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Malignant neoplasm is a condition that encompasses many disease states. One unifying theme of these broadly diverse diseases is the requirement for angiogenesis, the formation of new blood vessels, as a prerequisite for tumor growth and metastasis.¹ Because abnormal cell proliferation requires sufficient blood supply, one method for slowing tumor growth may be altering tumor vasculature with angiogenic inhibitors.

The Center for Drug Discovery, headed by Dr. Phillip Bowen, in collaboration with Dr. Jack Arbiser at the Emory University School of Medicine, have recently discovered that the natural compound, solenopsin A, is an effective inhibitor of angiogenesis. Solenopsin A's potent antiangiogenic activity was first identified using SVR proliferation assays. To probe the mode of action in which solenopsin A was inhibiting angiogenesis, further studies were conducted in cells. These studies suggest that solenopsin A is suppressing the phosphatidylinositol-3-kinase (PI3K)/Akt pathway by inhibiting a step in the signaling cascade upstream PI3K.²

The ultimate goal of this work is to develop an antiangiogenic drug based on solenopsin A. Our immediate research objective, however, is to explore structure-activity relationships for benzamide, ether, and amine side chain analogs of solenopsin A. Benzamide analogs of solenopsin A were first synthesized and screened for antiangiogenic activity using the SVR assays conducted at the Emory SOM. The assay results indicate that none of the analogs tested were antiangiogenic. To further explore

the structural effects on angiogenic inhibition, ether side chain analogs were synthesized with varying alkyl chain lengths and the benzamide derivatives, previously synthesized, were reduced to amine side chain analogs. To assess the effect of *n*-alkyl chain length on angiogenic inhibition, one long-chain analog was also generated. These ether, amine, and long-chain analogs are under consideration for testing using the SVR assays.

SYNTHETIC DERIVATIVES OF SOLENOPSIN A

by

Michael Robert Baker

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Approved by

Committee Co-Chair

Committee Co-Chair

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APPROVAL PAGE

This thesis has been approved by the following committee of the Faculty of The Graduate School at The University of North Carolina at Greensboro.

Committee Co-Chairs _____
J. Phillip Bowen

Gregory M. Raner

Committee Members _____
Kimberly S. Petersen

Ethan W. Taylor

Date of Acceptance by Committee

Date of Final Oral Examination

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TABLE OF ABBREVIATIONS

| | |
|------------------------|---------------------------------|
| EtOAc..... | ethyl acetate |
| LAH..... | lithium aluminum hydride |
| <i>t</i> -BuOK..... | potassium <i>tert</i> -butoxide |
| THF..... | tetrahydrofuran |
| MeOH..... | methanol |
| Et ₂ O..... | diethyl ether |
| DCM..... | dichloromethane |

CHAPTER I

INTRODUCTION

Solenopsis Invicta

The red imported fire ant, *Solenopsis invicta*, is native to southern Brazil, Paraguay, and northern Argentina, but they have spread globally now to inhabit the United States, Canada, Australia, and China.³ These red imported fire ants, referred to as RIFAs, are known to have a strong, painful sting which in rare cases has resulted in anaphylactic shock and human death.⁴ More common human reactions to the stings include dermal necrosis, edema, and the formation of sterile pustules.⁵ *S. invicta* administers its sting by locking onto its prey with its crablike arms and repetitively inserting its stinger. This allows for maximum delivery of its potent venom and immobilizes its prey. While stinging, the ant also releases chemical signals to attract nearby ants to the prey.

Since *S. invicta*'s introduction to the U.S. in the 1920s, it has become a growing pest problem particularly in the south and midwest. With few natural predators in North America, RIFAs have rapidly grown in numbers and now infest over 310 million acres of land, in 12 states, with the majority being in agricultural and residential areas.⁶ *S. invicta* is highly aggressive and colonies typically consist of 200,000 workers which swarm if threatened. This has significantly disturbed the biodiversity of many ecosystems by

driving away or killing native ground dwelling species, such as lizards and ground-nesting birds.⁷ *S. invicta*'s nesting in areas where colonies are more dense has caused additional ecological problems with land erosion, resulting largely from collapses of soil due to underground tunneling.⁸

In the U.S., the economic impact of *S. invicta* is estimated to be \$6 billion annually, with agricultural and energy sectors being most affected.⁹ The infestation of agricultural lands frequently leads to crop destruction and the killing of livestock.¹⁰ *S. invicta*, for unknown reasons, is attracted to electrical sources and often damages electrical equipment by crawling into the wiring and causing shortages. Many power companies have reoccurring difficulties with *S. invicta* infesting power distribution systems and converter boxers in residential areas.¹⁰ Farmers also report of *S. invicta* infestation of the electrical wiring of large farm equipment, which costs Texas farmers \$190 million in equipment damages in 1998.¹¹ Unfortunately the economic impact of *S. invicta* is not attributed to industry alone. The same survey in 1998, found Texan households spent \$526 million on pesticides aimed solely at fire ant control and in damage repairs.¹¹

The Solenopsin Alkaloids

The alkaloids produced in the poison glands of *S. invicta* make up over 99% of the venom and function as defensive compounds. They consist of a mixture of 2-methyl

6-*n*-alkyl (or -alkenyl) disubstituted piperidines and have also been shown to possess antibacterial, hemolytic, fungicidal, cytotoxic, and insecticidal properties.^{4, 12-14} The solenopsin alkaloids are classified depending on the length of their alkyl chain and the relative configuration of their substituents at the 2 and 6 positions of their piperidine rings (Figure 1).

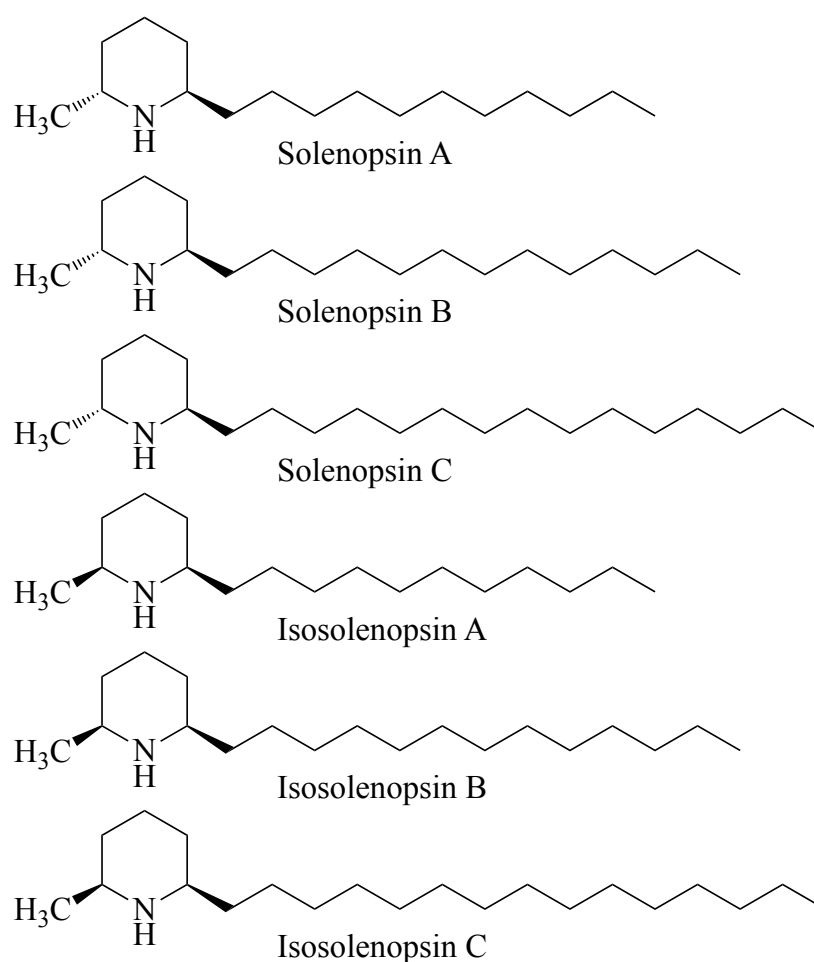
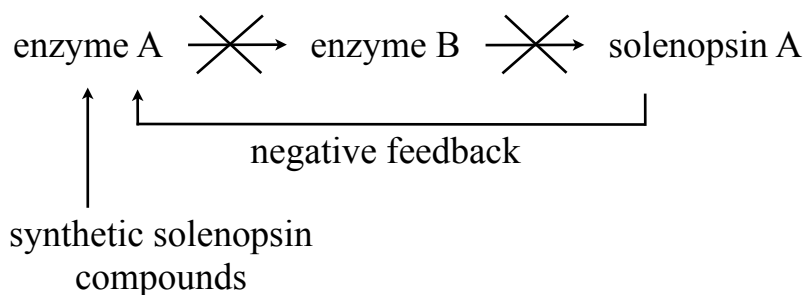


Figure 1. Alkaloid Components in the Venom of *S. invicta*

The solenopsins have a *trans* configuration about the 2 and 6 position of the piperidine ring, while the isosolenopsins are in *cis* configuration about the 2 and 6 positions. The stereochemistry of the *trans* solenopsins are specifically *2R,6R* and the *cis* isosolenopsins are *2R,6S*.¹⁵ Seven pairs of diastereoisomers have been identified with both *trans* and *cis* configurations about the piperidine ring.^{16,17} The first identified diastereoisomers were given trivial names based on the number of carbons in their alkyl chains. These compounds are classified as A, B, and C having 11, 13, and 15 carbon alkyl chains, respectively (Figure 1). The majority of the venom consists of the *trans* alkaloids, while the *cis* isoforms are present only in trace amounts. The biological functions of the venom components have therefore been accredited to the *trans* isomers in numerous studies.^{18 - 20}

A recent study was conducted in an attempt to control *S. invicta*'s growing population.² The goal of this project was to generate compounds that would inhibit the ant from producing the *trans* isomers, including solenopsin A. Without the ability to produce its most abundant, defensive alkaloids, *S. invicta* would naturally fall prey to another organism. The production of the solenopsins was hypothesized to be regulated by a negative feedback mechanism in its poison glands (Scheme 1). Compounds were generated in an attempt to mimic the structure of solenopsin A in hopes they would interfere in the enzymatic regulation pathway to prevent its production.



Scheme 1. Enzymatic Inhibition of Solenopsin A Production

The synthesized compounds (Figure 2) were tested using the SVR angiogenesis assay.² Angiogenesis, the formation of blood vessels from preexisting vasculature, is a process that begins in the endothelial cells of the vasculature. SVR assays using *ras*-transformed endothelial cells are an effective and widely-used screening tool to probe a compound's ability to inhibit angiogenesis in the initial stages of endothelial cell proliferation. While none of the synthetic compounds were found to be antiangiogenic, solenopsin A did prove to be an effective inhibitor of angiogenesis.²

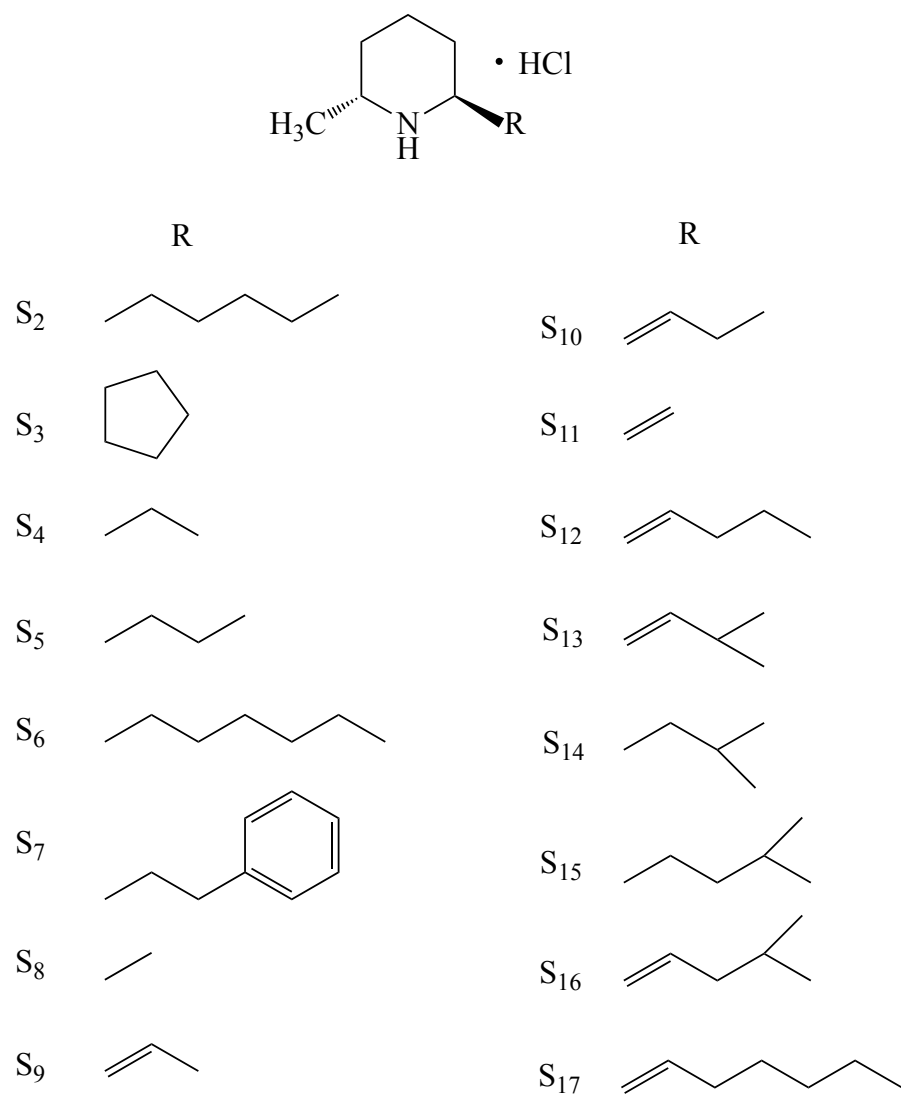


Figure 2. Synthetic Analogs (from Previous Study) Subject to SVR Assays

CHAPTER II

BACKGROUND

The ability to inhibit angiogenesis has potentially important applications for the treatment of patients with various types of cancer.²¹ Angiogenic inhibitors have already been effective in treating ovarian cancer,²¹ breast cancer,²² prostate cancer,²³ and approximately 70 other diseases. Cancer, termed medically as malignant neoplasms, is a general term describing many disease states when abnormal cells divide without control. The diversity of these disease states has made the development of treatment therapies difficult to achieve. Interestingly, more than 85% of all cancer related deaths are attributed to solid tumors.²⁴ Tumorigenesis, the formation of solid tumors, is then one unifying characteristic in this broadly diverse condition, making it an attractive physiological process to target.

Tumor-Induced Angiogenesis

While tumorigenesis involves many biochemical processes, angiogenesis is the major prerequisite for tumor growth and metastasis, and therefore constitutes a pivotal point in the control of cancer progression. Dividing malignant cells must stimulate

angiogenesis in order to continue dividing.²⁵ Angiogenesis is required because the rapidly dividing cells must obtain a sufficient supply of oxygen and nutrients while also being able to efficiently remove waste. The vascularization of the malignant cell mass allows for continued, accelerated growth, and facilitates metastasis, the spread of cancerous cells to other areas of the body through both the lymphatic and circulatory systems.²⁵

Tumor-induced angiogenesis is initiated once the cell mass reaches 2 mm in diameter and begins to secrete pro-angiogenic growth factors which bind their receptor tyrosine kinases (RTKs) on the surface of the endothelial cells of nearby vasculature. The primary RTKs involved in tumor-induced angiogenesis are the vascular endothelial growth factor receptors (VEGFRs).²⁶ The VEGFR family of receptors consists of VEGFR-1, -2, and -3, with VEGFR-2 having the most significant role in angiogenesis. The pro-angiogenic growth factors affecting these receptors are the VEGF ligands (VEGF-A, -B, -C, -D, and -E), with the most active and abundant isoform being VEGF-A (VEGF165) often referred to simply as VEGF.²⁷

Upon secretion from the tumorous cell mass, VEGF binds to 2 proximal VEGFR-2 on the surface of the endothelial cells of nearby vasculature. Once bound, the receptors dimerize leading to autophosphorylation and subsequent activation of their tyrosine kinase domains. Interaction of these kinase domains with intracellular substrate peptides activate a number of intracellular transduction pathways. The major substrate peptide overstimulated in tumor-induced angiogenesis is phosphatidylinositol-3-kinase

(PI3K). Once phosphorylated, PI3K then initiates a cascade of signaling events ultimately leading to the activation of Akt (or protein kinase B, PKB). This is known as the PI3K/Akt pathway and is amplified in a number of malignancies. Akt, once activated in the vascular endothelial cells, stimulates endothelial cell growth and proliferation which leads to the directional growth of blood vessels toward the malignant cell mass.²⁸

The PI3K-Akt Pathway

Angiogenesis and tumorigenesis are predominately mediated through the increased activity of the PI3K/Akt intracellular pathway thus making it an attractive pharmacologic target.²⁹ This pathway regulates cell survival, apoptosis, proliferation, cytoskeletal rearrangement, and protein synthesis, and is over expressed in both vascular endothelial cells and tumorous cells.²⁵ While the tyrosine kinase domain of VEGFR-2 induces PI3K activation in the vascular cells as described, other RTK activators of PI3K involved in tumorigenesis and tumor-induced angiogenesis include the fibroblast growth factor receptors (FGFR), platelet-derived growth factor receptors (PDGFR), insulin-like growth factor 1 receptors (IGF-1R), and the epidermal growth factor receptors (EGFRs).²⁵

FGFR is activated by fibroblast growth factor (FGF) and is involved in embryonic development, the regulation of angiogenesis in well developed tissues, and in wound repair. Platelet-derived growth factor (PDGF) is the ligand of PDGFR and is also

involved in wound repair, as well as the regulation of cellular development and proliferation.³⁰ Insulin-like growth factor 1 (IGF-1) binds IGF-1R and through activation of PI3K, regulates series of intracellular signaling pathways that lead to cell growth and regulation of metabolism. EGFRs are over expressed in the cells of solid tumors, specifically family members ErbB1 and HER1.³¹ This up-regulation overstimulates EGFR signaling which is initiated extracellularly by transforming growth factor 1 (TGF-1) or endogenously by epidermal growth factor (EGF), and is observed in colon and lung cancers.³²

The amplification of intracellular signaling by these RTKs, beginning with PI3K activation, is a common occurrence in many malignancies and is outlined below (Figure 3). Once activated, PI3K then phosphorylates phosphatidylinositol-3,4-diphosphate (PIP2) to produce phosphatidylinositol-3,4,5-triphosphate (PIP3), which acts as a second messenger leading to the subsequent activation of PKD1 and Akt. Akt regulates protein synthesis, cell cycle progression, cellular metabolism, anti-apoptosis, and is the major downstream target of PI3K.³³ The up-regulation of both PI3K and Akt, as well as mutations of PI3K have been observed in a number of malignancies, while inhibition of these second messengers has been shown to suppress both tumor growth and angiogenesis. Mutations of PI3K have been found to occur in gastric, breast, ovarian, hepatocellular carcinoma, and colorectal cancers.^{34, 35}

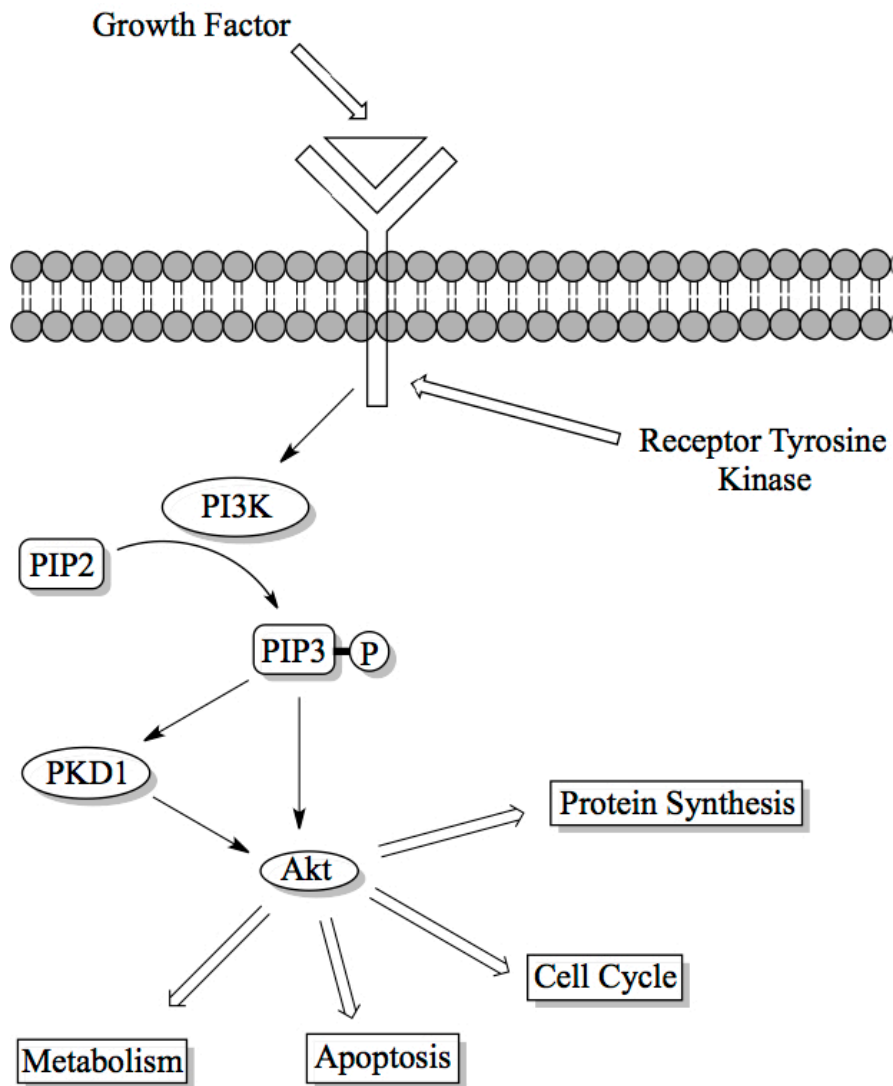


Figure 3. The PI3K/Akt Pathway

Small-Molecular-Weight Angiogenic Inhibitors

Up-regulation of VEGF, the most potent stimulator of tumor-induced angiogenesis, leads to overstimulation of signaling through VEGFR-2, while up-regulation of EGFRs, leads to amplification of EGF signaling in tumorigenesis. The

receptors for EGF and VEGF are therefore popular targets for angiogenic inhibition, with drug-protein interactions occurring both extracellularly, effecting ligand binding domains, and intracellularly, acting on receptor/protein tyrosine kinases. The direct binding of inhibitors to growth factors is another method of inhibition.²⁴

EGFR inhibition has been achieved with gefitinib (Iressa®)³⁶ and erlotinib (Tarceva®),³⁷ while EGFR protein tyrosine kinase (PTK) inhibition has also been achieved with salicylamide analogs (Figure 4). These analogs also suppress VEGFR signaling by their direct interaction with VEGF. The drug PTK787/ZK222584 is an receptor tyrosine kinase inhibitor of VEGFR-1,-2, and -3, and PDGFR (Figure 4).³⁸ Imatinib mesylate (Gleevec®) is also a receptor tyrosine kinase inhibitor of PDGFR beta and EGFR (Figure 4).³⁹

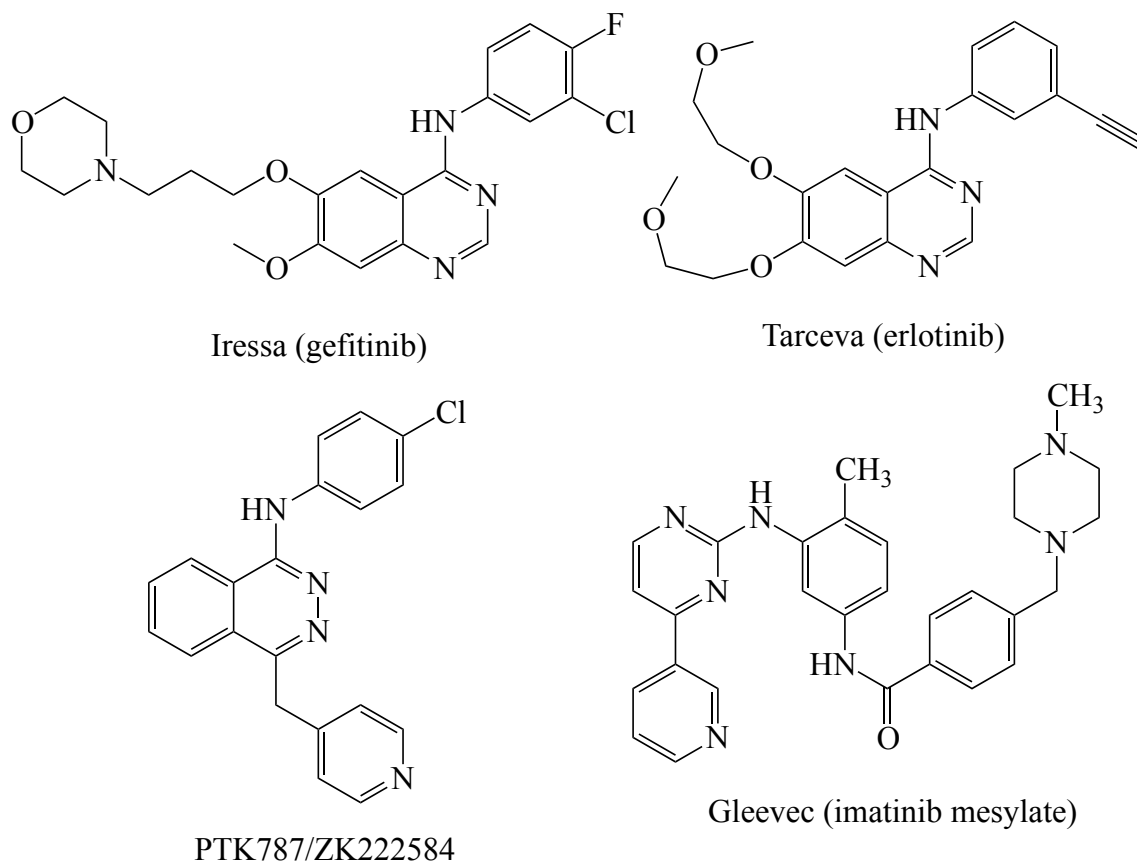


Figure 4. Tyrosine Receptor Inhibitors

Genistein has been shown to suppress both EGFR signaling in tumor cells, by inhibiting EGFR PTK, and VEGFR signaling in vascular endothelial cells, by binding directly to VEGF (Figure 5).⁴⁰ Bevacizumab (Avastin[®]), although not a small-molecule, also suppresses VEGFR signaling by interacting directly with VEGF to inhibit receptor binding. Bevacizumab was the first angiogenic inhibitor to be approved by the U.S. Food and Drug Administration (F.D.A.) in 2003.²⁴

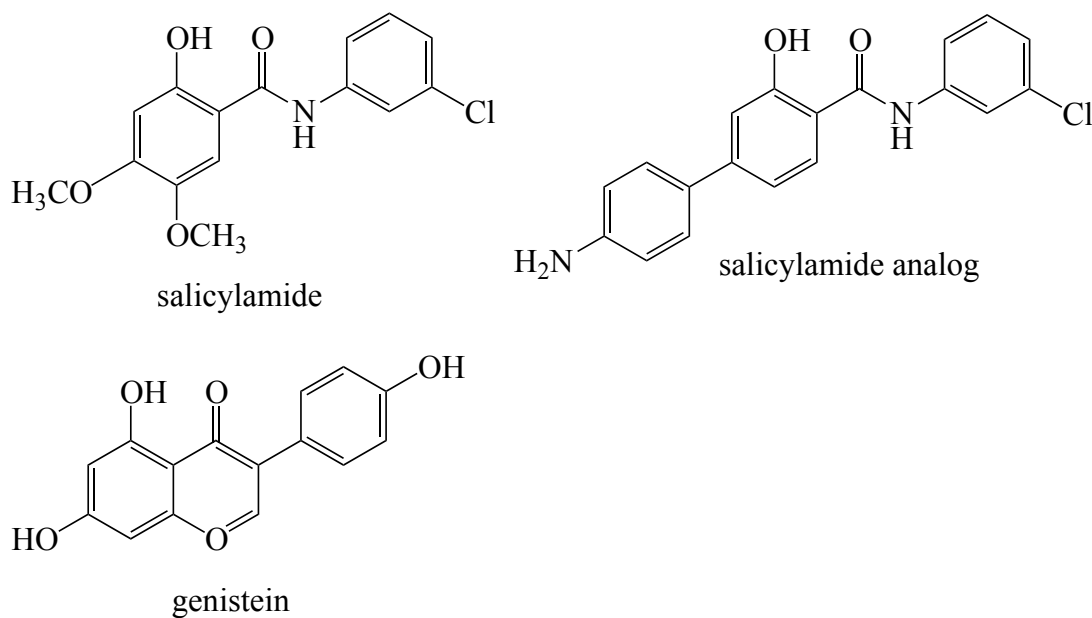
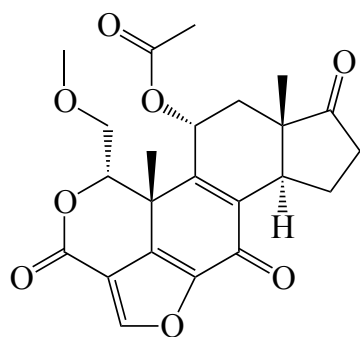
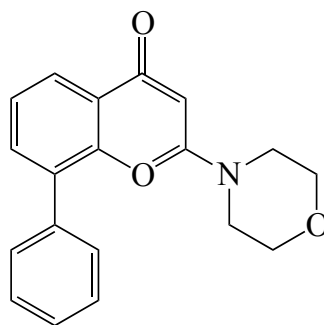


Figure 5. VEGF Inhibitors

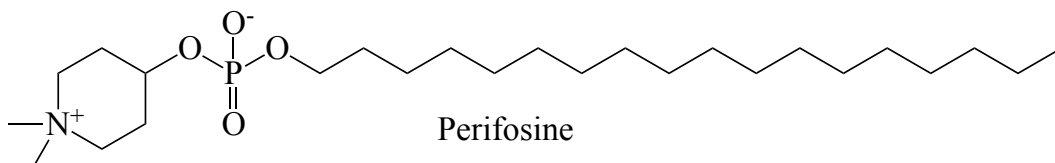
PI3K and Akt inhibitors are also currently being used to treat various types of cancer. The fungal metabolite, wortmannin, and the ether phospholipid, perifosine (Figure 6), both inhibit PI3K and represent the diversity of small-molecule angiogenic inhibitors, even among those acting on the same protein.^{41, 42} Perifosine has been shown to inhibit tumor growth in a number of malignancies while also enhancing the effectiveness of both chemotherapeutic drugs and radiation therapy.⁴³ Miltefosine, another phospholipid ether (Figure 6), and perifosine also have inhibitory effects directly on Akt.⁴⁴ The PI3K inhibitor, LY294002 (Figure 6), induces G1 cell cycle arrest and inhibits cell proliferation in ovarian cancer.⁴⁵



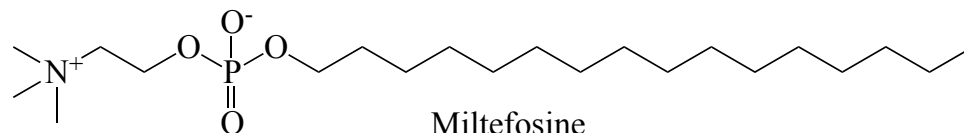
Wortmannin



LY294002



Perifosine

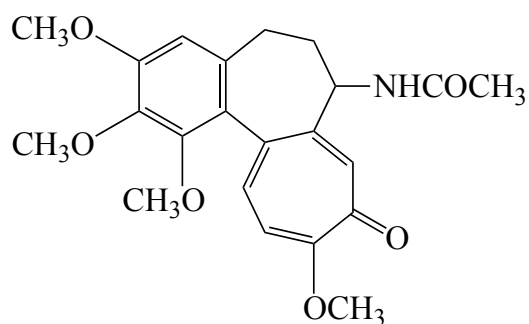


Miltefosine

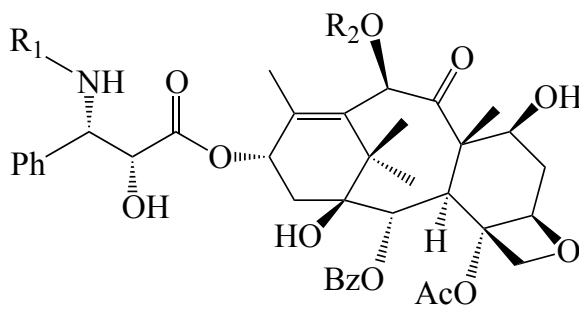
Figure 6. PI3K/Akt Inhibitors

Because microtubule formation, via tubulin polymerization, is essential for spindle formation and the separation of chromosomes required for cellular division, tubulin inhibitors, specifically those blocking polymerization/depolymerization, have been shown to inhibit the division/proliferation of malignant cells, as well as induce apoptosis. The drug colchicine (Figure 7), has been shown to inhibit tubulin polymerization leading to malignant cell death, while paclitaxel and docetaxel (Figure 7) inhibit depolymerization by binding to microtubules thus preventing malignant cellular

division.⁴⁶ Other promising tubulin inhibitors are the phenyl-3-(2-chloroethyl)ureas (CEUs) which have been shown to inhibit tumor cell and vascular endothelial cell proliferation. These compounds include 4-iodo-[3-(2-chloro-ethyl)ureido]phenyl (ICEU) and 4-tert-butyl-[3-(2-chloroethyl)ureido]phenyl (tBCEU) (Figure 7), and inhibit tumorigenesis and angiogenesis, by inhibiting microtubule formation via their formation of a covalent bond at the Cys239 of tubulin. They have also been shown to be antiangiogenic by inhibiting bFGF.²⁴

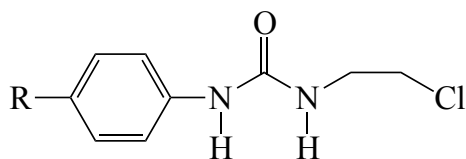


colchicine



Paclitaxel: $R_1 = \text{Bz}$; $R_2 = \text{Ac}$

Docetaxel: $R_1 = \text{Boc}$; $R_2 = \text{H}$



ICEU, $R = \text{I}$

tBCEU, $R = t\text{-Butyl}$

Figure 7. Tubulin Inhibitors

Previous Studies on Solenopsin A

SVR angiogenic assays evaluate a compound's ability to inhibit the proliferation of *ras*-transformed murine endothelial cells. The *ras* gene is often mutated in a number of malignancies leading to overactive Ras proteins which effectively control cell proliferation and cellular growth.⁴⁷ Solenopsin A was administered at concentrations of 1, 3, and 6 micrograms/ml into wells containing 10,000 SVR cells. After 48 hrs they were counted and compared to a control to assess inhibition of proliferation. The results showed solenopsin A inhibited cell proliferation in a dose-dependent manner (Figure 8).² Following these initial assays, a series of studies were conducted to assess solenopsin's mode of action.

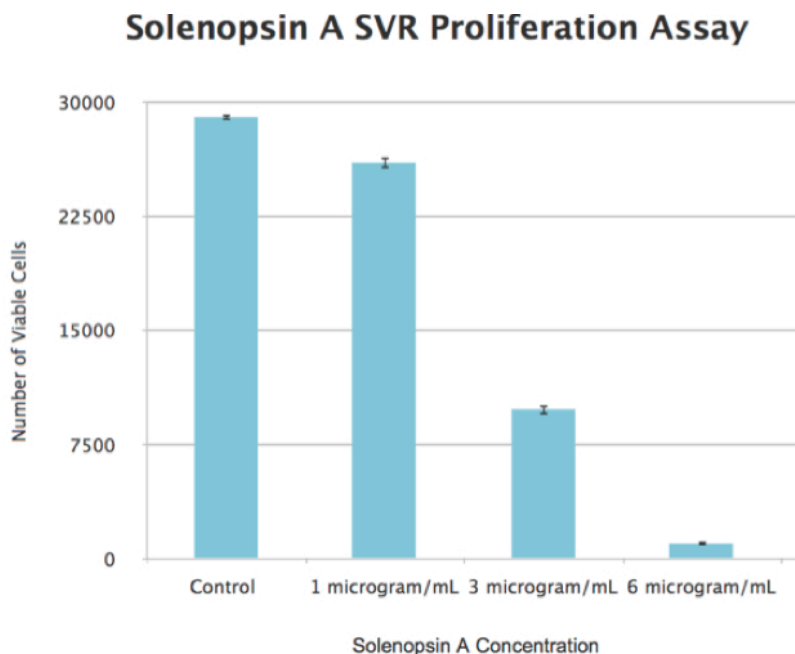


Figure 8. Previous SVR Assay Results of Solenopsin A

First, a series of *in vitro* studies were conducted to elucidate which protein(s) were being inhibited by solenopsin. This was done by screening solenopsin A against 28 protein kinases including PI3K, purified PI3K, and Akt. Solenopsin A was found to have no effect on PI3K. By measuring the production of PIP3 from PIP2 in a cell-free assay, solenopsin was also found to have no effect on purified recombinant PI3K p110alpha/p85alpha, nor did solenopsin inhibit PKD1 activity, one of several activators of Akt. Akt activity was, however, selectively inhibited by 50% when assayed with solenopsin A at a concentration of 10 micro-molar.²

A FOXO1a export assay on cells treated with solenopsin A was used to assess Akt inhibition in cells. Akt, the major downstream target of PI3K, phosphorylates numerous proteins involved in angiogenesis including the forkhead family of transcription factors (FKHD/FOXO). Once the FOXO transcription factors are phosphorylated by Akt, they are exported from the nucleus to the cytoplasm. Solenopsin was found to inhibit the transport of FOXO1a into the cytoplasm which suggested Akt inhibition. PI3K/Akt inhibition assays were also conducted in cells to determine the effects on PDGF stimulated signaling and insulin induced signaling. PDGF signaling was unaffected by solenopsin, however solenopsin did prevent PI3K activation in insulin stimulated cells without affecting IRS1 phosphorylation. Solenopsin's inhibition of PI3K, and subsequent downstream signaling events, without inhibiting IRS1 strongly suggested it was suppressing the PI3K/Akt pathway by inhibiting a step downstream of IRS1 and upstream of PI3K.²

CHAPTER III

RESULTS AND DISCUSSION

In order to explore the structure-activity relationships of solenopsin A analogs and their ability to inhibit angiogenesis, four groups of synthetic derivatives were generated. The compounds synthesized were based on the organic structure of our lead compound solenopsin A, in combination with common structural features of effective F.D.A. approved angiogenic inhibitors and those in clinical development. Consideration was given to features including common functional groups, aromatic/aliphatic regions, functional groups of attachment between aromatic/aliphatic regions, and alkyl chain length. Because the specific affecter protein(s) of solenopsin A has yet to be determined, we explored the commonalities of protein-specific angiogenic inhibitors including tyrosine kinase inhibitors, VEGF inhibitors, and tubulin inhibitors. Using this approach, four groups of compounds were synthesized including benzamide analogs, an amine analog, ether analogs, and a long-chain *n*-alkyl analog (Figure 9).

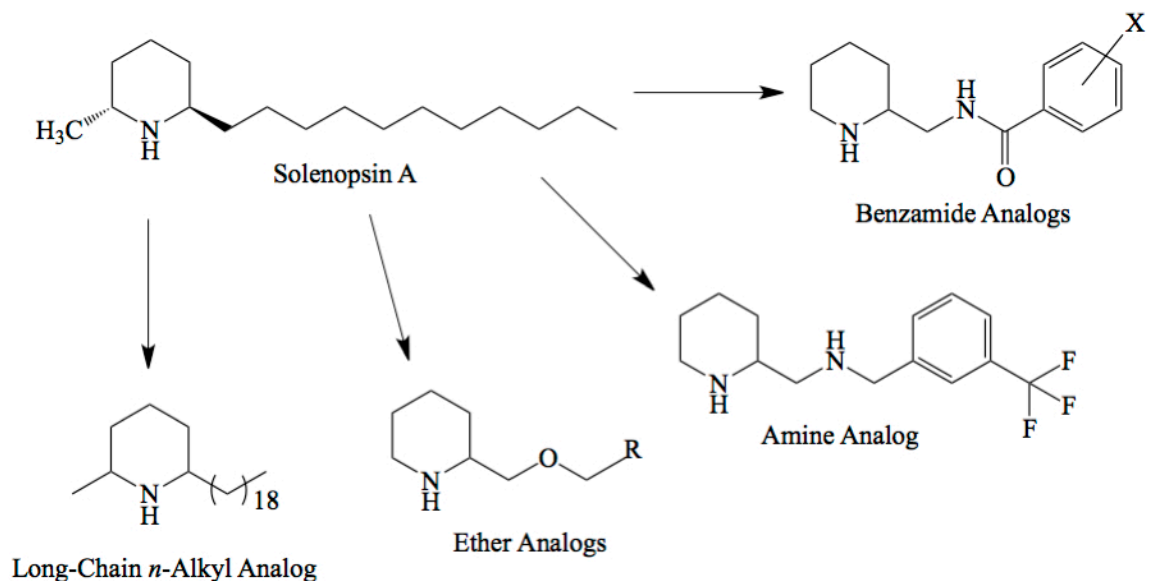


Figure 9. Overview of Synthesis

Synthesis of Solenopsin A Analogs

The major structural similarity of effective small-molecule angiogenic inhibitors consists of two aromatic regions connected by amide and/or amine functional groups.²⁴ The amide functional group of attachment can be seen in the tyrosine kinase inhibitor Gleevec (Figure 4) and the VEGF inhibitors salicylamide, salicylamide analog (Figure 5), and Bay 43-9006 (Figure 10). The tubulin inhibitors ICEU, tBCEU, LY295501, Sulofenur, and diarylsulfonylurea-2b also possess the amide functional group of attachment (Figures 7, 10). Several of these inhibitors, including Gleevec (Figure 4), salicylamide and salicylamide analog (Figure 5), and diarylsulfonylurea-2b (Figure 10), also contain benzamides as a common structural feature. This structural commonality

was introduced into our lead compound to generate the benzamide analogs by replacement of the undecyl side chain at the 6 position of solenopsin A's piperidine ring with substituted benzamides, and elimination of the methyl group at the 2 position of solenopsin A.

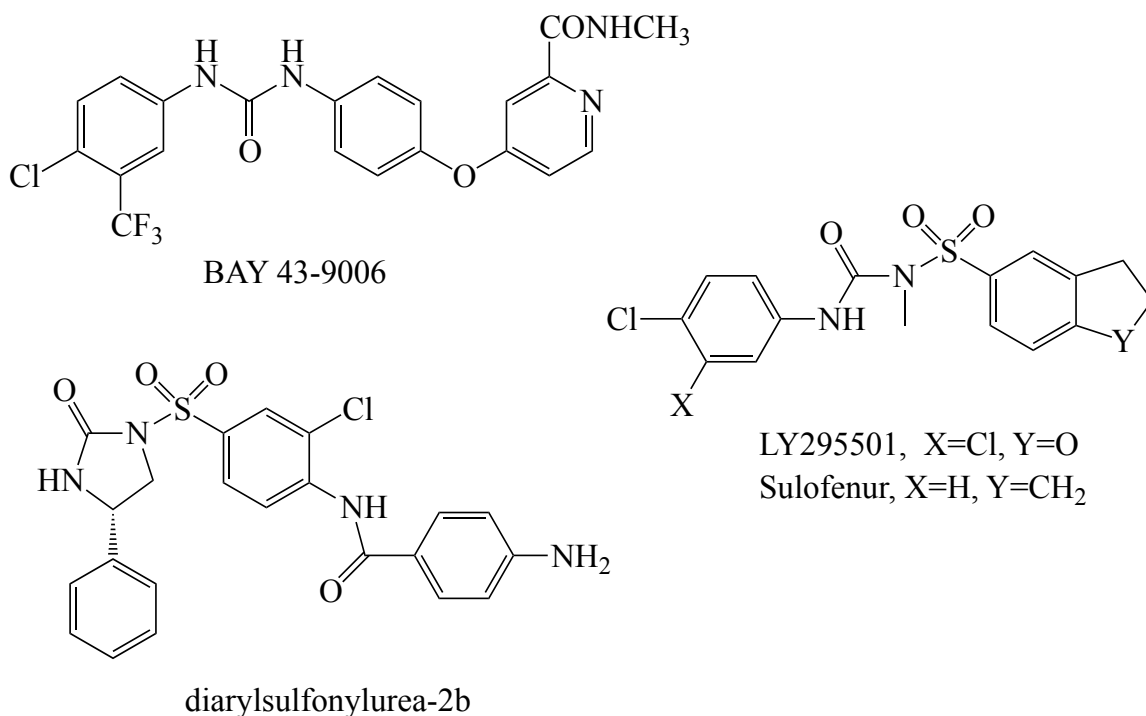
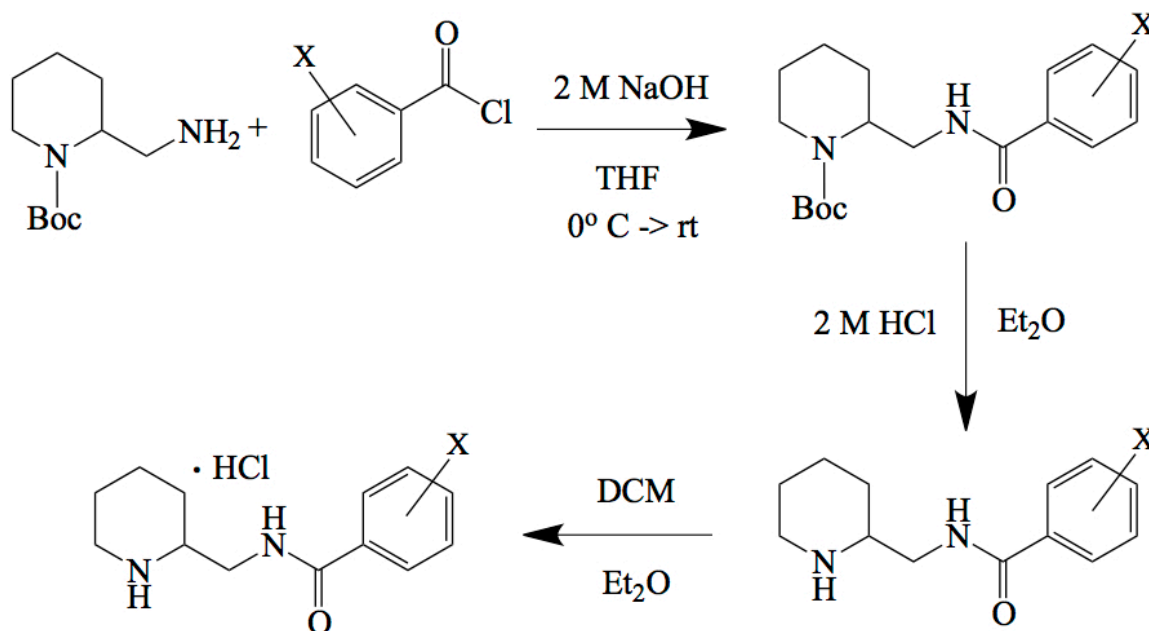


Figure 10. Small-Molecule Inhibitors with Amide Functional Groups

The benzamide compounds were synthesized as indicated in Scheme 2 via acyl exchange reactions in an aprotic solvent. 1-Boc-2-(aminomethyl)piperidine was reacted with various benzoyl chloride reagents to generate the compounds. 1-Boc-2-(aminomethyl)piperidine was chosen as the starting material due to the stability of the

Boc protecting group, while the benzoyl chloride reactant was varied depending on the desired product.



Scheme 2. Synthesis of Benzamide Analogs

The selectivity of the nucleophilic attack on the carbonyl carbon of the benzoyl chloride by the nitrogen of the primary amine resulted in few side products and relatively high yields. Initial attempts showed using 1.5 equivalents of the Boc protected starting material and 1.0 equivalent of benzoyl chloride resulted in the highest yields (60% - 80%). The reactions were conducted by chilling a solution of 1-Boc-2-(aminomethyl)piperidine in THF in an ice bath, followed by the addition of NaOH (2.0 M). The solution was stirred and the benzoyl chloride reagent was added. The reaction was allowed to reach room temperature while stirring for 2.5 hrs. The reaction mixture

was then extracted and the organic layer dried over MgSO_4 . Mixtures were purified using either column chromatography or preparative TLC. Solvents were evaporated following purification, and deprotection of the resulting residue was achieved by adding HCl in ether. Evaporation of solvents yielded the final product as either a white precipitate or as a yellowish, oily residue. To insure the stability of the compounds, several benzamide analogs were converted into water soluble hydrochloride salts following acidic removal of the Boc protecting group. This was achieved by dissolving the oil residues in a minimal amount of DCM and adding anhydrous ether (or anhydrous hexane) drop wise until precipitation occurred. The benzamide compounds that were generated are shown in Figure 11.

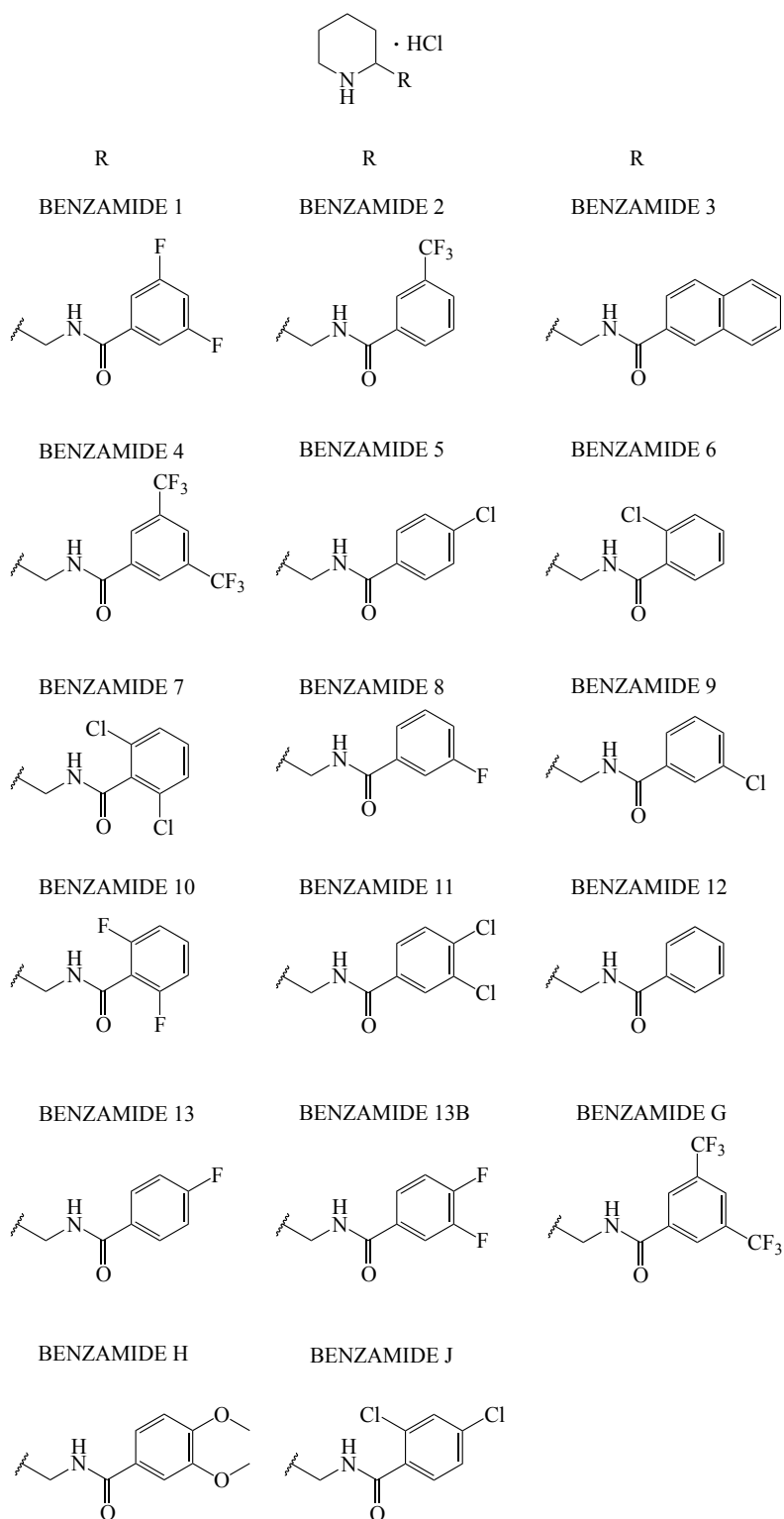
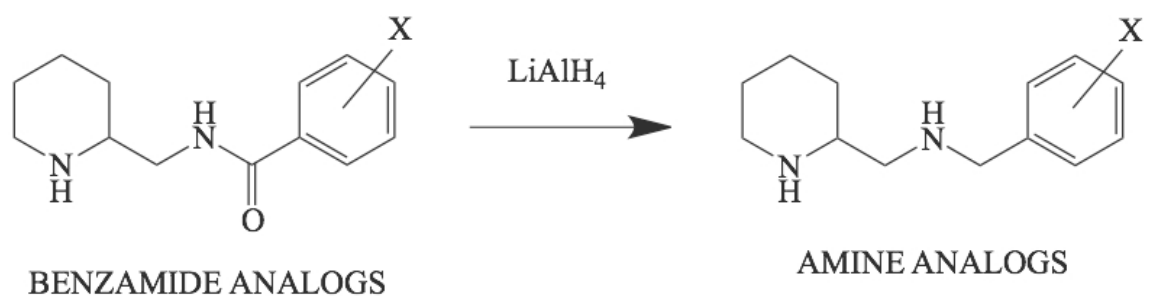


Figure 11. Synthetic Benzamide Analogs of Solenopsin A

The synthetic benzamide derivatives of solenopsin A were assayed against SVR endothelial cells and none were found to be antiangiogenic. Following these assays, the benzamide analogs were reduced from their amide connecting group to generate diamine analogs, as described in Scheme 3. The amine analogs were synthesized based on the tyrosine kinase inhibitors Iressa, Tarceva, PTK787/ZK222584, and Gleevec which all possess amine function groups of attachment (Figure 4). Although several of the benzamide analogs (Benzamides 1-6) were reduced, only Amine 2 could be isolated (Figure 12).



Scheme 3. Synthesis of Amine Analogs

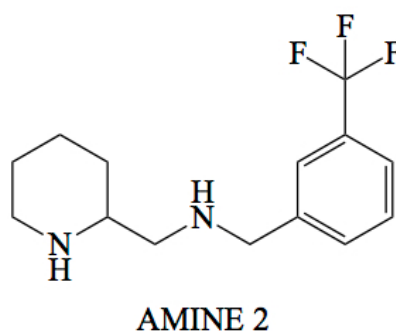


Figure 12. Synthetic Amine Analog of Solenopsin A

The benzamide analogs were first dissolved in anhydrous THF (or ether) under argon pressure, followed by the addition of 5 eq of lithium aluminum hydride. The reactions were refluxed for 12 - 24 hrs, and TLC analysis was used to confirm reaction completion using 5% - 10% MeOH in dichloromethane as the solvent system. The excess LAH was then quenched using either a Rochelle's salt solution or by conducting a Fieser workup.

When an aluminum emulsion resulted following the initial reaction, the emulsion was separated using a saturated aqueous solution of Rochelle's salt (sodium potassium tartrate). This was achieved by cooling the reaction solution to 0 °C and quenching the excess LAH by adding ethyl acetate drop wise while stirring. The ice bath was then removed and the Rochelle's salt solution was added to the reaction mixture and stirred for 1 -3 hrs. The reaction mixture was then diluted with DCM and extracted. The solvents were evaporated in the rotovap to yield the final amine product. Initial attempts at extraction often resulted in reformation of the emulsion which resulted in loss of the amine product.

A Fieser workup was also conducted on several of the benzamide reductions. This was done by quenching the initial reaction mixture by slowly adding 0.2 ml of H₂O, followed by the addition of 0.2 ml of 15% NaOH, followed by 0.6 ml of H₂O. The solution was then stirred on ice for 1 hr and resulted in formation of a granular salt. The salts were removed by vacuum filtration and washed with ether. The reaction mixture

was diluted with DCM and extracted. Solvents were evaporated in the rotovap to yield the final amine product.

The ether analogs were generated by removal of the 2-methyl of solenopsin A's piperidine ring and substitution at the 6 position with ether functional groups of attachment, having *n*-alkyl chain lengths of 2 and 3 carbons. A benzoyl protected alcohol starting material was reacted with alkyl iodides of various alkyl chain length to generate the ether compounds (Figure 13). The starting material's piperidine nitrogen was protected with the benzoyl group to allow for more accurate TLC analysis and because of its stability in basic solutions.

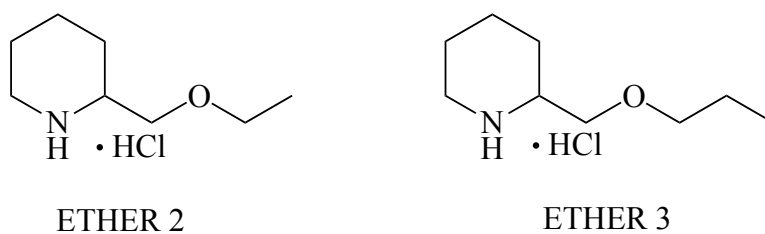
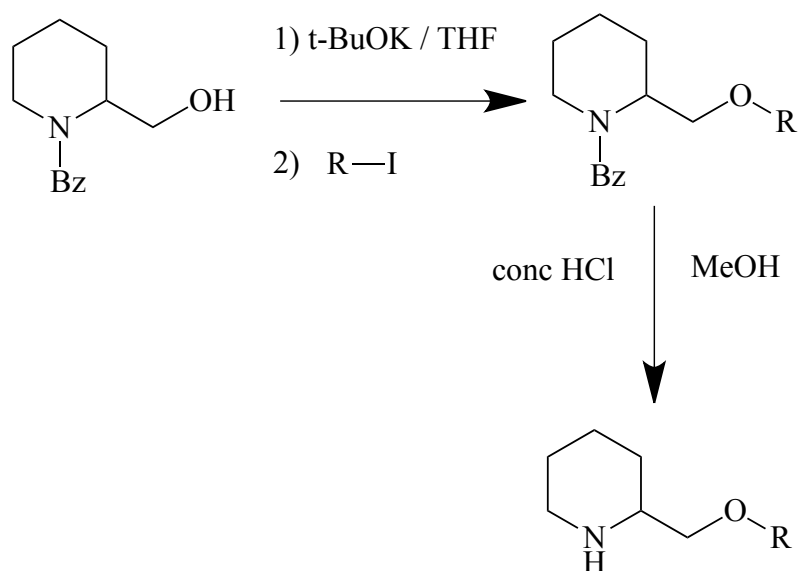


Figure 13. Synthetic Ether Analogs of Solenopsin A

The ether analogs were synthesized using base catalyzed ether formation reactions, followed by acidic deprotection as indicated in Scheme 4. This was achieved by first dissolving the benzoyl protected starting material, $C_{13}H_{12}NO_2$, in anhydrous THF on ice, while under Ar pressure. *t*-BuOK (2 eq) was then added followed by the addition of the alkyl iodide (2 eq) and the reaction was stirred for 2 - 4 hrs. The reaction mixture was diluted with DCM, extracted, and dried over $MgSO_4$. The drying agent was removed

by gravity filtration and TLC analysis assessed reaction completion. The reaction mixture was then purified by column chromatography using a solvent system of hexanes:EtOAc. Solvents were evaporated and removal of the benzoyl protecting group was achieved by adding MeOH to the resulting residue, followed by the addition of concentrated HCl. The reaction was refluxed for 24 hrs and the reaction solution neutralized with NaHCO₃ and the extracted. The organic layer was dried and gravity filtered to remove the drying agent. Solvents were removed in the rotovap to yield the final product as a brown precipitate.



Scheme 4. Synthesis of Ether Analogs

One long-chain *n*-alkyl derivative of solenopsin A was generated (Figure 14) by the reduction of 2-methyl-6-nonadecylpyridine to form 2-methyl-6-nonadecylpiperidine

as described in scheme 5. Five attempts at reduction, using increased reaction times, finally resulted in isolation of the desired product (Figure 14).

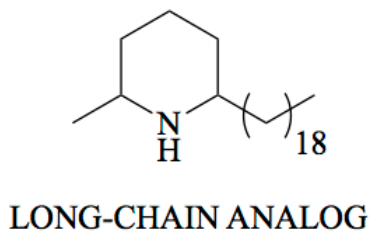
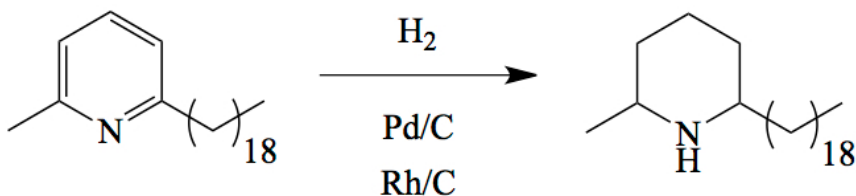


Figure 14. Synthetic Long-Chain *n*-Alkyl Analog of Solenopsin A

The reduction was achieved via catalytic hydrogenation by adding Pd/C and Rh/C to a solution of 2-methyl-6-nonadecylpyridine in absolute ethanol. The reaction was shaken under 55 psi for 30 hr in a parr hydrogenator. Catalyst were removed by filtering through a plug of celite using 5% MeOH in DCM. The reaction mixture was then purified by column chromatography using a minimal amount of silica and varying the solvent system between 5-10% MeOH in DCM, depending on separation during TLC analysis.



Scheme 5. Hydrogenation of 2-methyl-6-nonadecylpyridine

Experimental Data

General Procedures. All reagents, catalysts, and starting materials were purchased from Sigma Aldrich. Extractions of initial reaction mixtures were conducted using DCM unless stated otherwise. Following extraction, TLC analysis was done using Whatmen silica gel plates and reaction completion was determined by visualization under UV light. Purification, using silica gel 60 (230 - 400 mesh), was achieved by filtration through a plug of silica, column chromatography, or preparative TLC. All solvents were reagent grade, and the solvent systems were 1:1 ethyl acetate to hexanes unless otherwise indicated. ^1H NMR of protected analogs were taken after purification and before deprotecting. Final products were concentrated by evaporation of solvents using a rotary evaporator and subject to ^1H NMR and mass spectrometry to confirm purity.

3,5-difluoro-*N*-(piperidin-2-ylmethyl)benzamide (BENZAMIDE 1). A solution of *tert*-butyl 2-(aminomethyl)piperidine-1-carboxylate (50 mg, 0.234 mmol, 1.5 eq) in DCM (3.0 mL) was chilled in ice bath, followed by the addition of NaOH (2.0 M, 1.0 ml). The solution was stirred and 3,5-difluorobenzoyl chloride (0.0014 ml, 0.115 mmol, 1.0 eq) was added. The reaction was allowed to reach room temperature as the mixture continued stirring for 2.5 hrs. The reaction mixture was then diluted by the addition of DCM (10 ml) and extracted with water (2 x 1.0 ml). The DCM was dried over MgSO_4 (1.0 g) and filtered through a plug of Silica gel. The DCM was then evaporated using a

rotovap for 4.0 hrs and the residue which formed was dried under vacuum for 2 days.

Deprotection was conducted by adding HCl in ether (2.0 M, 2.0 ml) to the residue and stirring for 3 days at room temperature. Ether was then evaporated using the rotovap to yield 25 mg of product, as a white precipitate (86%).

***N*-(piperidin-2-ylmethyl)-3-(trifluoromethyl)benzamide hydrochloride**

(BENZAMIDE 2). *tert*-butyl 2-(aminomethyl)piperidine-1-carboxylate (100 mg, 0.467 mmol, 1.5 eq) in THF (1 ml), was chilled in an ice bath, followed by the addition of NaOH (2.0 M, 0.9 ml). The solution was stirred and 3-(trifluoromethyl)benzoyl chloride (0.0047 ml, 0.311 mmol, 1.0 eq) was added. The reaction mixture was allowed to reach room temperature while stirring overnight. The mixture was then diluted by the addition of dichloromethane (15 ml) and extracted with water (2 x 3.0 ml). The DCM layer was dried over MgSO₄ (1.0 g) and filtered through a plug of Silica gel. Solvents were removed by rotary evaporation. TLC analysis confirmed further purification was needed and this was accomplished using preparative TLC. The residue was dissolved in DCM (2.0 ml) and applied to a preparative TLC plate. A 1:1 hexane:EtOAc solvent system allowed for optimal separation of the reaction mixture. The desired portion was scraped from the TLC plate and stirred in EtOAc (25.0 ml) for 30 min. The EtOAc was then vacuum filtered to remove Silica and evaporated in the rotovap to form an oil residue. The residue was dried under vacuum for 2 days. Deprotection was conducted by adding HCl in ether (2.0 M, 3.0 ml) to the residue and stirring at room temperature overnight.

Ether was then evaporated using the rotovap overnight without heat which formed 52 mg of an oil product (58.5%). The residue was converted into a water soluble hydrochloride salt to insure stability of the compound. This was achieved by dissolving the oil residue in a minimal amount of dichloromethane (~0.0020 ml) and adding anhydrous ether drop wise until precipitation occurred. Solvents were then removed by rotary evaporation and purity assessed via ^1H NMR.

***N*-(piperidin-2-ylmethyl)-2-naphthamide hydrochloride (BENZAMIDE 3).** A

solution of *tert*-butyl 2-(aminomethyl)piperidine-1-carboxylate (100 mg, 0.467 mmol, 1.5 eq) in THF (1 ml) was chilled in an ice bath, followed by the addition of NaOH (2.0 M, 0.5 ml). The solution was stirred and beta-naphthoyl chloride (59.1 mg, 0.311 mmol, 1.0 eq) was added. The reaction was allowed to reach room temperature while stirring for 4 hrs. The reaction mixture was then diluted by the addition of ether (20 ml) and extracted with water (2 x 1.0 ml). The ether was dried over MgSO_4 (1.0 g), and the mixture was purified by column chromatography. The solvents were then removed by rotary evaporation and the residue which formed was dried under vacuum overnight.

Deprotection was conducted by adding HCl in ether (2.0 M, 3.0 ml) to the residue and stirring at room temperature overnight. Ether was then evaporated using the rotovap to leave an oily residue (47 mg, 56.6%). To achieve solid product, the residue was dissolved in DCM (0.0020 ml) and anhydrous ether was added drop wise. The precipitate was then stored under vacuum overnight.

***N*-(piperidin-2-ylmethyl)-3,5-bis(trifluoromethyl)benzamide hydrochloride**

(BENZAMIDE 4 & BENZAMIDE G). A solution of *tert*-butyl 2-(aminomethyl)piperidine-1-carboxylate (100 mg, 0.467 mmol, 1.5 eq) in THF (1.0 ml) was chilled on ice, followed by the addition of NaOH (2.0 M, 0.5 ml). The solution was stirred while adding 3,5-bis(trifluoromethyl)benzoyl chloride (0.0057 ml, 0.311 mmol, 1.0 eq). The reaction ran for 4 hrs and was maintained at 0 °C. The reaction mixture was then diluted by the addition of DCM (10 ml) and extracted with water (2 x 1.0 ml). The DCM was dried over MgSO₄ (1.0 g) and filtered through a plug of Silica gel. The reaction mixture was purified by column chromatography using 1:1 hexane:ethyl acetate solvent system. Solvents were removed by rotary evaporation and the residue was kept under vacuum for 2 days. The Boc protecting group was removed by adding HCl in ether (2.0 M, 2.0 ml) to the residue and stirring for 3 days at room temperature. Ether was then evaporated using the rotovap and a white precipitate (40 mg, 36%) was formed.

4-chloro-*N*-(piperidin-2-ylmethyl)benzamide hydrochloride (BENZAMIDE 5). *tert*-butyl 2-(aminomethyl)piperidine-1-carboxylate (100 mg, 0.467 mmol, 1.5 eq) was dissolved in THF (1.0 mL) and NaOH (2.0 M, 0.5 ml) was added. The solution was stirred and cooled in an ice bath while adding 4-chlorobenzoyl chloride (0.0040 ml, 0.311 mmol, 1.0 eq). The reaction was allowed to reach room temperature while running overnight. The reaction mixture was then diluted by the addition of DCM (10 ml) and extracted with water (2 x 1.0 ml). The DCM was dried over MgSO₄ (1.0 g) and filtered

through a plug of Silica gel. Further purification was achieved by column chromatography using 1:1 hexane:ethyl acetate solvent system. The residue was kept under vacuum for 2 days to remove solvents. The Boc protecting group was removed by addition of HCl in ether (2.0 M, 2.0 ml) to the residue and stirring for 3 days at room temperature. Ether was removed by storing under vacuum for 24 hrs, and a white precipitate (51mg, 64.9%) was formed .

2-chloro-*N*-(piperidin-2-ylmethyl)benzamide hydrochloride (BENZAMIDE 6).

NaOH (2.0 M, 0.5 ml) was added to a solution of *tert*-butyl 2-(aminomethyl)piperidine-1-carboxylate (100 mg, 0.467 mmol, 1.5 eq) dissolved in THF (1.0 ml). The solution was stirred and cooled in an ice bath while adding 2-chlorobenzoyl chloride (0.0040 ml, 0.311 mmol, 1.0 eq). The reaction was allowed to reach room temperature while running overnight. The reaction mixture was then diluted by the addition of DCM (10 ml) and extracted with water (2 x 1.0 ml). The DCM was dried over MgSO₄ (1.0 g) and filtered through a plug of Silica gel. Solvents were removed by rotary evaporation and TLC analysis confirmed that further purification was needed. The residue was dissolved in DCM (2.0 ml) and applied to a preparative TLC plate. A 1:1 hexane:EtOAc solvent system allowed for optimal separation of the reaction mixture. The desired portion was scraped from the TLC plate and stirred in EtOAc (15.0 ml) for 30 min. Following vacuum filtration the EtOAc was evaporated in the rotovap and the residue was kept under vacuum for 2 days. HCl in ether (2.0 M, 2.0 ml) was added to the residue and

stirred for 3 days at room temperature. Ether was removed by storing under vacuum overnight, and a white precipitate (56 mg, 71.4%) was formed.

2,6-dichloro-*N*-(piperidin-2-ylmethyl)benzamide (BENZAMIDE 7). *tert*-butyl 2-(aminomethyl)piperidine-1-carboxylate (100 mg, 0.467 mmol, 1.5 eq) was dissolved in THF (1.0 ml) and NaOH (2.0 M, 0.5 ml) was added. The solution was stirred and cooled in an ice bath and 2,6-dichlorobenzoyl chloride (0.0045 ml, 0.311 mmol, 1.0 eq) was added. The reaction was allowed to reach room temperature while running for 12 hrs. DCM (10 ml) was then added to dilute the reaction mixture following extraction with water (2 x 1.0 ml). The DCM was dried over MgSO₄ (1.0 g) and filtered through a plug of Silica gel. TLC analysis confirmed that further purification was needed. The reaction mixture was concentrated via rotary evaporation and dissolved in DCM (1.0 ml) before being applied to a preparative TLC plate. A 1:1 hexane:EtOAc solvent system allowed for optimal separation of the reaction mixture. The desired portion was scraped from the TLC plate and stirred in EtOAc (15.0 ml) for 30 min. Silica was removed by vacuum filtration and the EtOAc was evaporated in the rotovap. The residue was kept under vacuum for 2 days. HCl in ether (2.0 M, 2.0 ml) was then added to the residue and stirred for 3 days at room temperature. The ether was removed by storing under vacuum overnight, and a white precipitate (30 mg, 33.6%) was formed.

3-fluoro-*N*-(piperidin-2-ylmethyl)benzamide (BENZAMIDE 8). NaOH (2.0 M, 0.5 ml) was added to a solution of *tert*-butyl 2-(aminomethyl)piperidine-1-carboxylate (100 mg, 0.467 mmol, 1.5 eq) dissolved in THF (1.0 ml). The solution was stirred and cooled in an ice bath and 3-fluorobenzoyl chloride (0.0038 ml, 0.311 mmol, 1.0 eq) was added. The reaction was allowed to reach room temperature while running overnight. The reaction mixture was then diluted with DCM (10 ml) and extracted with water (2 x 1.0 ml). The DCM was dried over MgSO₄ (1.0 g) and gravity filtered to remove the drying agent. The reaction mixture was concentrated via rotary evaporation and dissolved in DCM (1.0 ml). Purification was achieved by prep TLC using a 1:1 hexane:EtOAc solvent system. Solvents were evaporated in the rotovap and the residue was kept under vacuum for 2 days. HCl in ether (2.0 M, 2.0 ml) was then added to the residue and stirred for 3 days at room temperature. Ether was removed by storing under vacuum overnight, and a white precipitate (50 mg, 68.0%) was formed.

3-chloro-*N*-(piperidin-2-ylmethyl)benzamide hydrochloride (BENZAMIDE 9). *tert*-butyl 2-(aminomethyl)piperidine-1-carboxylate (100 mg, 0.467 mmol, 1.5 eq) was dissolved in THF (1.0 ml) and NaOH (2.0 M, 0.5 ml) was added. The solution was stirred and cooled on ice while 3-chlorobenzoyl chloride (0.0040 ml, 0.311 mmol, 1.0 eq) was added. The reaction was allowed to reach room temperature while running for 12hrs. DCM (10 ml) was then added to dilute the reaction mixture following extraction with water (2 x 1.0 mL). The DCM was dried over MgSO₄ (1.0 g) and vacuum filtered to

remove the drying agent. The reaction mixture was concentrated via rotary evaporation. Purification was achieved by prep TLC using a 1:1 hexane:EtOAc solvent system. Solvents were evaporated in the rotovap and the residue was kept under vacuum for 2 days. HCl in ether (2.0 M, 2.0 ml) was then added to the residue and stirred for 3 days at room temperature. Ether was removed by storing under vacuum and 55 mg of an oil product formed (70.0%). To achieve solid product, the residue was dissolved in DCM (0.0020 ml) and anhydrous ether was added drop wise. The precipitate was then stored under vacuum overnight.

2,6-difluoro-*N*-(piperidin-2-ylmethyl)benzamide (BENZAMIDE 10). To a solution of *tert*-butyl 2-(aminomethyl)piperidine-1-carboxylate (100 mg, 0.467 mmol, 1.5 eq) dissolved in THF (1.0 ml), NaOH (2.0 M, 0.5 ml) was added. The solution was stirred and cooled on ice while 2,6-difluorobenzoyl chloride (0.0039 ml, 0.311 mmol, 1.0 eq) was added. The reaction was allowed to reach room temperature while running overnight. The reaction mixture was diluted with DCM (10 ml) and extracted with water (2 x 1.0 ml). The DCM was dried over MgSO₄ (1.0 g) and vacuum filtered to remove the drying agent. The reaction mixture was concentrated via rotary evaporation and subject to prep TLC using a 1:1 hexane:EtOAc solvent system. The solvents were removed by rotary evaporation and the residue was kept under vacuum for 2 days. TLC analysis confirmed that further purification was required. This was achieved by column chromatography. Deprotection was achieved using HCl in ether (2.0 M, 2.0 ml) while

stirring for 3 days at room temperature. The ether was removed by storing under vacuum overnight, and 41 mg of a white solid product was formed (51.8%).

3,4-dichloro-*N*-(piperidin-2-ylmethyl)benzamide hydrochloride (BENZAMIDE 11).

tert-butyl 2-(aminomethyl)piperidine-1-carboxylate (100 mg, 0.467 mmol, 1.5 eq) was dissolved in THF (1.0 ml) and NaOH (2.0 M, 0.5 ml) was added. The solution was stirred on ice while 3,4-dichlorobenzoyl chloride (65.14 mg, 0.311 mmol, 1.0 eq) was added. The reaction was allowed to reach room temperature while running overnight. DCM (10 ml) was then added to dilute the reaction mixture following extraction with water (2 x 1.0 ml). The DCM was dried over MgSO₄ (1.0 g) and vacuum filtered to remove the drying agent. The reaction mixture was concentrated by rotary evaporation. Purification was achieved by column chromatography. Solvents were evaporated in the rotovap and the residue was kept under vacuum for 2 days. HCl in ether (2.0 M, 2.0 ml) was then added to the residue and stirred for 3 days at room temperature to remove the boc protecting group. Ether was evaporated by storing under vacuum overnight, and a white solid formed (67 mg, 75.0%).

***N*-(piperidin-2-ylmethyl)benzamide hydrochloride (BENZAMIDE 12).** NaOH (2.0 M, 0.5 mL) was added to a solution of *tert*-butyl 2-(aminomethyl)piperidine-1-carboxylate (100 mg, 0.467 mmol, 1.5 eq) in THF (1.0 ml). The solution was stirred on ice while benzoyl chloride (0.0040 ml, 0.311 mmol, 1.0 eq) was added. The reaction was

allowed to reach room temperature while running overnight. DCM (10 ml) was then added to dilute the reaction mixture following extraction with water (2 x 1.0 ml). The DCM was dried over MgSO₄ (1.0 g) and vacuum filtered to remove the drying agent. The reaction mixture was concentrated by rotary evaporation and purification was achieved by prep TLC using a 1:1 hexane:EtOAc solvent system. Solvents were evaporated in the rotovap and the residue was kept under vacuum for 2 days. HCl in ether (2.0 M, 2.0 ml) was then added to the residue and stirred for 3 days at room temperature. Ether was evaporated by storing under vacuum overnight resulting in a white solid product (25 mg, 36.8%).

4-fluoro-*N*-(piperidin-2-ylmethyl)benzamide (BENZAMIDE 13). To a solution of *tert*-butyl 2-(aminomethyl)piperidine-1-carboxylate (100 mg, 0.467 mmol, 1.5 eq) dissolved in THF (1.0 ml), NaOH (2.0 M, 0.5 ml) was added. The solution was stirred on ice while 4-fluorobenzoyl chloride (0.0040 ml, 0.311 mmol, 1.0 eq) was added. The reaction was allowed to reach room temperature while running overnight. DCM (10 ml) was then added to dilute the reaction mixture following extraction with water (2 x 1.0 ml). The DCM was dried over MgSO₄ (1.0 g) and vacuum filtered to remove the drying agent. The reaction mixture was concentrated by rotary evaporation and purified using prep TLC. Solvents were removed by rotary evaporation and the residue was kept under vacuum for 2 days. HCl in ether (2.0 M, 2.0 ml) was then added to the residue and the reaction

mixture stirred for 3 days at room temperature. Ether was removed by storing under vacuum overnight to form a white solid product (58 mg, 78.9%).

3,4-difluoro-*N*-(piperidin-2-ylmethyl)benzamide hydrochloride (BENZAMIDE 13B).

NaOH (2.0 M, 0.5 ml) was added to a solution of *tert*-butyl 2-(aminomethyl)piperidine-1-carboxylate (100 mg, 0.467 mmol, 1.5 eq) dissolved in THF (1.0 ml). The solution was stirred on ice while 3,4-diflorobenzoyl chloride (64 mg, 0.311 mmol, 1.0 eq) was added. The reaction was allowed to reach room temperature while running overnight. DCM (10 ml) was then added to dilute the reaction mixture following extraction with water (2 x 1.0 ml). The DCM was dried over MgSO₄ (1.0 g) and gravity filtered to remove the drying agent. The reaction mixture was concentrated by rotary evaporation. Purification was achieved by column chromatography and solvents were evaporated in the rotovap. The residue was kept under vacuum for 2 days. HCl in ether (2.0 M, 2.0 ml) was then added to the residue and stirred for 3 days at room temperature to remove the boc protecting group. Ether was evaporated by storing under vacuum for 2 days and a white solid product (50 mg, 63.2%) was formed.

3,4-dimethoxy-*N*-(piperidin-2-ylmethyl)benzamide (BENZAMIDE H).

A solution of *tert*-butyl 2-(aminomethyl)piperidine-1-carboxylate (100 mg, 0.467 mmol, 1.5 eq) was dissolved in THF (1.0 ml) and NaOH (2.0 M, 0.5 ml) was added. The solution was stirred on ice while 3,4-dimethoxybenzoyl chloride (62.39 mg, 0.311 mmol, 1.0 eq) was

added. The reaction was allowed to reach room temperature while running overnight. DCM (10 ml) was then added to dilute the reaction mixture following extraction with water (2 x 1.0 ml). The DCM was dried over MgSO₄ (1.0 g) and vacuum filtered to remove the drying agent. The reaction mixture was concentrated by rotary evaporation. Purification was achieved by column chromatography. Solvents were evaporated in the rotovap and the residue was kept under vacuum for 2 days. HCl in ether (2.0 M, 2.0 ml) was then added to the residue and stirred for 3 days at room temperature to remove the boc protecting group. Ether was evaporated by storing under vacuum overnight and a white solid (70 mg, 80.8%) was formed.

2,4-dichloro-*N*-(piperidin-2-ylmethyl)benzamide (BENZAMIDE J). *tert*-butyl 2-(aminomethyl)piperidine-1-carboxylate (100 mg, 0.467 mmol, 1.5 eq) was dissolved in THF (1.0 ml) followed by the addition of NaOH (2.0 M, 0.5 ml). The solution was stirred on ice while 2,4-dichlorobenzoyl chloride (0.0038 ml, 0.311 mmol, 1.0 eq) was added. The reaction was kept at 0 °C and stirred for 4 hrs. DCM (10 ml) was then added to dilute the reaction mixture following extraction with water (2 x 1.0 ml). The DCM was dried over MgSO₄ (1.0 g) and vacuum filtered to remove the drying agent. The reaction mixture was concentrated by rotary evaporation and purified using prep TLC. Solvents were removed by rotary evaporation and the residue was kept under vacuum for 2 days. HCl in ether (2.0 M, 2.0 ml) was then added to the residue and the reaction mixture

stirred for 3 days at room temperature. Ether was evaporated by storing under vacuum overnight to form a white solid product (47 mg, 52.6%).

2-(((3-(trifluoromethyl)benzyl)amino)methyl)piperidin-1-ium (AMINE 2). The previously synthesized compound, *N*-(piperidin-2-ylmethyl)-3-(trifluoromethyl)-benzamide (BENZAMIDE 2) (21 mg), was suspended in 6.8 ml of anhydrous ether. Lithium aluminum hydride (19 mg) was added to the solution and the reaction was refluxed for 24 hrs. TLC analysis assess reaction completion using 5 % MeOH in dichloromethane as the solvent system. The reaction solution was chilled to 0 °C and quenched by slowly adding ethyl acetate drop wise while stirring. An aluminum emulsion resulted. To the emulsified reaction mixture, 5 drops of DI H₂O were added, followed by 5 drops of 2M NaOH, followed by 15 drops of DI H₂O while stirring in an ice bath for 1 hr. Separation of the emulsion resulted in formation of a granular salt. The reaction mixture was filtered under vacuum to remove salts and washed with ether. The reaction mixture was then diluted further with DCM (10 ml). The complete removal of H₂O was achieved by drying 5 times over MgSO₄ (1.0 g) and followed by vacuum filtration to remove the drying agent. The evaporation of solvents in the rotovap yielded 10 mg of the final amine product.

2-(ethoxymethyl)piperidin-1-ium (ETHER 2). The benzoyl protected starting material, C₁₃H₁₂NO₂, (310 mg, 1.41 mmol) was dissolved in 5 ml of anhydrous THF on ice while

under Ar pressure. *t*-BuOK (317 mg, 2.82 mmol, 2 eq) was then added, followed by the addition of of iodoethane (0.664 cc, 2.82 mmol, 2 eq). The reaction solution was stirred for 2.5 hrs and then extracted with 10 ml of DCM & washed twice with 5 ml of DI H₂O. The organic layer was dried over 1 g of MgSO₄ and gravity filtered to removed the drying agent. TLC analysis assessed reaction completion. The reaction mixture was then purified by column chromatography using a 1:1, hexanes:EtOAc solvent system. Solvents were removed via rotatory evaporation. Deprotection was achieved by adding 5 ml of MeOH to the resulting residue, followed by the addition of 2.5 ml of conc HCl and refluxing for 30 hrs. Following reflux, the reaction solution was neutralized by the addition of NaHCO₃ until litmus testing indicated basicity of the solution. The organic layer was then extraction with 10 ml of DCM, washed twice with 5 ml of DI H₂O, and dried over 1 g of MgSO₄. Gravity filtration removed drying agent and solvents were removed in the rotovap to yield final product as a brownish precipitate.

2-(propoxymethyl)piperidine (ETHER 3). The benzoyl protected starting material, C₁₃H₁₂NO₂, (310 mg, 1.41 mmol) was dissolved in 5 ml of anhydrous THF on ice while under Ar pressure. *t*-BuOK (317 mg, 2.82 mmol, 2 eq) was then added, followed by the addition of of iodopropane (0.274 cc, 2.82 mmol, 2 eq). The reaction solution was stirred for 2.5 hrs and then extracted with 10 ml of DCM & washed twice with 5 ml of DI H₂O. The organic layer was dried over 1 g of MgSO₄ and gravity filtered to removed the drying agent. TLC analysis assessed reaction completion. The reaction mixture was then

purified by column chromatography using a 1:1, hexanes:EtOAc solvent system.

Solvents were removed via rotatory evaporation. Deprotection was achieved by adding 5 ml of MeOH to the resulting residue, followed by the addition of 2.5 ml of conc HCl and refluxing for 30 hrs. Following reflux, the reaction solution was neutralized by the addition of NaHCO₃ until basicity of the solution was pH of 8. The organic layer was then extraction with 10 ml of DCM, washed twice with 5 ml of DI H₂O, and dried over 1 g of MgSO₄. Gravity filtration removed drying agent and solvents were removed in the rotovap to yield final product as a brownish precipitate.

2-methyl-6-nonadecylpiperidine (Long-Chain Analog). To a solution of 2-methyl-6-nonadecylpyridine (70 mg) dissolved in absolute ethanol (8 ml), Pd/C (20 mg) was added followed by the addition of Rh/C (50 mg). The solution was then shaken under 55 psi for 30 hrs. To remove the catalyst, the reaction mixture was filtered through a plug of celite using 5% MeOH in DCM as the solvent. The reaction mixture was then purified by column chromatography using a minimal amount of silica. The solvent system which allowed for optimal separation was 7% MeOH in DCM. Solvents were removed in the rotovap to yield 20 mg (28.6%) of product as a yellowish oil.

Biological Data

The following data is from SVR cell proliferation assays treated with the synthetic analogs of solenopsin A (Figure 15). Hydrochloride salt forms of the benzamide analogs were diluted with 1 ml of EtOH each and then diluted again with 5 micro liters into 5 ml of media. The endothelial cells were treated for 24 hours and then washed and counted.

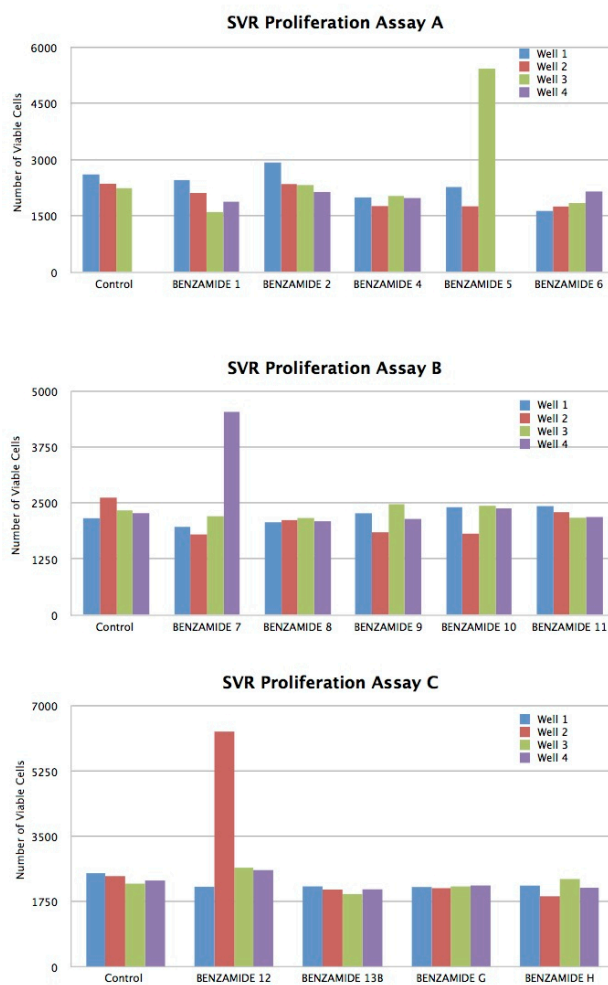


Figure 15. SVR Assay Results for Benzamide Analogs

The SVR angiogenesis assays confirm that none of the synthetic benzamide compounds were anti-angiogenic. For purposes of comparison the antiangiogenic bioactivity of solenopsin A, subject to the SVR assay, is again presented (Figure 16). Given the main structural feature of solenopsin A is the undecyl side-chain at the 6 position of its piperidine ring, it is likely the structural component responsible for the observed antiangiogenic activity. The synthetic long-chain *n*-alkyl analog, 2-methyl-6-nonadecylpiperidine, is currently under consideration for testing in the SVR angiogenic assay.

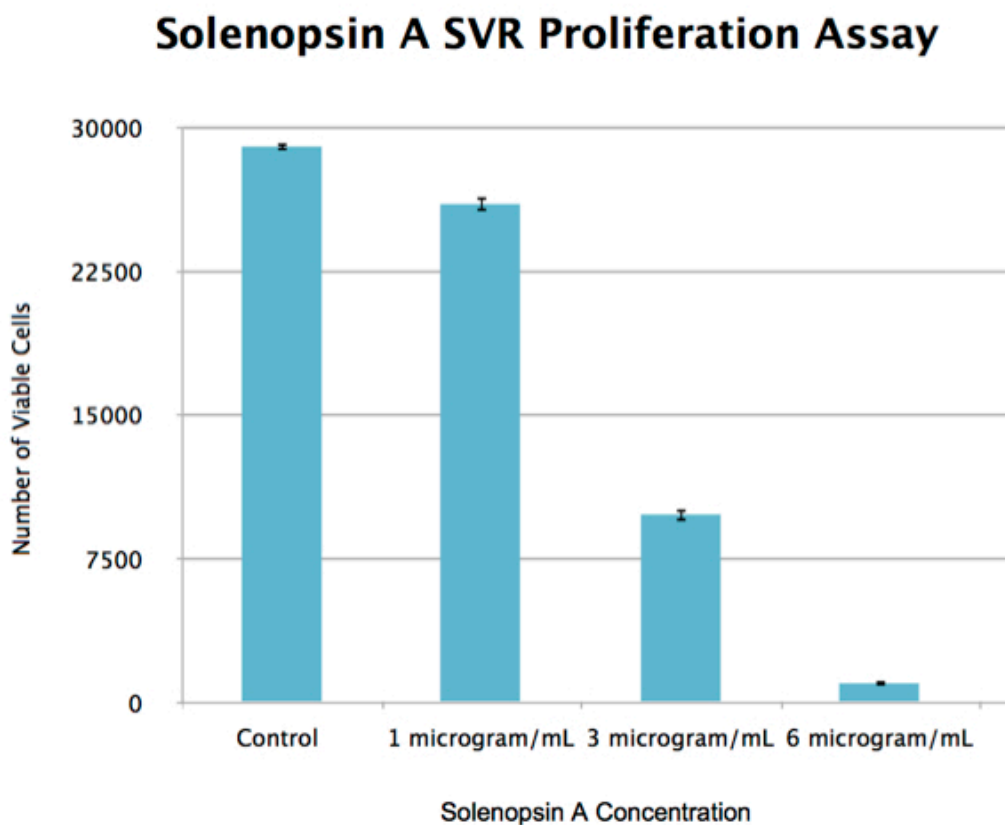


Figure 16. Previous SVR Assay Results of Solenopsin A

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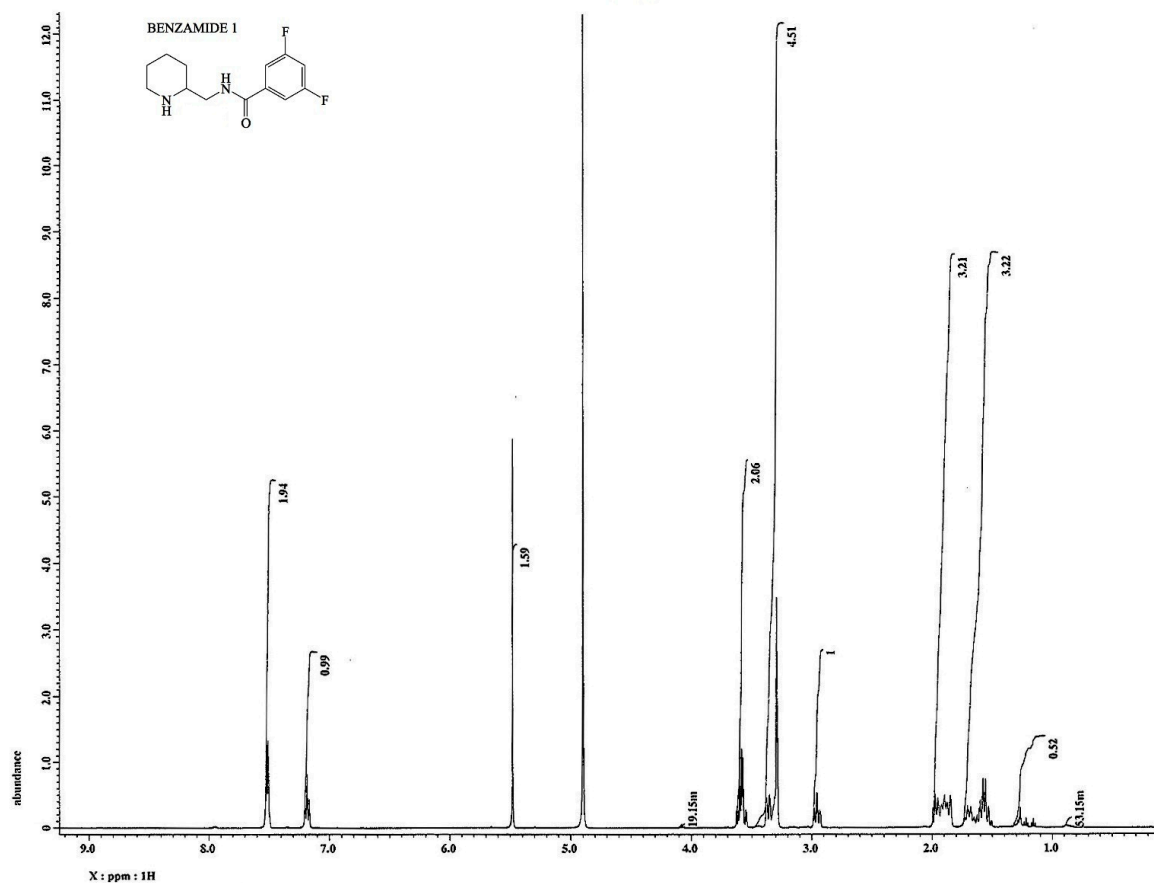
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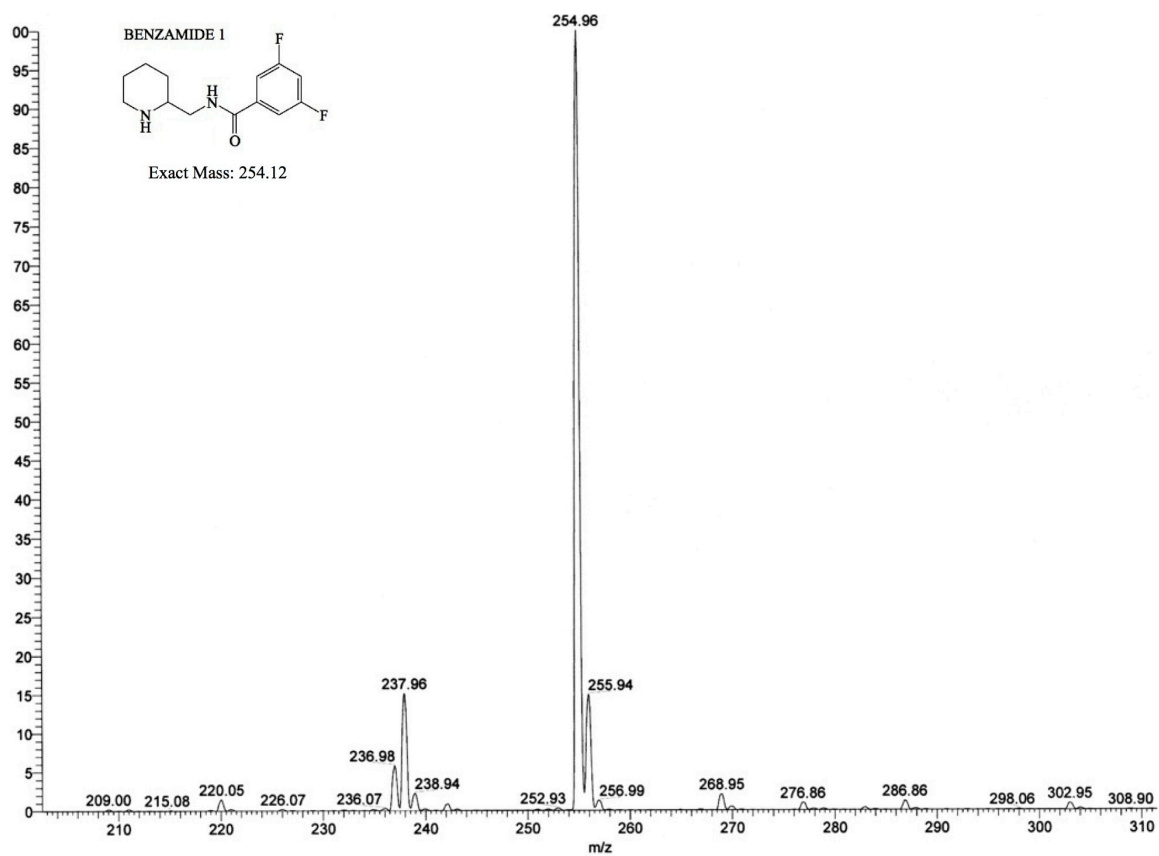
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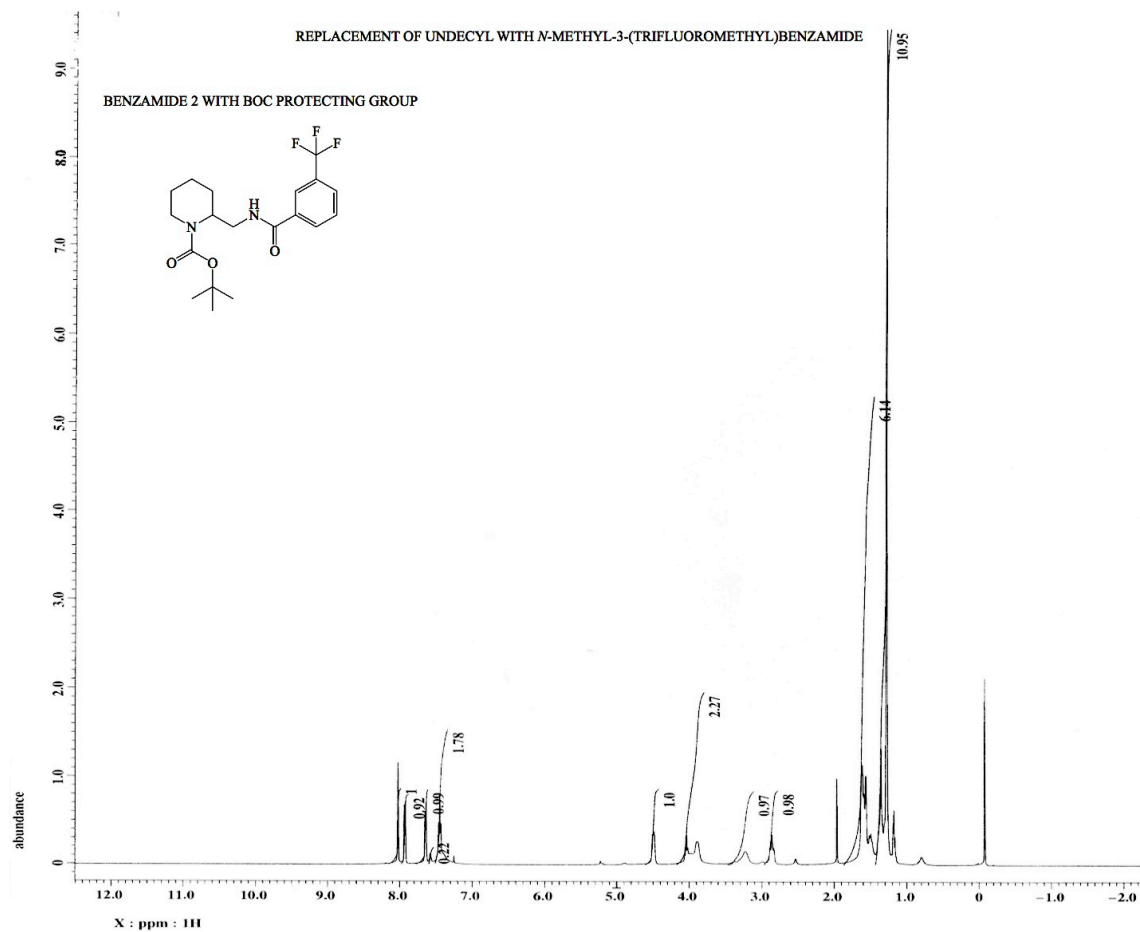
APPENDIX A

REPRESENTATIVE ^1H NMR AND MASS SPECTRA

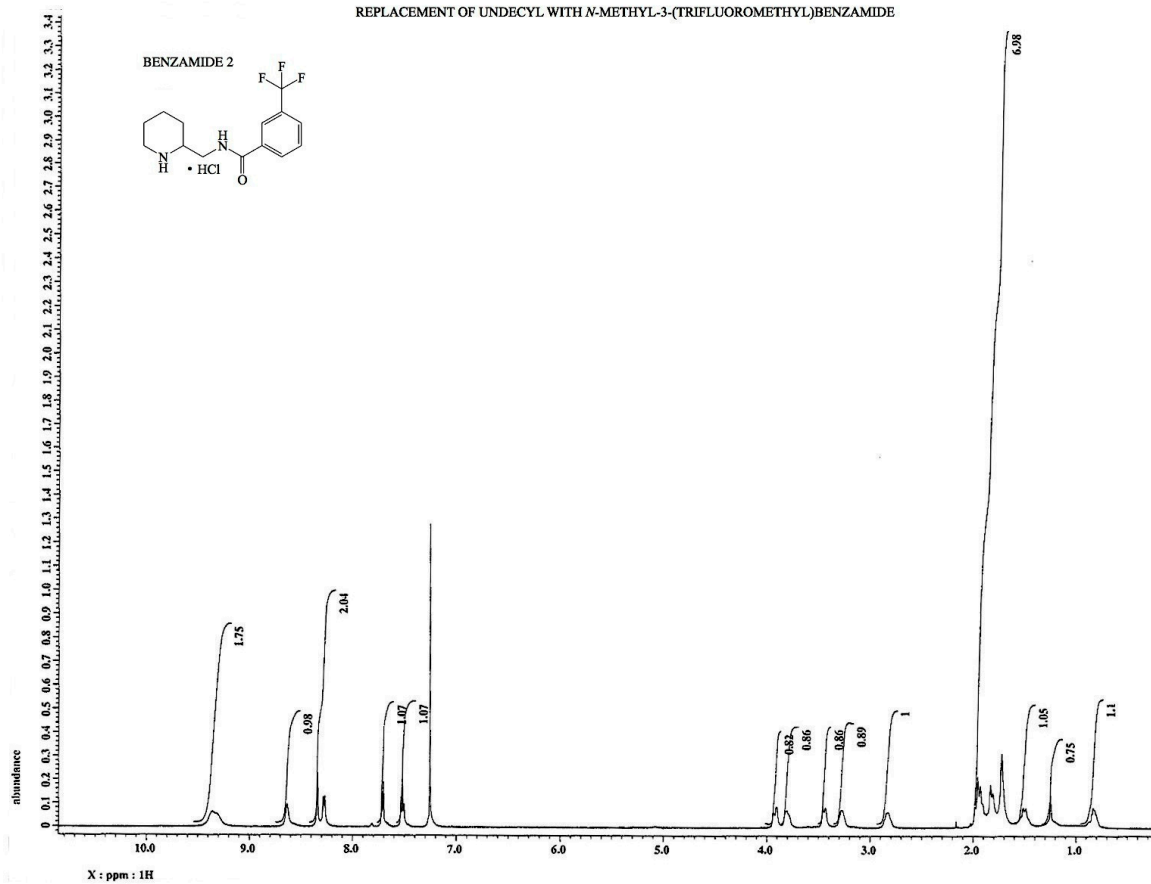
REPLACEMENT OF UNDECYL WITH 3,5-DIFLUORO-N-METHYLBENZAMIDE

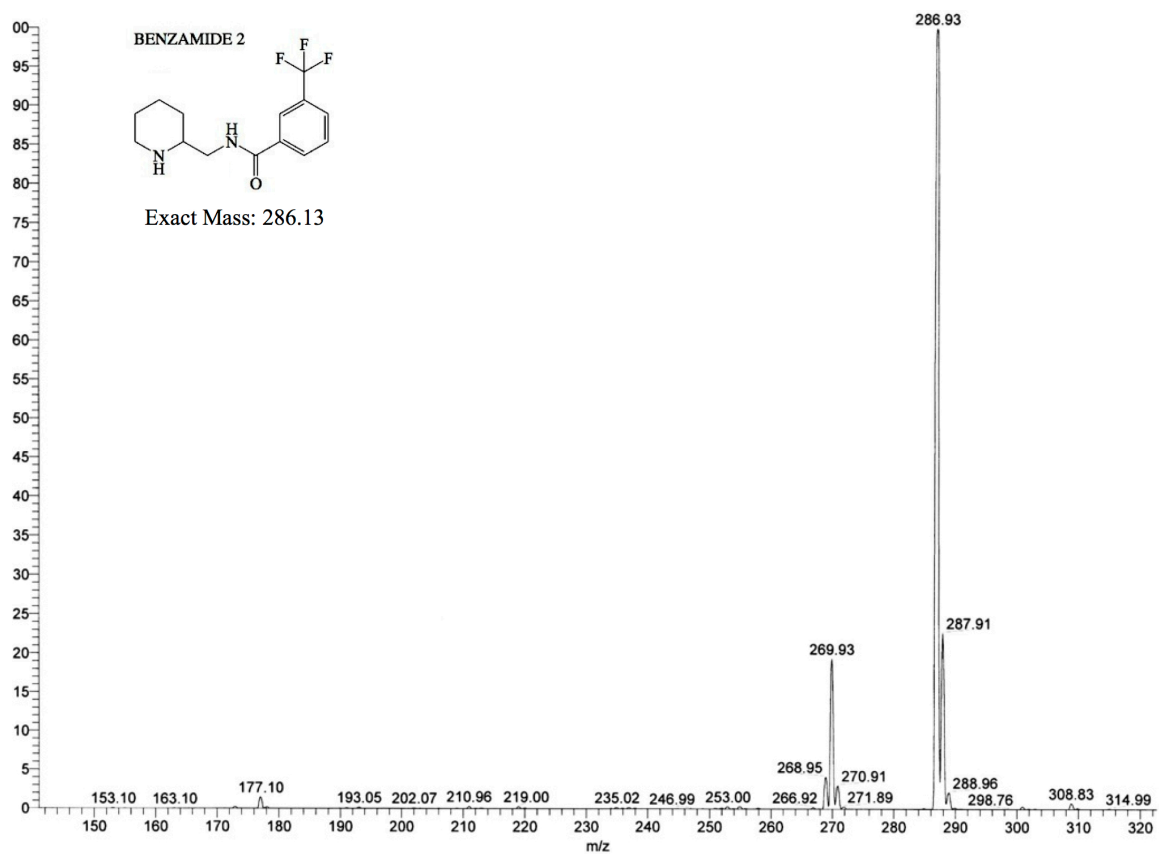


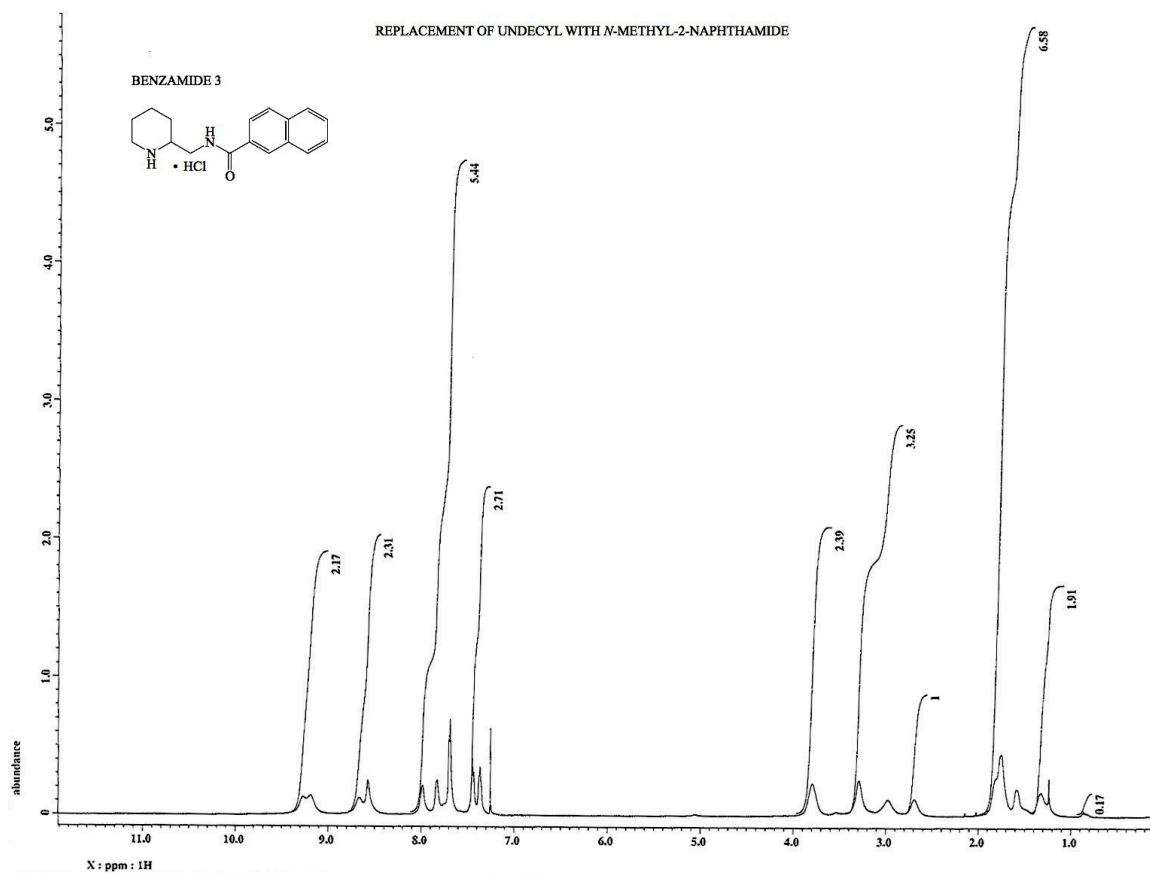


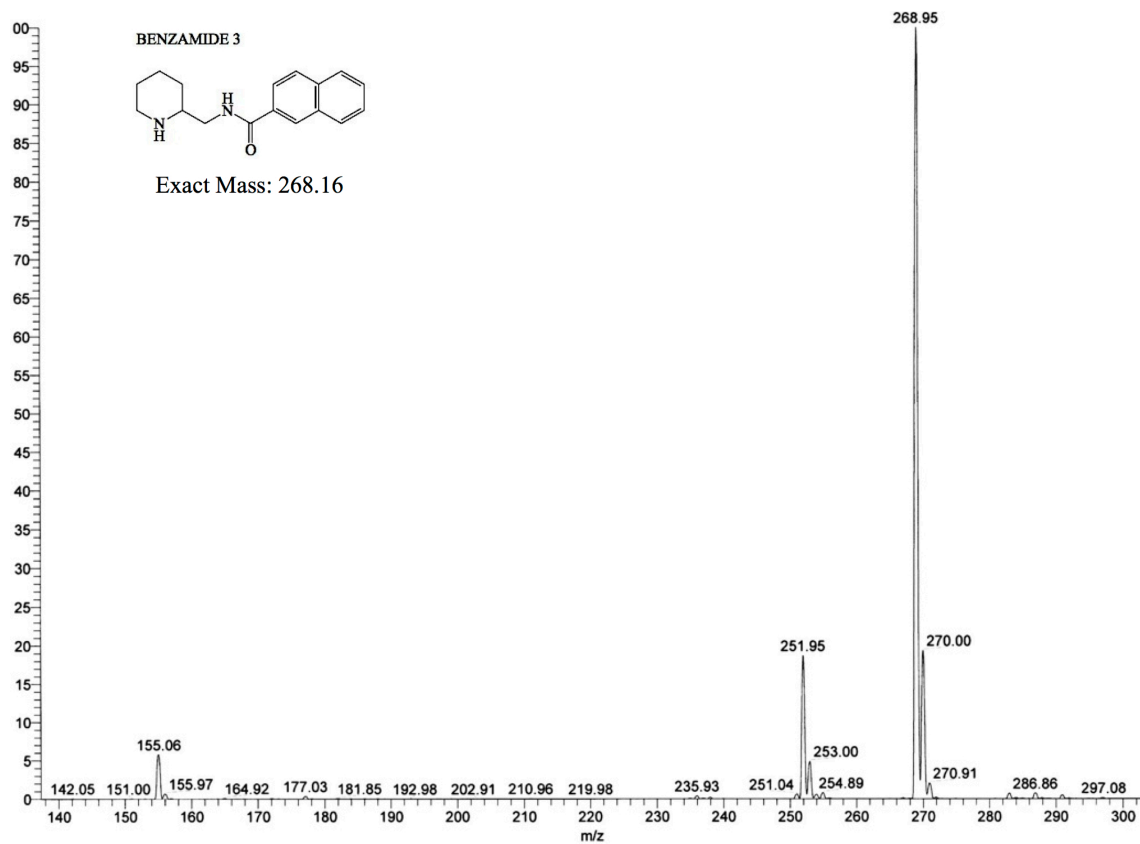


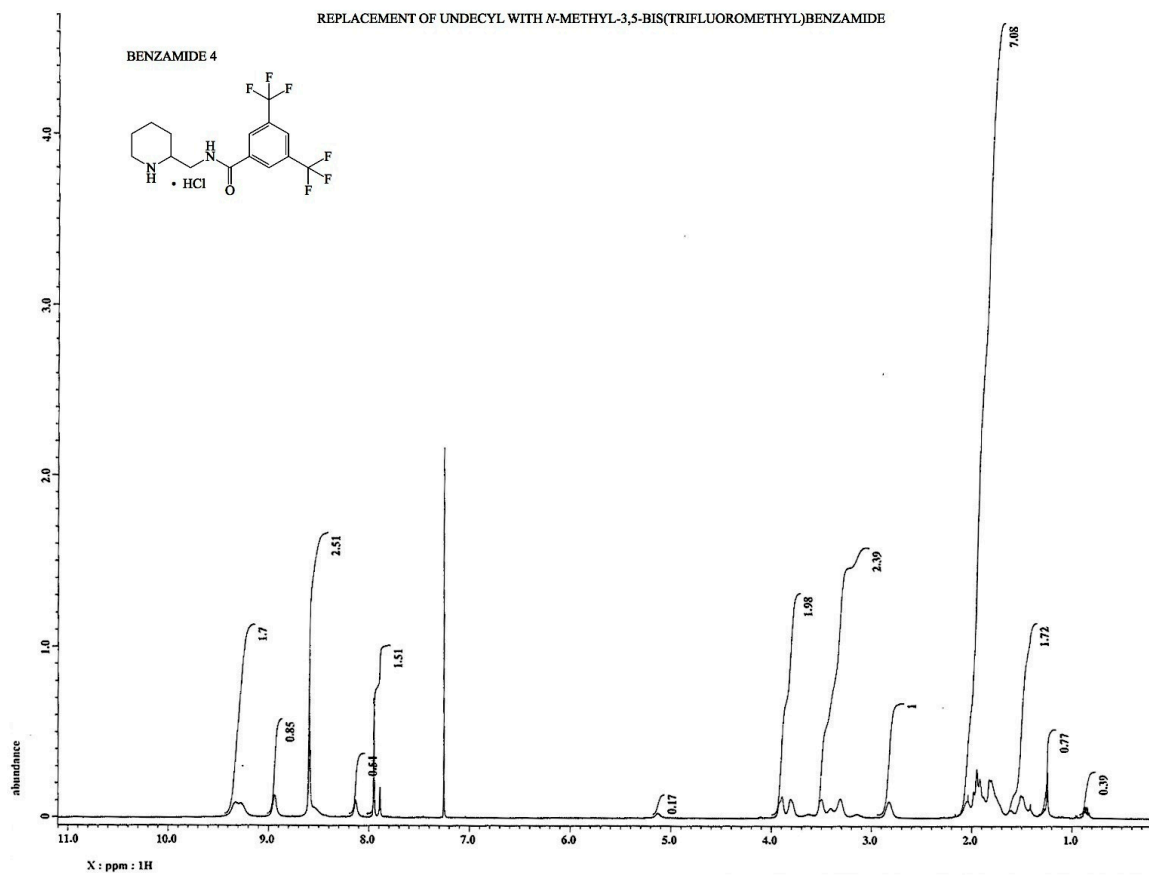
REPLACEMENT OF UNDECYL WITH N-METHYL-3-(TRIFLUOROMETHYL)BENZAMIDE

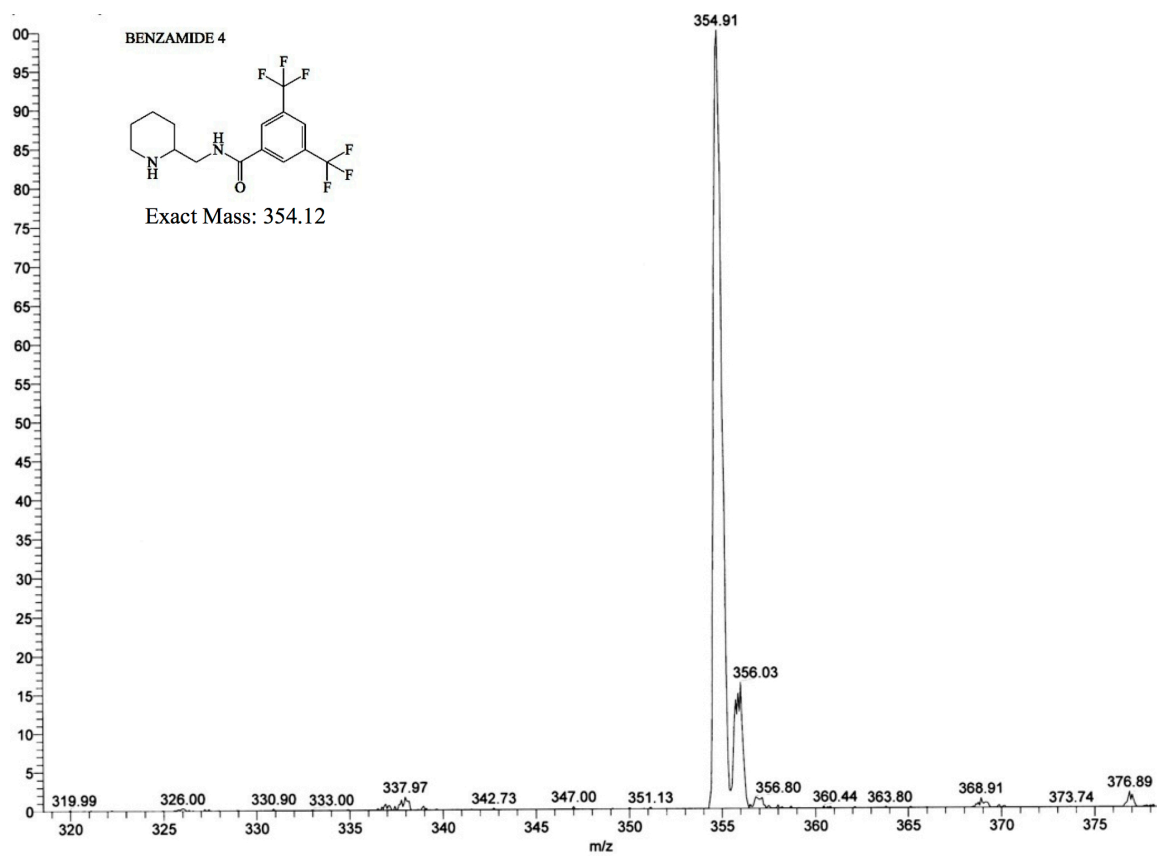


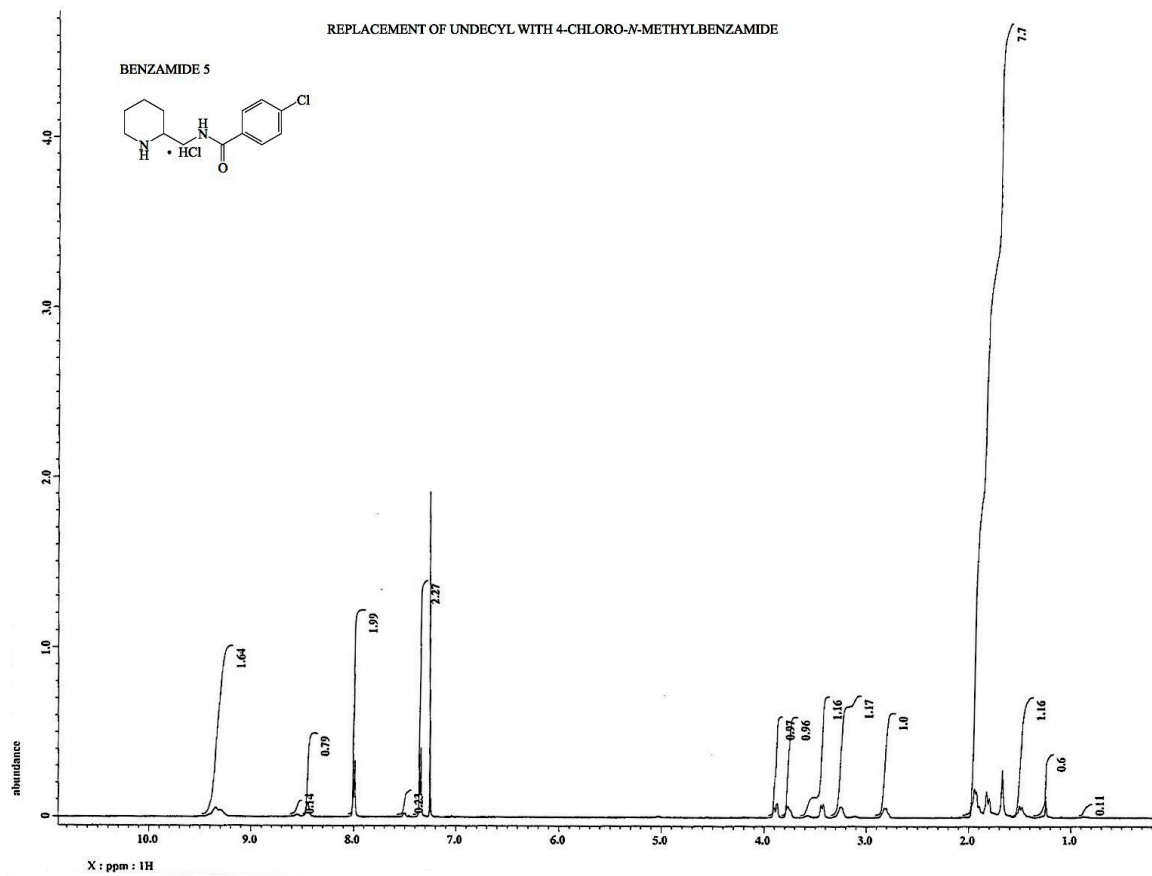


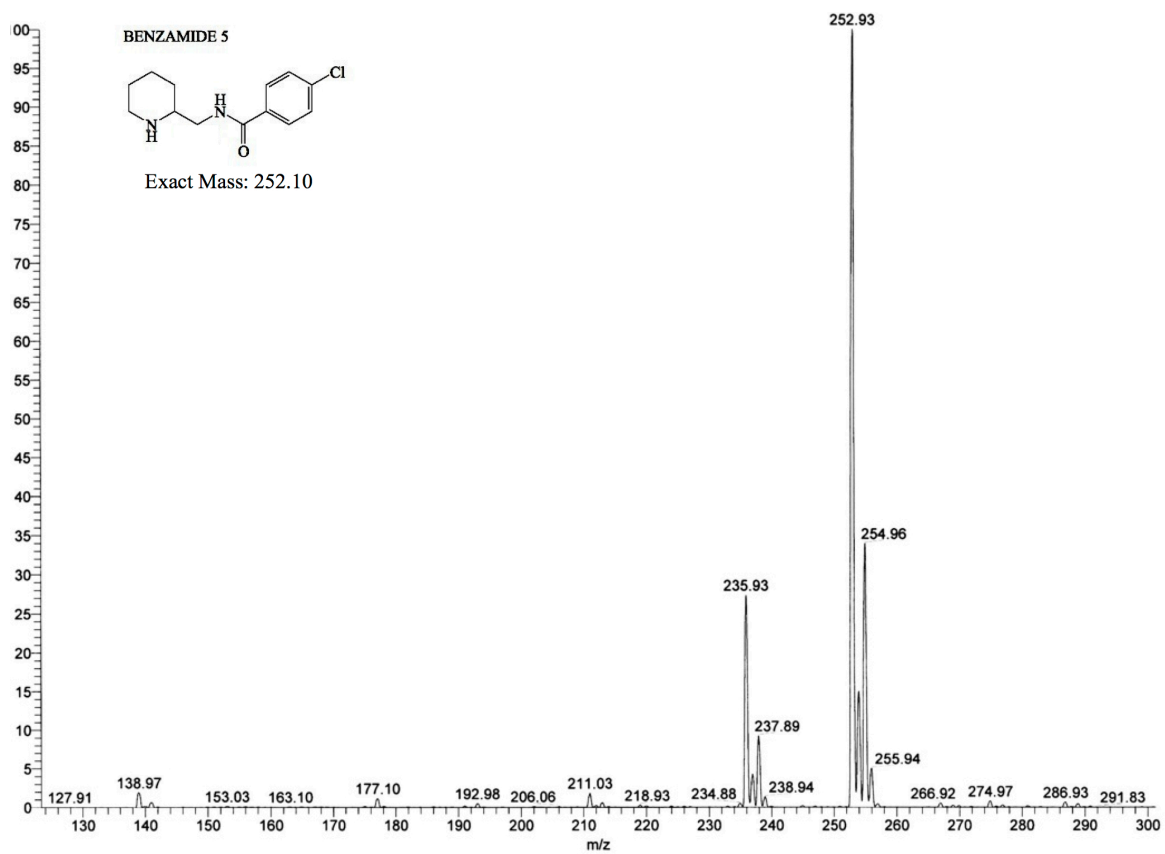




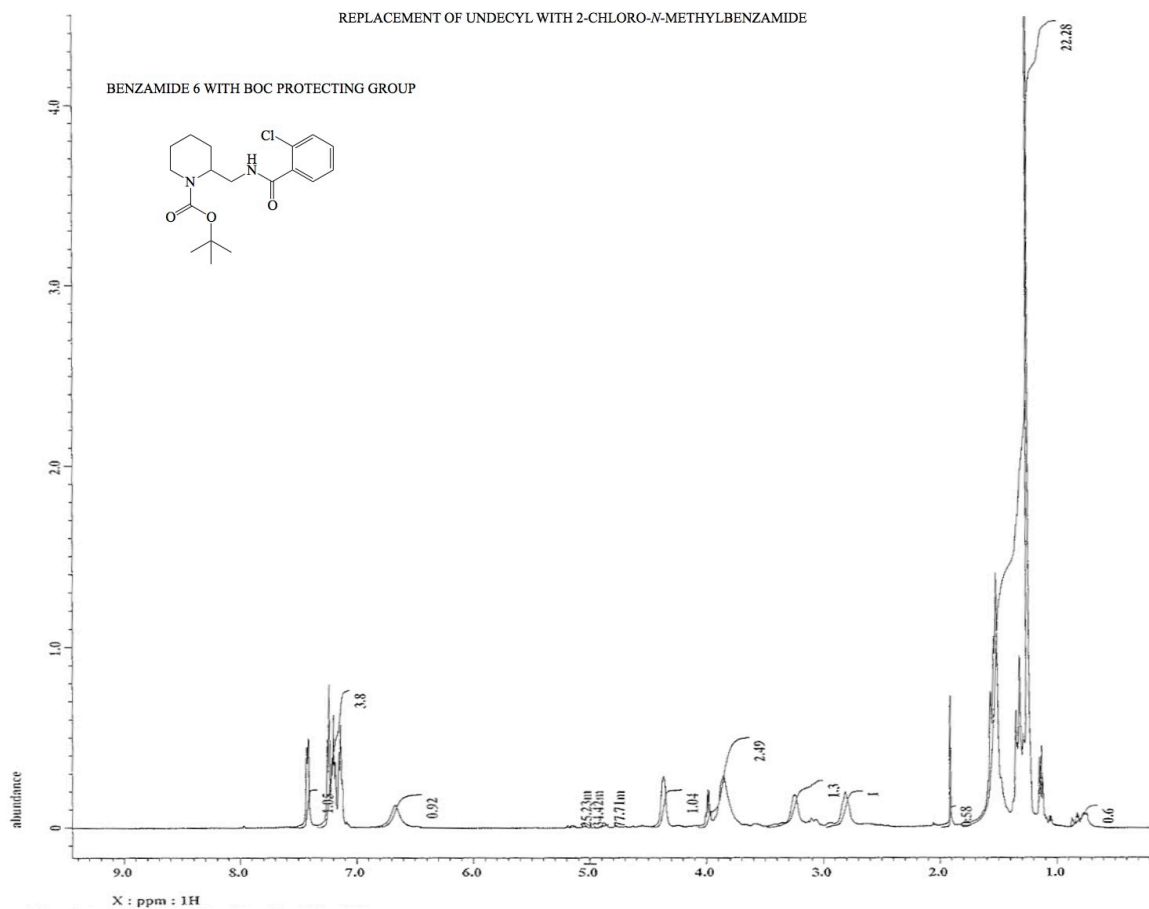


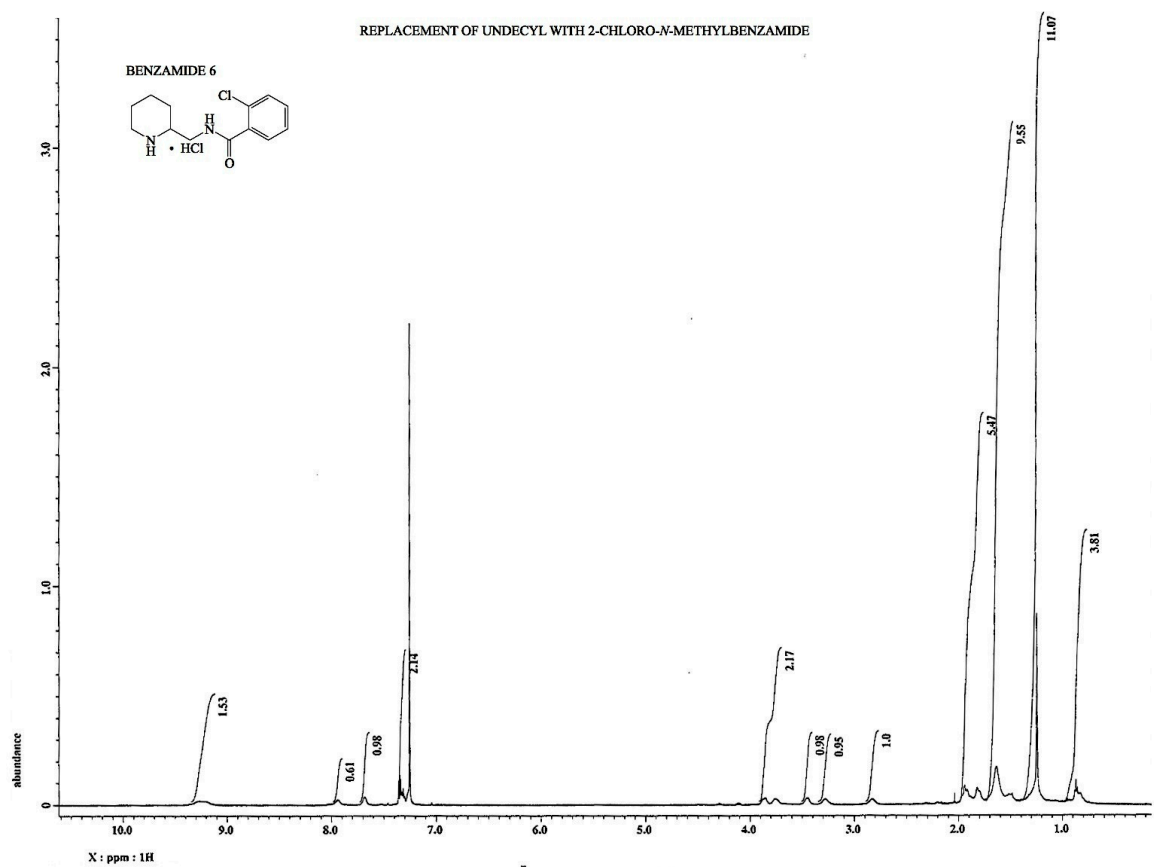




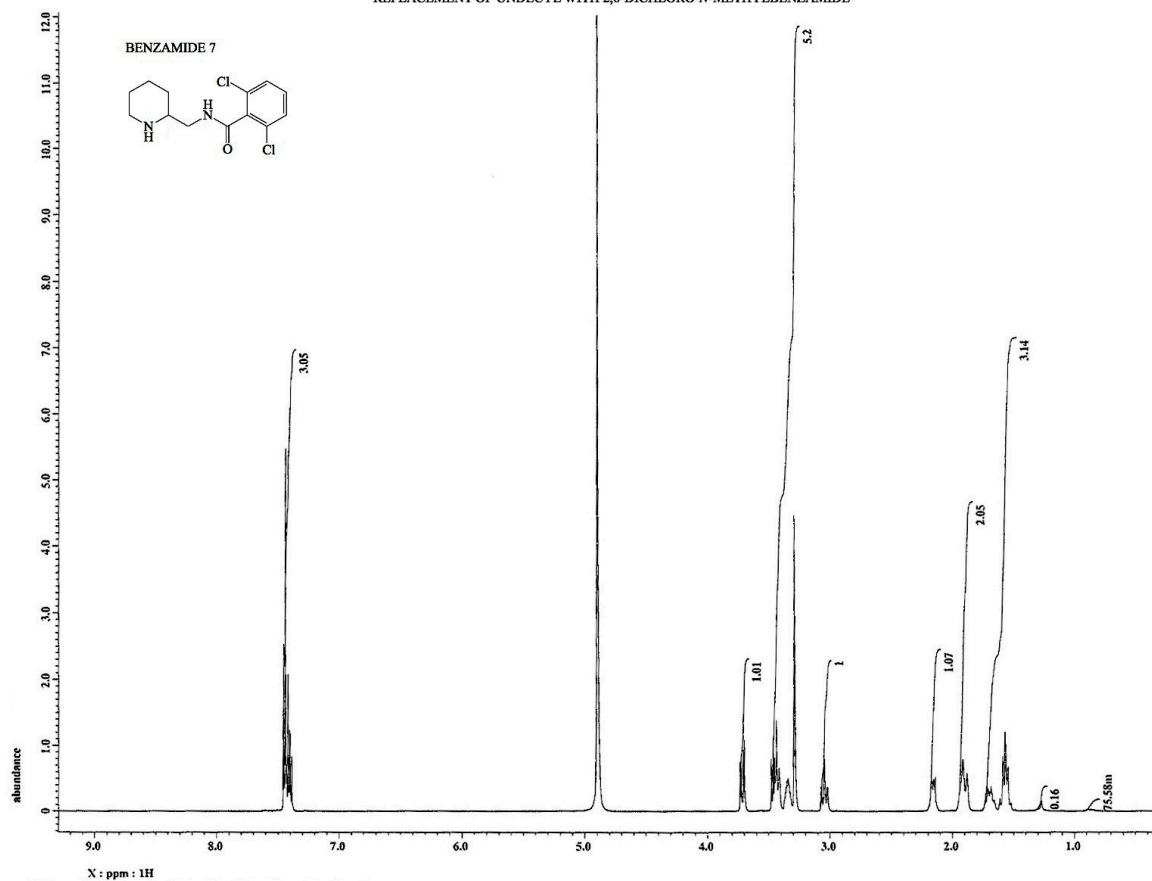


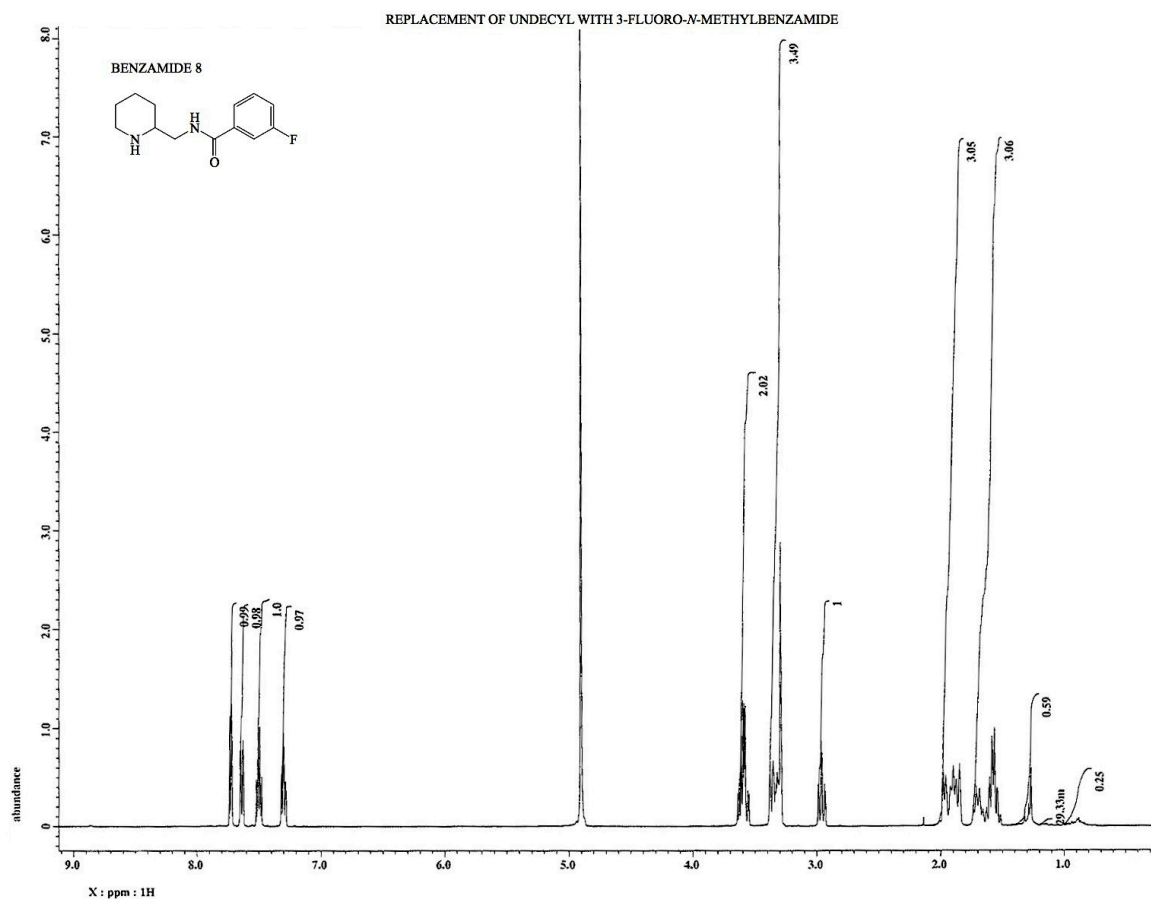
REPLACEMENT OF UNDECYL WITH 2-CHLORO-N-METHYLBENZAMIDE

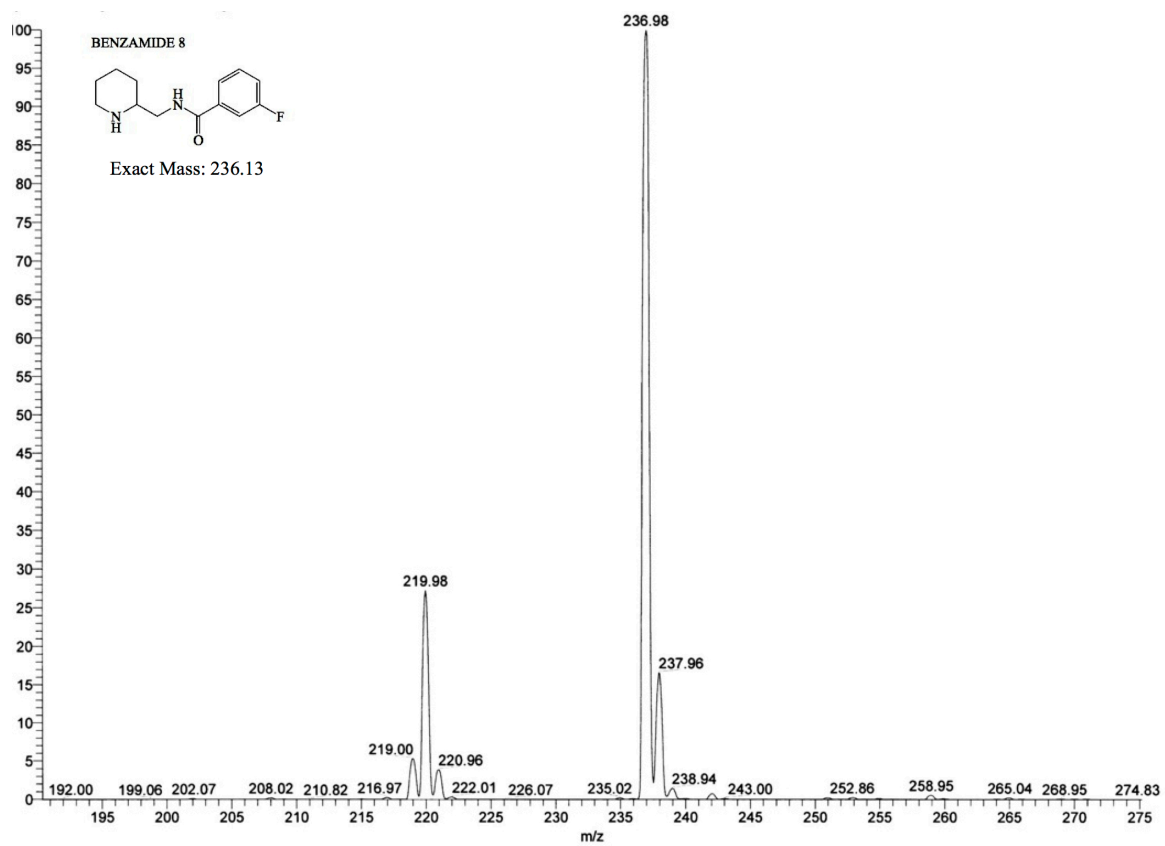


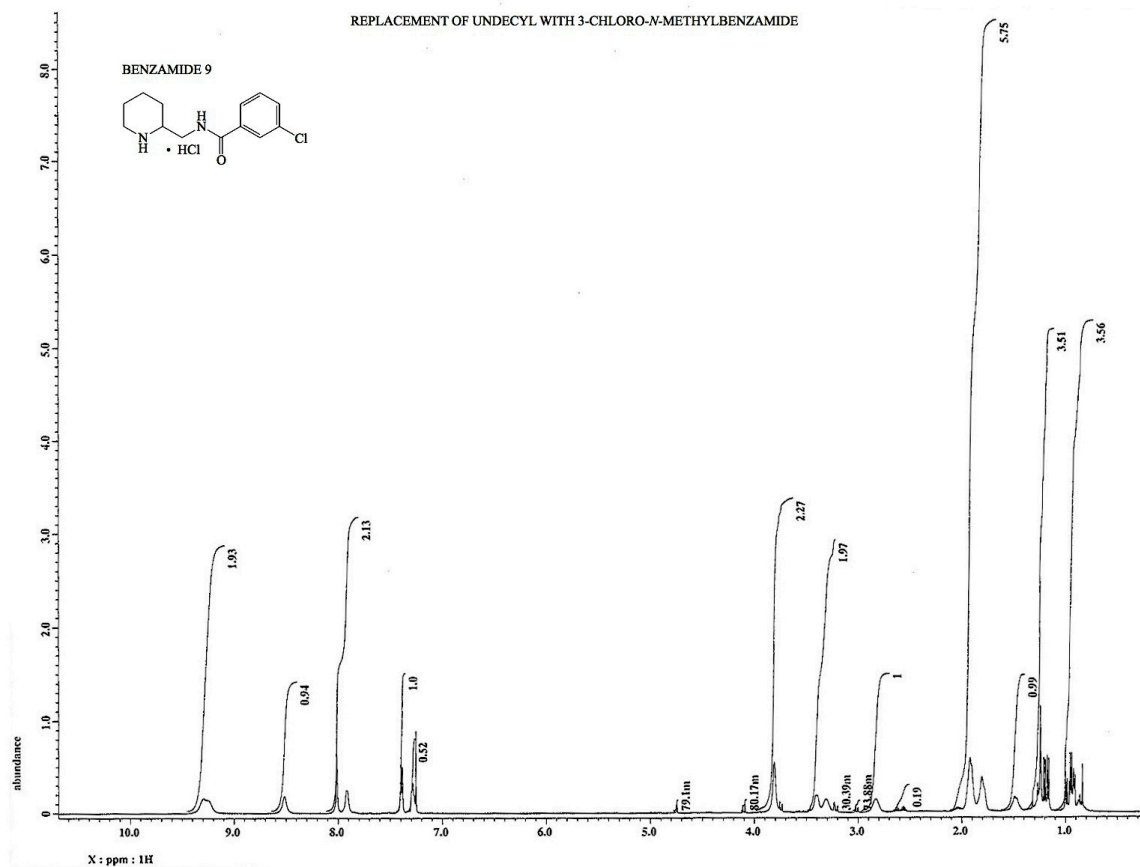


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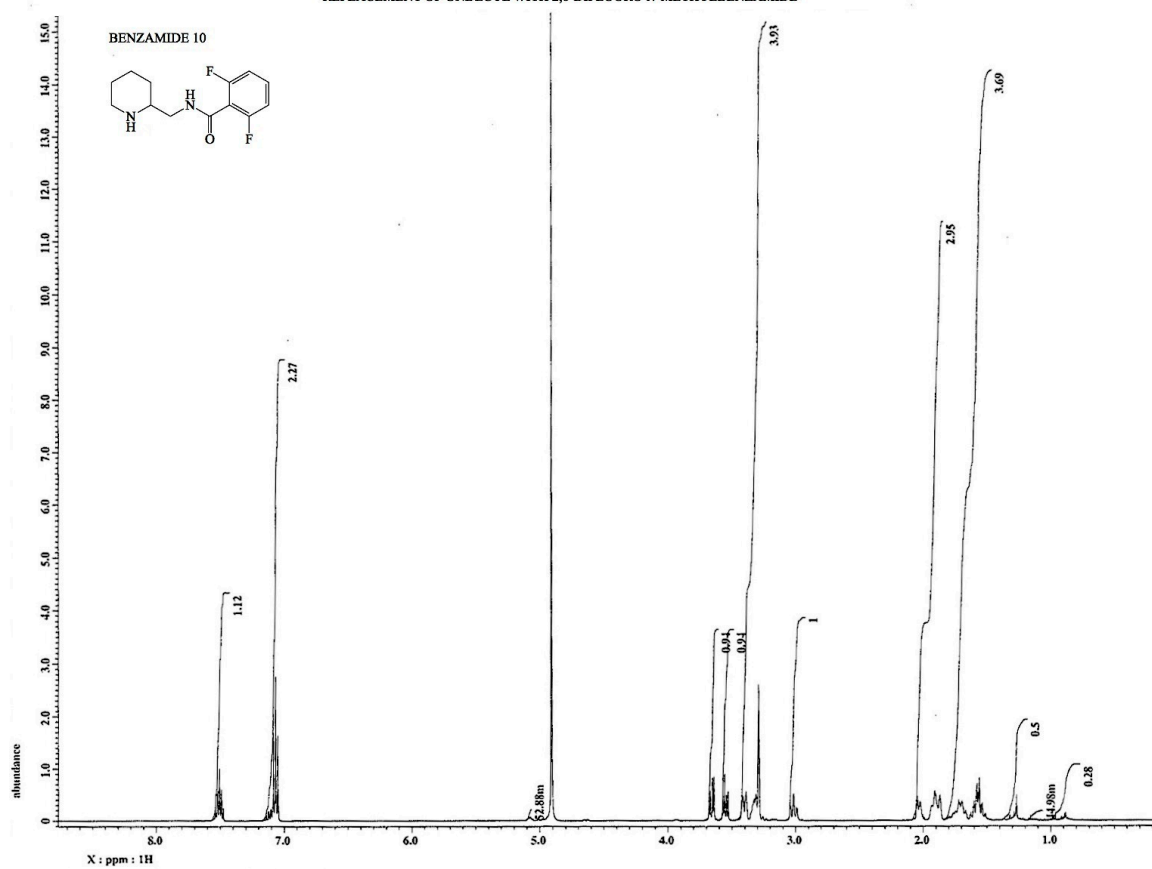


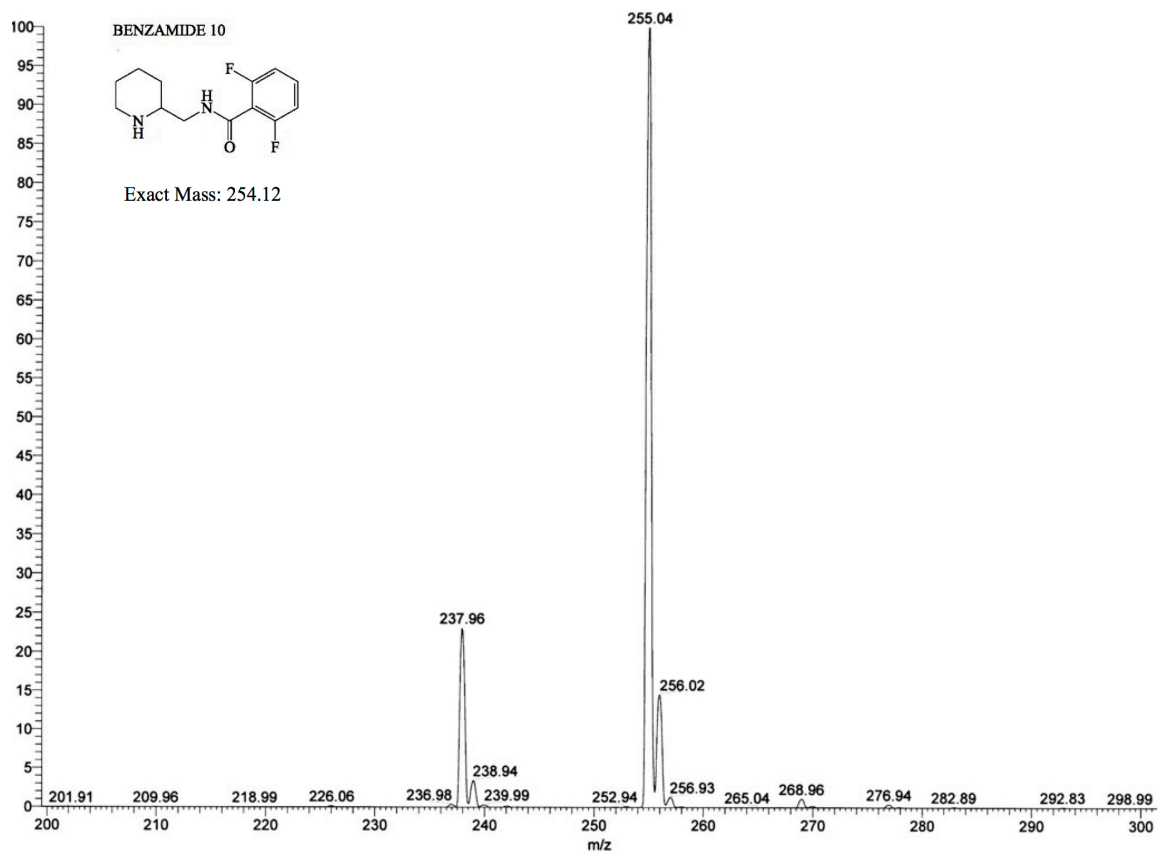


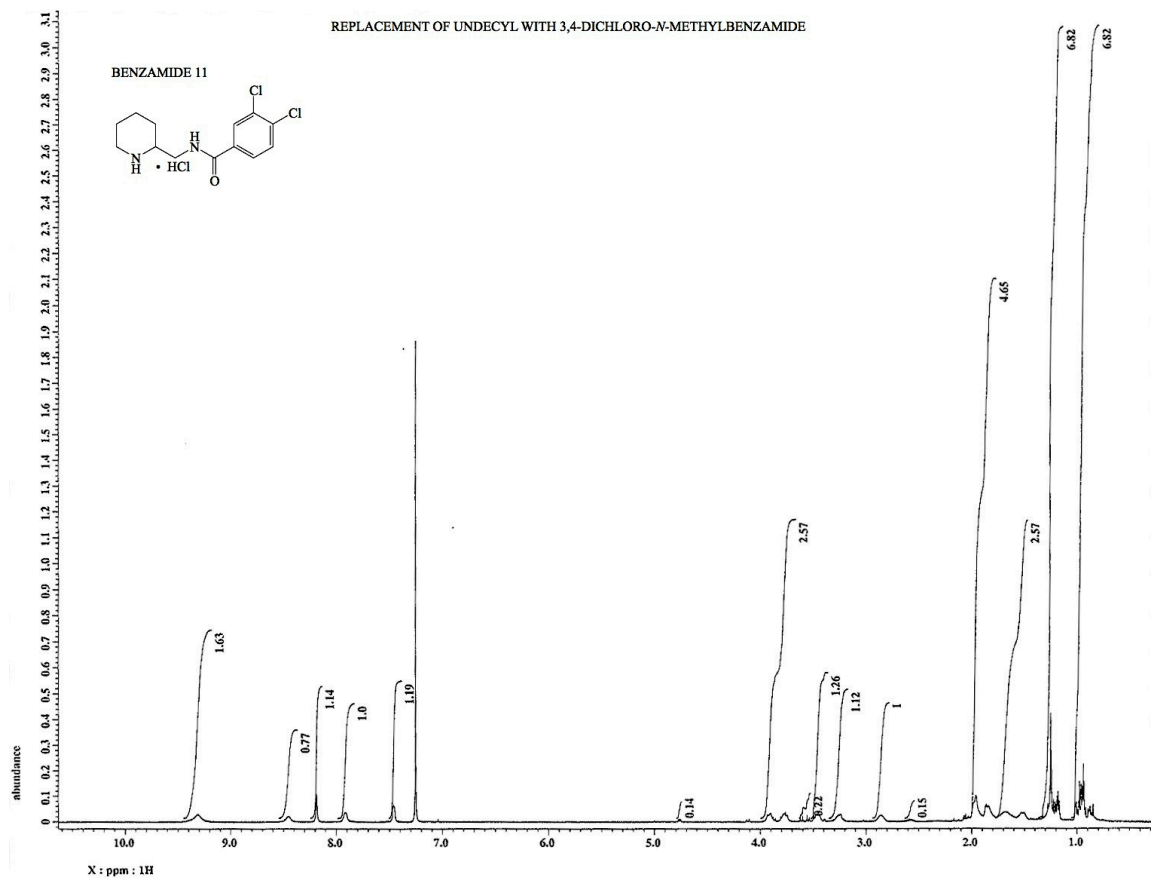


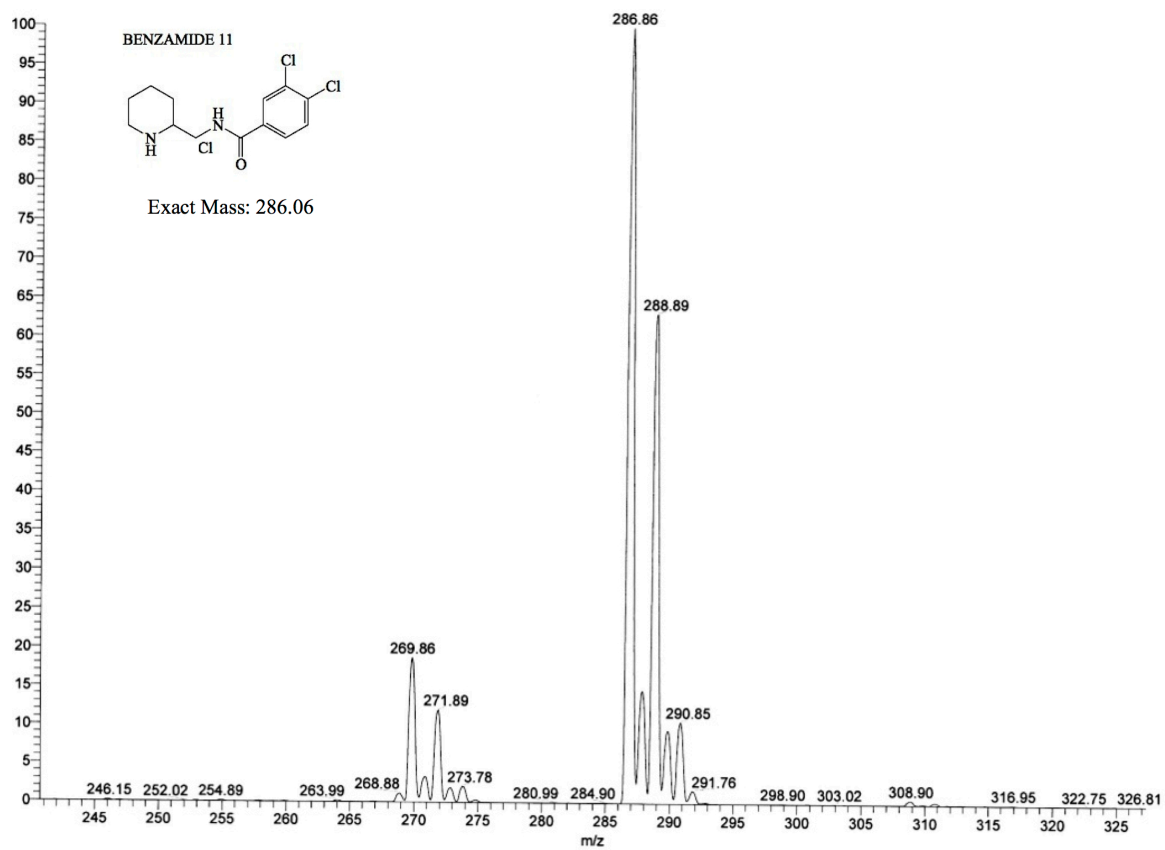


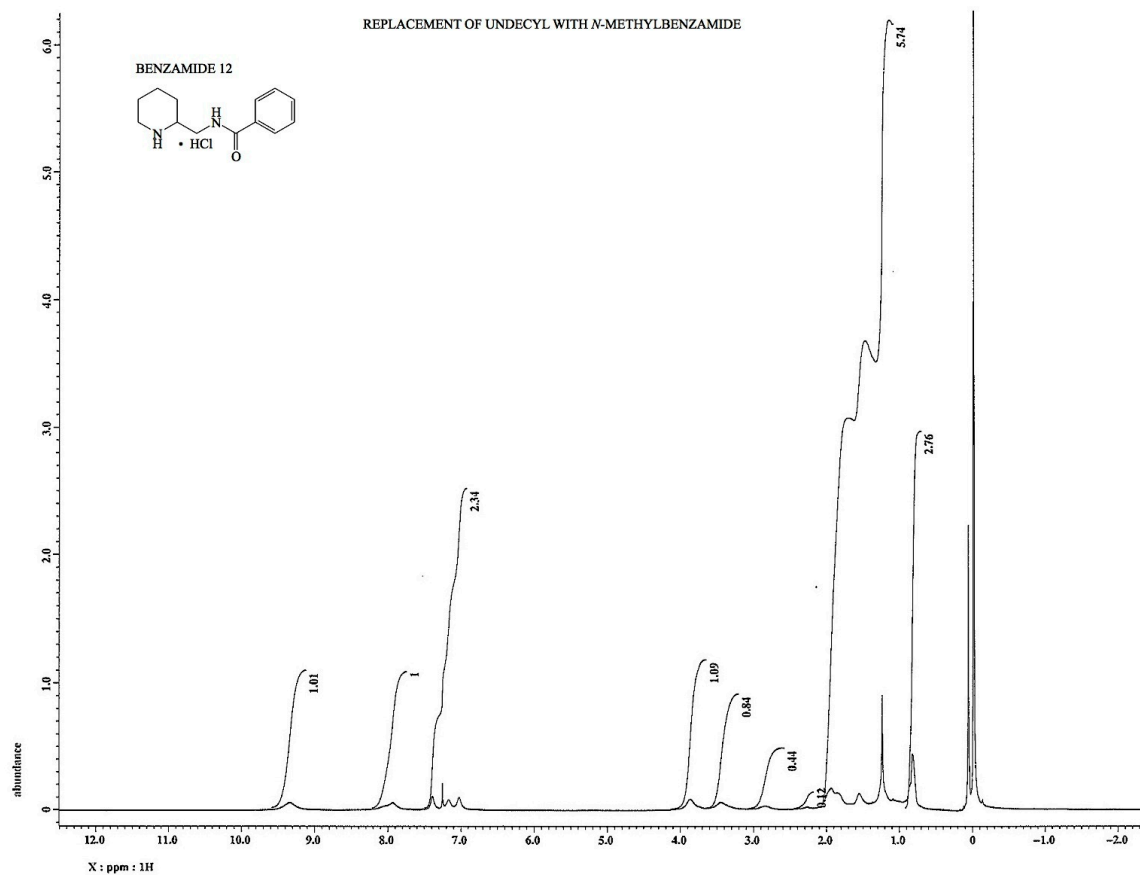
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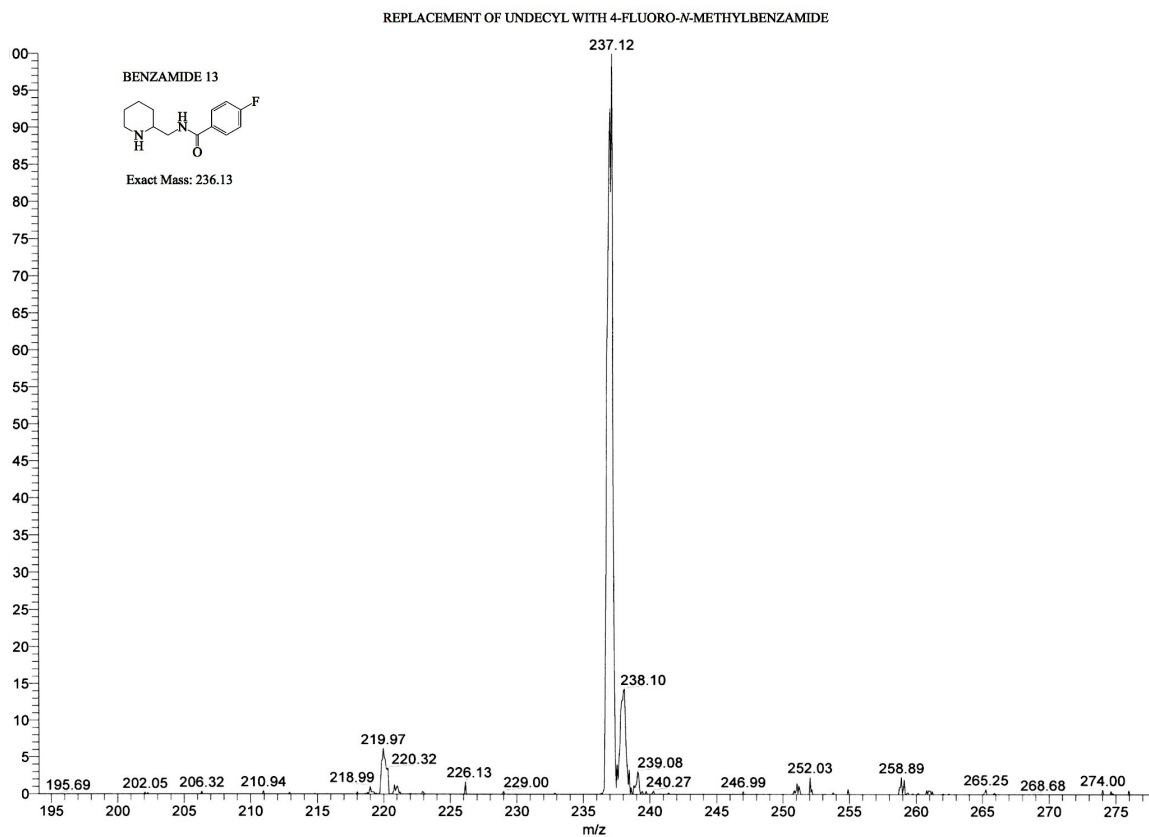


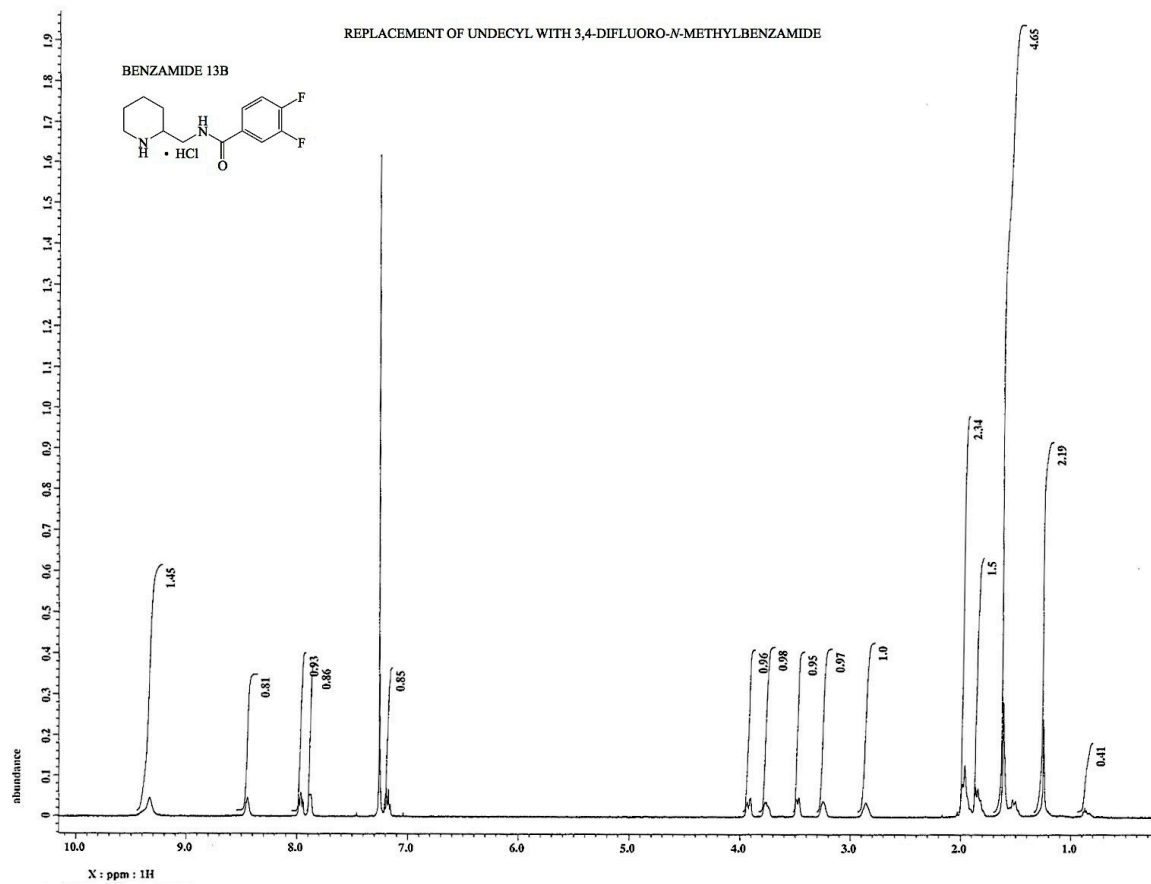


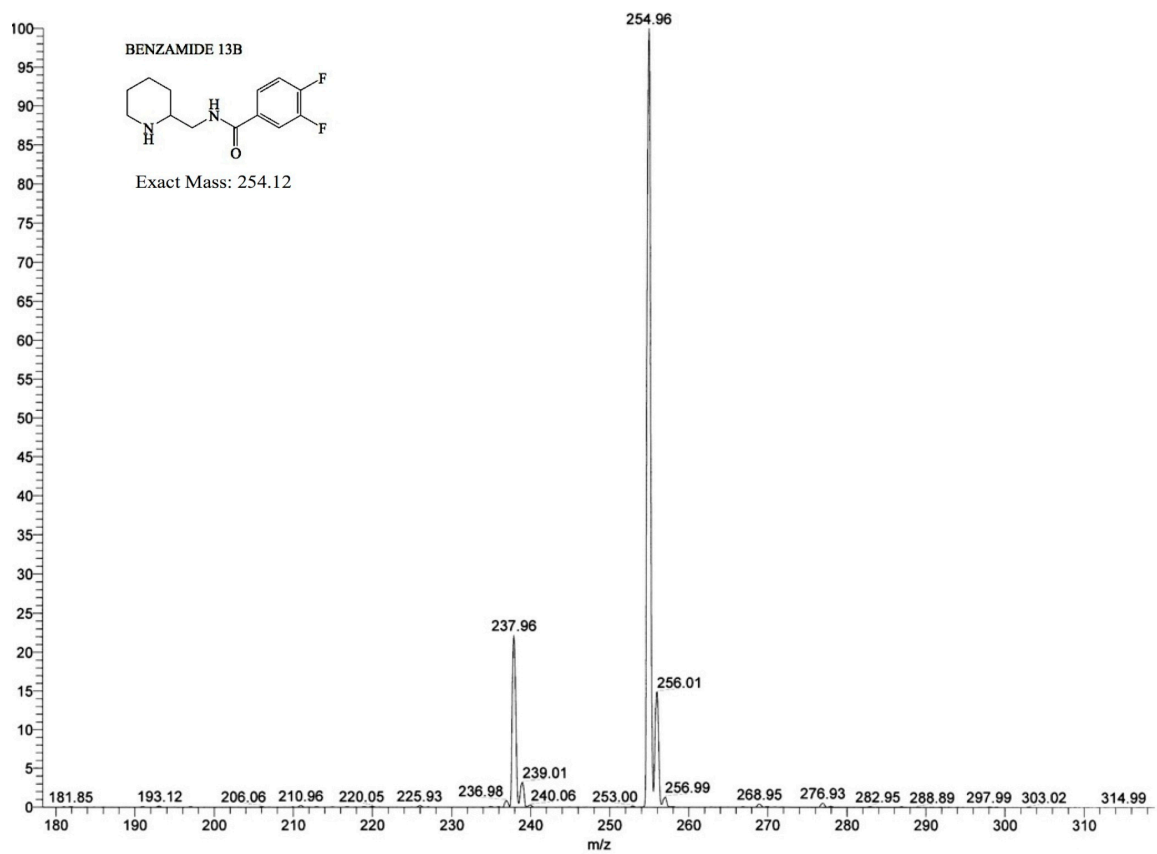




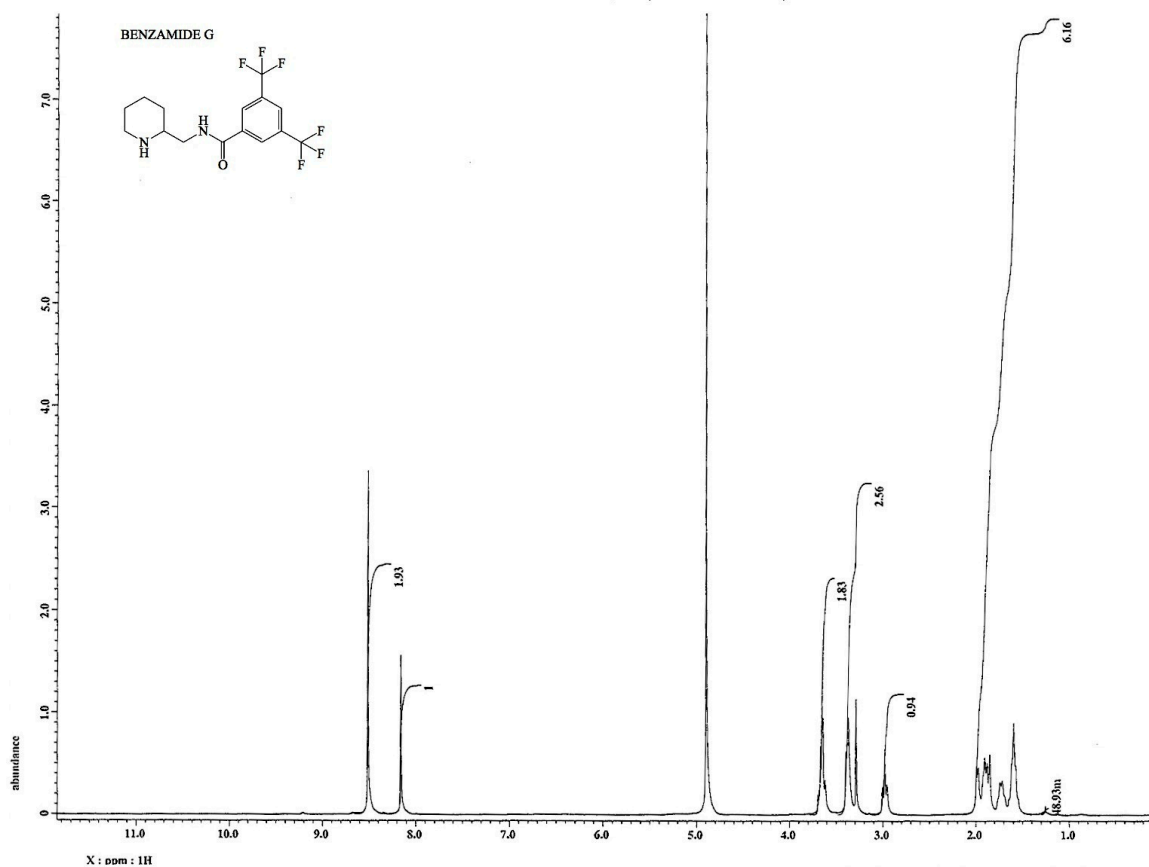


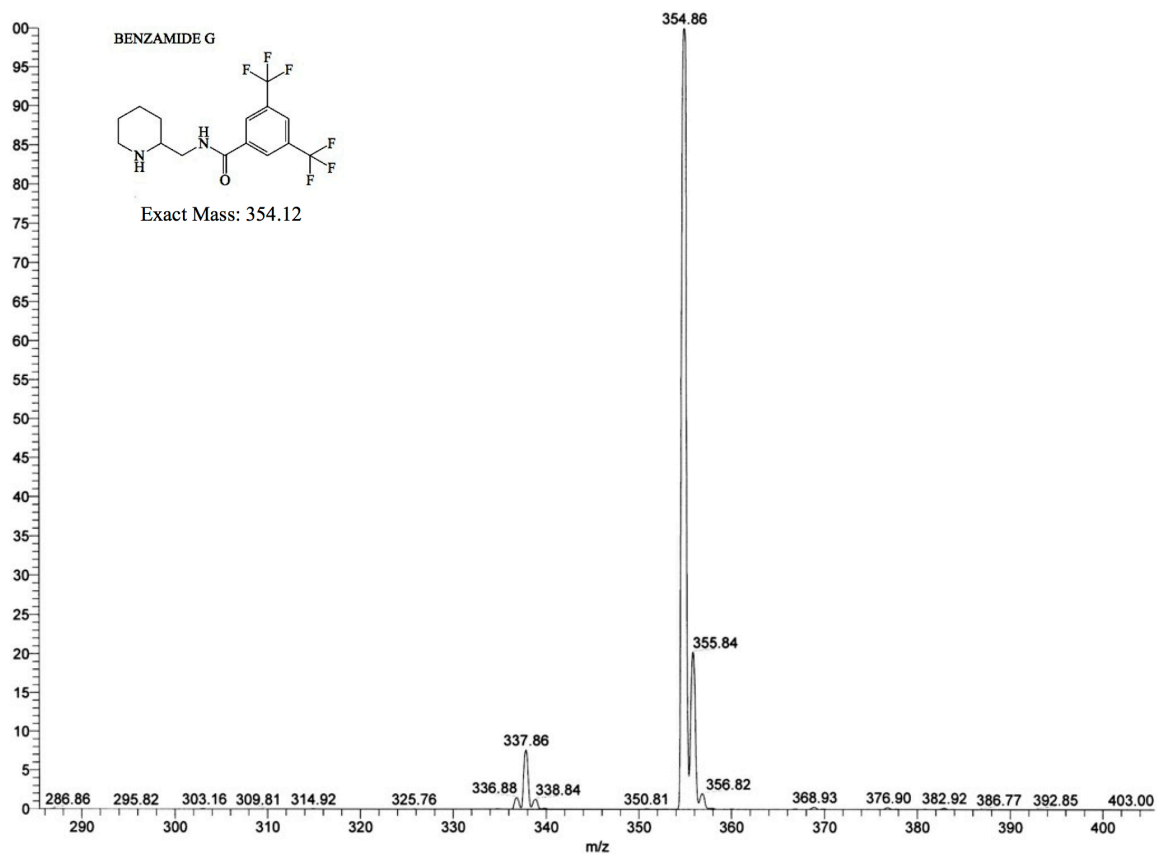






REPLACEMENT OF UNDECYL WITH *N*-METHYL-3,5-BIS(TRIFLUOROMETHYL)BENZAMIDE





REPLACEMENT OF UNDECYL WITH 3,4-DIMETHOXY-N-METHYLBENZAMIDE

