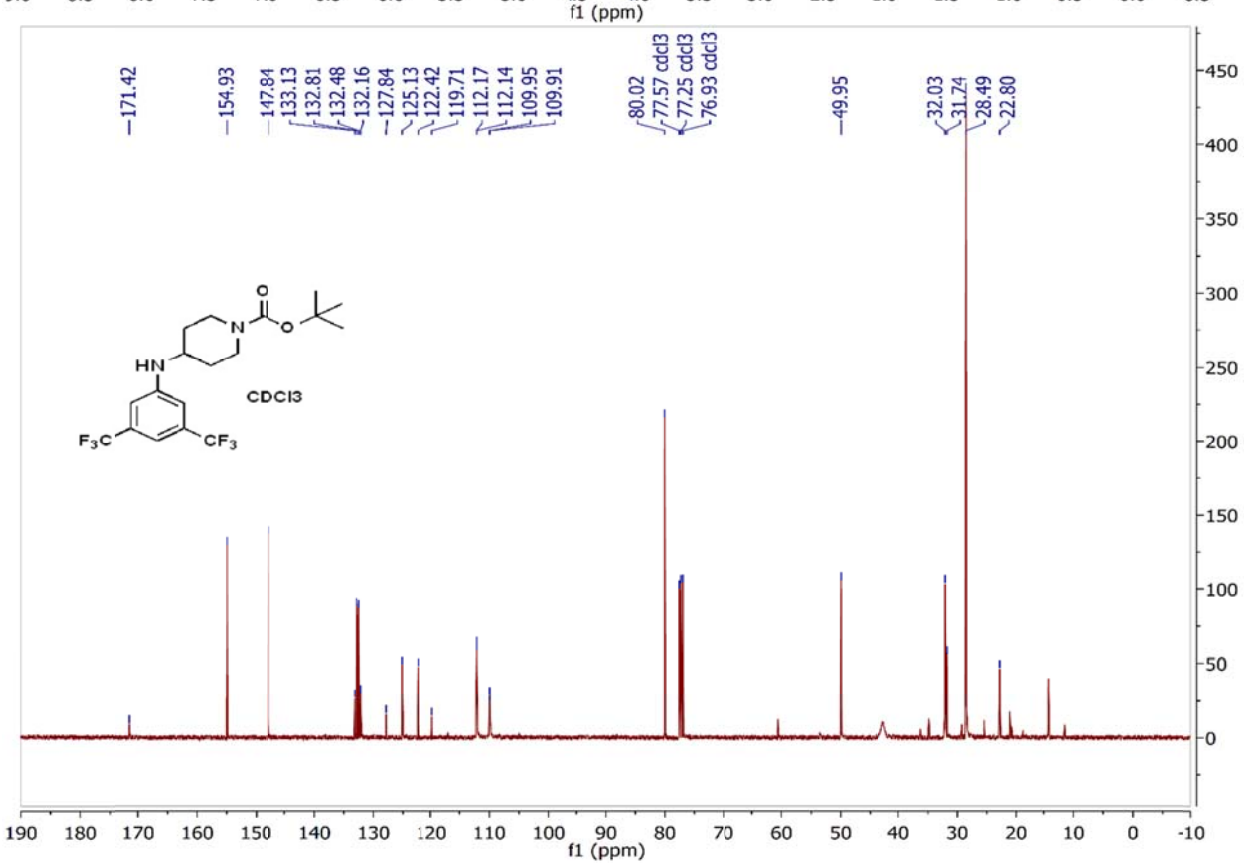


^{1}H NMR and ^{13}C NMR of compound 2.47

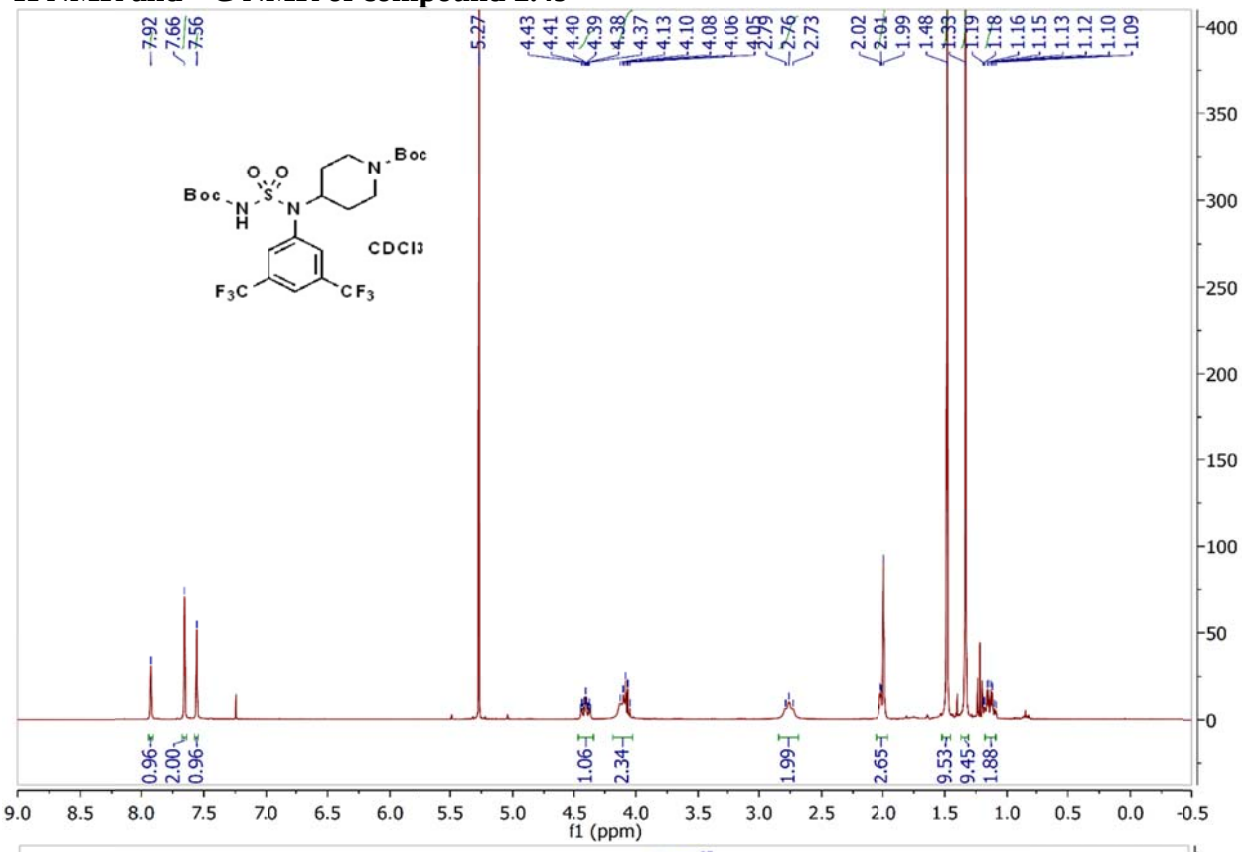


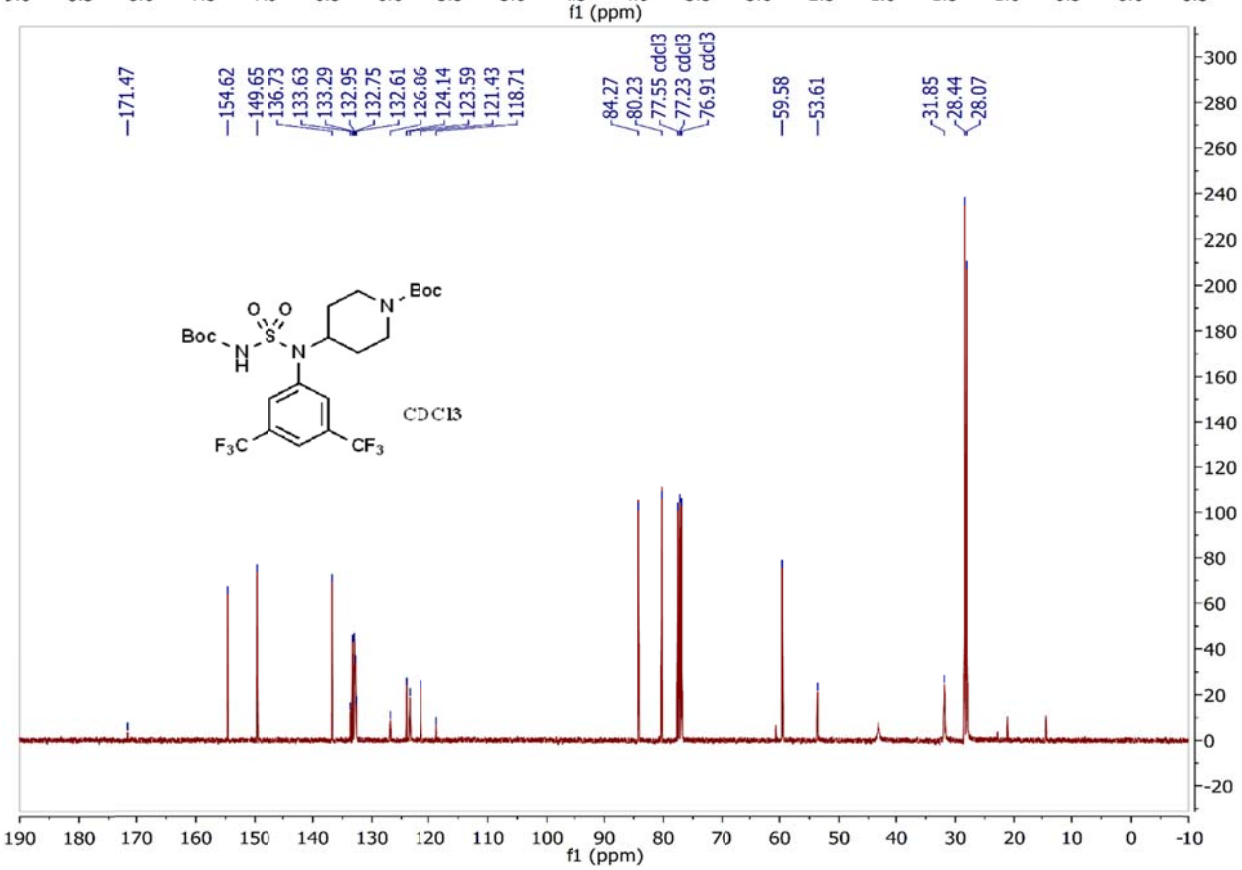
^{1}H NMR and ^{13}C NMR of compound 2.48



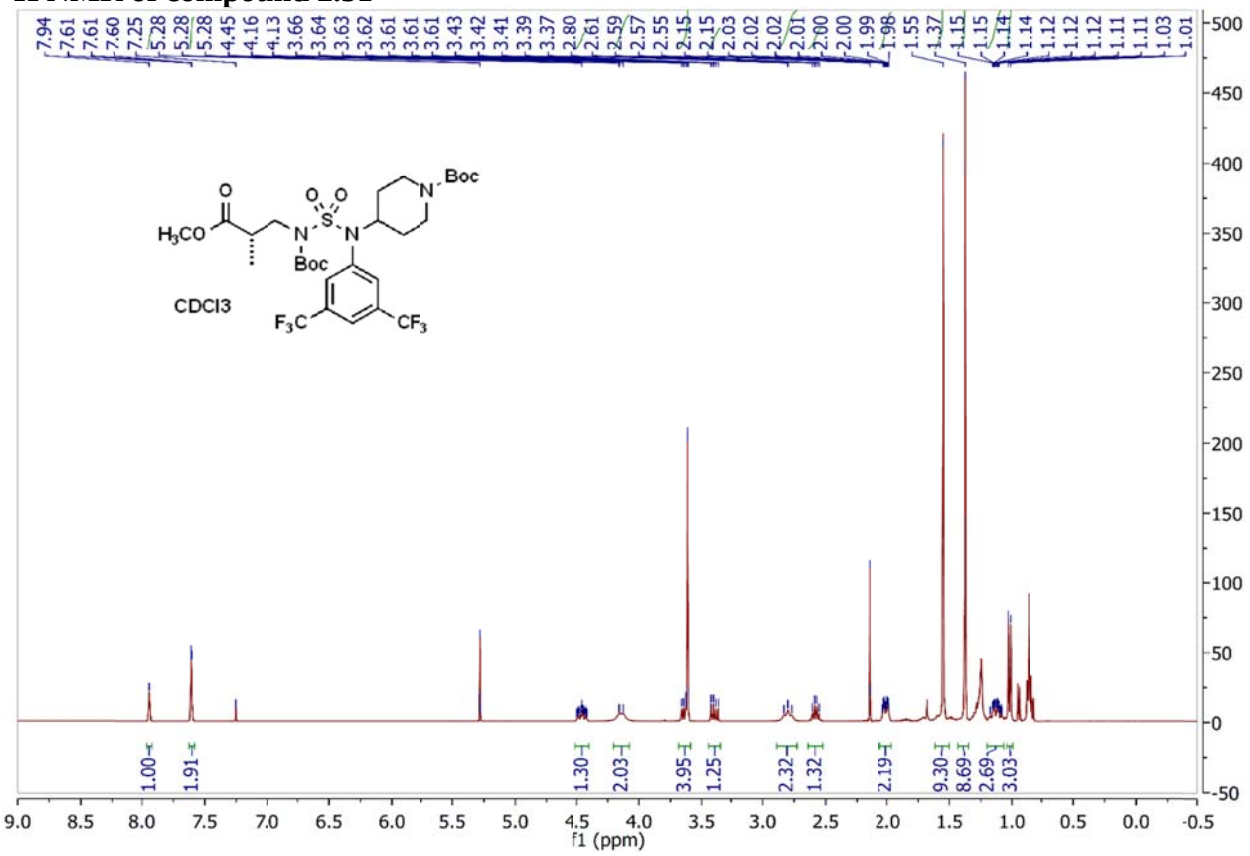


^{1}H NMR and ^{13}C NMR of compound 2.49

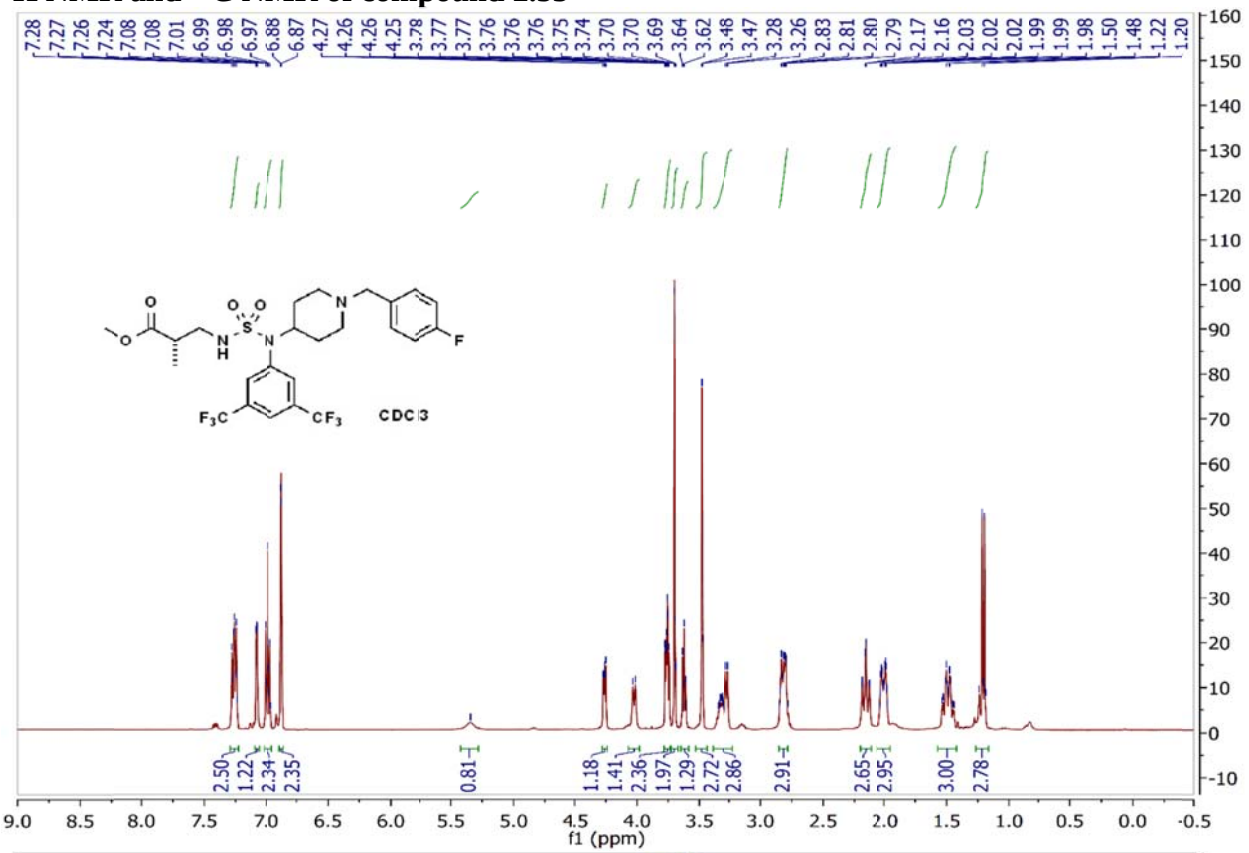


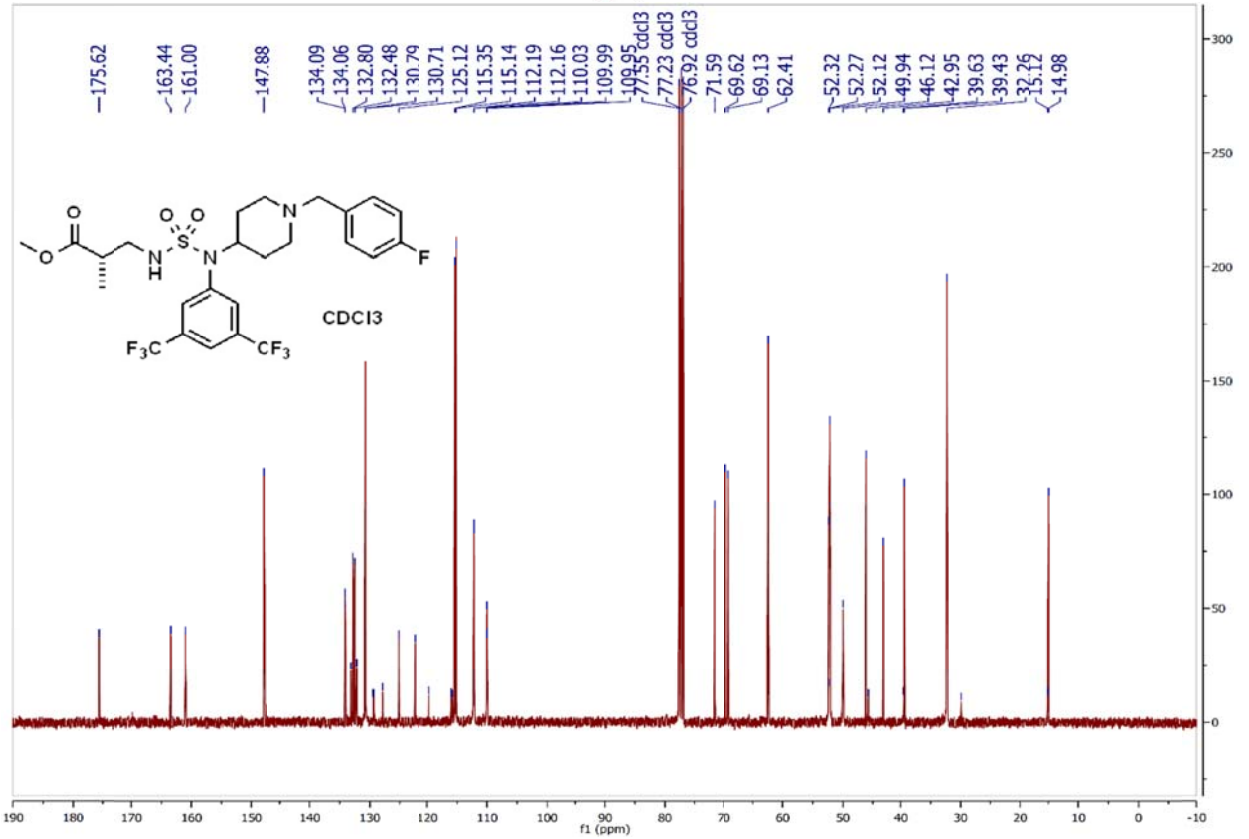


^{1}H NMR of compound 2.51

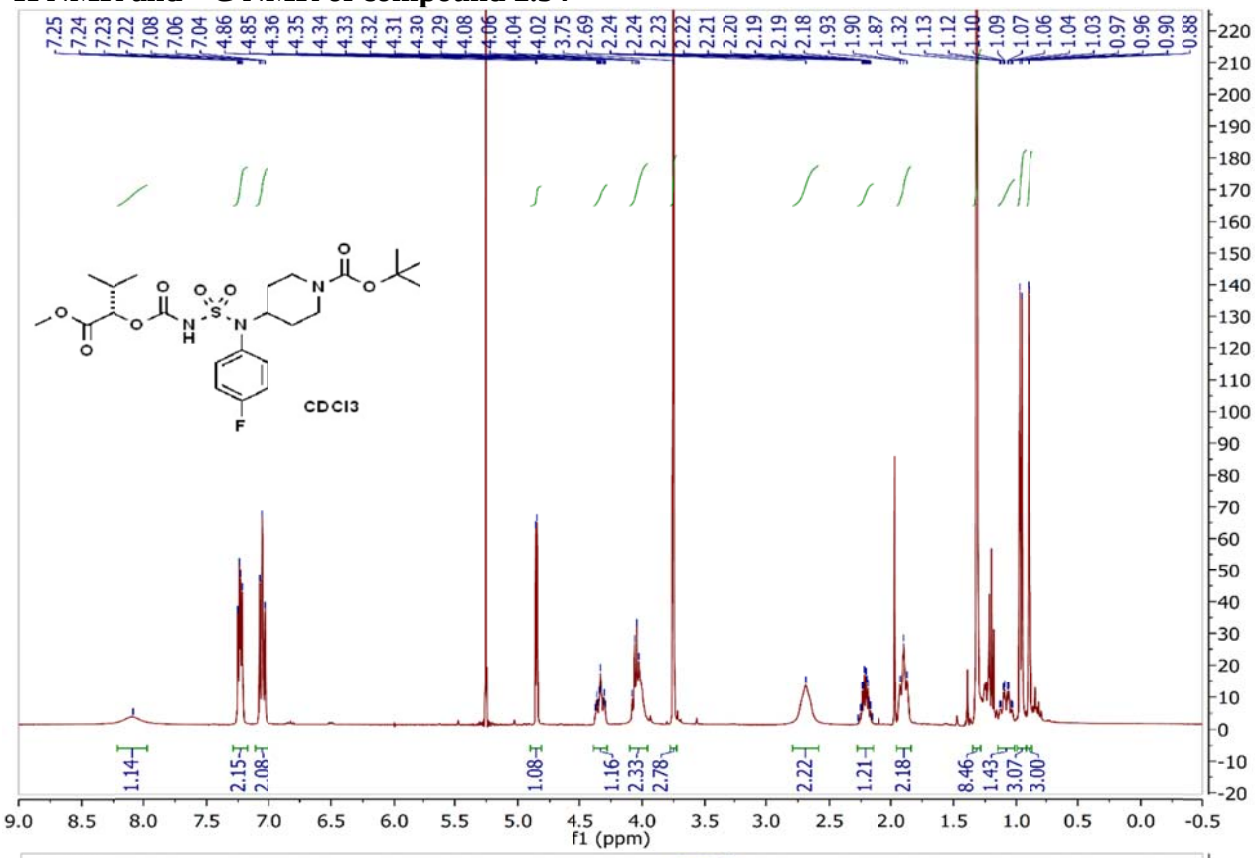


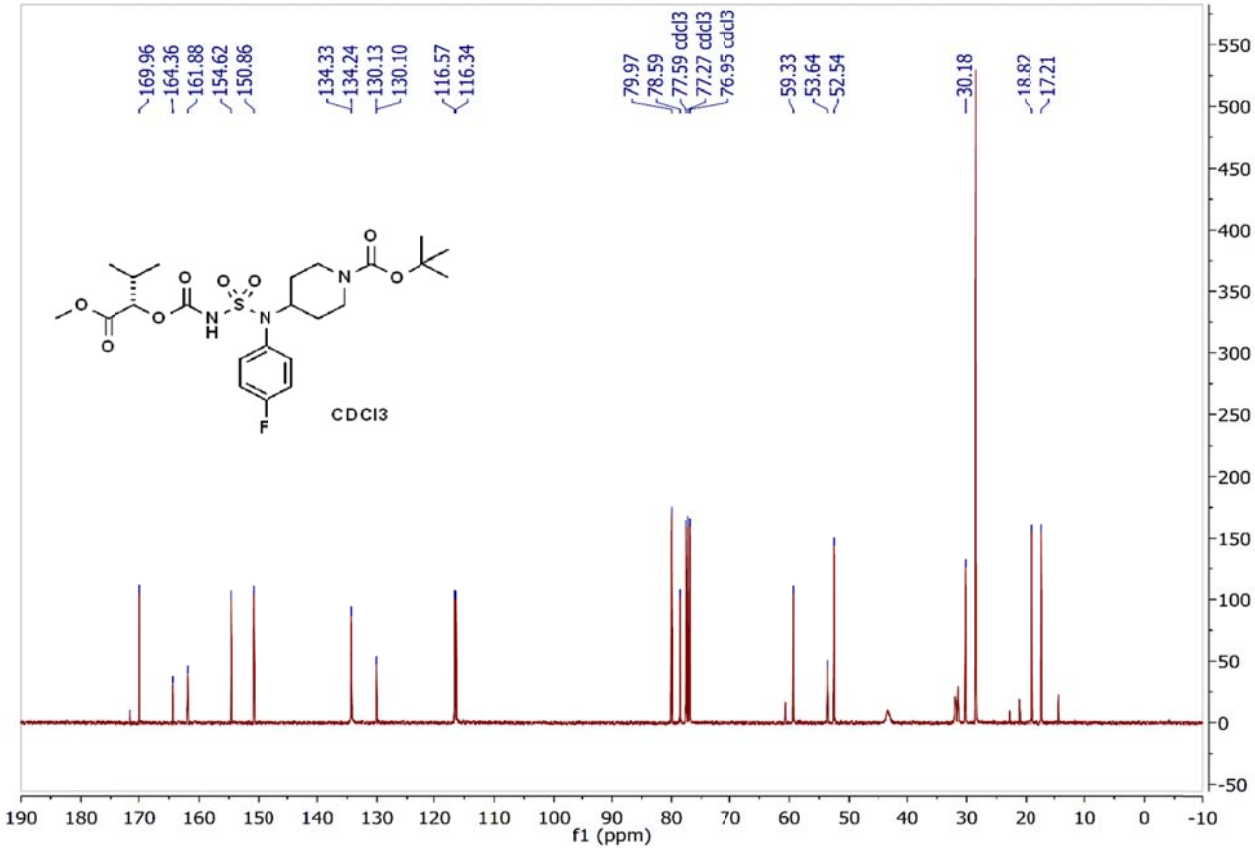
^{1}H NMR and ^{13}C NMR of compound 2.53



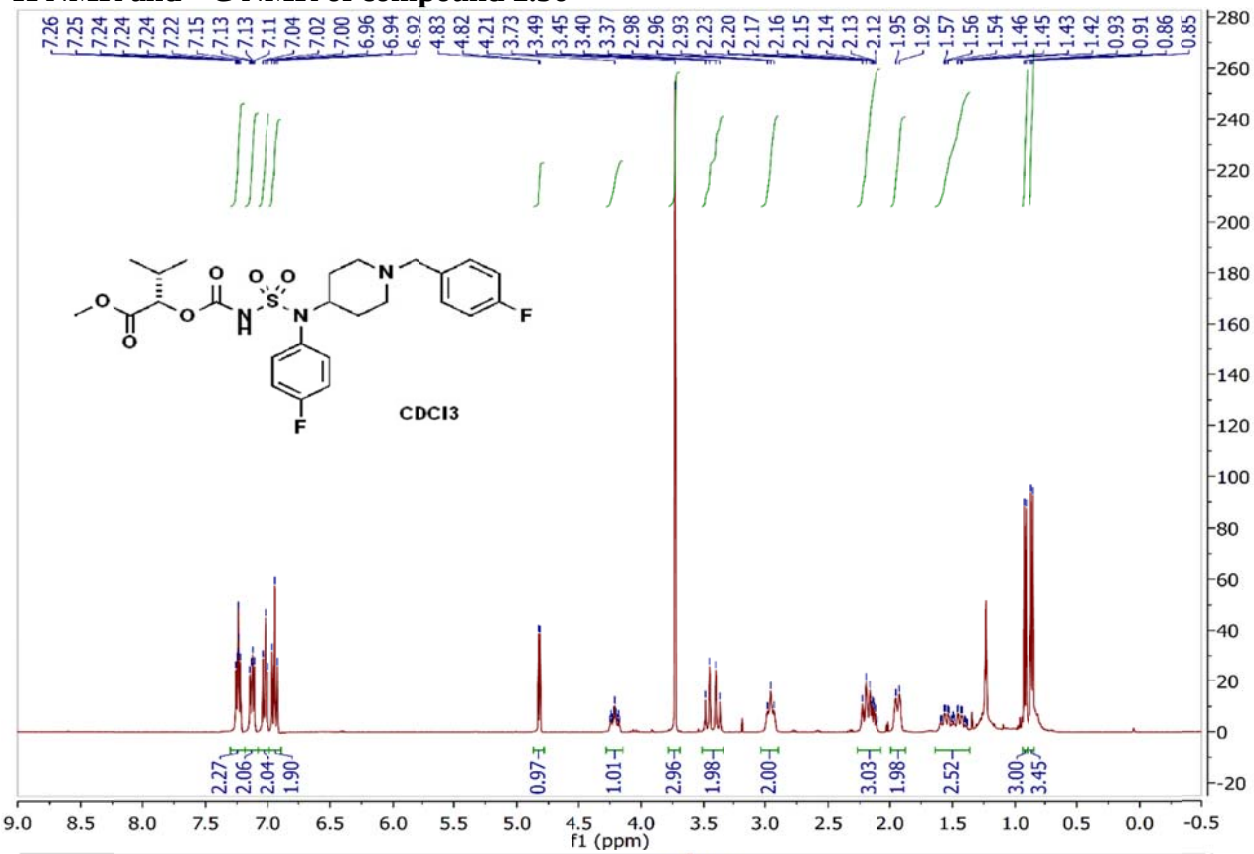


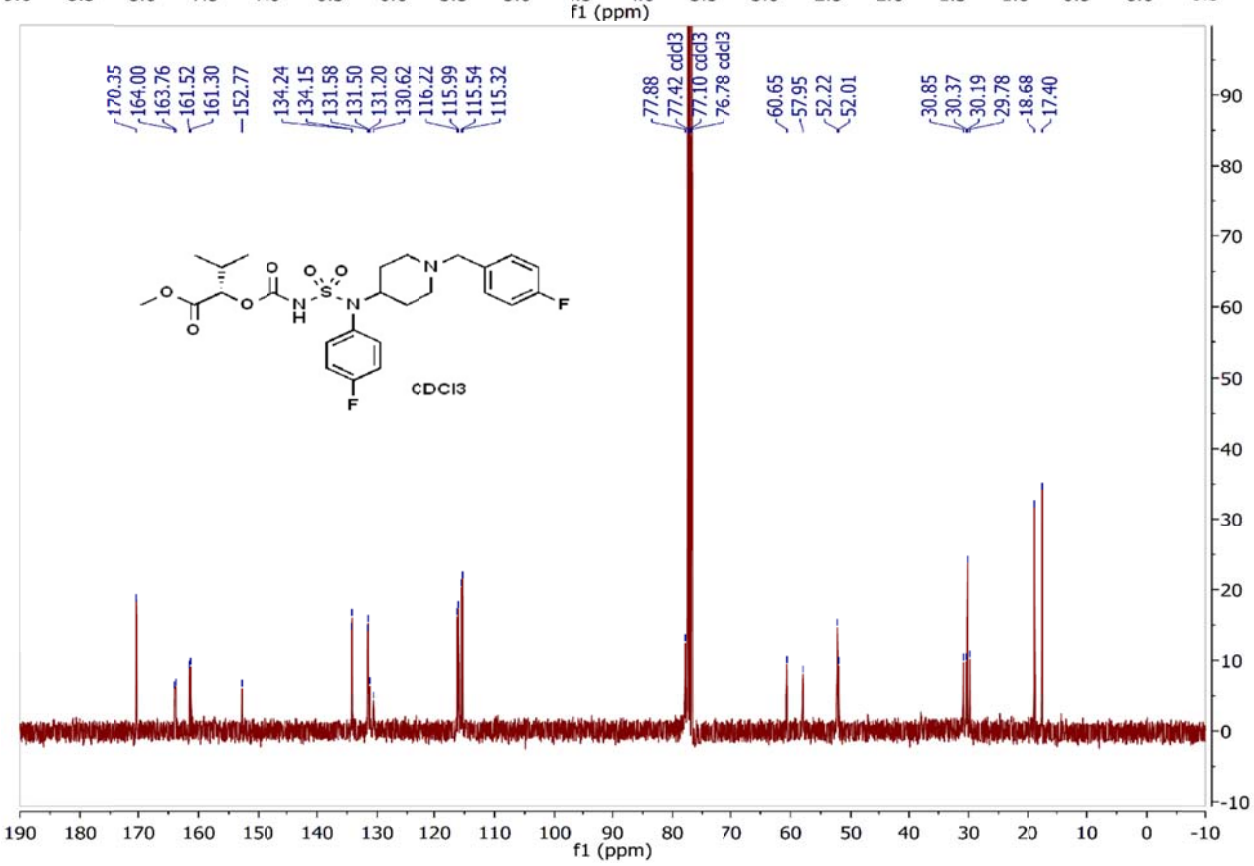
^{1}H NMR and ^{13}C NMR of compound 2.54



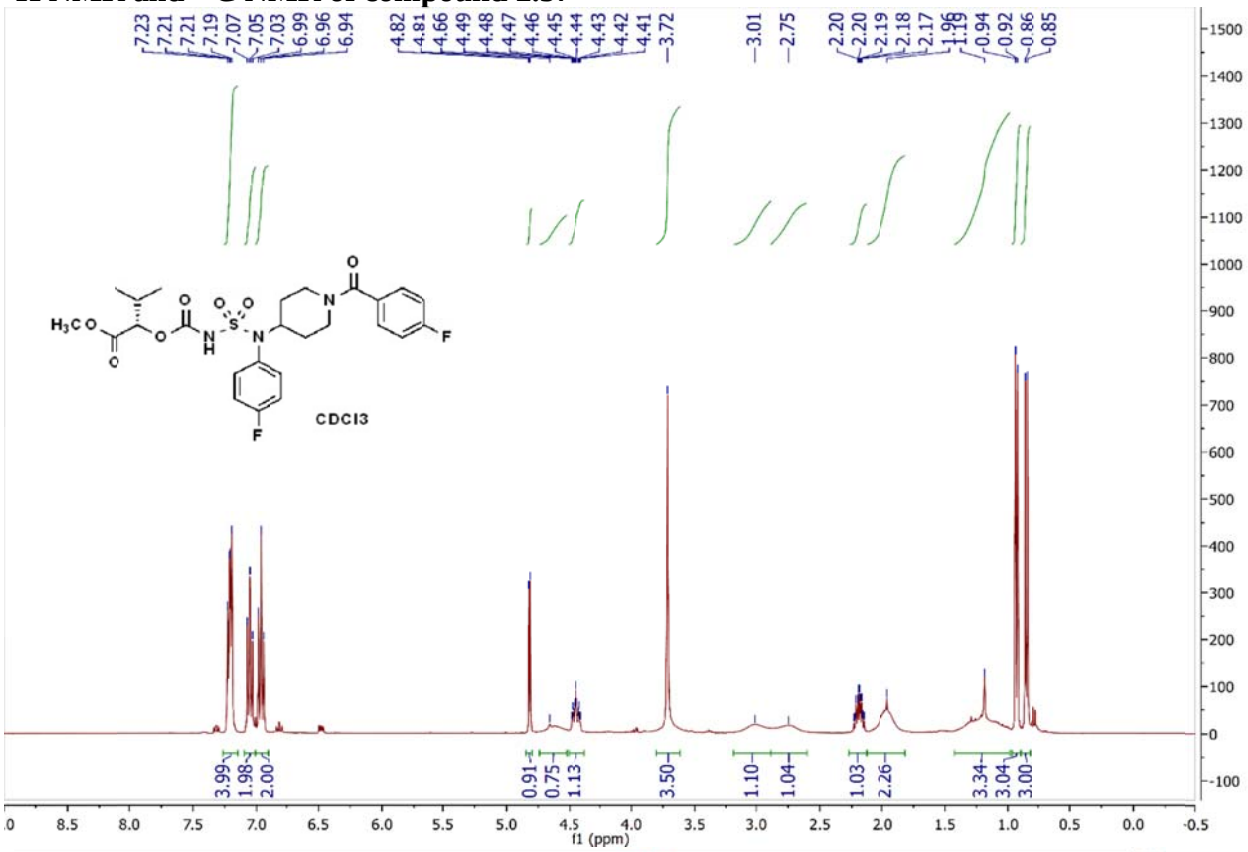


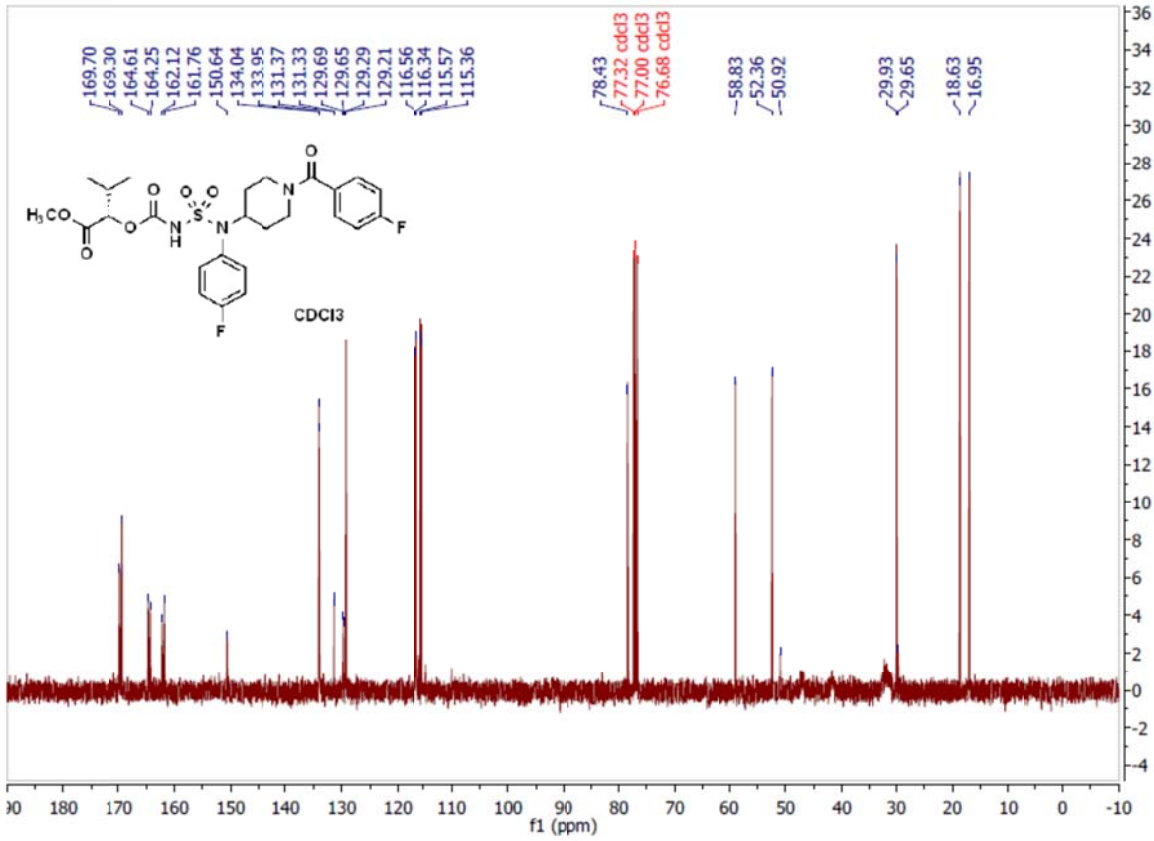
^{1}H NMR and ^{13}C NMR of compound 2.56





^{1}H NMR and ^{13}C NMR of compound 2.57





5.4. Appendix B

One and five dose experimental data from 60 cell line

-Tdp1 related compounds-

One dose experimental data of compound 2.7 (NSC 750772)

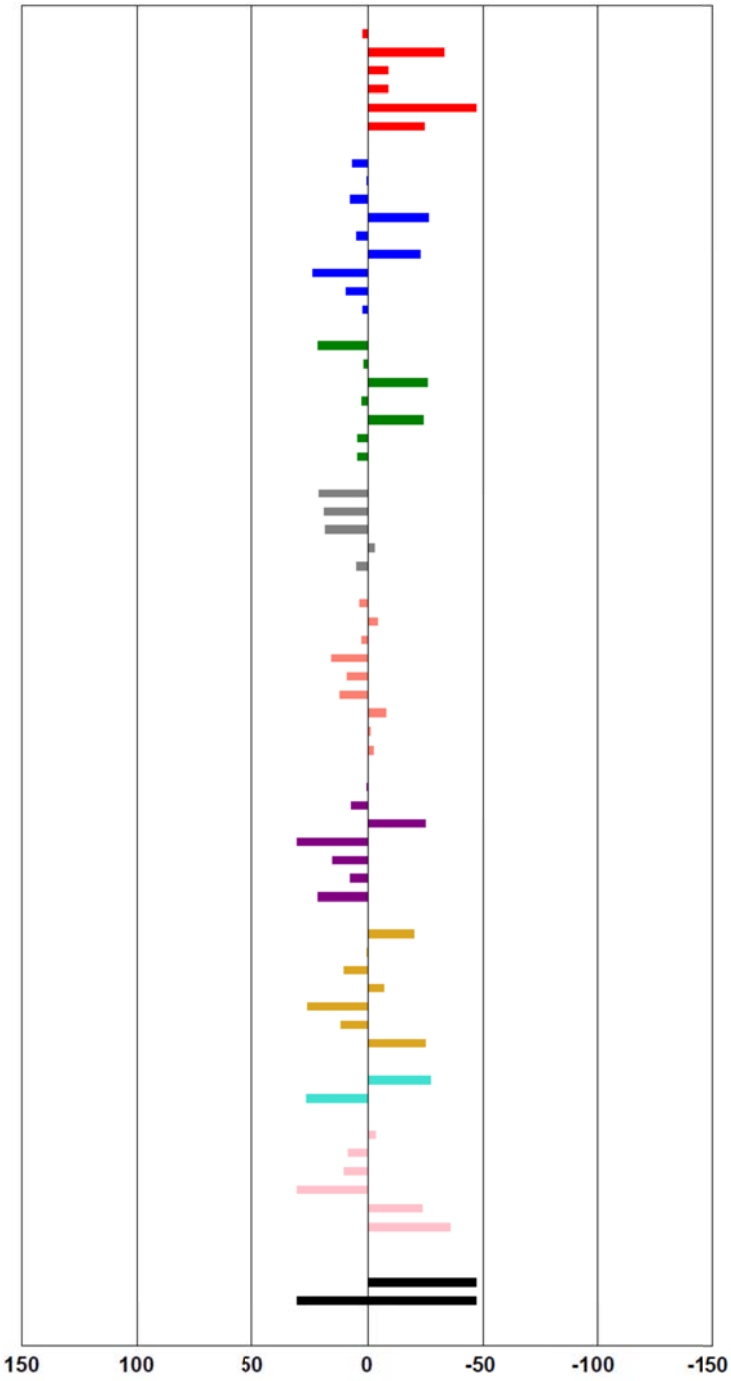
Developmental Therapeutics Program

One Dose Mean Graph

NSC: 750772 / 1	Conc: 1.00E-5 Molar	Test Date: Jul 20, 2009
Experiment ID: 0907OS88		Report Date: Feb 15, 2012

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	84.78
HL-60(TB)	48.99
K-562	74.02
MOLT-4	74.05
RPMI-8226	35.19
SR	57.56
Non-Small Cell Lung Cancer	
A549/ATCC	89.28
EKVX	83.18
HOP-62	90.22
HOP-92	55.98
NCI-H226	87.61
NCI-H23	59.42
NCI-H322M	105.98
NCI-H460	91.81
NCI-H522	84.55
Colon Cancer	
COLO 205	103.87
HCC-2998	84.37
HCT-116	56.17
HCT-15	85.15
HT29	58.22
KM12	86.88
SW-620	87.18
CNS Cancer	
SF-268	103.45
SF-295	101.32
SF-539	100.98
SNB-75	79.57
U251	87.39
Melanoma	
LOX IMVI	86.19
MALME-3M	78.56
M14	85.40
MDA-MB-435	98.14
SK-MEL-2	91.47
SK-MEL-28	94.74
SK-MEL-5	74.74
UACC-257	81.46
UACC-62	80.31
Ovarian Cancer	
IGROV1	82.97
OVCAR-3	89.49
OVCAR-4	57.14
OVCAR-5	112.96
OVCAR-8	97.86
NCI/ADR-RES	89.91
SK-OV-3	103.87
Renal Cancer	
786-0	61.98
A498	82.85
ACHN	92.53
CAKI-1	75.54
RXF 393	108.43
TK-10	94.03
UO-31	57.18
Prostate Cancer	
PC-3	54.73
DU-145	108.61
Breast Cancer	
MCF7	79.26
MDA-MB-231/ATCC	90.88
HS 578T	92.95
BT-549	112.58
T-47D	58.45
MDA-MB-468	46.57
Mean	82.41
Delta	47.22
Range	77.77

Mean Growth Percent - Growth Percent



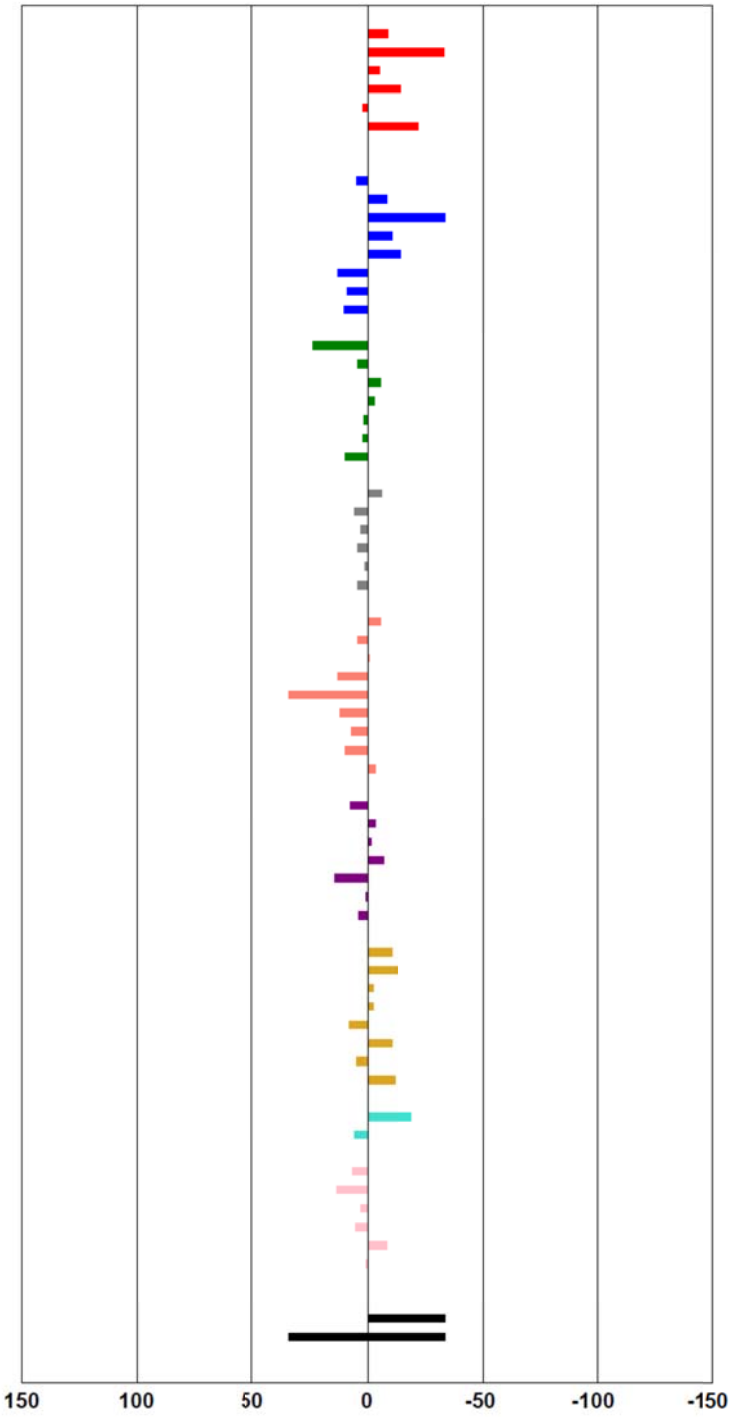
One dose experimental data of compound 2.15 (NSC 750710)

Developmental Therapeutics Program

One Dose Mean Graph

NSC: 750710 / 1	Conc: 1.00E-5 Molar	Test Date: Jul 06, 2009
Experiment ID: 0907OS76		Report Date: Feb 15, 2012

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	92.08
HL-60(TB)	67.24
K-562	95.78
MOLT-4	85.92
RPMI-8226	102.88
SR	78.56
Non-Small Cell Lung Cancer	
A549/ATCC	100.72
EKVX	105.48
HOP-62	92.52
HOP-92	67.08
NCI-H226	90.36
NCI-H23	86.12
NCI-H322M	113.49
NCI-H460	109.81
NCI-H522	110.83
Colon Cancer	
COLO 205	124.29
HCC-2998	105.33
HCT-116	95.59
HCT-15	97.97
HT29	102.39
KM12	102.93
SW-620	110.44
CNS Cancer	
SF-268	95.08
SF-295	106.38
SF-539	104.01
SNB-19	105.16
SNB-75	102.26
U251	105.08
Melanoma	
LOX IMVI	95.34
MALME-3M	104.97
M14	100.15
MDA-MB-435	113.52
SK-MEL-2	134.43
SK-MEL-28	112.89
SK-MEL-5	107.83
UACC-257	110.59
UACC-62	97.74
Ovarian Cancer	
IGROV1	108.37
OVCAR-3	97.84
OVCAR-4	99.47
OVCAR-5	94.02
OVCAR-8	115.14
NCI/ADR-RES	101.57
SK-OV-3	104.57
Renal Cancer	
786-0	90.44
A498	87.34
ACHN	98.43
CAKI-1	98.71
RXF 393	108.76
SN12C	90.42
TK-10	105.70
UO-31	89.30
Prostate Cancer	
PC-3	81.41
DU-145	106.57
Breast Cancer	
MCF7	107.39
MDA-MB-231/ATCC	114.12
HS 578T	103.82
BT-549	105.99
T-47D	92.73
MDA-MB-468	101.57
Mean	100.52
Delta	33.54
Range	67.35



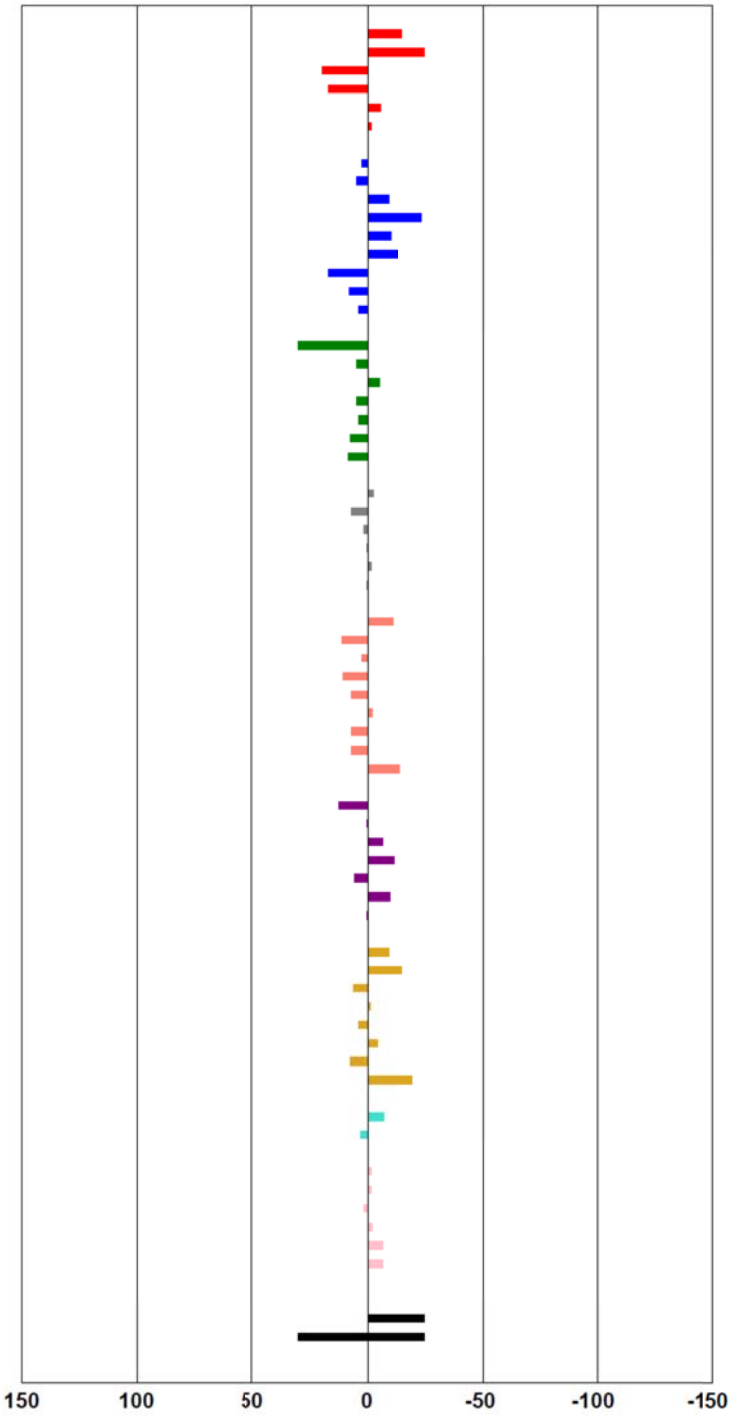
One dose experimental data of compound 2.16 (NSC 750711)

Developmental Therapeutics Program

One Dose Mean Graph

NSC: 750711 / 1	Conc: 1.00E-5 Molar	Test Date: Jul 06, 2009
Experiment ID: 0907OS76		Report Date: Feb 15, 2012

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	87.89
HL-60(TB)	78.38
K-562	122.76
MOLT-4	120.31
RPMI-8226	98.03
SR	101.84
Non-Small Cell Lung Cancer	
A549/ATCC	105.59
EKVX	107.94
HOP-62	94.34
HOP-92	79.47
NCI-H226	93.42
NCI-H23	89.79
NCI-H322M	120.12
NCI-H460	111.35
NCI-H522	107.34
Colon Cancer	
COLO 205	132.86
HCC-2998	108.13
HCT-116	98.43
HCT-15	108.13
HT29	107.28
KM12	110.61
SW-620	111.47
CNS Cancer	
SF-268	100.77
SF-295	110.52
SF-539	105.05
SNB-19	103.50
SNB-75	101.53
U251	103.79
Melanoma	
LOX IMVI	92.34
MALME-3M	114.43
M14	105.85
MDA-MB-435	113.77
SK-MEL-2	110.50
SK-MEL-28	101.20
SK-MEL-5	110.14
UACC-257	110.31
UACC-62	89.03
Ovarian Cancer	
IGROV1	115.54
OVCAR-3	103.60
OVCAR-4	97.11
OVCAR-5	91.93
OVCAR-8	108.87
NCI/ADR-RES	93.79
SK-OV-3	103.56
Renal Cancer	
786-0	94.24
A498	87.86
ACHN	109.50
CAKI-1	102.24
RXF 393	107.22
SN12C	99.22
TK-10	110.58
UO-31	83.54
Prostate Cancer	
PC-3	96.41
DU-145	106.40
Breast Cancer	
MCF7	101.56
MDA-MB-231/ATCC	101.73
HS 578T	105.12
BT-549	101.38
T-47D	96.85
MDA-MB-468	96.73
Mean	103.06
Delta	24.58
Range	54.48



One dose experimental data of compound 2.21 (NSC 750706)

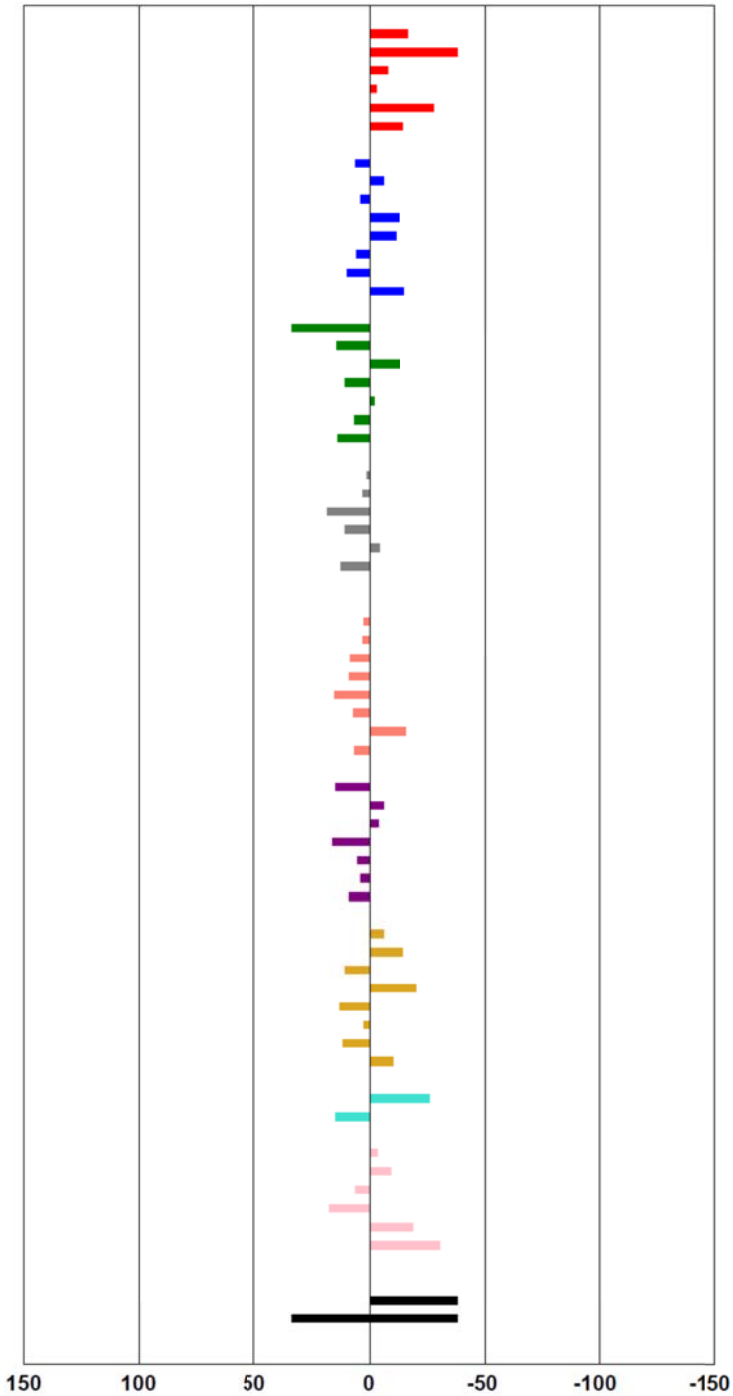
Developmental Therapeutics Program

One Dose Mean Graph

NSC: 750706 / 1	Conc: 1.00E-5 Molar	Test Date: Jul 06, 2009
Experiment ID: 0907OS76		Report Date: Feb 15, 2012

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	64.95
HL-60(TB)	43.58
K-562	74.58
MOLT-4	79.52
RPMI-8226	53.82
SR	67.45
Non-Small Cell Lung Cancer	
A549/ATCC	88.22
EKVX	76.33
HOP-62	86.05
HOP-92	69.38
NCI-H226	70.98
NCI-H322M	87.82
NCI-H460	92.01
NCI-H522	66.88
Colon Cancer	
COLO 205	115.29
HCC-2998	96.30
HCT-116	68.92
HCT-15	92.75
HT29	80.46
KM12	88.50
SW-620	95.98
CNS Cancer	
SF-268	83.34
SF-295	85.14
SF-539	100.29
SNB-19	92.77
SNB-75	77.97
U251	94.41
Melanoma	
LOX IMVI	82.20
MALME-3M	84.51
M14	85.28
MDA-MB-435	90.56
SK-MEL-2	90.80
SK-MEL-28	97.30
SK-MEL-5	89.14
UACC-257	65.79
UACC-62	88.86
Ovarian Cancer	
IGROV1	96.87
OVCAR-3	76.33
OVCAR-4	78.35
OVCAR-5	97.82
OVCAR-8	87.26
NCI/ADR-RES	86.19
SK-OV-3	90.92
Renal Cancer	
786-0	76.43
A498	67.17
ACHN	92.90
CAKI-1	61.45
RXF 393	95.01
SN12C	84.48
TK-10	93.73
UO-31	72.35
Prostate Cancer	
PC-3	55.71
DU-145	96.55
Breast Cancer	
MCF7	79.06
MDA-MB-231/ATCC	72.99
HS 578T	88.41
BT-549	99.45
T-47D	63.00
MDA-MB-468	51.53
Mean	81.90
Delta	38.22
Range	71.61

Mean Growth Percent - Growth Percent



One dose experimental data of compound 2.22 (NSC 747166)

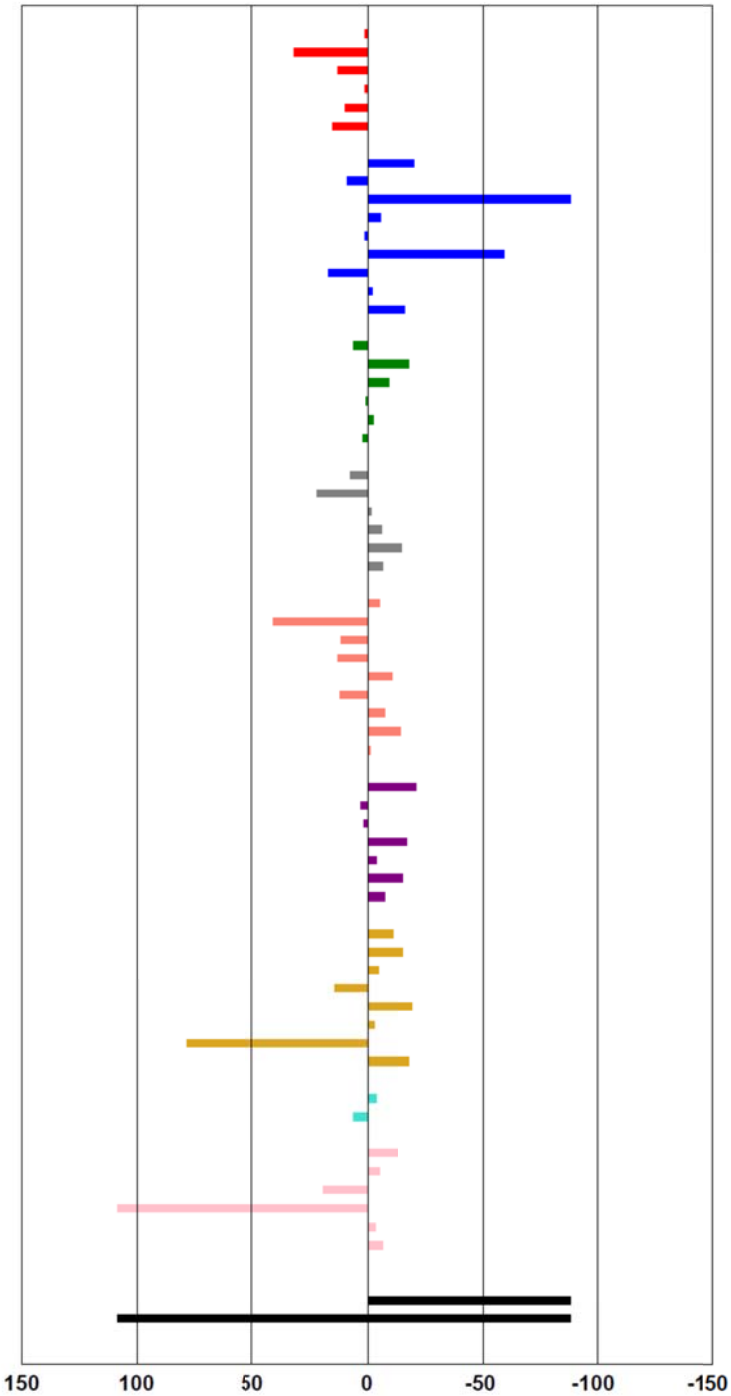
Developmental Therapeutics Program

One Dose Mean Graph

NSC: 747166 / 1	Conc: 1.00E-5 Molar	Test Date: Mar 03, 2008
Experiment ID: 0803OS30		Report Date: Feb 12, 2012

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	101.95
HL-60(TB)	132.14
K-562	113.79
MOLT-4	102.26
RPMI-8226	110.53
SR	115.78
Non-Small Cell Lung Cancer	
A549/ATCC	80.49
EKVX	109.58
HOP-62	12.34
HOP-92	95.48
NCI-H226	101.98
NCI-H23	41.77
NCI-H322M	117.85
NCI-H460	98.77
NCI-H522	84.10
Colon Cancer	
COLO 205	107.17
HCT-116	82.52
HCT-15	91.90
HT29	101.54
KM12	98.57
SW-620	102.84
CNS Cancer	
SF-268	108.31
SF-295	122.74
SF-539	99.48
SNB-19	94.90
SNB-75	85.46
U251	94.46
Melanoma	
LOX IMVI	95.94
MALME-3M	141.40
M14	112.52
MDA-MB-435	113.80
SK-MEL-2	90.45
SK-MEL-28	112.75
SK-MEL-5	93.72
UACC-257	86.11
UACC-62	99.83
Ovarian Cancer	
IGROV1	79.44
OVCAR-3	103.91
OVCAR-4	102.59
OVCAR-5	83.53
OVCAR-8	97.07
NCI/ADR-RES	85.04
SK-OV-3	93.46
Renal Cancer	
786-0	90.28
A498	85.27
ACHN	96.46
CAKI-1	114.97
RXF 393	80.97
SN12C	98.17
TK-10	179.28
UO-31	82.61
Prostate Cancer	
PC-3	97.35
DU-145	106.83
Breast Cancer	
MCF7	87.71
MDA-MB-231/ATCC	95.85
HS 578T	119.94
BT-549	208.78
T-47D	97.53
MDA-MB-468	94.73
Mean	100.53
Delta	88.29
Range	196.44

Mean Growth Percent - Growth Percent



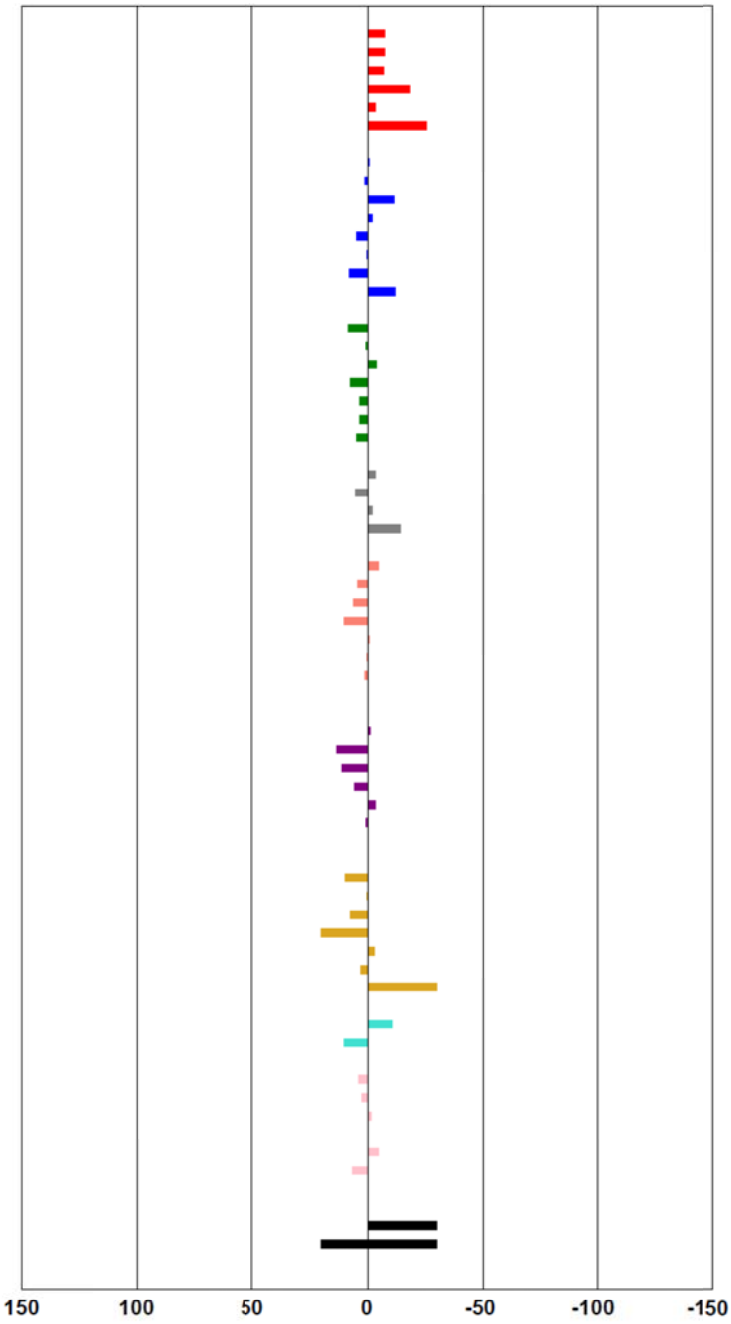
One dose experimental data of compound 2.23 (NSC 767523)

Developmental Therapeutics Program

One Dose Mean Graph

NSC: D-767523 / 1	Conc: 1.00E-5 Molar	Test Date: Sep 24, 2012
Experiment ID: 1209OS49		Report Date: May 05, 2013

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	90.36
HL-60(TB)	90.23
K-562	90.72
MOLT-4	78.52
RPMI-8226	94.21
SR	71.73
Non-Small Cell Lung Cancer	
A549/ATCC	97.13
HOP-62	98.78
HOP-92	86.45
NCI-H226	95.89
NCI-H23	102.21
NCI-H322M	97.73
NCI-H460	105.31
NCI-H522	85.98
Colon Cancer	
COLO 205	106.10
HCC-2998	98.28
HCT-116	93.96
HCT-15	104.96
HT29	101.00
KM12	101.13
SW-620	102.31
CNS Cancer	
SF-268	94.45
SF-539	102.59
SNB-19	95.55
SNB-75	82.79
Melanoma	
LOX IMVI	92.81
MALME-3M	101.85
M14	103.48
MDA-MB-435	107.55
SK-MEL-28	96.79
SK-MEL-5	98.03
UACC-62	98.87
Ovarian Cancer	
IGROV1	97.57
OVCAR-3	96.75
OVCAR-4	110.54
OVCAR-5	108.57
OVCAR-8	103.27
NCI/ADR-RES	94.41
SK-OV-3	98.51
Renal Cancer	
786-0	97.27
A498	107.38
ACHN	98.02
CAKI-1	105.09
RXF 393	117.58
SN12C	94.75
TK-10	100.72
UO-31	67.24
Prostate Cancer	
PC-3	87.26
DU-145	107.77
Breast Cancer	
MCF7	101.47
MDA-MB-231/ATCC	100.04
HS 578T	96.09
BT-549	97.44
T-47D	93.19
MDA-MB-468	104.29
Mean	97.34
Delta	30.10
Range	50.44



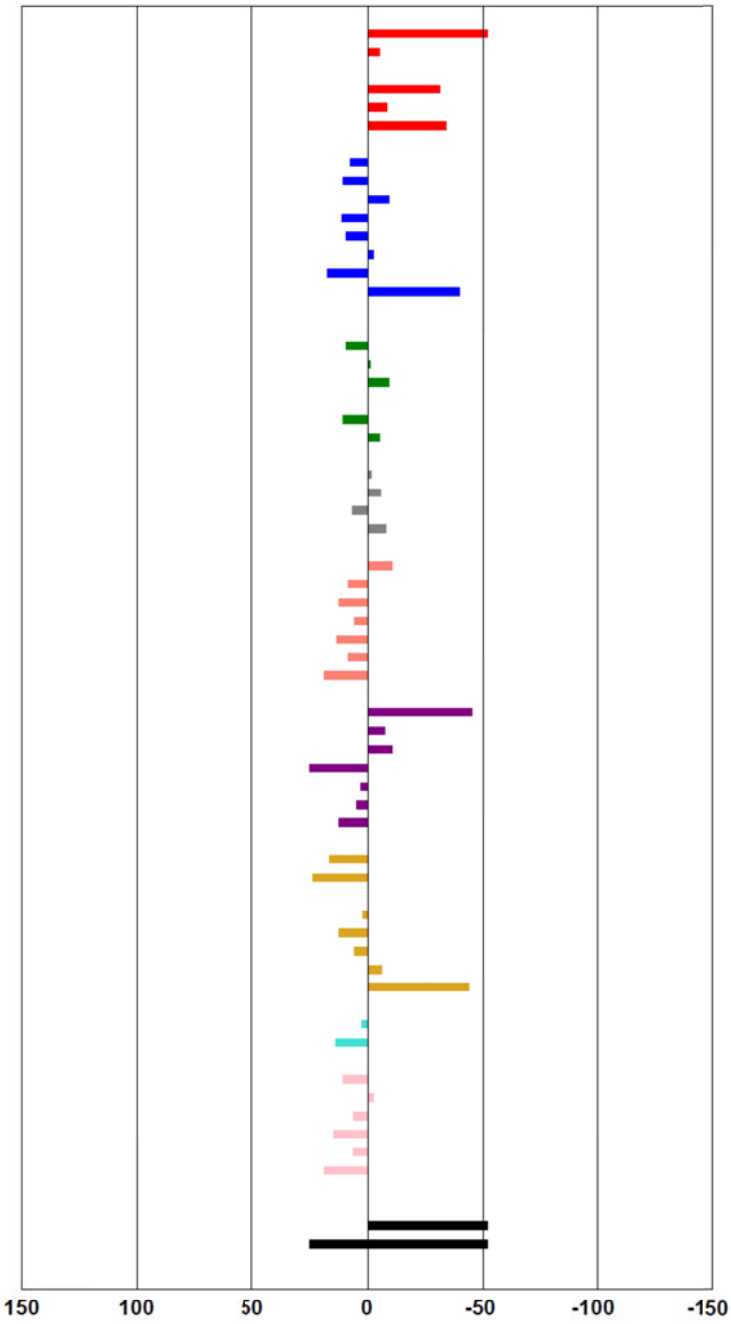
One dose experimental data of compound 2.24 (NSC 767524)

Developmental Therapeutics Program

One Dose Mean Graph

NSC: D-767524 / 1	Conc: 1.00E-5 Molar	Test Date: Sep 24, 2012
Experiment ID: 1209OS49		Report Date: May 05, 2013

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	33.20
HL-60(TB)	80.00
K-562	85.01
MOLT-4	53.55
RPMI-8226	77.30
SR	50.71
Non-Small Cell Lung Cancer	
A549/ATCC	92.54
HOP-62	95.74
HOP-92	76.41
NCI-H226	96.41
NCI-H23	94.33
NCI-H322M	82.93
NCI-H460	102.46
NCI-H522	45.22
Colon Cancer	
COLO 205	85.13
HCC-2998	94.50
HCT-116	84.34
HCT-15	76.07
HT29	85.24
KM12	95.90
SW-620	80.10
CNS Cancer	
SF-268	83.58
SF-539	79.72
SNB-19	91.85
SNB-75	77.57
Melanoma	
LOX IMVI	75.02
MALME-3M	93.77
M14	97.78
MDA-MB-435	90.75
SK-MEL-28	98.26
SK-MEL-5	93.78
UACC-62	103.58
Ovarian Cancer	
IGROV1	39.79
OVCAR-3	78.02
OVCAR-4	74.56
OVCAR-5	109.93
OVCAR-8	88.25
NCI/ADR-RES	89.91
SK-OV-3	97.42
Renal Cancer	
786-0	101.47
A498	108.50
ACHN	85.30
CAKI-1	87.20
RXF 393	97.55
SN12C	90.87
TK-10	79.43
UO-31	40.96
Prostate Cancer	
PC-3	87.77
DU-145	98.95
Breast Cancer	
MCF7	96.00
MDA-MB-231/ATCC	82.58
HS 578T	91.23
BT-549	99.97
T-47D	91.24
MDA-MB-468	104.01
Mean	84.99
Delta	51.79
Range	76.73



One dose experimental data of compound 2.25 (NSC 750707)

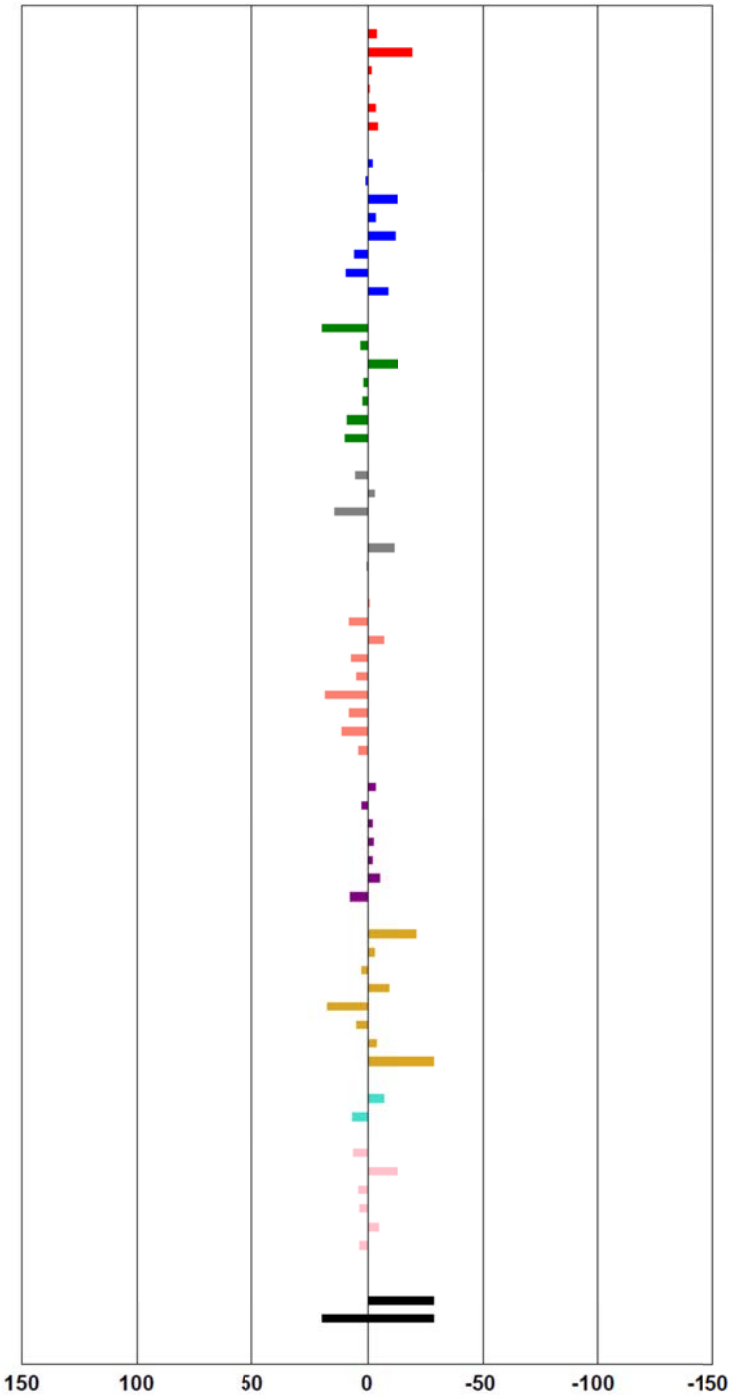
Developmental Therapeutics Program

One Dose Mean Graph

NSC: 750707 / 1	Conc: 1.00E-5 Molar	Test Date: Jul 06, 2009
Experiment ID: 0907OS76		Report Date: Feb 15, 2012

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	95.27
HL-60(TB)	78.96
K-562	97.18
MOLT-4	98.44
RPMI-8226	95.54
SR	94.89
Non-Small Cell Lung Cancer	
A549/ATCC	96.83
EKVX	99.55
HOP-62	86.18
HOP-92	95.46
NCI-H226	86.98
NCI-H322M	104.58
NCI-H460	108.10
NCI-H522	90.45
Colon Cancer	
COLO 205	118.35
HCC-2998	101.73
HCT-116	85.85
HCT-15	100.43
HT29	100.97
KM12	107.52
SW-620	108.35
CNS Cancer	
SF-268	103.92
SF-295	95.97
SF-539	112.97
SNB-19	98.52
SNB-75	87.44
U251	99.30
Melanoma	
LOX IMVI	98.22
MALME-3M	106.75
M14	92.00
MDA-MB-435	105.73
SK-MEL-2	103.54
SK-MEL-28	116.79
SK-MEL-5	106.57
UACC-257	109.95
UACC-62	102.94
Ovarian Cancer	
IGROV1	95.55
OVCAR-3	101.22
OVCAR-4	96.74
OVCAR-5	96.39
OVCAR-8	96.86
NCI/ADR-RES	94.03
SK-OV-3	106.47
Renal Cancer	
786-0	77.49
A498	95.92
ACHN	101.24
CAKI-1	89.54
RXF 393	115.93
SN12C	103.53
TK-10	95.13
UO-31	69.57
Prostate Cancer	
PC-3	91.97
DU-145	105.56
Breast Cancer	
MCF7	105.00
MDA-MB-231/ATCC	86.17
HS 578T	102.59
BT-549	102.09
T-47D	94.39
MDA-MB-468	102.14
Mean	98.52
Delta	28.95
Range	48.58

Mean Growth Percent - Growth Percent



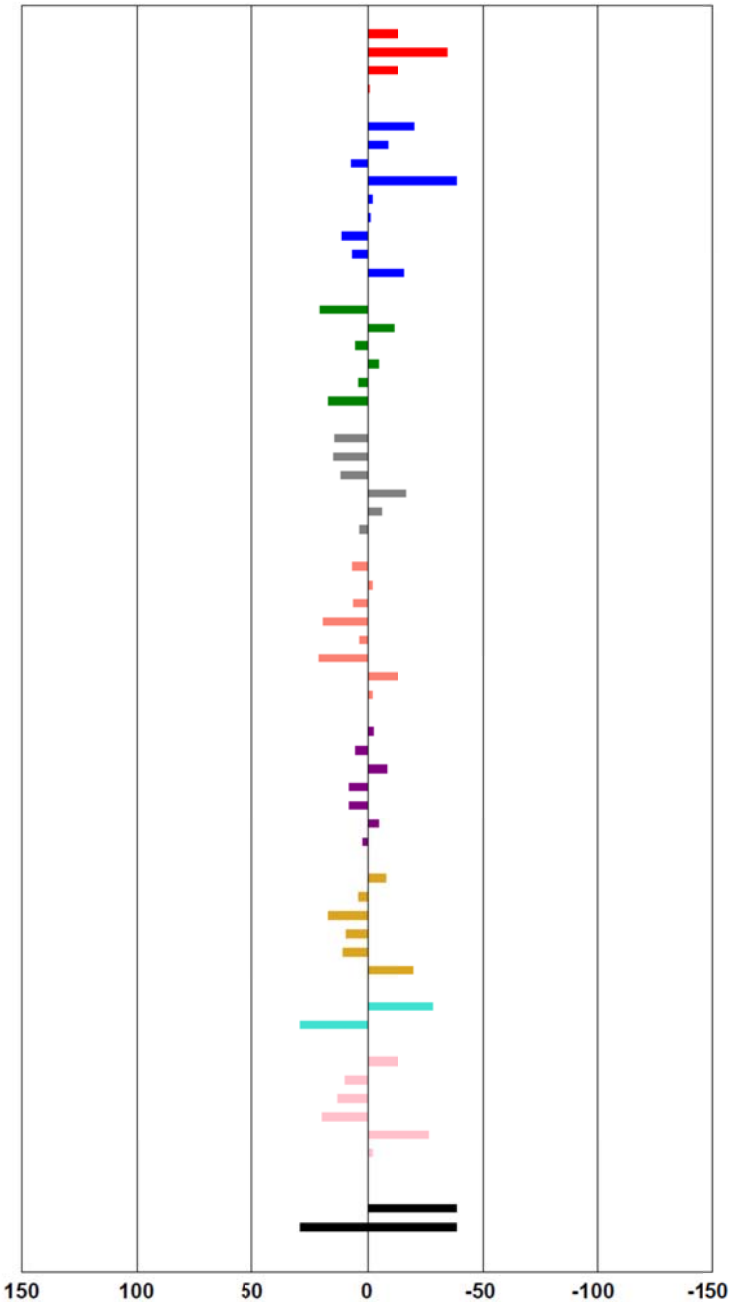
One dose experimental data of compound 2.30 (NSC 749205)

Developmental Therapeutics Program

One Dose Mean Graph

NSC: 749205 / 1	Conc: 1.00E-5 Molar	Test Date: Dec 08, 2008
Experiment ID: 0812OS07		Report Date: Feb 15, 2012

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	65.07
HL-60(TB)	43.70
MOLT-4	65.50
SR	77.98
Non-Small Cell Lung Cancer	
A549/ATCC	57.84
EKVX	69.81
HOP-62	85.39
HOP-92	39.58
NCI-H226	76.87
NCI-H23	77.33
NCI-H322M	89.52
NCI-H460	85.07
NCI-H522	62.55
Colon Cancer	
COLO 205	98.80
HCT-116	67.40
HCT-15	83.74
HT29	74.07
KM12	82.52
SW-620	95.26
CNS Cancer	
SF-268	92.83
SF-295	93.26
SF-539	89.85
SNB-19	61.33
SNB-75	72.53
U251	82.00
Melanoma	
LOX IMVI	85.30
MALME-3M	76.66
M14	84.55
MDA-MB-435	97.48
SK-MEL-2	81.94
SK-MEL-28	99.21
SK-MEL-5	65.58
UACC-62	76.58
Ovarian Cancer	
IGROV1	76.41
OVCAR-3	83.86
OVCAR-4	70.51
OVCAR-5	86.52
OVCAR-8	86.56
NCI/ADR-RES	74.12
SK-OV-3	80.56
Renal Cancer	
786-0	70.85
A498	82.37
ACHN	95.39
SN12C	87.77
TK-10	89.31
UO-31	58.59
Prostate Cancer	
PC-3	50.15
DU-145	107.51
Breast Cancer	
MCF7	65.53
MDA-MB-231/ATCC	88.01
HS 578T	91.33
BT-549	98.08
T-47D	51.56
MDA-MB-468	76.49
Mean	78.32
Delta	38.74
Range	67.93



One dose experimental data of compound 2.34 (NSC 749201)

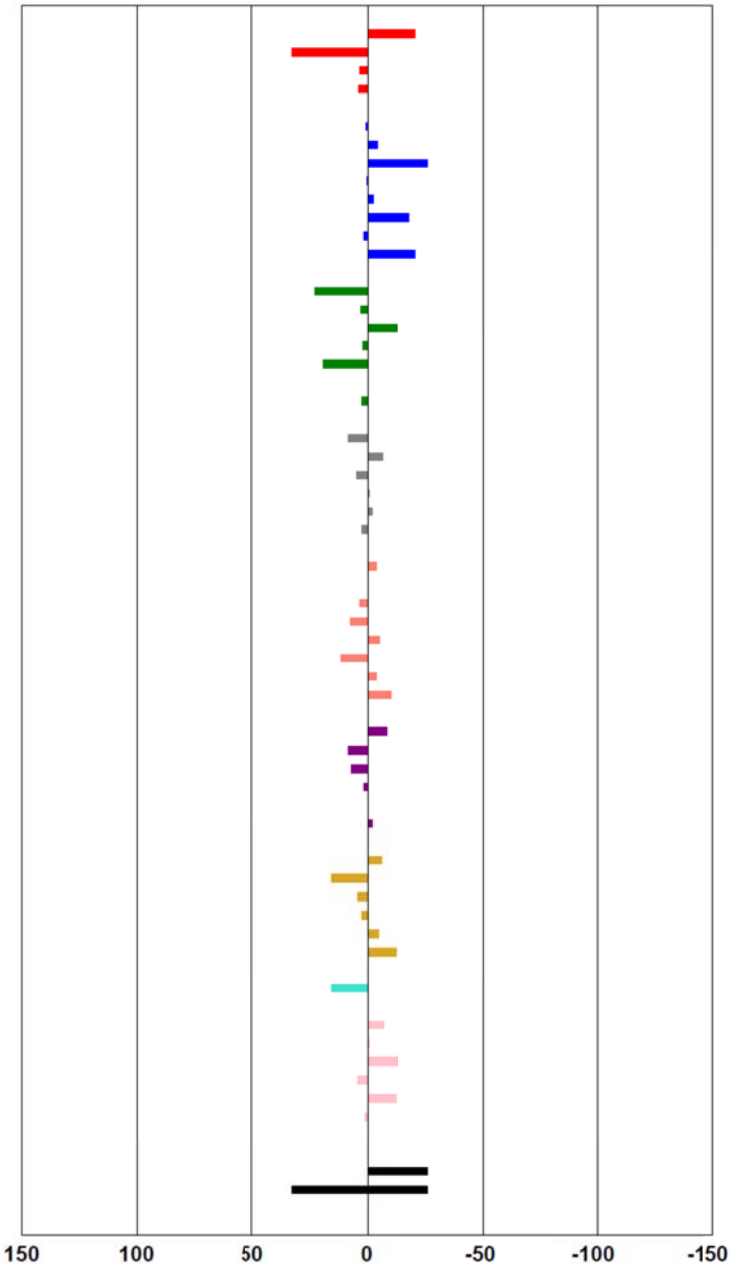
Developmental Therapeutics Program

One Dose Mean Graph

NSC: 749201 / 1	Conc: 1.00E-5 Molar	Test Date: Dec 08, 2008
Experiment ID: 0812OS07		Report Date: Feb 12, 2012

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	77.21
HL-60(TB)	130.53
MOLT-4	101.41
SR	101.91
Non-Small Cell Lung Cancer	
EKVX	98.56
HOP-62	93.98
HOP-92	71.49
NCI-H226	98.54
NCI-H23	95.75
NCI-H322M	79.88
NCI-H460	99.56
NCI-H522	77.16
Colon Cancer	
COLO 205	120.70
HCC-2998	100.94
HCT-116	85.59
HCT-15	100.30
HT29	116.88
KM12	98.13
SW-620	100.53
CNS Cancer	
SF-268	106.47
SF-295	91.82
SF-539	102.91
SNB-19	97.35
SNB-75	96.20
U251	100.41
Melanoma	
LOX IMVI	94.50
MALME-3M	97.76
M14	101.43
MDA-MB-435	105.45
SK-MEL-2	92.93
SK-MEL-28	109.27
SK-MEL-5	94.57
UACC-62	88.27
Ovarian Cancer	
IGROV1	90.05
OVCAR-3	106.58
OVCAR-4	104.86
OVCAR-5	99.55
NCI/ADR-RES	97.84
SK-OV-3	96.31
Renal Cancer	
786-0	91.95
A498	113.44
ACHN	102.31
SN12C	100.50
TK-10	93.57
UO-31	85.77
Prostate Cancer	
DU-145	113.36
Breast Cancer	
MCF7	91.15
MDA-MB-231/ATCC	97.55
HS 578T	84.44
BT-549	102.46
T-47D	86.04
MDA-MB-468	99.47
Mean	97.81
Delta	26.32
Range	59.04

Mean Growth Percent - Growth Percent



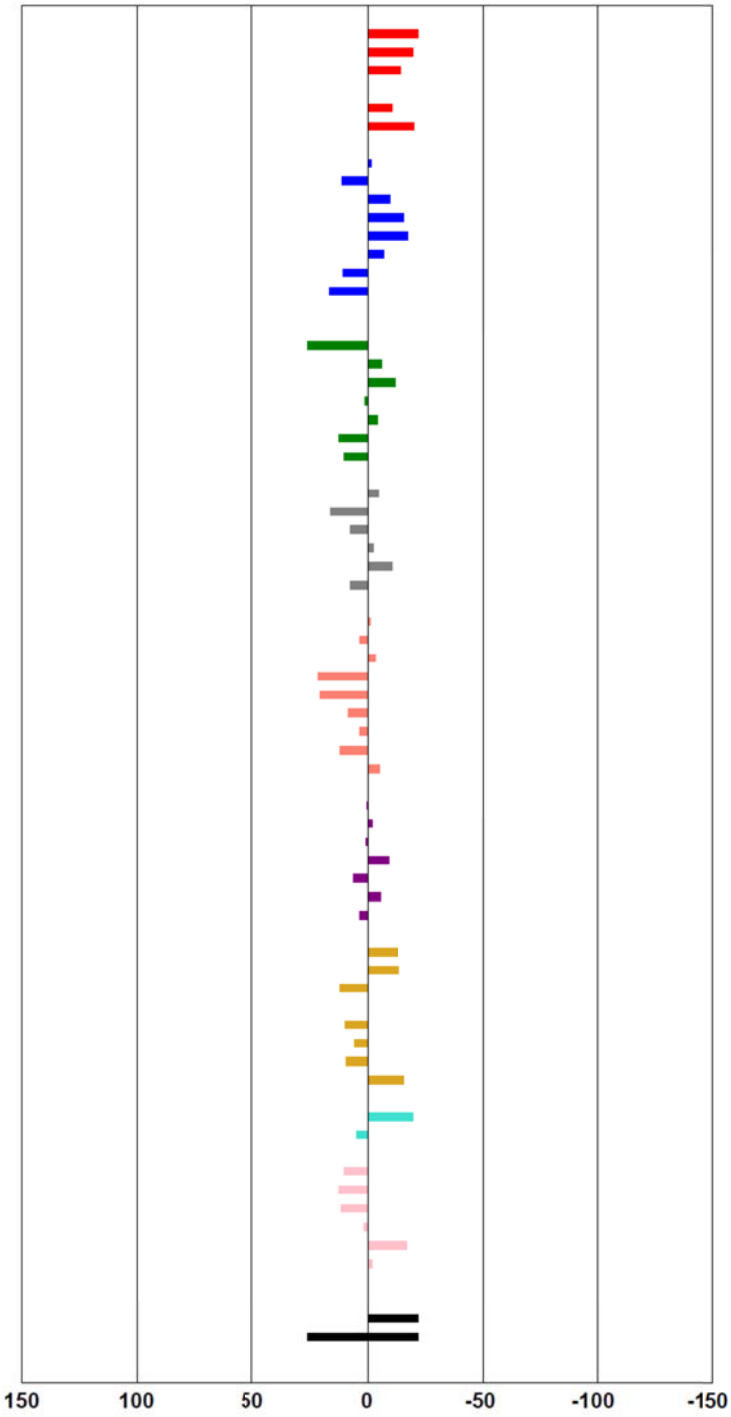
One dose experimental data of compound 2.38 (NSC 750715)

Developmental Therapeutics Program

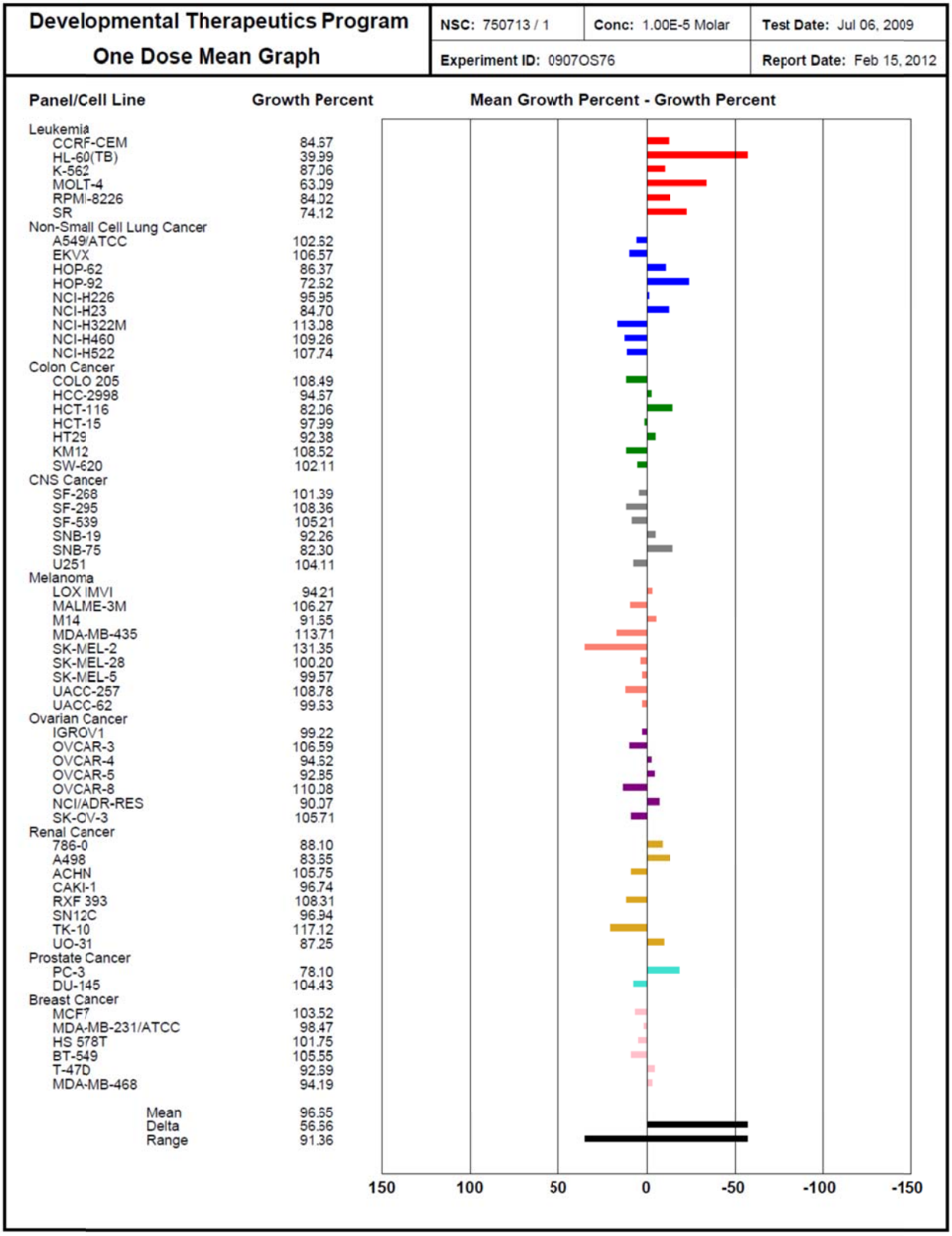
One Dose Mean Graph

NSC: 750715 / 1	Conc: 1.00E-5 Molar	Test Date: Jul 06, 2009
Experiment ID: 0907OS76		Report Date: Feb 15, 2012

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	76.86
HL-60(TB)	78.76
K-562	84.33
MOLT-4	98.90
RPMI-8226	88.52
SR	78.52
Non-Small Cell Lung Cancer	
A549/ATCC	97.78
EKVX	109.87
HOP-62	89.76
HOP-92	83.05
NCI-H226	81.01
NCI-H23	92.22
NCI-H322M	109.42
NCI-H460	115.23
NCI-H522	98.70
Colon Cancer	
COLO 205	124.74
HCC-2998	93.12
HCT-116	87.47
HCT-15	100.29
HT29	94.71
KM12	111.25
SW-620	108.93
CNS Cancer	
SF-268	94.52
SF-295	115.06
SF-539	106.40
SNB-19	96.50
SNB-75	88.53
U251	106.51
Melanoma	
LOX IMVI	97.95
MALME-3M	102.39
M14	95.84
MDA-MB-435	120.12
SK-MEL-2	119.58
SK-MEL-28	107.27
SK-MEL-5	102.25
UACC-257	110.93
UACC-62	93.97
Ovarian Cancer	
IGROV1	99.32
OVCAR-3	97.25
OVCAR-4	99.59
OVCAR-5	90.20
OVCAR-8	105.16
NCI/ADR-RES	93.75
SK-OV-3	102.47
Renal Cancer	
786-0	85.88
A498	84.91
ACHN	110.96
CAKI-1	98.99
RXF 393	108.53
SN12C	104.54
TK-10	108.08
UO-31	82.72
Prostate Cancer	
PC-3	78.84
DU-145	103.98
Breast Cancer	
MCF7	109.29
MDA-MB-231/ATCC	111.36
HS 578T	110.59
BT-549	100.70
T-47D	81.53
MDA-MB-468	96.92
Mean	98.79
Delta	21.93
Range	47.88



One dose experimental data of compound 2.40 (NSC 750713)



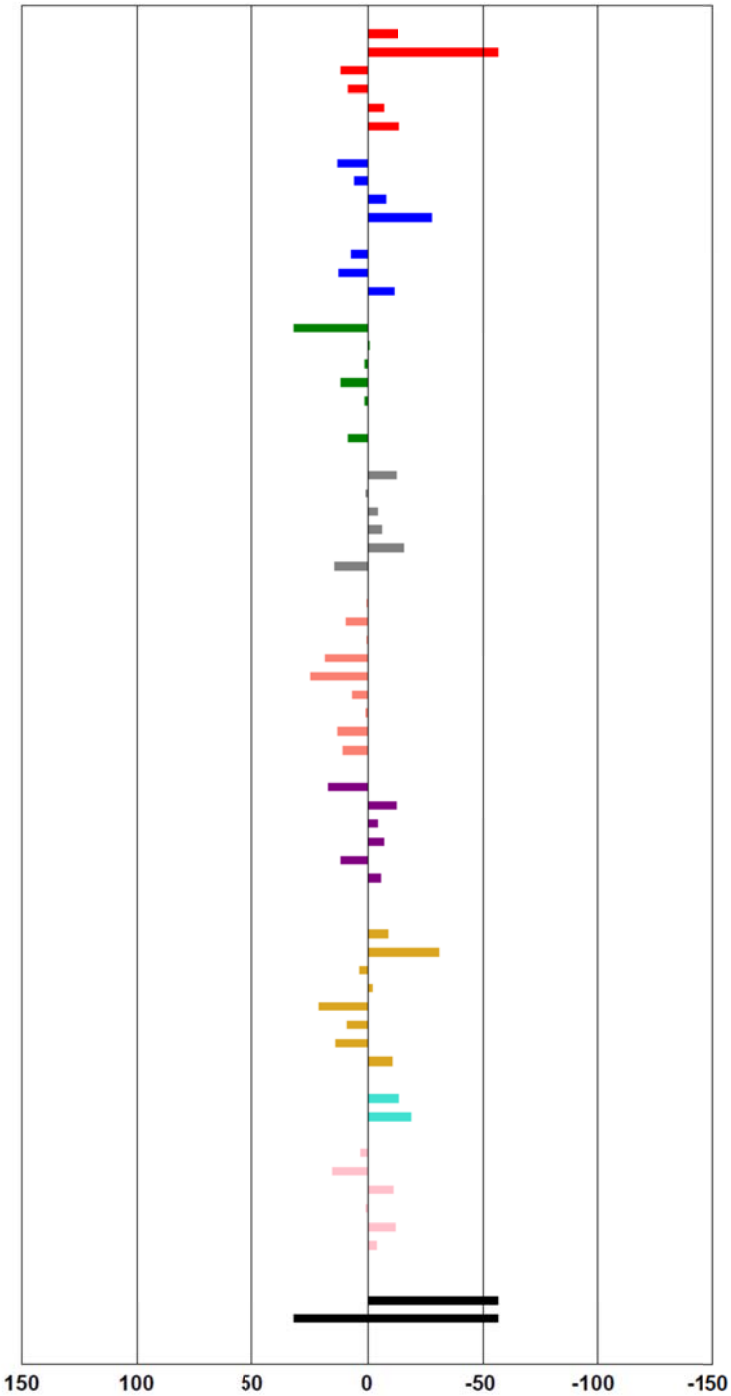
One dose experimental data of compound 2.42 (NSC 750714)

Developmental Therapeutics Program

One Dose Mean Graph

NSC: 750714 / 1	Conc: 1.00E-5 Molar	Test Date: Jul 06, 2009
Experiment ID: 0907OS76		Report Date: Feb 15, 2012

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	86.21
HL-60(TB)	43.07
K-562	111.22
MOLT-4	108.26
RPMI-8226	93.06
SR	85.93
Non-Small Cell Lung Cancer	
A549/ATCC	112.52
EKVX	105.50
HOP-62	92.03
HOP-92	71.61
NCI-H226	99.48
NCI-H322M	106.51
NCI-H460	111.89
NCI-H522	88.28
Colon Cancer	
COLO 205	131.06
HCC-2998	99.14
HCT-116	101.01
HCT-15	111.31
HT29	101.15
KM12	99.75
SW-620	108.00
CNS Cancer	
SF-268	87.52
SF-295	100.40
SF-539	95.82
SNB-19	93.61
SNB-75	83.73
U251	113.83
Melanoma	
LOX IMVI	100.03
MALME-3M	108.82
M14	100.14
MDA-MB-435	117.87
SK-MEL-2	124.03
SK-MEL-28	106.11
SK-MEL-5	100.32
UACC-257	112.42
UACC-62	110.16
Ovarian Cancer	
IGROV1	116.58
OVCAR-3	87.51
OVCAR-4	95.54
OVCAR-5	92.73
OVCAR-8	111.03
NCI/ADR-RES	94.28
SK-OV-3	99.53
Renal Cancer	
786-0	91.25
A498	68.51
ACHN	103.23
CAKI-1	97.89
RXF 393	120.35
SN12C	108.57
TK-10	113.46
UO-31	89.40
Prostate Cancer	
PC-3	85.99
DU-145	80.52
Breast Cancer	
MCF7	102.59
MDA-MB-231/ATCC	114.57
HS 578T	89.06
BT-549	100.55
T-47D	88.14
MDA-MB-468	96.06
Mean	99.49
Delta	56.42
Range	87.99



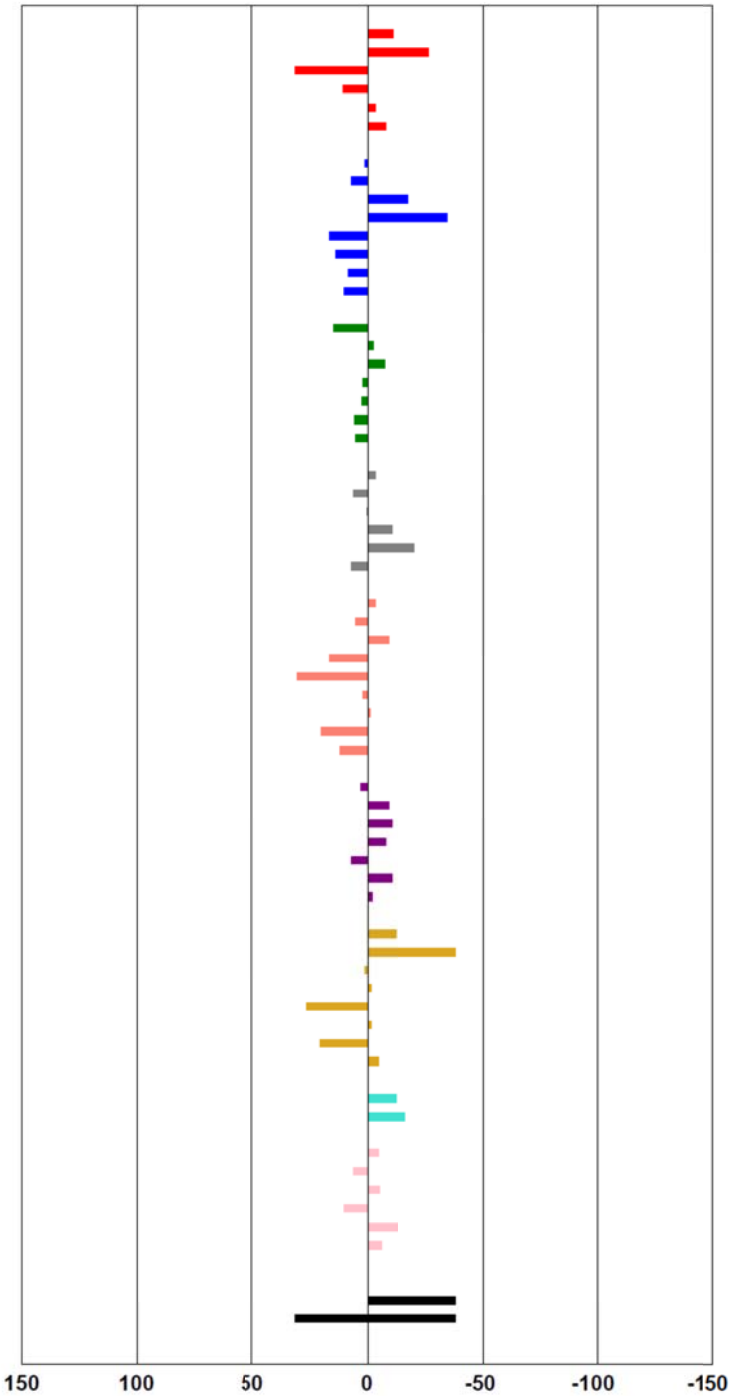
One dose experimental data of compound 2.43 (NSC 750712)

Developmental Therapeutics Program

One Dose Mean Graph

NSC: 750712 / 1	Conc: 1.00E-5 Molar	Test Date: Jul 06, 2009
Experiment ID: 0907OS76		Report Date: Feb 15, 2012

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	89.90
HL-60(TB)	74.10
K-562	131.82
MOLT-4	111.42
RPMI-8226	97.48
SR	93.41
Non-Small Cell Lung Cancer	
A549/ATCC	101.99
EKVX	107.74
HOP-62	82.82
HOP-92	65.91
NCI-H226	117.10
NCI-H322M	114.34
NCI-H460	109.25
NCI-H522	110.93
Colon Cancer	
COLO 205	115.38
HCC-2998	98.72
HCT-116	93.51
HCT-15	102.76
HT29	103.48
KM12	106.57
SW-620	106.24
CNS Cancer	
SF-268	97.54
SF-295	106.81
SF-539	101.41
SNB-19	90.38
SNB-75	80.26
U251	107.86
Melanoma	
LOX IMVI	97.85
MALME-3M	106.06
M14	91.59
MDA-MB-435	117.04
SK-MEL-2	130.87
SK-MEL-28	103.11
SK-MEL-5	99.75
UACC-257	120.57
UACC-62	112.75
Ovarian Cancer	
IGROV1	104.07
OVCAR-3	91.83
OVCAR-4	90.34
OVCAR-5	93.41
OVCAR-8	107.71
NCI/ADR-RES	90.61
SK-OV-3	98.78
Renal Cancer	
786-0	88.56
A498	62.35
ACHN	102.27
CAKI-1	99.22
RXF 393	126.94
SN12C	99.53
TK-10	121.23
UO-31	96.37
Prostate Cancer	
PC-3	88.56
DU-145	84.35
Breast Cancer	
MCF7	96.19
MDA-MB-231/ATCC	106.78
HS 578T	95.95
BT-549	110.92
T-47D	87.80
MDA-MB-468	94.82
Mean	100.54
Delta	38.29
Range	69.47



One dose experimental data of compound 2.47 (NSC 764209)

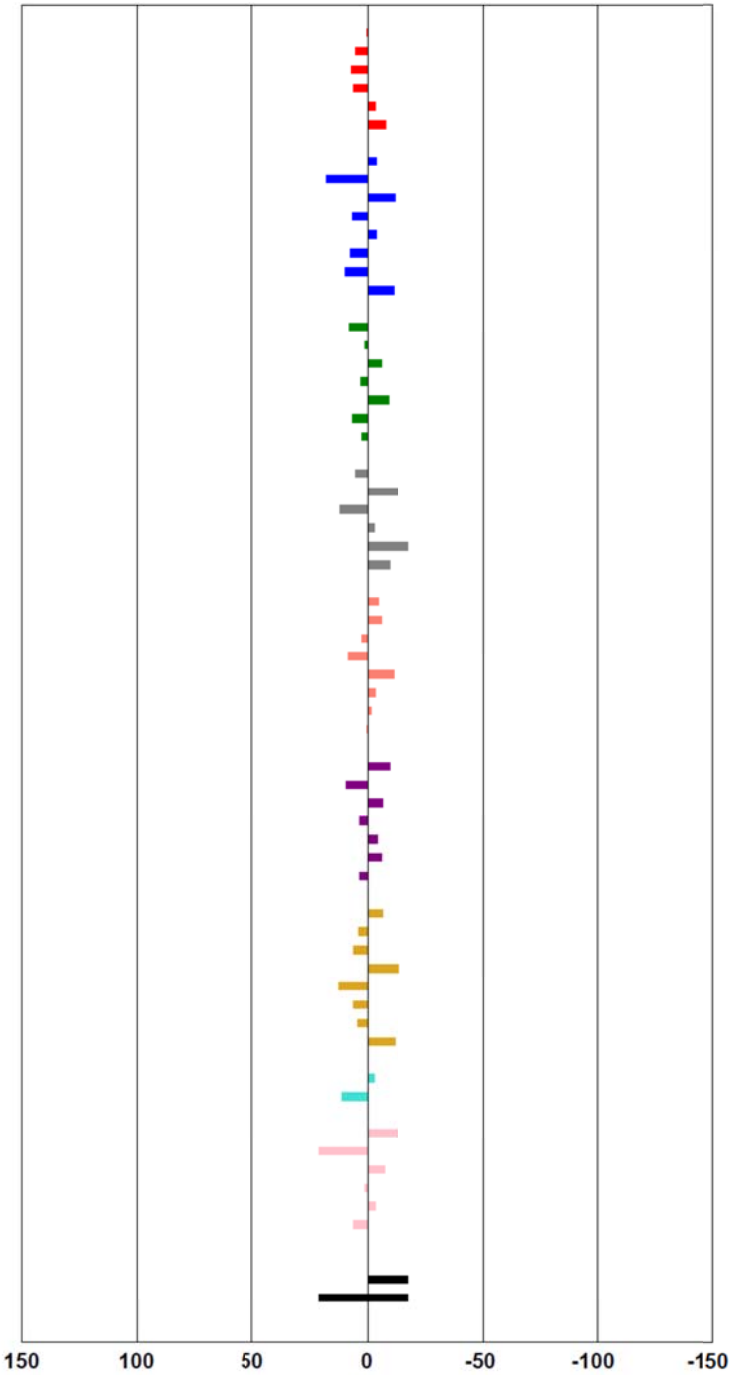
Developmental Therapeutics Program

One Dose Mean Graph

NSC: D-764209 / 1	Conc: 1.00E-5 Molar	Test Date: Mar 19, 2012
Experiment ID: 1203OS38		Report Date: May 05, 2013

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	106.00
HL-60(TB)	110.57
K-562	112.58
MOLT-4	111.56
RPMI-8226	102.16
SR	97.89
Non-Small Cell Lung Cancer	
A549/ATCC	101.84
HOP-62	123.16
HOP-92	93.92
NCI-H226	112.09
NCI-H23	102.04
NCI-H322M	112.89
NCI-H460	115.33
NCI-H522	94.09
Colon Cancer	
COLO 205	113.55
HCC-2998	106.88
HCT-116	99.59
HCT-15	108.35
HT29	96.70
KM12	112.01
SW-620	107.91
CNS Cancer	
SF-268	110.57
SF-295	92.21
SF-539	117.22
SNB-19	102.82
SNB-75	87.42
U251	96.15
Melanoma	
MALME-3M	100.87
M14	99.57
MDA-MB-435	108.31
SK-MEL-2	114.07
SK-MEL-28	94.36
SK-MEL-5	102.10
UACC-257	103.87
UACC-62	105.55
Ovarian Cancer	
IGROV1	95.94
OVCAR-3	114.54
OVCAR-4	99.19
OVCAR-5	109.17
OVCAR-8	101.52
NCI/ADR-RES	99.59
SK-OV-3	109.14
Renal Cancer	
786-0	99.13
A498	109.47
ACHN	111.58
CAKI-1	91.54
RXF 393	117.78
SN12C	111.54
TK-10	109.70
UO-31	93.85
Prostate Cancer	
PC-3	102.58
DU-145	116.59
Breast Cancer	
MCF7	92.73
MDA-MB-231/ATCC	126.22
HS 578T	98.35
BT-549	106.75
T-47D	102.21
MDA-MB-468	111.55
Mean	105.30
Delta	17.88
Range	38.80

Mean Growth Percent - Growth Percent



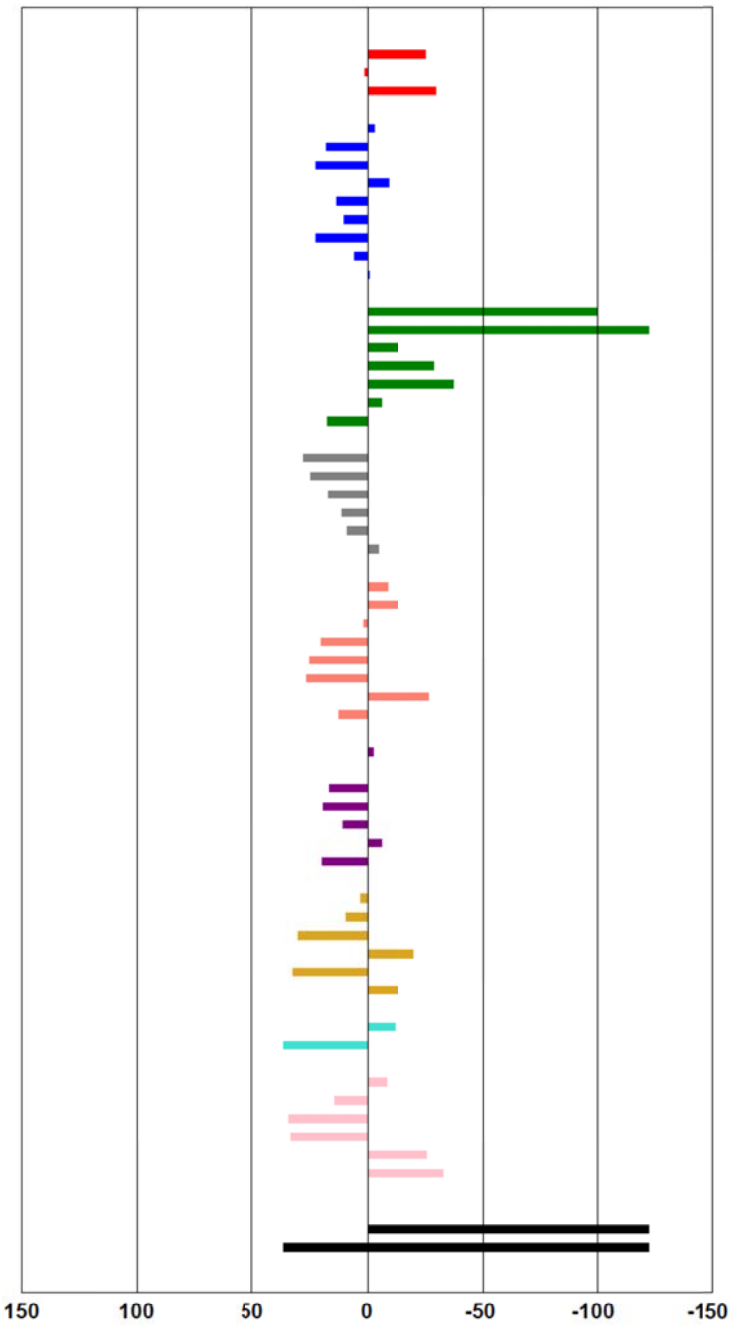
One dose experimental data of compound 2.53 (NSC 749204)

Developmental Therapeutics Program

One Dose Mean Graph

NSC: 749204 / 1	Conc: 1.00E-5 Molar	Test Date: Dec 08, 2008
Experiment ID: 0812OS07		Report Date: Feb 12, 2012

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	56.54
HL-60(TB)	31.29
MOLT-4	58.22
SR	26.73
Non-Small Cell Lung Cancer	
A549/ATCC	53.96
EKVX	74.50
HOP-62	79.02
HOP-92	47.80
NCI-H226	70.09
NCI-H23	66.78
NCI-H322M	79.04
NCI-H460	62.34
NCI-H522	56.14
Colon Cancer	
COLO 205	-43.19
HCC-2998	-65.26
HCT-116	43.37
HCT-15	27.86
HT29	19.49
KM12	51.02
SW-620	73.90
CNS Cancer	
SF-268	84.38
SF-295	81.14
SF-539	73.55
SNB-19	67.57
SNB-75	65.45
U251	52.28
Melanoma	
LOX IMVI	48.20
MALME-3M	43.83
M14	58.50
MDA-MB-435	76.81
SK-MEL-2	81.71
SK-MEL-28	82.87
SK-MEL-5	29.94
UACC-62	69.15
Ovarian Cancer	
IGROV1	54.72
OVCAR-3	56.73
OVCAR-4	73.41
OVCAR-5	76.08
OVCAR-8	67.33
NCI/ADR-RES	50.97
SK-OV-3	76.24
Renal Cancer	
786-0	59.73
A498	65.97
ACHN	86.39
SN12C	36.84
TK-10	88.98
UO-31	43.98
Prostate Cancer	
PC-3	45.36
DU-145	92.88
Breast Cancer	
MCF7	48.59
MDA-MB-231/ATCC	70.95
HS 578T	90.74
BT-549	89.53
T-47D	31.11
MDA-MB-468	23.52
Mean	56.55
Delta	121.91
Range	158.14



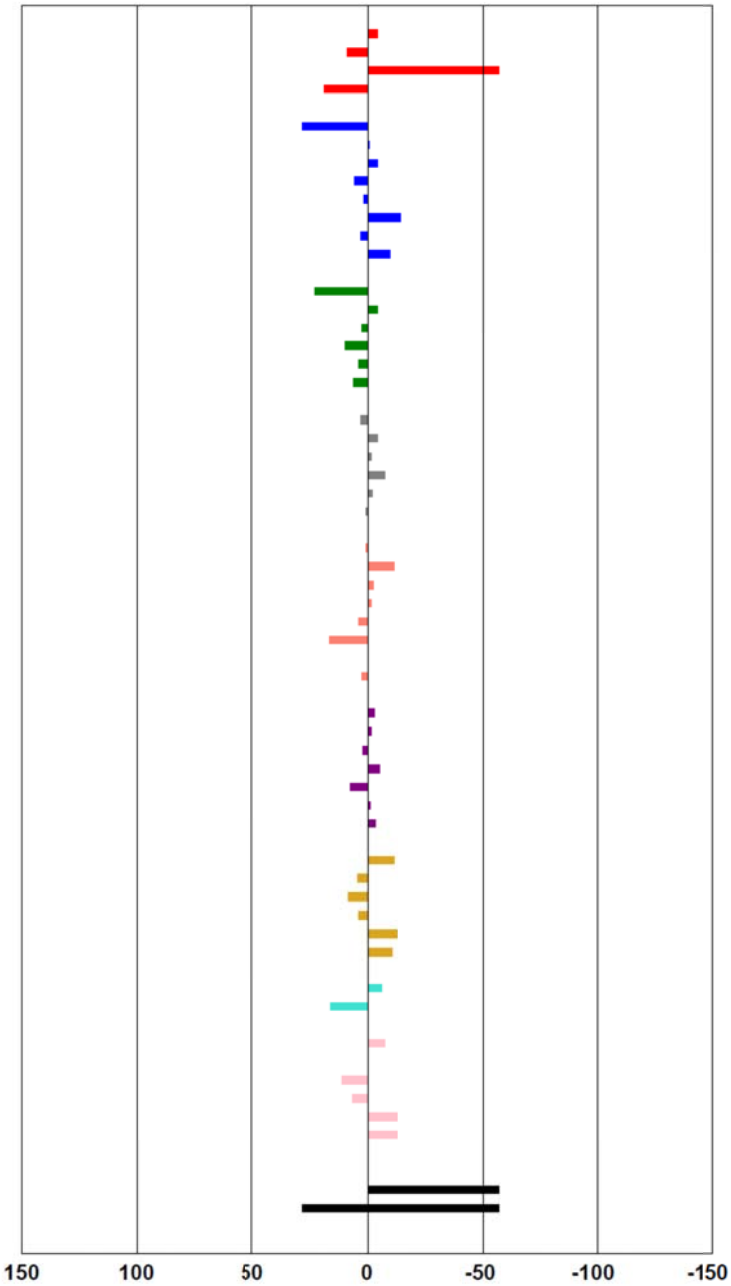
One dose experimental data of compound 2.56 (NSC 749203)

Developmental Therapeutics Program

One Dose Mean Graph

NSC: 749203 / 1	Conc: 1.00E-5 Molar	Test Date: Dec 08, 2008
Experiment ID: 0812OS07		Report Date: Feb 12, 2012

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	94.42
HL-60(TB)	107.05
MOLT-4	41.46
SR	116.72
Non-Small Cell Lung Cancer	
A549/ATCC	126.10
EKVX	97.78
HOP-62	94.41
NCI-H226	103.83
NCI-H23	100.15
NCI-H322M	83.44
NCI-H460	101.43
NCI-H522	88.96
Colon Cancer	
COLO 205	121.13
HCT-116	94.25
HCT-15	100.82
HT29	107.90
KM12	102.16
SW-620	104.55
CNS Cancer	
SF-268	101.17
SF-295	94.17
SF-539	97.02
SNB-19	91.13
SNB-75	96.56
U251	99.11
Melanoma	
LOX IMVI	99.02
MALME-3M	87.05
M14	96.15
MDA-MB-435	97.02
SK-MEL-2	102.03
SK-MEL-28	114.50
SK-MEL-5	98.14
UACC-62	100.97
Ovarian Cancer	
IGROV1	95.55
OVCAR-3	96.97
OVCAR-4	100.47
OVCAR-5	93.35
OVCAR-8	105.73
NCI/ADR-RES	97.42
SK-OV-3	94.99
Renal Cancer	
786-0	87.09
A498	102.54
ACHN	106.50
SN12C	102.22
TK-10	85.52
UO-31	88.01
Prostate Cancer	
PC-3	92.46
DU-145	114.28
Breast Cancer	
MCF7	91.11
MDA-MB-231/ATCC	98.39
HS 578T	109.47
BT-549	104.94
T-47D	85.93
MDA-MB-468	85.81
Mean	98.11
Delta	56.55
Range	84.54



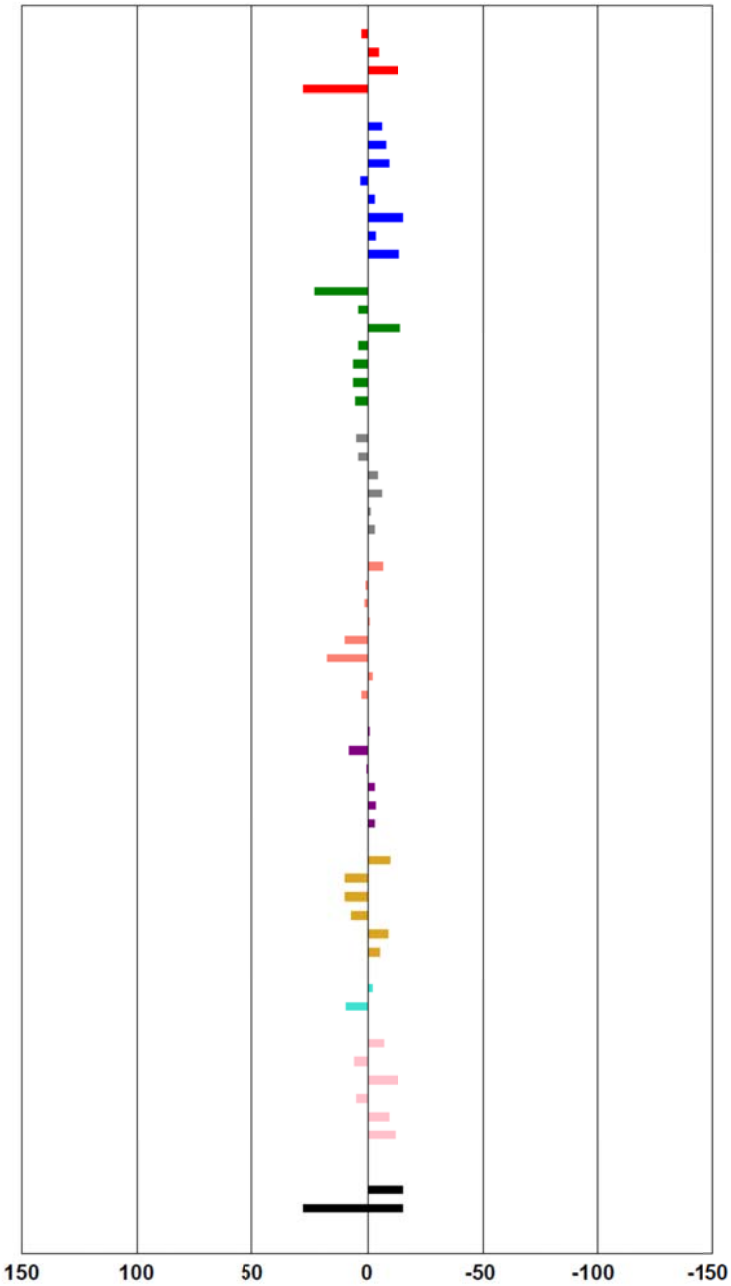
One dose experimental data of compound 2.57 (NSC 749202)

Developmental Therapeutics Program

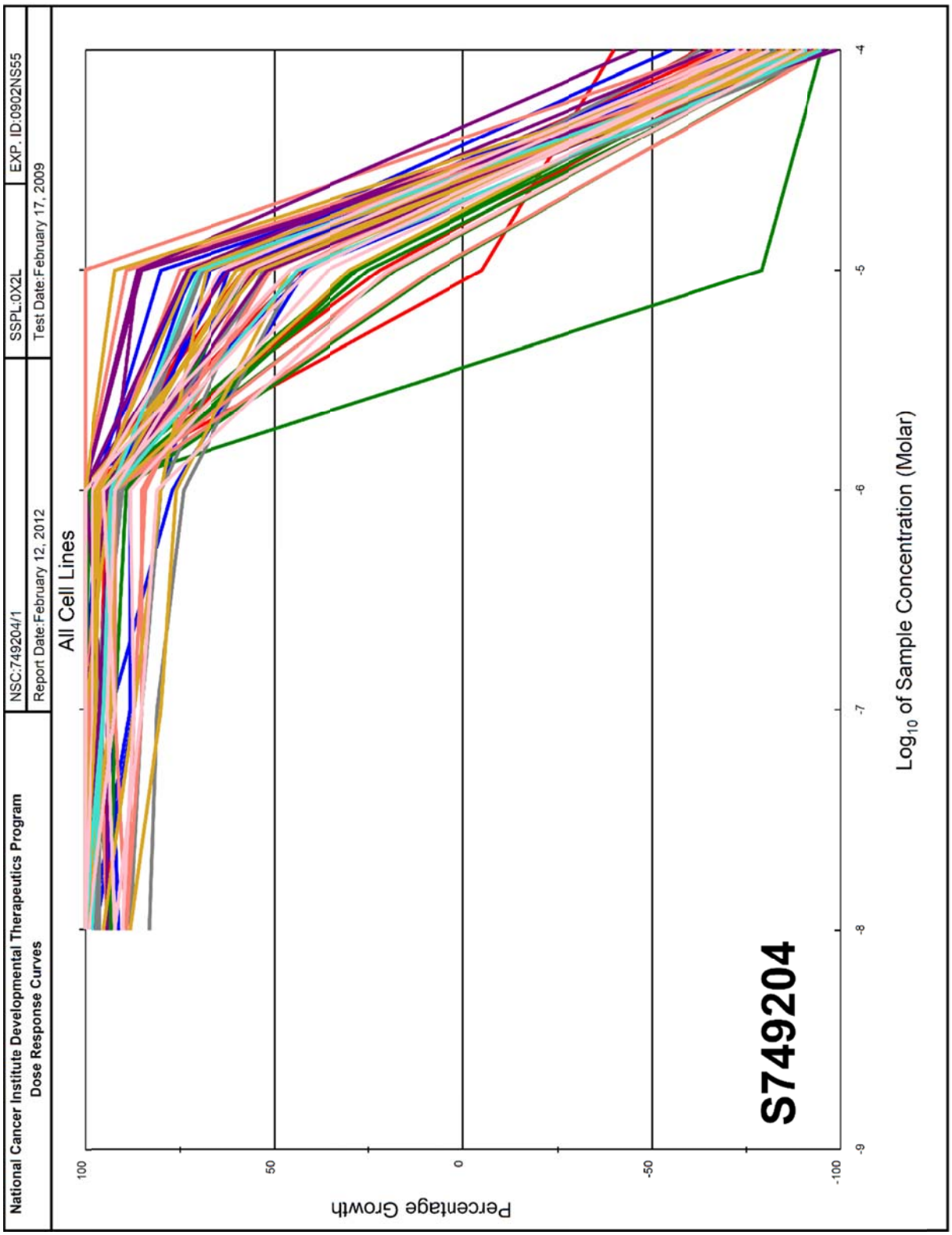
One Dose Mean Graph

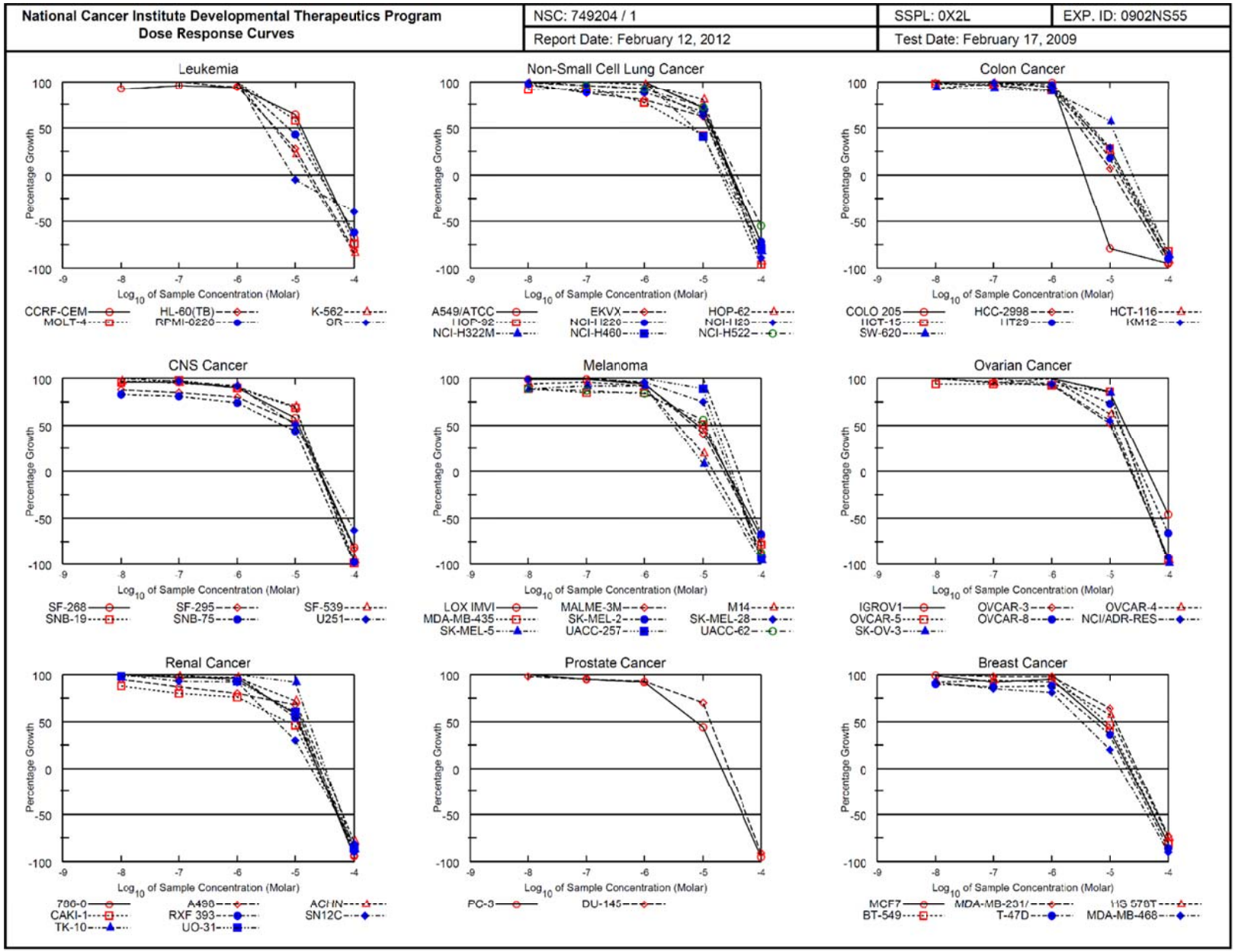
NSC: 749202 / 1	Conc: 1.00E-5 Molar	Test Date: Dec 08, 2008
Experiment ID: 0812OS07		Report Date: Feb 12, 2012

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	102.22
HL-60(TB)	95.13
MOLT-4	86.07
SR	127.35
Non-Small Cell Lung Cancer	
EKVX	94.01
HOP-62	92.10
HOP-92	90.74
NCI-H226	102.71
NCI-H23	97.07
NCI-H322M	83.92
NCI-H460	96.71
NCI-H522	85.95
Colon Cancer	
COLO 205	122.16
HCC-2998	103.78
HCT-116	85.29
HCT-15	103.58
HT29	105.97
KM12	105.73
SW-620	104.92
CNS Cancer	
SF-268	104.59
SF-295	103.46
SF-539	95.52
SNB-19	93.96
SNB-75	98.87
U251	96.94
Melanoma	
LOX IMVI	93.31
MALME-3M	100.38
M14	100.92
MDA-MB-435	99.23
SK-MEL-2	109.46
SK-MEL-28	116.99
SK-MEL-5	97.74
UACC-62	102.16
Ovarian Cancer	
IGROV1	99.33
OVCAR-3	107.84
OVCAR-4	100.24
OVCAR-5	97.17
NCI/ADR-RES	96.56
SK-OV-3	97.05
Renal Cancer	
786-0	90.40
A498	109.55
ACHN	109.43
SN12C	106.86
TK-10	91.34
UO-31	94.94
Prostate Cancer	
PC-3	98.06
DU-145	108.98
Breast Cancer	
MCF7	92.88
MDA-MB-231/ATCC	105.22
HS 578T	86.45
BT-549	104.55
T-47D	90.54
MDA-MB-468	87.97
Mean	99.52
Delta	15.50
Range	43.43



Five dose experimental data of compound 2.53 (NSC 749204)



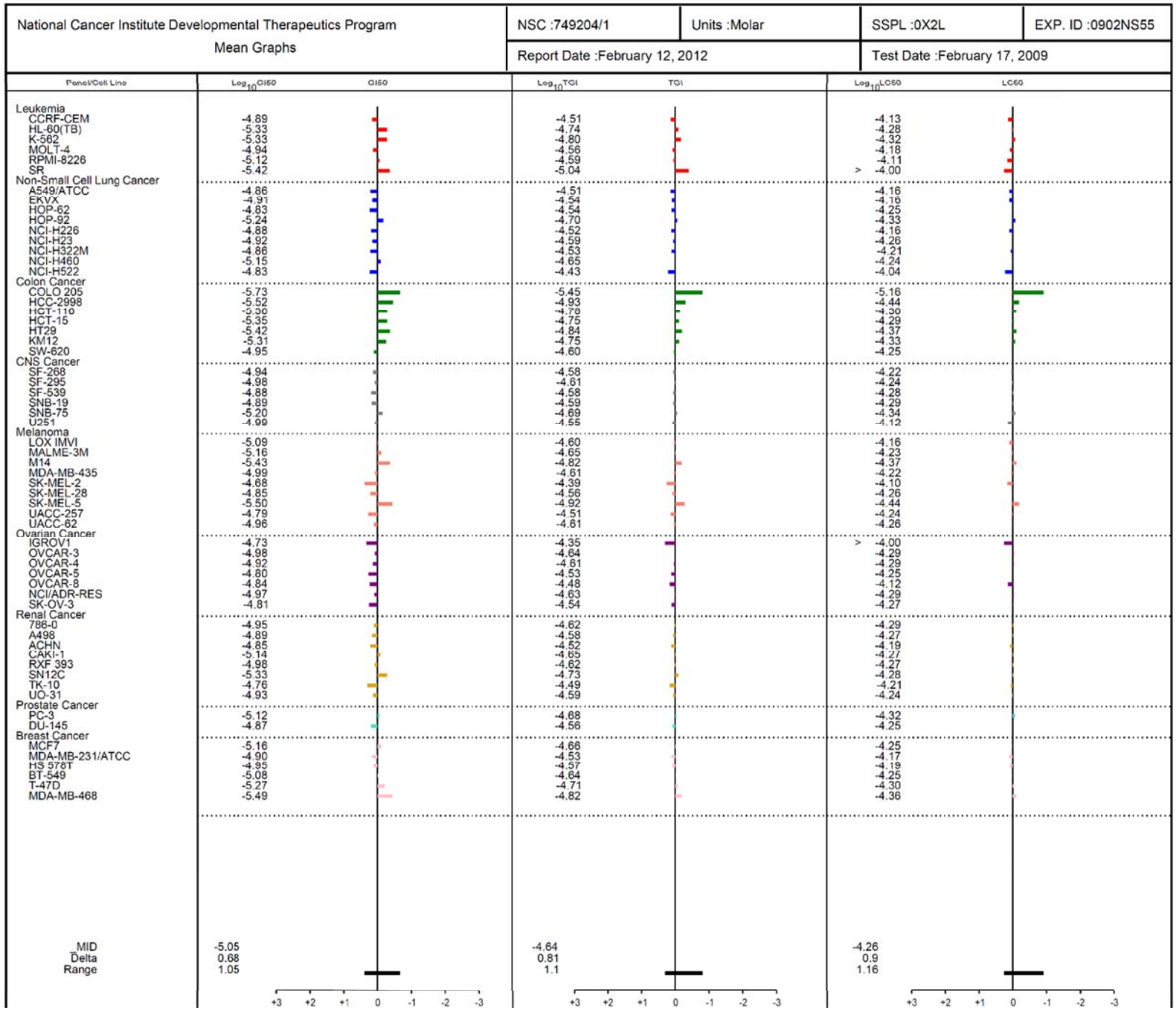

National Cancer Institute Developmental Therapeutics Program
Dose Response Curves
NSC: 749204 / 1
Report Date: February 12, 2012
SSPL: 0X2L
Test Date: February 17, 2009
EXP. ID: 0902NS55
Leukemia
Non-Small Cell Lung Cancer
Colon Cancer
CNS Cancer
Melanoma
Ovarian Cancer
Renal Cancer
Prostate Cancer
Breast Cancer
Percentage Growth
Log10 of Sample Concentration (Molar)
CCRF-CEM
HL-60(TB)
K-562
MOLT-4
RPMI-8226
SR
A549/ATCC
EKVX
HOP-62
NCI-H322M
NCI-H460
NCI-H522
COLO 205
HCC-2998
HCT-116
SW-620
SF-268
SF-295
SF-539
SNB-19
SNB-75
U251
LOX IMVI
MALME-3M
M14
MDA-MB-435
SK-MEL-2
SK-MEL-28
SK-MEL-5
UACC-257
UACC-62
IGROV1
OVCAR-3
OVCAR-4
OVCAR-5
OVCAR-8
NCI/ADR-RES
SK-OV-3
786-0
A498
ACHN
CAKI-1
RXF 393
SN12C
TK-10
UO-31
PC-3
DU-145
MCF7
MDA-MB-231/
HS 578T
BT-549
T-47D
MDA-MB-468

National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

NSC : 749204 / 1	Experiment ID : 0902NS55	Test Type : 08	Units : Molar
Report Date : February 12, 2012	Test Date : February 17, 2009	QNS :	MC :
COMI : LSC-JHJ-I-150-1 (81538)	Stain Reagent : SRB Dual-Pass Related	SSPL : 0X2L	

Log10 Concentration

Panel/Cell Line	Time Zero	Mean Optical Densities Ctrl	-8.0	-7.0	-6.0	-5.0	-4.0	Percent Growth -8.0	-7.0	-6.0	-5.0	-4.0	GI50	TGI	LC50
Leukemia															
CCRF-CEM	0.568	1.798	1.699	1.753	1727	1.354	0.185	92	96	94	64	-68	1.28E-5	307E-5	7.36E-5
HL-60(TB)	0.660	1.253	1.323	1.276	1218	0.829	0.129	112	104	94	28	-81	4.69E-6	182E-5	5.25E-5
K-562	0.229	1.171	1.239	1.284	1232	0.433	0.037	107	112	107	22	-84	4.63E-6	160E-5	4.78E-5
MOLT-4	0.524	1.392	1.440	1.526	1673	1.026	0.135	105	115	132	58	-74	1.15E-5	274E-5	6.54E-5
RPMI-8226	0.559	1.672	1.712	1.761	1714	1.033	0.214	104	108	104	43	-62	7.57E-6	256E-5	7.72E-5
SR	0.208	0.513	0.526	0.581	0598	0.199	0.125	102	120	126	-5	-40	3.81E-6	922E-6	> 1.00E-4
Non-Small Cell Lung Cancer															
A549/ATCC	0.242	1.163	1.207	1.228	1178	0.895	0.065	105	107	102	71	-73	1.40E-5	310E-5	6.91E-5
EKVX	0.628	1.445	1.421	1.342	1282	1.133	0.176	97	87	80	62	-72	1.22E-5	289E-5	6.84E-5
HOP-62	0.512	1.153	1.138	1.178	1132	1.027	0.034	98	104	97	80	-93	1.50E-5	290E-5	5.62E-5
HOP-92	0.836	1.267	1.229	1.236	1167	1.015	0.033	91	93	77	42	-96	5.76E-6	200E-5	4.62E-5
NCI-H226	0.727	1.463	1.449	1.373	1377	1.218	0.201	98	88	88	67	-72	1.32E-5	302E-5	6.91E-5
NCI-H23	0.532	1.764	1.753	1.714	1678	1.307	0.058	99	96	93	63	-89	1.22E-5	259E-5	5.53E-5
NCI-H322M	0.646	1.480	1.559	1.519	1493	1.246	0.118	110	105	102	72	-82	1.39E-5	294E-5	6.21E-5
NCI-H460	0.206	2.115	2.211	2.143	2112	0.997	0.044	105	101	100	41	-79	7.13E-6	221E-5	5.77E-5
NCI-H522	0.310	1.859	1.908	1.804	1739	1.424	0.141	103	96	92	72	-55	1.49E-5	370E-5	9.18E-5
Colon Cancer															
COLO 205	0.275	0.897	0.959	0.943	0890	0.057	0.015	110	107	99	-79	-95	1.88E-6	359E-6	6.85E-6
HCC-2998	0.744	2.842	2.828	2.755	2624	0.889	0.039	99	96	90	7	-95	3.01E-6	117E-5	3.62E-5
HCT-116	0.199	1.484	1.399	1.452	1416	0.525	0.016	93	98	95	25	-92	4.41E-6	164E-5	4.37E-5
HCT-15	0.448	2.365	2.325	2.304	2181	0.981	0.079	98	97	90	28	-82	4.42E-6	179E-5	5.08E-5
HT29	0.137	0.967	0.976	0.980	0918	0.285	0.014	101	102	94	18	-90	3.78E-6	146E-5	4.25E-5
KM12	0.205	1.064	1.094	1.053	1037	0.456	0.023	103	99	97	29	-89	4.92E-6	176E-5	4.68E-5
SW-620	0.223	1.279	1.205	1.191	1168	0.820	0.031	93	92	89	57	-86	1.11E-5	249E-5	5.58E-5
CNS Cancer															
SF-268	0.406	1.430	1.389	1.394	1325	1.004	0.079	96	96	90	58	-81	1.15E-5	263E-5	6.02E-5
SF-295	0.773	1.903	1.768	1.735	1679	1.370	0.133	88	85	80	53	-83	1.05E-5	245E-5	5.72E-5
SF-539	0.529	1.740	1.703	1.679	1636	1.375	0.023	97	95	91	70	-96	1.32E-5	264E-5	5.29E-5
SNB-19	0.666	1.645	1.607	1.623	1548	1.345	0.009	96	98	90	69	-99	1.30E-5	258E-5	5.13E-5
SNB-75	0.612	1.170	1.073	1.064	1025	0.857	0.012	83	81	74	44	-98	6.24E-6	204E-5	4.58E-5
U251	0.177	1.047	1.061	1.022	0980	0.620	0.065	102	97	92	51	-63	1.02E-5	279E-5	7.65E-5
Melanoma															
LOX IMVI	0.231	1.611	1.593	1.594	1531	0.860	0.072	99	99	94	46	-69	8.10E-6	250E-5	6.84E-5
MALME-3M	0.783	1.449	1.451	1.448	1415	1.058	0.179	100	100	95	41	-77	6.89E-6	223E-5	5.90E-5
M14	0.315	1.245	1.188	1.206	1167	0.494	0.031	94	96	92	19	-90	3.75E-6	150E-5	4.28E-5
MDA-MB-435	0.429	1.744	1.593	1.542	1547	1.098	0.093	89	85	85	51	-78	1.02E-5	248E-5	6.04E-5
SK-MEL-2	0.369	0.827	0.888	0.896	0917	0.854	0.122	113	115	119	106	-67	2.10E-5	410E-5	7.98E-5
SK-MEL-28	0.504	1.412	1.401	1.421	1380	1.182	0.035	99	101	96	75	-93	1.40E-5	278E-5	5.53E-5
SK-MEL-5	0.358	1.647	1.511	1.547	1550	0.462	0.014	89	92	92	8	-96	3.19E-6	120E-5	3.61E-5
UACC-257	0.837	1.584	1.648	1.650	1596	1.501	0.060	109	109	102	89	-93	1.64E-5	308E-5	5.81E-5
UACC-62	0.522	2.012	1.848	1.816	1777	1.353	0.069	89	87	84	56	-87	1.10E-5	246E-5	5.51E-5
Ovarian Cancer															
IGROV1	0.198	1.371	1.640	1.682	1549	1.208	0.107	123	126	115	86	-46	1.88E-5	449E-5	> 1.00E-4
OVCAR-3	0.405	1.242	1.243	1.209	1187	0.843	0.032	100	96	93	52	-92	1.04E-5	230E-5	5.11E-5
OVCAR-4	0.459	1.433	1.442	1.392	1435	1.067	0.017	101	95	100	62	-96	1.19E-5	247E-5	5.10E-5
OVCAR-5	0.383	0.945	0.914	0.910	0909	0.870	0.014	94	94	93	86	-96	1.58E-5	297E-5	5.58E-5
OVCAR-8	0.228	0.929	0.937	0.968	0954	0.738	0.078	101	106	104	73	-66	1.46E-5	334E-5	7.67E-5
NCI/ADR-RES	0.344	1.259	1.255	1.263	1203	0.843	0.023	100	100	94	55	-93	1.07E-5	234E-5	5.09E-5
SK-OV-3	0.450	1.125	1.182	1.144	1133	1.025	0.006	108	103	101	85	-99	1.55E-5	290E-5	5.43E-5
Renal Cancer															
786-0	0.947	2.397	2.418	2.354	2344	1.784	0.055	101	97	96	58	-94	1.12E-5	240E-5	5.11E-5
A498	0.846	1.509	1.475	1.422	1380	1.294	0.051	95	87	80	68	-94	1.29E-5	262E-5	5.34E-5
ACHN	0.340	1.337	1.342	1.320	1304	1.058	0.073	101	98	97	72	-79	1.40E-5	300E-5	6.45E-5
CAKI-1	0.722	1.845	1.712	1.621	1580	1.235	0.104	88	80	76	46	-86	7.23E-6	223E-5	5.36E-5
RXF 393	0.685	1.284	1.346	1.358	1318	1.006	0.075	110	112	106	54	-89	1.06E-5	237E-5	5.32E-5
SN12C	0.335	1.231	1.228	1.168	1158	0.600	0.063	100	93	92	30	-81	4.69E-6	185E-5	5.23E-5
TK-10	0.877	1.375	1.388	1.414	1385	1.337	0.114	102	108	102	92	-87	1.72E-5	327E-5	6.21E-5
UO-31	0.259	1.269	1.251	1.292	1203	0.866	0.040	98	102	93	60	-85	1.17E-5	260E-5	5.76E-5
Prostate Cancer															
PC-3	0.349	1.125	1.140	1.090	1061	0.694	0.017	102	95	92	44	-95	7.61E-6	208E-5	4.75E-5
DU-145	0.337	1.401	1.379	1.346	1323	1.086	0.032	98	95	93	70	-91	1.34E-5	274E-5	5.60E-5
Breast Cancer															
MCF7	0.295	1.592	1.575	1.487	1524	0.833	0.058	99	92	95	41	-81	6.91E-6	219E-5	5.62E-5
MDA-MB-231/ATCC	0.326	0.927	0.930	0.918	0913	0.711	0.087	100	98	98	64	-73	1.27E-5	293E-5	6.77E-5
HS 578T	0.630	1.152	1.110	1.120	1109	0.927	0.161	92	94	92	57	-75	1.13E-5	271E-5	6.51E-5
BT-549	0.995	1.483	1.518	1.533	1487	1.220	0.186	106	109	100	46	-81	8.34E-6	229E-5	5.67E-5
T-47D	0.740	1.533	1.458	1.436	1443	1.026	0.102	90	87	88	36	-86	5.34E-6	196E-5	5.04E-5
MDA-MB-468	0.456	1.207	1.147	1.097	1067	0.608	0.046	92	85	81	20	-90	3.25E-6	153E-5	4.34E-5


National Cancer Institute Developmental Therapeutics Program
Mean Graphs
NSC :749204/1
Units :Molar
SSPL :0X2L
EXP. ID :0902NS55
Report Date :February 12, 2012
Test Date :February 17, 2009
Panel/Cell Line
Log10GI50
GI50
Log10TGI
TGI
Log10LC50
LC50
Leukemia
CCRF-CEM -4.89 -4.51 -4.13
HL-60(TB) -5.33 -4.74 -4.28
K-562 -5.33 -4.80 -4.32
MOLT-4 -4.94 -4.56 -4.18
RPMI-8226 -5.12 -4.59 -4.11
SR -5.42 -5.04 > -4.00
Non-Small Cell Lung Cancer
A549/ATCC -4.86 -4.51 -4.16
EKVX -4.91 -4.54 -4.16
HOP-62 -4.83 -4.54 -4.25
HOP-92 -5.24 -4.70 -4.33
NCI-H226 -4.88 -4.52 -4.16
NCI-H23 -4.92 -4.59 -4.26
NCI-H322M -4.86 -4.53 -4.21
NCI-H460 -5.15 -4.65 -4.24
NCI-H522 -4.83 -4.43 -4.04
Colon Cancer
COLO 205 -5.73 -5.45 -5.16
HCC-2998 -5.52 -4.93 -4.44
HCT-116 -5.36 -4.79 -4.36
HCT-15 -5.35 -4.75 -4.29
HT29 -5.42 -4.84 -4.37
KM12 -5.31 -4.75 -4.33
SW-620 -4.95 -4.60 -4.25
CNS Cancer
SF-268 -4.94 -4.58 -4.22
SF-295 -4.98 -4.61 -4.24
SF-539 -4.88 -4.58 -4.28
SNB-19 -4.89 -4.59 -4.29
SNB-75 -5.20 -4.69 -4.34
U251 -4.99 -4.55 -4.12
Melanoma
LOX IMVI -5.09 -4.60 -4.16
MALME-3M -5.16 -4.65 -4.23
M14 -5.43 -4.82 -4.37
MDA-MB-435 -4.99 -4.61 -4.22
SK-MEL-2 -4.68 -4.39 -4.10
SK-MEL-28 -4.85 -4.56 -4.26
SK-MEL-5 -5.50 -4.92 -4.44
UACC-257 -4.79 -4.51 -4.24
UACC-62 -4.96 -4.61 -4.26
Ovarian Cancer
IGROV1 -4.73 -4.35 > -4.00
OVCAR-3 -4.98 -4.64 -4.29
OVCAR-4 -4.92 -4.61 -4.29
OVCAR-5 -4.80 -4.53 -4.25
OVCAR-8 -4.84 -4.48 -4.12
NCI/ADR-RES -4.97 -4.63 -4.29
SK-OV-3 -4.81 -4.54 -4.27
Renal Cancer
786-0 -4.95 -4.62 -4.29
A498 -4.89 -4.58 -4.27
ACHN -4.85 -4.52 -4.19
CAKI-1 -5.14 -4.65 -4.27
RXF 393 -4.98 -4.62 -4.27
SN12C -5.33 -4.73 -4.28
TK-10 -4.76 -4.49 -4.21
UO-31 -4.93 -4.59 -4.24
Prostate Cancer
PC-3 -5.12 -4.68 -4.32
DU-145 -4.87 -4.56 -4.25
Breast Cancer
MCF7 -5.16 -4.66 -4.25
MDA-MB-231/ATCC -4.90 -4.53 -4.17
HS 578T -4.95 -4.57 -4.19
BT-549 -5.08 -4.64 -4.25
T-47D -5.27 -4.71 -4.30
MDA-MB-468 -5.49 -4.82 -4.36
MID -5.05 -4.64 -4.26
Delta 0.68 0.81 0.9
Range 1.05 1.1 1.16
+3 +2 +1 0 -1 -2 -3

5.5. Appendix C

One and five dose experimental data from 60 cell line

-Cyclic sulfamide compounds-

One dose experimental data of compound 4.21 (NSC 764190)

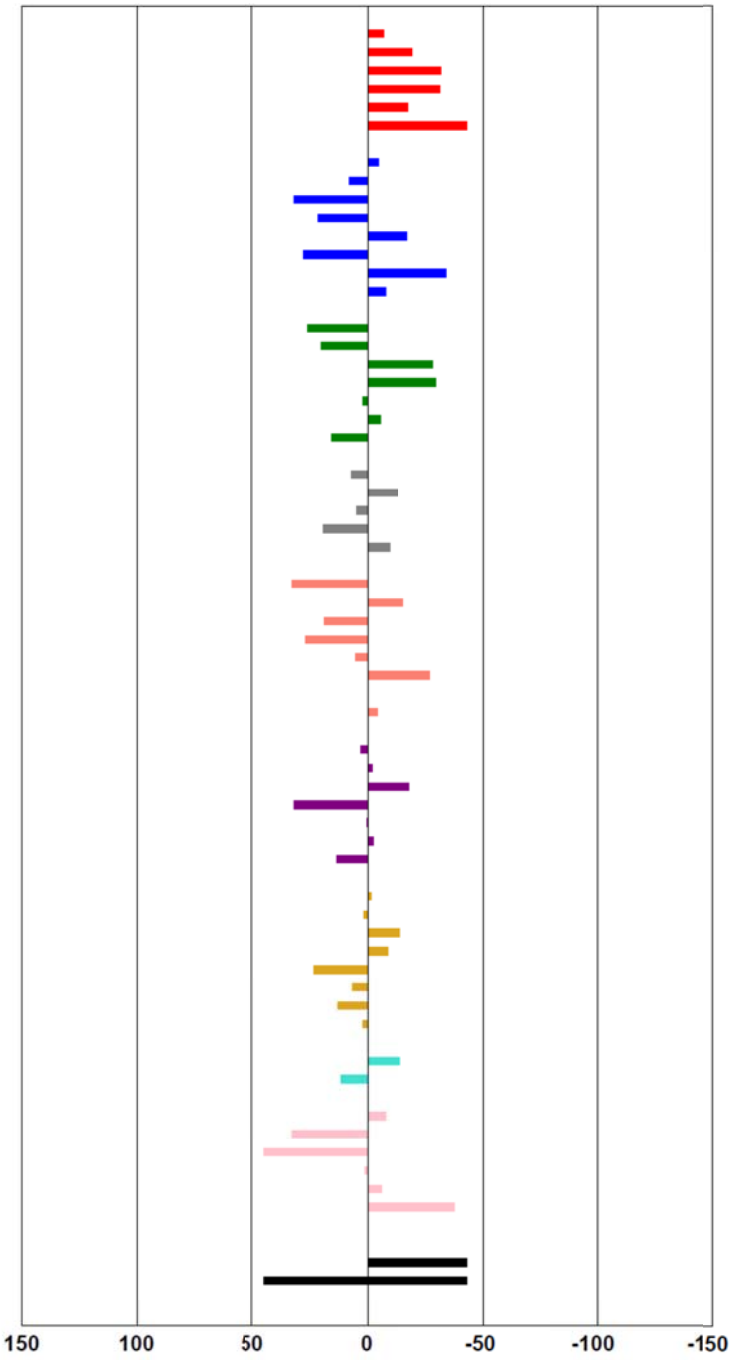
Developmental Therapeutics Program

One Dose Mean Graph

NSC: D-764190 / 1	Conc: 1.00E-5 Molar	Test Date: Mar 19, 2012
Experiment ID: 1203OS38		Report Date: May 09, 2013

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	48.50
HL-60(TB)	35.73
K-562	22.97
MOLT-4	23.72
RPMI-8226	37.48
SR	11.87
Non-Small Cell Lung Cancer	
A549/ATCC	50.80
HOP-62	62.98
HOP-92	86.58
NCI-H226	76.36
NCI-H23	37.91
NCI-H322M	82.88
NCI-H460	20.82
NCI-H522	47.49
Colon Cancer	
COLO 205	80.98
HCC-2998	75.36
HCT-116	26.78
HCT-15	25.36
HT29	57.41
KM12	49.96
SW-620	70.58
CNS Cancer	
SF-268	62.25
SF-295	41.82
SF-539	59.87
SNB-19	74.07
U251	45.72
Melanoma	
MALME-3M	87.71
M14	39.38
MDA-MB-435	73.86
SK-MEL-2	81.79
SK-MEL-28	60.47
SK-MEL-5	27.89
UACC-257	55.24
UACC-62	51.05
Ovarian Cancer	
IGROV1	58.05
OVCAR-3	53.26
OVCAR-4	37.00
OVCAR-5	86.88
OVCAR-8	55.54
NCI/ADR-RES	52.89
SK-OV-3	68.49
Renal Cancer	
786-0	53.83
A498	57.01
ACHN	41.03
CAKI-1	46.76
RXF 393	78.05
SN12C	61.78
TK-10	68.15
UO-31	57.14
Prostate Cancer	
PC-3	40.87
DU-145	66.80
Breast Cancer	
MCF7	47.43
MDA-MB-231/ATCC	87.54
HS 578T	99.55
BT-549	56.36
T-47D	49.45
MDA-MB-468	17.38
Mean	55.00
Delta	43.13
Range	87.78

Mean Growth Percent - Growth Percent



One dose experimental data of compound 4.22 (NSC 751486)

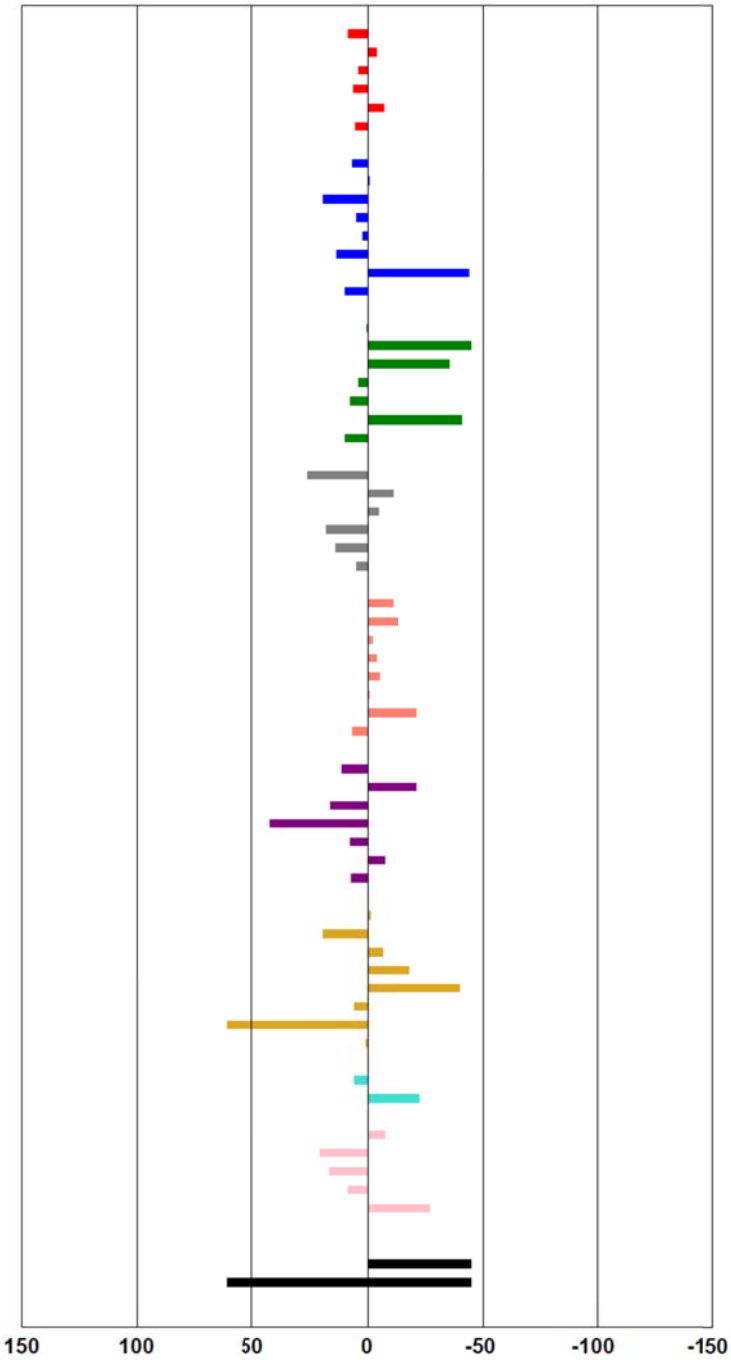
Developmental Therapeutics Program

One Dose Mean Graph

NSC: 751486 / 1	Conc: 1.00E-5 Molar	Test Date: Nov 09, 2009
Experiment ID: 0911OS45		Report Date: Feb 15, 2012

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	7.73
HL-60(TB)	-4.17
K-562	3.12
MOLT-4	5.55
RPMI-8226	-7.20
SR	4.45
Non-Small Cell Lung Cancer	
A549/ATCC	6.12
EKVX	-1.30
HOP-62	18.38
HOP-92	4.20
NCI-H23	1.32
NCI-H322M	12.53
NCI-H460	-44.82
NCI-H522	9.21
Colon Cancer	
COLO 205	-0.27
HCC-2998	-45.75
HCT-116	-36.49
HCT-15	3.16
HT29	6.95
KM12	-41.58
SW-620	9.22
CNS Cancer	
SF-268	25.28
SF-295	-11.23
SF-539	-5.16
SNB-19	17.04
SNB-75	133.1
U251	4.33
Melanoma	
LOX IMVI	-11.35
MALME-3M	-14.17
M14	-2.40
MDA-MB-435	-4.20
SK-MEL-2	-5.58
SK-MEL-5	-1.02
UACC-257	-22.12
UACC-62	5.81
Ovarian Cancer	
IGROV1	10.35
OVCAR-3	-22.03
OVCAR-4	15.38
OVCAR-5	41.06
OVCAR-8	7.06
NCI/ADR-RES	-7.86
SK-OV-3	6.58
Renal Cancer	
786-0	-1.43
A498	18.54
ACHN	-7.05
CAKI-1	-19.13
RXF 393	-40.80
SN12C	5.15
TK-10	60.35
UO-31	0.00
Prostate Cancer	
PC-3	4.89
DU-145	-23.44
Breast Cancer	
MDA-MB-231/ATCC	-7.98
HS 578T	19.57
BT-549	15.57
T-47D	7.87
MDA-MB-468	-27.71
Mean	-.81
Delta	44.94
Range	106.10

Mean Growth Percent - Growth Percent



One dose experimental data of compound 4.23 (NSC 751478)

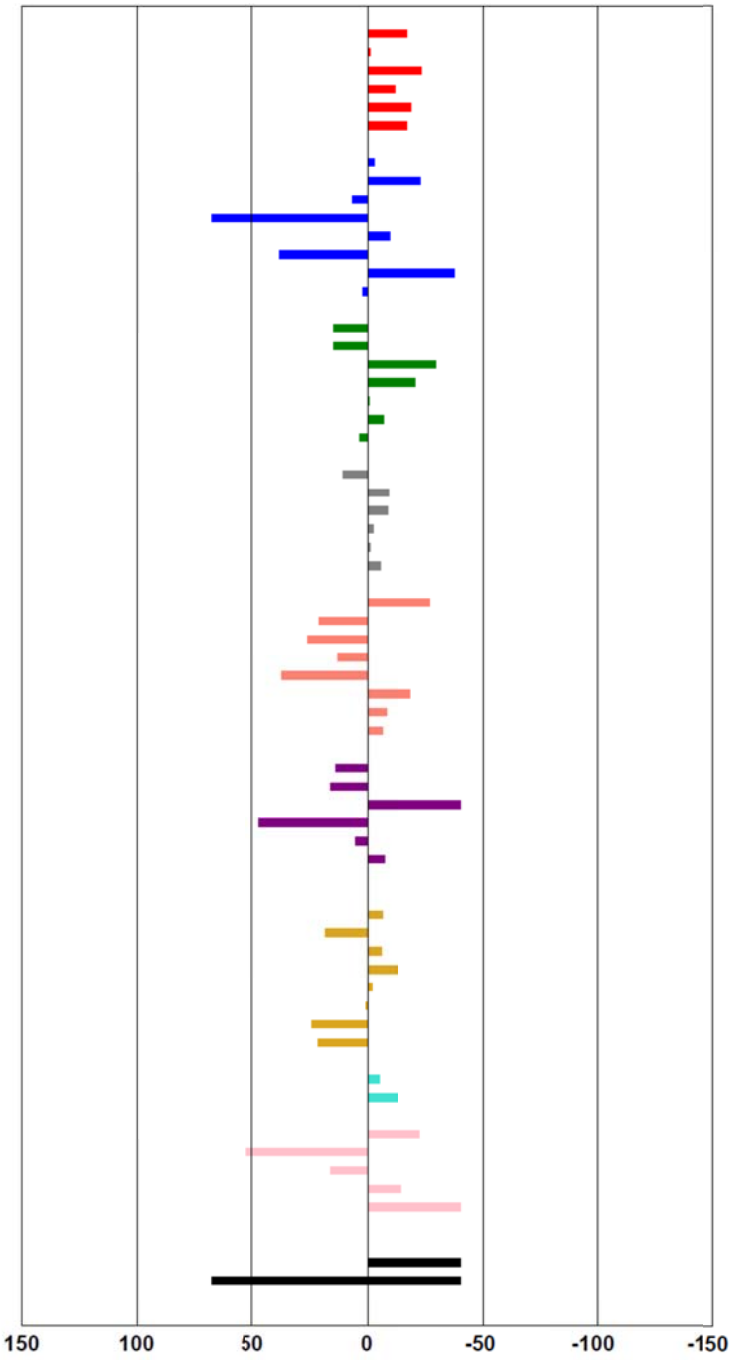
Developmental Therapeutics Program

One Dose Mean Graph

NSC: 751478 / 1	Conc: 1.00E-5 Molar	Test Date: Nov 09, 2009
Experiment ID: 0911OS45		Report Date: May 09, 2013

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	29.49
HL-60(TB)	45.99
K-562	23.35
MOLT-4	35.44
RPMI-8226	27.88
SR	29.56
Non-Small Cell Lung Cancer	
A549/ATCC	44.14
EKVX	24.02
HOP-62	53.53
HOP-92	114.43
NCI-H23	37.58
NCI-H322M	84.89
NCI-H460	9.31
NCI-H522	49.28
Colon Cancer	
COLO 205	61.75
HCC-2998	61.42
HCT-116	17.02
HCT-15	26.08
HT29	46.58
KM12	40.22
SW-620	50.61
CNS Cancer	
SF-268	57.80
SF-295	38.01
SF-539	38.52
SNB-19	44.90
SNB-75	46.27
U251	41.45
Melanoma	
LOX IMVI	19.61
MALME-3M	67.81
M14	72.50
MDA-MB-435	60.07
SK-MEL-2	84.06
SK-MEL-5	28.27
UACC-257	39.09
UACC-62	40.76
Ovarian Cancer	
IGROV1	60.50
OVCAR-3	62.80
OVCAR-4	6.50
OVCAR-5	93.51
OVCAR-8	52.43
NCI/ADR-RES	40.01
SK-OV-3	47.03
Renal Cancer	
786-0	40.61
A498	65.42
ACHN	41.23
CAKI-1	33.75
RXF 393	45.18
SN12C	47.82
TK-10	71.05
UO-31	68.14
Prostate Cancer	
PC-3	42.04
DU-145	34.26
Breast Cancer	
MDA-MB-231/ATCC	24.21
HS 578T	98.95
BT-549	63.07
T-47D	32.49
MDA-MB-468	6.52
Mean	46.84
Delta	40.32
Range	107.91

Mean Growth Percent - Growth Percent



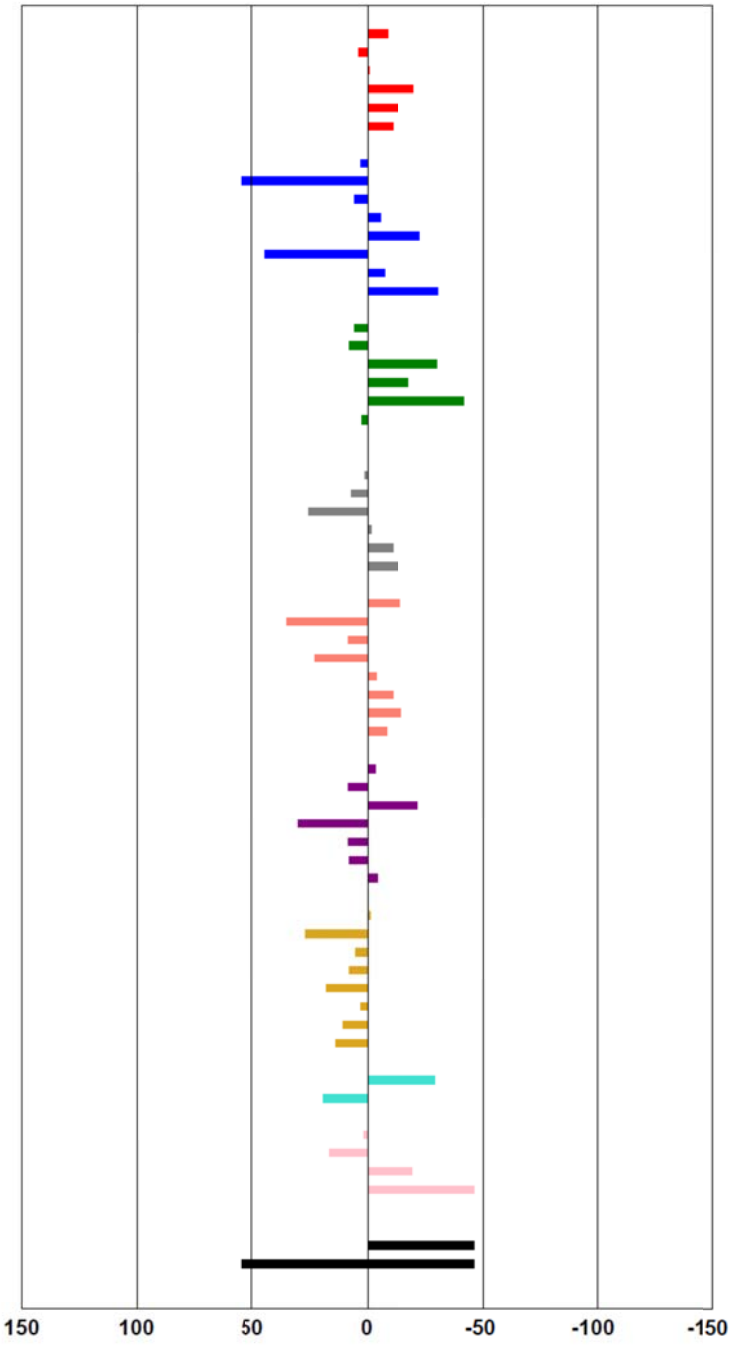
One dose experimental data of compound 4.27 (NSC 751468)

Developmental Therapeutics Program

One Dose Mean Graph

NSC: 751468 / 1	Conc: 7.50E-6 Molar	Test Date: Nov 09, 2009
Experiment ID: 0911OS45		Report Date: Feb 15, 2012

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	77.42
HL-60(TB)	89.96
K-562	85.33
MOLT-4	65.92
RPMI-8226	72.45
SR	75.23
Non-Small Cell Lung Cancer	
A549/ATCC	89.05
EKVX	140.80
HOP-62	91.45
HOP-92	80.33
NCI-H23	63.04
NCI-H322M	129.84
NCI-H460	78.74
NCI-H522	55.30
Colon Cancer	
COLO 205	91.53
HCC-2998	93.58
HCT-116	55.58
HCT-15	68.08
HT29	44.03
KM12	88.44
SW-620	85.83
CNS Cancer	
SF-268	87.18
SF-295	93.07
SF-539	111.06
SNB-19	84.58
SNB-75	75.34
U251	72.36
Melanoma	
LOX IMVI	71.50
MALME-3M	120.76
M14	94.37
MDA-MB-435	108.39
SK-MEL-2	82.54
SK-MEL-5	75.07
UACC-257	71.15
UACC-62	77.83
Ovarian Cancer	
IGROV1	82.56
OVCAR-3	94.53
OVCAR-4	63.95
OVCAR-5	115.49
OVCAR-8	94.16
NCI/ADR-RES	93.82
SK-OV-3	82.03
Renal Cancer	
786-0	85.08
A498	112.48
ACHN	91.31
CAKI-1	93.73
RXF 393	103.49
SN12C	89.15
TK-10	96.46
UO-31	99.55
Prostate Cancer	
PC-3	56.40
DU-145	105.13
Breast Cancer	
MDA-MB-231/ATCC	87.83
HS 578T	102.28
T-47D	66.39
MDA-MB-468	39.55
Mean	85.77
Delta	46.12
Range	101.15



One dose experimental data of compound 4.28 (NSC 751469)

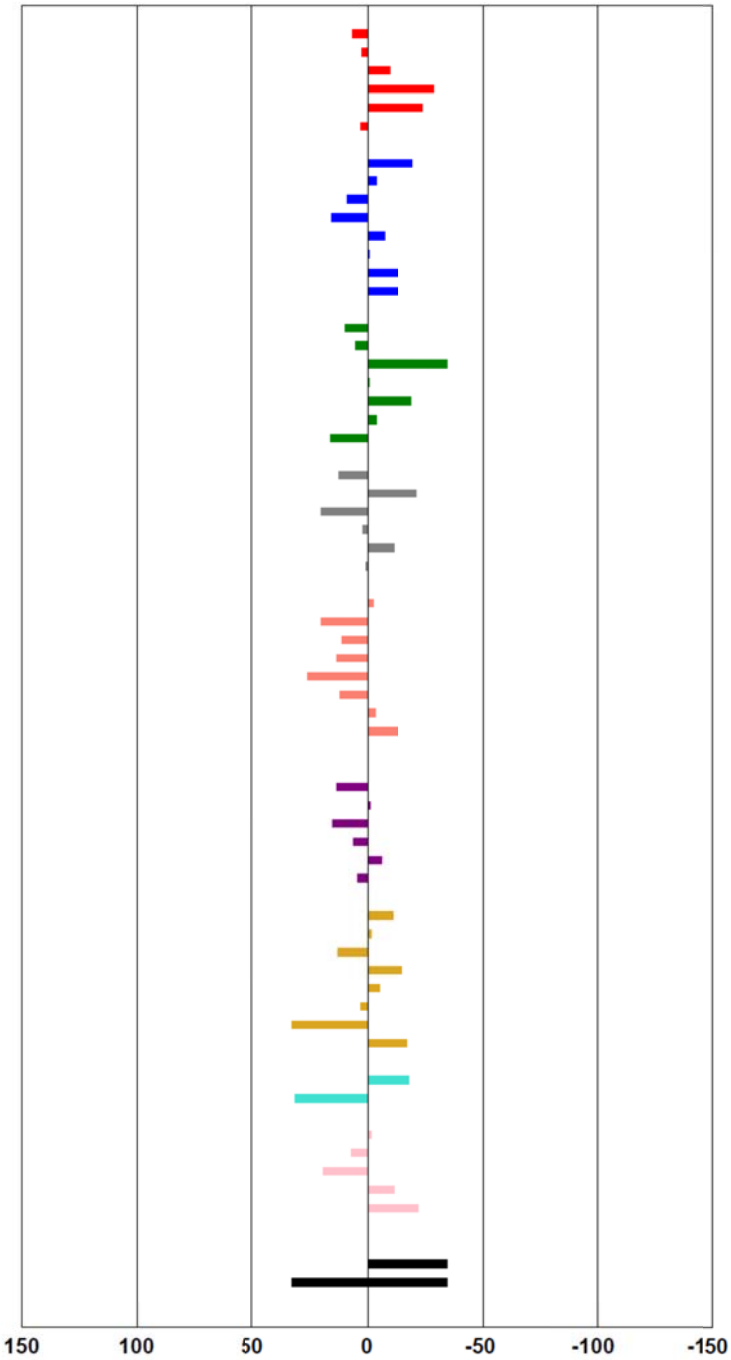
Developmental Therapeutics Program

One Dose Mean Graph

NSC: 751469 / 1	Conc: 2.00E-6 Molar	Test Date: Nov 09, 2009
Experiment ID: 0911OS45		Report Date: Feb 15, 2012

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	88.10
HL-60(TB)	83.96
K-562	71.75
MOLT-4	52.29
RPMI-8226	57.41
SR	84.32
Non-Small Cell Lung Cancer	
A549/ATCC	61.57
EKVX	77.56
HOP-62	89.99
HOP-92	97.09
NCI-H23	74.35
NCI-H322M	80.91
NCI-H460	68.09
NCI-H522	68.09
Colon Cancer	
COLO 205	91.05
HCC-2998	86.43
HCT-116	46.61
HCT-15	80.82
HT29	62.18
KM12	77.75
SW-620	97.40
CNS Cancer	
SF-268	93.73
SF-295	59.81
SF-539	101.19
SNB-19	83.71
SNB-75	70.29
U251	82.39
Melanoma	
LOX IMVI	79.07
MALME-3M	101.49
M14	92.44
MDA-MB-435	94.86
SK-MEL-2	107.32
SK-MEL-5	93.32
UACC-257	78.11
UACC-62	67.87
Ovarian Cancer	
IGROV1	81.11
OVCAR-3	94.57
OVCAR-4	80.57
OVCAR-5	96.29
OVCAR-8	87.36
NCI/ADR-RES	75.36
SK-OV-3	85.58
Renal Cancer	
786-0	70.75
A498	79.91
ACHN	94.28
CAKI-1	66.15
RXF 393	76.61
SN12C	84.36
TK-10	113.74
UO-31	63.81
Prostate Cancer	
PC-3	62.95
DU-145	112.35
Breast Cancer	
MDA-MB-231/ATCC	80.04
HS 578T	88.42
BT-549	100.27
T-47D	70.27
MDA-MB-468	59.03
Mean	81.18
Delta	34.57
Range	67.13

Mean Growth Percent - Growth Percent



One dose experimental data of compound 4.29 (NSC 751470)

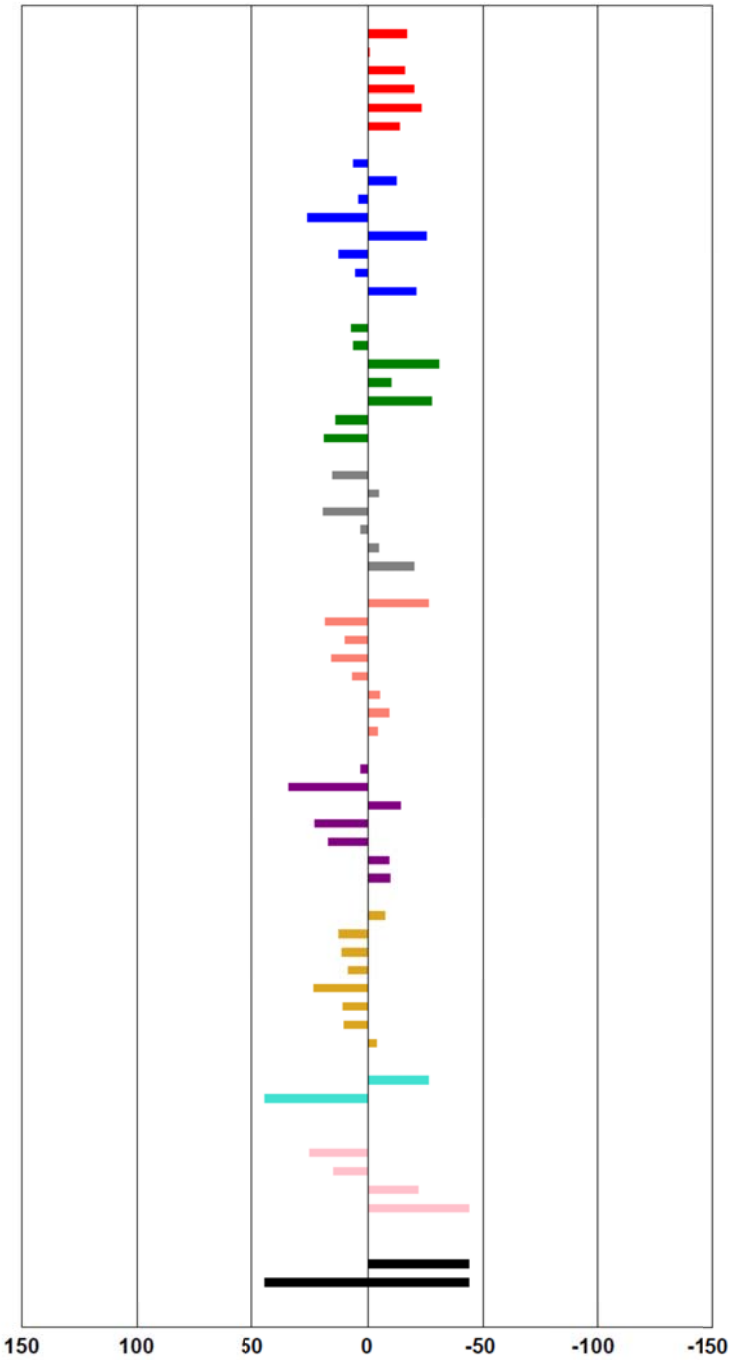
Developmental Therapeutics Program

One Dose Mean Graph

NSC: 751470 / 1	Conc: 5.00E-6 Molar	Test Date: Nov 09, 2009
Experiment ID: 0911OS45		Report Date: Feb 15, 2012

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	62.40
HL-60(TB)	78.96
K-562	62.96
MOLT-4	59.30
RPMI-8226	56.11
SR	65.36
Non-Small Cell Lung Cancer	
A549/ATCC	85.88
EKVX	67.50
HOP-62	83.39
HOP-92	105.35
NCI-H23	53.89
NCI-H322M	91.87
NCI-H460	84.96
NCI-H522	58.16
Colon Cancer	
COLO 205	86.61
HCC-2998	85.82
HCT-116	48.53
HCT-15	69.81
HT29	51.42
KM12	93.26
SW-620	98.10
CNS Cancer	
SF-268	94.73
SF-295	75.22
SF-539	98.77
SNB-19	82.80
SNB-75	75.11
U251	59.20
Melanoma	
LOX IMVI	52.97
MALME-3M	97.81
M14	89.57
MDA-MB-435	95.30
SK-MEL-2	86.43
SK-MEL-5	74.49
UACC-257	70.57
UACC-62	75.47
Ovarian Cancer	
IGROV1	82.47
OVCAR-3	113.21
OVCAR-4	64.94
OVCAR-5	102.24
OVCAR-8	96.58
NCI/ADR-RES	70.87
SK-OV-3	70.40
Renal Cancer	
786-0	72.50
A498	91.97
ACHN	90.47
CAKI-1	87.88
RXF 393	102.74
SN12C	90.06
TK-10	89.71
UO-31	75.97
Prostate Cancer	
PC-3	53.06
DU-145	123.55
Breast Cancer	
MDA-MB-231/ATCC	79.58
HS 578T	104.57
BT-549	94.39
T-47D	57.30
MDA-MB-468	35.50
Mean	79.44
Delta	43.94
Range	88.05

Mean Growth Percent - Growth Percent



One dose experimental data of compound 4.37c (NSC 764189)

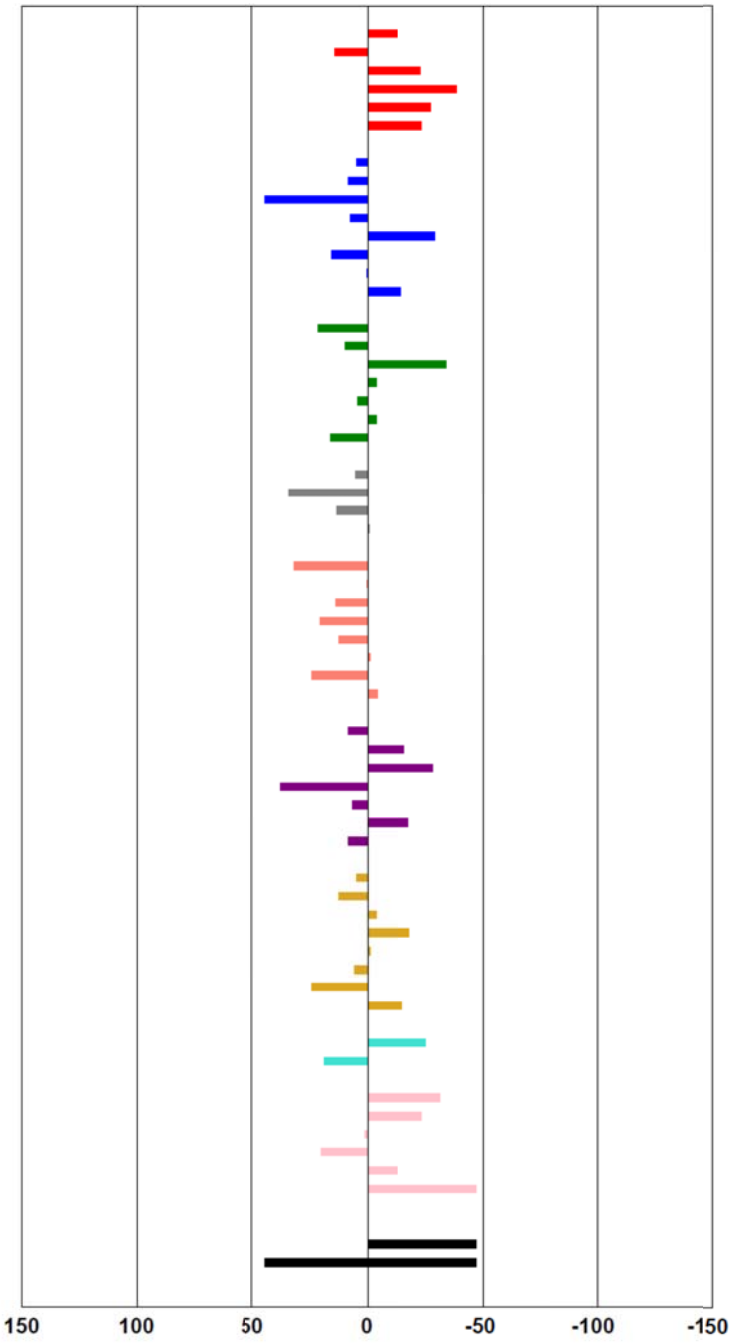
Developmental Therapeutics Program

One Dose Mean Graph

NSC: D-764189 / 1	Conc: 1.00E-5 Molar	Test Date: Mar 19, 2012
Experiment ID: 1203OS38		Report Date: Sep 19, 2012

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	52.98
HL-60(TB)	79.36
K-562	41.89
MOLT-4	26.35
RPMI-8226	37.59
SR	41.77
Non-Small Cell Lung Cancer	
A549/ATCC	70.01
HOP-62	73.89
HOP-92	109.35
NCI-H226	72.98
NCI-H23	35.88
NCI-H322M	80.99
NCI-H460	65.81
NCI-H522	50.39
Colon Cancer	
COLO 205	86.50
HCC-2998	74.98
HCT-116	31.01
HCT-15	61.53
HT29	69.76
KM12	61.54
SW-620	81.23
CNS Cancer	
SF-268	70.39
SF-539	99.00
SNB-19	78.70
U251	64.82
Melanoma	
MALME-3M	96.53
M14	65.52
MDA-MB-435	78.83
SK-MEL-2	85.83
SK-MEL-28	77.81
SK-MEL-5	64.49
UACC-257	89.34
UACC-62	61.26
Ovarian Cancer	
IGROV1	73.61
OVCAR-3	49.37
OVCAR-4	36.57
OVCAR-5	102.81
OVCAR-8	71.98
NCI/ADR-RES	47.57
SK-OV-3	73.77
Renal Cancer	
786-0	70.14
A498	77.50
ACHN	61.87
CAKI-1	46.78
RXF 393	64.41
SN12C	70.88
TK-10	89.25
UO-31	49.91
Prostate Cancer	
PC-3	39.75
DU-145	83.87
Breast Cancer	
MCF7	33.87
MDA-MB-231/ATCC	41.59
HS 578T	66.74
BT-549	85.05
T-47D	52.84
MDA-MB-468	18.03
Mean	65.12
Delta	47.09
Range	91.32

Mean Growth Percent - Growth Percent



One dose experimental data of compound 4.44 (NSC 751477)

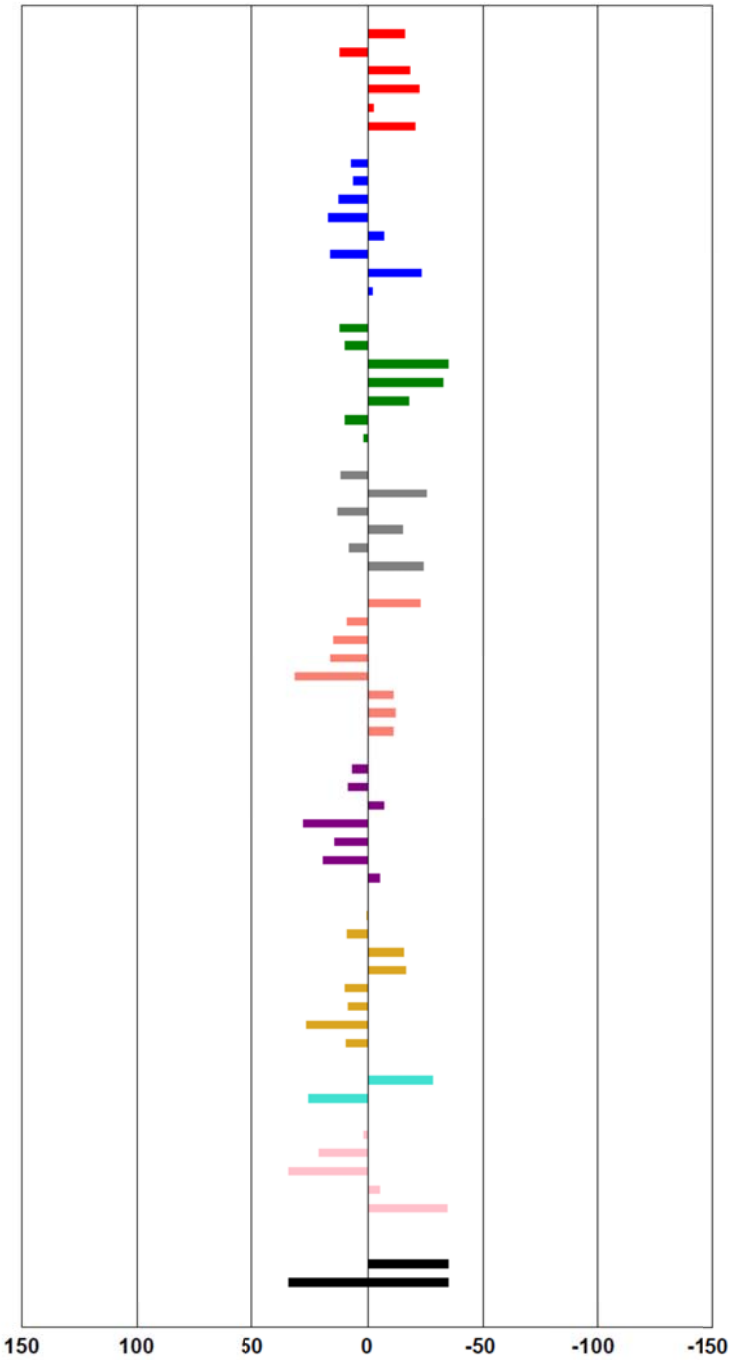
Developmental Therapeutics Program

One Dose Mean Graph

NSC: 751477 / 1	Conc: 3.75E-6 Molar	Test Date: Nov 09, 2009
Experiment ID: 0911OS45		Report Date: Feb 15, 2012

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	60.20
HL-60(TB)	88.52
K-562	58.00
MOLT-4	54.02
RPMI-8226	74.20
SR	55.81
Non-Small Cell Lung Cancer	
A549/ATCC	83.59
EKVX	82.59
HOP-62	89.11
HOP-92	93.21
NCI-H23	69.56
NCI-H322M	92.36
NCI-H460	52.72
NCI-H522	74.52
Colon Cancer	
COLO 205	88.50
HCC-2998	86.19
HCT-116	41.25
HCT-15	43.59
HT29	58.34
KM12	86.13
SW-620	78.21
CNS Cancer	
SF-268	87.96
SF-295	50.82
SF-539	89.59
SNB-19	61.12
SNB-75	84.45
U251	51.95
Melanoma	
LOX IMVI	53.21
MALME-3M	85.56
M14	91.36
MDA-MB-435	92.52
SK-MEL-2	107.84
SK-MEL-5	65.59
UACC-257	64.84
UACC-62	65.54
Ovarian Cancer	
IGROV1	83.12
OVCAR-3	84.95
OVCAR-4	69.58
OVCAR-5	104.22
OVCAR-8	90.53
NCI/ADR-RES	95.59
SK-OV-3	71.57
Renal Cancer	
786-0	76.77
A498	85.61
ACHN	60.41
CAKI-1	59.48
RXF 393	86.48
SN12C	85.16
TK-10	102.76
UO-31	85.79
Prostate Cancer	
PC-3	47.97
DU-145	101.83
Breast Cancer	
MDA-MB-231/ATCC	78.50
HS 578T	97.22
BT-549	110.24
T-47D	71.52
MDA-MB-468	41.58
Mean	76.40
Delta	35.15
Range	68.99

Mean Growth Percent - Growth Percent



One dose experimental data of compound 4.56 (NSC 751472)

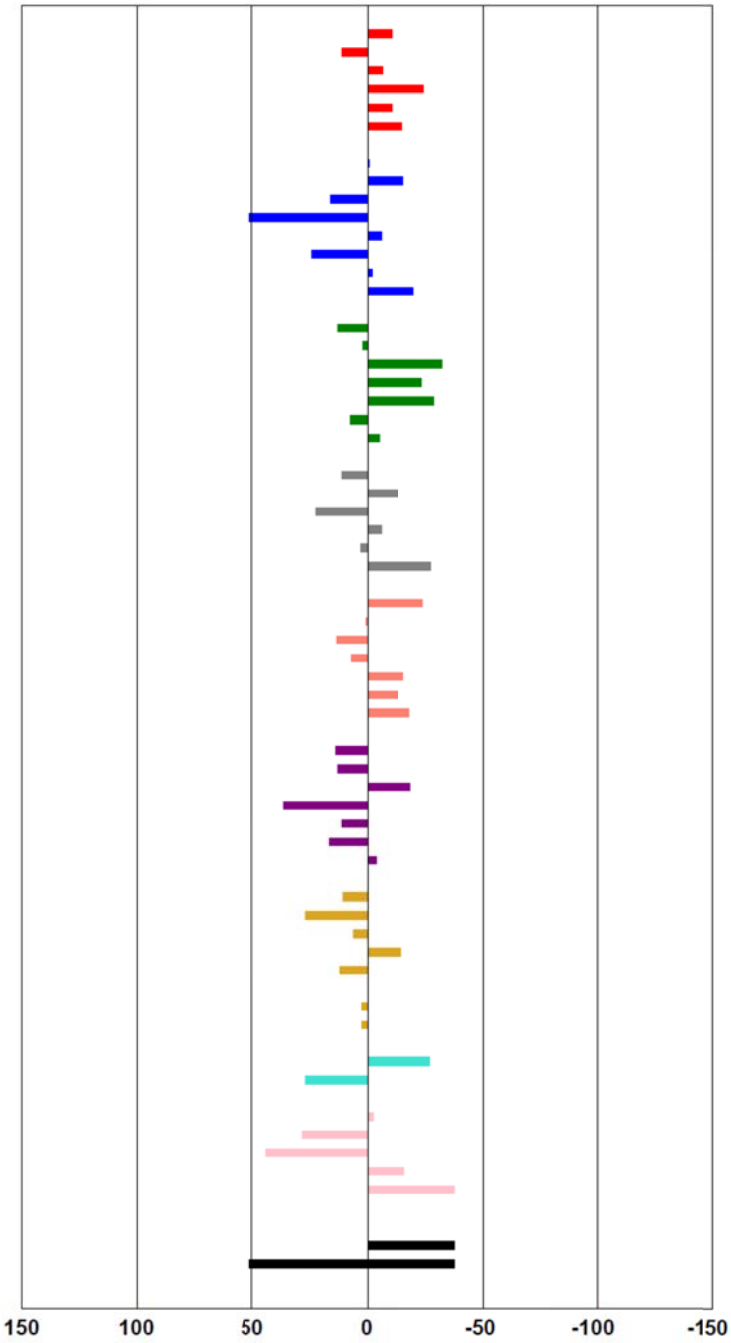
Developmental Therapeutics Program

One Dose Mean Graph

NSC: 751472 / 1	Conc: 5.00E-6 Molar	Test Date: Nov 09, 2009
Experiment ID: 0911OS45		Report Date: Feb 15, 2012

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	71.37
HL-60(TB)	92.83
K-562	75.53
MOLT-4	57.43
RPMI-8226	71.39
SR	66.53
Non-Small Cell Lung Cancer	
A549/ATCC	81.23
EKVX	66.11
HOP-62	97.77
HOP-92	132.58
NCI-H23	76.17
NCI-H322M	105.57
NCI-H460	79.85
NCI-H522	61.90
Colon Cancer	
COLO 205	94.75
HCC-2998	84.06
HCT-116	49.41
HCT-15	58.12
HT29	52.86
KM12	89.33
SW-620	76.91
CNS Cancer	
SF-268	92.71
SF-295	68.25
SF-539	104.00
SNB-19	76.18
SNB-75	84.59
U251	54.07
Melanoma	
LOX IMVI	57.51
MALME-3M	82.53
M14	95.06
MDA-MB-435	88.82
SK-MEL-5	66.09
UACC-257	68.50
UACC-62	63.48
Ovarian Cancer	
IGROV1	95.35
OVCAR-3	94.54
OVCAR-4	62.93
OVCAR-5	117.93
OVCAR-8	93.02
NCI/ADR-RES	98.18
SK-OV-3	78.00
Renal Cancer	
786-0	92.31
A498	108.50
ACHN	88.20
CAKI-1	67.20
RXF 393	93.50
SN12C	81.59
TK-10	84.39
UO-31	84.43
Prostate Cancer	
PC-3	54.56
DU-145	108.41
Breast Cancer	
MDA-MB-231/ATCC	79.35
HS 578T	109.95
BT-549	125.44
T-47D	65.79
MDA-MB-468	44.15
Mean	81.54
Delta	37.49
Range	88.53

Mean Growth Percent - Growth Percent



One dose experimental data of compound 4.61 (NSC 751473)

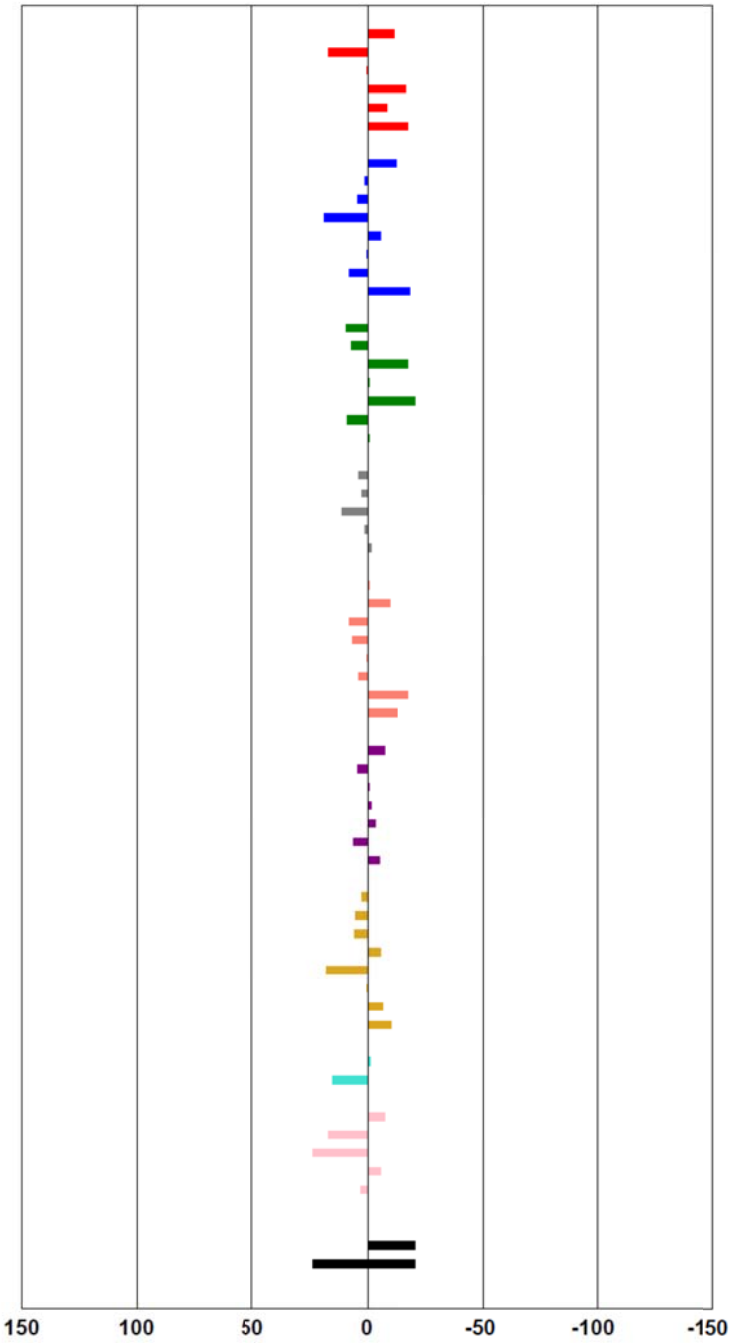
Developmental Therapeutics Program

One Dose Mean Graph

NSC: 751473 / 1	Conc: 1.25E-6 Molar	Test Date: Nov 09, 2009
Experiment ID: 0911OS45		Report Date: Feb 15, 2012

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	91.16
HL-60(TB)	119.38
K-562	102.89
MOLT-4	85.27
RPMI-8226	94.18
SR	84.36
Non-Small Cell Lung Cancer	
A549/ATCC	90.38
EKVX	103.56
HOP-62	106.58
HOP-92	121.00
NCI-H23	96.87
NCI-H322M	102.98
NCI-H460	110.36
NCI-H522	83.56
Colon Cancer	
COLO 205	111.80
HCC-2998	109.47
HCT-116	84.44
HCT-15	102.07
HT29	81.52
KM12	111.17
SW-620	101.82
CNS Cancer	
SF-268	106.27
SF-295	105.13
SF-539	113.25
SNB-75	103.81
U251	101.07
Melanoma	
LOX IMVI	101.81
MALME-3M	93.20
M14	110.47
MDA-MB-435	108.98
SK-MEL-2	102.74
SK-MEL-5	106.18
UACC-257	84.34
UACC-62	89.94
Ovarian Cancer	
IGROV1	95.24
OVCAR-3	106.76
OVCAR-4	102.09
OVCAR-5	100.93
OVCAR-8	99.20
NCI/ADR-RES	108.55
SK-OV-3	97.47
Renal Cancer	
786-0	105.21
A498	107.83
ACHN	108.03
CAKI-1	96.87
RXF 393	120.30
SN12C	102.78
TK-10	96.03
UO-31	92.36
Prostate Cancer	
PC-3	101.56
DU-145	117.48
Breast Cancer	
MDA-MB-231/ATCC	95.28
HS 578T	119.32
BT-549	126.01
T-47D	97.22
MDA-MB-468	105.55
Mean	102.22
Delta	20.50
Range	44.39

Mean Growth Percent - Growth Percent



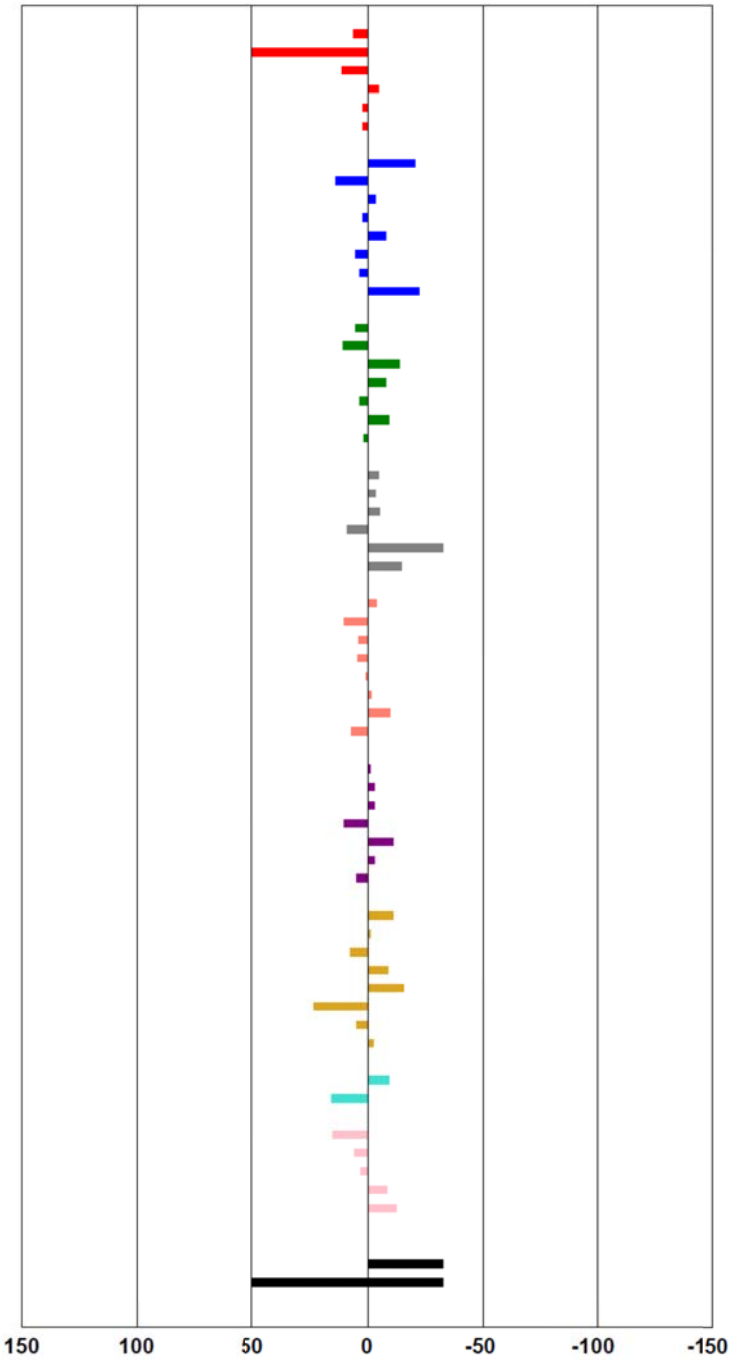
One dose experimental data of compound 4.62a (NSC 751479)

Developmental Therapeutics Program

One Dose Mean Graph

NSC: 751479 / 1	Conc: 1.00E-5 Molar	Test Date: Nov 09, 2009
Experiment ID: 0911OS45		Report Date: Feb 15, 2012

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	107.18
HL-60(TB)	150.32
K-562	111.92
MOLT-4	96.39
RPMI-8226	102.92
SR	102.86
Non-Small Cell Lung Cancer	
A549/ATCC	79.96
EKVX	114.44
HOP-62	97.85
HOP-92	102.85
NCI-H23	93.32
NCI-H322M	106.11
NCI-H460	104.47
NCI-H522	78.21
Colon Cancer	
COLO 205	106.05
HCC-2998	111.48
HCT-116	86.52
HCT-15	93.27
HT29	104.14
KM12	91.87
SW-620	102.56
CNS Cancer	
SF-268	96.23
SF-295	97.70
SF-539	95.71
SNB-19	109.59
SNB-75	67.93
U251	85.50
Melanoma	
LOX IMVI	97.09
MALME-3M	111.01
M14	104.79
MDA-MB-435	105.10
SK-MEL-2	101.55
SK-MEL-5	99.50
UACC-257	91.47
UACC-62	107.59
Ovarian Cancer	
IGROV1	99.78
OVCAR-3	98.02
OVCAR-4	97.90
OVCAR-5	111.21
OVCAR-8	90.14
NCI/ADR-RES	98.15
SK-OV-3	105.76
Renal Cancer	
786-0	90.16
A498	99.80
ACHN	108.31
CAKI-1	92.18
RXF 393	84.89
SN12C	123.94
TK-10	105.51
UO-31	98.55
Prostate Cancer	
PC-3	91.90
DU-145	116.42
Breast Cancer	
MDA-MB-231/ATCC	116.03
HS 578T	106.51
BT-549	103.81
T-47D	92.91
MDA-MB-468	88.78
Mean	100.54
Delta	32.71
Range	82.39



One dose experimental data of compound 4.62b (NSC 751483)

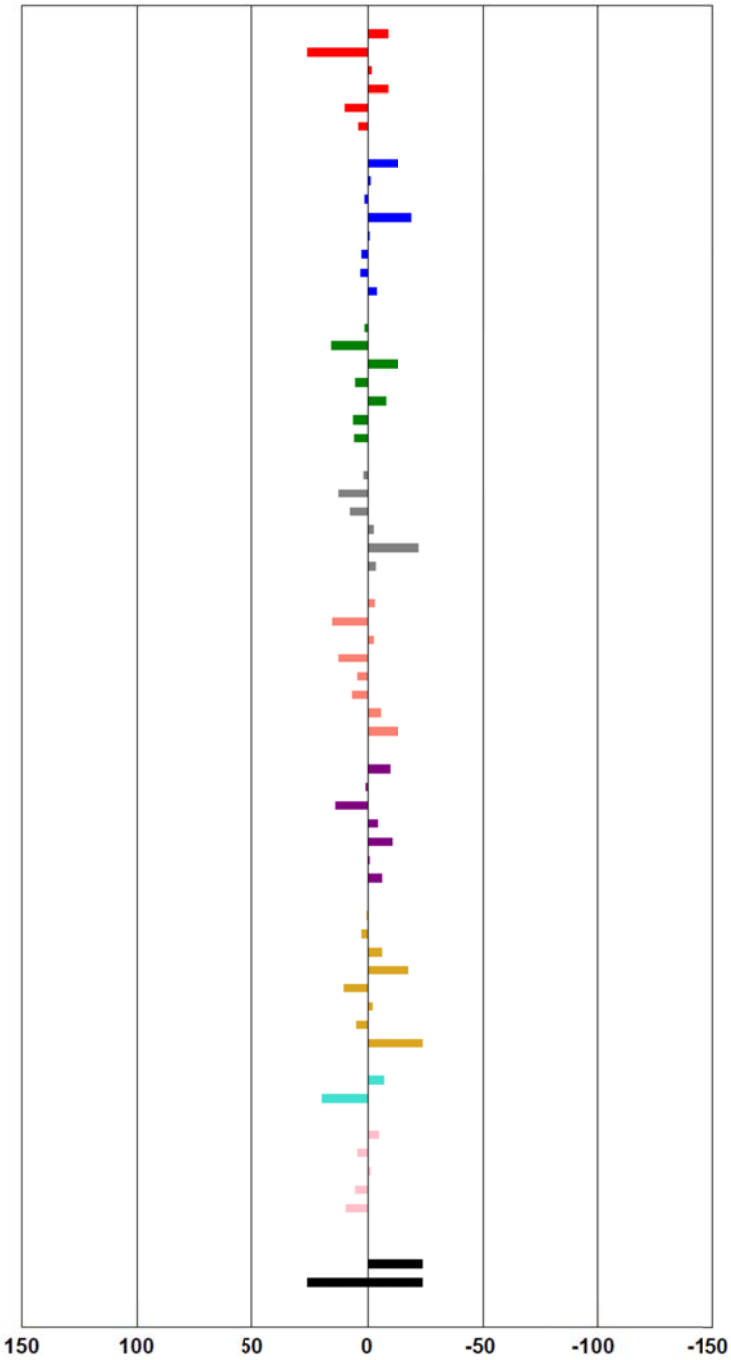
Developmental Therapeutics Program

One Dose Mean Graph

NSC: 751483 / 1	Conc: 1.00E-5 Molar	Test Date: Nov 09, 2009
Experiment ID: 0911OS45		Report Date: Feb 15, 2012

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	91.32
HL-60(TB)	125.47
K-562	98.54
MOLT-4	91.18
RPMI-8226	109.53
SR	103.80
Non-Small Cell Lung Cancer	
A549/ATCC	86.86
EKVX	98.87
HOP-62	101.01
HOP-92	80.76
NCI-H23	99.50
NCI-H322M	102.53
NCI-H460	102.86
NCI-H522	96.29
Colon Cancer	
COLO 205	101.34
HCC-2998	115.46
HCT-116	86.71
HCT-15	105.32
HT29	92.38
KM12	105.98
SW-620	105.59
CNS Cancer	
SF-268	101.37
SF-295	112.17
SF-539	107.53
SNB-19	97.56
SNB-75	77.42
U251	96.47
Melanoma	
LOX IMVI	96.97
MALME-3M	115.01
M14	97.54
MDA-MB-435	112.05
SK-MEL-2	104.14
SK-MEL-5	106.55
UACC-257	94.44
UACC-62	86.30
Ovarian Cancer	
IGROV1	90.35
OVCAR-3	100.47
OVCAR-4	113.78
OVCAR-5	95.90
OVCAR-8	89.46
NCI/ADR-RES	99.17
SK-OV-3	94.04
Renal Cancer	
786-0	100.24
A498	102.50
ACHN	93.92
CAKI-1	81.87
RXF 393	109.98
SN12C	98.11
TK-10	104.70
UO-31	75.56
Prostate Cancer	
PC-3	93.19
DU-145	119.49
Breast Cancer	
MDA-MB-231/ATCC	95.30
HS 578T	104.08
BT-549	98.87
T-47D	104.95
MDA-MB-468	109.00
Mean	99.59
Delta	24.13
Range	49.91

Mean Growth Percent - Growth Percent



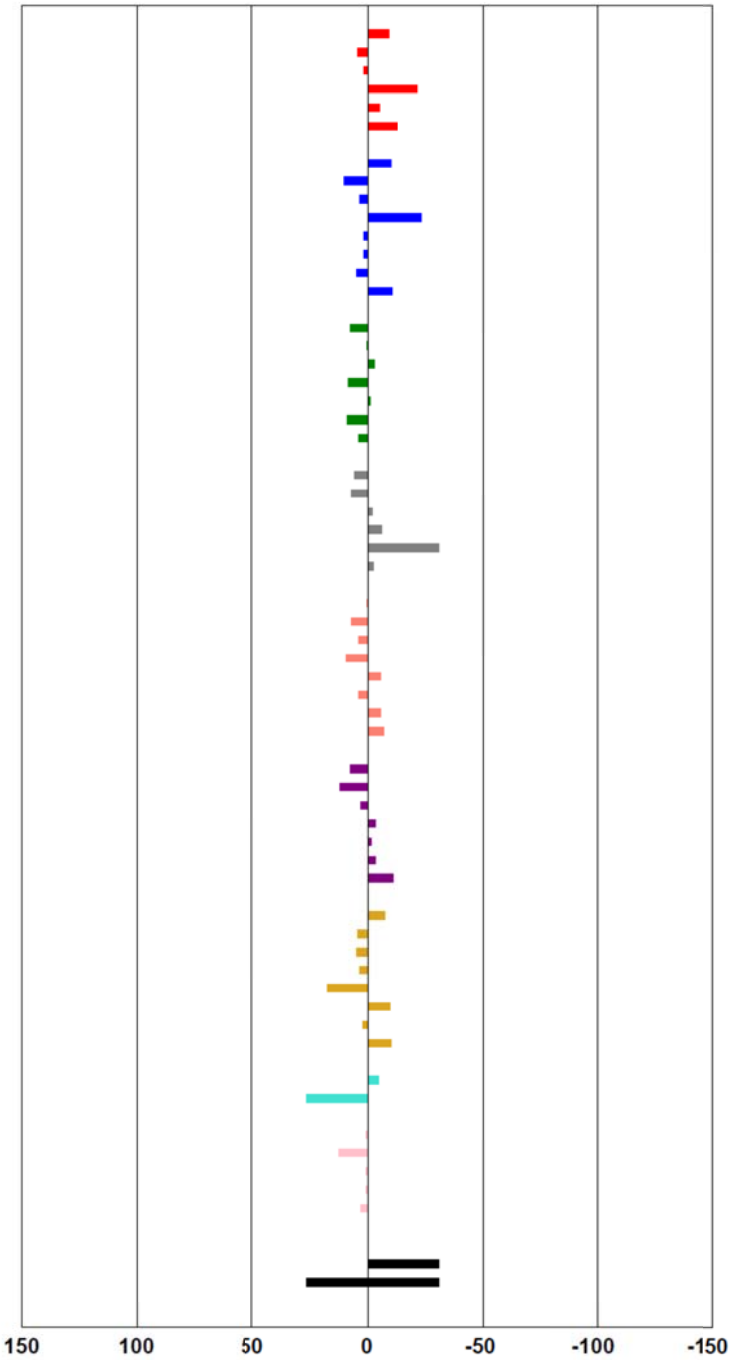
One dose experimental data of compound 4.63 (NSC 751467)

Developmental Therapeutics Program

One Dose Mean Graph

NSC: 751467 / 1	Conc: 1.20E-6 Molar	Test Date: Nov 09, 2009
Experiment ID: 0911OS45		Report Date: Feb 15, 2012

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	91.94
HL-60(TB)	105.06
K-562	102.38
MOLT-4	78.82
RPMI-8226	95.71
SR	88.53
Non-Small Cell Lung Cancer	
A549/ATCC	91.04
EKVX	111.12
HOP-62	104.28
HOP-92	77.41
NCI-H23	102.48
NCI-H322M	102.36
NCI-H460	105.73
NCI-H522	90.77
Colon Cancer	
COLO 205	108.27
HCC-2998	101.13
HCT-116	98.18
HCT-15	109.05
HT29	100.02
KM12	109.73
SW-620	104.94
CNS Cancer	
SF-268	106.73
SF-295	107.83
SF-539	98.85
SNB-19	95.14
SNB-75	69.49
U251	98.76
Melanoma	
LOX IMVI	101.35
MALME-3M	108.10
M14	104.91
MDA-MB-435	110.34
SK-MEL-2	95.50
SK-MEL-5	104.53
UACC-257	95.50
UACC-62	94.01
Ovarian Cancer	
IGROV1	108.23
OVCAR-3	112.83
OVCAR-4	103.70
OVCAR-5	97.56
OVCAR-8	99.53
NCI/ADR-RES	97.59
SK-OV-3	90.23
Renal Cancer	
786-0	93.83
A498	105.35
ACHN	105.71
CAKI-1	104.34
RXF 393	118.09
SN12C	91.54
TK-10	103.09
UO-31	90.79
Prostate Cancer	
PC-3	96.54
DU-145	127.06
Breast Cancer	
MDA-MB-231/ATCC	101.88
HS 578T	113.28
BT-549	101.80
T-47D	101.47
MDA-MB-468	103.95
Mean	100.58
Delta	31.19
Range	57.57



One dose experimental data of compound 4.64 (NSC 764191)

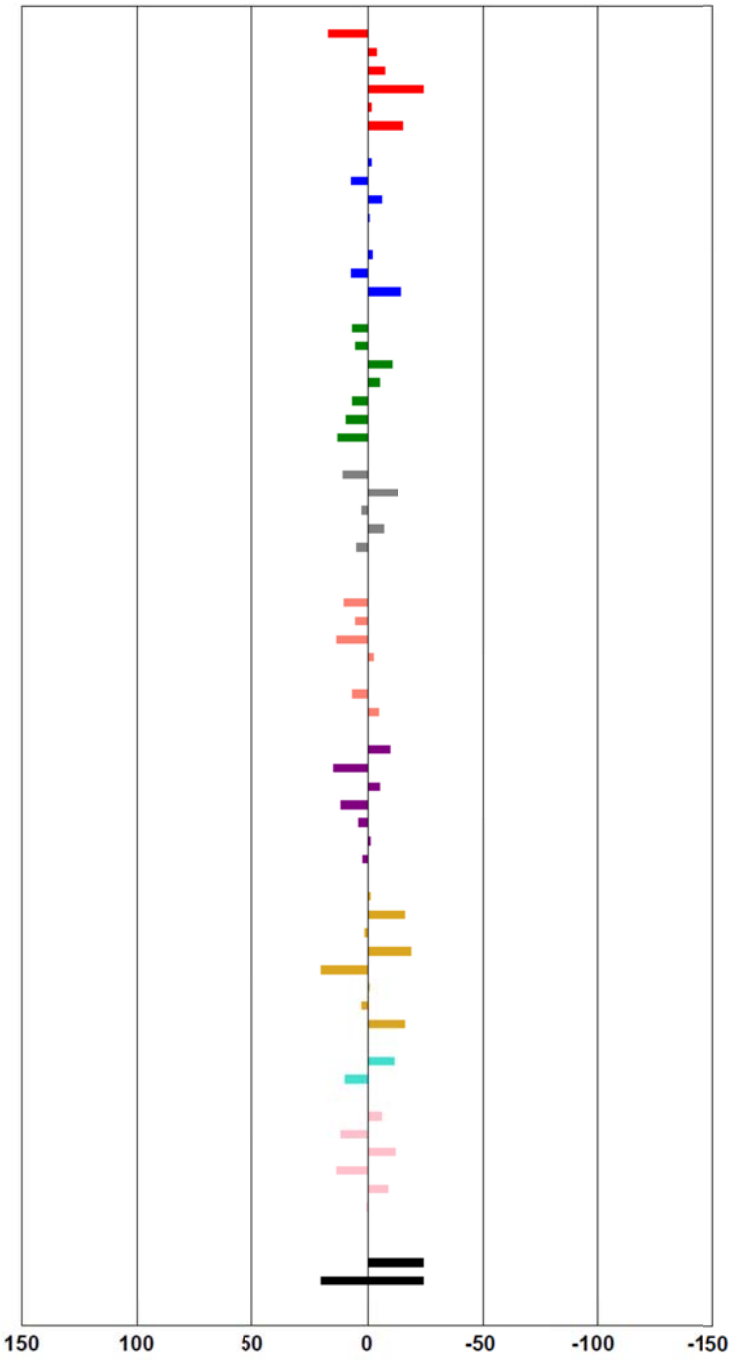
Developmental Therapeutics Program

One Dose Mean Graph

NSC: D-764191 / 1	Conc: 1.00E-5 Molar	Test Date: Mar 19, 2012
Experiment ID: 1203OS38		Report Date: May 09, 2013

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	113.40
HL-60(TB)	92.82
K-562	89.56
MOLT-4	71.87
RPMI-8226	95.13
SR	80.88
Non-Small Cell Lung Cancer	
A549/ATCC	95.30
HOP-62	103.59
HOP-92	90.53
NCI-H226	96.16
NCI-H23	96.74
NCI-H322M	94.91
NCI-H460	103.53
NCI-H522	81.81
Colon Cancer	
COLO 205	103.25
HCC-2998	101.90
HCT-116	86.38
HCT-15	91.77
HT29	103.14
KM12	105.78
SW-620	109.25
CNS Cancer	
SF-268	107.35
SF-295	83.18
SF-539	99.04
SNB-19	89.98
U251	101.23
Melanoma	
MALME-3M	96.49
M14	106.85
MDA-MB-435	101.73
SK-MEL-2	109.96
SK-MEL-28	94.25
SK-MEL-5	96.58
UACC-257	103.19
UACC-62	91.93
Ovarian Cancer	
IGROV1	87.40
OVCAR-3	111.41
OVCAR-4	91.61
OVCAR-5	107.94
OVCAR-8	100.55
NCI/ADR-RES	95.77
SK-OV-3	98.96
Renal Cancer	
786-0	95.73
A498	80.04
ACHN	98.09
CAKI-1	77.24
RXF 393	116.41
SN12C	95.99
TK-10	99.11
UO-31	80.30
Prostate Cancer	
PC-3	85.53
DU-145	106.28
Breast Cancer	
MCF7	90.56
MDA-MB-231/ATCC	108.16
HS 578T	85.08
BT-549	109.73
T-47D	88.27
MDA-MB-468	97.06
Mean	96.44
Delta	24.57
Range	44.54

Mean Growth Percent - Growth Percent



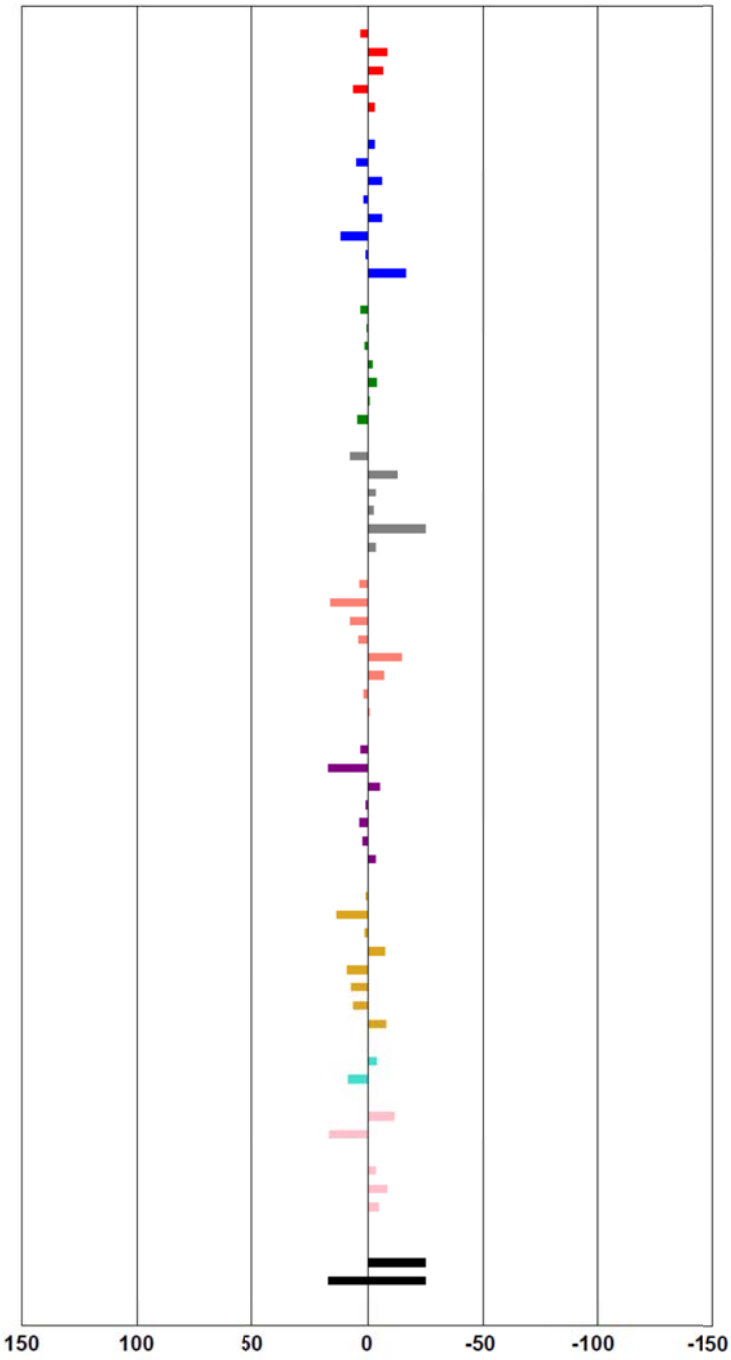
One dose experimental data of compound 4.65 (NSC 764192)

Developmental Therapeutics Program

One Dose Mean Graph

NSC: D-764192 / 1	Conc: 1.00E-5 Molar	Test Date: Mar 19, 2012
Experiment ID: 1203OS38		Report Date: May 09, 2013

Panel/Cell Line	Growth Percent
Leukemia	
HL-60(TB)	106.76
K-562	95.50
MOLT-4	97.41
RPMI-8226	109.79
SR	100.77
Non-Small Cell Lung Cancer	
A549/ATCC	100.79
HOP-62	108.42
HOP-92	97.56
NCI-H226	105.43
NCI-H23	97.83
NCI-H322M	115.22
NCI-H460	104.36
NCI-H522	86.52
Colon Cancer	
COLO 205	106.77
HCC-2998	103.99
HCT-116	104.54
HCT-15	101.54
HT29	100.03
KM12	102.98
SW-620	107.83
CNS Cancer	
SF-268	110.96
SF-295	91.08
SF-539	100.30
SNB-19	101.15
SNB-75	78.05
U251	100.21
Melanoma	
MALME-3M	106.99
M14	119.51
MDA-MB-435	110.83
SK-MEL-2	107.26
SK-MEL-28	88.36
SK-MEL-5	96.53
UACC-257	105.20
UACC-62	102.98
Ovarian Cancer	
IGROV1	106.53
OVCAR-3	120.37
OVCAR-4	98.36
OVCAR-5	104.23
OVCAR-8	106.89
NCI/ADR-RES	105.84
SK-OV-3	100.50
Renal Cancer	
786-0	104.15
A498	116.98
ACHN	104.55
CAKI-1	96.27
RXF 393	112.45
SN12C	110.57
TK-10	109.89
UO-31	95.90
Prostate Cancer	
PC-3	99.81
DU-145	111.80
Breast Cancer	
MCF7	92.41
MDA-MB-231/ATCC	119.82
HS 578T	103.25
BT-549	100.28
T-47D	95.59
MDA-MB-468	99.05
Mean	103.32
Delta	25.27
Range	42.32



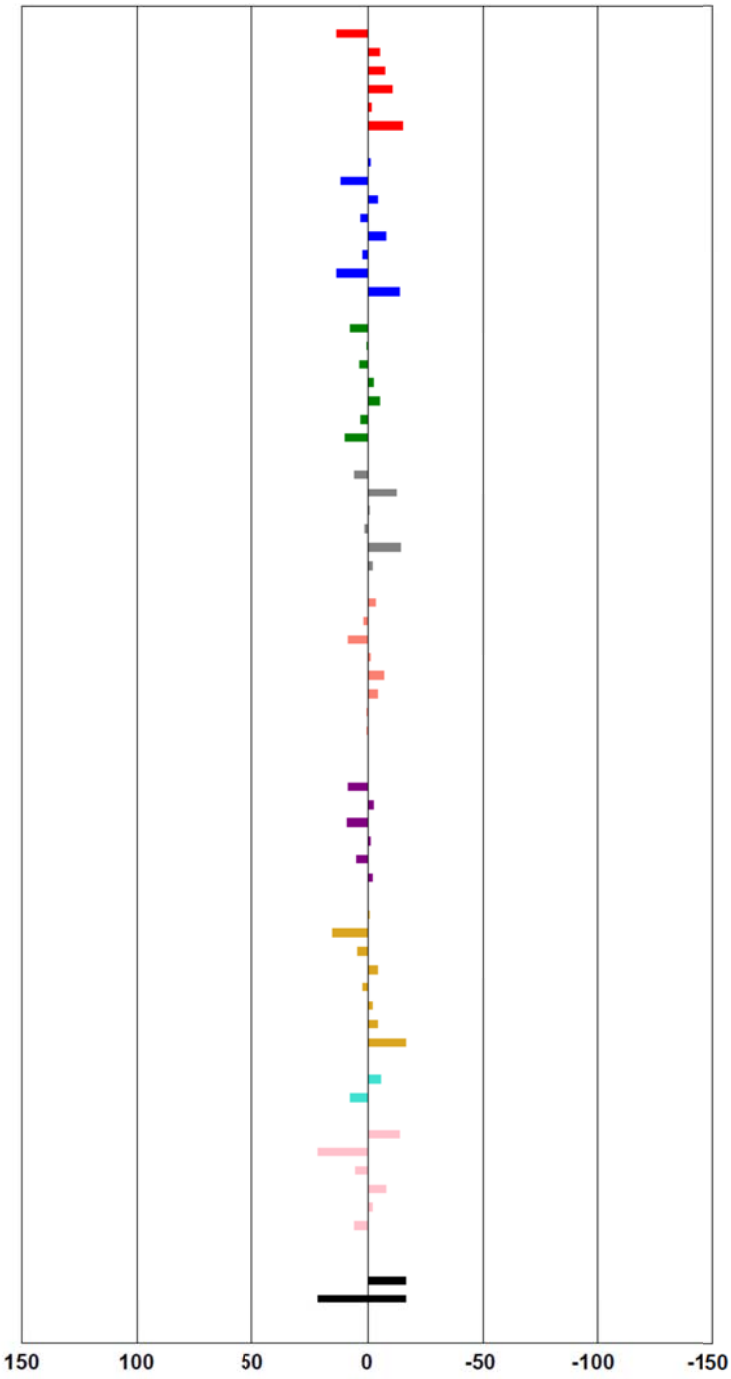
One dose experimental data of compound 4.66 (NSC 764193)

Developmental Therapeutics Program

One Dose Mean Graph

NSC: D-764193 / 1	Conc: 1.00E-5 Molar	Test Date: Mar 19, 2012
Experiment ID: 1203OS38		Report Date: May 09, 2013

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	118.08
HL-60(TB)	99.54
K-562	97.52
MOLT-4	94.18
RPMI-8226	103.35
SR	89.07
Non-Small Cell Lung Cancer	
A549/ATCC	103.88
HOP-62	116.11
HOP-92	100.37
NCI-H226	107.56
NCI-H23	97.14
NCI-H322M	106.73
NCI-H460	118.10
NCI-H522	90.14
Colon Cancer	
COLO 205	112.20
HCC-2998	104.80
HCT-116	108.10
HCT-15	102.17
HT29	99.45
KM12	107.71
SW-620	114.41
CNS Cancer	
SF-268	110.14
SF-295	92.54
SF-539	104.29
SNB-19	105.84
SNB-75	90.03
U251	102.90
Melanoma	
MALME-3M	101.41
M14	106.33
MDA-MB-435	112.85
SK-MEL-2	103.86
SK-MEL-28	97.88
SK-MEL-5	100.36
UACC-257	104.96
UACC-62	104.93
Ovarian Cancer	
IGROV1	104.61
OVCAR-3	113.05
OVCAR-4	102.38
OVCAR-5	113.33
OVCAR-8	103.55
NCI/ADR-RES	109.49
SK-OV-3	102.92
Renal Cancer	
786-0	104.04
A498	119.58
ACHN	109.00
CAKI-1	100.55
RXF 393	106.73
SN12C	102.59
TK-10	100.78
UO-31	87.73
Prostate Cancer	
PC-3	99.34
DU-145	112.05
Breast Cancer	
MCF7	90.26
MDA-MB-231/ATCC	125.96
HS 578T	110.11
BT-549	96.98
T-47D	102.97
MDA-MB-468	110.29
Mean	104.45
Delta	16.72
Range	38.23



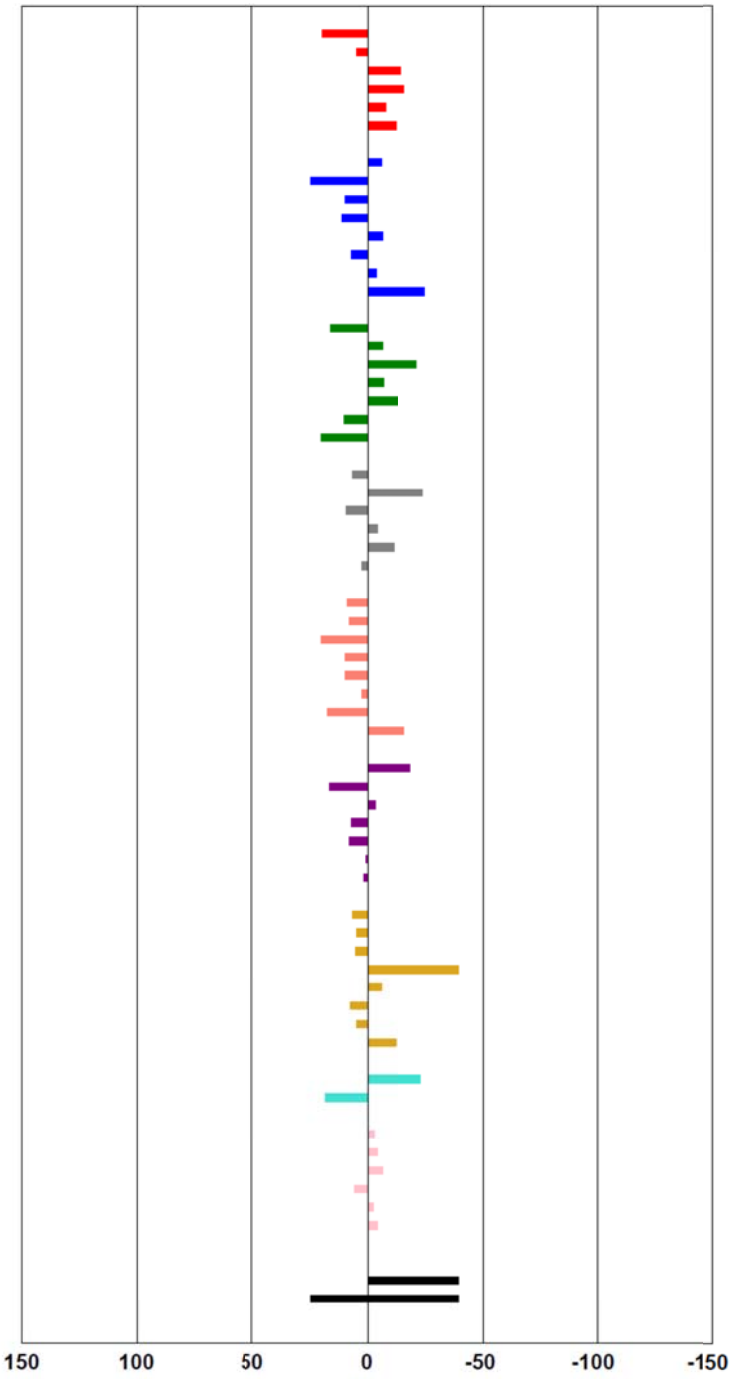
One dose experimental data of compound 4.67 (NSC 764194)

Developmental Therapeutics Program

One Dose Mean Graph

NSC: D-764194 / 1	Conc: 1.00E-5 Molar	Test Date: Mar 19, 2012
Experiment ID: 1203OS38		Report Date: May 09, 2013

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	102.84
HL-60(TB)	88.10
K-562	68.31
MOLT-4	67.16
RPMI-8226	75.55
SR	71.04
Non-Small Cell Lung Cancer	
A549/ATCC	77.39
HOP-62	107.76
HOP-92	93.05
NCI-H226	94.42
NCI-H23	76.85
NCI-H322M	90.40
NCI-H460	79.55
NCI-H522	58.31
Colon Cancer	
COLO 205	99.21
HCC-2998	76.77
HCT-116	61.83
HCT-15	76.29
HT29	70.01
KM12	93.34
SW-620	103.18
CNS Cancer	
SF-268	89.97
SF-295	58.92
SF-539	92.38
SNB-19	79.25
SNB-75	71.86
U251	85.52
Melanoma	
MALME-3M	91.88
M14	91.13
MDA-MB-435	103.31
SK-MEL-2	92.71
SK-MEL-28	93.05
SK-MEL-5	85.86
UACC-257	100.52
UACC-62	66.94
Ovarian Cancer	
IGROV1	64.48
OVCAR-3	99.59
OVCAR-4	79.87
OVCAR-5	90.42
OVCAR-8	91.05
NCI/ADR-RES	83.86
SK-OV-3	84.83
Renal Cancer	
786-0	89.94
A498	87.83
ACHN	88.34
CAKI-1	43.31
RXF 393	77.42
SN12C	90.57
TK-10	87.98
UO-31	71.01
Prostate Cancer	
PC-3	59.81
DU-145	101.13
Breast Cancer	
MCF7	80.54
MDA-MB-231/ATCC	79.13
HS 578T	76.83
BT-549	89.07
T-47D	80.85
MDA-MB-468	79.29
Mean	82.97
Delta	39.56
Range	64.45



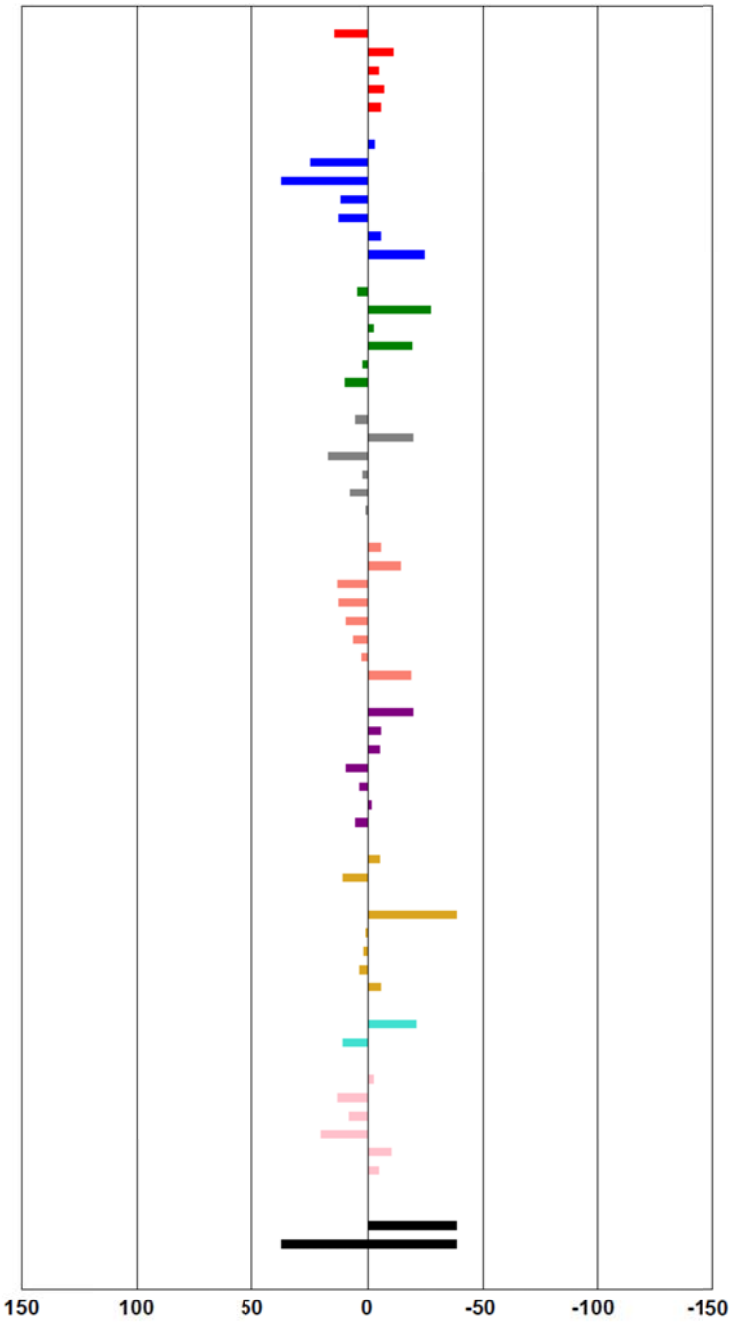
One dose experimental data of compound 4.68 (NSC 764195)

Developmental Therapeutics Program

One Dose Mean Graph

NSC: D-764195 / 1	Conc: 1.00E-5 Molar	Test Date: Mar 19, 2012
Experiment ID: 1203OS38		Report Date: May 09, 2013

Panel/Cell Line	Growth Percent
Leukemia	
HL-60(TB)	99.50
K-562	74.56
MOLT-4	80.82
RPMI-8226	78.56
SR	79.89
Non-Small Cell Lung Cancer	
A549/ATCC	82.45
HOP-62	109.44
HOP-92	122.20
NCI-H226	96.71
NCI-H322M	97.76
NCI-H460	79.52
NCI-H522	60.31
Colon Cancer	
HCC-2998	89.52
HCT-116	57.52
HCT-15	83.06
HT29	65.34
KM12	87.48
SW-620	94.97
CNS Cancer	
SF-268	90.54
SF-295	65.06
SF-539	101.92
SNB-19	87.50
SNB-75	92.53
U251	86.13
Melanoma	
MALME-3M	79.72
M14	70.61
MDA-MB-435	97.89
SK-MEL-2	97.58
SK-MEL-28	94.26
SK-MEL-5	91.47
UACC-257	87.97
UACC-62	65.92
Ovarian Cancer	
IGROV1	64.92
OVCAR-3	79.59
OVCAR-4	80.17
OVCAR-5	94.54
OVCAR-8	88.49
NCI/ADR-RES	83.98
SK-OV-3	90.39
Renal Cancer	
786-0	80.40
A498	95.90
ACHN	85.08
CAKI-1	46.29
RXF 393	86.18
SN12C	86.83
TK-10	88.77
UO-31	79.59
Prostate Cancer	
PC-3	63.55
DU-145	96.01
Breast Cancer	
MCF7	82.96
MDA-MB-231/ATCC	97.95
HS 578T	92.96
BT-549	105.22
T-47D	75.36
MDA-MB-468	80.85
Mean	85.01
Delta	38.72
Range	75.91



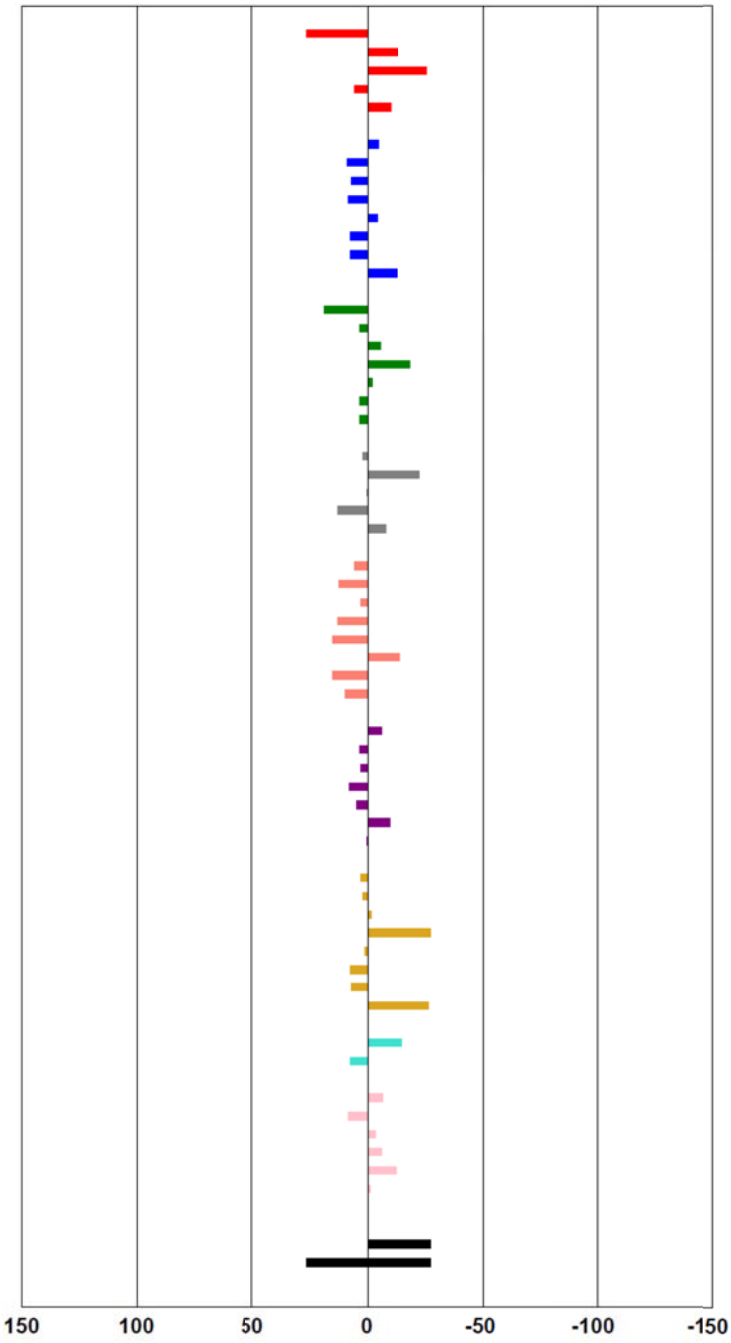
One dose experimental data of compound 4.69 (NSC 764196)

Developmental Therapeutics Program

One Dose Mean Graph

NSC: D-764196 / 1	Conc: 1.00E-5 Molar	Test Date: Mar 19, 2012
Experiment ID: 1203OS38		Report Date: May 09, 2013

Panel/Cell Line	Growth Percent
Leukemia	
HL-60(TB)	127.74
K-562	87.90
MOLT-4	75.53
RPMI-8226	107.27
SR	91.42
Non-Small Cell Lung Cancer	
A549/ATCC	96.90
HOP-62	110.40
HOP-92	108.55
NCI-H226	109.59
NCI-H23	97.55
NCI-H322M	108.74
NCI-H460	109.01
NCI-H522	89.06
Colon Cancer	
COLO 205	120.08
HCC-2998	105.08
HCT-116	95.98
HCT-15	82.78
HT29	99.50
KM12	104.89
SW-620	104.94
CNS Cancer	
SF-268	103.54
SF-295	78.85
SF-539	101.89
SNB-19	114.21
U251	93.56
Melanoma	
MALME-3M	107.21
M14	113.61
MDA-MB-435	104.35
SK-MEL-2	114.17
SK-MEL-28	116.59
SK-MEL-5	87.01
UACC-257	116.40
UACC-62	110.95
Ovarian Cancer	
IGROV1	95.39
OVCAR-3	104.76
OVCAR-4	104.35
OVCAR-5	109.45
OVCAR-8	106.08
NCI/ADR-RES	91.99
SK-OV-3	101.56
Renal Cancer	
786-0	104.70
A498	103.76
ACHN	99.93
CAKI-1	73.57
RXF 393	102.58
SN12C	108.90
TK-10	108.59
UO-31	74.54
Prostate Cancer	
PC-3	86.34
DU-145	109.05
Breast Cancer	
MCF7	94.97
MDA-MB-231/ATCC	109.95
HS 578T	98.43
BT-549	95.55
T-47D	89.41
MDA-MB-468	100.47
Mean	101.26
Delta	27.59
Range	54.17



One dose experimental data of compound 4.70 (NSC 764197)

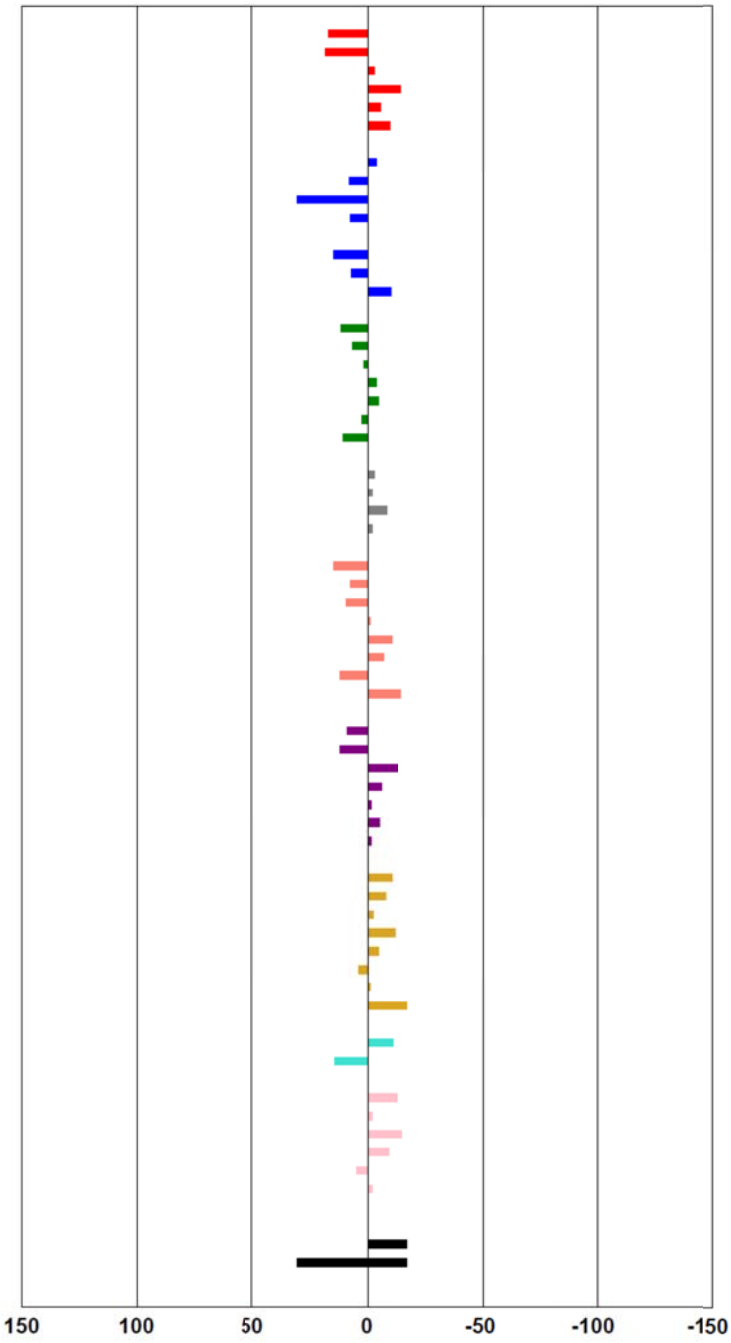
Developmental Therapeutics Program

One Dose Mean Graph

NSC: D-764197 / 1	Conc: 1.00E-5 Molar	Test Date: Mar 19, 2012
Experiment ID: 1203OS38		Report Date: May 09, 2013

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	116.78
HL-60(TB)	118.10
K-562	97.07
MOLT-4	85.18
RPMI-8226	94.11
SR	90.51
Non-Small Cell Lung Cancer	
A549/ATCC	96.20
HOP-62	107.89
HOP-92	129.96
NCI-H226	107.31
NCI-H23	99.80
NCI-H322M	114.44
NCI-H460	107.00
NCI-H522	89.73
Colon Cancer	
COLO 205	111.09
HCC-2998	106.38
HCT-116	101.40
HCT-15	96.08
HT29	95.35
KM12	102.55
SW-620	110.49
CNS Cancer	
SF-268	96.90
SF-539	98.01
SNB-19	91.55
U251	97.79
Melanoma	
MALME-3M	114.53
M14	107.01
MDA-MB-435	108.95
SK-MEL-2	98.58
SK-MEL-28	89.54
SK-MEL-5	93.09
UACC-257	111.73
UACC-62	85.01
Ovarian Cancer	
IGROV1	108.50
OVCAR-3	111.76
OVCAR-4	86.17
OVCAR-5	93.72
OVCAR-8	98.53
NCI/ADR-RES	94.70
SK-OV-3	98.40
Renal Cancer	
786-0	89.43
A498	92.12
ACHN	97.37
CAKI-1	87.92
RXF 393	95.02
SN12C	103.72
TK-10	98.84
UO-31	82.35
Prostate Cancer	
PC-3	88.99
DU-145	113.81
Breast Cancer	
MCF7	87.26
MDA-MB-231/ATCC	97.70
HS 578T	84.57
BT-549	90.71
T-47D	104.50
MDA-MB-468	97.98
Mean	99.55
Delta	17.20
Range	47.61

Mean Growth Percent - Growth Percent



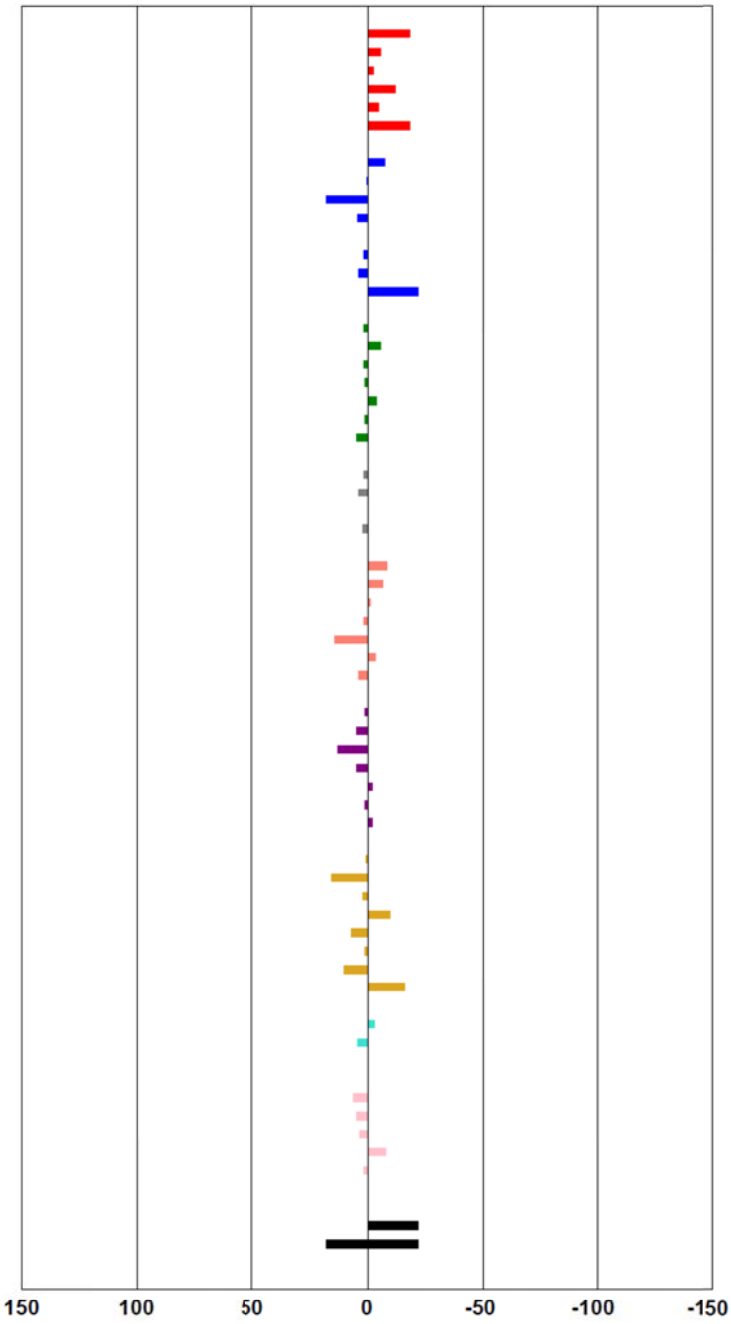
One dose experimental data of compound 4.71 (NSC 767525)

Developmental Therapeutics Program

One Dose Mean Graph

NSC: D-767525 / 1	Conc: 1.00E-5 Molar	Test Date: Sep 24, 2012
Experiment ID: 1209OS49		Report Date: May 09, 2013

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	83.17
HL-60(TB)	96.78
K-562	99.54
MOLT-4	90.32
RPMI-8226	97.79
SR	83.55
Non-Small Cell Lung Cancer	
A549/ATCC	94.90
HOP-62	102.40
HOP-92	119.76
NCI-H226	106.70
NCI-H23	102.20
NCI-H322M	103.83
NCI-H460	106.09
NCI-H522	79.70
Colon Cancer	
COLO 205	103.80
HCC-2998	96.52
HCT-116	103.71
HCT-15	103.34
HT29	98.56
KM12	103.34
SW-620	107.00
CNS Cancer	
SF-268	103.85
SF-539	105.87
SNB-19	102.26
SNB-75	104.20
Melanoma	
LOX IMVI	94.17
MALME-3M	96.03
M14	101.30
MDA-MB-435	103.73
SK-MEL-28	116.41
SK-MEL-5	98.95
UACC-62	106.10
Ovarian Cancer	
IGROV1	103.54
OVCAR-3	107.15
OVCAR-4	114.96
OVCAR-5	106.74
OVCAR-8	100.36
NCI/ADR-RES	103.41
SK-OV-3	100.41
Renal Cancer	
786-0	102.89
A498	117.70
ACHN	104.13
CAKI-1	92.73
RXF 393	108.98
SN12C	103.21
TK-10	112.27
UO-31	85.38
Prostate Cancer	
PC-3	99.56
DU-145	106.48
Breast Cancer	
MCF7	101.86
MDA-MB-231/ATCC	108.17
HS 578T	106.74
BT-549	105.96
T-47D	94.59
MDA-MB-468	104.02
Mean	101.95
Delta	22.25
Range	40.06



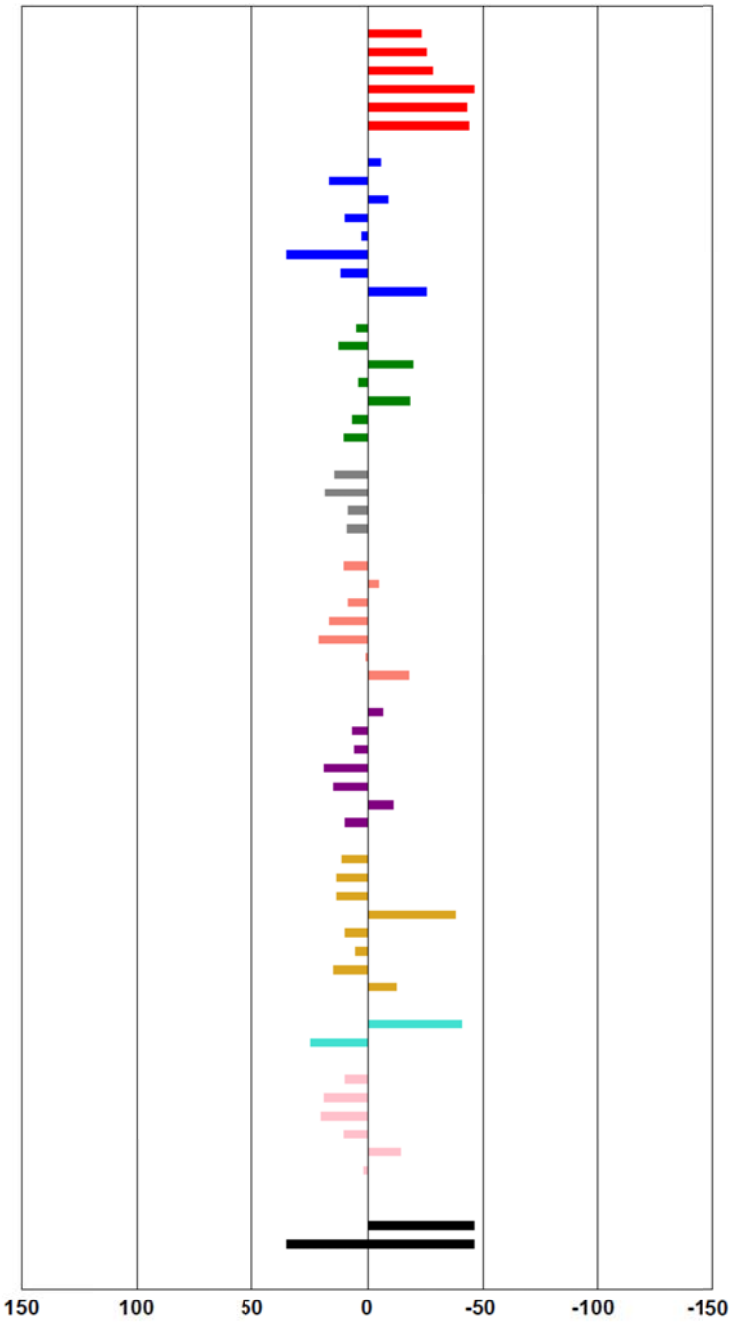
One dose experimental data of compound 4.72 (NSC 767526)

Developmental Therapeutics Program

One Dose Mean Graph

NSC: D-767526 / 1	Conc: 1.00E-5 Molar	Test Date: Sep 24, 2012
Experiment ID: 1209OS49		Report Date: May 09, 2013

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	57.02
HL-60(TB)	54.58
K-562	52.08
MOLT-4	34.17
RPMI-8226	37.57
SR	36.58
Non-Small Cell Lung Cancer	
A549/ATCC	75.16
HOP-62	97.11
HOP-92	72.15
NCI-H226	90.41
NCI-H23	83.23
NCI-H322M	115.22
NCI-H460	92.31
NCI-H522	54.76
Colon Cancer	
COLO 205	85.58
HCC-2998	92.85
HCT-116	60.37
HCT-15	84.76
HT29	62.01
KM12	87.07
SW-620	90.99
CNS Cancer	
SF-268	94.58
SF-539	98.71
SNB-19	89.01
SNB-75	89.50
Melanoma	
LOX IMVI	90.55
MALME-3M	76.23
M14	88.89
MDA-MB-435	97.13
SK-MEL-28	101.61
SK-MEL-5	81.56
UACC-62	62.37
Ovarian Cancer	
IGROV1	74.39
OVCAR-3	87.03
OVCAR-4	86.21
OVCAR-5	99.21
OVCAR-8	95.40
NCI/ADR-RES	69.83
SK-OV-3	90.29
Renal Cancer	
786-0	91.54
A498	94.14
ACHN	94.05
CAKI-1	42.19
RXF 393	90.17
SN12C	86.05
TK-10	95.24
UO-31	68.77
Prostate Cancer	
PC-3	39.75
DU-145	105.20
Breast Cancer	
MCF7	90.15
MDA-MB-231/ATCC	99.20
HS 578T	100.52
BT-549	90.96
T-47D	66.07
MDA-MB-468	82.26
Mean	80.46
Delta	46.29
Range	81.05



One dose experimental data of compound 4.73 (NSC 767527)

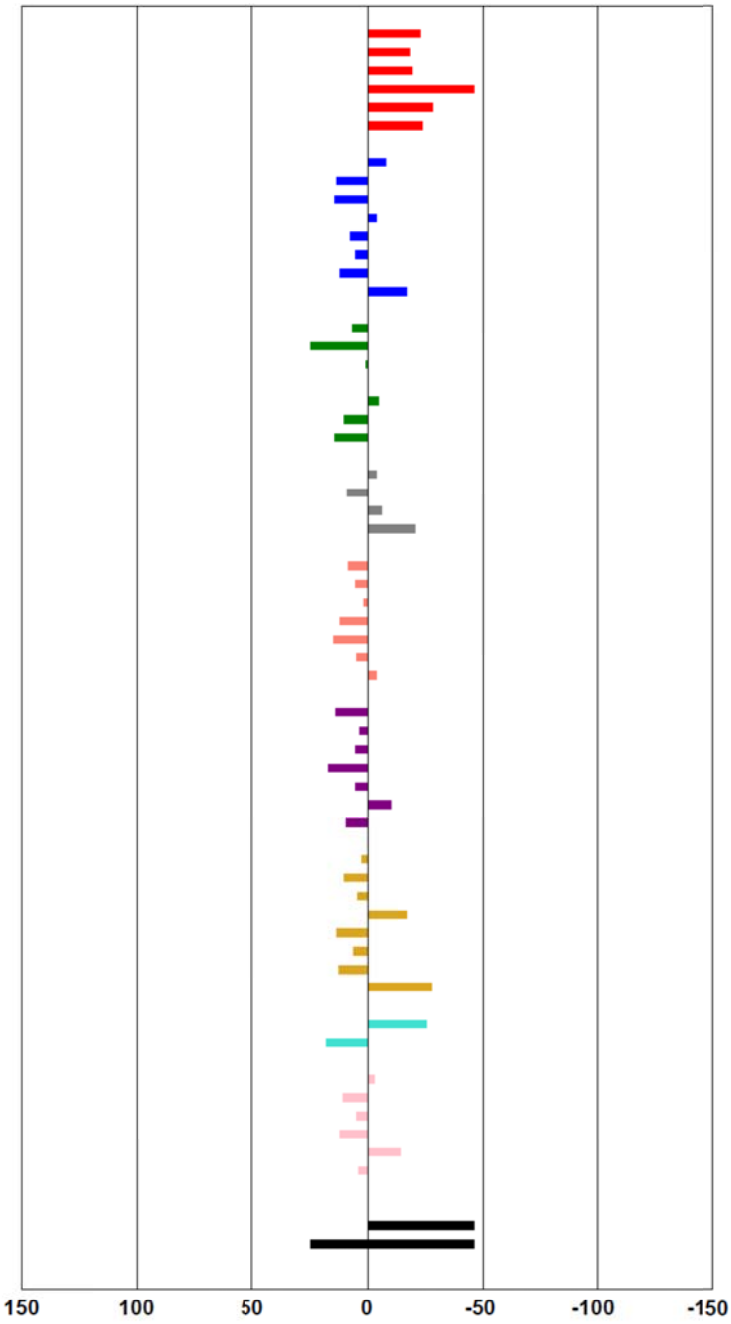
Developmental Therapeutics Program

One Dose Mean Graph

NSC: D-767527 / 1	Conc: 1.00E-5 Molar	Test Date: Sep 24, 2012
Experiment ID: 1209OS49		Report Date: May 09, 2013

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	62.87
HL-60(TB)	67.45
K-562	66.37
MOLT-4	39.80
RPMI-8226	57.52
SR	62.16
Non-Small Cell Lung Cancer	
A549/ATCC	78.43
HOP-62	99.35
HOP-92	100.18
NCI-H226	82.41
NCI-H23	93.51
NCI-H322M	91.49
NCI-H460	98.05
NCI-H522	68.81
Colon Cancer	
COLO 205	92.80
HCC-2998	110.48
HCT-116	86.97
HCT-15	86.13
HT29	81.73
KM12	96.23
SW-620	100.35
CNS Cancer	
SF-268	82.53
SF-539	94.91
SNB-19	80.27
SNB-75	65.31
Melanoma	
LOX IMVI	94.29
MALME-3M	91.55
M14	87.56
MDA-MB-435	98.19
SK-MEL-28	100.70
SK-MEL-5	90.75
UACC-62	82.52
Ovarian Cancer	
IGROV1	99.95
OVCAR-3	89.51
OVCAR-4	91.24
OVCAR-5	102.78
OVCAR-8	91.49
NCI/ADR-RES	76.10
SK-OV-3	95.19
Renal Cancer	
786-0	88.74
A498	96.03
ACHN	90.58
CAKI-1	68.79
RXF 393	99.42
SN12C	92.03
TK-10	98.37
UO-31	58.05
Prostate Cancer	
PC-3	60.29
DU-145	103.59
Breast Cancer	
MCF7	83.16
MDA-MB-231/ATCC	96.59
HS 578T	90.84
BT-549	97.90
T-47D	71.13
MDA-MB-468	90.07
Mean	85.89
Delta	46.09
Range	70.58

Mean Growth Percent - Growth Percent



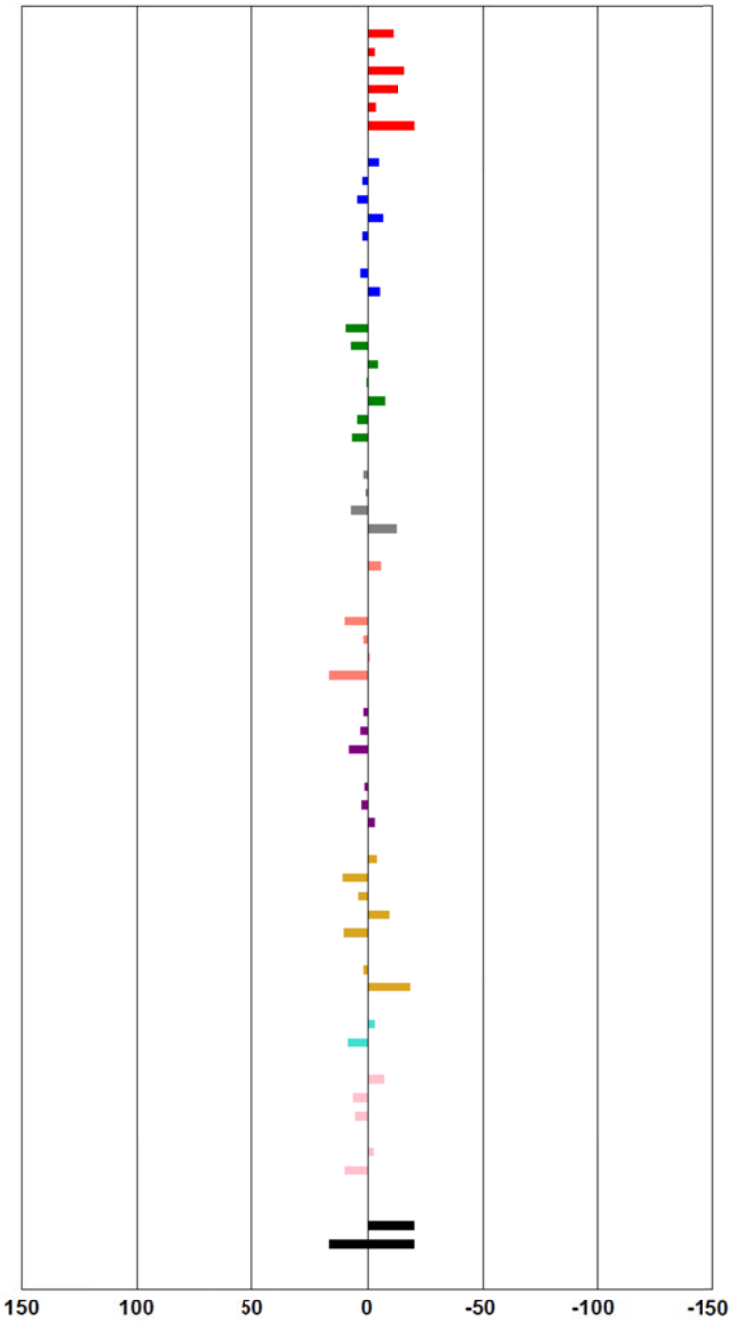
One dose experimental data of compound 4.74 (NSC 767528)

Developmental Therapeutics Program

One Dose Mean Graph

NSC: D-767528 / 1	Conc: 1.00E-5 Molar	Test Date: Sep 24, 2012
Experiment ID: 1209OS49		Report Date: May 09, 2013

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	92.29
HL-60(TB)	100.00
K-562	86.92
MOLT-4	89.71
RPMI-8226	99.59
SR	82.41
Non-Small Cell Lung Cancer	
A549/ATCC	98.21
HOP-62	105.24
HOP-92	107.30
NCI-H226	96.51
NCI-H23	105.25
NCI-H322M	103.09
NCI-H460	105.79
NCI-H522	97.94
Colon Cancer	
COLO 205	112.31
HCC-2998	109.82
HCT-116	98.75
HCT-15	103.31
HT29	95.93
KM12	107.38
SW-620	109.76
CNS Cancer	
SF-268	104.87
SF-539	103.72
SNB-19	109.80
SNB-75	90.73
Melanoma	
LOX IMVI	97.72
MALME-3M	102.58
M14	102.84
MDA-MB-435	112.53
SK-MEL-28	104.46
SK-MEL-5	102.54
UACC-62	119.28
Ovarian Cancer	
IGROV1	104.55
OVCAR-3	106.09
OVCAR-4	111.06
OVCAR-5	102.76
OVCAR-8	104.18
NCI/ADR-RES	105.47
SK-OV-3	100.37
Renal Cancer	
786-0	99.12
A498	113.33
ACHN	106.77
CAKI-1	93.78
RXF 393	113.06
SN12C	102.74
TK-10	104.55
UO-31	83.96
Prostate Cancer	
PC-3	100.33
DU-145	111.37
Breast Cancer	
MCF7	96.15
MDA-MB-231/ATCC	109.05
HS 578T	108.15
BT-549	102.82
T-47D	100.50
MDA-MB-468	112.54
Mean	102.75
Delta	20.34
Range	36.87



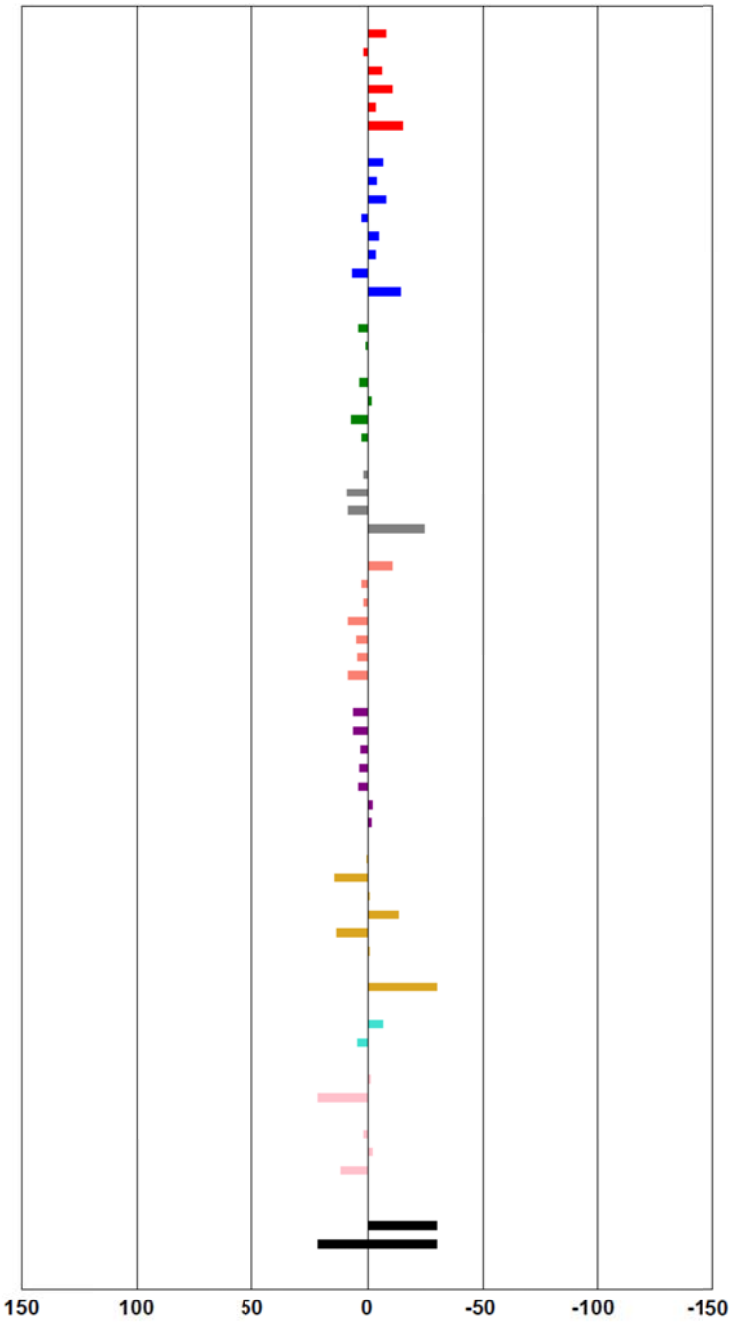
One dose experimental data of compound 4.75 (NSC 767529)

Developmental Therapeutics Program

One Dose Mean Graph

NSC: D-767529 / 1	Conc: 1.00E-5 Molar	Test Date: Sep 24, 2012
Experiment ID: 1209OS49		Report Date: May 09, 2013

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	93.61
HL-60(TB)	102.81
K-562	95.25
MOLT-4	90.95
RPMI-8226	97.85
SR	85.38
Non-Small Cell Lung Cancer	
A549/ATCC	94.86
HOP-62	97.57
HOP-92	93.73
NCI-H226	103.82
NCI-H23	96.53
NCI-H322M	98.03
NCI-H460	107.59
NCI-H522	86.29
Colon Cancer	
COLO 205	105.14
HCC-2998	101.85
HCT-116	101.15
HCT-15	104.70
HT29	99.91
KM12	108.48
SW-620	103.84
CNS Cancer	
SF-268	102.72
SF-539	109.89
SNB-19	109.48
SNB-75	76.18
Melanoma	
LOX IMVI	90.90
MALME-3M	103.58
M14	103.15
MDA-MB-435	109.76
SK-MEL-28	106.11
SK-MEL-5	105.71
UACC-62	109.70
Ovarian Cancer	
IGROV1	107.42
OVCAR-3	107.20
OVCAR-4	104.06
OVCAR-5	104.57
OVCAR-8	105.10
NCI/ADR-RES	99.50
SK-OV-3	100.04
Renal Cancer	
786-0	101.57
A498	115.57
ACHN	100.91
CAKI-1	87.17
RXF 393	114.51
SN12C	100.94
TK-10	101.09
UO-31	70.87
Prostate Cancer	
PC-3	95.09
DU-145	105.39
Breast Cancer	
MCF7	100.36
MDA-MB-231/ATCC	122.73
HS 578T	101.08
BT-549	103.00
T-47D	99.48
MDA-MB-468	112.77
Mean	101.04
Delta	30.17
Range	51.96



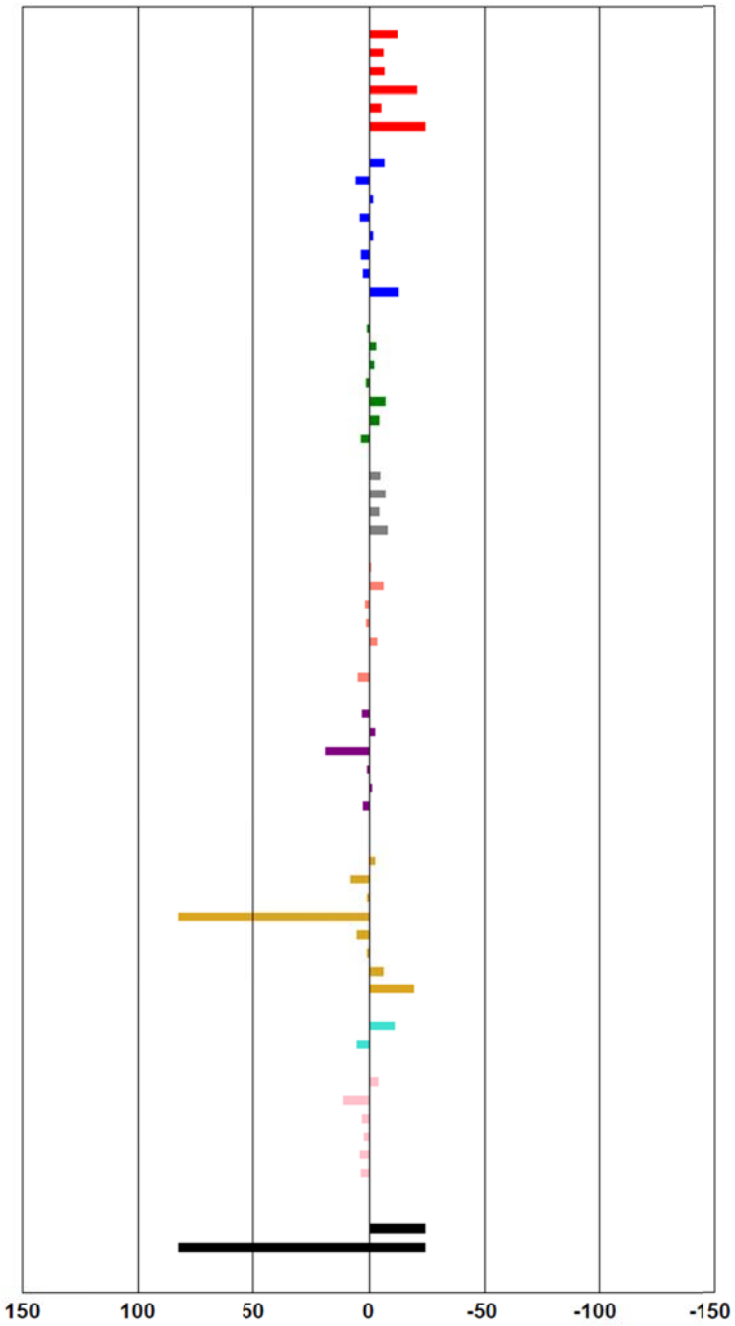
One dose experimental data of compound 4.76 (NSC 767530)

Developmental Therapeutics Program

One Dose Mean Graph

NSC: D-767530 / 1	Conc: 1.00E-5 Molar	Test Date: Sep 24, 2012
Experiment ID: 1209OS49		Report Date: May 09, 2013

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	92.74
HL-60(TB)	98.94
K-562	98.79
MOLT-4	83.99
RPMI-8226	100.03
SR	80.47
Non-Small Cell Lung Cancer	
A549/ATCC	98.47
HOP-62	110.50
HOP-92	103.52
NCI-H226	108.88
NCI-H23	103.37
NCI-H322M	108.54
NCI-H460	107.44
NCI-H522	91.95
Colon Cancer	
COLO 205	105.94
HCC-2998	102.35
HCT-116	103.13
HCT-15	106.27
HT29	98.30
KM12	100.69
SW-620	108.28
CNS Cancer	
SF-268	100.58
SF-539	98.02
SNB-19	100.80
SNB-75	97.46
Melanoma	
LOX IMVI	104.43
MALME-3M	98.89
M14	106.79
MDA-MB-435	106.10
SK-MEL-28	101.55
SK-MEL-5	104.95
UACC-62	109.80
Ovarian Cancer	
IGROV1	107.88
OVCAR-3	102.50
OVCAR-4	123.34
OVCAR-5	105.66
OVCAR-8	103.86
NCI/ADR-RES	107.34
SK-OV-3	104.96
Renal Cancer	
786-0	102.51
A498	112.98
ACHN	105.90
CAKI-1	187.29
RXF 393	110.28
SN12C	105.75
TK-10	99.09
UO-31	85.34
Prostate Cancer	
PC-3	94.00
DU-145	110.02
Breast Cancer	
MCF7	101.24
MDA-MB-231/ATCC	115.88
HS 578T	107.81
BT-549	106.85
T-47D	108.86
MDA-MB-468	108.31
Mean	104.72
Delta	24.25
Range	106.82



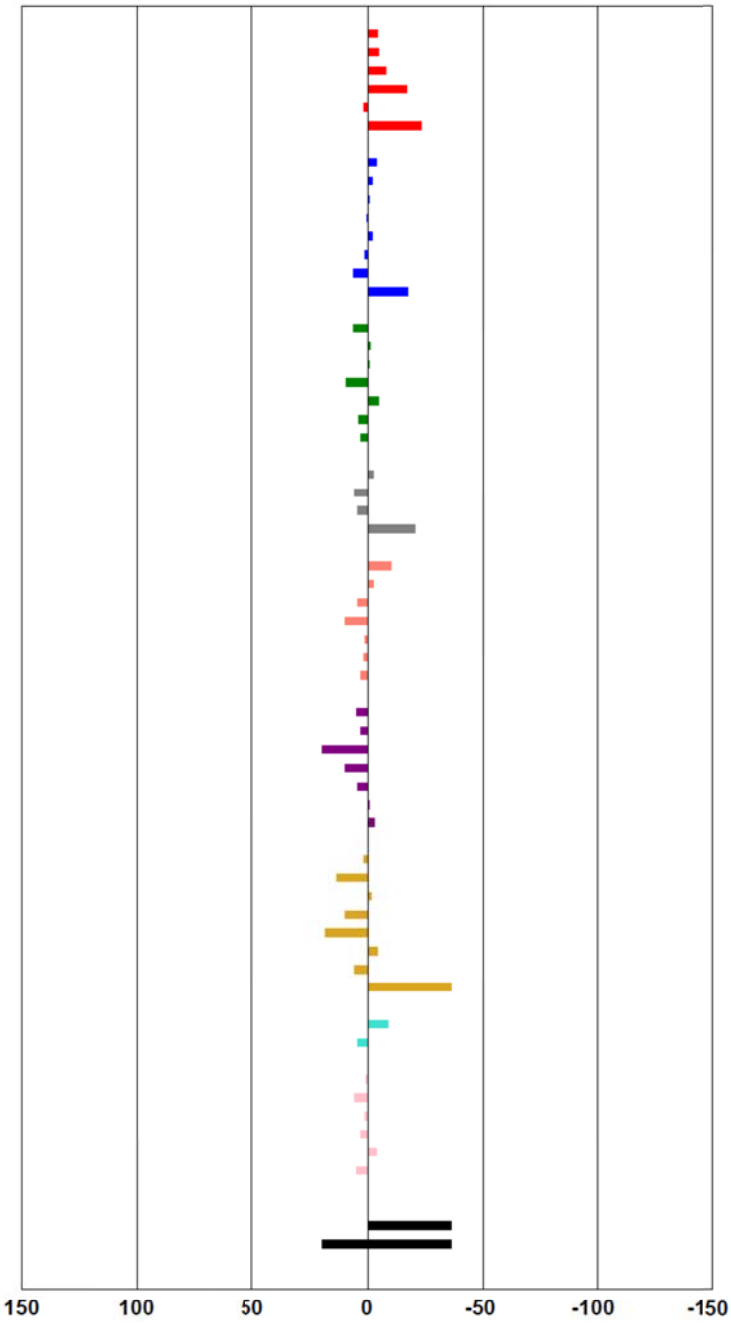
One dose experimental data of compound 4.77 (NSC 767531)

Developmental Therapeutics Program

One Dose Mean Graph

NSC: D-767531 / 1	Conc: 1.00E-5 Molar	Test Date: Sep 24, 2012
Experiment ID: 1209OS49		Report Date: May 09, 2013

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	96.85
HL-60(TB)	96.23
K-562	93.20
MOLT-4	83.15
RPMI-8226	102.31
SR	77.13
Non-Small Cell Lung Cancer	
A549/ATCC	97.29
HOP-62	99.01
HOP-92	100.17
NCI-H226	101.08
NCI-H23	98.79
NCI-H322M	102.15
NCI-H460	107.01
NCI-H522	82.54
Colon Cancer	
COLO 205	106.89
HCC-2998	99.57
HCT-116	100.16
HCT-15	109.81
HT29	96.11
KM12	104.76
SW-620	103.82
CNS Cancer	
SF-268	98.40
SF-539	106.31
SNB-19	105.10
SNB-75	79.57
Melanoma	
LOX IMVI	90.84
MALME-3M	98.28
M14	104.90
MDA-MB-435	110.24
SK-MEL-28	102.05
SK-MEL-5	102.24
UACC-62	103.50
Ovarian Cancer	
IGROV1	105.40
OVCAR-3	103.71
OVCAR-4	120.00
OVCAR-5	110.51
OVCAR-8	105.06
NCI/ADR-RES	100.23
SK-OV-3	97.91
Renal Cancer	
786-0	102.61
A498	114.08
ACHN	99.42
CAKI-1	110.26
RXF 393	119.03
SN12C	96.74
TK-10	106.24
UO-31	64.25
Prostate Cancer	
PC-3	92.01
DU-145	105.08
Breast Cancer	
MCF7	101.43
MDA-MB-231/ATCC	106.39
HS 578T	102.17
BT-549	103.76
T-47D	97.19
MDA-MB-468	105.40
Mean	100.51
Delta	36.26
Range	55.75



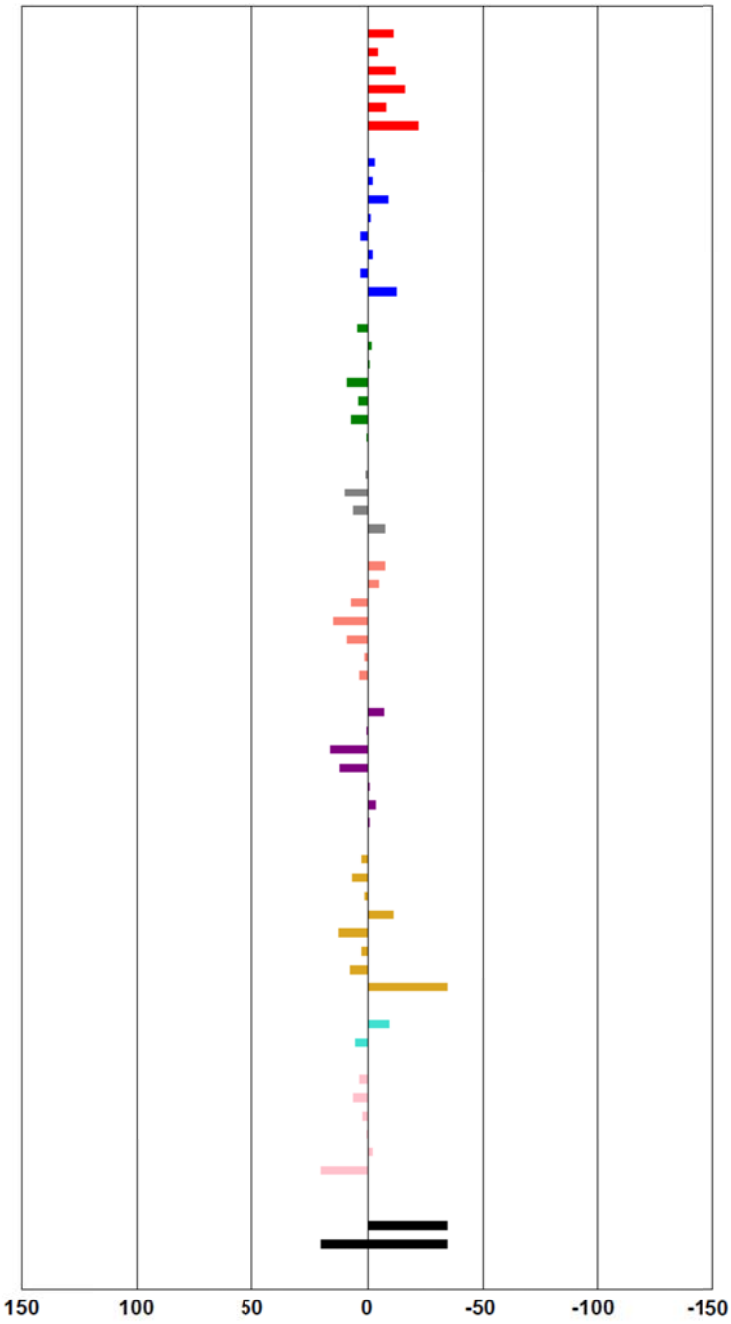
One dose experimental data of compound 4.78 (NSC 767532)

Developmental Therapeutics Program

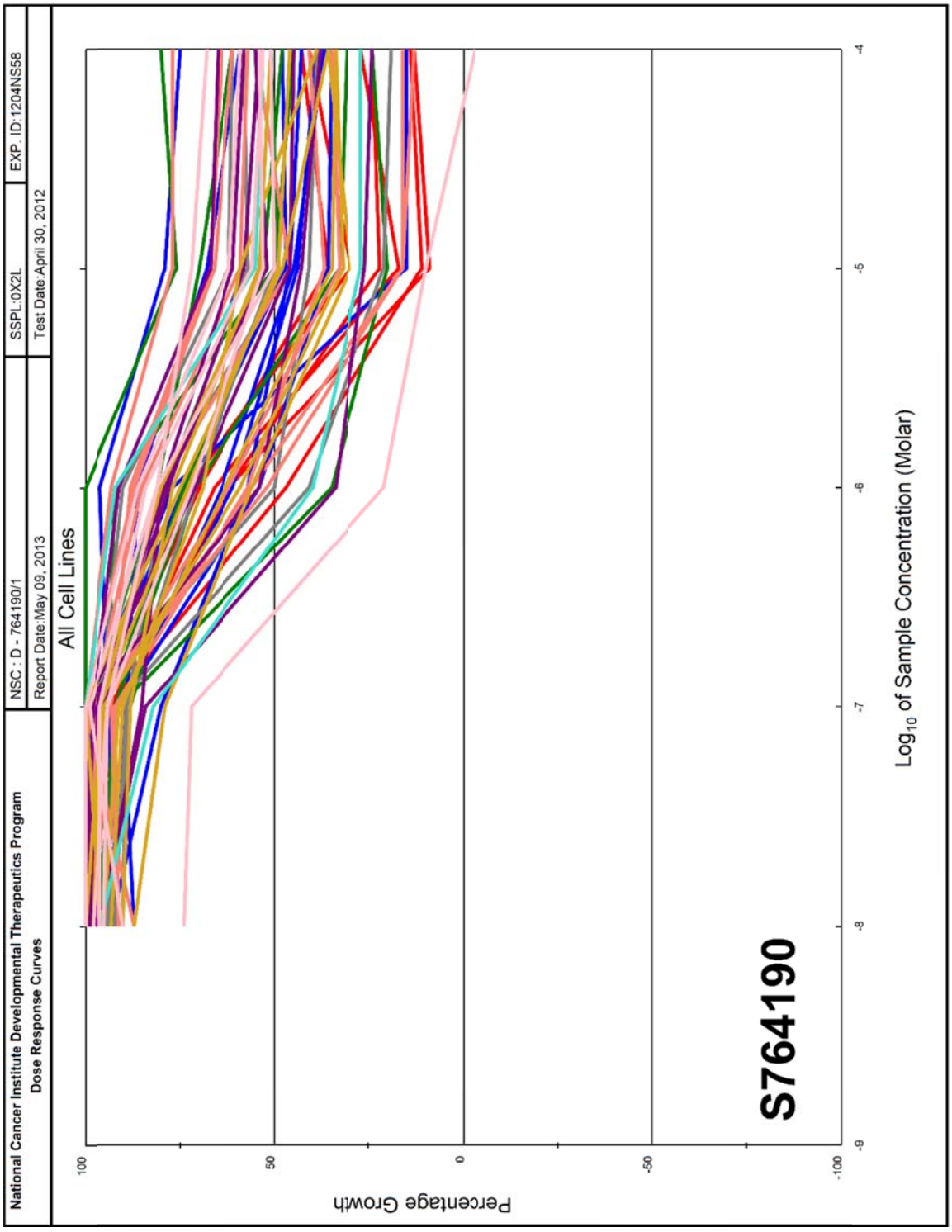
One Dose Mean Graph

NSC: D-767532 / 1	Conc: 1.00E-5 Molar	Test Date: Sep 24, 2012
Experiment ID: 1209OS49		Report Date: May 09, 2013

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	88.52
HL-60(TB)	95.41
K-562	87.61
MOLT-4	82.80
RPMI-8226	91.59
SR	77.01
Non-Small Cell Lung Cancer	
A549/ATCC	96.53
HOP-62	97.43
HOP-92	90.88
NCI-H226	98.40
NCI-H23	102.29
NCI-H322M	97.54
NCI-H460	102.38
NCI-H522	87.38
Colon Cancer	
COLO 205	103.97
HCC-2998	98.02
HCT-116	99.15
HCT-15	108.30
HT29	103.25
KM12	106.45
SW-620	100.02
CNS Cancer	
SF-268	100.29
SF-539	109.16
SNB-19	105.59
SNB-75	92.32
Melanoma	
LOX IMVI	92.33
MALME-3M	95.03
M14	106.35
MDA-MB-435	113.93
SK-MEL-28	108.44
SK-MEL-5	100.58
UACC-62	102.75
Ovarian Cancer	
IGROV1	92.78
OVCAR-3	99.72
OVCAR-4	115.23
OVCAR-5	111.59
OVCAR-8	99.04
NCI/ADR-RES	96.08
SK-OV-3	98.96
Renal Cancer	
786-0	102.09
A498	106.00
ACHN	100.76
CAKI-1	88.76
RXF 393	111.53
SN12C	101.88
TK-10	106.75
UO-31	64.80
Prostate Cancer	
PC-3	90.39
DU-145	104.52
Breast Cancer	
MCF7	102.76
MDA-MB-231/ATCC	105.56
HS 578T	101.50
BT-549	99.97
T-47D	97.46
MDA-MB-468	119.30
Mean	99.27
Delta	34.47
Range	54.50

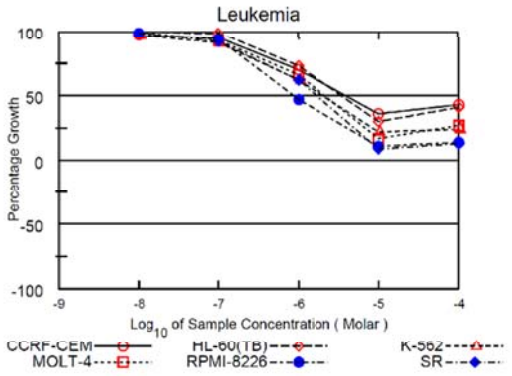


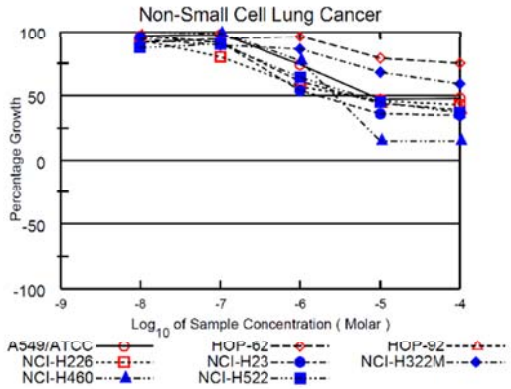
Five dose experimental data of compound 4.21 (NSC 764190)

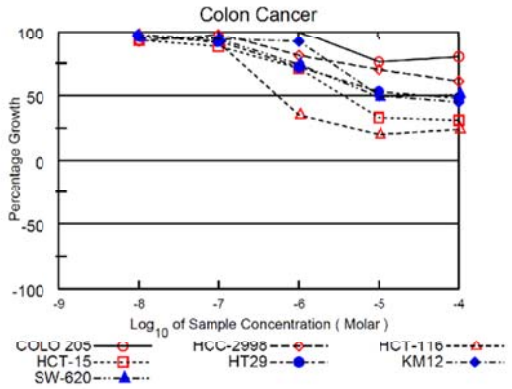


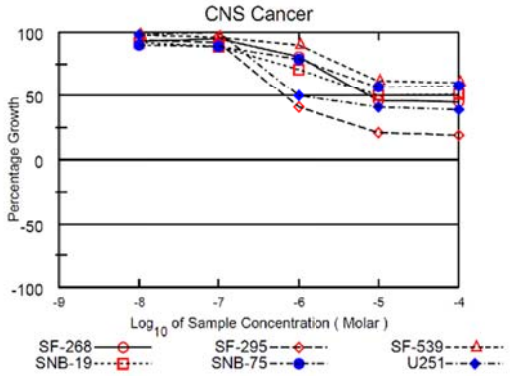
National Cancer Institute Developmental Therapeutics Program
Dose Response Curves

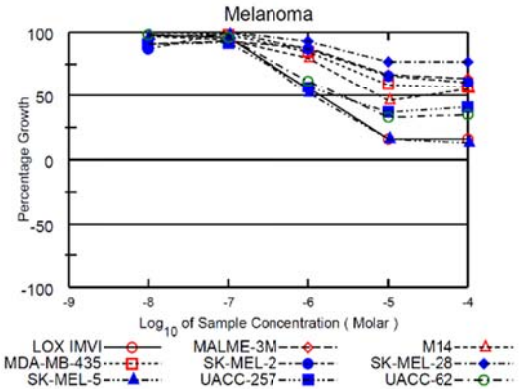
NSC: D - 764190 / 1	SSPL: 0X2L	EXP. ID: 1204NS58
Report Date: May 09, 2013	Test Date: April 30, 2012	

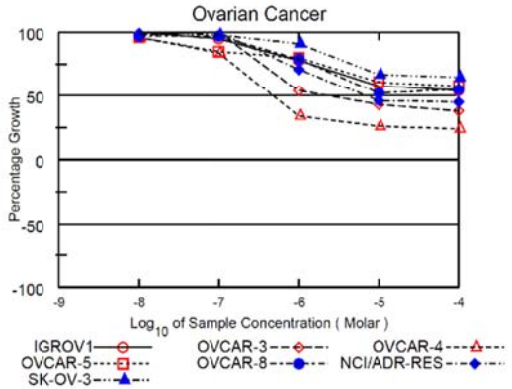


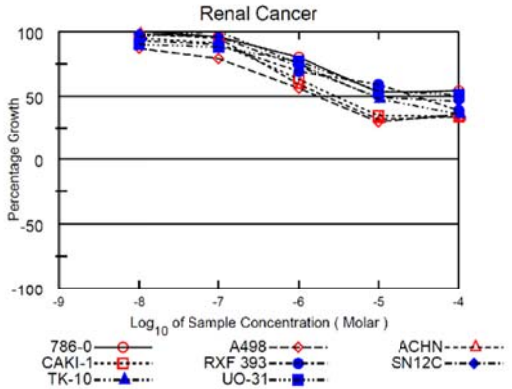


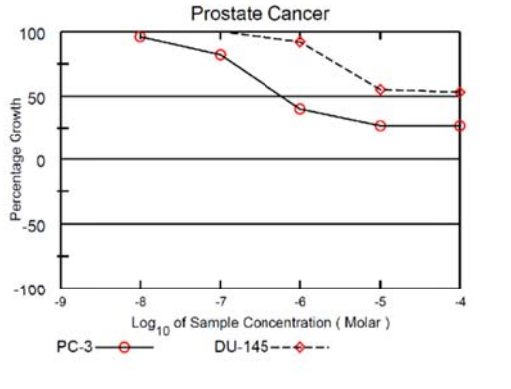


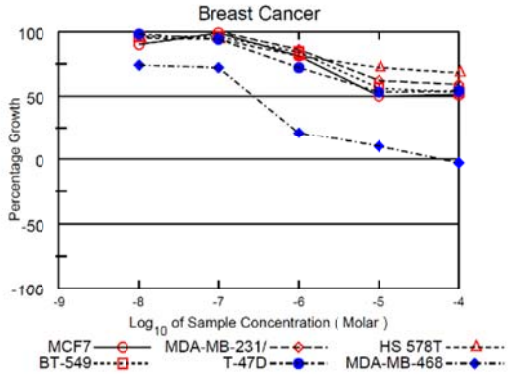












National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

NSC : D - 764190 / 1	Experiment ID : 1204NS58	Test Type : 08	Units : Molar
Report Date : May 09, 2013	Test Date : April 30, 2012	QNS :	MC :
COMI : LSC-KU-JJ-II-134-1 (91319)	Stain Reagent : SRB Dual-Pass Related	SSPL : 0X2L	

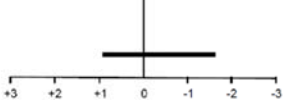
			Log10 Concentration											
	Time		Mean Optical Densities					Percent Growth						
Panel/Cell Line	Zero	Ctrl	-8.0	-7.0	-6.0	-5.0	-4.0	-8.0	-7.0	-6.0	-5.0	-4.0	GI50	TGI	LC50
Leukemia															
CCRF-CEM	0.703	2.623	2.566	2.539	2046	1.395	1.537	97	95	70	36	43	3.85E-6	> 1.00E-4	> 1.00E-4
HL-60(TB)	0.718	2.622	2.615	2.600	2115	1.285	1.507	100	99	73	30	41	3.43E-6	> 1.00E-4	> 1.00E-4
K-562	0.265	1.739	1.696	1.611	1183	0.584	0.616	97	91	62	22	24	2.00E-6	> 1.00E-4	> 1.00E-4
MOLT-4	0.512	1.901	1.895	1.809	1432	0.753	0.884	100	93	66	17	27	2.15E-6	> 1.00E-4	> 1.00E-4
RPMI-8226	0.882	2.369	2.353	2.280	1579	1.047	1.095	99	94	47	11	14	8.59E-7	> 1.00E-4	> 1.00E-4
SR	0.510	1.773	1.761	1.671	1292	0.618	0.679	99	92	62	9	13	1.66E-6	> 1.00E-4	> 1.00E-4
Non-Small Cell Lung Cancer															
A549/ATCC	0.256	1.450	1.415	1.427	1142	0.816	0.830	97	98	74	47	48	7.67E-6	> 1.00E-4	> 1.00E-4
HOP-62	0.357	0.933	0.883	0.901	0914	0.813	0.793	91	94	96	79	75	> 1.00E-4	> 1.00E-4	> 1.00E-4
HOP-92	1.100	1.471	1.460	1.439	1325	1.264	1.245	97	91	61	44	39	4.40E-6	> 1.00E-4	> 1.00E-4
NCI-H226	0.549	1.348	1.292	1.186	1000	0.918	0.888	93	80	57	46	43	4.35E-6	> 1.00E-4	> 1.00E-4
NCI-H23	0.524	1.519	1.479	1.427	1060	0.885	0.872	96	91	54	36	35	1.66E-6	> 1.00E-4	> 1.00E-4
NCI-H322M	0.723	1.609	1.558	1.512	1488	1.324	1.243	94	89	86	68	59	> 1.00E-4	> 1.00E-4	> 1.00E-4
NCI-H460	0.368	2.742	2.742	2.708	2206	0.725	0.723	100	99	77	15	15	2.75E-6	> 1.00E-4	> 1.00E-4
NCI-H522	0.829	1.749	1.630	1.656	1422	1.242	1.169	87	90	64	45	37	5.46E-6	> 1.00E-4	> 1.00E-4
Colon Cancer															
COLO 205	0.277	1.073	1.108	1.094	1091	0.886	0.914	104	102	102	76	80	> 1.00E-4	> 1.00E-4	> 1.00E-4
HCC-2998	0.442	1.641	1.541	1.622	1414	1.286	1.170	92	98	81	70	61	> 1.00E-4	> 1.00E-4	> 1.00E-4
HCT-116	0.174	1.593	1.550	1.473	0668	0.465	0.515	97	91	35	20	24	5.35E-7	> 1.00E-4	> 1.00E-4
HCT-15	0.280	1.393	1.324	1.265	1072	0.645	0.621	93	88	71	33	31	3.51E-6	> 1.00E-4	> 1.00E-4
HT29	0.313	1.540	1.488	1.440	1200	0.959	0.907	96	92	72	53	48	4.26E-5	> 1.00E-4	> 1.00E-4
KM12	0.208	1.143	1.127	1.091	1065	0.674	0.630	98	94	92	50	45	9.92E-6	> 1.00E-4	> 1.00E-4
SW-620	0.368	2.384	2.281	2.256	1856	1.355	1.389	95	94	74	49	51	.	> 1.00E-4	> 1.00E-4
CNS Cancer															
SF-268	0.553	1.683	1.607	1.634	1469	1.080	1.066	93	95	81	46	45	7.85E-6	> 1.00E-4	> 1.00E-4
SF-295	1.189	2.860	2.755	2.730	1874	1.536	1.511	94	92	41	21	19	6.67E-7	> 1.00E-4	> 1.00E-4
SF-539	0.887	2.213	2.186	2.167	2091	1.719	1.700	98	96	90	62	61	> 1.00E-4	> 1.00E-4	> 1.00E-4
SNB-19	0.466	1.307	1.239	1.218	1062	0.887	0.898	92	89	71	50	51	> 1.00E-4	> 1.00E-4	> 1.00E-4
SNB-75	0.533	1.145	1.087	1.077	1017	0.882	0.890	90	89	79	57	58	> 1.00E-4	> 1.00E-4	> 1.00E-4
U251	0.355	1.720	1.688	1.750	1044	0.910	0.886	98	102	50	41	39	1.11E-6	> 1.00E-4	> 1.00E-4
Melanoma															
LOX IMVI	0.274	2.115	2.079	2.034	1301	0.570	0.563	98	96	56	16	16	1.40E-6	> 1.00E-4	> 1.00E-4
MALME-3M	0.670	1.404	1.340	1.352	1314	1.161	1.141	91	93	88	67	64	> 1.00E-4	> 1.00E-4	> 1.00E-4
M14	0.347	1.379	1.345	1.318	1169	0.821	0.920	97	94	80	46	55	.	> 1.00E-4	> 1.00E-4
MDA-MB-435	0.473	2.138	2.128	2.104	1893	1.457	1.426	100	98	85	59	57	> 1.00E-4	> 1.00E-4	> 1.00E-4
SK-MEL-2	0.971	1.699	1.604	1.721	1605	1.448	1.414	87	103	87	66	61	> 1.00E-4	> 1.00E-4	> 1.00E-4
SK-MEL-28	0.464	1.273	1.300	1.288	1213	1.088	1.089	103	102	93	77	77	> 1.00E-4	> 1.00E-4	> 1.00E-4
SK-MEL-5	0.503	2.555	2.503	2.506	1571	0.827	0.773	97	98	52	16	13	1.14E-6	> 1.00E-4	> 1.00E-4
UACC-257	0.722	1.713	1.625	1.638	1286	1.091	1.130	91	92	57	37	41	2.23E-6	> 1.00E-4	> 1.00E-4
UACC-62	0.960	2.305	2.274	2.246	1800	1.406	1.425	98	96	62	33	35	2.66E-6	> 1.00E-4	> 1.00E-4
Ovarian Cancer															
IGROV1	0.620	1.843	1.852	1.785	1571	1.331	1.278	101	95	78	58	54	> 1.00E-4	> 1.00E-4	> 1.00E-4
OVCAR-3	0.545	1.393	1.382	1.408	1007	0.911	0.866	98	101	54	43	38	2.34E-6	> 1.00E-4	> 1.00E-4
OVCAR-4	0.661	1.535	1.505	1.395	0962	0.889	0.875	96	84	34	26	24	4.83E-7	> 1.00E-4	> 1.00E-4
OVCAR-5	0.531	1.470	1.429	1.326	1281	1.108	1.073	96	85	80	61	58	> 1.00E-4	> 1.00E-4	> 1.00E-4
OVCAR-8	0.320	1.393	1.363	1.364	1176	0.880	0.916	97	97	79	52	55	> 1.00E-4	> 1.00E-4	> 1.00E-4
NCI/ADR-RES	0.556	1.829	1.815	1.969	1462	1.144	1.132	99	111	71	46	45	7.03E-6	> 1.00E-4	> 1.00E-4
SK-OV-3	0.340	0.883	0.894	0.875	0835	0.705	0.694	101	98	91	67	65	> 1.00E-4	> 1.00E-4	> 1.00E-4
Renal Cancer															
786-0	0.509	2.003	2.015	1.953	1709	1.305	1.316	100	96	80	53	54	> 1.00E-4	> 1.00E-4	> 1.00E-4
A498	1.232	1.873	1.794	1.738	1596	1.428	1.461	87	79	56	30	36	1.77E-6	> 1.00E-4	> 1.00E-4
ACHN	0.279	1.249	1.233	1.200	0848	0.589	0.608	98	95	59	32	34	2.10E-6	> 1.00E-4	> 1.00E-4
CAKI-1	0.628	2.289	2.198	2.139	1667	1.206	1.186	95	91	63	35	34	2.83E-6	> 1.00E-4	> 1.00E-4
RXF 393	0.555	1.020	0.987	0.972	0874	0.828	0.738	93	90	69	59	39	2.80E-5	> 1.00E-4	> 1.00E-4
SN12C	0.590	2.401	2.370	2.333	1912	1.485	1.423	98	96	73	49	46	9.42E-6	> 1.00E-4	> 1.00E-4
TK-10	0.896	1.472	1.447	1.505	1328	1.171	1.103	96	106	75	48	36	8.26E-6	> 1.00E-4	> 1.00E-4
UO-31	0.685	1.815	1.700	1.680	1555	1.300	1.263	90	88	77	54	51	> 1.00E-4	> 1.00E-4	> 1.00E-4
Prostate Cancer															
PC-3	0.624	1.953	1.905	1.719	1156	0.986	0.978	96	82	40	27	27	5.80E-7	> 1.00E-4	> 1.00E-4
DU-145	0.374	1.338	1.340	1.346	1260	0.902	0.882	100	101	92	55	53	> 1.00E-4	> 1.00E-4	> 1.00E-4
Breast Cancer															
MCF7	0.264	1.498	1.376	1.490	1262	0.882	0.897	90	99	81	50	51	> 1.00E-4	> 1.00E-4	> 1.00E-4
MDA-MB-231/ATCC	0.515	1.280	1.340	1.317	1176	0.988	0.963	108	105	86	62	59	> 1.00E-4	> 1.00E-4	> 1.00E-4
HS 578T	1.090	1.835	1.801	1.789	1695	1.625	1.595	95	94	81	72	68	> 1.00E-4	> 1.00E-4	> 1.00E-4
BT-549	0.700	1.733	1.696	1.698	1572	1.278	1.248	96	96	84	56	53	> 1.00E-4	> 1.00E-4	> 1.00E-4
T-47D	0.624	1.338	1.321	1.294	1138	1.001	1.007	98	94	72	53	54	> 1.00E-4	> 1.00E-4	> 1.00E-4
MDA-MB-468	0.596	1.051	0.932	0.923	0690	0.643	0.579	74	72	21	10	-3	2.67E-7	6.01E-5	> 1.00E-4

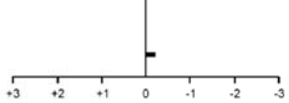
National Cancer Institute Developmental Therapeutics Program

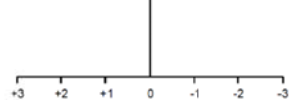
Mean Graphs

NSC : D - 764190/1	Units :Molar	SSPL :0X2L	EXP. ID :1204NS58
Report Date :May 09, 2013		Test Date :April 30, 2012	

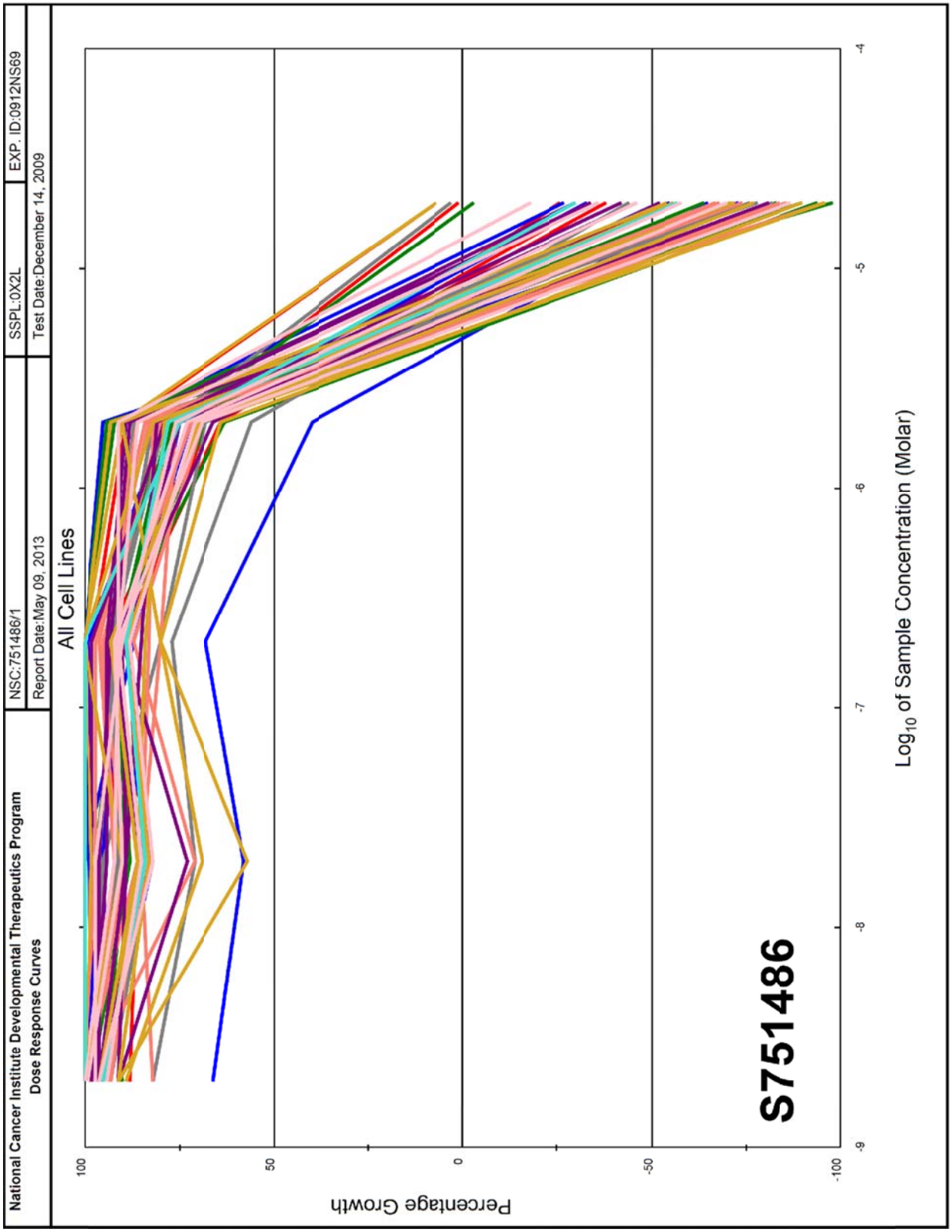
Panel/Cell Line	Log_{10}GI50	GI50	Log_{10}TGI	TGI	Log_{10}LC50	LC50
Leukemia						
CCRF-CEM	-5.41		> -4.00		> -4.00	
HL-60(TB)	-5.46		> -4.00		> -4.00	
K-562	-5.70		> -4.00		> -4.00	
MOLT-4	-5.67		> -4.00		> -4.00	
RPMI-8226	-6.07		> -4.00		> -4.00	
SR	-5.78		> -4.00		> -4.00	
Non-Small Cell Lung Cancer						
A549/ATCC	-5.12		> -4.00		> -4.00	
HOP-62	> -4.00		> -4.00		> -4.00	
HOP-92	-5.36		> -4.00		> -4.00	
NCI-H226	-5.36		> -4.00		> -4.00	
NCI-H23	-5.78		> -4.00		> -4.00	
NCI-H322M	> -4.00		> -4.00		> -4.00	
NCI-H460	-5.56		> -4.00		> -4.00	
NCI-H522	-5.26		> -4.00		> -4.00	
Colon Cancer						
COLO 205	> -4.00		> -4.00		> -4.00	
HCC-2998	> -4.00		> -4.00		> -4.00	
HCT-116	-6.27		> -4.00		> -4.00	
HCT-15	-5.45		> -4.00		> -4.00	
HT29	-4.37		> -4.00		> -4.00	
KM12	-5.00		> -4.00		> -4.00	
SW-620			> -4.00		> -4.00	
CNS Cancer						
SF-268	-5.11		> -4.00		> -4.00	
SF-295	-6.18		> -4.00		> -4.00	
SF-539	> -4.00		> -4.00		> -4.00	
SNB-19	> -4.00		> -4.00		> -4.00	
SNB-75	> -4.00		> -4.00		> -4.00	
U251	-5.96		> -4.00		> -4.00	
Melanoma						
LOX IMVI	-5.85		> -4.00		> -4.00	
MALME-3M	> -4.00		> -4.00		> -4.00	
M14			> -4.00		> -4.00	
MDA-MB-435	> -4.00		> -4.00		> -4.00	
SK-MEL-2	> -4.00		> -4.00		> -4.00	
SK-MEL-28	> -4.00		> -4.00		> -4.00	
SK-MEL-5	-5.94		> -4.00		> -4.00	
UACC-257	-5.65		> -4.00		> -4.00	
UACC-62	-5.57		> -4.00		> -4.00	
Ovarian Cancer						
IGROV1	> -4.00		> -4.00		> -4.00	
OVCAR-3	-5.63		> -4.00		> -4.00	
OVCAR-4	-6.32		> -4.00		> -4.00	
OVCAR-5	> -4.00		> -4.00		> -4.00	
OVCAR-8	> -4.00		> -4.00		> -4.00	
NCI/ADR-RES	-5.15		> -4.00		> -4.00	
SK-OV-3	> -4.00		> -4.00		> -4.00	
Renal Cancer						
786-0	> -4.00		> -4.00		> -4.00	
A498	-5.75		> -4.00		> -4.00	
ACHN	-5.68		> -4.00		> -4.00	
CAKI-1	-5.55		> -4.00		> -4.00	
RXF 393	-4.55		> -4.00		> -4.00	
SN12C	-6.03		> -4.00		> -4.00	
TK-10	-5.08		> -4.00		> -4.00	
UO-31	> -4.00		> -4.00		> -4.00	
Prostate Cancer						
PC-3	-6.24		> -4.00		> -4.00	
DU-145	> -4.00		> -4.00		> -4.00	
Breast Cancer						
MCF7	> -4.00		> -4.00		> -4.00	
MDA-MB-231/ATCC	> -4.00		> -4.00		> -4.00	
HS 578T	> -4.00		> -4.00		> -4.00	
BT-549	> -4.00		> -4.00		> -4.00	
T-47D	> -4.00		> -4.00		> -4.00	
MDA-MB-468	-6.57		-4.22		> -4.00	
MID	-4.94		-4.0		-4.0	
Delta	1.63		0.22		0	
Range	2.57		0.22		0.0	





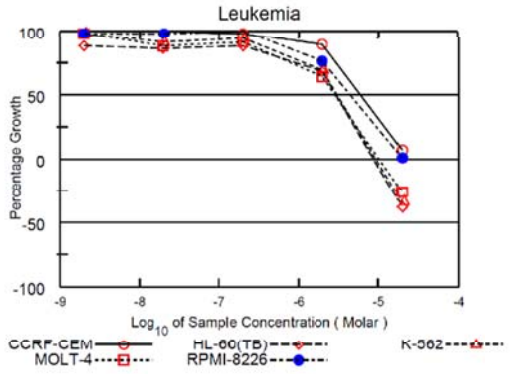


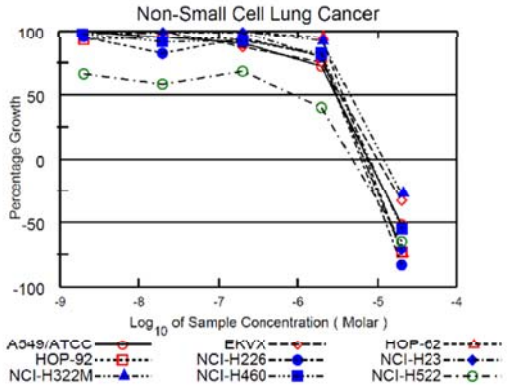
Five dose experimental data of compound 4.22 (NSC 751486)

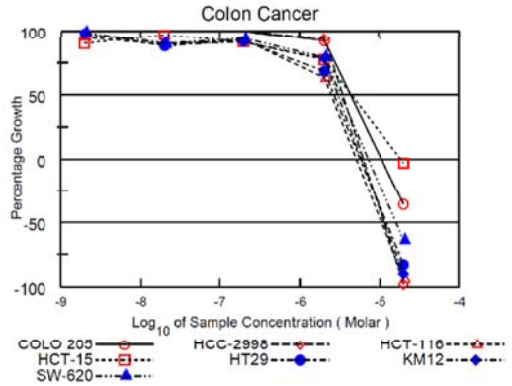


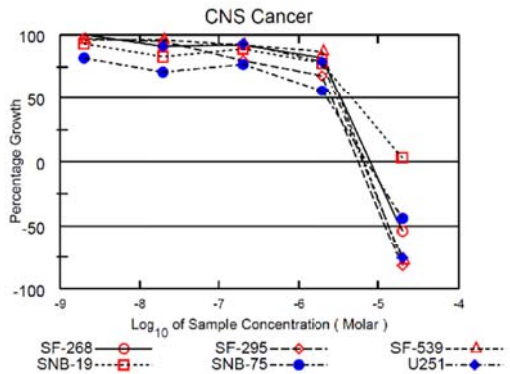
National Cancer Institute Developmental Therapeutics Program
Dose Response Curves

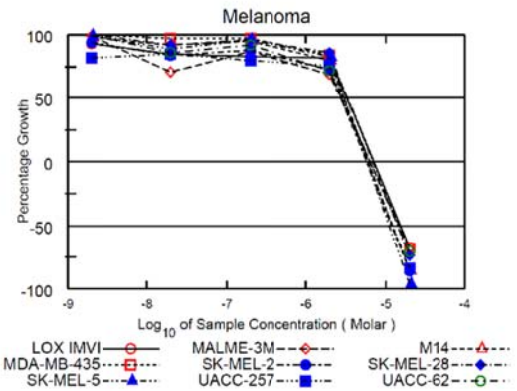
NSC: 751486 / 1	SSPL: 0X2L	EXP. ID: 0912NS69
Report Date: May 09, 2013	Test Date: December 14, 2009	

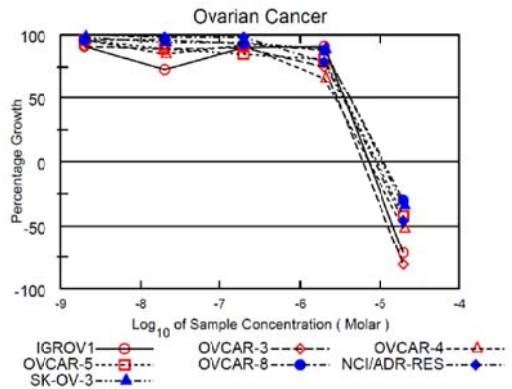


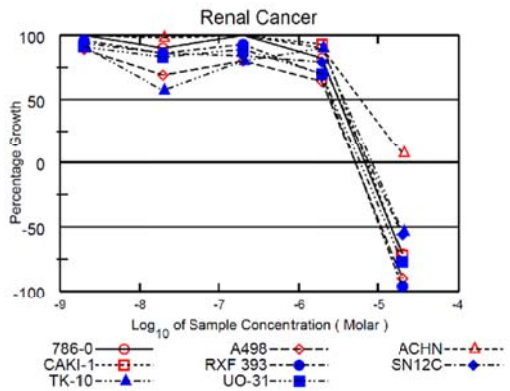


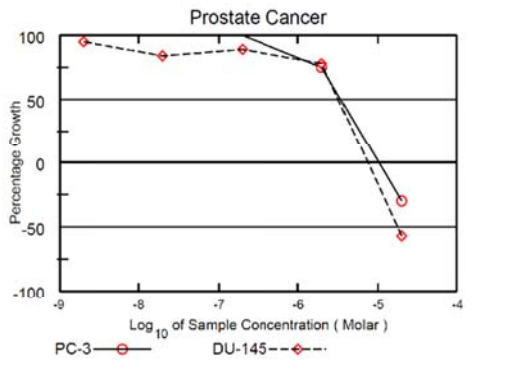


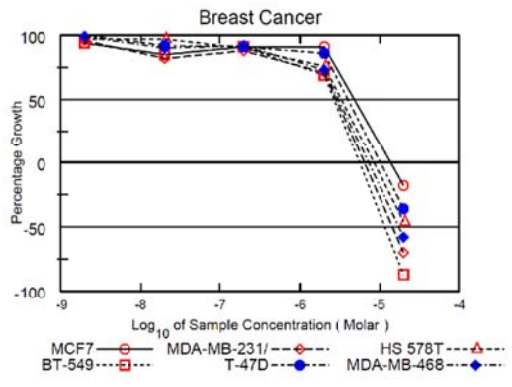








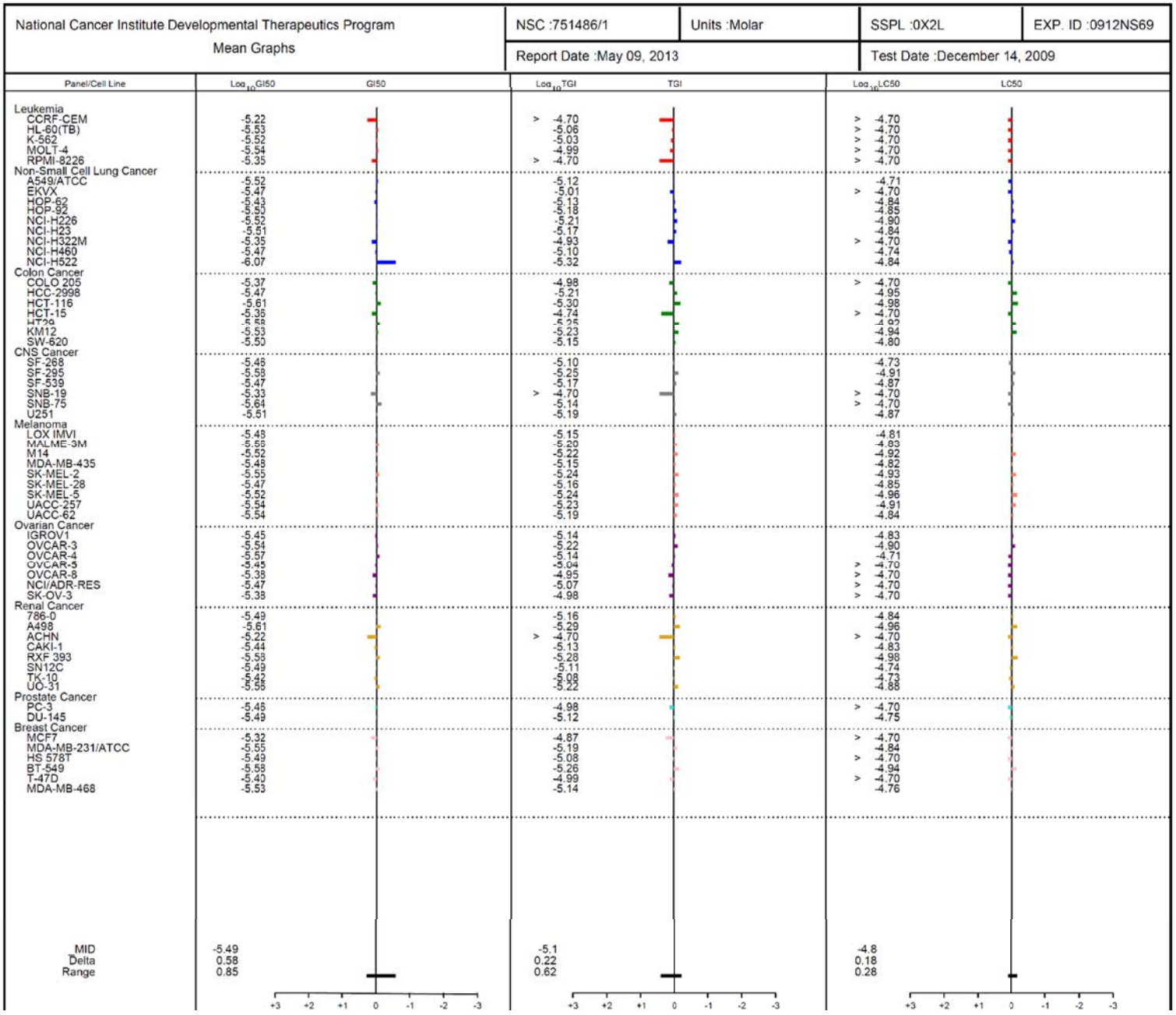




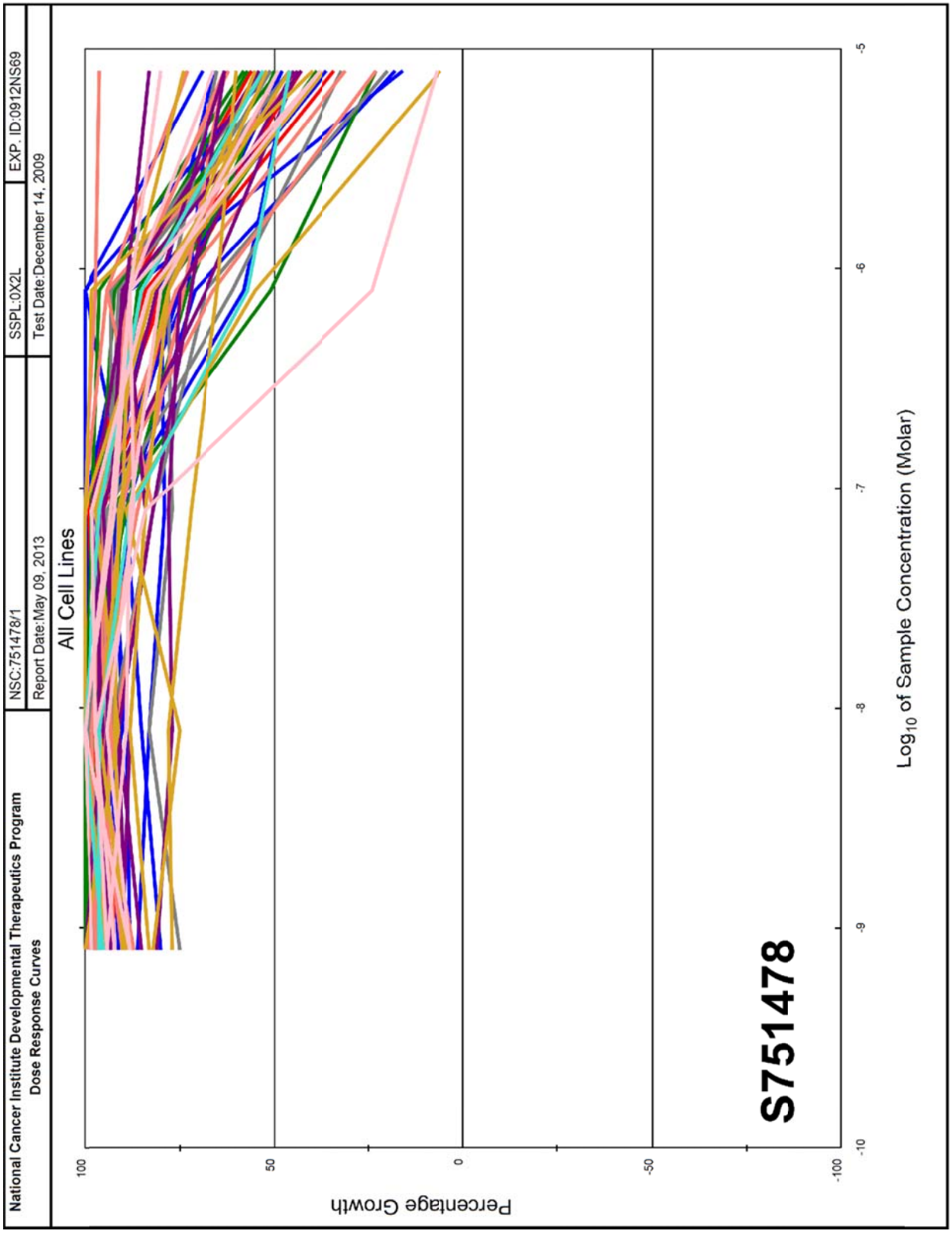
National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

NSC : 751486 / 1	Experiment ID : 0912NS69	Test Type : 08	Units : Molar
Report Date : May 09, 2013	Test Date : December 14, 2009	QNS :	MC :
COMI : LSC-KU-JJ-II-140-1 (91146)	Stain Reagent : SRB Dual-Pass Related	SSPL : 0X2L	

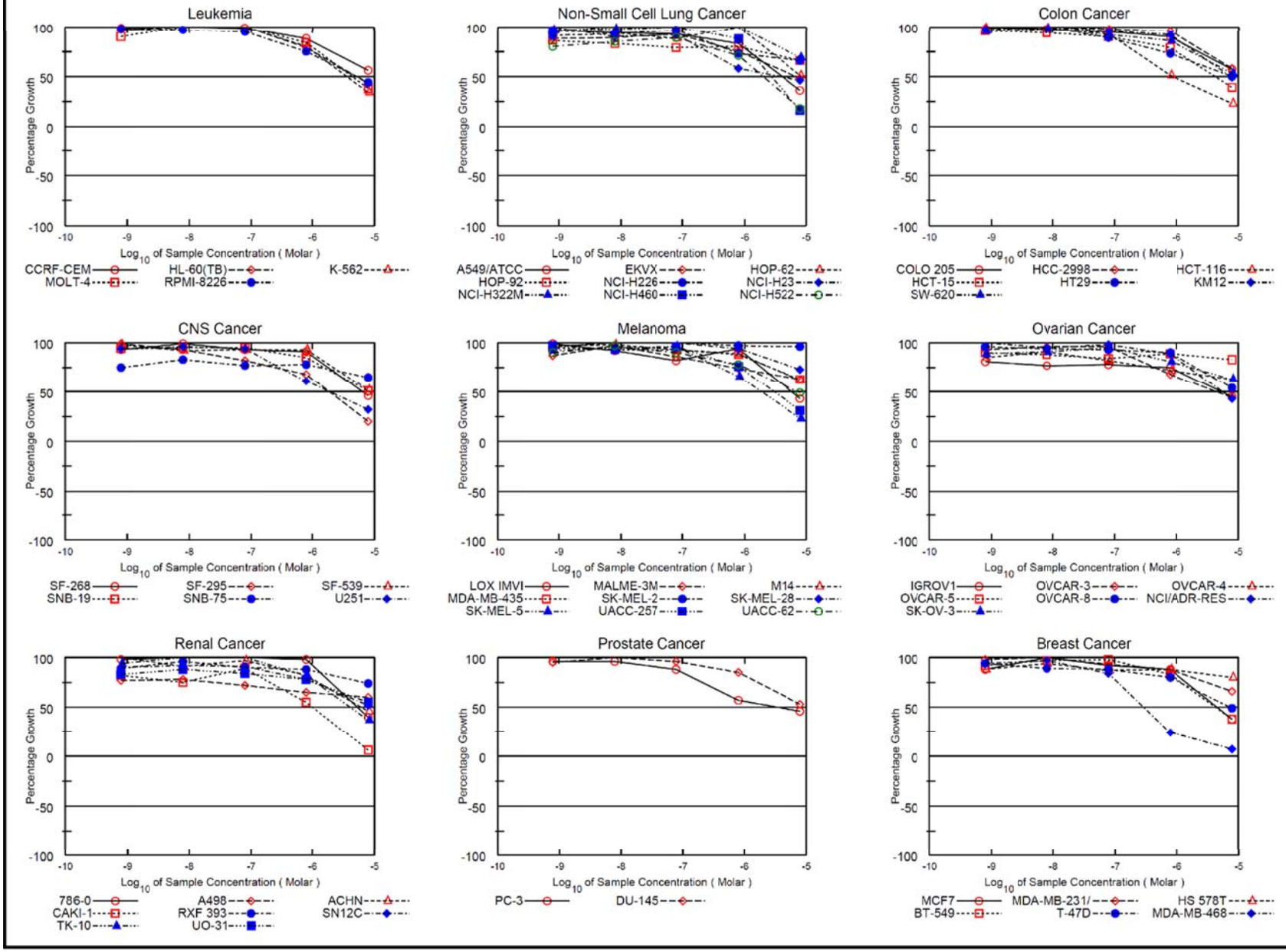
			Log10 Concentration												
	Time		Mean Optical Densities					Percent Growth							
Panel/Cell Line	Zero	Ctrl	-8.7	-7.7	-6.7	-5.7	-4.7	-8.7	-7.7	-6.7	-5.7	-4.7	GI50	TGI	LC50
Leukemia															
CCRF-CEM	0.349	1.650	1.647	1.656	1620	1.503	0.442	100	100	98	89	7	5.96E-6	> 2.00E-5	> 2.00E-5
HL-60(TB)	0.717	2.673	2.438	2.401	2440	2.053	0.441	88	86	88	68	-38	2.97E-6	8.72E-6	> 2.00E-5
K-562	0.257	1.660	1.633	1.531	1572	1.221	0.171	98	91	94	69	-34	3.05E-6	9.38E-6	> 2.00E-5
MOLT-4	0.598	1.955	1.932	1.790	1837	1.467	0.440	98	88	91	64	-26	2.86E-6	1.02E-5	> 2.00E-5
RPMI-8226	0.685	2.365	2.332	2.329	2369	1.966	0.710	98	98	100	76	1	4.49E-6	> 2.00E-5	> 2.00E-5
Non-Small Cell Lung Cancer															
A549/ATCC	0.353	1.107	1.131	1.072	1030	0.893	0.171	103	95	90	72	-52	2.99E-6	7.62E-6	1.94E-5
EKVX	0.633	1.603	1.689	1.609	1482	1.369	0.421	108	100	87	75	-33	3.43E-6	9.86E-6	> 2.00E-5
HOP-62	0.259	0.825	0.893	0.837	0845	0.800	0.069	112	102	103	95	-74	3.71E-6	7.34E-6	1.45E-5
HOP-92	1.146	2.033	1.975	1.993	1974	1.859	0.308	93	95	93	80	-73	3.15E-6	6.68E-6	1.41E-5
NCI-H226	0.670	1.443	1.403	1.305	1396	1.285	0.114	95	82	94	80	-83	3.04E-6	6.17E-6	1.25E-5
NCI-H23	0.402	1.253	1.245	1.253	1251	1.077	0.116	98	99	99	79	-71	3.11E-6	6.71E-6	1.45E-5
NCI-H322M	0.461	0.872	0.959	0.898	0883	0.838	0.335	121	106	103	92	-27	4.48E-6	1.18E-5	> 2.00E-5
NCI-H460	0.241	1.933	1.897	1.774	1801	1.625	0.108	98	91	92	82	-55	3.41E-6	7.91E-6	1.83E-5
NCI-H522	0.593	0.822	0.743	0.726	0748	0.684	0.210	66	58	68	40	-65	8.47E-7	4.79E-6	1.45E-5
Colon Cancer															
COLO 205	0.195	1.363	1.401	1.412	1377	1.276	0.125	103	104	101	92	-36	4.26E-6	1.05E-5	> 2.00E-5
HCC-2998	0.747	1.797	1.848	1.808	1848	1.738	0.018	105	101	105	94	-98	3.41E-6	6.20E-6	1.13E-5
HCT-116	0.239	1.655	1.590	1.506	1559	1.137	0.014	95	89	93	63	-94	2.43E-6	5.05E-6	1.05E-5
HCT-15	0.243	1.625	1.493	1.578	1507	1.312	0.235	90	97	91	77	-3	4.37E-6	1.82E-5	> 2.00E-5
HT29	0.153	0.599	0.592	0.545	0563	0.456	0.026	98	88	92	68	-83	2.63E-6	5.64E-6	1.21E-5
KM12	0.247	1.315	1.291	1.211	1216	1.083	0.025	98	90	91	78	-90	2.94E-6	5.84E-6	1.16E-5
SW-620	0.205	1.169	1.157	1.070	1109	0.965	0.074	99	90	94	79	-64	3.18E-6	7.12E-6	1.59E-5
CNS Cancer															
SF-268	0.389	1.221	1.222	1.148	1151	1.071	0.180	100	91	92	82	-54	3.44E-6	8.03E-6	1.88E-5
SF-295	0.751	1.474	1.455	1.436	1326	1.240	0.139	97	95	80	68	-82	2.62E-6	5.68E-6	1.23E-5
SF-539	0.602	2.012	1.959	1.953	1904	1.835	0.133	96	96	92	87	-78	3.37E-6	6.76E-6	1.36E-5
SNB-19	0.492	1.584	1.506	1.394	1465	1.341	0.522	93	83	89	78	3	4.69E-6	> 2.00E-5	> 2.00E-5
SNB-75	0.519	1.053	0.961	0.900	0935	0.823	0.291	82	71	77	56	-44	2.31E-6	7.28E-6	> 2.00E-5
U251	0.273	1.215	1.212	1.134	1153	1.016	0.066	100	91	93	79	-76	3.07E-6	6.47E-6	1.36E-5
Melanoma															
LOX IMVI	0.105	0.600	0.568	0.528	0515	0.511	0.035	93	85	83	82	-67	3.28E-6	7.09E-6	1.53E-5
MALME-3M	0.844	1.419	1.415	1.255	1344	1.241	0.269	99	71	87	69	-68	2.75E-6	6.37E-6	1.47E-5
M14	0.297	1.005	0.993	0.951	0972	0.866	0.041	98	92	95	80	-86	3.04E-6	6.07E-6	1.21E-5
MDA-MB-435	0.349	1.193	1.199	1.166	1164	1.056	0.110	101	97	97	84	-68	3.33E-6	7.10E-6	1.51E-5
SK-MEL-2	0.714	1.044	1.026	0.992	1005	0.957	0.097	94	84	88	73	-86	2.80E-6	5.76E-6	1.18E-5
SK-MEL-28	0.451	1.374	1.416	1.303	1341	1.243	0.119	104	92	96	86	-74	3.35E-6	6.90E-6	1.42E-5
SK-MEL-5	0.409	2.182	2.172	1.984	2112	1.874	0.017	99	89	96	83	-96	3.05E-6	5.80E-6	1.11E-5
UACC-257	0.559	0.993	0.915	0.929	0907	0.887	0.088	82	85	80	76	-84	2.89E-6	5.93E-6	1.22E-5
UACC-62	0.662	1.960	1.991	1.784	1859	1.599	0.195	102	86	92	72	-71	2.86E-6	6.40E-6	1.43E-5
Ovarian Cancer															
IGROV1	0.479	1.057	1.007	0.902	1000	1.004	0.135	91	73	90	91	-72	3.57E-6	7.24E-6	1.47E-5
OVCAR-3	0.536	1.373	1.298	1.281	1288	1.166	0.103	91	89	90	75	-81	2.90E-6	6.07E-6	1.27E-5
OVCAR-4	0.347	0.633	0.622	0.589	0610	0.535	0.168	96	85	92	66	-52	2.72E-6	7.26E-6	1.94E-5
OVCAR-5	0.488	1.192	1.162	1.113	1087	1.059	0.283	96	89	85	81	-42	3.57E-6	9.10E-6	> 2.00E-5
OVCAR-8	0.228	0.780	0.758	0.759	0744	0.713	0.161	96	96	93	88	-30	4.20E-6	1.12E-5	> 2.00E-5
NCI/ADR-RES	0.283	0.945	0.970	0.933	0934	0.804	0.154	104	98	98	79	-46	3.40E-6	8.58E-6	> 2.00E-5
SK-OV-3	0.460	1.157	1.141	1.112	1115	1.081	0.304	98	94	94	89	-34	4.15E-6	1.06E-5	> 2.00E-5
Renal Cancer															
786-0	0.387	1.419	1.474	1.317	1449	1.235	0.111	105	90	103	82	-71	3.24E-6	6.86E-6	1.45E-5
A498	0.736	1.533	1.444	1.283	1373	1.243	0.071	89	69	80	64	-90	2.45E-6	5.18E-6	1.09E-5
ACHN	0.348	1.567	1.588	1.542	1572	1.441	0.430	102	98	100	90	7	6.01E-6	> 2.00E-5	> 2.00E-5
CAKI-1	0.678	1.105	1.148	1.237	1246	1.077	0.191	110	131	133	93	-72	3.65E-6	7.33E-6	1.47E-5
RXF 393	0.649	1.235	1.211	1.151	1196	1.053	0.028	96	86	93	69	-96	2.60E-6	5.24E-6	1.05E-5
SN12C	0.519	1.847	1.763	1.656	1635	1.563	0.229	94	86	84	79	-56	3.26E-6	7.68E-6	1.81E-5
TK-10	0.374	0.565	0.548	0.483	0527	0.545	0.171	91	57	80	90	-54	3.77E-6	8.38E-6	1.86E-5
UO-31	0.569	1.157	1.106	1.054	1095	0.979	0.130	91	83	89	70	-77	2.73E-6	5.96E-6	1.31E-5
Prostate Cancer															
PC-3	0.397	1.583	1.625	1.591	1595	1.295	0.278	103	100	101	75	-30	3.48E-6	1.04E-5	> 2.00E-5
DU-145	0.309	1.145	1.103	1.013	1053	0.959	0.133	95	84	89	78	-57	3.21E-6	7.55E-6	1.78E-5
Breast Cancer															
MCF7	0.204	1.185	1.129	1.039	1097	1.102	0.167	94	85	91	91	-18	4.77E-6	1.36E-5	> 2.00E-5
MDA-MB-231/ATCC	0.449	1.129	1.102	1.010	1047	0.931	0.137	96	82	88	71	-70	2.82E-6	6.39E-6	1.45E-5
HS 578T	0.514	1.142	1.122	1.122	1077	0.992	0.277	97	97	90	76	-46	3.27E-6	8.38E-6	> 2.00E-5
BT-549	0.792	1.399	1.365	1.306	1342	1.211	0.101	94	85	91	69	-87	2.65E-6	5.53E-6	1.16E-5
T-47D	0.443	1.089	1.089	1.034	1032	0.998	0.284	100	92	91	86	-36	3.95E-6	1.02E-5	> 2.00E-5
MDA-MB-468	0.536	1.329	1.324	1.249	1263	1.113	0.223	99	90	92	73	-58	2.98E-6	7.17E-6	1.73E-5


National Cancer Institute Developmental Therapeutics Program
Mean Graphs
NSC :751486/1
Units :Molar
SSPL :0X2L
EXP. ID :0912NS69
Report Date :May 09, 2013
Test Date :December 14, 2009
Panel/Cell Line
Log10GI50
GI50
Log10TGI
TGI
Log10LC50
LC50
Leukemia
CCRF-CEM -5.22 > -4.70 > -4.70
HL-60(TB) -5.53 -5.06 > -4.70
K-562 -5.52 -5.03 > -4.70
MOLT-4 -5.54 -4.99 > -4.70
RPMI-8226 -5.35 > -4.70 > -4.70
Non-Small Cell Lung Cancer
A549/ATCC -5.52 -5.12 -4.71
EKVX -5.47 -5.01 > -4.70
HOP-62 -5.43 -5.13 -4.84
HOP-92 -5.50 -5.18 -4.85
NCI-H226 -5.52 -5.21 -4.90
NCI-H23 -5.51 -5.17 -4.84
NCI-H322M -5.35 -4.93 > -4.70
NCI-H460 -5.47 -5.10 -4.74
NCI-H522 -6.07 -5.32 -4.84
Colon Cancer
COLO 205 -5.37 -4.98 > -4.70
HCC-2998 -5.47 -5.21 -4.95
HCT-116 -5.61 -5.30 -4.98
HCT-15 -5.36 -4.74 > -4.70
HT29 -5.58 -5.25 -4.93
KM12 -5.53 -5.23 -4.94
SW-620 -5.50 -5.15 -4.80
CNS Cancer
SF-268 -5.46 -5.10 -4.73
SF-295 -5.58 -5.25 -4.91
SF-539 -5.47 -5.17 -4.87
SNB-19 -5.33 > -4.70 > -4.70
SNB-75 -5.64 -5.14 > -4.70
U251 -5.51 -5.19 -4.87
Melanoma
LOX IMVI -5.48 -5.15 -4.81
MALME-3M -5.56 -5.20 -4.83
M14 -5.52 -5.22 -4.92
MDA-MB-435 -5.48 -5.15 -4.82
SK-MEL-2 -5.55 -5.24 -4.93
SK-MEL-28 -5.47 -5.16 -4.85
SK-MEL-5 -5.52 -5.24 -4.96
UACC-257 -5.54 -5.23 -4.91
UACC-62 -5.54 -5.19 -4.84
Ovarian Cancer
IGROV1 -5.45 -5.14 -4.83
OVCAR-3 -5.54 -5.22 -4.90
OVCAR-4 -5.57 -5.14 -4.71
OVCAR-5 -5.45 -5.04 > -4.70
OVCAR-8 -5.38 -4.95 > -4.70
NCI/ADR-RES -5.47 -5.07 > -4.70
SK-OV-3 -5.38 -4.98 > -4.70
Renal Cancer
786-0 -5.49 -5.16 -4.84
A498 -5.61 -5.29 -4.96
ACHN -5.22 > -4.70 > -4.70
CAKI-1 -5.44 -5.13 -4.83
RXF 393 -5.59 -5.28 -4.98
SN12C -5.49 -5.11 -4.74
TK-10 -5.42 -5.08 -4.73
UO-31 -5.56 -5.22 -4.88
Prostate Cancer
PC-3 -5.46 -4.98 > -4.70
DU-145 -5.49 -5.12 -4.75
Breast Cancer
MCF7 -5.32 -4.87 > -4.70
MDA-MB-231/ATCC -5.55 -5.19 -4.84
HS 578T -5.49 -5.08 > -4.70
BT-549 -5.58 -5.26 -4.94
T-47D -5.40 -4.99 > -4.70
MDA-MB-468 -5.53 -5.14 -4.76
MID -5.49 -5.1 -4.8
Delta 0.58 0.22 0.18
Range 0.85 0.62 0.28
+3 +2 +1 0 -1 -2 -3

Five dose experimental data of compound 4.23 (NSC 751478)



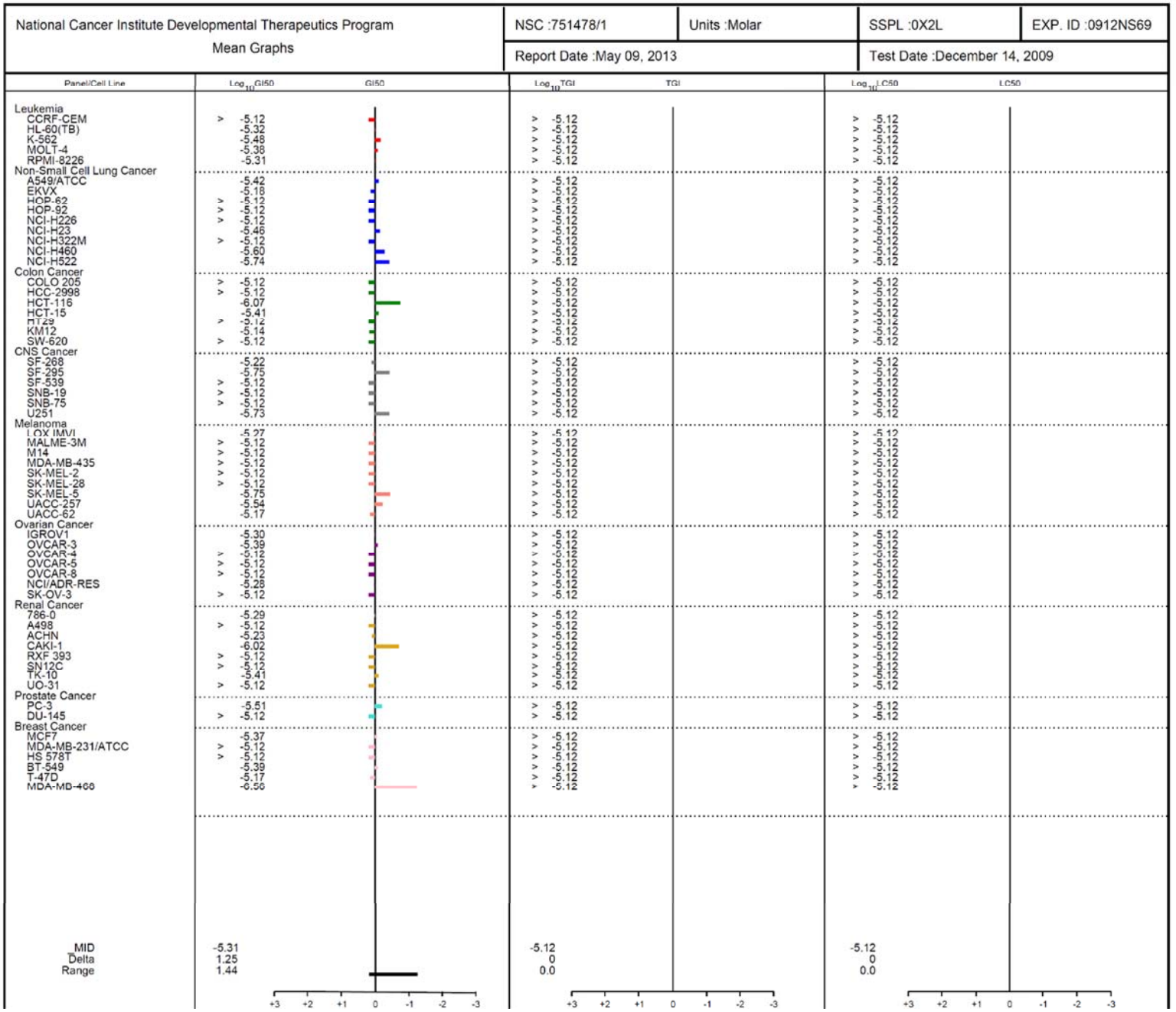
National Cancer Institute Developmental Therapeutics Program Dose Response Curves	NSC: 751478 / 1	SSPL: 0X2L	EXP. ID: 0912NS69
	Report Date: May 09, 2013	Test Date: December 14, 2009	



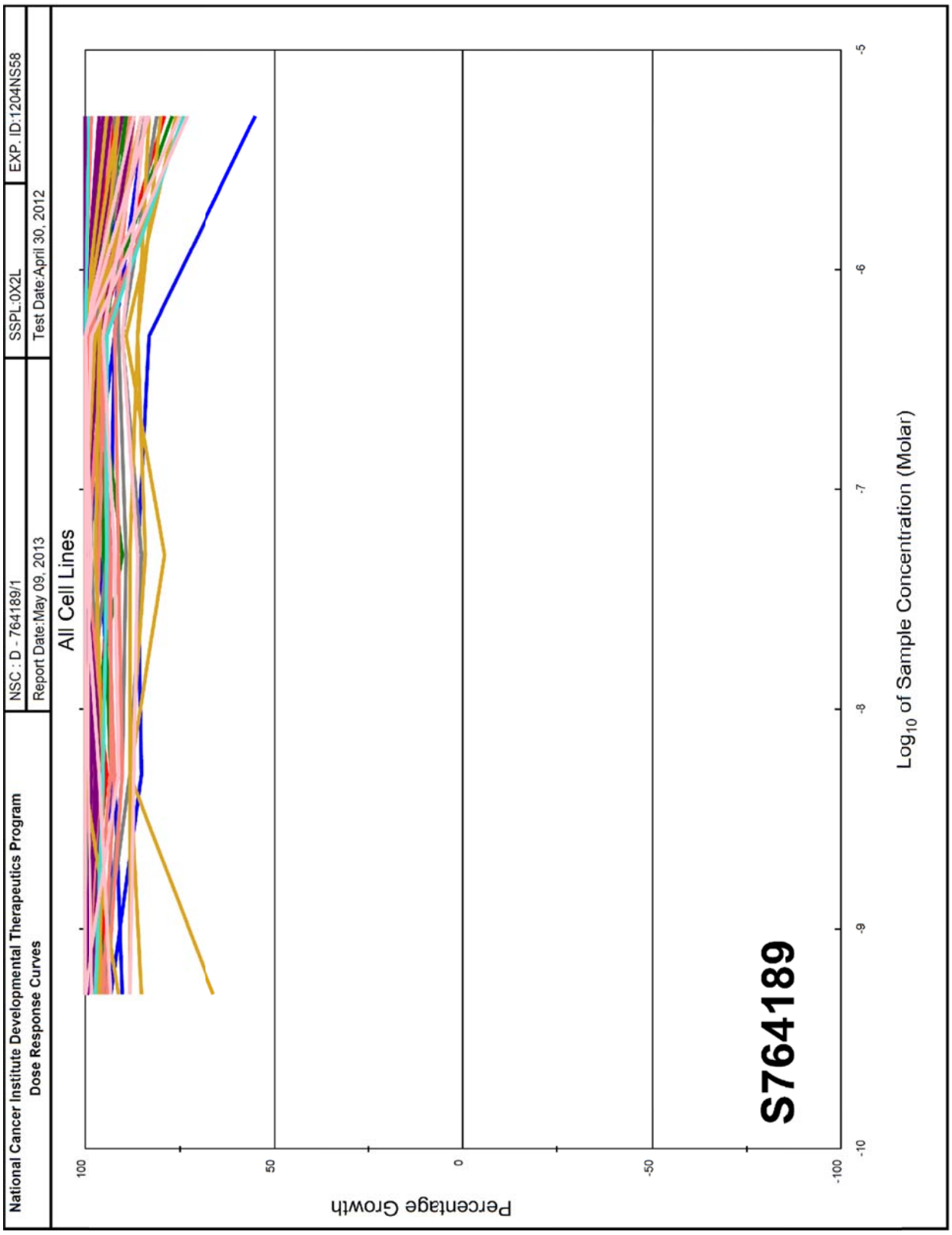
National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

NSC : 751478 / 1	Experiment ID : 0912NS69	Test Type : 08	Units : Molar
Report Date : May 09, 2013	Test Date : December 14, 2009	QNS :	MC :
COMI : LSC-KU-JJ-II-146-1 (91136)	Stain Reagent : SRB Dual-Pass Related	SSPL : 0X2L	

			Log10 Concentration												
	Time		Mean Optical Densities					Percent Growth							
Panel/Cell Line	Zero	Ctrl	-9.1	-8.1	-7.1	-6.1	-5.1	-9.1	-8.1	-7.1	-6.1	-5.1	GI50	TGI	LC50
Leukemia															
CCRF-CEM	0.349	1.575	1.560	1.610	1560	1.430	1.037	99	103	99	88	56	> 7.50E-6	> 7.50E-6	> 7.50E-6
HL-60(TB)	0.717	2.509	2.451	2.519	2529	2.141	1.483	97	101	101	79	43	4.75E-6	> 7.50E-6	> 7.50E-6
K-562	0.257	1.607	1.600	1.651	1696	1.342	0.710	100	103	107	80	34	3.34E-6	> 7.50E-6	> 7.50E-6
MOLT-4	0.598	1.936	1.800	2.003	2030	1.727	1.106	90	105	107	84	38	4.12E-6	> 7.50E-6	> 7.50E-6
RPMI-8226	0.685	2.350	2.324	2.292	2265	1.928	1.426	98	97	95	75	44	4.92E-6	> 7.50E-6	> 7.50E-6
Non-Small Cell Lung Cancer															
A549/ATCC	0.353	1.163	1.138	1.114	1103	1.022	0.646	97	94	93	83	36	3.78E-6	> 7.50E-6	> 7.50E-6
EKVX	0.633	1.595	1.482	1.485	1525	1.355	1.099	88	89	93	75	48	6.55E-6	> 7.50E-6	> 7.50E-6
HOP-62	0.259	0.871	0.849	0.834	0798	0.870	0.573	96	94	88	100	51	> 7.50E-6	> 7.50E-6	> 7.50E-6
HOP-92	1.146	1.989	1.869	1.847	1809	1.821	1.700	86	83	79	80	66	> 7.50E-6	> 7.50E-6	> 7.50E-6
NCI-H226	0.670	1.397	1.335	1.349	1376	1.204	1.153	91	93	97	73	66	> 7.50E-6	> 7.50E-6	> 7.50E-6
NCI-H23	0.402	1.290	1.294	1.200	1215	0.921	0.808	100	90	92	58	46	3.47E-6	> 7.50E-6	> 7.50E-6
NCI-H322M	0.461	0.898	0.886	0.889	0922	0.925	0.761	97	98	105	106	69	> 7.50E-6	> 7.50E-6	> 7.50E-6
NCI-H460	0.241	1.914	1.853	1.972	1937	1.721	0.501	96	103	101	88	16	2.53E-6	> 7.50E-6	> 7.50E-6
NCI-H522	0.593	0.842	0.791	0.805	0814	0.769	0.637	80	85	89	71	18	1.83E-6	> 7.50E-6	> 7.50E-6
Colon Cancer															
COLO 205	0.195	1.330	1.334	1.313	1274	1.215	0.841	100	98	95	90	57	> 7.50E-6	> 7.50E-6	> 7.50E-6
HCC-2998	0.747	1.829	1.818	1.871	1812	1.789	1.375	99	104	98	96	58	> 7.50E-6	> 7.50E-6	> 7.50E-6
HCT-116	0.239	1.533	1.521	1.523	1444	0.908	0.536	99	99	93	51	23	8.44E-7	> 7.50E-6	> 7.50E-6
HCT-15	0.243	1.635	1.576	1.553	1501	1.339	0.783	96	94	90	79	39	3.92E-6	> 7.50E-6	> 7.50E-6
HT29	0.153	0.617	0.635	0.635	0567	0.494	0.385	104	104	89	73	50	> 7.50E-6	> 7.50E-6	> 7.50E-6
KM12	0.247	1.233	1.208	1.246	1193	1.162	0.735	97	101	96	92	49	7.19E-6	> 7.50E-6	> 7.50E-6
SW-620	0.205	1.213	1.174	1.200	1139	1.072	0.747	96	98	92	86	53	> 7.50E-6	> 7.50E-6	> 7.50E-6
CNS Cancer															
SF-268	0.389	1.213	1.153	1.205	1159	1.141	0.767	93	99	93	91	46	6.08E-6	> 7.50E-6	> 7.50E-6
SF-295	0.751	1.585	1.573	1.527	1436	1.319	0.922	99	93	82	68	20	1.80E-6	> 7.50E-6	> 7.50E-6
SF-539	0.602	1.927	1.904	1.824	1832	1.839	1.295	98	92	93	93	52	> 7.50E-6	> 7.50E-6	> 7.50E-6
SNB-19	0.492	1.482	1.423	1.433	1434	1.338	0.993	94	95	95	85	51	> 7.50E-6	> 7.50E-6	> 7.50E-6
SNB-75	0.519	0.943	0.841	0.876	0848	0.855	0.796	75	83	77	78	65	> 7.50E-6	> 7.50E-6	> 7.50E-6
U251	0.273	1.230	1.169	1.192	1171	0.860	0.584	94	96	94	61	32	1.84E-6	> 7.50E-6	> 7.50E-6
Melanoma															
LOX IMVI	0.105	0.674	0.669	0.630	0570	0.640	0.347	99	92	82	94	43	5.38E-6	> 7.50E-6	> 7.50E-6
MALME-3M	0.844	1.474	1.395	1.456	1384	1.310	1.244	87	97	86	74	63	> 7.50E-6	> 7.50E-6	> 7.50E-6
M14	0.297	0.925	0.905	0.913	0884	0.859	0.688	97	98	93	89	62	> 7.50E-6	> 7.50E-6	> 7.50E-6
MDA-MB-435	0.349	1.283	1.240	1.218	1219	1.178	0.943	95	93	93	88	63	> 7.50E-6	> 7.50E-6	> 7.50E-6
SK-MEL-2	0.714	1.071	1.055	1.113	1084	1.060	1.058	96	112	104	97	96	> 7.50E-6	> 7.50E-6	> 7.50E-6
SK-MEL-28	0.451	1.253	1.220	1.280	1250	1.209	1.036	96	103	100	94	73	> 7.50E-6	> 7.50E-6	> 7.50E-6
SK-MEL-5	0.409	2.277	2.133	2.189	2203	1.646	0.843	92	95	96	66	23	1.79E-6	> 7.50E-6	> 7.50E-6
UACC-257	0.559	0.993	0.979	0.961	0975	0.890	0.696	97	93	96	76	31	2.89E-6	> 7.50E-6	> 7.50E-6
UACC-62	0.662	1.935	1.809	1.901	1804	1.655	1.282	90	97	90	78	49	6.75E-6	> 7.50E-6	> 7.50E-6
Ovarian Cancer															
IGROV1	0.479	1.103	0.988	0.964	0970	0.950	0.761	81	77	78	75	45	5.06E-6	> 7.50E-6	> 7.50E-6
OVCAR-3	0.536	1.274	1.219	1.246	1247	1.035	0.857	93	96	96	68	44	4.03E-6	> 7.50E-6	> 7.50E-6
OVCAR-4	0.347	0.641	0.596	0.613	0588	0.560	0.532	85	91	82	72	63	> 7.50E-6	> 7.50E-6	> 7.50E-6
OVCAR-5	0.488	1.135	1.074	1.056	1034	1.064	1.024	90	88	84	89	83	> 7.50E-6	> 7.50E-6	> 7.50E-6
OVCAR-8	0.228	0.795	0.775	0.762	0754	0.737	0.532	96	94	93	90	54	> 7.50E-6	> 7.50E-6	> 7.50E-6
NCI/ADR-RES	0.283	0.963	0.960	0.930	0951	0.890	0.573	100	95	98	89	43	5.23E-6	> 7.50E-6	> 7.50E-6
SK-OV-3	0.460	1.172	1.095	1.110	1139	1.040	0.910	89	91	95	81	63	> 7.50E-6	> 7.50E-6	> 7.50E-6
Renal Cancer															
786-0	0.387	1.419	1.394	1.447	1427	1.404	0.804	98	103	101	98	40	5.12E-6	> 7.50E-6	> 7.50E-6
A498	0.736	1.583	1.385	1.397	1346	1.284	1.245	77	78	72	65	60	> 7.50E-6	> 7.50E-6	> 7.50E-6
ACHN	0.348	1.552	1.560	1.449	1514	1.333	0.905	101	91	97	82	46	5.88E-6	> 7.50E-6	> 7.50E-6
CAKI-1	0.678	1.223	1.132	1.092	1166	0.981	0.709	82	75	89	55	6	9.49E-7	> 7.50E-6	> 7.50E-6
RXF 393	0.649	1.237	1.171	1.211	1181	1.165	1.084	89	96	90	88	74	> 7.50E-6	> 7.50E-6	> 7.50E-6
SN12C	0.519	1.801	1.676	1.702	1679	1.525	1.190	90	92	91	78	52	> 7.50E-6	> 7.50E-6	> 7.50E-6
TK-10	0.374	0.593	0.580	0.631	0594	0.554	0.456	94	117	100	82	37	3.90E-6	> 7.50E-6	> 7.50E-6
UO-31	0.569	1.249	1.132	1.169	1140	1.096	0.946	83	88	84	78	55	> 7.50E-6	> 7.50E-6	> 7.50E-6
Prostate Cancer															
PC-3	0.397	1.543	1.496	1.500	1413	1.051	0.922	96	96	88	57	46	3.06E-6	> 7.50E-6	> 7.50E-6
DU-145	0.309	1.083	1.047	1.088	1057	0.973	0.723	95	100	96	85	53	> 7.50E-6	> 7.50E-6	> 7.50E-6
Breast Cancer															
MCF7	0.204	1.223	1.105	1.231	1147	1.110	0.589	88	100	92	88	38	4.27E-6	> 7.50E-6	> 7.50E-6
MDA-MB-231/ATCC	0.449	1.094	1.079	1.101	1052	1.014	0.875	98	101	93	88	66	> 7.50E-6	> 7.50E-6	> 7.50E-6
HS 578T	0.514	1.184	1.102	1.146	1099	1.102	1.052	88	94	87	88	80	> 7.50E-6	> 7.50E-6	> 7.50E-6
BT-549	0.792	1.330	1.288	1.316	1320	1.237	0.998	92	97	98	83	38	4.09E-6	> 7.50E-6	> 7.50E-6
T-47D	0.443	1.175	1.133	1.098	1089	1.027	0.800	94	89	88	80	49	6.81E-6	> 7.50E-6	> 7.50E-6
MDA-MB-468	0.536	1.323	1.280	1.306	1198	0.725	0.592	94	97	84	24	7	2.74E-7	> 7.50E-6	> 7.50E-6

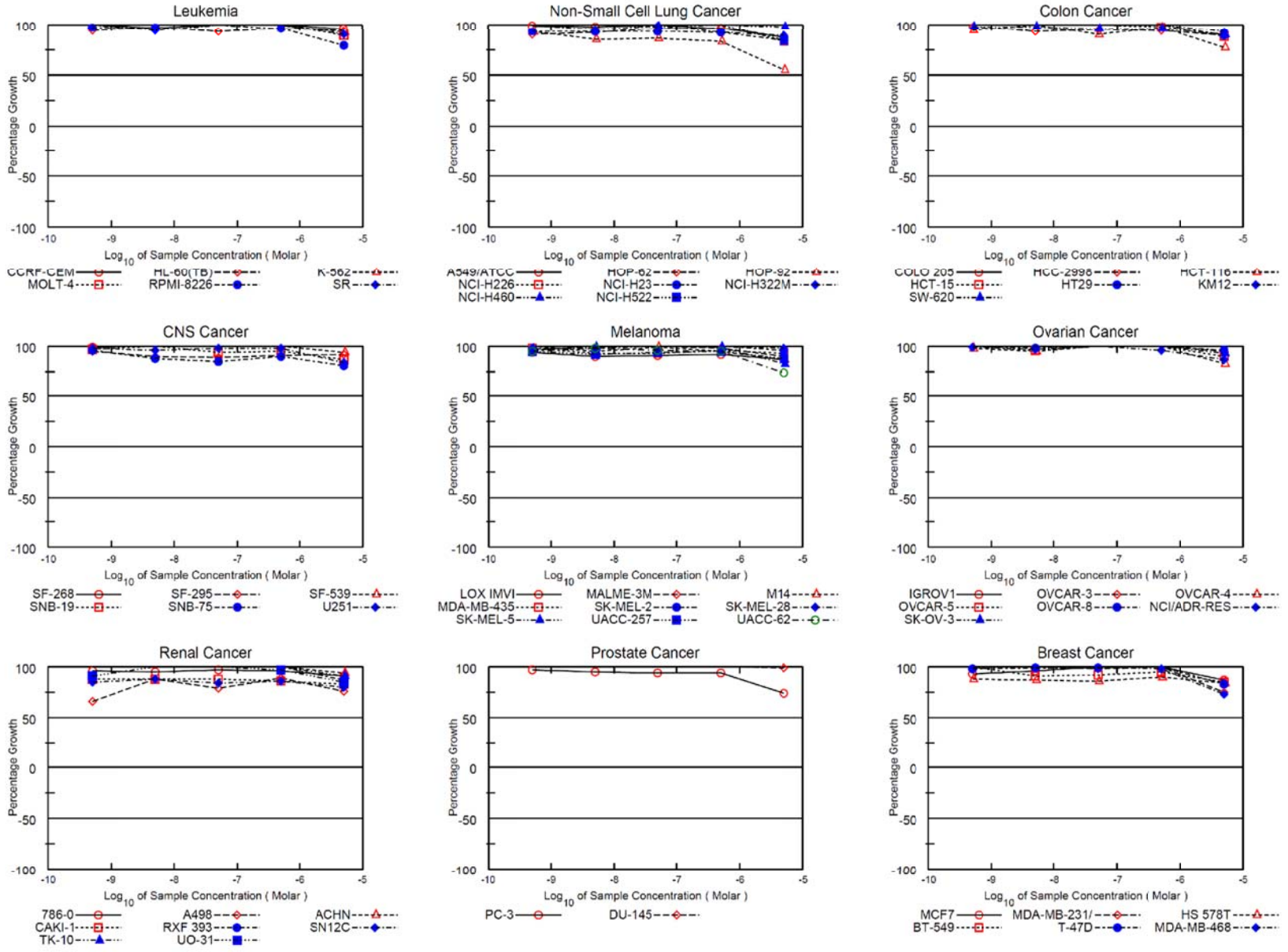

National Cancer Institute Developmental Therapeutics Program
Mean Graphs
NSC :751478/1
Units :Molar
SSPL :0X2L
EXP. ID :0912NS69
Report Date :May 09, 2013
Test Date :December 14, 2009
Panel/Cell Line
Log10GI50
GI50
Log10TGI
TGI
Log10LC50
LC50
Leukemia
CCRF-CEM > -5.12
HL-60(TB) -5.32
K-562 -5.48
MOLT-4 -5.38
RPMI-8226 -5.31
Non-Small Cell Lung Cancer
A549/ATCC -5.42
EKVX -5.18
HOP-62 > -5.12
HOP-92 > -5.12
NCI-H226 > -5.12
NCI-H23 -5.46
NCI-H322M > -5.12
NCI-H460 -5.60
NCI-H522 -5.74
Colon Cancer
COLO 205 > -5.12
HCC-2998 > -5.12
HCT-116 -6.07
HCT-15 -5.41
HT29 > -5.12
KM12 -5.14
SW-620 > -5.12
CNS Cancer
SF-268 -5.22
SF-295 -5.75
SF-539 > -5.12
SNB-19 > -5.12
SNB-75 > -5.12
U251 -5.73
Melanoma
LOX IMVI -5.27
MALME-3M > -5.12
M14 > -5.12
MDA-MB-435 > -5.12
SK-MEL-2 > -5.12
SK-MEL-28 > -5.12
SK-MEL-5 -5.75
UACC-257 -5.54
UACC-62 -5.17
Ovarian Cancer
IGROV1 -5.30
OVCAR-3 -5.39
OVCAR-4 > -5.12
OVCAR-5 > -5.12
OVCAR-8 > -5.12
NCI/ADR-RES -5.28
SK-OV-3 > -5.12
Renal Cancer
786-0 -5.29
A498 > -5.12
ACHN -5.23
CAKI-1 -6.02
RXF 393 > -5.12
SN12C > -5.12
TK-10 -5.41
UO-31 > -5.12
Prostate Cancer
PC-3 -5.51
DU-145 > -5.12
Breast Cancer
MCF7 -5.37
MDA-MB-231/ATCC > -5.12
HS 578T > -5.12
BT-549 -5.39
T-47D -5.17
MDA-MB-468 -6.56
MID -5.31 -5.12 -5.12
Delta 1.25 0 0
Range 1.44 0.0 0.0
+3 +2 +1 0 -1 -2 -3

Five dose experimental data of compound 4.37c (NSC 764189)



National Cancer Institute Developmental Therapeutics Program
Dose Response Curves

NSC: D - 764189 / 1	SSPL: 0X2L	EXP. ID: 1204NS58
Report Date: May 09, 2013	Test Date: April 30, 2012	



National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

NSC : D - 764189 / 1	Experiment ID : 1204NS58	Test Type : 08	Units : Molar
Report Date : May 09, 2013	Test Date : April 30, 2012	QNS :	MC :
COMI : LSC-KU-JJ-II-164-1 (91318)	Stain Reagent : SRB Dual-Pass Related	SSPL : 0X2L	

			Log10 Concentration											
	Time		Mean Optical Densities					Percent Growth						
Panel/Cell Line	Zero	Ctrl	-9.3	-8.3	-7.3	-6.3	-5.3	-9.3	-8.3	-7.3	-6.3	-5.3	GI50	TGI	LC50
Leukemia															
CCRF-CEM	0.703	2.711	2.724	2.655	2.721	2.725	2.622	101	97	100	101	96	> 5.00E-6	> 5.00E-6	> 5.00E-6
HL-60(TB)	0.718	2.840	2.722	2.781	2.681	2.765	2.718	94	97	93	96	94	> 5.00E-6	> 5.00E-6	> 5.00E-6
K-562	0.265	1.952	2.048	2.006	2.011	2.045	1.821	106	103	103	106	92	> 5.00E-6	> 5.00E-6	> 5.00E-6
MOLT-4	0.512	2.043	2.095	2.084	2.129	2.093	1.870	103	103	106	103	89	> 5.00E-6	> 5.00E-6	> 5.00E-6
RPMI-8226	0.882	2.372	2.327	2.323	2.375	2.314	2.064	97	97	100	96	79	> 5.00E-6	> 5.00E-6	> 5.00E-6
SR	0.510	1.893	1.923	1.809	1.950	1.933	1.763	102	94	104	102	90	> 5.00E-6	> 5.00E-6	> 5.00E-6
Non-Small Cell Lung Cancer															
A549/ATCC	0.256	1.477	1.469	1.449	1.509	1.424	1.313	99	98	103	96	87	> 5.00E-6	> 5.00E-6	> 5.00E-6
HOP-62	0.357	0.955	0.895	0.905	0928	0.936	0.879	90	92	96	97	87	> 5.00E-6	> 5.00E-6	> 5.00E-6
HOP-92	1.100	1.465	1.441	1.413	1414	1.405	1.303	93	85	86	83	55	> 5.00E-6	> 5.00E-6	> 5.00E-6
NCI-H226	0.549	1.344	1.356	1.344	1367	1.381	1.206	101	100	103	105	83	> 5.00E-6	> 5.00E-6	> 5.00E-6
NCI-H23	0.524	1.514	1.445	1.445	1443	1.436	1.358	93	93	93	92	84	> 5.00E-6	> 5.00E-6	> 5.00E-6
NCI-H322M	0.723	1.555	1.569	1.515	1547	1.492	1.460	102	95	99	92	89	> 5.00E-6	> 5.00E-6	> 5.00E-6
NCI-H460	0.368	2.727	2.740	2.813	2682	2.794	2.679	101	104	98	103	98	> 5.00E-6	> 5.00E-6	> 5.00E-6
NCI-H522	0.829	1.912	1.914	1.954	1989	1.912	1.744	100	104	107	100	84	> 5.00E-6	> 5.00E-6	> 5.00E-6
Colon Cancer															
COLO 205	0.277	1.103	1.212	1.189	1219	1.217	1.132	113	110	114	114	103	> 5.00E-6	> 5.00E-6	> 5.00E-6
HCC-2998	0.442	1.525	1.500	1.450	1469	1.466	1.404	98	93	95	94	89	> 5.00E-6	> 5.00E-6	> 5.00E-6
HCT-116	0.174	1.595	1.509	1.659	1456	1.559	1.266	94	104	90	97	77	> 5.00E-6	> 5.00E-6	> 5.00E-6
HCT-15	0.280	1.383	1.425	1.418	1464	1.360	1.253	104	103	107	98	88	> 5.00E-6	> 5.00E-6	> 5.00E-6
HT29	0.313	1.709	1.708	1.821	1799	1.766	1.554	100	108	106	104	89	> 5.00E-6	> 5.00E-6	> 5.00E-6
KM12	0.208	1.124	1.167	1.166	1189	1.131	1.062	105	105	107	101	93	> 5.00E-6	> 5.00E-6	> 5.00E-6
SW-620	0.368	2.464	2.418	2.432	2384	2.372	2.263	98	98	96	96	90	> 5.00E-6	> 5.00E-6	> 5.00E-6
CNS Cancer															
SF-268	0.553	1.717	1.706	1.786	1786	1.833	1.751	99	106	106	110	103	> 5.00E-6	> 5.00E-6	> 5.00E-6
SF-295	1.189	2.864	2.784	2.695	2682	2.716	2.728	95	90	89	91	92	> 5.00E-6	> 5.00E-6	> 5.00E-6
SF-539	0.887	2.175	2.186	2.189	2257	2.293	2.095	101	101	106	109	94	> 5.00E-6	> 5.00E-6	> 5.00E-6
SNB-19	0.466	1.273	1.247	1.285	1227	1.237	1.170	97	101	94	95	87	> 5.00E-6	> 5.00E-6	> 5.00E-6
SNB-75	0.533	1.114	1.092	1.045	1027	1.053	1.002	96	88	85	90	81	> 5.00E-6	> 5.00E-6	> 5.00E-6
U251	0.355	1.764	1.737	1.710	1742	1.736	1.537	98	96	98	98	84	> 5.00E-6	> 5.00E-6	> 5.00E-6
Melanoma															
LOX IMVI	0.274	2.103	1.991	1.926	1944	1.963	1.863	94	90	91	92	87	> 5.00E-6	> 5.00E-6	> 5.00E-6
MALME-3M	0.670	1.383	1.357	1.387	1408	1.371	1.312	96	101	104	98	90	> 5.00E-6	> 5.00E-6	> 5.00E-6
M14	0.347	1.369	1.391	1.316	1362	1.322	1.235	102	95	99	95	87	> 5.00E-6	> 5.00E-6	> 5.00E-6
MDA-MB-435	0.473	2.153	2.120	2.029	2027	2.090	2.032	98	93	93	96	93	> 5.00E-6	> 5.00E-6	> 5.00E-6
SK-MEL-2	0.971	1.903	2.022	2.072	2061	2.080	1.873	112	118	116	118	96	> 5.00E-6	> 5.00E-6	> 5.00E-6
SK-MEL-28	0.464	1.273	1.264	1.265	1287	1.309	1.264	98	98	101	104	98	> 5.00E-6	> 5.00E-6	> 5.00E-6
SK-MEL-5	0.503	2.562	2.498	2.532	2583	2.544	2.208	97	99	101	99	83	> 5.00E-6	> 5.00E-6	> 5.00E-6
UACC-257	0.722	1.734	1.680	1.651	1679	1.681	1.613	95	92	94	95	88	> 5.00E-6	> 5.00E-6	> 5.00E-6
UACC-62	0.960	2.192	2.127	2.172	2145	2.136	1.871	95	98	96	95	74	> 5.00E-6	> 5.00E-6	> 5.00E-6
Ovarian Cancer															
IGROV1	0.620	1.775	1.873	1.888	1878	1.874	1.805	108	110	109	109	103	> 5.00E-6	> 5.00E-6	> 5.00E-6
OVCAR-3	0.545	1.407	1.505	1.502	1485	1.532	1.360	111	111	109	114	95	> 5.00E-6	> 5.00E-6	> 5.00E-6
OVCAR-4	0.661	1.515	1.499	1.470	1542	1.535	1.369	98	95	103	102	83	> 5.00E-6	> 5.00E-6	> 5.00E-6
OVCAR-5	0.531	1.367	1.373	1.338	1476	1.377	1.282	101	96	113	101	90	> 5.00E-6	> 5.00E-6	> 5.00E-6
OVCAR-8	0.320	1.409	1.412	1.391	1420	1.410	1.364	100	98	101	100	96	> 5.00E-6	> 5.00E-6	> 5.00E-6
NCI/ADR-RES	0.556	1.773	1.768	1.744	1842	1.727	1.622	99	97	105	96	87	> 5.00E-6	> 5.00E-6	> 5.00E-6
SK-OV-3	0.340	0.847	0.846	0.865	0.891	0.871	0.814	100	104	109	105	93	> 5.00E-6	> 5.00E-6	> 5.00E-6
Renal Cancer															
786-0	0.509	2.019	1.953	1.949	1967	1.959	1.887	96	95	97	96	91	> 5.00E-6	> 5.00E-6	> 5.00E-6
A498	1.232	1.973	1.726	1.891	1823	1.894	1.796	66	88	79	89	76	> 5.00E-6	> 5.00E-6	> 5.00E-6
ACHN	0.279	1.270	1.303	1.320	1333	1.327	1.208	103	105	106	106	94	> 5.00E-6	> 5.00E-6	> 5.00E-6
CAKI-1	0.628	2.389	2.186	2.177	2170	2.151	2.095	88	88	88	86	83	> 5.00E-6	> 5.00E-6	> 5.00E-6
RXF 393	0.555	1.015	1.033	1.051	1042	1.020	0.959	104	107	105	101	87	> 5.00E-6	> 5.00E-6	> 5.00E-6
SN12C	0.590	2.453	2.178	2.234	2157	2.199	2.080	85	88	84	86	80	> 5.00E-6	> 5.00E-6	> 5.00E-6
TK-10	0.896	1.623	1.662	1.719	1691	1.642	1.563	105	112	109	102	91	> 5.00E-6	> 5.00E-6	> 5.00E-6
UO-31	0.685	1.754	1.662	1.766	1759	1.727	1.596	91	101	100	97	85	> 5.00E-6	> 5.00E-6	> 5.00E-6
Prostate Cancer															
PC-3	0.624	1.927	1.886	1.860	1852	1.853	1.593	97	95	94	94	74	> 5.00E-6	> 5.00E-6	> 5.00E-6
DU-145	0.374	1.369	1.432	1.444	1528	1.484	1.357	106	108	116	111	99	> 5.00E-6	> 5.00E-6	> 5.00E-6
Breast Cancer															
MCF7	0.264	1.413	1.334	1.367	1413	1.425	1.270	93	96	100	101	87	> 5.00E-6	> 5.00E-6	> 5.00E-6
MDA-MB-231/ATCC	0.515	1.244	1.311	1.256	1228	1.251	1.065	109	102	98	101	75	> 5.00E-6	> 5.00E-6	> 5.00E-6
HS 578T	1.090	1.864	1.768	1.761	1754	1.790	1.742	88	87	86	90	84	> 5.00E-6	> 5.00E-6	> 5.00E-6
BT-549	0.700	1.715	1.715	1.624	1635	1.666	1.564	100	91	92	95	85	> 5.00E-6	> 5.00E-6	> 5.00E-6
T-47D	0.624	1.353	1.336	1.342	1343	1.368	1.226	98	99	99	102	83	> 5.00E-6	> 5.00E-6	> 5.00E-6
MDA-MB-468	0.596	1.013	1.018	1.023	1042	1.006	0.900	101	102	107	98	73	> 5.00E-6	> 5.00E-6	> 5.00E-6

National Cancer Institute Developmental Therapeutics Program

Mean Graphs

NSC : D - 764189/1 | Units :Molar | SSPL :0X2L | EXP. ID :1204NS58

Report Date :May 09, 2013 | Test Date :April 30, 2012

Panel/Cell Line	Log_{10}GI50	GI50	Log_{10}TGI	TGI	Log_{10}LC50	LC50
Leukemia						
CCRF-CEM	> -5.30		> -5.30		> -5.30	
HL-60(TB)	> -5.30		> -5.30		> -5.30	
K-562	> -5.30		> -5.30		> -5.30	
MOLT-4	> -5.30		> -5.30		> -5.30	
RPMI-8226	> -5.30		> -5.30		> -5.30	
SR	> -5.30		> -5.30		> -5.30	
Non-Small Cell Lung Cancer						
A549/ATCC	> -5.30		> -5.30		> -5.30	
HOP-62	> -5.30		> -5.30		> -5.30	
HOP-92	> -5.30		> -5.30		> -5.30	
NCI-H226	> -5.30		> -5.30		> -5.30	
NCI-H23	> -5.30		> -5.30		> -5.30	
NCI-H322M	> -5.30		> -5.30		> -5.30	
NCI-H460	> -5.30		> -5.30		> -5.30	
NCI-H522	> -5.30		> -5.30		> -5.30	
Colon Cancer						
COLO 205	> -5.30		> -5.30		> -5.30	
HCC-2998	> -5.30		> -5.30		> -5.30	
HCT-116	> -5.30		> -5.30		> -5.30	
HCT-15	> -5.30		> -5.30		> -5.30	
HT29	> -5.30		> -5.30		> -5.30	
KM12	> -5.30		> -5.30		> -5.30	
SW-620	> -5.30		> -5.30		> -5.30	
CNS Cancer						
SF-268	> -5.30		> -5.30		> -5.30	
SF-295	> -5.30		> -5.30		> -5.30	
SF-539	> -5.30		> -5.30		> -5.30	
SNB-19	> -5.30		> -5.30		> -5.30	
SNB-75	> -5.30		> -5.30		> -5.30	
U251	> -5.30		> -5.30		> -5.30	
Melanoma						
LOX IMVI	> -5.30		> -5.30		> -5.30	
MALME-3M	> -5.30		> -5.30		> -5.30	
M14	> -5.30		> -5.30		> -5.30	
MDA-MB-435	> -5.30		> -5.30		> -5.30	
SK-MEL-2	> -5.30		> -5.30		> -5.30	
SK-MEL-28	> -5.30		> -5.30		> -5.30	
SK-MEL-5	> -5.30		> -5.30		> -5.30	
UACC-257	> -5.30		> -5.30		> -5.30	
UACC-62	> -5.30		> -5.30		> -5.30	
Ovarian Cancer						
IGROV1	> -5.30		> -5.30		> -5.30	
OVCAR-3	> -5.30		> -5.30		> -5.30	
OVCAR-4	> -5.30		> -5.30		> -5.30	
OVCAR-5	> -5.30		> -5.30		> -5.30	
OVCAR-8	> -5.30		> -5.30		> -5.30	
NCI/ADR-RES	> -5.30		> -5.30		> -5.30	
SK-OV-3	> -5.30		> -5.30		> -5.30	
Renal Cancer						
786-0	> -5.30		> -5.30		> -5.30	
A498	> -5.30		> -5.30		> -5.30	
ACHN	> -5.30		> -5.30		> -5.30	
CAKI-1	> -5.30		> -5.30		> -5.30	
RXF 393	> -5.30		> -5.30		> -5.30	
SN12C	> -5.30		> -5.30		> -5.30	
TK-10	> -5.30		> -5.30		> -5.30	
UO-31	> -5.30		> -5.30		> -5.30	
Prostate Cancer						
PC-3	> -5.30		> -5.30		> -5.30	
DU-145	> -5.30		> -5.30		> -5.30	
Breast Cancer						
MCF7	> -5.30		> -5.30		> -5.30	
MDA-MB-231/ATCC	> -5.30		> -5.30		> -5.30	
HS 578T	> -5.30		> -5.30		> -5.30	
BT-549	> -5.30		> -5.30		> -5.30	
T-47D	> -5.30		> -5.30		> -5.30	
MDA-MB-468	> -5.30		> -5.30		> -5.30	
MID	-5.3		-5.3		-5.3	
Delta	0		0		0	
Range	0.0		0.0		0.0	
		+3 +2 +1 0 -1 -2 -3		+3 +2 +1 0 -1 -2 -3		+3 +2 +1 0 -1 -2 -3

5.6. References

1. Le Bourdonnec, B.; Leister, L. K.; Ajello, C. A.; Cassel, J. A.; Seida, P. R.; O'Hare, H.; Gu, M.; Chu, G. H.; Tuthill, P. A.; DeHaven, R. N.; Dolle, R. E., Discovery of a series of aminopiperidines as novel NOS inhibitors. *Bioorganic & Medicinal Chemistry Letters* **2008,** *18*, 336-343.