



12-2015

Design and Synthesis of New Non-Steroidal Anti-Inflammatory Drugs with Anti-Cancer Activity on Colon Rectal Cancer Cell Lines HCT-116 and Caco-2

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**DESIGN AND SYNTHESIS OF NEW NON-STEROIDAL ANTI-
INFLAMMATORY DRUGS WITH ANTI-CANCER ACTI-
VITY ON COLON RECTAL CANCER CELL
LINES HCT-116 AND Caco-2**

by

Wanda Ivette Rodríguez Rivera

A dissertation submitted to the Graduate College
in partial fulfillment of the requirements
for the degree of Doctor of Philosophy
Chemistry
Western Michigan University
December 2015

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Wanda Ivette Rodríguez Rivera, Ph.D.

Western Michigan University, 2015

Colon rectal cancer is one of the most common types of cancer and is the third leading cause of cancer related deaths among western countries. Current chemotherapy treatments are highly toxic and mostly result in only a low percentage of tumor reduction; therefore an effective treatment with low toxicity is needed.

In this study, different innovative new COX-2 inhibitors were synthesized using the frame of biologically active chalcones to which active COX-2 pharmacophores SO_2CH_3 , SO_2NH_2 , $\text{SO}_2\text{NHCOCH}_3$ were added. Additionally, the effect of different alkyl chain lengths and the effect of different electron donors on the binding of the active sites of these compounds with the COX-2 enzyme were measured.

In total, 25 different compounds were synthesized. It was found that the drugs were non-selective towards the COX-2 or COX-1 enzyme. The lack of selectivity towards inhibition didn't affect the effectiveness of the compounds to inhibit the growth of cancer cells indicating that there is more than just the inhibition of the COX-2 involved in the process of inhibiting colon cancer tumors.

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1. Background

Chronic pain is one of the leading causes of disability in the United States and worldwide. Consequently, it generates sales of over five billion dollars in non-steroidal anti-inflammatory drugs per year (NSAIDs) [1]. However, the long term use of Non-Steroidal Anti-Inflammatory drugs (NSAIDs) has been linked to gastrointestinal problems like bleeding, development of ulcers and renal liabilities.[2]

1.1 Mechanism of action of arachidonic acid

NSAIDs work by interfering with the mechanism of arachidonic acid pathways. One of the metabolic pathways of arachidonic acid is catalyzed by cyclo-oxygenase (COX) isozymes. COX enzymes convert arachidonic acid into prostaglandins, which are responsible for inflammation [3]. An illustration of how the arachidonic acid is converted to prostaglandins is shown in Figure 1. Arachidonic acid is metabolized via one of three distinct signaling pathways; cyclooxygenase (COX), lipoxygenase (LOX), and P-450 epoxygenase. Cyclooxygenase (COX) enzymes catalyze the first step in the synthesis of prostaglandins from arachidonic acid [7]. This step adds molecules of O₂ to the arachidonic acid, which begins a set of reactions that eventually produce the prostaglandins PGE₂, PGD₂, PGF₂, PGI₂ and TXA₂.

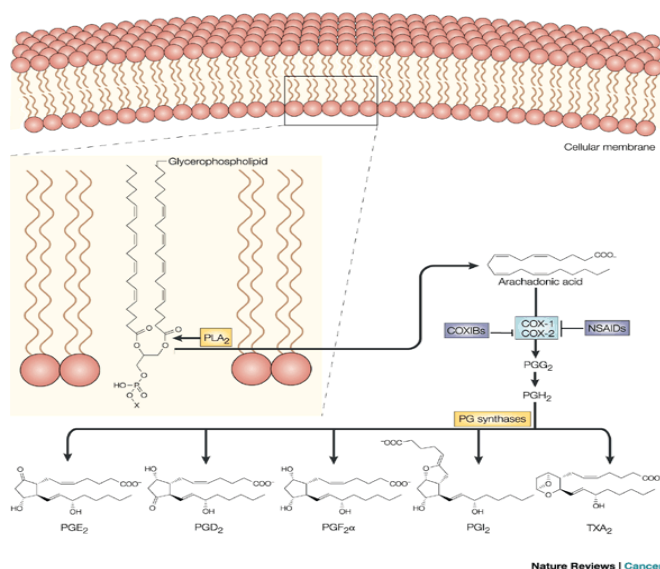


Figure 1. Mechanism of Action of Prostaglandin

1.1.1 Aspirin - metabolic pathway

Aspirin is a common non-steroidal anti-inflammatory drug that inhibits COX enzymes COX-1 and COX-2 irreversibly. It inhibits through the acetylation of the amino acid Ser530 hydroxyl functional group on the main binding site of COX-1 and COX-2. The acetyl group blocks the enzyme from attaching which prevents the oxidation of the arachidonic acid [8]. This acetylation is thought to result from the initial binding of this group with a carboxylic acid group (COOH) from Arg120 residue near the mouth of the COX binding site. It seems that the therapeutic effects of aspirin are due to the inhibition of the enzyme COX-2, while the ulcerogenic and anti-thrombotic effects are attributed to the inhibition of the enzyme COX-1 [8,9]. A description of the structural differences and function of each enzyme is given below.

1.2 Types of COX enzymes

In the 1990's, two isoforms of the COX enzyme [10], COX-1 and COX- 2 were discovered. COX-1 was found to be present in many tissues, such as stomach, kidney, and platelets [10,1], and shown to be important in the maintenance of physiological

functions in the body and keeping vascular homeostasis [13,1]. It was also shown to exhibit cytoprotective properties. COX-2 was found to respond to inflammation, pain and oncogenesis [11,12]. Studies showed the COX-2 enzyme to be induced by mitogenic and proinflammatory stimuli linking its involvement to inflammatory processes associated with injuries [1,14]. It was also discovered that the COX-2 enzyme is responsible for birth contractions, inflammation, hormones and various types of cancer [91,92,93]. Since it is believed that conventional Non-Steroidal Anti-Inflammatory drugs (NSAIDs) inhibit both enzymes causing gastrointestinal distress in many individuals, new research towards a selective COX- 2 drug was undertaken.

1.2.1 Structural differences of COX-1 and COX-2 enzymes

In order to design one molecule selective towards COX-2 and not COX-1, the structural differences between these two enzymes COX-2 and COX-1 must be taken into consideration. One of the main structural differences between COX-1 and COX-2 is the large enzymatic, pocket active site, which exists within the COX-2 enzyme.

This site exists where the amino acid valine is present at the 523 position which provides a smaller side chain that accommodates sulfur groups. On the other hand, the active site of the COX-1 has an isoleucine amino acid at the 523 position, which is a bigger side chain. This side chain prevents the binding of the inhibitor to the active site [13]. Additionally, it has been reported that the replacement of His513 in the COX-1 by the Arg513 found in COX-2 participates in the H-bonding network of the COX-2 binding site [9]. The access of the ligands to the secondary pocket is controlled by the amino acids histidine (His90), glutamine (Gln192), and tyrosine (Tyr355) [15]. Figure 2 below shows a schematic representation of the COX-1 and COX-2 enzymes. The side pocket mentioned is clearly illustrated.

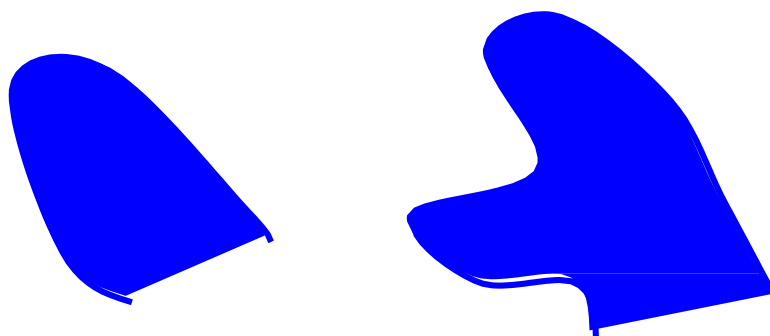


Figure 2. Schematic Representation of COX-1 and COX-2 Enzymes

Researchers have shown that a time dependent inhibition occurs when a ligand drug is bound to Arg513 [16]. Additionally, it has been reported that the combined volume of COX-2 is 25% larger than the volume of COX-1 with a volume of 394 \AA^3 and 316 \AA^3 for the COX-2 and COX-1, respectively [17].

1.3 Structural examples of common Selective COX-2 inhibitors

Common selective COX-2 inhibitor drugs are small molecules consisting of a carboxylic central ring, surrounded by two aryl moieties [18]. In other studies, the central ring was substituted with a benzofuran moiety. Benzofuran derivatives have been reported to have anti- inflammatory properties [19,20] and to possibly provide relief without gastrointestinal side effects [21,22]. Most common COX-2 inhibitor compounds are designed with a SO_2 group.

1.3.1 COX-2 pharmacophores

SO_2 groups are commonly known as COX-2 pharmacophores due to their ability to selectively bind to the side pocket of the COX-2 enzyme. These groups are densely rich in electrons, which tightly bind to the amino acids of the side pocket, in some cases forming hydrogen bonds.

The most common COX-2 pharmacophores are SO_2CH_3 and SO_2NH_2 . Most recently, NHSO_2CH_3 , N_3 and $\text{SO}_2\text{NHCOCH}_3$ have been introduced as more selective

COX-2 pharmacophores. Specifically, it is known that the COX-2 pharmacophore $\text{SO}_2\text{NHCOCH}_3$ is a more reactive acetylating agent than the more common and simple amide SO_2NH_2 [23].

1.3.2 Types of binding in active site

As mentioned previously, these COX-2 pharmacophores are designed to selectively bind to the active site of the COX-2 enzyme to avoid the production of prostaglandins hormones from the COX- 2 enzyme, but not COX-1 enzyme. The blockage of the active site of the COX-2 enzyme can occur inside the site of pocket or outside the site or pocket. Primarily, the intention of most designs is to block the active site from the inside, as shown in Figure 3.

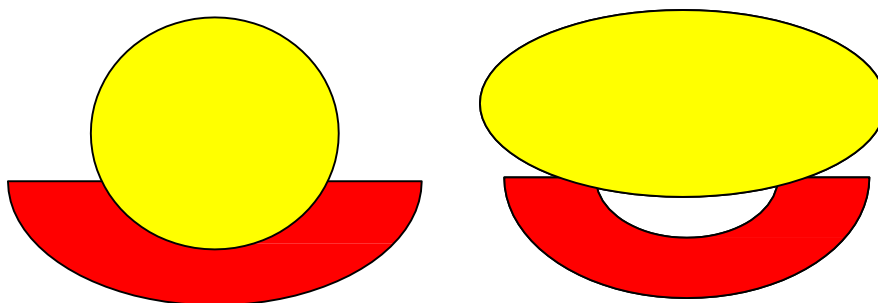


Figure 3. Schematic Representation of Blockage of the Active Site of the Enzyme

1.3.3 Cardiovascular effects of Common COX-2 inhibitors

The development of COX-2 selective drugs has been done, resulting in the release of Rofecoxib and Celecoxib drugs, followed by valdecoxib and etoricoxib [14, 24, 25, 26]. The selectivity of these drugs refers to the measure of the relative ratio of concentrations at which the enzyme is inhibited in a ratio COX-1/COX-2, IC_{50} concentration.

However, Rofecoxib and Valdecoxib were removed from the market due to adverse cardiovascular effects [1,27]. This has caused an increasing concern regarding their use in patients at risk for an adverse cardiovascular event [27] and initiated the

need to design and evaluate new structural templates that can be selective towards COX-2 without adverse cardiovascular effects. It is believed that a possible explanation for an increased incidence of prothrombotic episode with these drugs is due to the lower level of vasodilator and platelet aggregation inhibitor prostacyclin (PGI₂) in conjunction with a higher level of the platelet activator and aggregator thromboxane A₂ (T_xA₂) [10].

1.4 NSAIDS as anticancer remedy

Besides their well-known anti-inflammatory properties, NSAIDs have proven to have anticancer effects on different types of cancer [28, 29, 30]. Sulindac is a non-steroidal anti-inflammatory drug known for its ability to reduce the size of colon rectal tumors in patients with familial adenomatous polyposis (FAP). However its effect is incomplete and reversible [30, 31] so unfortunately the tumor can grow back once the drug is no longer taken.

Colorectal cancer is among the leading cause of malignancy deaths in the western countries. The yearly incidence is approximately 1.0 million, of whom 50,000 people die [32]. Most strategies are focusing on prevention, early detection and improvement of current therapy. However, the high costs of early detection and prevention techniques make the treatment inaccessible to high-risk populations [32].

Population studies have shown that regular use of NSAIDs in combination with selective COX-2 inhibitors reduces the incidence and mortality of cancer [33]. Furthermore the combination of COX-2 inhibitors with anticancer drugs like doxorubicin, bleomycin, vincristine and 5-fluorouracil has been effective for anticancer activity in humans [42,43]. Celecoxib is a very well-known NSAID that has selectivity towards COX-2, which is currently under study for anti-cancer therapy [41].

Oxaliplatin, a third generation anticancer drug derivative of cisplatin, offers another alternative to cisplatin and has been shown to be effective against some colon cancer tumors that are resistant to cisplatin. The action of oxaliplatin is due to the formation of cross links between two adjacent guanine residues or a guanine and an

adenine of DNA that results in the blockage of replication and transcription. This drug is used in combination with celecoxib and etodolac, two well-known COX-2 inhibitors, to measure the cytotoxicity in human colon cancer.

It was found that the combination of etodolac, celecoxib and oxaliplatin increased the reduction of surviving protein, growth inhibition and death of human colon cancer cells. The surviving protein is a cancer colon marker, as it is a member of the inhibitor apoptosis family. This is a promising indication that the combination of oxaliplatin with COX-2 inhibitors could be used as an anticancer therapy treatment [90].

1.4.1 Cancer apoptosis suppression

One of the main hallmarks of cancer is the inhibition of cell apoptosis. Apoptosis is programmed cell death and is an important mechanism to maintaining homeostasis during the growth and development of cells. It is also important to cell survival and fitness as it prevents genetically modified cells from multiplying. Genetically modified cells cause activation of the mitochondrial pathway to release pro-apoptotic factors like cytochrome-c, leading to cell death [32]. Signaling pathways of several anti-apoptotic proteins, have been identified that have a role in the regulation of apoptosis, e.g., APC, p53, NFkB [35, 36, 37]. In carcinogenesis, there is a survival and expansion of malignant or genetically modified cells. Together with this expansion of altered cells there is evidence of the expression of COX-2 enzyme, which seems to be a part of the regulating process of carcinogenesis.

1.4.2 Expression COX-2 in tumors

The process, by which COX-2 is expressed in tumor growth, causing the cells to avoid the process of apoptosis, occurs in three different stages. The first stage is the induction of angiogenic factors such as VEGF. The second stage is anti-apoptosis. The third stage is the development of a malignant tumor that can produce metastasis throughout the body [38,39,40]. From a close look of this process, it is evident that the

expression of COX-2 is linked to the suppression of apoptosis in cells, which ultimately leads to the development of tumors [40].

The COX-2 enzyme is expressed in high concentrations in tumor cells and this happens with different kinds of tumors. Sano et al stated that COX-2 is overexpressed in a high percentage of colorectal polyps and cancer [34]. While the exact role of the COX-2 enzyme in the regulation of apoptosis is not known [32], it is known that presence of COX-2 results in the suppression of apoptosis and therefore plays a role in the uncontrolled growth of cells [37].

2. Introduction

2.1 Design new COX-2 inhibitor compounds

Design of new compounds selective towards COX-2 enzyme without gastrointestinal effects or any cardiovascular effects is imperative. Researchers have tested the inhibition capacity of compounds with more than one benzene group and different COX-2 pharmacophores.

Pharmacophores are the groups responsible for interactions between amino acids and enzymes. Two pharmacophores groups have been widely proven to be effective towards the inhibition and selectivity of COX-2, SO_2CH_3 and SO_2NH_2 groups. Other groups that have been tested are N_3 , NHSO_2CH_3 , and most recently $\text{SO}_2\text{NHCOCH}_3$. This last pharmacophore is known for being a more reactive acetylating agent of enzyme serine hydroxyls than simple amides [94]. $\text{SO}_2\text{NHCOCH}_3$ can serve a dual role; as an acetylating agent and pro-drug. In most studies the compounds are tested with different electron withdrawing groups on the benzene group next to the benzene group containing the pharmacophores (SO_2CH_3 , SO_2NH_2 , $\text{SO}_2\text{NHCOCH}_3$, etc.). The groups with different electron withdrawing properties commonly used are H, Cl, F, CH_3 and OCH_3 . In most cases the compound that was carrying the hydrogen group demonstrated the highest capacity of enzyme inhibition [77]. For other designed compounds, the groups with highest hydrogen acceptor capacity, like OCH_3 , were found to be highly selective

and potent towards inhibition of COX-2 [78]. However, in other designed compounds with just two benzene rings, the methyl provided the most potent inhibitory capacity [78]. The template design used in this study is the same template design used by Edward Knauss in one of his studies [79], see Figure 4. While studies have been carried out / done with the substituent as a phenyl ring [80], very few studies showing the effect of the alkyl chain length of the compound on enzyme inhibition capacity have been performed. The observed trend is that as the alkyl chain increases, the inhibitory and selective capacity increases, but at some point there is no additional effect on inhibition with larger alkyl chain lengths substituents [81].

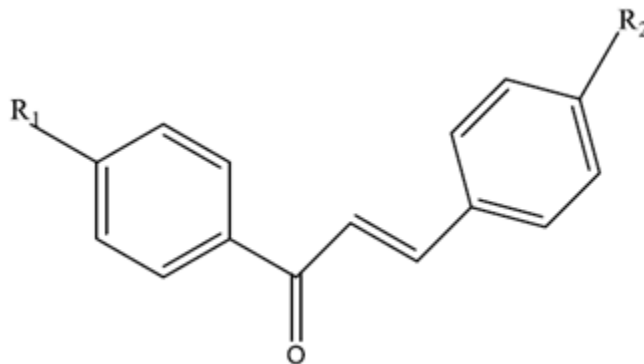


Figure 4. Chalcone Backbone Frame Used to Synthesize Compounds [96]

Besides its advantage of not being linked to non-gastrointestinal damage, potent inhibitory COX-2 products have shown to be effective in the treatment of cancer, which is another application that can be considered [82,83]. Scientists have found that tumors contain a high amount of COX-2 enzyme and the inhibition of COX-2 enzyme appears to have an effect in shrinking the tumors.

2.2 Objective

The aim of this study is to develop molecules that provide an inhibition and selectivity towards COX-2, and can be tested toward the inhibition of cell growth and cell apoptosis of colon cancer cells HCT-116 and Caco-2.

2.3 Chalcones

Chalcones, are natural products that are abundantly present in ferns, fruits, vegetables, spices, tea and soy based products [9]. Chalcones (1,3-diaryl-2-propen-1-ones), belong to the flavonoid family, which consists of two aromatic rings that are joined by a three-carbon α,β -unsaturated carbonyl system [10].

Chalcones have been reported to have bactericidal, antifungal and insecticidal activity. Most recently, there have been some reports about the activity of chalcones against cancer [10]. A dehydrochalcone isolated from *Pityrogramma calomelanos* was found to be cytotoxic and tumor reducing [11]. For anti-inflammatory properties, chalcones work by inhibiting the enzyme cyclo-oxygenase (COX).

2.4 Studies

In this work, four different projects were developed. In the first study, different families of drugs were synthesized using different COX-2 pharmacophores in order to measure their ability to decrease the activity of COX-2 over the COX-1 enzyme. Compounds with different alkyl chain lengths at the four positions of the second benzyl group were synthesized to produce families of COX-2 pharmacophores; SO_2CH_3 , SO_2NH_2 , $\text{SO}_2\text{NHCOCH}_3$. The alkyl chain length was varied from two carbons to four carbons to add bulkiness to the compound and to avoid interaction with COX-1 enzyme. Among the structural differences between COX-1 and COX-2 enzyme is the hydrophilic side pocket of COX-2, which adds bulkiness to the active site.

A general form of the structure of the compounds to be synthesized in the first study is presented in Figure 4. As mentioned previously, COX-1 is responsible for physiological functions in the body, while COX-2 responds to induced damages, like injuries and chronic illnesses [2, 1, 3, 4].

Adverse gastrointestinal irritations, ulcerations, and renal liabilities have been attributed

to COX- 1 inhibition when being used over long periods of time at high doses [5]. The structural differences between COX-2 and COX-1 are taken into consideration in order to design one molecule selective towards COX-2 and not COX-1. The large hydrophilic side pocket on the COX-2 is distinctive from the COX-1 enzyme. Within the side pocket of COX-2 we find that the amino acid valine at the 523 position has a smaller side chain that accommodates sulfur groups. Also, the active site of COX-1 has an isoleucine amino acid at the 523 position, this bigger side chain prevents the binding of the inhibitor on the active site [4]. These differences were exploited when designing the new COX-2 selective compounds in this study.

Auto docking software allows the modeling of the compound against the active site of the enzyme. In the Auto docking program the protein and the ligand are held fixed in a conformational space.

2.5 General Reactions

The reactions of aldol, acetylation, protection of OH group, and conversion from 4-acetylbenzenesulfonylchloride to 4-acetylbenzenesulfonamide will be explained in detail in the subsections below.

2.5.1 Aldol reaction

In an aldol reaction an enolate of an aldehyde or ketone reacts at the α -carbon with the carbonyl of another molecule under basic or acidic conditions to obtain a β -hydroxy aldehyde or ketone [95]. As shown in Figure 5, the mechanism of the aldol reaction can be explained in three steps. The first step is the formation of the enolate ion. The second step is the nucleophilic addition of the enolate to the carbonyl group of the second aldehyde. The final step is the protonation of the molecule, which completes the nucleophilic addition.

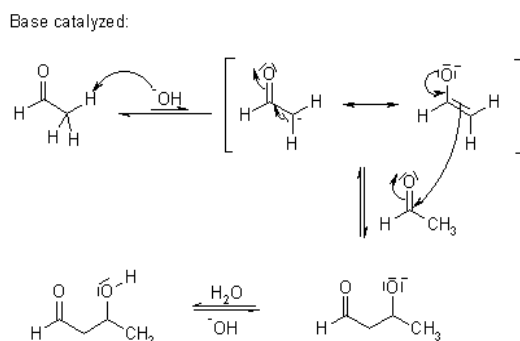


Figure 5. Aldol Reaction Mechanism [71]

2.5.2 Acetylation reaction

The IUPAC (International Union of Pure and Applied Chemistry) nomenclature describes the acetyl chloride reaction that introduces an acetyl functional group into a chemical compound. As shown in Figure 6, the amine reacts with the acetyl compound leaving hydrochloric acid as a byproduct.

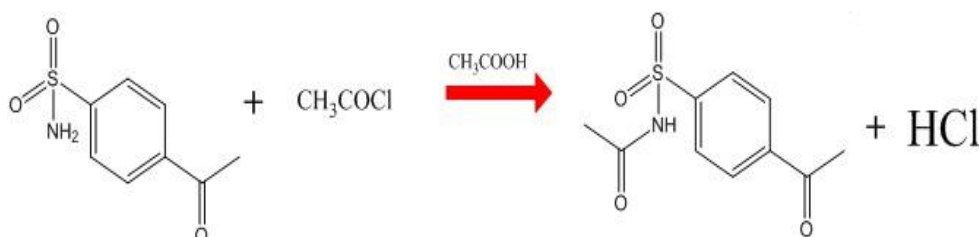


Figure 6. Reaction with Acetyl Chloride 2.5.2 Acetylation reaction

2.5.3 Protection OH group reaction

There are a variety of considerations that need to be taken before choosing a compound to protect the OH groups on a molecule. Since the pH of the reaction will influence the bases, nucleophiles and electrophiles in the reaction it must be carefully controlled. A functional group that is stable under the conditions must therefore be

chosen.

Examples of protecting groups that can be used are methoxymethyl ether (MOM ether), tetrahydropyranyl ether (THP ether), t-butyl ether, allyl ether, benzyl ether, t-butyltrimethylsilyl ether (TBDMS ether) t-butyltriphenylsilyl ether, acetic acid ester, pivalic acid ester, benzoic acid ester, acetonide, and benzylidene acetal [72]. As shown in Figure 7, the OH group can be protected by using benzyl bromide in a basic environment. In the paper in which this reaction path was reported, the compound with the OH group to be protected was added to a flask using methanol as the solvent. Sodium carbonate was then added to create a basic environment [84]. The reaction was then left to reflux overnight and allowed to cool to room temperature. Once the mixture was cooled, the sodium carbonate was removed by filtration and the compound with solvent was evaporated using a rotary evaporator. After this step, no further purification was performed.

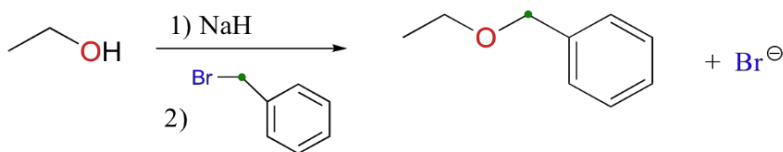


Figure 7. OH Protection Reaction

2.5.4 Conversion of 4-acetylbenzenesulfonyl chloride to 4-acetylbenzenesulfonamide

In Figure 8, the conversion of 4-Acetylbenzenesulfonyl chloride to 4-acetylbenzenesulfonamide is shown. In this reaction, the amine (NH₂) reacts with Cl, releasing HCl as a byproduct and a sulfonamide is generated.

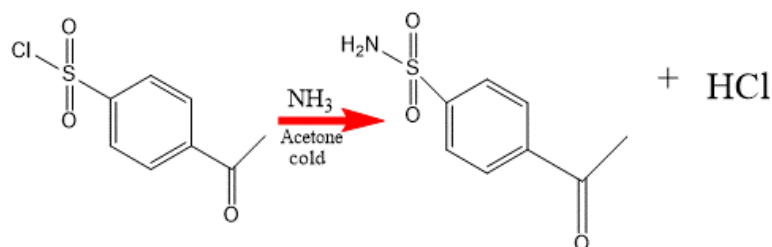


Figure 8. Conversion of 4-Acetylbenzenesulfonyl Chloride to 4-Acetylbenzenesulfonamide

A more detailed description of the steps used to prepare this compound can be found in the Methodology chapter of this work under the subheading of SO₂NH₂ reactions.

2.5.5 Oxidation of methyl group to carboxylic acid

The oxidation of a methyl group to produce carboxylic acid can be done by mixing 2 mmol of the organic compound of interest with 12 mmol of KMnO₄ and 24 mL of a 0.5 N NaOH solution. After combining all materials, the mixture should be stirred at an elevated temperature of 60 °C for approximately 6 hours [88]. Figure 9 shows this reaction.

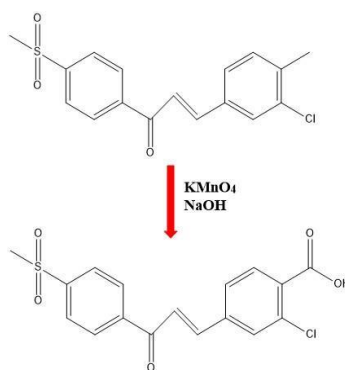


Figure 9. Oxidation of the methyl group of an alkylbenzene using KMnO₄

3. Methodology

3.1 Study 1 reactions

Materials

All chemical reagents were acquired through Sigma Aldrich and used without further purification.

A total of nine different compounds were synthesized in the first study. Three different sets of families of compounds were synthesized in which the R group on the fourth position was varied from ethyl, propyl to butyl. Additionally the R group on the left was changed by adding different sulfoxides during the experimental process. Some of the sulfoxides used were, SO_2CH_3 , SO_2NH_2 and $\text{SO}_2\text{NHCOCH}_3$. The 9 synthesized compounds obtained are shown Figure 10.

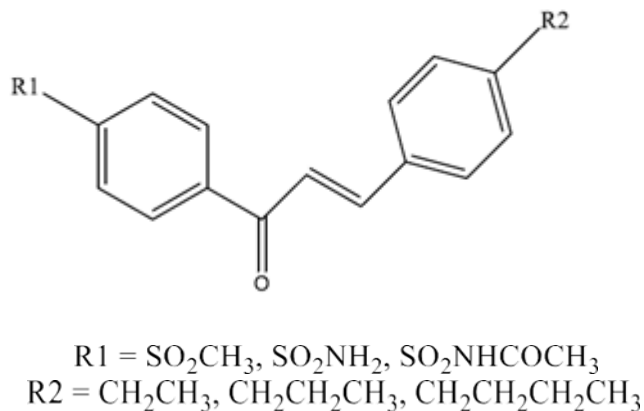


Figure 10. Schematic Representation of Compounds Synthesized in Study # 1

3.1.1 SO_2CH_3 family of compounds

Compounds containing SO_2CH_3 groups were synthesized in one single step by use of the aldol reaction [90]. Figure 10 shows the general reaction used. The functional groups interacting with each other were a ketone and an aldehyde. To do

this reaction 1 mmol of the corresponding ketone and aldehyde were mixed with approximately 4 mL of methanol and 100 mg of sodium hydroxide. The reaction mixture was stirred until a precipitate was obtained. To obtain the precipitate, the reaction time could vary from 30 minutes to 9 hours depending on the compound used.

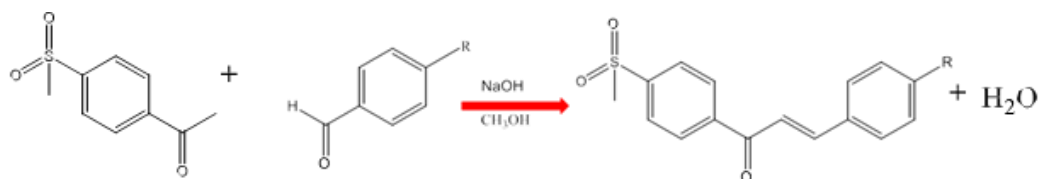


Figure 11. Claisen Schmidh [90] Representation Reaction for SO₂CH₃ Family of Compounds

Once obtained, the precipitate was filtered and washed with cold methanol. The precipitate was then purified by recrystallization. A mixture of ethyl acetate and hexane solvent of different solvent ratios, ethanol, methanol and dichloromethane were then used to achieve the recrystallization of each precipitate with the ethyl, propyl and butyl group, respectively. The recovery yield fluctuated between approximately 30 to 44 percent for this family of compounds. The purified compounds were then analyzed using NMR technique and Mass Spectrometry. The characterization of these techniques is explained in more detail in the NMR and Mass Spectrometry sections of this work. Crystals were attempted to be grown for each compound. When a good size crystal was obtained, crystallography analysis was performed.

3.1.2 SO₂NH₂ family of compounds

Compounds with the SO₂NH₂ were synthesized in two steps, the first step being the conversion of SO₂Cl to SO₂NH₂ reaction and the second step the aldol reaction. The first step is the only one described in this subsection as the second step was previously outlined. Figure 12 shows the two step reaction. In the first step, 4-acetylbenzenesulfonyl

chloride was reacted with ammonia in acetone.

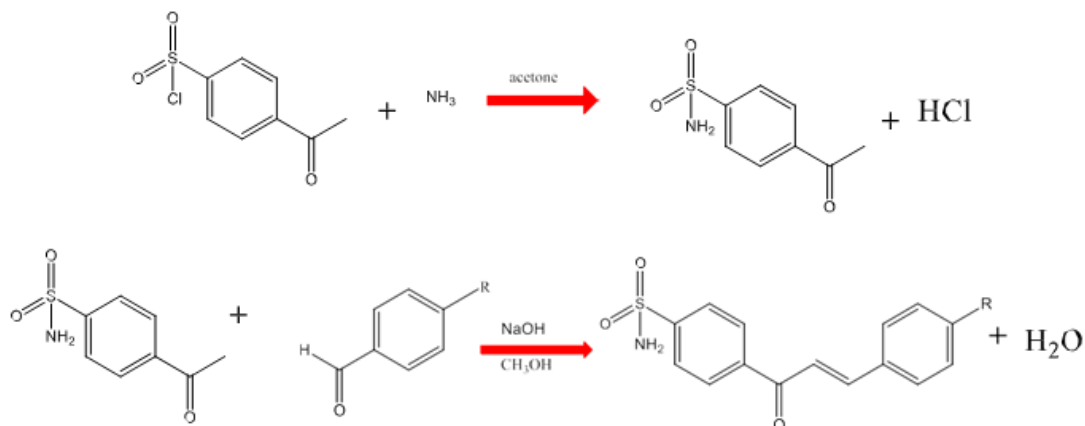


Figure 12. Two Step Representation Reaction for SO₂NH₂
Family of Compounds

Specifically, about 2 g of 4-acetylbenzenesulfonyl chloride were mixed with approximately 4 mL of 28% ammonia in 15 mL of acetone. To do the reaction, the 4-acetylbenzenesulfonyl chloride was mixed with the acetone and cooled to zero degrees Celsius. To lower the temperature, a bath mixture of crushed dry ice with ice and acetone was prepared. The solution of ammonia at 28% was then added slowly, drop-by-drop under constant stirring. The 4-acetylbenzenesulfonyl chloride soon changed from dark brown to a yellow-white solution. After adding all the ammonia, the solution was transferred to an ice bath and stir for approximately 10 minutes. This was done to assure that the maximum amount of product was obtained. Later, the precipitate was filtered and washed with water. The final product was produced at approximately a 90% yield. The second step is described in the subsection above. The final product was purified with methanol using the recrystallization technique. The final yield was about 40% for all products produced by this method. The purified compounds were analyzed using NMR technique and Mass Spectrometry. Crystals were grown for each compound and when a good size crystal was obtained, a crystallographic analysis was performed.

3.1.3 SO₂NHCOCH₃ family of compounds

Compounds with the SO₂NHCOCH₃ were synthesized in three steps, as shown in Figure 13. The first step is explained in subsection SO₂NH₂. In the second step, the sulfonamide group SO₂NH₂ was acetylated with acetyl chloride in acetic acid. For this reaction, about 0.95 mmol of 4-acetylbenzenesulfonamide was mixed with 2 mL of glacial acetic acid and 14.0 mmol of acetyl chloride. The acetyl chloride was added to the flask under a nitrogen inert environment. The reaction mixture was refluxed for about an hour after which time it was left to reach room temperature. The excess acetyl chloride was then evaporated using a rotary evaporator. The organic phase was then extracted with water and 30 mL of ethyl acetate. The organic phase was dried with Na₂SO₄, to yield a pale yellow syrup. When needed, the compound was purified in a silica gel column with a 2:1 ratio of ethyl acetate to hexane. The solvent was evaporated using a rotary evaporator. The third step was to perform the aldol reaction explained in subsection SO₂CH₃ of this work. The compounds were purified by recrystallization using ethanol and methanol. The recovery yield of the compounds was about 40%. The purified compounds were analyzed by using NMR and Mass Spectrometry. When crystals were grown, they were analyzed using crystallographic technique.

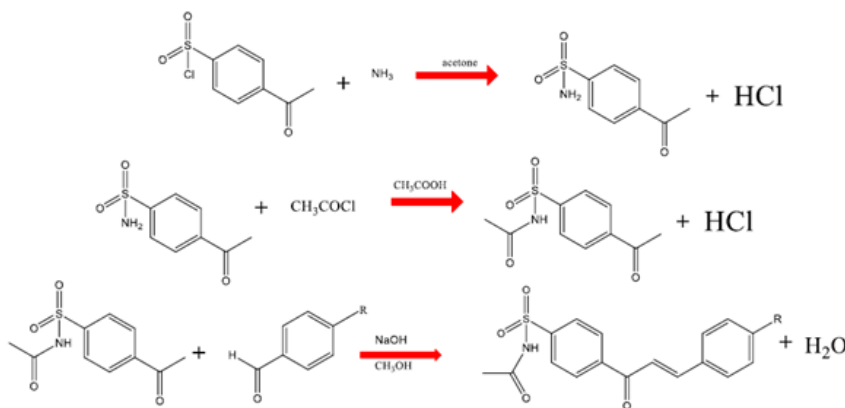


Figure 13. Three Step Representation Reaction for SO₂NHCOCH₃ Family of Compounds

3.2 Study 2 reactions

In the second study the influence of different electron donors at the fourth position of the benzene group on the active site of the enzyme were analyzed using the $\text{SO}_2\text{NHCOCH}_3$ family group. The electron donors analyzed were F, Cl, OCH_3 , $\text{OCH}_2\text{CH}_3(\text{C}_6\text{H}_6)$. Four different compounds were synthesized. The compounds were synthesized in three steps, as shown in Figure 14.

The steps to synthesize the compounds followed those previously discussed in this work. The compounds were purified using recrystallization techniques with mixtures of different solvents like ethanol, hexane, ethyl acetate, methanol, and isopropyl alcohol. Proton NMR, ^{13}C NMR and Mass Spectrometry were done to verify the purity of the compounds. Additionally, crystal growth was attempted by using different techniques like solvent evaporation, liquid-liquid diffusion and vapor diffusion. These techniques are explained in more detail with the crystallography section of this work. When a good size crystal was obtained, crystallographic analysis was performed.

3.3 Study 3 reactions

In this third study, nine different compounds were attempted to be synthesized. The compounds were similar to those obtained in the first study, but in this study a methyl group was added on the fourth position of the benzene group and different electron donors are added on the fifth position. As shown in Figure 14, the compounds were synthesized following the reactions already explained in study 1. The compounds were purified by recrystallization using ethyl acetate: hexane and methanol and characterized with ESI-Mass Spectrometry, ^1H -NMR and C_{13} NMR. When a good size crystal was obtained, crystallography analysis was performed.

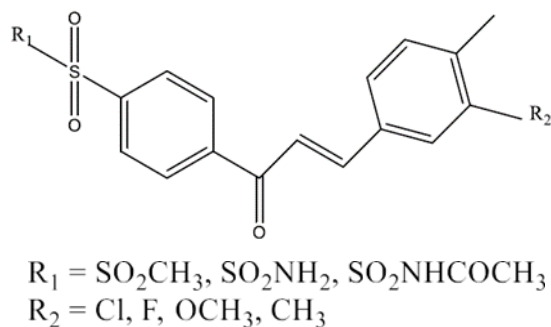


Figure 14. Schematic Representation of Compounds Synthesized in Study # 3

3.4 Study 4 reactions

In the fourth study, the compounds from the third study were used and a carboxylic acid group added. The carboxylic group was added to enable the hydrogen bonding of these groups with the amino acids on the active site of the pocket. Since the oxygens in the carboxylic acid provide a high electron density, which can have a stronger electrostatic interaction with the amino acids, stronger bonding at the active site was expected.

The carboxylic acid was added by oxidation of a methyl group by combining 2 mmol of the organic compound to be oxidized with 12 mmol of KMnO_4 or approximately 2 grams of KMnO_4 and 24 mL of a 0.5 N NaOH solution. All the reagents were stir and heated at 60 °C for approximately 6 hours [88,89]. The reaction was then quenched with acetone and the insoluble material removed by filtration. Next, the solution was diluted with approximately 150 mL of milli-Q water and acidified to a pH 3.0 using a 5% HCl solution. The organic phase was extracted with dichloromethane and washed with water. Any residual water was then removed with sodium sulfate (Na_2SO_4). The solvent was evaporated in a rotary evaporator [88, 89]. A schematic representation of the synthesis is shown in Figure 15. The compounds were purified by recrystallization using ethyl acetate: hexane and methanol, and characterized with ESI-

Mass Spectrometry, ^1H -NMR and ^{13}C NMR. To promote the growth of new crystals, different growing techniques were used like solvent evaporation, vapor diffusion and liquid-liquid diffusion. For more information about these crystal growing techniques, refer to the crystallography section of this work. When a good size crystal was obtained, crystallographic analysis was performed.

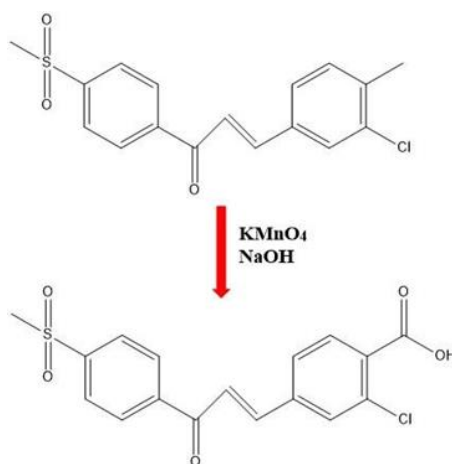


Figure 15. Schematic Representation of the Oxidation of a Methyl Group to Carboxylic Acid

3.5 Melting point analysis

Through melting point analysis, the temperature at which a solid turns into a liquid is measured. Since the melting point of materials is unique this method is an easy way to determine the identity of a compound. A melting point analyzer was used to record the melting point of the compounds. The temperature range of the equipment used enabled testing up to 300 degrees Celsius to be performed.

Compounds with the SO_2CH_3 pharmacophore family group were analyzed by raising the temperature slowly while watching the compound through the magnifying lenses until it melted. Compounds with the SO_2NH_2 and $\text{SO}_2\text{NHCOCH}_3$ pharmacophore families were analyzed by placing the compound on the melting point analyzer while

hot, with the temperature close to the expected melting point of the compound. Extreme care had to be taken since many of the compounds will form supramolecular structures in a solid state, or form hydrogen bonds. Care also had to be taken because if the temperature was raised too slowly, the compound would oxidize with the oxygen in the environment before the melting point could be measured.

Due to the temperature limitations of the instrument, the melting point of a few compounds could not be measured. The melting point of these compounds was higher than the range of the scale on the melting point analyzer.

3.6 NMR technique

The NMR instrument used to analyze the samples was a JEOL Bruker instrument. A schematic representation of the instrument is provided in Figure 16.

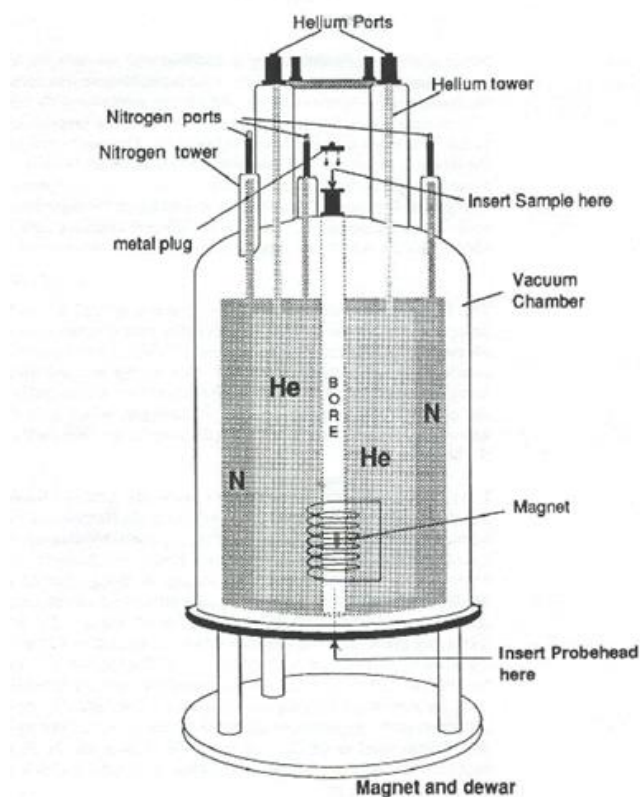


Figure 16. Representative Image of an NMR [85].

When running an NMR, the sample is positioned in a magnetic field and excited via pulsations in the radio frequency input circuit. An NMR spectrum, which is a plot of the radio frequency applied against absorption, is obtained. A signal in the spectrum is referred to as resonance and the frequency of a signal is known as chemical shift [73]. Nuclei with an odd mass or odd atomic number have a “nuclear spin”, this includes ^1H and ^{13}C nuclei. The spin nuclei are sufficiently different that an NMR can differentiate one particular isotope of a particular element [73]. When there is no magnetic field the spins are randomly oriented but when a field is applied the spins are aligned or opposed. This is depicted in Figure 17 below.

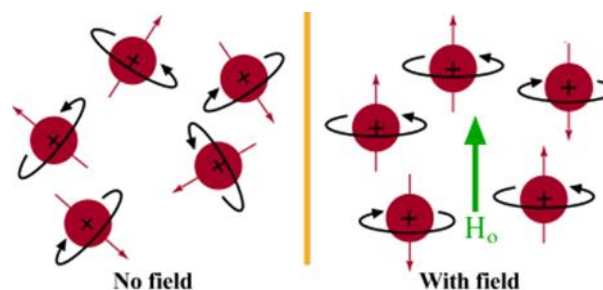


Figure 17. Representation of Spin with and without Magnetic Field [73]

3.7 Mass spectrometry

In mass spectrometry a charged particle passes through a magnetic field and is deflected through a circular path on a radius that is proportional to the mass to charge ratio, m/e . By use of this method, the molecular weight of a compound can be determined.

3.7.1 Electrospray ionization mass spectrometry

Non-volatile organic compounds (heavier compounds) are used for analysis with electrospray ionization technique. With this technique, the sample is mixed with solvent and introduced into a vessel, called a capillary. A high voltage is applied to the solvent, which charges the molecules in the solvent. The spraying process can be streamlined by

using a nebulizing gas. As shown in Figure 18, a high voltage is applied to the solvent, which charges the molecules in the solvent.

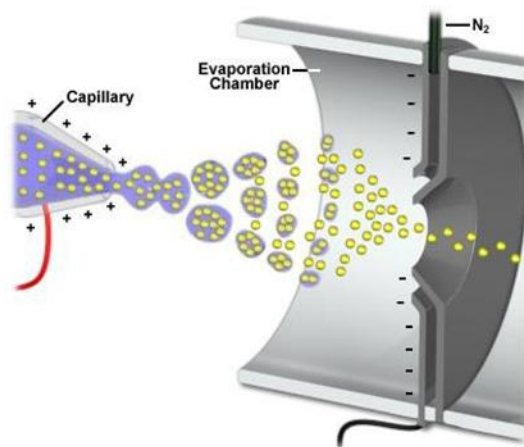


Figure 18. Electrospray Ionization [74]

Since they are charged with the same charge once they are pushed through the nozzle, they repel against each other violently. The solvent eventually evaporates, forcing the charges in the molecules, which now are ions, to draw closer together. The ions are solvated by the solvent molecules and a radical ion is formed before either a positive or negative charge ion is made [86]. This process repeats itself until all of the solvent evaporates and the droplets have split up to the point of a single charge molecule [74]. The ions are then analyzed in a mass analyzer. Once in the mass analyzer a spectrum is generated from the molecular ion or parent ion, which is the ion that represents the molecular weight of the compound being analyzed.

3.8 Crystallography

Crystallography studies the arrangement of atoms in single crystal form. In this technique, the crystal is bombarded with x-rays, where the atoms inside the crystal absorb the X-rays and diffract the light according to the structure lattice of the crystal. The diffraction provides information on the structure. Crystals group together forming

lattice systems, there are seven different lattice systems triclinic, monoclinic, orthorhombic, rhombohedral, tetragonal, hexagonal, and cubic, see Figure 18. Crystal systems can include simple cubic (P), body centered cubic (I) or face centered cubic (F) [75].

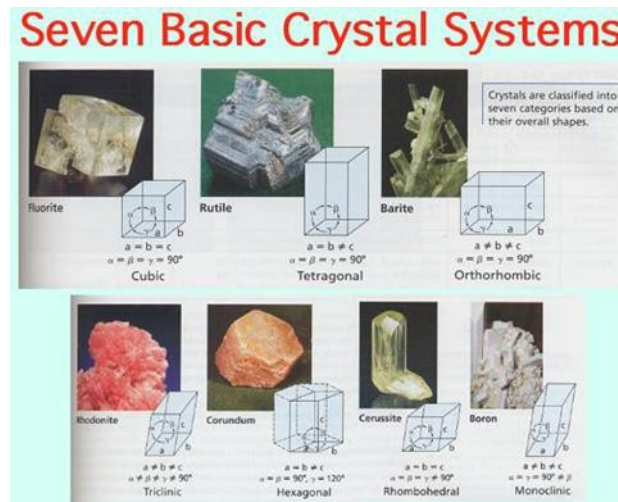
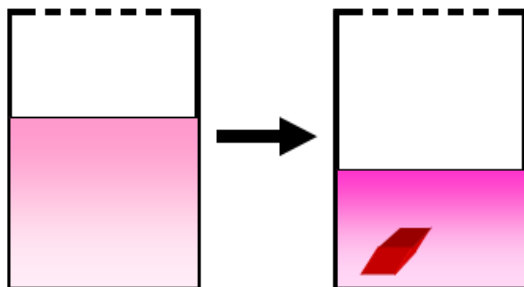


Figure 19. Crystal Structure System [75]

3.8.1 Crystal growing techniques

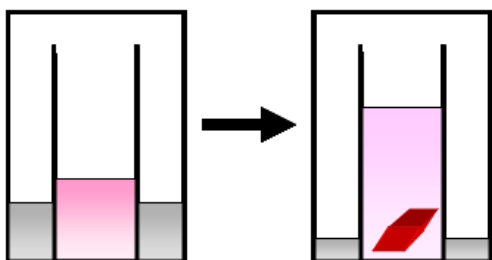
For optimal crystal growth different techniques are used, but before attempting to grow a crystal the compound should be purified to a purity of 75% or higher. The more pure the compound, the better chances of growing a good crystal. A description of different crystal growing techniques used is provided below.

- **Solvent Evaporation**



This is the simplest technique to grow. In this method the compound is dissolved in a solvent to create a nearly saturated solution. The solvent is then evaporated slowly to allow the crystals to grow.

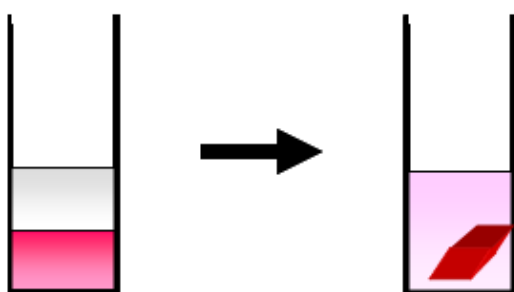
- **Vapor Diffusion**



This technique requires the use of two different solvents with different boiling points and two different vials. One solvent is a material in which the compound doesn't dissolve and is more volatile with a lower boiling point than the solvent in which the compound gets dissolved.

- **Liquid-Liquid Diffusion**

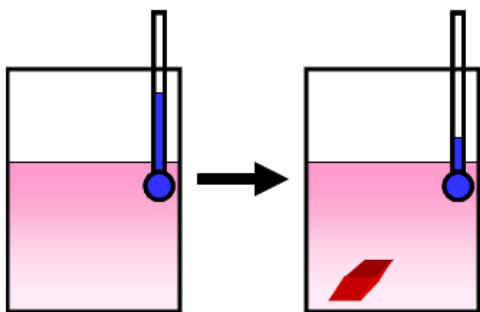
This technique doesn't require solvents of different boiling points to be used, but it



requires solvents of different densities to be used. The solvent with the highest density is placed on top of the other solvent. This technique requires high skill, the solvent at the top has to be added slowly, so it won't mix right away with the solvent at the bottom. The idea is for the solvents to diffuse

slowly together. To facilitate the pouring of the solvents some technicians freeze the lower solvent before pouring the upper layer solvent.

- **Solvent Cooling**



This technique was used with all of the compounds synthesized, as an initial purification process. The main process was to find a solvent in which the compound of interest was insoluble at room temperature but soluble at high temperature. The solvent is warmed at high temperature and the

compound to be purified is added to the solution. Once the solution starts to cool down slowly a precipitate would be formed and if it precipitates slowly you can get a crystal.

3.9 MTT cell proliferation analysis

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) [44] is widely used for toxicity, cell viability and cell proliferation analysis on different kinds of cells [45, 46, 47], among those cancer cells.

MTT gives a yellowish solution color that turns to violet-blue formazan upon reduction by dehydrogenase and other reducing agents from metabolically active cells, see Figure 20.

It is currently thought that the amount of formazan is directly proportional to the amount of living cells [47] and when the analysis gives a yellow or crystal clear color the color is proportional to the amount of dead cells present.

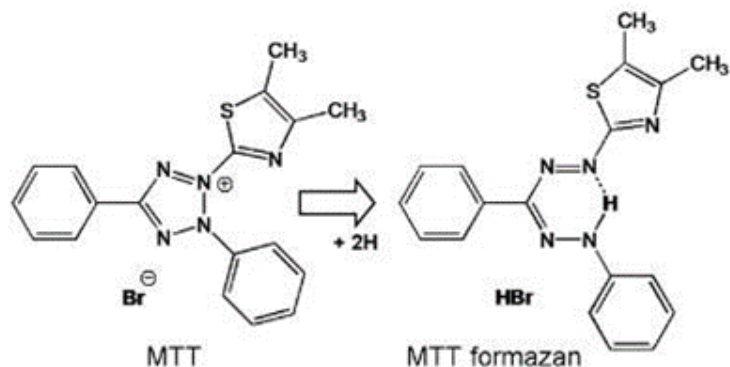


Figure 20. MTT Reduction [48]

In this study, the HCT-116 cells and Caco-2 were sub cultured and allowed to grow until there was approximately 500, 000 cells per plate. Please refer to section on cell culture within this work for information on how to grow, subculture and count cells that are alive.

The cells were allowed let to attach to the microplate inside an incubator at 37 °C and 5% of CO₂ for 24 hours [52, 53, 54]. Each well in the cell had 100 µL of the medium, counting for approximately 5000 cells per well. After 24 hours had passed, 100 µL of each compound was added to each well. The concentration of the compounds was varied accordingly to the study. For Caco-2 cells 90 µL of the compound was added plus 10 µL of Fetal Bovine Serum. Caco-2 cells need 20 % of FBS or incubation [55], contrary to HCT-116 cells that need just 10% FBS [56].

To develop the MTT, a solution of 12 mg of MTT in 12 mL of Phosphate Buffered Saline (PBS) solution was prepared. For a procedure on how to prepare the saline solution see Appendix C. This solution was diluted to a final concentration of 200 mM. The final concentration of the MTT solution was, if needed, adjusted according to the brightness and clarity of colors the analyst would like to see. The final solution was filtered using a sterile syringe of 0.2 µm mesh and the tube covered with aluminum foil [52, 53, 54]. The MTT indicator gave a yellow/white color for well plates with dead

cells and purple color with living cells. The absorbance was measured using a microplate reader at 575 nm.

3.9.1 Cell culture

To grow the cells, certain general procedures had to be followed. First, the solutions had to be warmed to 37 °C, which the temperature where the cells have optimal growth. At a different temperature the cells would not grow and die. Second, the working area had to be sterile, which was accomplished by wiping down the work area with a solution of 70% ethanol. Care was also taken to constantly sterilize any gloves worn with 70% ethanol. By taking these measures, cells were successfully grown in the incubator at 37 °C and 5% CO₂.

The cells were subculture initially by washing away the dead cells with a solution of PBS. The procedure of how to make the PBS solution can found in Appendix C. The cells were detached using trypsin. The amount of trypsin added varied according to the size of the flask. The flask with trypsin was shaken gently and incubated for 6 minutes at 37 °C, an additional incubation time is not recommended as it can cause damage to the cells. Once the cells were detached, they were counted under a microscope using a hemocytometer. For easy counting the cells, the cells were colored with a Trypitan blue solution. This solution helped to distinguish between the cells that were alive and dead.

The hemocytometer has four different regions in which one may count the cells. For this study, approximately 500,000 cells or 5,000 cells per plate were needed. To calculate the total amount of cells in the flask the following formula was used:

$$\text{Total cells/mL} = \text{Total cells counted} * (\text{dilution factor} / \# \text{ of squares}) * 10,000 \text{ cells/mL}$$

To continue growing the cells, the flask was placed back in the incubator at 37 °C and 5% CO₂ and the medium changed every two or three days. The amount of cells within the flask was not allowed to surpass 80% to prevent them from overlapping, which would cause them to start dying instead of continuously growing.

3.10 Cytolysis analysis

Cytolysis occurs when the membrane of the cell erupts due to extra fluid buildup from an osmotic imbalance. Here the cells erupted going through purification. The number of dead cells that went through cytolysis was counted after an incubation period of 96 hours.

This analysis enabled the affect of foreign substances on cell to be determined and to verify if cytolysis, by any extent, is an indication of rapid or slow cell death. Other cells died without undergoing cytolysis [76].

4 Results

Section 4.1, 4.2 and 4.3 describe the results obtained from the different studies with a brief description of each study performed. A total of 25 new compounds with potential biological activity were synthesized, along with two previously synthesized compounds obtained from study number 4 with carboxylic acid groups. The compounds are described in each study by their IUPAC names and their effect on cancer cells discussed. A list of all the synthesized compounds with their respect to their IUPAC names is provided in Appendix N.

4.1 Study #1

4.1.1 Brief description

In the first study, the effect of the carbon chain at the fourth position on one of the benzyl groups was studied. The purpose of this study was to observe the effect of different chain lengths with different SO₂ pharmacophores, SO₂CH₃, SO₂NH₂, SO₂NHCOCH₃. A total of nine different compounds were synthesized. All compounds were successfully synthesized and purified according the methods described in Methodology chapter, study #3. The compounds were characterized using ¹H NMR, ¹³C NMR, ESI- Mass Spectrometry, crystallography and melting point analysis. All the spectra and data can be found in the Appendix. The results obtained from the more

effective compounds are discussed here.

The compounds were initially tested at a concentration of 400 μ M for colon cancer cell lines Caco-2 and HCT-116. Caco-2 selectively expresses the enzyme COX-2, while HCT-116 is a colon cancer cell line that does not express COX-2. Both cell lines were put to the test to determine if there was any indication that cancer cell lines expressing COX-2 were going to be more effectively killed or cell proliferation stopped. All compounds were effective at stopping cell proliferation and killing cancer cells at a concentration of 400 μ M. A more detailed quantitative study was done through the Fluofarma company to verify the previous calculated results and to determine the effectiveness of the compounds at a low concentration. The analysis was done for the HCT-116 cell line. A Caco-2 cell line will be done in a future study. The quantitative analysis was done over periods of 24, 48 and 96 hours. The percentage of lysed cells was calculated at 96 hours. Molecular modeling analysis of the compounds was done using autodock and docking server which integrated autodock modeling software with other programs [57, 58, 59, 60, 61]. This analysis estimated the energy binding of the compounds together with different electrostatic interactions like hydrogen bonding, hydrophilic interactions, hydrophobic interactions, among others. The results of the cell analysis were compared with the docking model results to look for possible explanations, behaviors, and if a relationship between COX-2 selectivity and anti-cancer activity existed. A small trial on enzyme selectivity for the COX-1/COX-2 compound was done at 40 μ M.

4.2 Cell proliferation analysis on HCT-116 and Caco-2 at 400 μ M

Figures 21 and 22 below show the proliferation of cell lines Caco-2, which expresses the COX-2 enzyme and HCT-116 cells not COX-2 enzyme. On this initial analysis the compounds were incubated for 96 hours at a concentration of 400 μ M. From these figures it is clear that the compounds were effective in stopping the cell proliferation of the cells on both cell lines. However, the compounds were more effective on HCT-116 cells than Caco-2 cells. Compounds 11 and 14 with

identification codes of $\text{SO}_2\text{NHCOCH}_3\text{Cl}$ and $\text{SO}_2\text{NHCOCH}_3\text{OBenzyl}$ were very effective at stopping the proliferation of the cancerous cells. Compound 12 with identification of $\text{SO}_2\text{NHCOCH}_3\text{F}$ was not very effective at stopping the cell proliferation of the cancerous cells after 96 hours of incubation. The electronegativity of the electron donor F is 3.98 while the electronegativity of the electron donor Cl is 3.16. The high electronegative chlorine (Cl), but less electronegative than F was able to stop the proliferation of the cells much more efficiently. Obviously, there is a difference in interaction between the COX-2 enzyme and Caco-2 and HCT-116 cancerous cells lines. Differences between the compounds identified as $\text{SO}_2\text{NHCOCH}_3\text{F}$, $\text{SO}_2\text{NHCOCH}_3\text{Cl}$, and $\text{SO}_2\text{NHCOCH}_3\text{Obenzyl}$ are also observed.

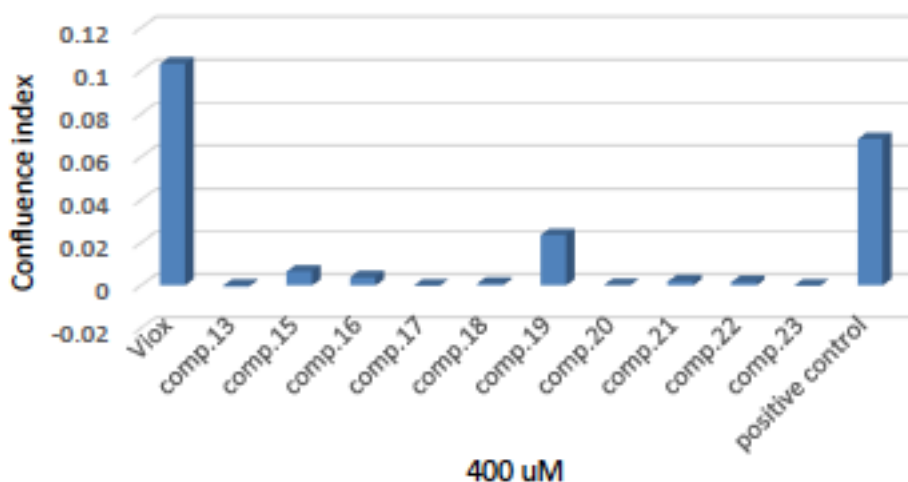


Figure 21. Study #1 - Confluence index after 96 hours on HCT-116 line

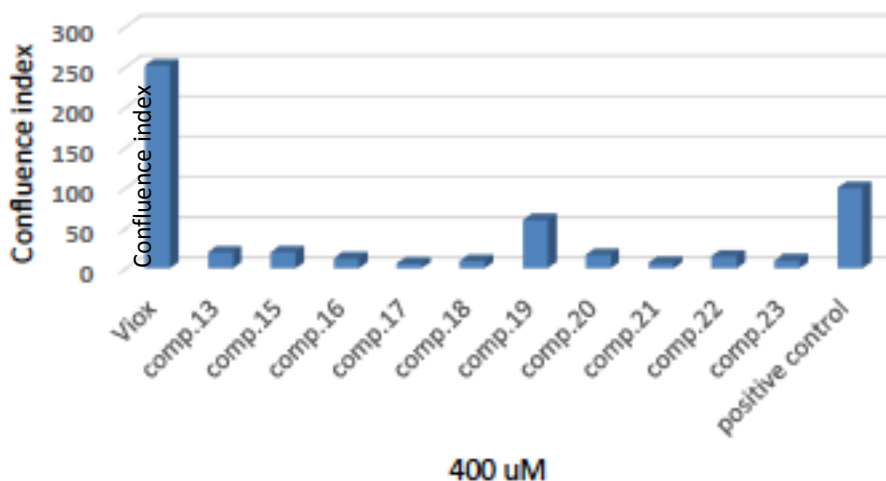


Figure 22. Study #1- Confluence index after 96 hours on Caco-2 cell line

4.3 Comparative study of cell proliferation analysis between cell lines HCT-116 and Caco-2 at 400 µM

A comparative study between Caco-2 colon rectal cancer cells, which expresses COX-2 enzyme, and HCT-116 colon rectal cancer cells, which do not express the COX-2 enzyme, was performed at a concentration of 400 µM. Quantitative and kinetic analysis of Caco-2 at a lower concentration were not performed by the Fluofarma Company due to time and funding limitations. Further studies should be performed at some time.

Figure 23 shows the compounds to be effective at stopping the proliferation of both cells, but they are more effective at stopping the proliferation of HCT-116 than Caco-2 cells. Compound 2 and compound 8 with identification codes of $\text{SO}_2\text{CH}_3\text{CH}_3\text{Cl}$ and $\text{SO}_2\text{CH}_3\text{CH}_3\text{F}$, respectively were not able to stop the proliferation of Caco-2 cells. Similar findings were also found for compounds 9, 6, 7 and 3 with identification codes of $\text{SO}_2\text{NHCOCH}_3\text{CH}_3\text{Cl}$, $\text{SO}_2\text{NH}_2\text{CH}_3\text{CH}_3$, $\text{SO}_2\text{NH}_2\text{CH}_3\text{Cl}$ and $\text{SO}_2\text{NH}_2\text{CH}_3\text{F}$. Compounds 4 and 5, with identification codes of $\text{SO}_2\text{CH}_3\text{CH}_3\text{F}$ and $\text{SO}_2\text{NH}_2\text{CH}_3\text{OCH}_3$, respectively, were also not very effective towards inhibiting the growth of Caco-2 cells.

However, all these compounds were effective at stopping the proliferation of HCT-116 cells. Viox, the NSAID compound used as the control also did not show any effectiveness at stopping the proliferation of the cells. This could be due to the variation in effectiveness of non-steroidal compound from one carcinogenic cell to another.

Our data nullifies the hypothesis that COX-2 expressing tumor cancer cells are more likely to shrink in size and to be able to avoid metastasis if selective COX-2 is used as NASID inhibitors. The compounds designed with known COX-2 pharmacophore groups that would tightly bind to the active site of the enzyme, didn't show any selectivity towards cancerogenous cells that express COX-2 enzyme. The compounds however, showed efficacy at stopping the cell proliferation and killing the cells on both cells line. Out of the total amount of cells that were subjected to apoptosis, 20% underwent cytolysis. The cytolysis was noted during the cell proliferation analysis.

To further study the relationship between COX-2 enzyme and compound selectivity towards the enzyme, to understand the mechanisms of binding, and determine the kind of interactions that may occur, molecular modeling analyses were performed utilizing different software programs. The software programs used are discussed in more detail in the AutoDock section of the methodology chapter.

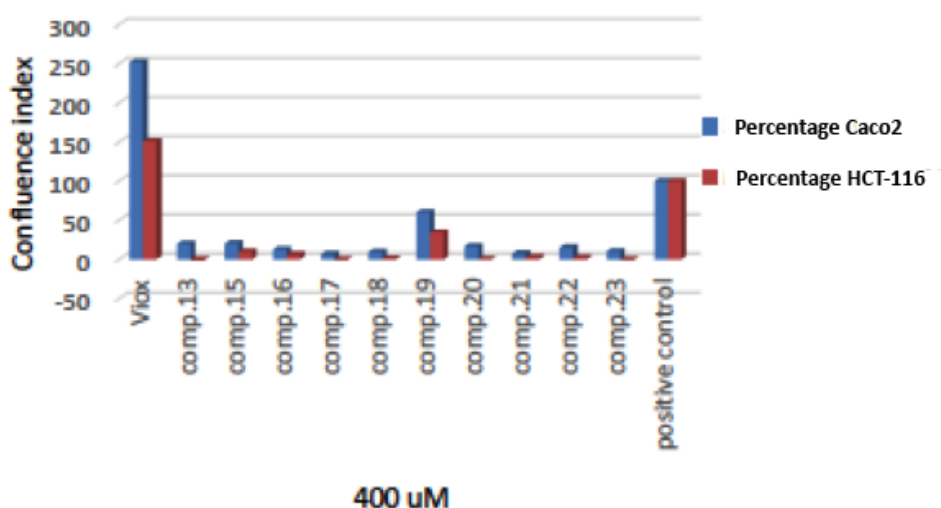


Figure 23. Study #1- Comparative cell proliferation of HCT-116 and Caco-2 cell lines

4.3. 1 Introduction Fluofarma studies

A more in depth study was done by utilizing the services of the Fluofarma Services Company. Through this company a detailed quantitative and kinetic analysis of the incubation of the cells were completed at a concentration of 10 μ M and 400 μ M. The proliferation of cells was measured after 24, 48 and 96 hours. The percentage of lysed cells was calculated at 96 hours and the amount of live cells counted at 96 hours. Compounds in the medium that precipitated at a concentration of 400 μ M were excluded from the analysis. The culture medium used followed the guidelines of the American Type Culture Collection (ATCC) for HCT-116 cell line colorectal carcinoma. Staurosporine, originally isolated in 1977, is a natural product with demonstrated anti-cancer cell proliferation and induction of apoptosis activity [64, 65]. Due to its well-known anticancer properties, Staurosporine was used as the control for the experiments carried out by Fluofarma.

4.4 Cell proliferation analysis on HCT-116 at 10 μ M

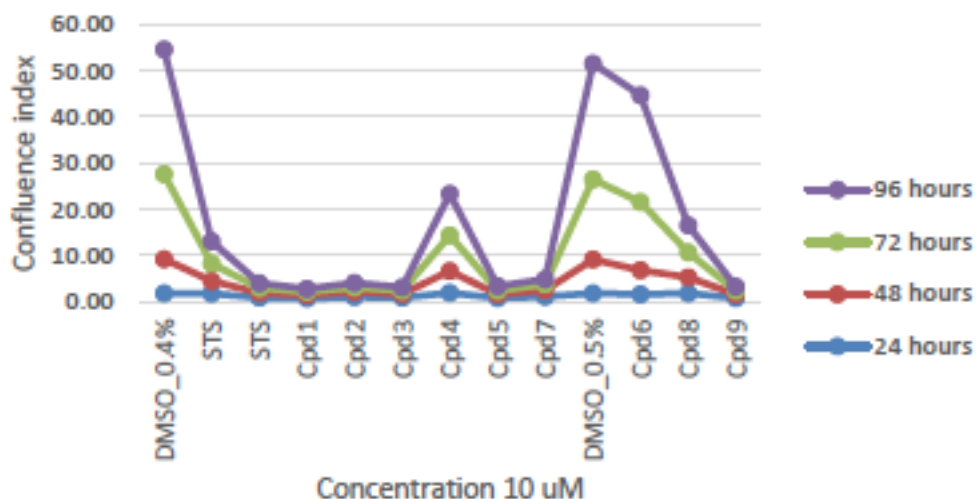


Figure 24. Study #1 - Confluence index on HCT-116 cells

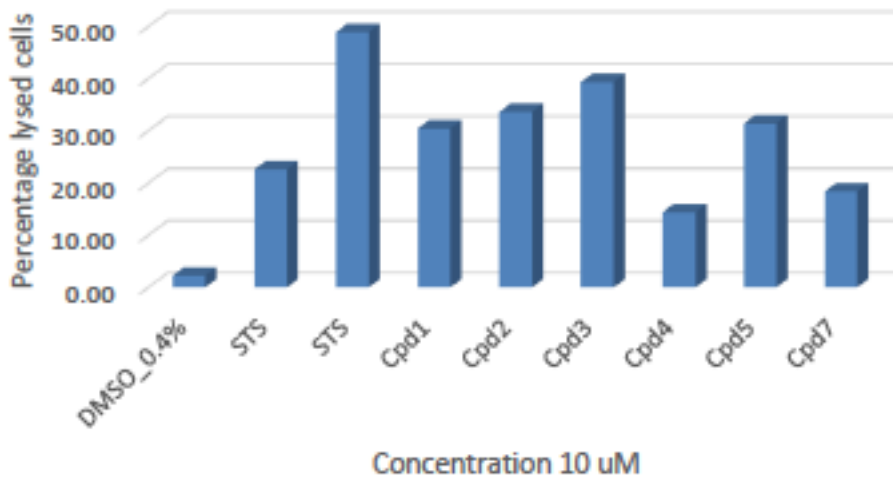


Figure 25. Study #1 - Percentage of lysed cells on HCT-116 cell lines

4.4.1 Kinetic Analysis

The ability of the cells to growth over time and to observe any indication of growth stunt at times was measured every hour. The compounds were compared against the

control group. As shown in Figure 26 the incubation with compound 15 did not inhibit the growth of the HCT-116 cells. These findings agree with the previously reported data.

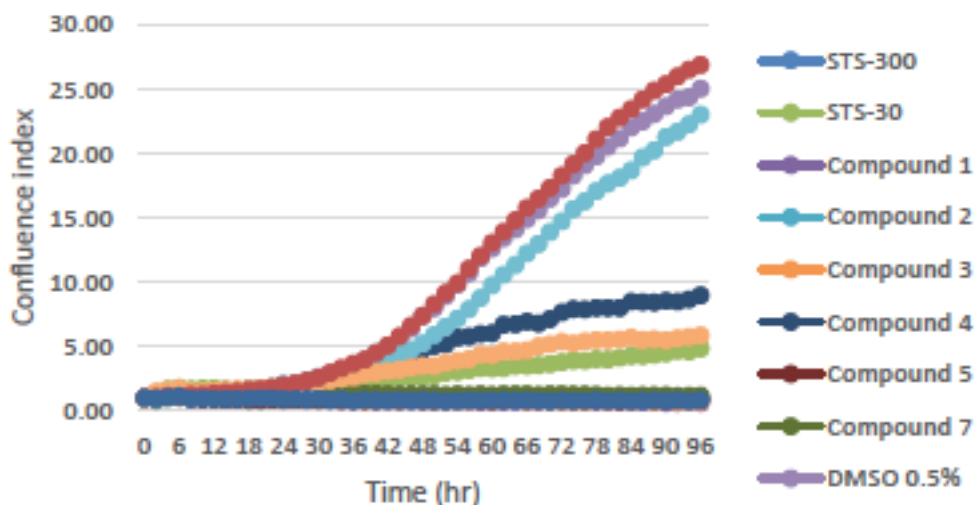


Figure 26. Study #1- Confluence index over time

The growth of cells using compound 15 is very similar to that of the control group. The other compounds were quite effective at stopping the proliferation of the cells at a concentration of 400 μ M and 10 μ M with some of the compounds showing promise at maybe working lower concentrations (in the nanomolar range). Compounds 17 and 16 showed an excellent effectiveness towards stopping the proliferations of cells. Compounds 21, were able to stop the proliferation of cells quite well, and after 48 hours of incubation a slow increase in cells occurs.

The effectiveness of compounds 21, show list was decreasing over time as we can see the proliferation of the cells increasing. Still it was quite effective when compared with the control group and the compounds were able to control the growth for at a ratio of three fold.

4.5 Modeling analysis of compounds with COX-2 enzyme

A COX-2 enzyme was downloaded from the protein database bank and modified as explained in the AutoDock section of the methodology chapter. The enzyme was selected based on completeness of protein structure. A grid calculation was made on the

active site of the enzyme and the compound was modeled against the active site. The torsion bonds of the compounds were selected before performing the molecular modeling analysis. The flexibility of the amino acid residues in the protein was taken into consideration when running the docking models.

Each docking experiment was derived from 100 different runs that were set to terminate after a maximum of 2,500,000 energy evaluations. The population size was set at 150. Translational, quaternion and torsion steps were used as default method of 0.2 Å, 5 and 5, respectively. The free energy of binding of each conformation of the compounds against the enzyme was calculated with units of kcal/mol, together with the energy of the van der Waals, hydrogen bonding and desolvation energy forces. The amino acid residues that interacted with the compound were calculated as hydrogen bonding type interactions, hydrophobic interactions, halogen and other kind of interactions.

Compounds with identification codes of SO₂CH₃CH₃Cl and SO₂NH₂CH₃Cl and IUPAC names of (E)-3-(3-chloro-4-methylphenyl)-1-(4-methylsulfonyl)prop-2-en-1-one and (E)-4-(3-(3-chloro-4-methylphenyl)acryloyl)benzenesulfonamide respectively; showed the lowest amount of energy binding with -6.65 Kcal/mol for both of them. The van der Waals, hydrogen bond and desolvation energy of these compounds was calculated as -7.67 Kcal/mol and -8.04 Kcal/mol for SO₂CH₃CH₃Cl and SO₂NH₂CH₃Cl, respectively.

An in-depth analysis of the interaction of the kind of interactions of the amino acids with the compounds was made. All interaction data from all of the compounds can be found in the Appendix. The compound with identification code SO₂CH₃CH₃Cl showed hydrophobic, halogen and other types of interactions. For better identification and interpretation, each compound was labeled with a number from 1 through 22. Please refer to Figures 27 and 28 to follow the explanation of the interactions of the compound to follow. Carbon 7 and carbon 6 showed a hydrophobic interaction with Tyr 55, tyrosine, a non-polar amino acid with a hydroxyl group; while carbon 5, carbon 17 and sulfur 16 had a hydrophobic interaction with Pro 35, proline, a non-polar amino acid. The halogen Cl with numbering 1 has a halogen interaction with Ala 33, alanine, a non-

polar amino acid. Other interactions occur with amino acids. For example, Ser 38, serine, a polar uncharged amino acid interacts with C1. Gln 54, glutamine, is a polar uncharged amino acid that interacts with C10 and O9. Lys 56, lysine, is a polar, positively charged amino acid that interacts with O 19. GLU 67, glutamic acid, is a polar charged amino acid that interacts with C15, C13, C14 and O18.

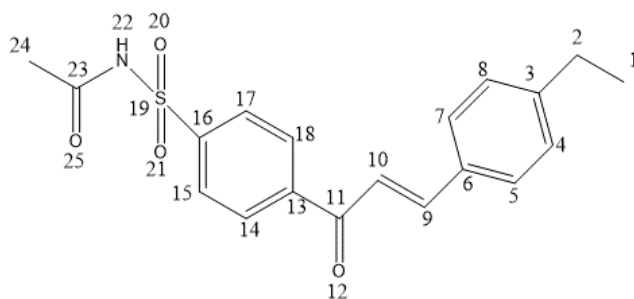


Figure 27. Schematic Representation of Labeled Atoms of SO₂NHCOCH₃CH₂CH₃ Compound

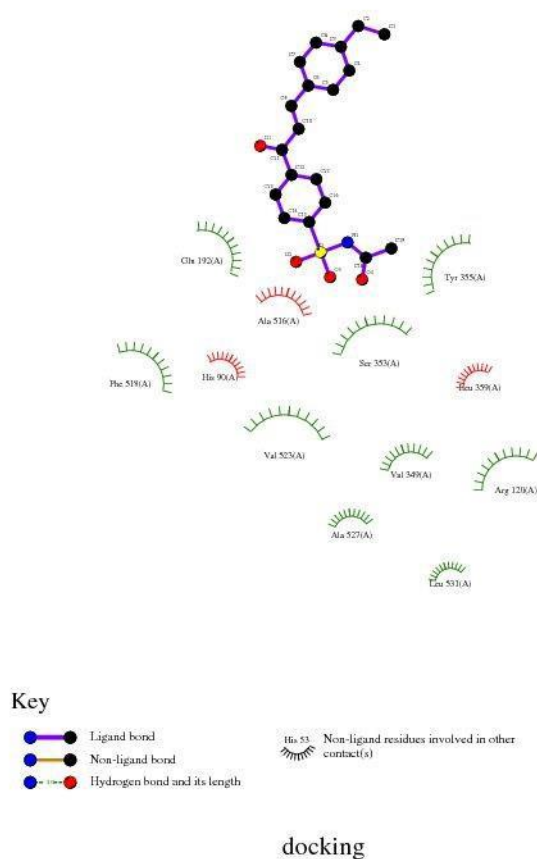


Figure 28. Study #1- Docking image SO₂NHCOCH₃CH₂CH₃

From the main interactions it is observed that all of the interactions occurred with hydrophobic residues. These data will be compared with the data from study 2 and 3 to observe any patterns of behavior.

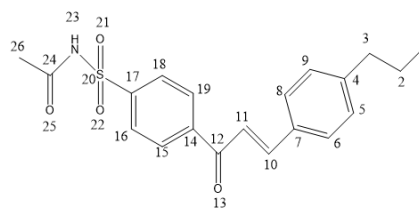


Figure 29. Schematic Representation of Labeled Atoms of SO₂NHCOCH₃CH₂CH₃

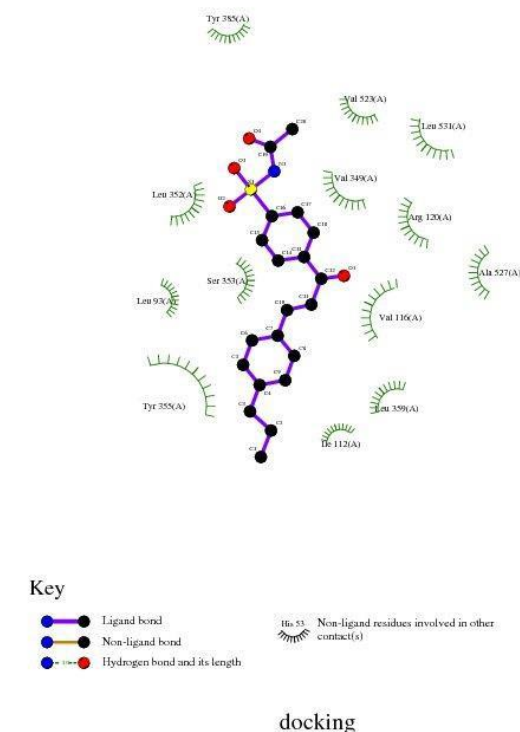


Figure 30. Study #1 - Docking image of SO₂NHCOCH₃CH₂CH₂CH₃

Interactions of the compound with identification code SO₂NH₂CH₃Cl were studied in depth, as this compound also showed a low free energy binding similar to that of the compound with identification code SO₂CH₃CH₃Cl. The energy interactions of this compound occur between the hydrogen bond of the nitrogen (N 17) with Tyr 55. Observation of the crystal packed asymmetric structure of this compound reveals that it forms hydrogen bonds between the hydrogen of the nitrogen with the oxygen of the sulfoxide group. Hydrophobic interactions between the ligand and the enzyme took place with the amino acids Pro 35, proline a non-polar amino acid and Tyr 55. C5, C15 and C16 had a hydrophobic interaction with Pro 35 and C7 and C6 had a hydrophobic interaction with Tyr 55. As with the previous compound, Ala 33, which had a halogen interaction with chlorine (Cl). Other interactions occur with amino acids Ser 38 and C1.

Amino acid Glu 67 and O 18, C 13, C 14; amino acid Lys 56 and N 1, H 12, H 13; lastly amino acid Gln 54 and C 8, C 10, O 17.

4.7 Study # 2

4.7.1 Brief description

In the second study, different electron donor groups (F, Cl, OCH₃, OBenzyl) were added to a derivate of chalcone with two aryl groups that holds the innovative pharmacophore group SO₂NHCOCH₃ . The purpose of this study was to observe the effect of different electron donors groups using the pharmacophore group SO₂NHCOCH₃ on the selectivity of COX-2 enzyme and apoptosis of colon cancer apoptosis of colon cancer cells. A total of four compounds were synthesized. The identification codes of the synthesized compounds are SO₂NHCOCH₃F, SO₂NHCOCH₃Cl, SO₂NHCOCH₃OCH₃, SO₂NHCOCH₃OBenzyl and IUPAC names of (E)- N – ((4 – (3-(4-fluorophenyl) acryloyl) phenyl) sulfonyl) acetamide; (E)- N – ((4 – (3-(4- chlorophenyl) acryloyl) phenyl) sulfonyl) acetamide; (E)- N – ((4 – (3-(4- methoxyphenyl) acryloyl) phenyl) sulfonyl) acetamide; (E)- N – ((4 – (3-(4- (benzyloxy)phenyl) acryloyl) phenyl) sulfonyl) acetamide respectively. Compound with identification code SO₂NHCOCH₃OCH₃ and IUPAC name (E)- N – ((4 – (3-(4- methoxyphenyl) acryloyl) phenyl) sulfonyl) acetamide was not initially analyzed due to accidental contamination on the day the compounds were being prepared for analysis, but all the compounds were successfully synthesized, purified and crystallized. Information on the synthesis and purification of the materials can be found in the methodology chapter, section Study#3 reactions, SO₂NH₂ synthesis, Acetate synthesis, OH synthesis and aldol reaction (Claisen Schmidt reaction). The compounds were characterized using ¹H NMR, ¹³C NMR, ESI- Mass Spectrometry, crystallography and melting point analysis. All the spectra and data can be found in the Appendix. The results for the more effective compounds are discussed here.

The compounds were initially tested at a concentration of 400 µM for colon

cancer cell lines Caco-2 and HCT-116. Caco-2 expresses the enzyme COX-2, while HCT-116 is a colon cancer cell line that doesn't express COX-2. Both cell lines were put to the test to see if there was any indication that cancer cell lines expressing COX-2 were going to be more effectively killed or stopped. All the compounds were demonstrated to be effective at stopping cell proliferation and killing cancer cells at the 400 μ M concentration. A more detailed quantitative study was done through Fluofarma, to verify the previous calculated results and to determine the effectiveness of the compounds at a low concentration. The analysis was done for HCT-116 cell line. It is recommended that the Caco-2 cell line be done in a future study. The quantitative analysis was done over periods of 24, 48 and 96 hours. The percentage of lysed cells was calculated at 96 hours. "Lysed cells" refers to cytolysis analysis in which the cell membrane erupts. For more information on cytolysis analysis refer to the methodology chapter, cytoanalysis section of this work.

Molecular modeling analysis of the compounds was done using AutoDock and docking server which integrated AutoDock modeling software with other programs [57, 58, 59, 60, 61]. For more information on molecular docking analysis, refer to the Methodology chapter, section modeling analysis.

This analysis estimated the energy binding of the compounds together with different electrostatic interactions like hydrogen bonding, hydrophilic interactions, hydrophobic interactions, among others. The results of the cell analysis were compared with the docking models results to look for possible explanations, behaviors, and if there is any relationship between COX-2 selectivity and anti-cancer activity. A small trial on enzyme selectivity for the COX-1/COX-2 compound was done at 40 μ M.

4.7.2 Cell proliferation analysis on Caco-2 and HCT-116 cell line at 400 μ M

Figures 31 and 32 show the cell proliferation of Caco-2 cell lines. Once again, the Caco-2 expresses the COX-2 enzyme and HCT-116 cell does not. In this initial analysis the compounds were incubated for 96 hours at a concentration of 400 μ M. In Figure 31 it is seen that the compounds were effective at stopping the cell proliferation

of the cells on both cell lines. However, the compounds were more effective on HCT-116 cell line than Caco-2 cell line. Compounds 11 and 14 with identification codes of $\text{SO}_2\text{NHCOCH}_3\text{Cl}$ and $\text{SO}_2\text{NHCOCH}_3\text{OBenzyl}$ were very effective at stopping the cell proliferation of the cancerous cells. Compound 12 with identification of $\text{SO}_2\text{NHCOCH}_3\text{F}$ was not very effective at stopping the proliferation of the cancerous cells after 96 hours of incubation. The electronegativity of the electron donor F is 3.98 while the electronegativity of the electron donor Cl is 3.16. The high electronegative chlorine (Cl) was able to stop the proliferation of the cells much more efficiently than the more electronegative F. Obviously, there was a difference in interaction of this compound with the COX-2 enzyme and with cancerous cells lines Caco-2 and HCT-116. This was between compounds with identification code $\text{SO}_2\text{NHCOCH}_3\text{F}$, $\text{SO}_2\text{NHCOCH}_3\text{Cl}$, and $\text{SO}_2\text{NHCOCH}_3\text{OBenzyl}$.

The molecular modeling docking interaction of the compounds against the COX-2 enzyme was studied and compared for differences and to see how slight changes in electronegative donors affected the effectiveness of a particular compound. The protein COX-2 was taken from the protein databank. More information about the docking analysis and the protein can be found in the modeling analysis section.

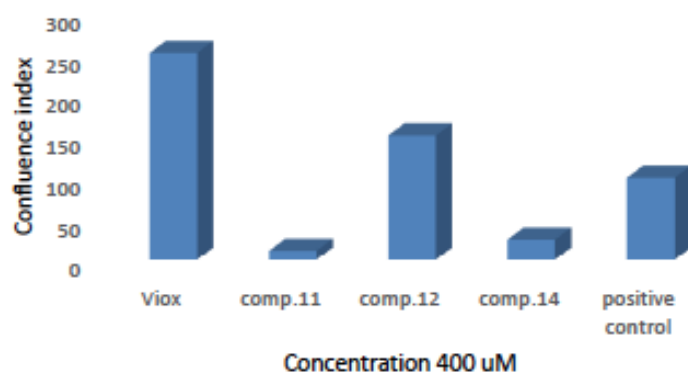


Figure 31. Study #2- Confluence index after 96 hours of incubation on Caco-2 cell line

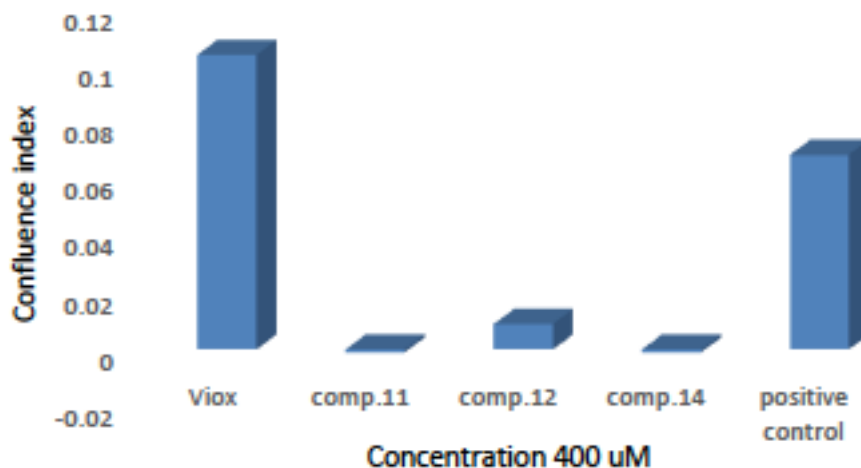


Figure 32. Study #2- Confluence index after 96 hours of incubation on Caco-2 cell line

Viox, a very effective COX-2 inhibitor didn't demonstrate any sign of inhibiting the cell proliferation of the cells HCT-116 and Caco-2 at a concentration of 100 µM. The concentration of 100 µM was selected due to the proven effectiveness as an anticancer agent demonstrated in other studies.

4.8 Comparative study of cell proliferation analysis between cell lines HCT-116 and Caco-2 at 400 µM

A comparative study between Caco-2 colon rectal cancer cells which expresses COX-2 enzyme and HCT-116 colon rectal cancer cells which doesn't express COX-2 enzyme was performed at a concentration of 400 µM. Quantitative and kinetic analysis of Caco-2 at a lower concentration was not performed through Fluofarma Company at the moment due to limited time and funding.

From Figure 33, it is clear that the compounds are effective at stopping the cell proliferation of both cells, but were more effective at stopping the proliferation of HCT-116 than Caco-2 cells. Compound 2 and compound 8 with identification codes of $\text{SO}_2\text{CH}_3\text{CH}_3\text{Cl}$ and $\text{SO}_2\text{CH}_3\text{CH}_3\text{F}$, respectively were not able to stop the proliferation of Caco-2 cells as well as compound 9, 6, 7 and 3 with identification codes of $\text{SO}_2\text{NHCOCH}_3\text{CH}_3\text{Cl}$, $\text{SO}_2\text{NH}_2\text{CH}_3\text{CH}_3$, $\text{SO}_2\text{NH}_2\text{CH}_3\text{Cl}$ and $\text{SO}_2\text{NH}_2\text{CH}_3\text{F}$.

Compounds 4 and 5 with identification codes of $\text{SO}_2\text{CH}_3\text{CH}_3\text{F}$ and $\text{SO}_2\text{NH}_2\text{CH}_3\text{OCH}_3$ respectively, also were not very effective towards Caco-2 cells. All of these compounds were effective at stopping the proliferation of HCT-116. Vioxx, the NSAID compound used as the control did not show any effectiveness at stopping the proliferation of the cells. This could be due to the variable effectiveness of non-steroidal compounds on different cancer cells.

Our data nullifies the hypothesis that COX-2 expressing tumor cancer cells are more likely to shrink in size and be able to avoid metastasis if selective COX-2 NSAID inhibitors are used. The compounds designed with known COX-2 pharmacophore groups that would tightly bind to the active site of the enzyme, didn't show any selectivity towards cancer cells that express COX-2 enzyme. The compounds however, showed efficacy at stopping cell proliferation and killing the cells on both cell lines. The percentage of cells that underwent cytolysis analysis was not high (around 20%).

To further look for any relationship between COX-2 enzyme and selectivity of the compounds towards the enzyme, understand the binding, and any kind of interactions that may occur, a molecular modeling analysis was made using different kinds of software programs. The software programs are discussed in more detailed in the AutoDock section of the methodology chapter.

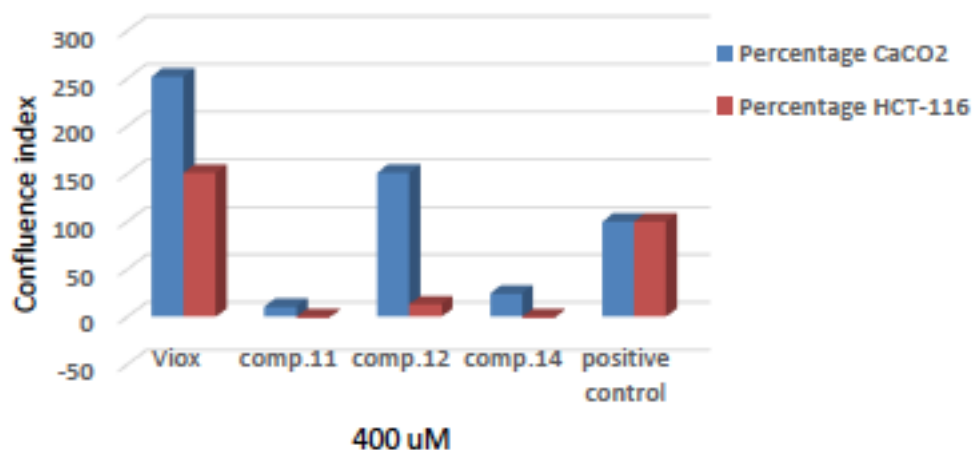


Figure 33. Study #2- Comparative cell proliferation study between HCT-116 and Caco-2 cell lines

4.8.1 Introduction Fluofarma studies

A more in-depth study was done through the Fluofarma Services Company. With this company a detailed quantitative and kinetic analyses of the incubation of the cell was done at a concentration of 10 μM and 400 μM . The proliferation of cells was measured at 24, 48 and 96 hours. The percentage of lysed cells was calculated at 96 hours and the amount of cells counted at 96 hours. Compounds that precipitated at a concentration of 400 μM in the medium were excluded from the analysis. The culture medium used followed the guidelines of the American Type Culture Collection (ATCC) for HCT-116 cell line colorectal carcinoma. Staurosporine, originally isolated in 1977, is a natural product with demonstrated anti-cancer cell proliferation and induction of apoptosis activity [64.65]. Due to its well-known anticancer properties, Staurosporine was used as a control for the experiments carried out by Fluofarma.

4.8.1.1 Kinetic cell proliferation analysis on HCT-116 cell line at 400 μM and 10 μM

Figure 34 depicts the confluence index at 24, 48, 72, and 96 hours for compounds synthesized in the third study at a concentration of 10 μM . The compounds were tested against HCT-116 cell line. Effectiveness of the compounds against the Caco-2 cell line was not tested. From Figure 34 it is clear that compounds 10, 11, 12 and 13 were very effective at stopping cell proliferation. These compounds are coded as: 10- $\text{SO}_2\text{NHCOCH}_3\text{OCH}_3$, 11- $\text{SO}_2\text{NHCOCH}_3\text{Cl}$, 12- $\text{SO}_2\text{NHCOCH}_3\text{F}$ and 13- $\text{SO}_2\text{NHCOCH}_3\text{OBenzyl}$; with IUPAC names of 10- (E)-N-((4-(3-(4-methoxyphenyl)acryloyl)phenyl)sulfonyl)acetamide, 11-(E)-N-((4-(3-(4-chlorophenyl)acryloyl)phenyl)sulfonyl)acetamide, 12-(E)-N-((4-(3-(4-fluorophenyl)acryloyl)phenyl)sulfonyl)acetamide, 13-(E)-N-((4-(3-(4-benzoyloxy)phenyl)acryloyl)phenyl)sulfonyl)acetamide.

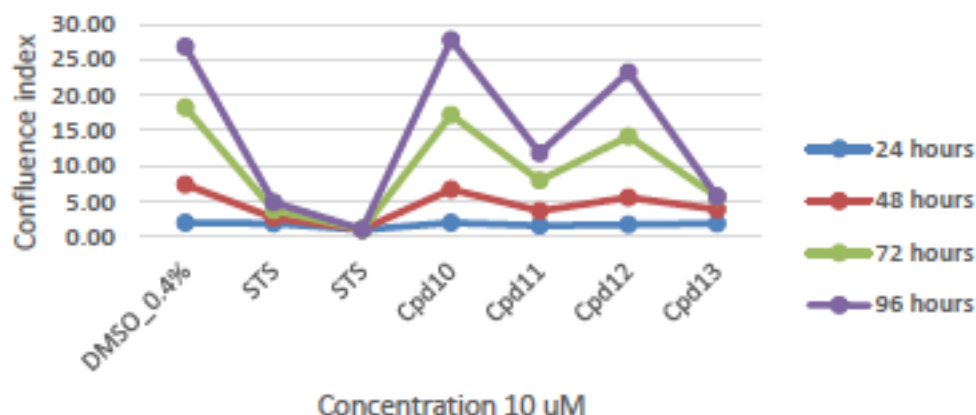


Figure 34. Study #2- Confluence index of compounds on HCT-116 cell line at different hours

Unlike the results found in the initial analysis after 96 hours of incubation, the compound with identification $\text{SO}_2\text{NHCOCH}_3\text{F}$ was not the less effective compound, but along with compound $\text{SO}_2\text{NHCOCH}_3\text{OCH}_3$, both compounds were not effective at stopping the proliferation of cells in HCT-116 cell line at a concentration of 10 μM . However, all the compounds were very effective at stopping the proliferation of the cells at 24 hours of incubation and fairly effective at 48 hours. After 48 hours the effectiveness of compounds 10 and 12 showed a decreased rate of killing the colon cancer cells of cell line HCT-116.

Results at concentrations higher than 10 μM , in the 250 μM or 400 μM are found in the Appendix. Results were not included for compounds that precipitated at these concentrations. The results agree with previous analysis realized by the student on HCT-116 carcinoma cell line at a concentration of 400 μM . All compounds were effective at stopping the cell proliferation at high concentration.

An analysis of the interactions of the compounds against the COX-2 enzyme was done to determine whether a relationship between interactions, inhibition of cell proliferation and or selectivity towards COX-2 could be found. Analysis at 400 μM for Caco-2 and HCT-116 cells did not show any selectivity towards the COX-2 enzyme. In

fact, the results showed that the compounds were effective at stopping the cell proliferation of both cancer cells Caco-2, which express the COX-2 enzyme and HCT-116, which does not express the COX-2 enzyme. Therefore, the compounds did not show any selectivity towards Caco-2 cells, which expresses the COX-2 enzyme. Furthermore, the compounds were able to stop cell proliferation of the HCT-116 cells more effectively than the proliferation of Caco-2 cells. The importance of performing the analysis to include the COX-2 enzyme is that the presence of COX-2 has been detected in more than 85% of the patients with colorectal cancer [66, 68, 69, 70], while patients with no colon tumors have undetectable levels of COX-2 [67]. This provides evidence that there is a correlation between expression of COX-2 in patients with colorectal cancer, although the expression of COX-2 can vary in different types of cancer.

Sulindac, a non-selective NSAID has proven to be effective towards colon rectal cancer [30] and it is believed that selective COX-2 inhibitors can be more effective towards colon cancer or other types of cancer. From the preliminary results of this work, it can be seen that the compounds are not selective towards the enzyme COX-2 as was expected. However, most of the compounds are effective at stopping the cell proliferation and at killing cancerous cells, thus showing that the compounds were very effective without being selective towards the COX-2 enzyme. Viox, a powerful non-steroidal compound selective towards COX-2, did not stop the cell proliferation. This finding shows the complexity of the mechanisms of apoptosis. In fact, several articles have shown contradictory evidence regarding the effectiveness of selective COX-2 inhibitors on the treatment against cancer.

However, regardless of whether COX-2 inhibitors can help in the processes of shrinking malign tumors or not, it is of common agreement an immediate and effective cure is needed.

4.8.1.2 Cytolysis of HCT-116 at 10 μ M

To better understand the process by which the cells go into apoptosis (programmed cell death), the percentage of cells in which the membrane erupted due

osmotic imbalance or cytolysis was calculated from the dead cells after 96 hours of incubation. The results in Figure 35 show that although the compounds were effective at stopping cell proliferation and killing of the cells, the cytolysis percentage calculated from the dead cells was not high. Compounds 10 and 12 resulted with less than 10% of lysed cells. The identification code of compounds 10 and 12 is $\text{SO}_2\text{NHCOCH}_3\text{OCH}_3$ and $\text{SO}_2\text{NHCOCH}_3\text{F}$, respectively. Compound 11 and compound 13 were able to induce cytolysis in close to 20% and around 25% percent of the cells respectively. The identification code of compounds 11 and 13 is $\text{SO}_2\text{NHCOCH}_3\text{Cl}$ and $\text{SO}_2\text{NHCOCH}_3\text{Obenzene}$, respectively.

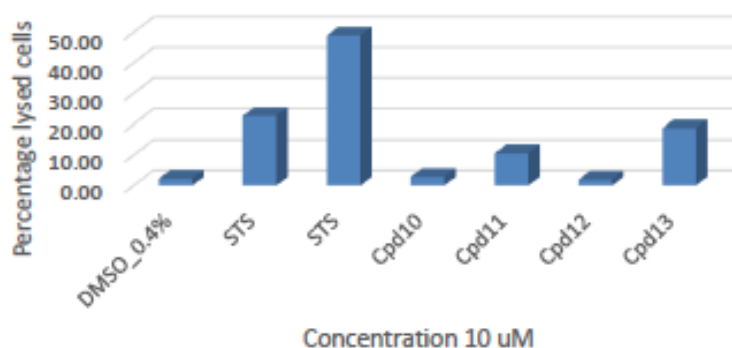


Figure 35. Study #2- Percentage of lysed cells on HCT-116 cell line after 96 hours of incubation

As stated previously, the compound with identification $\text{SO}_2\text{NHCOCH}_3\text{OCH}_3$ was not analyzed due to an accident right before the analysis. This compound will be analyzed in the future. Other reason to discriminate the compounds from analysis was precipitation of the compound at the measured concentration.

4.8.2 Kinetic analysis

The ability of the cells to growth over time and to observe any indication of growth stunting at a given time of cell growth was measured every hour. The compounds were compared against the control group. Figure 26 shows agreement with previously reported results. The incubation with compound 15 did not inhibit the growth of the

HCT-116 cells.

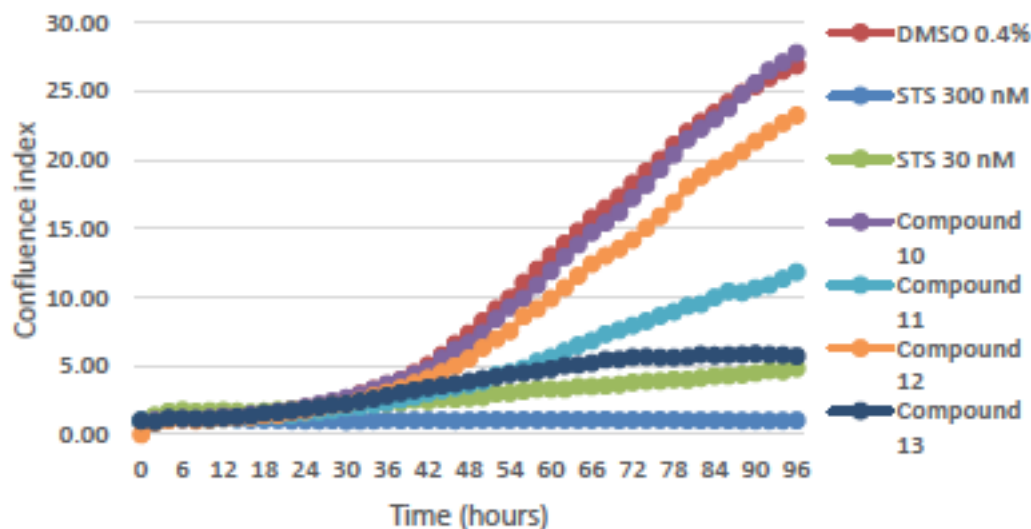


Figure 36. Study #2- Confluence index over time on HCT-116 cell line

The growth of cells using compound 15 is very similar to that of the control group. The other compounds were quite effective at stopping the proliferation of the cells at a concentration of 400 μ M and 10 μ M. Compounds 17, 16 compounds showed an excellent effectiveness towards stopping the proliferation of cells. Compound 21, was able to stop the proliferation of cells quite well and after 48 hours of incubation period a slow increase of cells is observed. Even though the effectiveness of compounds 21, show decreased over time, it was still quite effective, in comparison to the control group, to control the growth for a ratio of three fold.

4.9 Modeling analysis of compounds with COX-2 enzyme

A COX-2 enzyme was downloaded from the protein database bank and modified as explained in the AutoDock section of the methodology chapter. The enzyme was selected based on completeness of the protein structure along with other. A grid calculation was made on the active site of the enzyme and the compound was modeled against the active site. The torsions bonds of the compounds were selected before

performing the molecular modeling analysis. The flexibility of the amino acid residues in the protein was taken in consideration when running the docking models also.

Each docking experiment was derived from 100 different runs that were set to terminate after a maximum of 2500000 energy evaluations. The population size was set at 150. Translational, quaternion and torsion steps were used as default method of 0.2 Å, 5 and 5, respectively. The free energy of binding of each conformation of the compounds against the enzyme was calculated with units of kcal/mol, together with the energy of the van der Waals interaction, hydrogen bond and desolvation energy forces. The amino acids residues that interacted with the compound were calculated as hydrogen bond type of interaction, hydrophobic interaction, halogen and other kind of interaction.

Compound with identification code of SO₂NHCOCH₃F and IUPAC name of (E)-N-((4-(3-(4-fluorophenyl)acryloyl)phenyl)sulfonyl)acetamide showed the lowest amount of energy binding with -6.42 Kcal/mol. The van der Waals, hydrogen bond and desolvation energies of this compound was calculated as -7.83 Kcal/mol.

An in-depth analysis of the interaction of the kind of interactions of the amino acids with the compounds was made. All interaction data from all of the compounds can be found in the Appendix. Compound with identification code SO₂NHCOCH₃F showed hydrophobic, halogen, hydrogen bonding, π - π and other types of interactions. For better identification and interpretation, each compound was labeled with a number from 1 through 22. Please refer to Figure 37 while referring to the explanation of the interactions. Oxygen 7 showed a polar interaction with Ser 38, serine a polar uncharged amino acid; while nitrogen 5 formed hydrogen bonding with amino acid Pro 35, proline a non-polar amino acid. Carbon 9, 10 and 11 had a hydrophobic interaction with Tyr 55, tyrosine, a non-polar amino acid with a hydroxyl group. The carbon in position 1 also had a hydrophobic interaction with Tyr 55. The halogen F with numbering 22 had a halogen interaction with Glu 67, glutamic acid a polar charged amino acid. Carbons 4, 5, 6, 7, 8, 13 and 14 experienced a π - π bond interaction with amino acid Tyr 55. A π interaction is one in which the atomic orbitals overlap. A π - π bond interaction occurs when already overlapped orbitals from different compounds interact together. Other

types of interactions were experienced with amino acid Ser 38, and oxygens in position 6 and 7, carbon in position 2 also interacted with Ser 38. More interactions include Tyr 55, tyrosine with carbon 13, Lys 56, lysine with the halogen F, Glu 67, glutamic acid with carbons 11, 12, 13 and 14 and Val 156, valine with oxygen 3.

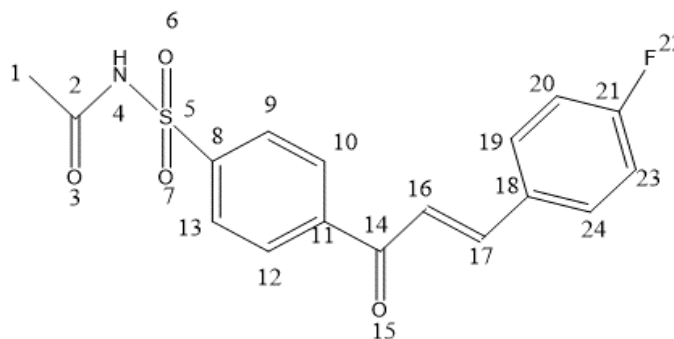


Figure 37- Schematic Representation of Labeled Atoms of SO₂NHCOCH₃F Compound

The main interactions of hydrogen bonding, polar, hydrophobic interactions and halogen interaction occur with a variety of non-polar and polar uncharged amino acids; contrary to the compounds of study # 3 that all interacted with non-polar amino acids. However, the same amino acids at the same position are the ones that are interacting with the compounds. For example: Tyrosine (Tyr) in position 55 is the one that interacts with the compounds, not another Tyrosine (Tyr) at a different position. The same behavior is observed with the other amino acids, giving us an idea of the amino acids in the active site with their respective positions. The interaction energies of hydrogen bond, halogen bond, hydrophobic and polar interactions were calculated in units of kcal/mol. The energy of the hydrophobic interaction with Cys 37 and Tyr 55 is -0.2684 and 1.1523 respectively. The hydrogen bond energy interaction was calculated to be -0.4612 with Pro 35. The halogen bond interaction with Glu 67 was calculated to be -1.4596. The polar interaction of Ser 38 was calculated to be -0.4679.

A docking diagram that shows the discussed interactions in the above paragraph

is shown below. From this figure the hydrogen bonding of Pro 35 and nitrogen and the other amino acids around the compound are clear.

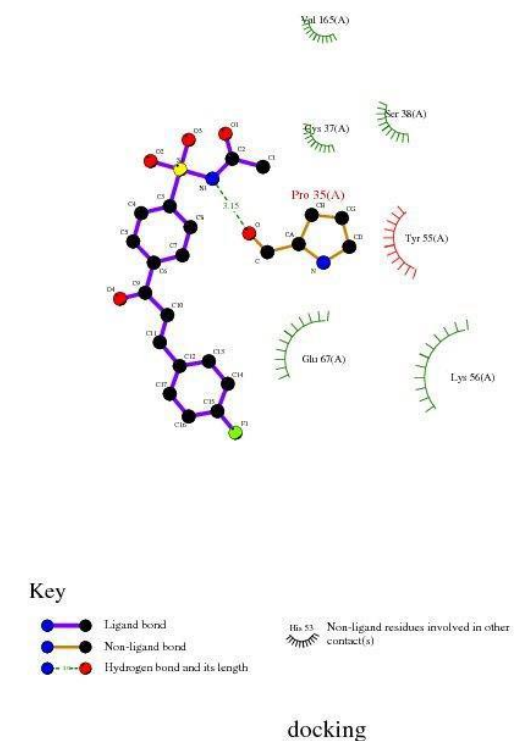


Figure 38. Study #2 -Docking image

A comparison of the interactions among all the compounds from all the studies will be discussed on the comparative section to look for any relationship between type of interaction and amino acid and elements that are most likely to have certain kind of interaction.

4.10 Study # 3

4.10.1 Brief description

In the third study, it was decided to add electron donor groups on the third position and a methyl group on the fourth position of the benzyl group. The purpose of this study was to observe the effect of different electron donor groups with a methyl on the fourth position and to see if there is any difference with the compounds with one electron donor group with no other substituent. A total of twelve compounds with different substituents were studied, four from each selective pharmacophore (SO_2CH_3 , SO_2NH_2 , $\text{SO}_2\text{NHCOCH}_3$) with its respective electron donor groups Cl, F, OCH_3 , CH_3). All compounds were successfully synthesized and purified, for more details on the synthesis process and purification please refer to the Methodology chapter, study #3. The compounds were characterized using ^1H NMR, ^{13}C NMR, ESI- Mass Spectrometry, crystallography and melting point analysis. All the spectra and data can be found in the Appendix. Data of the more effective compounds is discussed here.

The compounds were initially tested at a concentration of 400 μM for colon cancer cell lines Caco-2 and HCT-116. Caco-2 constitutively expresses the enzyme COX-2, while HCT-116 is a colon cancer cell line that doesn't express COX-2. Both cell lines were put to the test to see if there was any indication that cancer cell lines expressing COX-2 were going to be more effectively kill or stop the cell proliferation. All the compounds demonstrated to be effective at stopping cell proliferation and killing cancer cells at a concentration of 400 μM . A more detailed quantitative study was done through Fluofarma, to verify the previous calculated results and to determine the effectiveness of the compounds at a low concentration. The analysis was done for HCT-116 cell line, Caco-2 cell line will be done in a future study. The quantitative analysis was done over periods of 24, 48 and 96 hours. The percentage of lysed cells was calculated at 96 hours. "Lysed cells" refers to cytolysis analysis in which the cell membrane erupts.

Molecular modeling analysis of the compounds was done using AutoDock and

docking server which integrated AutoDock modeling software with other programs [57, 58, 59, 60, 61]. For more information on molecular docking analysis, refer to the Methodology chapter, section modeling analysis.

This analysis estimated the energy binding of the compounds together with different electrostatic interactions like hydrogen bonding, hydrophilic interactions, hydrophobic interactions, among others. The results of the cell analysis were compared with the docking models results to look for possible explanations, behaviors, and if there is any relationship between COX-2 selectivity and anti-cancer. A small trial on enzyme selectivity for the COX-1/COX-2 compound was done at 40 μ M.

4.10.2 Cell proliferation analysis on Caco-2 and HCT-116 cell line at 400 μ M

Figures 39 and 40 show the cell proliferation of cell lines Caco-2 that expresses COX-2 enzyme and HCT-116 cell that does not express COX-2 enzyme. On this initial analysis the compounds were incubated for 4 days or 96 hours at a concentration of 400 μ M. From Figures 39 and 40 it can be seen that the compounds were effective at stopping the cell proliferation of the cells on both cell lines. However, the compounds were more effective on HCT-116 cell line than Caco-2 cell line.

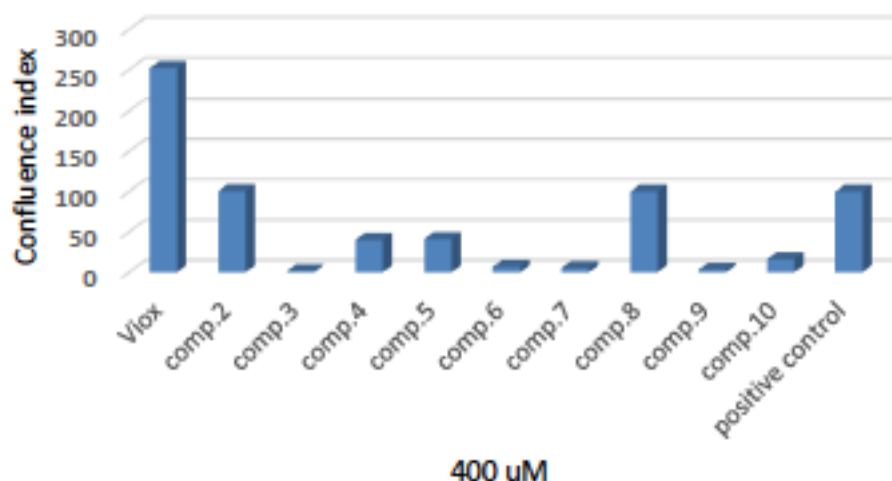


Figure 39. Study #3- Confluence index after 96 hours of incubation on cell line Caco-2

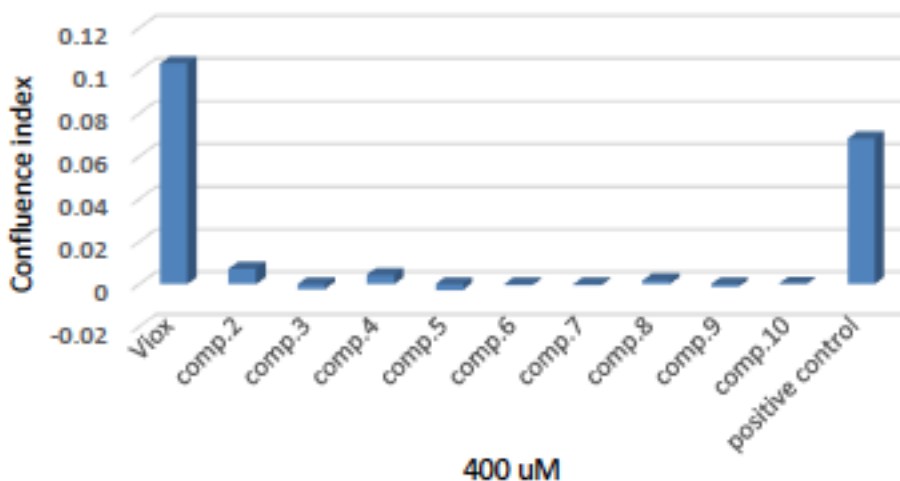


Figure 40- Study #3 - Confluence index after 96 hours of incubation on cell Line HCT- 116

As shown, for the HCT-116 cell line the proliferation of the compounds after 96 hours was zero percent. Viox, a very effective COX-2 inhibitor did not demonstrate any sign of inhibiting the proliferation of the HCT-116 and Caco-2 cells at a concentration of 100 μ M. The concentration of 100 μ M was selected due to the proven effectiveness as an anticancer agent demonstrated on other studies [99]. On Caco-2 cell line, compound two and eight didn't show any signs to inhibit the cell proliferation, further quantitative studies will be done to double check results. And compounds four and five were about fifty percent effective at a concentration of 400 μ M, with a percentage of 40 and 41 for compounds four and five respectively. Compounds three, six, seven, nine and ten are quite effective at inhibiting cell proliferation and killing the cells at the studied concentration of 400 μ M.

4.10.3 Introduction to Fluofarma studies

A more in-depth study was done through Fluofarma Services Company. With this company a detailed quantitative and kinetic analysis of the incubation of the cell was done at a concentration of 10 μ M and 400 μ M. The proliferation of cells was

measured at 24, 48 and 96 hours. The percentage of lysed cells was calculated at 96 hours and the amount of cells was counted at 96 hours. Compounds that precipitated at a concentration of 400 μ M in the medium were excluded from the analysis. The culture medium used following the guidelines of the American Type Culture Collection (ATCC) for HCT-116 cell line colorectal carcinoma. Staurosporine, originally isolated in 1977, is a natural product with demonstrated anti-cancer cell proliferation and induction of apoptosis activity [64.65]. Due to its well-known anticancer properties, Staurosporine was used as a control for the experiments carried out by Fluofarma.

4.10.3.1 Kinetic cell proliferation analysis on HCT-116 cell line at 400 μ M and 10 μ M

Figure 41 shows the confluence index at 24, 48, 72, and 96 hours for compounds in the third study at a concentration of 10 μ M. The compounds were tested against a HCT-116 cell line. From the figure it is clear that compounds 16, 17, 18, 20 and 24 were very effective at stopping the cell proliferation. These compounds are coded as: 16-SO₂NH₂CH₃Cl, 17- SO₂NH₂CH₃F, 18-SO₂NHCOCH₃CH₃Cl, 20-SO₂CH₃CH₃Cl and 24-SO₂CH₃CH₃F; with IUPAC names of 16- (E)-4- (3 – (3 – chloro-4-methylphenyl) acryloyl) benzenesulfonamide, 17- (E)- 4- (3 – (3 – fluoro-4-methylphenyl) acryloyl) benzenesulfonamide, 18- (E)-N-((4-(3-(3-chloro- 4-methylphenyl)acryloyl)phenyl) sulfonyl)acetamide, 20- (E)- 3- (3- chloro- 4-methylphenyl)- 1-(4-(methylsulfonyl) phenyl) prop-2-en-1-one, 24- (E)- 3- (3- fluoro-4-methylphenyl)-1-(4- (methylsulfonyl) phenyl)prop-2-en-1-one.

Compounds 19, 21 and 23 with identification codes of SO₂NHCOCH₃CH₃F, SO₂NHCOCH₃CH₃CH₃ and SO₂CH₃CH₃CH₃ showed very good anti cell proliferation activity, but they were not as effective as compounds 16, 17, 18, 20 and 24. The IUPAC names for these compounds are (E)- N- ((4- (3 – fluoro- 4- methylphenyl) acryloyl) phenyl) sulfonyl) acetamide, (E)- N- ((4- (3- (3,4 – dimethylphenyl) acryloyl) phenyl) sulfonyl) acetamide and (E)-3-(3, 4 – dimethylphenyl) -1- (4- (methylsulfonyl) phenyl) prop- 2 –en – 1-one respectively. Compound 15 with identification code of

SO₂NH₂CH₃OCH₃ and IUPAC name of (E) -4 – (3 – (4- methoxy- 3- methylphenyl) acryloyl) benzenesulfonamide did not show any anti-cell proliferation activity as shown on the results below. It compared with the control group. Compound 14 with identification code of SO₂NH₂CH₃CH₃ and IUPAC name for these compounds are (E) -4- (3-(3,4- dimethylphenyl) acryloyl) benzenesulfonamide showed some cell anti-proliferation activity but it was much less effective than compounds 16, 17, 18, 19, 20, 21 and 23.

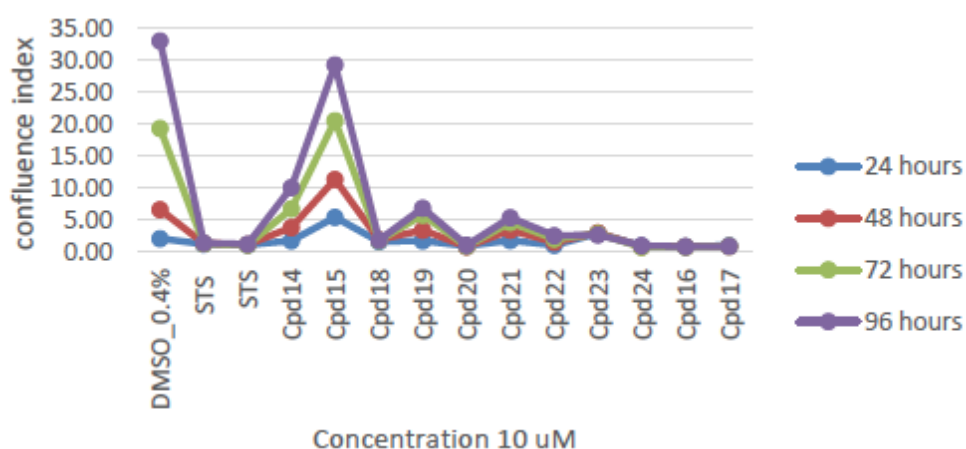


Figure 41. Study #3- Confluence index at different times on HCT-116 cell line

Analysis results at concentration higher than 10 µM, in the 250 µM or 400 µM are found in the appendix. Results were not included for compounds that precipitate at these concentrations. The results agree with previous analysis realized by the student on HCT-116 carcinoma cell line at a concentration of 400 µM. All the compounds were effective at stopping the cell proliferation at high concentration. The only compound not effective at stopping the cell proliferation at 10 µM was compound number 15, (E) -4 – (3 – (4-methoxy- 3- methylphenyl) acryloyl) benzenesulfonamide.

An analysis of the interactions of the compounds against the COX-2 enzyme was also done to look for any relationship between interactions, inhibition of cell

proliferation and or selectivity towards COX-2. Analysis at 400 μ M for Caco-2 and HCT-116 cells didn't show any selectivity towards COX-2 enzyme. In fact, the results showed that the compounds were effective at stopping the cell proliferation of both cancer cells Caco-2 that express the enzyme COX-2 and HCT-116 which doesn't express the enzyme COX-2. Therefore the compounds didn't show any selectivity towards Caco-2 cells which expresses the COX-2 enzyme. Furthermore, the compounds were able to stop the proliferation of the HCT-116 cells more effectively than the proliferation of Caco-2 cells. The importance of performing analysis which includes the enzyme COX-2, is because the presence of COX-2 has been detected in more than 85% of patients who present colorectal cancer [66,68, 69, 70], while patients with no colon tumors have undetectable levels of COX-2 [67]. Evidently, there is a correlation between expression of COX-2 in patients with colorectal cancer. Although, the expression of COX-2 can vary on different types of cancer throughout the body.

4.10.3.2 Cytolysis of HCT-116 at 10 μ M

To better understand the process by which the cells go into apoptosis programmed cell death, the percentage of cells in which the membrane erupted due osmotic imbalance or cytolysis was calculated from the death cells after 96 hours of incubation. These results are shown in Figures 42 and 43. Although the compounds were effective at stopping the cell proliferation of the cells and killing the cells, the cytolysis percentage calculated from the dead cells was not high. Compounds 14, 15, 19 and 21 resulted with less than 20% of lysed cells. The identification code of these compounds is $\text{SO}_2\text{NH}_2\text{CH}_3\text{CH}_3$, $\text{SO}_2\text{NH}_2\text{CH}_3\text{OCH}_3$, $\text{SO}_2\text{NHCOCH}_3\text{CH}_3\text{F}$, and $\text{SO}_2\text{NHCOCH}_3\text{CH}_3\text{CH}_3$, respectively. Compound 19 was able to increase the percentage of cytolysed cells by more than 40% at a concentration of 400 μ M, see results on appendix from 24 hours, while compound 21 showed a more gradual (slow) increase of cytolysis cells at 24 hours when using a concentration of 400 μ M. Compounds 14 and 15 with identification codes of $\text{SO}_2\text{NH}_2\text{CH}_3\text{CH}_3$ and $\text{SO}_2\text{NH}_2\text{CH}_3\text{OCH}_3$ were not

analyzed at 400 μ M because a precipitate formed while mixing the compounds with medium.

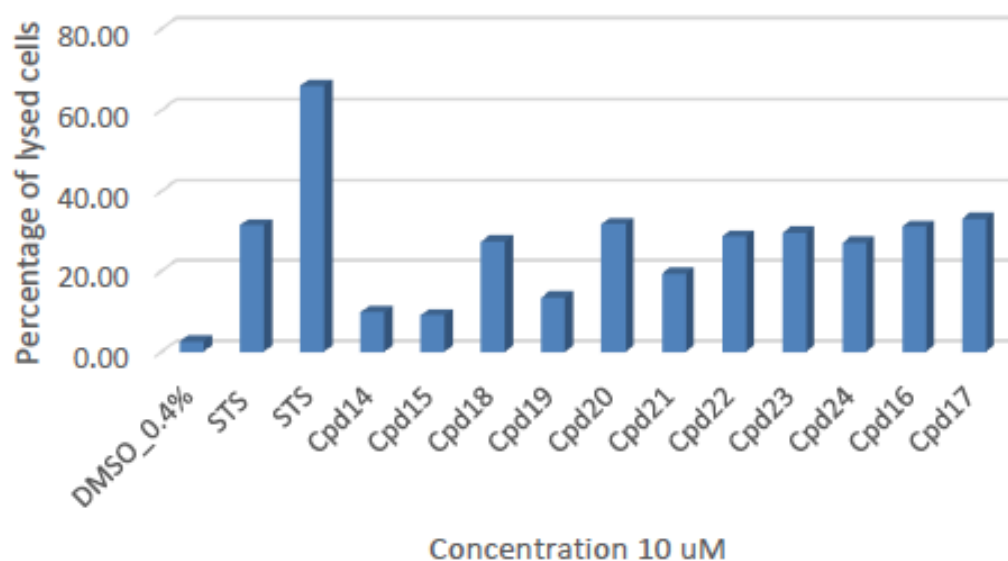


Figure 42. Study #3- Percentage of lysed cells on HCT-116 cell line after 96 hours of incubation

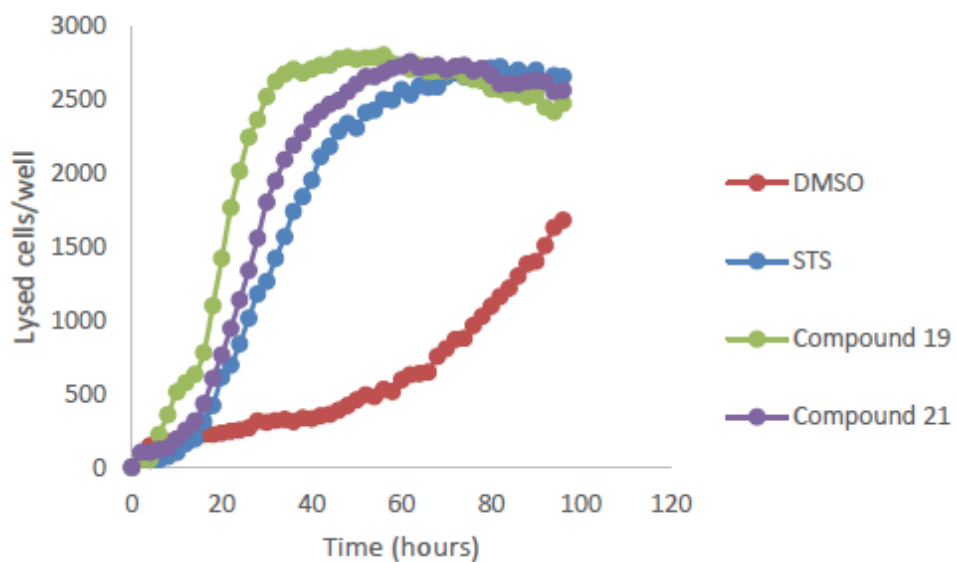


Figure 43. Study #3- Cytolysis over time of compounds 19 and 21

4.10.4 Kinetic Analysis

The ability of the cells to grow over time and the observation of any indication of growth stunt at certain amount of time were measured every hour. The compounds were compared against the control group. As seen in Figure 44 below the results agree with previously reported data that showed the incubation with compound 15 did not inhibit the growth of the HCT-116 cells.

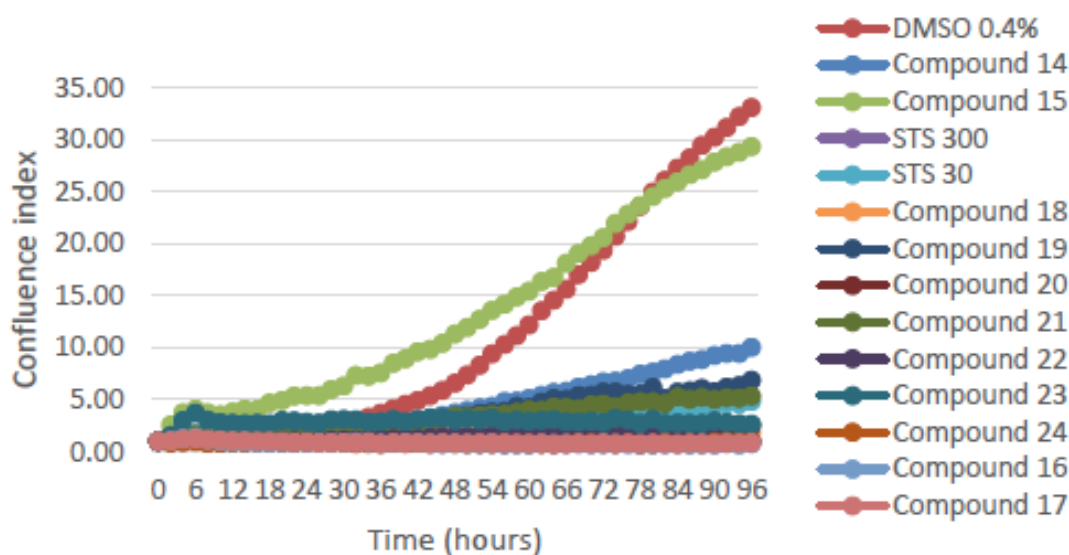


Figure 44. Study #3- Confluence index over time on HCT-116 cell line

The growth of cells using compound 15 is very similar to that of the control group. The other compounds were quite effective at stopping the proliferation of the cells at a concentration of 400 μ M and 10 μ M. Compounds 17, 16 list compounds showed an excellent effectiveness towards stopping the proliferations of cells.

Compounds 21, show were able to stop the proliferation of cells quite well and after 48 hours of incubation period slowed the increase in cells. Even though the effectiveness of the compounds 21 show decreased over time they were quite effective when compared with the control group, the compounds were able to control the growth.

4.11 Comparative study of cell proliferation analysis between cell lines HCT-116 and Caco-2 at 400 μ M

A comparative study between Caco-2 colon rectal cancer cells which expresses COX-2 enzyme and HCT-116 colon rectal cancer cells which does not express COX-2 enzyme was performed at a concentration of 400 μ M. Quantitative and kinetic analyses of Caco-2 at a lower concentration were not performed through Fluofarma company at the moment due to limited amount of time and limited funding but further studies will be done in a near future.

The results in Figure 45 show the compounds to effective in stopping the proliferation of both cells, but they were clearly more effective at stopping the proliferation of HCT-116 than Caco-2 cells. Compound 2 and compound 8 with identification codes of $\text{SO}_2\text{CH}_3\text{CH}_3\text{Cl}$ and $\text{SO}_2\text{CH}_3\text{CH}_3\text{F}$ respectively were not able to stop the proliferation of Caco-2 cells as wells as compound 9, 6, 7 and 3 with identification codes of $\text{SO}_2\text{NHCOCH}_3\text{CH}_3\text{Cl}$, $\text{SO}_2\text{NH}_2\text{CH}_3\text{CH}_3$, $\text{SO}_2\text{NH}_2\text{CH}_3\text{Cl}$ and $\text{SO}_2\text{NH}_2\text{CH}_3\text{F}$. Compounds 4 and 5 with identification codes of $\text{SO}_2\text{CH}_3\text{CH}_3\text{F}$ and $\text{SO}_2\text{NH}_2\text{CH}_3\text{OCH}_3$ respectively, also were not very effective towards Caco-2 cells. All of these compounds were effective at stopping the proliferation of HCT-116. Viox, the non-steroidal anti-inflammatory compound used as control did not show any effectiveness at stopping the proliferation of the cells. This could be due to the effectiveness a non-steroidal compound being able to act significantly differently from one non- carcenogenic cell to another.

Our data proves wrong the hypothesis written that states COX-2 expressing tumor cancer cells are more likely to be shrink in size and to be able to avoid metastasis if we used selective COX-2 non-steroidal anti-inflammatory drugs inhibitors. The compounds designed with known COX-2 pharmacophore groups that would tightly bind to the active site of the enzyme, didn't show any selectivity towards cancerogenous cells that express COX-2 enzyme. The compounds however, showed efficacy at stopping the cell proliferation and killing the cells on both cells line. The percentage of cells that

underwent cytotoxicity analysis was not high around 20%.

To further look for any relationship between COX-2 enzyme and selectivity of the compounds towards the enzyme, understand the binding, and any kind of interactions that may occur a molecular modeling analysis was made using different kinds of software programs. The software programs are discussed in more detailed in the AutoDock section of the methodology chapter.

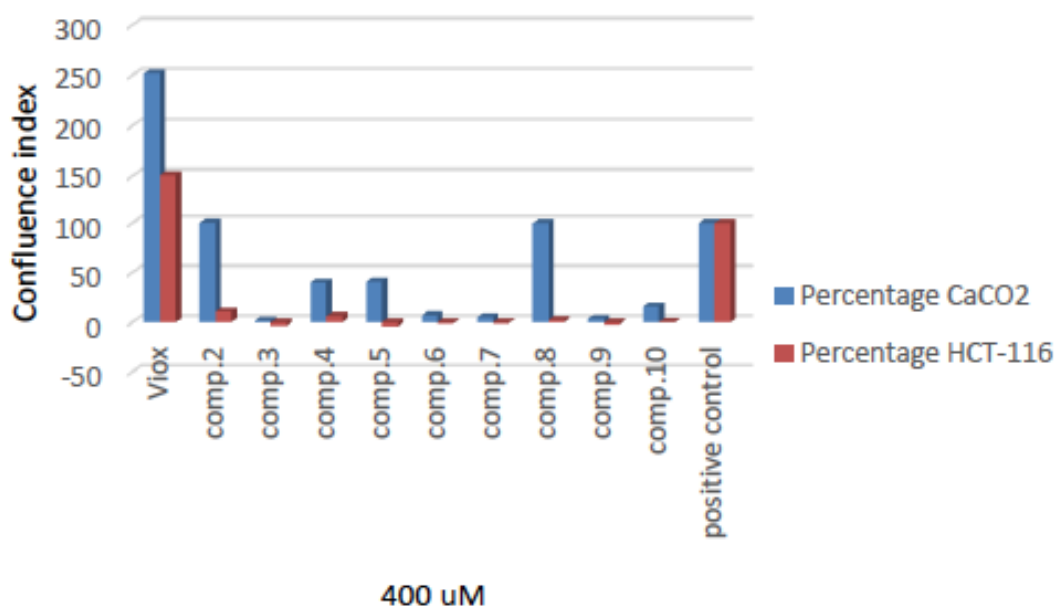


Figure 45. Study #3- Comparative cell proliferation analysis between HCT-116 and Caco-2 cell lines

4.12 Modeling analysis of compounds with COX-2 enzyme

A COX-2 enzyme was downloaded from the protein database bank and modified as explained in the AutoDock section of the methodology chapter. The enzyme was selected based on completeness of the protein structure along with other criteria. A grid calculation was made on the active site of the enzyme and the compound was modeled against the active site. The torsions bonds of the compounds were selected before performing the molecular modeling analysis. The flexibility of the amino acid residues

in the protein was taken in consideration when running the docking models also.

Each docking experiment was derived from 100 different runs that were set to terminate after a maximum of 2,500,000 energy evaluations. The population size was set at 150. Translational, quaternion and torsion steps were used as default method of 0.2 Å, 5 and 5 respectively. The free energy of binding of each conformation of the compounds against the enzyme was calculated with units of kcal/mol, together with the energy of the van der Waals, hydrogen bond and desolvation energy forces. The amino acids residues that interacted with the compound were calculated as hydrogen bond type of interaction, hydrophobic interaction, halogen and other kind of interaction.

Compounds with identification codes of $\text{SO}_2\text{CH}_3\text{CH}_3\text{Cl}$ and $\text{SO}_2\text{NH}_2\text{CH}_3\text{Cl}$ and IUPAC names of (E)-3-(3-chloro-4-methylphenyl)-1-(4-methylsulfonyl)prop-2-en-1-one and (E)-4-(3-(3-chloro-4-methylphenyl)acryloyl)benzenesulfonamide respectively; showed the lowest amount of energy binding with -6.65 Kcal/mol for both of them. The van der Waals, hydrogen bond and desolvation energy of these compounds was calculated as -7.67 Kcal/mol and -8.04 Kcal/mol for $\text{SO}_2\text{CH}_3\text{CH}_3\text{Cl}$ and $\text{SO}_2\text{NH}_2\text{CH}_3\text{Cl}$ respectively.

An in-depth analysis of the interactions with the different amino acids was made. All interaction data from all of the compounds can be found in the Appendix. The compound with identification code $\text{SO}_2\text{CH}_3\text{CH}_3\text{Cl}$ showed hydrophobic, halogen and other types of interactions. For better identification and interpretation each compound was labeled with a number from 1 through 22. Please refer to the figure below while explaining the interactions of the compound. Carbon 7 and carbon 6 showed a hydrophobic interaction with Tyr 55, tyrosine a non-polar amino acid with a hydroxyl group; while carbon 5, carbon 17 and sulfur 16 had a hydrophobic interaction with Pro 35, proline, a non-polar amino acid. The halogen Cl with numbering 1 has a halogen interaction with Ala 33, alanine, a non-polar amino acid. Other interactions occur with amino acids like Ser 38, serine a polar uncharged amino acid interacts with C1, Gln 54, glutamine a polar uncharged amino acid interacts with C10 and O9, Lys 56, lysine, a polar positively charged amino acid interacts with O 19 and GLU 67, glutamic

acid a polar charged amino acid that interacts with C15, C13, C14 and O18. For better visualization, see Figure 47.

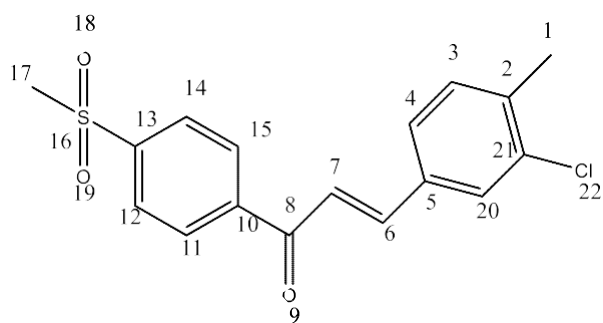


Figure 46. Study #3- Schematic representation of labeling of atoms

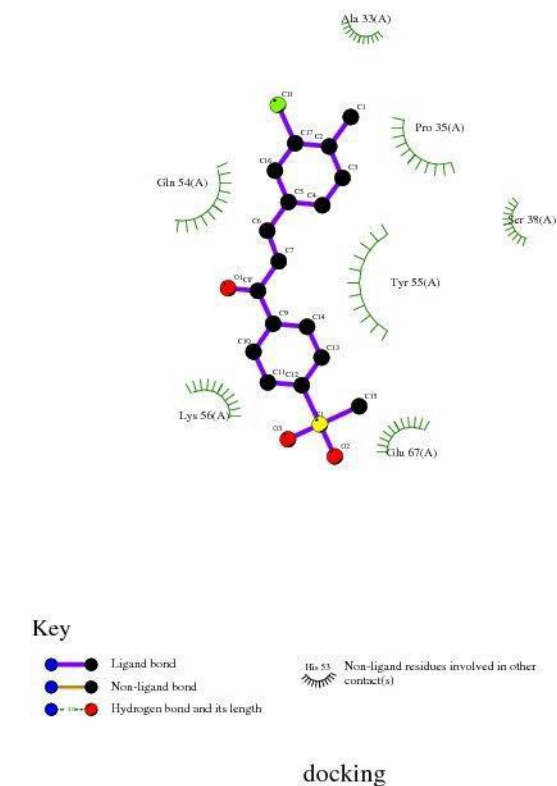


Figure 47. Study #3- Docking image SO₂CH₃CH₃Cl

From the main interactions we can observe all of the interactions happen with hydrophobic residues. These data will be compared with the data from study 1 and 2 to observe any pattern behaviors.

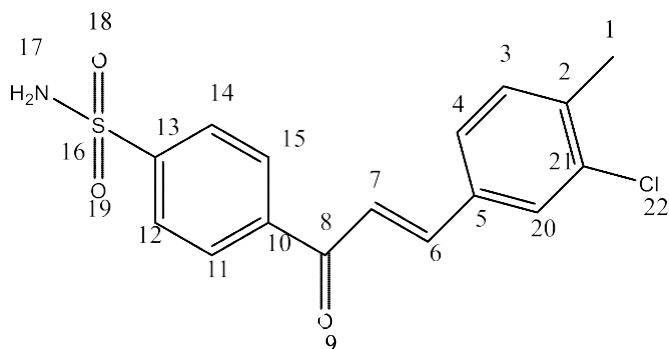


Figure 48. Study #3- Schematic representation of labeling of atoms

Interactions of compound with identification code $\text{SO}_2\text{NH}_2\text{CH}_3\text{Cl}$ were studied in depth, as this compound also showed a low free energy binding similar to that of compound with identification code $\text{SO}_2\text{CH}_3\text{CH}_3\text{Cl}$. The energy interactions of this compound are hydrogen bond of the nitrogen (N 17) with Tyr 55, tyrosine a non-polar amino acid with a hydroxyl group. The crystal packed asymmetric structure of this compound forms hydrogen bonding between the hydrogen of the nitrogen with the oxygen of the sulfoxide group. Hydrophobic interactions between the ligand and the enzyme took place with the amino acids Pro 35, proline a non-polar amino acid and Tyr 55, tyrosine a non-polar amino acid.

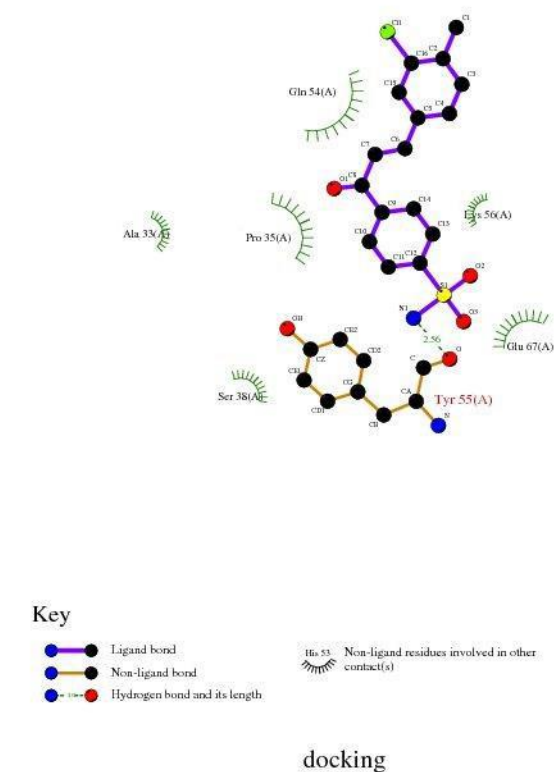


Figure 49. Study #3 - Docking image SO₂NH₂CH₃Cl

C5, C15 and C16 had a hydrophobic interaction with Pro 35 and C7 and C6 had a hydrophobic interaction with Tyr 55. As with the previous compound, Ala 33, alanine a non-polar amino acid had a halogen interaction with chlorine (Cl). Other interactions occur with amino acids Ser 38 and C1. Amino acid Glu 67 and O 18, C 13, C 14; amino acid Lys 56 and N 1, H 12, H 13; lastly amino acid Gln 54 and C 8, C 10, O 17.

4.13 Study # 4

4.13.1 Brief description

In study #4, the compounds from study #3 were oxidized with KMnO₄, to add a carboxylic acid group to the methyl of the benzyl on the third position. See figure below.

For more information on the different reactions of study #3 and study #4, including purification analysis, refer to the methodology chapter study #3 and study #4 reactions.

After analysis of the product, it was found the actual product was not the designed one and the KMnO_4 oxidation reaction will have preference to react with the double bond close to the carbonyl group than the single methyl group attached to the benzene group on the third position. The final product is shown on the figure below, a one benzyl compound with a carboxylic acid group to the right and the respective pharmacophore on the left. The final product is a previously synthesized compound and never synthesized before compound as it was the intended designed compound [50, 51]. Accidentally, a new simple, synthetic route with a yield of over 90% was discovered. For more information, see chapter, new synthetic route for the synthesis of p-4-methylsulfonyl benzoic acid. The products with the SO_2CH_3 and SO_2NH_2 were purified and analyzed. Further synthesis of compounds with $\text{SO}_2\text{NHCOCH}_3$ was not performed because the reaction did not proceed as expected, see Figure 50.

4.13.2 Characterization $\text{SO}_2\text{CH}_3\text{COOH}$ compound

The synthesized compound with the SO_2CH_3 group was analyzed using ^1H NMR, ^{13}C NMR, Mass Spectral and X-Ray crystallography analysis. The J values from the compound with the SO_2CH_3 pharmacophore group are as follows:

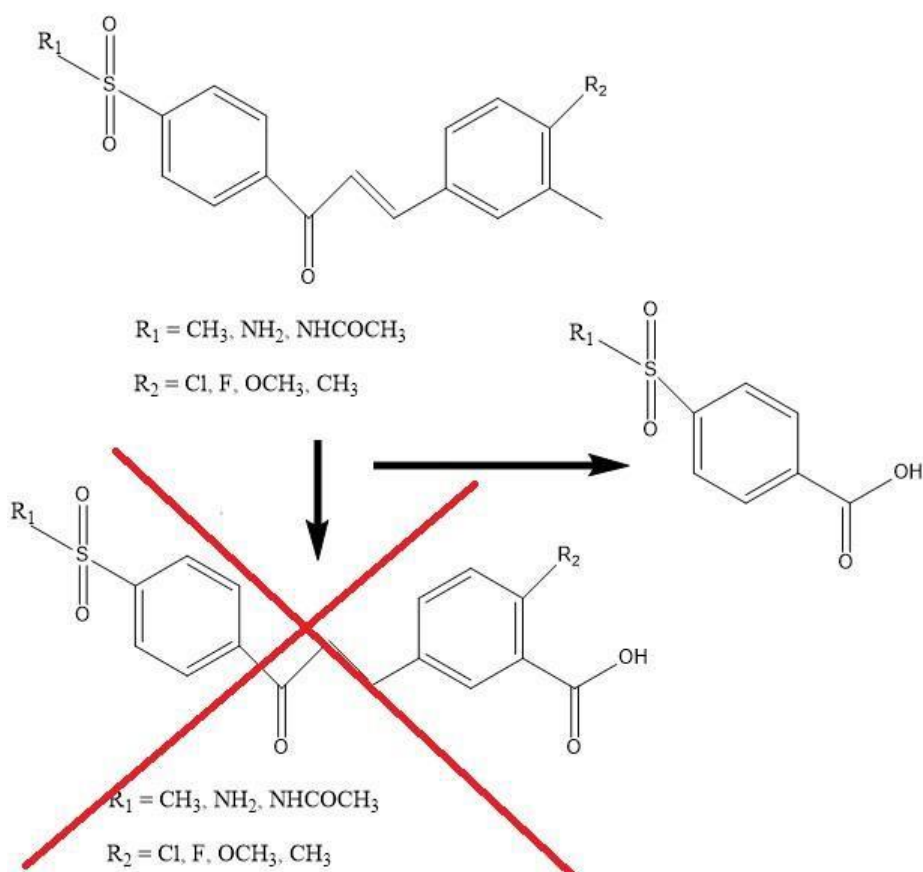


Figure 50. Schematic Representation of Achieved Product

The signal of the OH from the carboxylic acid group was at 12.71 ppm. The benzyl group showed typical signals of 8.01 and 8.11 ppm. The methyl group attached to the benzyl group was expressed in a singlet signal. The integration of the area of each peak clearly matched the compound with one benzyl group. To see the ^1H NMR spectrum of this compound go to Appendix, section A, study #4.

A ^{13}C NMR analysis was done on the compound and the signals clearly matched the compound. The Mass Spectrometry analysis of the compound was done at an estimated concentration of 10 μM with a solvent ratio of 70:30 (methanol:Milli-Q water) using positive ionization. The mass spectral analysis accurately demonstrated the molecular weight of the compound to be exactly 200 grams/mol and to further help determine the structure of the compound accurately. To see the spectral results, refer to

Appendix A, study #4 section.

The last characterization analysis was done using X-Ray crystallography analysis. This analysis accurately identifies the structure of the compound by measuring how each element diffracted the light. An image of the crystal structure is shown below. Here, we can see the carboxylic acid group, the benzyl group with its hydrogens and the SO_2CH_3 pharmacophore group. Melting point analysis was determined to be at 270°C , which agrees with previous analysis. With the identity of the compound clearly demonstrated, it was decided to test the compounds against anticancer effect of colon cancer lines Caco-2 and HCT-116. Where Caco-2 cell lines, respectively express COX-2 enzyme [50], while the HCT-116 cell line doesn't express the COX-2 enzyme. Refer to cell proliferation analysis on Caco-2 and HCT-116 section for discussion of results.

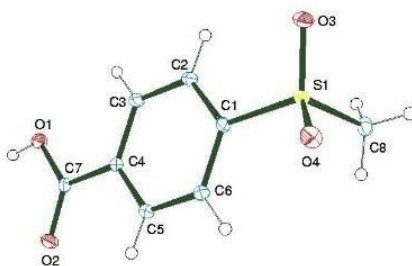


Figure 51. Study #4- Crystal structure of 4-(methylsulfonyl) benzoic acid

4.13.4 Characterization $\text{SO}_2\text{NH}_2\text{COOH}$ compound

The signal of the OH from the carboxylic acid group was at 12.71 ppm. The benzyl group showed typical signals of 7.94 and 8.01 ppm. The NH_2 group attached to the SO_2 group was expressed in a singlet signal. The integration of the area of each peak clearly matched the compound with one benzyl group. To see the ^1H NMR spectrum of this compound go to appendix section A, study #4. The ^{13}C NMR spectrum matched

the identity of the compound, see appendix section B, study #4. Crystals of this compound were grown and analyzed using X-ray crystallography and accurately identified.

With the identity of the compound clearly demonstrated, it was decided to test the compounds against anticancer effect of colon cancer lines Caco-2 and HCT-116. Where Caco-2 cell lines constitutively express COX-2 enzyme [50], while the HCT-116 cell line doesn't express the COX-2 enzyme. Refer to cell proliferation analysis on Caco-2 and HCT-116 section for a discussion of results of the results.

4.13.5 Cell proliferation analysis on Caco-2 and HCT-116 cell line at 400 μ M

Figure 52 shows the effectiveness of the compounds in study #4 in inhibiting the uncontrollable growth (angiogenesis) of cells. Compound 24 is the molecule with pharmacophore group SO_2CH_3 and compound 25 is the molecule with the SO_2NH_2 . The absorbance was read after 96 hours of incubation at 37 °C and 5% of CO_2 [52,53,54]. For more information on how to do the analysis, refer to the Methodology chapter, MTT analysis section.

The compounds were incubated at a concentration of 400 μ M and Vioxx was used as control due to its ability to selectively inhibit the enzyme COX-2. From previous studies [add reference] on proliferation analysis using Vioxx as a control, it was decided to run the initial analysis at a concentration of 100 μ M. Rofecoxib (Vioxx), the highly selective COX-2 inhibitor did not have any effect on the cell growth of the cells of Caco-2 or HCT-116. The publishing data regarding the effectiveness of selective COX-2 NSAIDs on the inhibition of angiogenesis and induction of apoptosis on cancer cells is conflicting. Some studies find a direct relationship between expression of COX-2 and tumor growth. By inhibiting the expression of COX-2, the production of prostaglandins that induce inflammation is stopped resulting in tumor shrinkage. But other studies have shown that the use of selective COX-2 NSAIDs do not have any effect on decreasing the uncontrolled cell multiplication or angiogenesis.

Figure 52 shows the compounds that did not demonstrate any inhibition of cell growth for either of the two cell lines. Compounds 24 and 25 did not demonstrate any anticancer effect after incubation for 96 hours for either Caco-2 or HCT-116 cell lines.

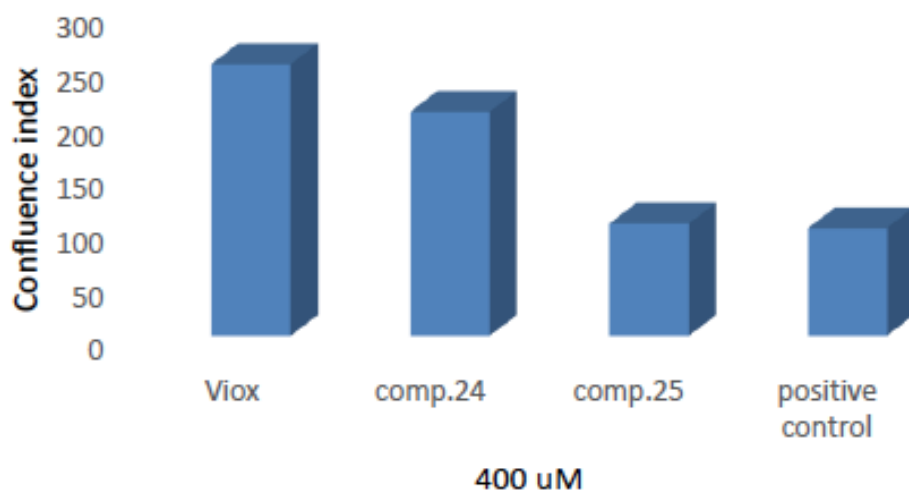


Figure 52. Study #4- Confluence index after 96 hours of incubation with Caco-2 cell line

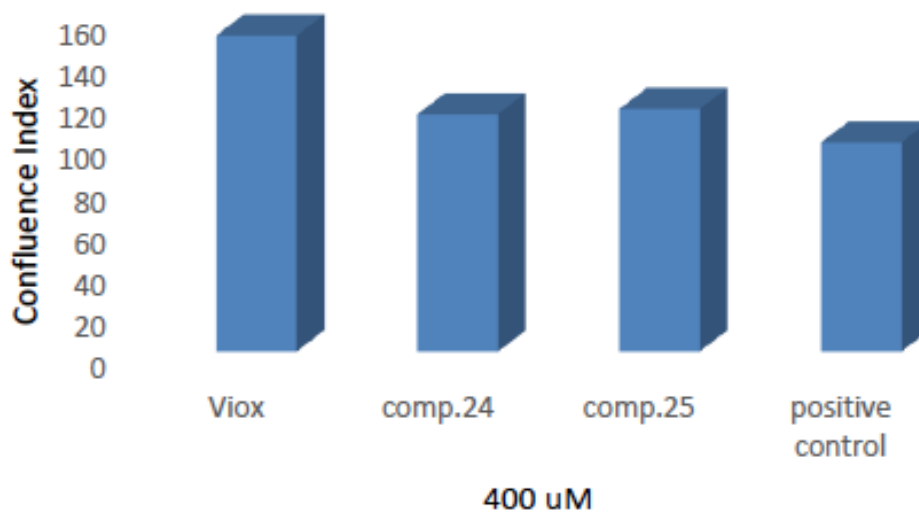


Figure 53. Study #4- Confluence index after 96 hours of incubation with HCT-116 cell line

Unexpectedly, the cells incubated with Viox showed more cell growth than the positive control. Since this is a colorimetric analysis, the slightest change in color can have a dramatic change in the results. As a preventive measure, for future measurements a widely known anticancer compound will be used on the tests. See the figure above.

4.14 Comparative study of cell proliferation analysis between cell lines HCT-116 and Caco-2 at 400 μ M

A comparative study between the effectiveness on anti-cell proliferation of the compounds on HCT-116 and Caco-2 cell lines was done. The compounds didn't show any inhibition for any of the cell lines. Further studies on docking modeling analysis against the activity site of the enzyme and inhibition analysis of COX-1 and COX-2 were cancelled as the compounds have proven not to have any anti-cancer properties or selectivity for COX-2 with the studies performed at a concentration of 400 μ M.

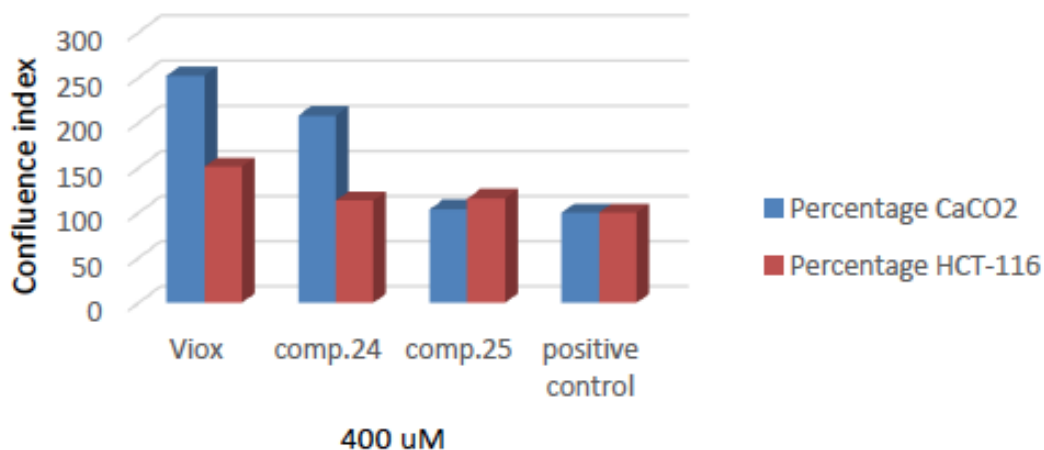


Figure 54. Study #4- Comparative cell proliferation study between HCT-116 and Caco-2 cell lines

It appears that compound 25 ($\text{SO}_2\text{NH}_2\text{COOH}$) was a slightly more efficient than compound 24, but neither of the two compounds were able to either stop the cell proliferation of the cells after 96 hours or induce apoptosis (dead cells). It can be concluded that besides the strong electron rich group of the carboxylic acid, the one benzyl group frame did not prove to have any biological activity and the chalcone frame with two benzene groups is needed to further continue testing the different hypothesis.

5. Comparative analysis- summary of all studies

A comparative study analysis table was done to look for any relationship among the compounds that exhibit the highest energy binding together with the highest efficiency at stopping the proliferation of cancer cells HCT-116 and Caco-2 within 96 hours of study time.

Comparative Analysis of Studies # 1, # 2, and # 3 on the effectiveness at stopping cell proliferation of HCT-116 and Caco-2 cells, and binding interactions with enzyme COX-2						
Study # 1	Ener. bind kcal/mol	Polar	Halogen	Inter. Hydrophobic bonding	Inter. – OH bond	Cell prolif.
SO ₂ NHCOCH ₃ CH ₂ C H ₃	-8.68	TYR 355	X	LEU359, PHE518	X	Not eff. 10UM Eff. 250UM
SO ₂ NHCOCH ₃ CH ₂ C H ₂ CH ₃	-8.66	X	X	LEU352, LEU359, LEU531, LEU93	X	More eff. 10UM
Study # 2						
SO ₂ NHCOCH ₃ F	-6.42	SER 38	GLU67	CYS37, TYR55	PRO35	Not eff. 10UM Eff. 400UM
Study # 3						
SO ₂ CH ₃ CH ₃ Cl	-6.65	X	ALA33	TYR55, PRO35	X	Very eff 10UM

The table above shows docking interactions of the compounds with the lowest energy binding from each study against cell proliferation analysis using colon cancer HCT-116. From the results of study number 1, we can observe that the compound with the lowest energy binding is not effective at stopping the proliferation of the cells at a

low concentration of 10 uM, but is effective at a concentration at 250 uM. Other compounds within the same study have proven to be effective at stopping the proliferation of cells with higher energy bindings indicating that there is no actual relationship between COX-2 selective inhibitors and effectiveness towards inhibiting the proliferation of cells. Additionally, on study #2 we observe the same behavior as in study #1. The compound with the lowest energy binding, in this case compound with identification code $\text{SO}_2\text{NHCOCH}_3\text{F}$ did not prove to be effective to stop the proliferation of cancer cells HCT-116 at a low concentration. This indicates that there is more within the relationship of COX-2 selective inhibitors effectiveness on killing cancer related cells. In study # 3, however the compound with the lowest energy binding also demonstrated to have a good effectiveness at killing cancerous cells at low concentration. Among the different electrostatics interactions studied within the docking analysis, the only pattern we can observe is that most amino acids that participate in hydrogen bonding, hydrophobic interactions, polar, halogen related interactions are non-polar amino acids.

As previously seen on the comparative analysis of cell expressing the enzyme COX-2 Caco-2 and the cell with no expression of COX-2 HCT-116 the compounds were equally selective to both cancer cells expressing COX-2 and cancer cells not expressing COX-2. Furthermore, Viox a powerful selective COX-2 inhibitor was not effective at stopping the proliferation of either cancer cells expressing the enzyme COX-2 Caco-2 or cancer cells not expressing the enzyme COX-2 HCT-116. This indicates that there is more regarding the effectiveness of COX-2 inhibitors at stopping the proliferation of cancer cells. Recent articles, report the effectiveness of COX-2 inhibitors when combine with known anticancer drugs like oxaliplatin and selective non-steroidal anti-inflammatory inhibitors selective towards COX-2. This combination proved to improve the death rate of colon cancer cells as reported [97]. In a different study of the role of COX-2 in the development of colon cancer, it was found that COX-2 was present in 80% of all human carcinomas. So Non- Steroidal anti-inflammatory drugs can play a significant role in the prevention of colon cancer cells. However, it was found that the

induction of COX-2 was not present at earliest stages, which its necessary if we want to use NSAIDS as a chemoprevention tool [98]. Nobuka, reported that there is no COX-2 expression in human aberrant crypt foci, the earliest recognizable form of premalignant lesion in the colon [99].

6. Conclusions

The following are general conclusions for the majority of the compounds synthesized and studied:

- The compounds were not selective to either the COX-1 or COX-2 enzyme.
- The lack of selectivity towards COX-2 didn't show any impediment or affect the ability of the compounds to inhibit the cell proliferation of colon cancer cells HCT-116 and Caco-2.
- Besides the ability to inhibit cell proliferation, the compounds were able to kill the cells over the studied incubation period of time.
- Most compounds cytolyse the cells HCT-116 and Caco-2 at an average of 20%; therefore most of the cells died without going through cytolysis.
- A new synthetic route was discovered for compound 4-(methylsulfonyl)-benzoic acid.
- All compounds were purified using recrystallization techniques and characterized by Mass Spectrometry, ^1H NMR, ^{13}C NMR, and melting point analysis.
- Crystal structures were determined for 18 of the 27 synthesized compounds. Additionally, crystal structures of the intermediates were obtained.
- Energy bindings in Kcal/mol were obtained for docking studies of the compounds against the active site of the COX-2 enzyme and energy of the different interactions like hydrogen bonding, hydrophobic, polar, halogen were obtained. The lowest energy binding was analyzed and compared against the cell proliferation analysis results.
- Compounds with identification codes $\text{SO}_2\text{NHCOCH}_3\text{F}$ from study number 2 had the lowest energy binding from the studied compound within study number 2. Two compounds from study number one had similar energy binding, the identification code of these compounds is as follow: $\text{SO}_2\text{NHCOCH}_3\text{CH}_2\text{CH}_3$,

SO₂NHCOCH₃CH₂CH₂CH₃. Additionally, two compounds from study number had similar energy binding. The identification code of the compounds is: SO₂CH₃CH₃Cl, SO₂NH₂CH₃Cl. All these compounds with exception of compounds SO₂NHCOCH₃F and SO₂NHCOCH₃CH₂CH₃ were able to strongly inhibit the cell proliferation of carcinogenic colon cancer cells HCT-116 and Caco-2 at a concentration of 10 µM. Compounds SO₂NHCOCH₃F and SO₂NHCOCH₃CH₂CH₃ were able to inhibit the proliferation of the cells at a stronger concentration.

In detail conclusions of the compounds:

- Compound 15 was not able to elicit any inhibition of cell proliferation at 10µM in these experimental conditions.
- Compound 14 was able to elicit a moderate inhibition of cell proliferation at 10µM.
- Compound 11 was able to elicit a moderate inhibition of cell proliferation at 10µM and a strong inhibition of cell proliferation at 400µM.
- Compounds 10 and 12 were able to elicit a strong inhibition of cell proliferation at 400µM
- Compounds 6 was able to elicit a strong inhibition of cell proliferation at 250µM.
- Compounds 1; 2; 3; 4; 5; 7; 8; 9; 13; 16; 17; 18; 19; 20; 21; 22; 23 and 24 were able to elicit a strong inhibition of cell proliferation at 10µM.
- Compounds 7; 11; 18 and 19 were able to increase the number of cytolysed cell from 24h at 400µM.

7.Future recommendations

Besides the good results that were obtained with the analysis, more studies should be done in order to have a complete set of data. Below are some recommendations for future analysis.

- To incubate the compounds with cell line HCT-116 and Caco-2 at lower concentrations to be able to determine the IC 50, which is the concentration where half of the cells are inhibited.
- To measure the toxicity of the compounds in normal healthy cells and perform a comparative study.
- To measure the toxicity of the compounds on different types of cancer cells.
- To test the cell proliferation of the cells against powerful COX-2 inhibitors at the same concentration as the runs made with the synthesized compounds.

8. Additional compounds

This chapter includes reactions of different compounds that may have biological activity but were not tested. Included here is the synthesis of hydroxamic acids and synthesis of $\text{MoO}_5(\text{HMPT})\text{H}_2\text{O}$, where HMPT is hexamethylphosphorous triamide. The image of HMPT is given in Figure 55.

8.1 Synthesis $\text{MoO}_5(\text{HMPT})\text{H}_2\text{O}$

Approximately 5 grams of MoO_3 (molybdenum trioxide) were mixed with 50mL of H_2O_2 (30% sodium peroxide) in a round flask at a temperature of 40°C . The solution was stirred for approximately 26 hours or until all of the molybdenum trioxide was dissolved. After complete dissolution, a crystal clear solution was obtained. The solution was cooled to a temperature between -5°C and 0°C . Then approximately 7.146 mL of HMPT was added drop-by-drop. The reaction was extremely exothermic, so drops were added one minute apart to keep the temperature at 0°C . A yellow precipitated was slowly formed. [63]

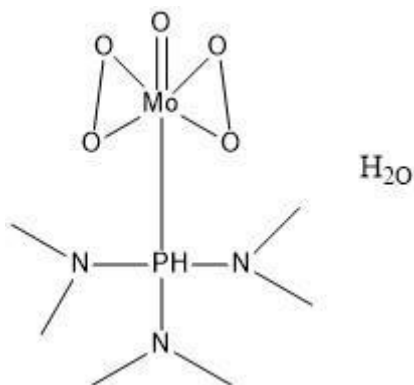


Figure 55. $\text{MoO}_5(\text{HMPT}) \cdot \text{H}_2\text{O}$

The precipitate was weighed with care since the compound is explosive. After weighing the precipitate steps were taken to make sure it remained in solution. The product was analyzed using the ^1H NMR technique, the chemical shift was around 2.80 ppm

8.2 Synthesis Metal Complex with acetaminophen

A metal complex between acetaminophen with $\text{MoO}_5(\text{HMPT}) \cdot \text{H}_2\text{O}$ was attempted. Approximately 0.252 grams of acetaminophen were mixed with 346 μL of BSA (Bis (trimethylsilylacetamide)) under a nitrogen atmosphere. The mixture was warm at 40°C for 5 minutes. Then the solution was diluted with 2.5mL of dichloromethane (DCM). A solution of 0.247 grams of $\text{MoO}_5(\text{HMPT})$ in 5mL dichloromethane (DCM) was added to the mixture. As soon as the solution was added, the mixture turned brown red in color. The mixture was left to stir overnight with 2 balloons of nitrogen to ensure a nitrogen atmosphere.

Analysis

NMR analyses were inconclusive. While it can be concluded that a reaction occurred the product could not be identified

Synthesis Hydroxamic Acid

The synthesis of the hydroxamic acids requires two steps. Initially an ester is synthesized from a carboxylic acid and an alcohol. To this mixture sulfuric acid (H_2SO_4) is added as a catalyst.

8.3.1 First synthesis Step of esters

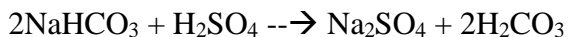
Two different esters were synthesized phenyl propionate and methyl phenyl butyrate. The procedure for the synthesis of each ester will now be explained.

Synthesis of Phenyl Propionate

Approximately 10 grams of phenyl propionic acid were mixed with 10 mL of methanol in a round flask with one opening. To this mixture 45 drops of sulfuric acid, as catalyst, were added. This solution was left to reflux for 3 hours. After refluxing, the solution was allowed to reach room temperature.

Purification

NaHCO₃ was added until the bubbles stopped or litmus paper color remained neutral. The NaHCO₃ removes the excess of H₂SO₄. See the reaction below.



Extraction

The extraction of the product was made with water and chloroform. Approximately 3* 30mL of chloroform was used for the extraction. The organic phase was dried over a filter of Na₂SO₄ anhydrous.

Analysis

Identification of the product was done with GC-MS and ¹H NMR. From the spectra it was determined that the product was pure. The ¹H NMR spectrum had a doublet at around 7.27 ppm, this shift indicates the phenyl group and two more doublets, one at 2.61 ppm and the other at 2.93 ppm. The GC-MS shows a peak at around 18.08 minutes with a molecular weight of 164, which is the weight of phenyl propionate.

8.3.2. Synthesis Methyl-Phenyl butyrate

Approximately 10 grams of phenyl butyric acid were mixed with 10mL of methanol in a round flask with one opening. To this mixture 45 drops of sulfuric acid as catalyst were added. This solution was left to reflux for 3 hours. After refluxing, it was allowed to reach room temperature. For purification and extraction, see subsection of purification and extraction with phenyl propionate.

Analysis

Identification of the product was done with GC-MS and ^1H NMR. From the spectra it was determined that the product was pure. The ^1H NMR spectrum had a doublet at around 7.28 ppm; this shift indicates the phenyl group and two more doublets, one at 2.68 ppm, the other at 2.37 ppm and 1.99 ppm. The GC-MS shows a peak at around 19.21 minutes with a molecular weight of 178, which is the weight of phenyl propionate.

8.3.3 Synthesis Methyl-Phenyl valerate

The diagram below shows the reaction for methyl-phenyl valerate. Approximately 10 grams of phenyl butyric acid were mixed with 10 mL of methanol in a round flask with one opening. To this mixture 45 drops of sulfuric acid, as catalyst, were added. The solution was left to reflux for 3 hours. After refluxing, it was allowed to reach room temperature. For purification and extraction; see subsection of purification and extraction with phenyl propionate.

Analysis

Identification of the product was done with GC-MS and ^1H NMR. From the spectra it was determined that the product was pure. The ^1H NMR spectrum has a doublet at around 7.28 ppm and 7.21 ppm, this shift indicates the phenyl group and two more doublets, one at 2.68 ppm, the other at 2.49 ppm and 1.67 ppm and one singlet at 3.67 ppm. The GC-MS shows a peak at around 19.96 minutes with a molecular weight

of 192, which is the weight of phenyl propionate.

8.3.4 Second step synthesis of Hydroxamic Acid from methyl-phenyl acetate

The general reaction of hydroxamic acid from methyl phenyl acetate is shown below. In a round flask with one opening about 2.2 grams of $\text{NH}_2\text{OH} \cdot \text{HCl}$ were dissolved in 10mL of methanol. To this solution approximately 1.8 grams of KOH were added. After precipitation of the KCl, approximately 0.011mol (1.55mL) of methyl phenyl acetate was added. This reaction mixture was left to stir at room temperature for 48 hours. Then 1.25M acetic acid was added to the solution until the pH reached 4. The solution was left standing at 4 °C overnight. The volume of the solution was reduced by using a rotary evaporator.

Extraction

The extraction was made with 10mL of H_2O and 10mL of chloroform. A saturated solution of NaHCO_3 was added until there were no more bubbles. The sample was then dried over a filter of Na_2SO_4 .

Column Separation

The sample was separated in a silica gel column with a solvent of 6:4 mol ratio of ethyl acetate/hexane. The solution was evaporated in a rotary evaporator and a white crystal solid was obtained. A visualization agent was added to the solution of 1 gram KMnO_4 with 2 grams of Na_2CO_3 in 100mL of H_2O to use with the TLC sheets.

Colorimetric titration for identification of hydroxamic acids

A colorimetric analysis for the presence of hydroxamic acid was made. Ferric chloride (FeCl_3) reacts with hydroxamic acid to produce a deep burgundy color. The solution was made by using 2.5 grams of FeCl_3 diluted in 57mL of methanol.

Analysis

¹H NMR analysis was done on the product. A spectrum of the compound is on the Appendix.

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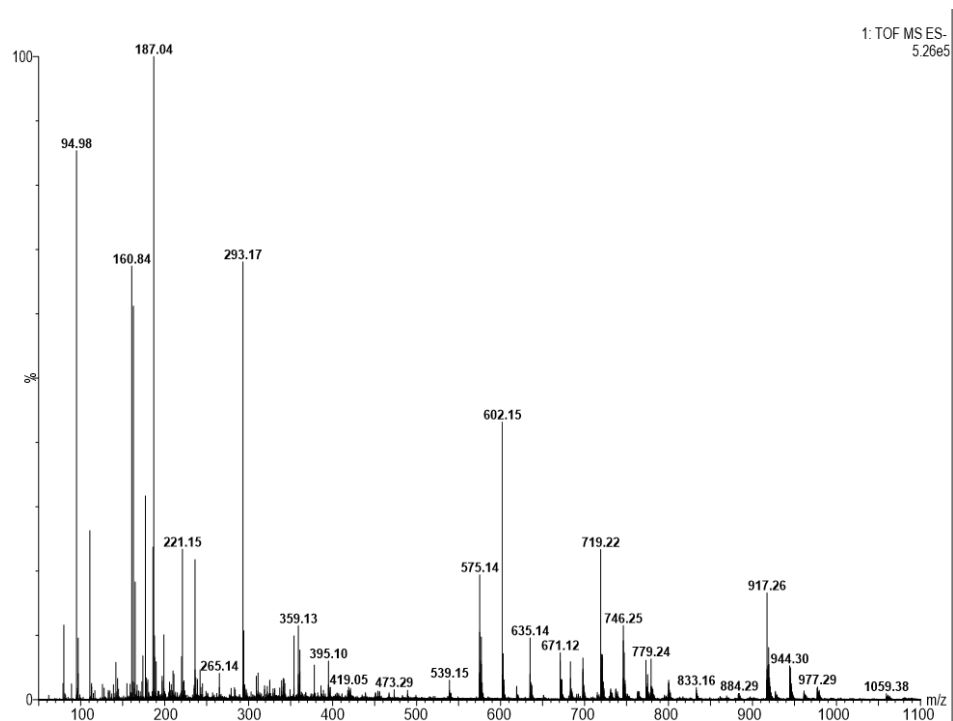
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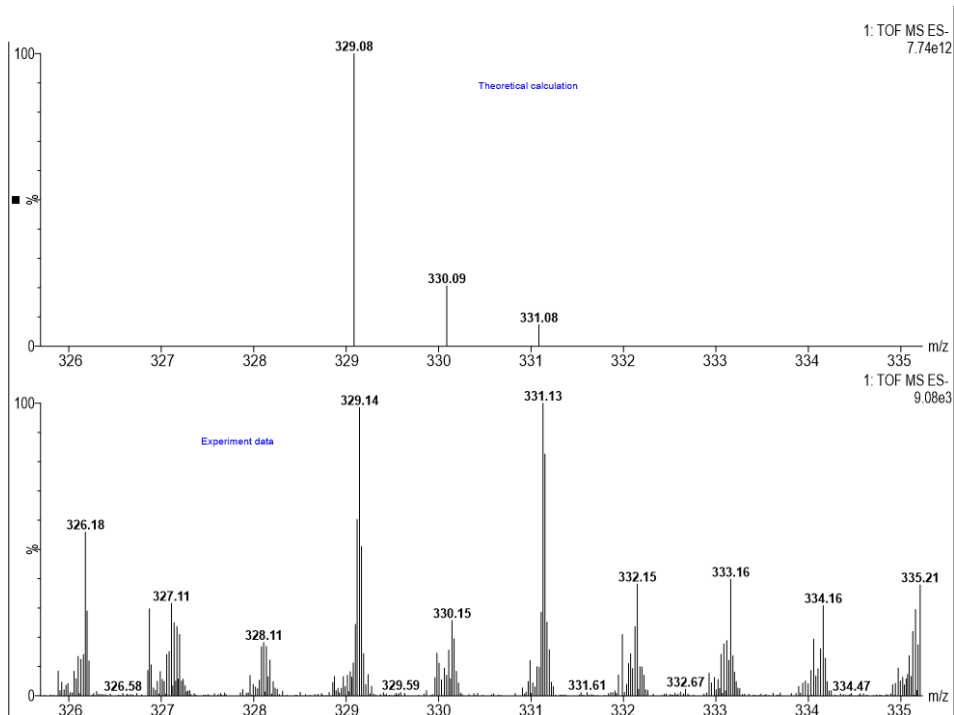
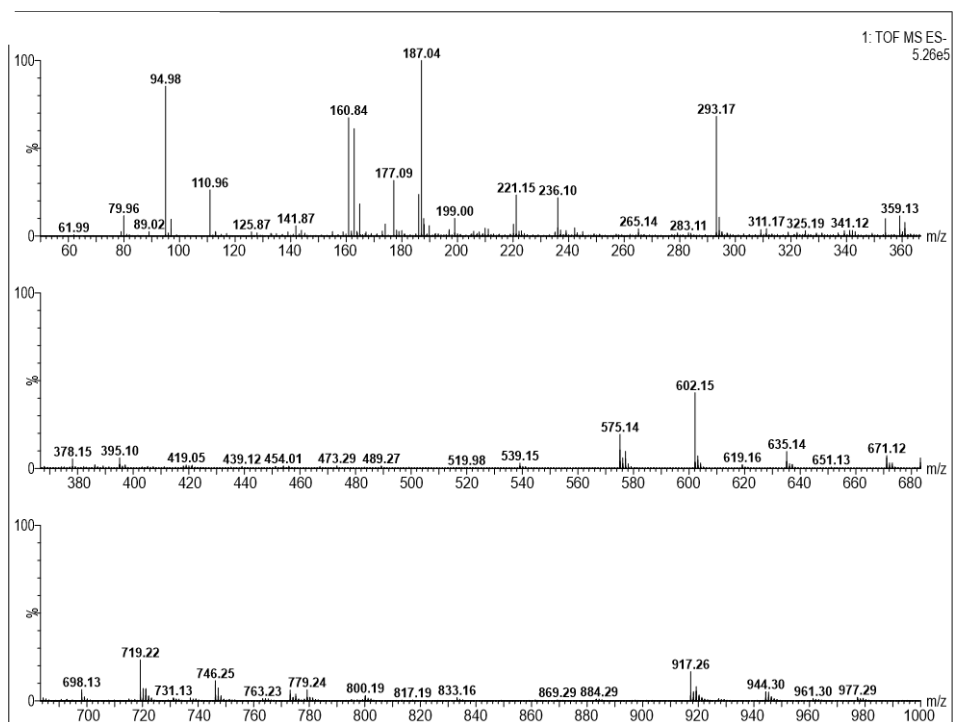
Appendix A

MS Spectra

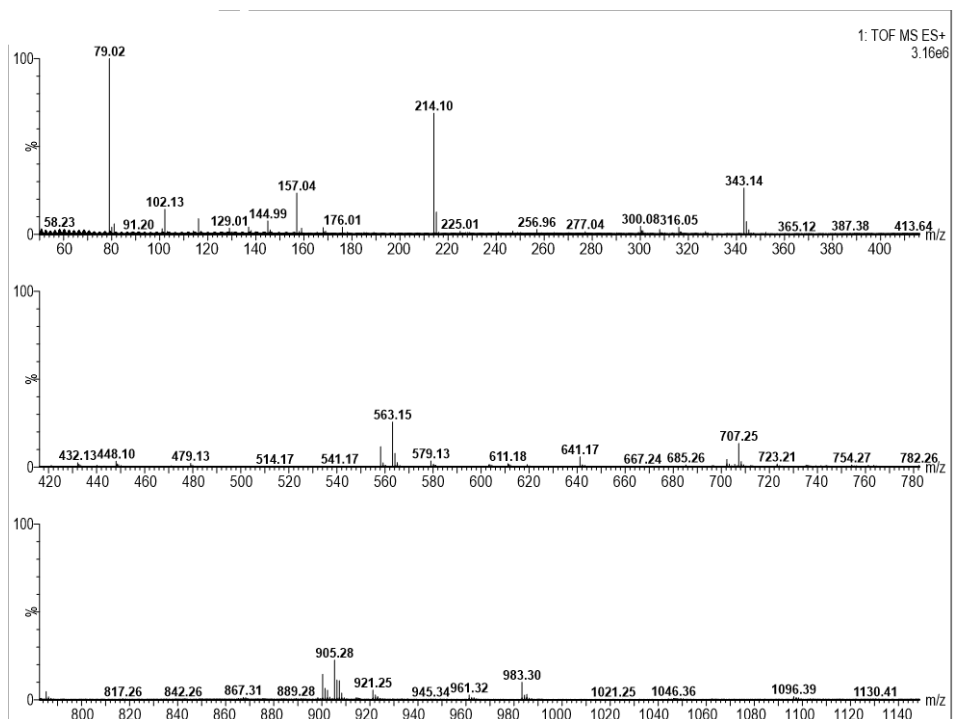
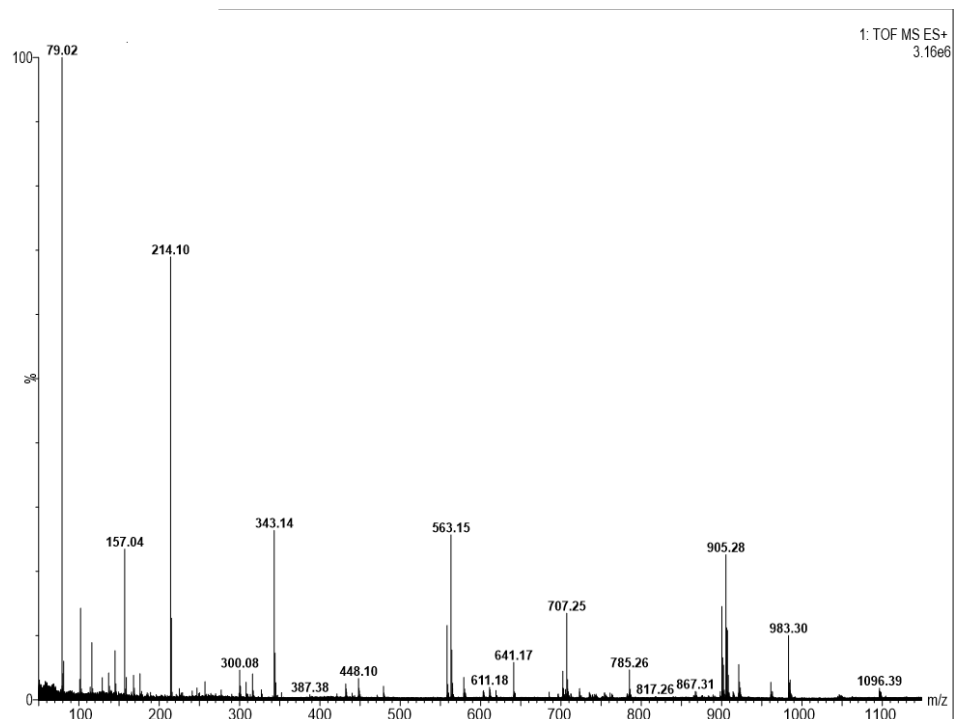
Study 1

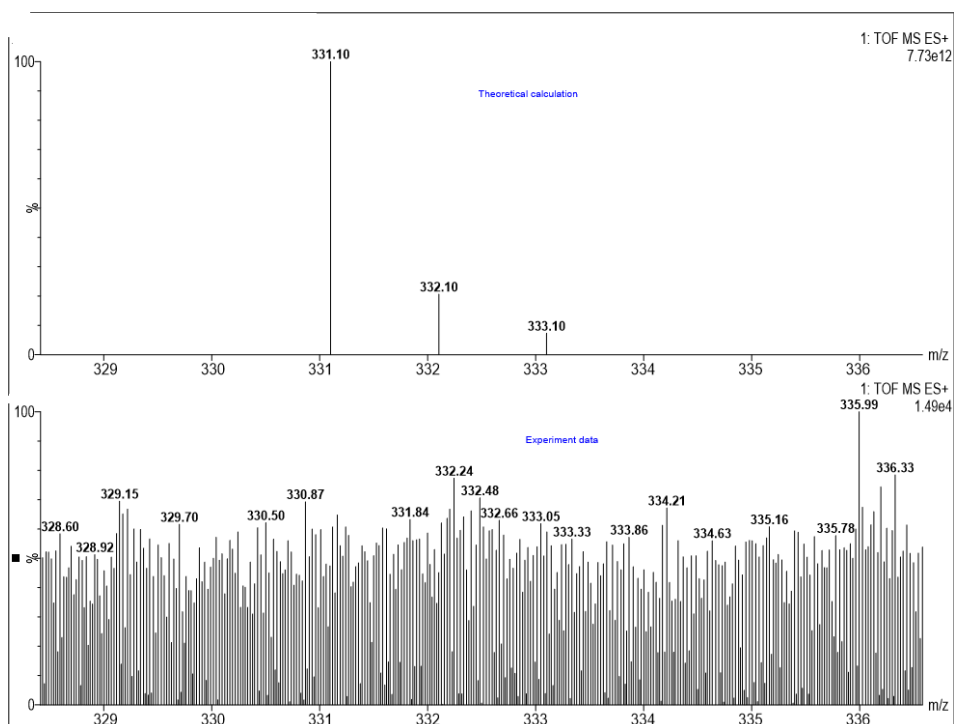
- SO_2CH_3 family – negative ionization
 - $\text{SO}_2\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$



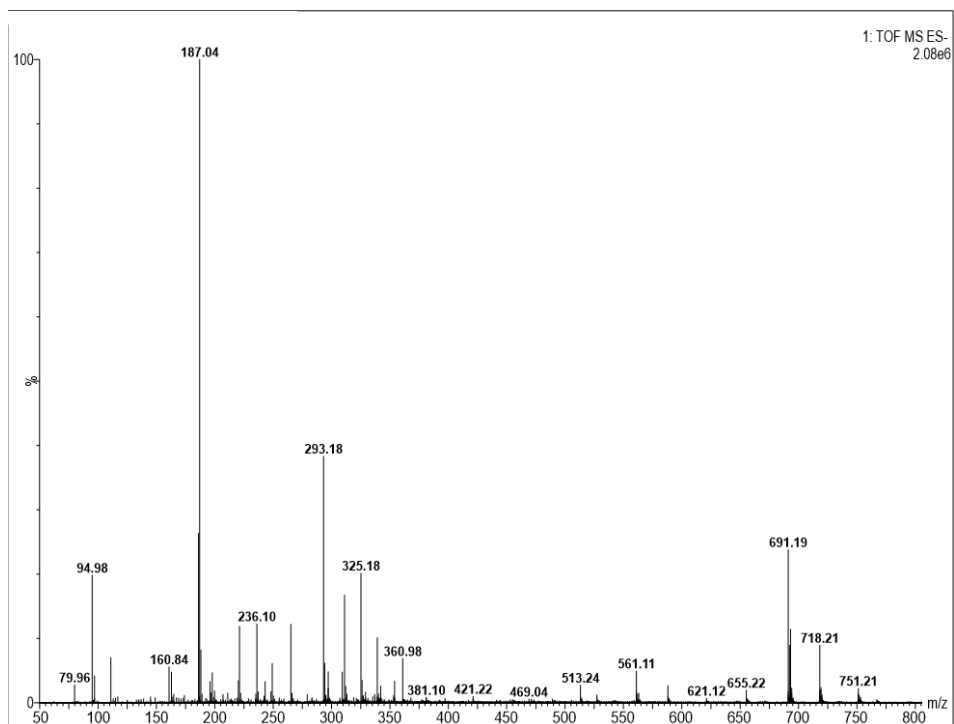


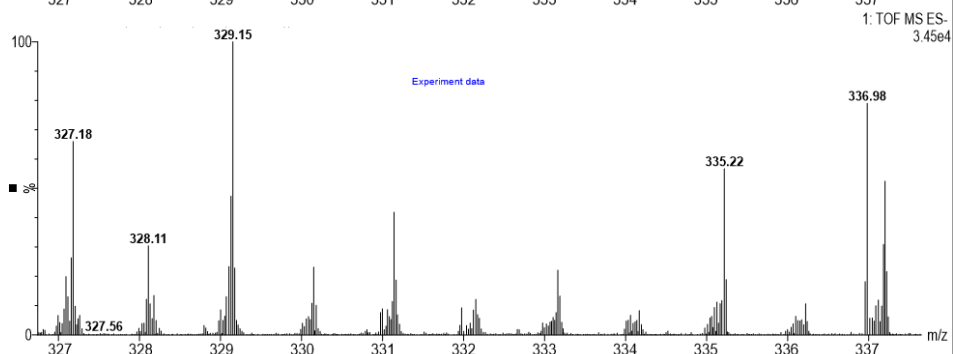
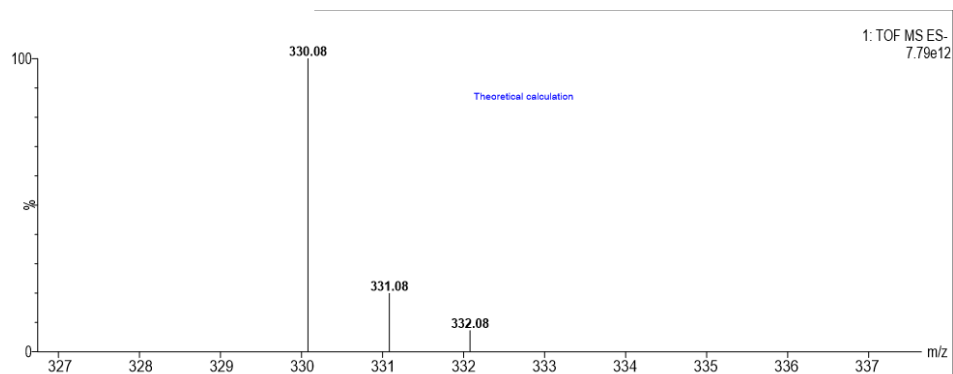
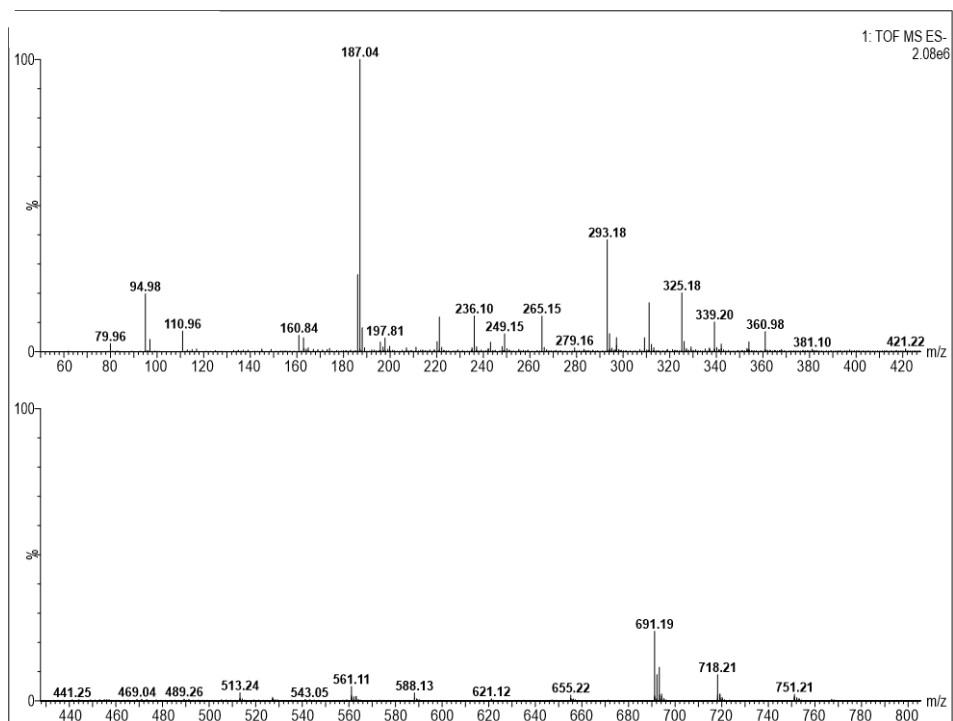
- SO_2CH_3 - positive ionization
 - $\text{SO}_2\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$



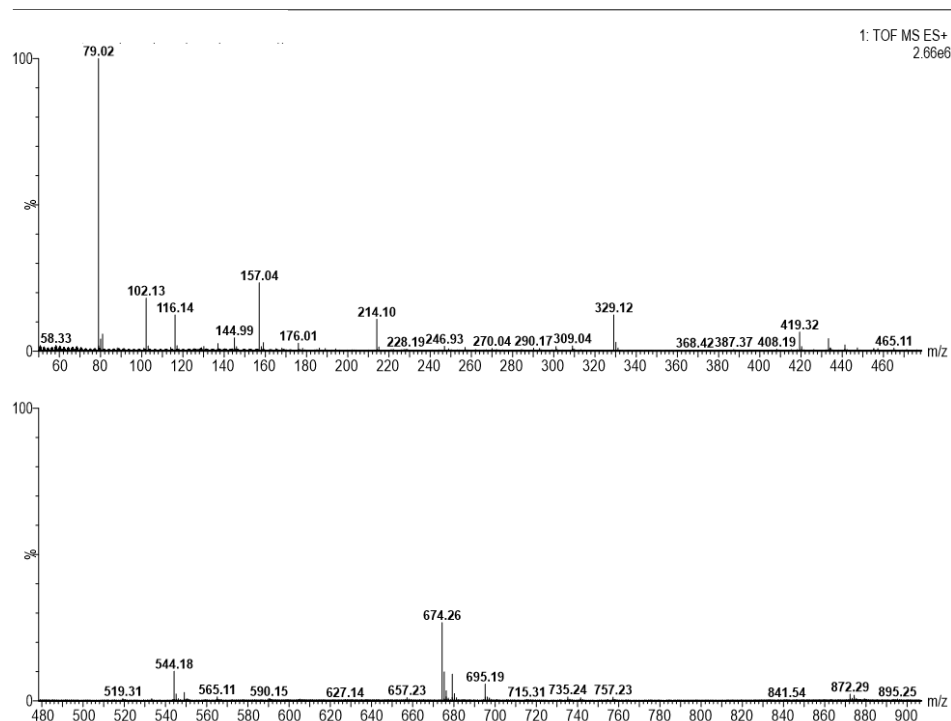
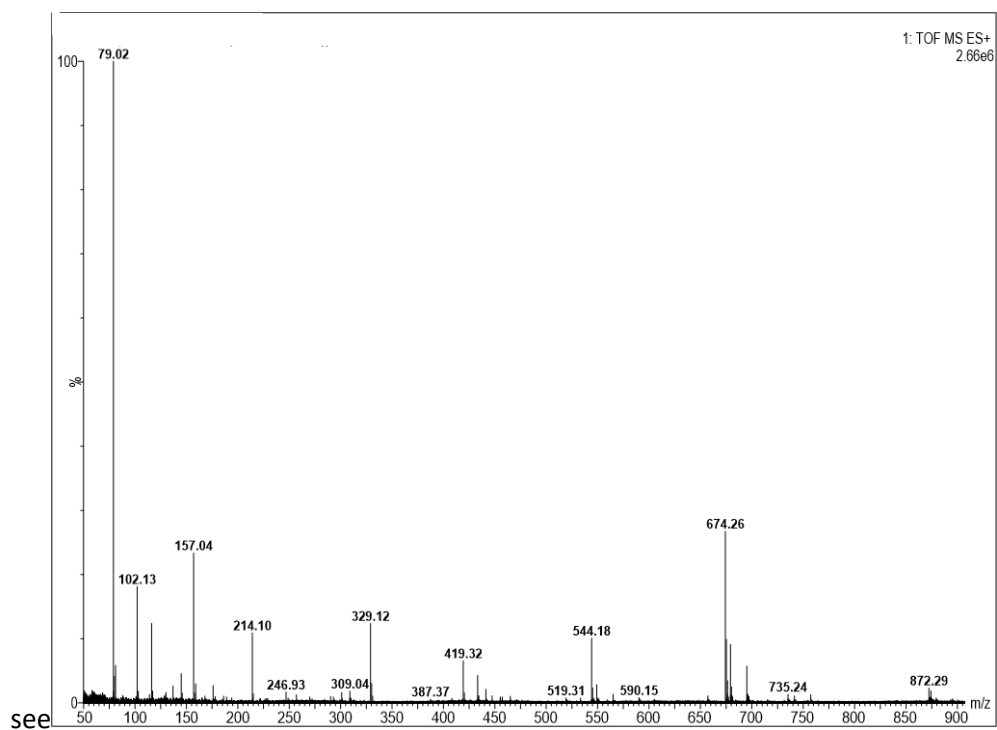


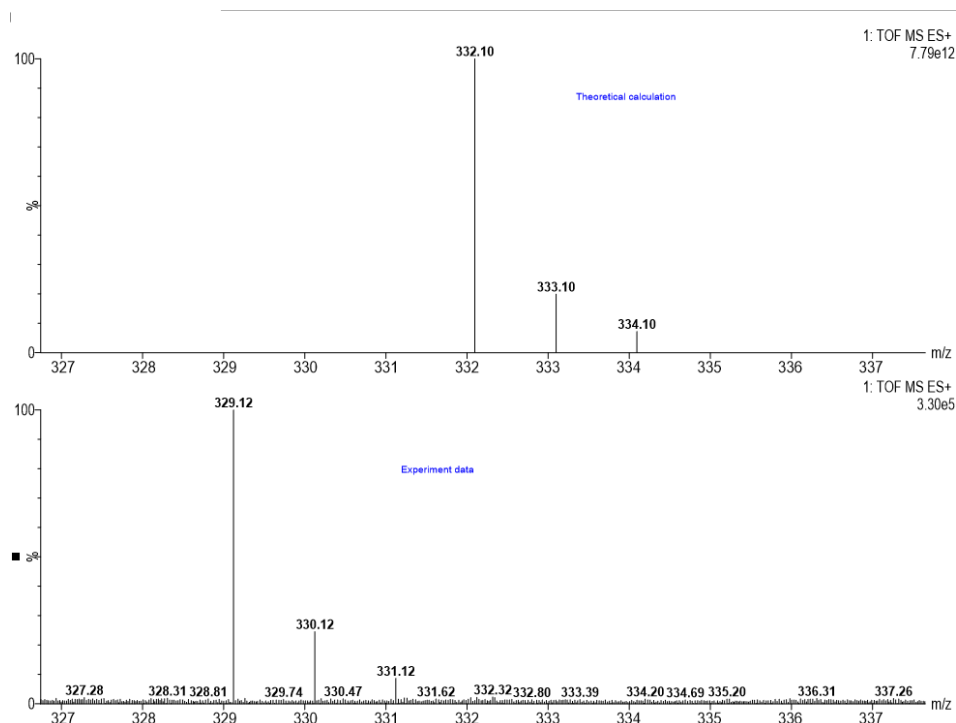
- SO_2CH_3 family – negative ionization
 - $\text{SO}_2\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_3$



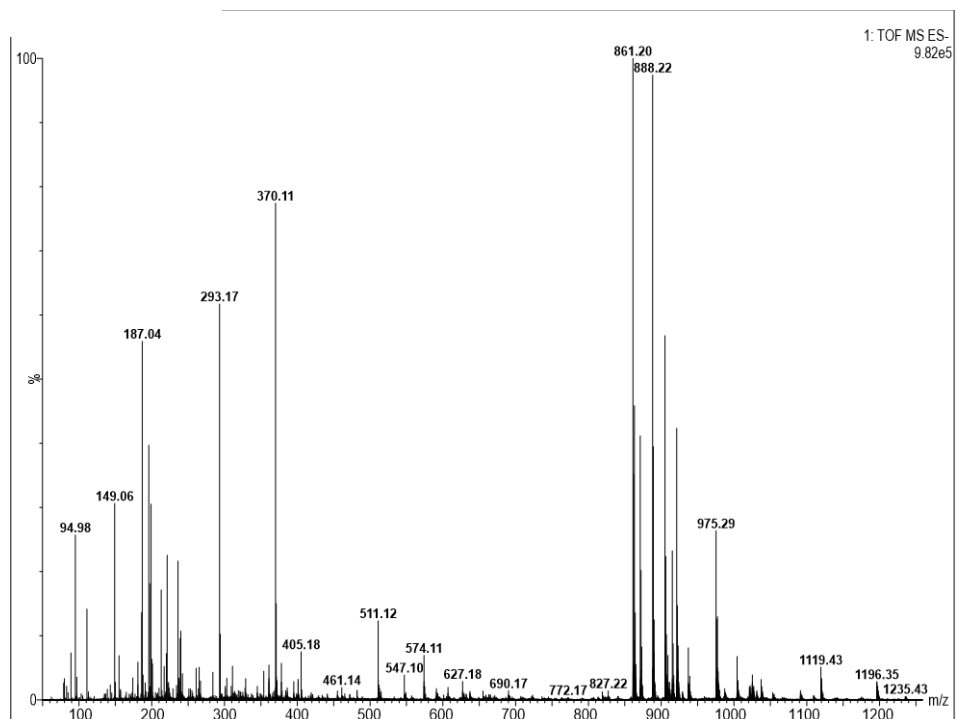


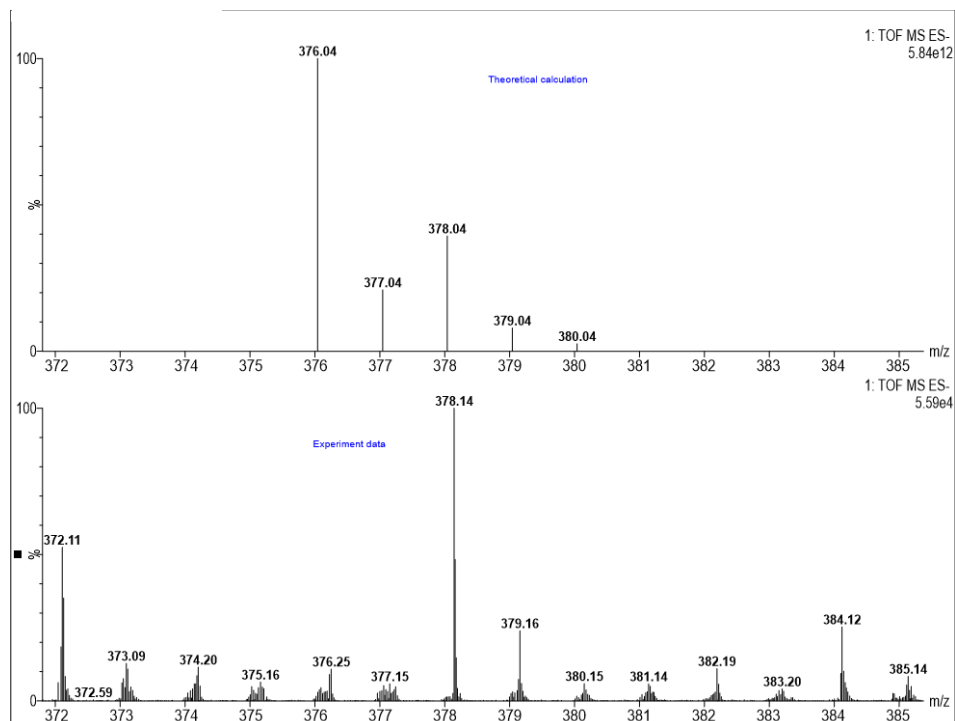
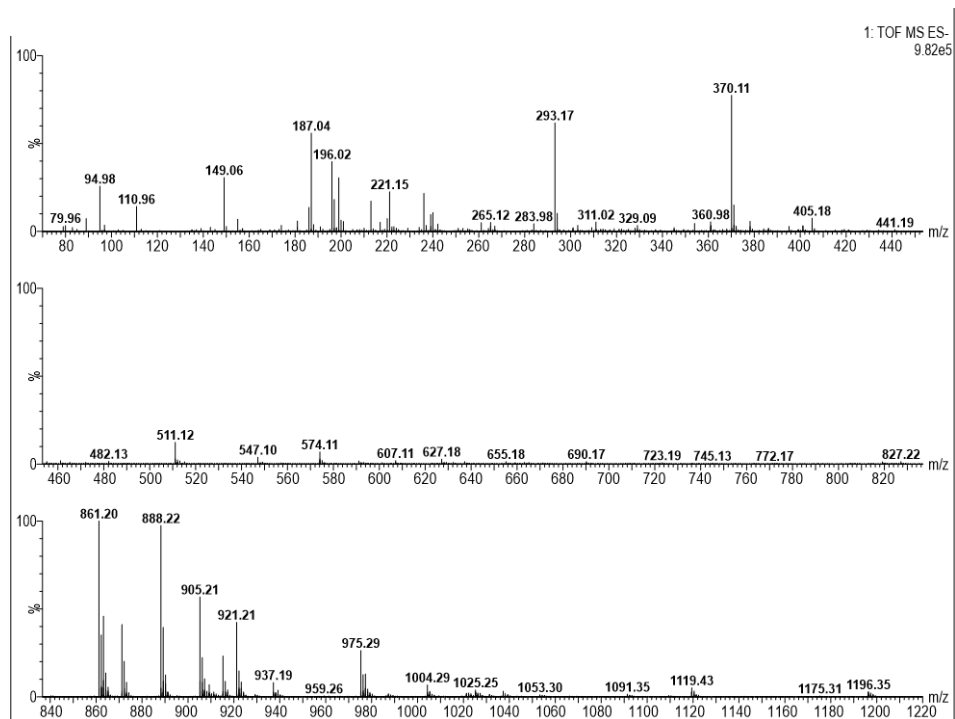
- SO_2CH_3 family – positive ionization
 - $\text{SO}_2\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_3$



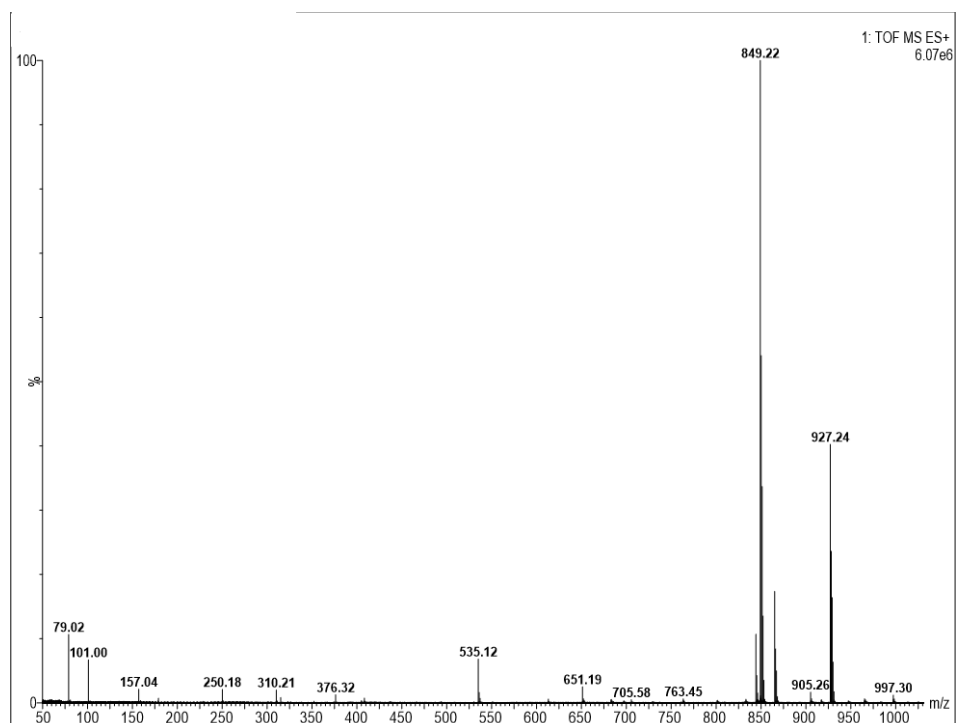


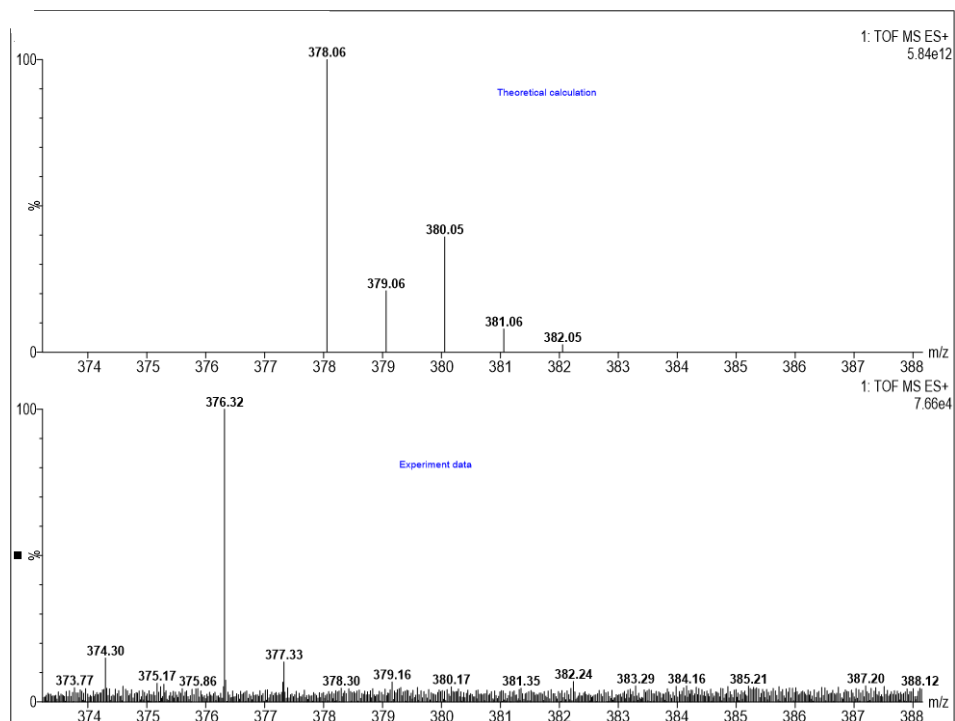
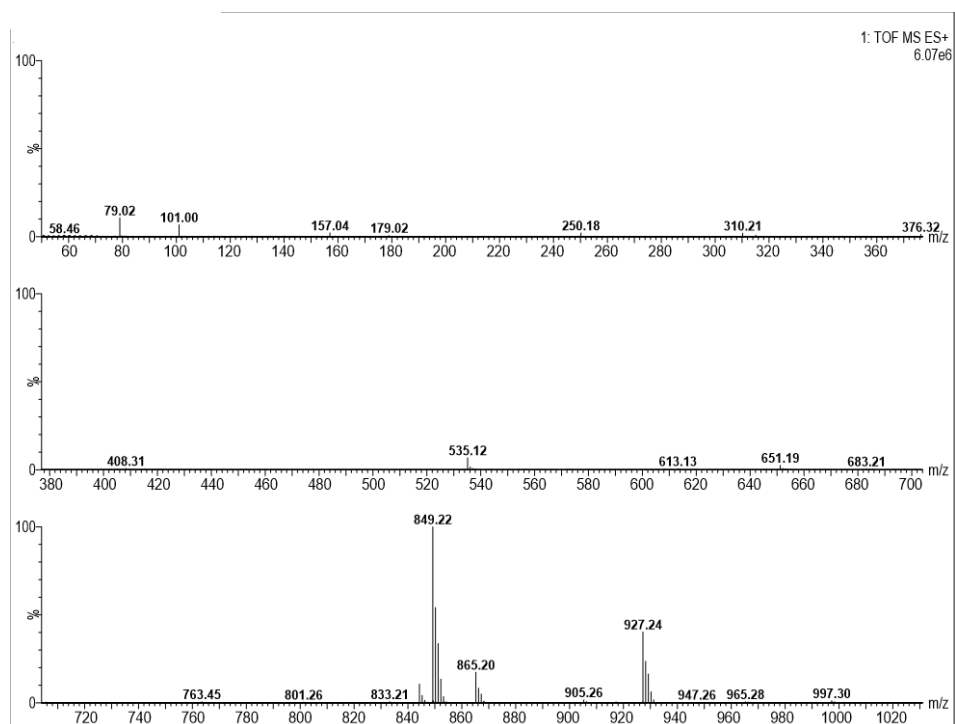
- SO_2CH_3 family – negative ionization
 - $\text{SO}_2\text{CH}_3\text{CH}_2\text{H}_3$



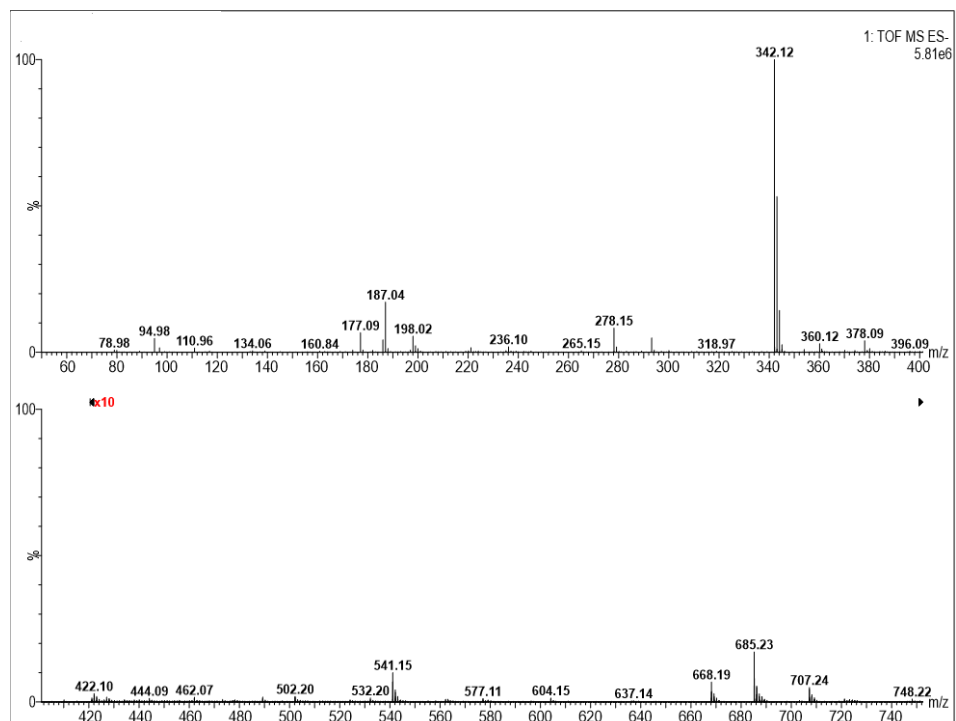
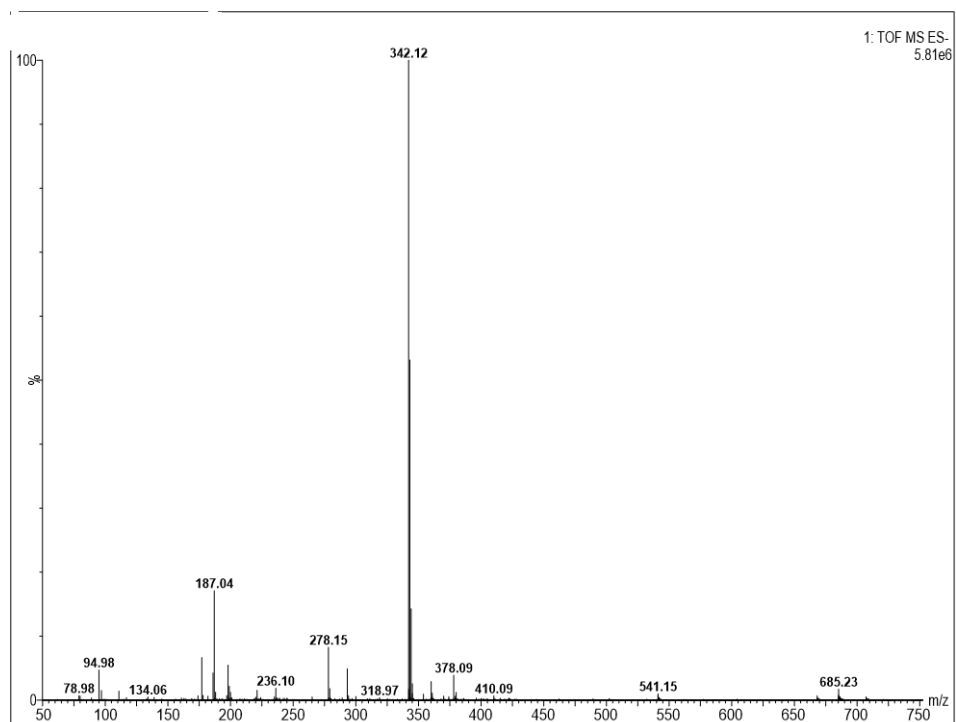


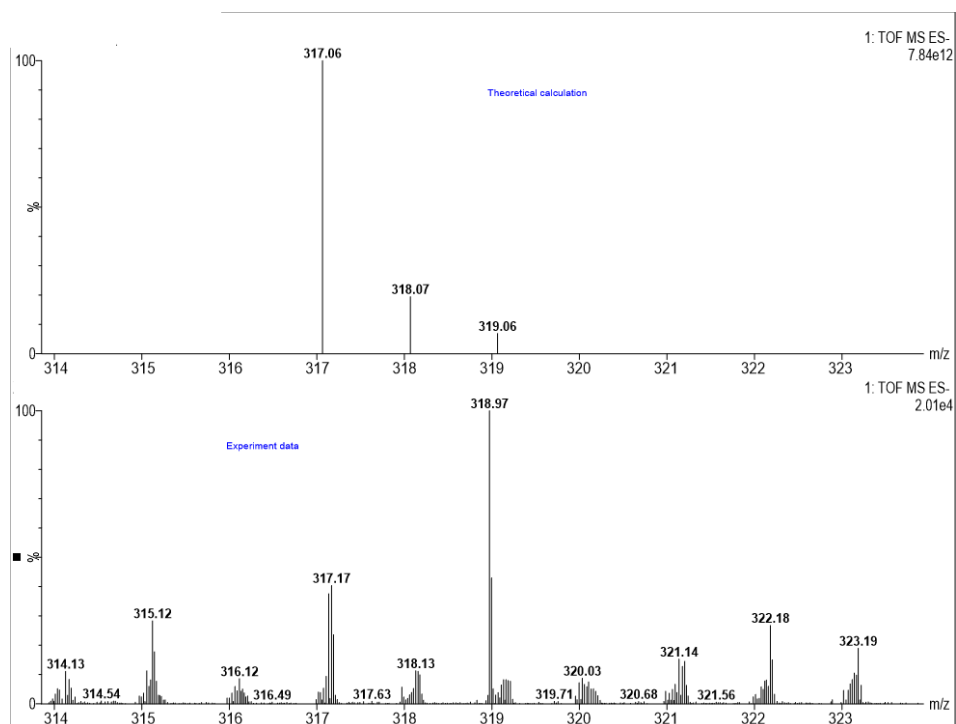
- SO_2CH_3 family – positive ionization
 - $\text{SO}_2\text{CH}_3\text{CH}_2\text{H}_3$



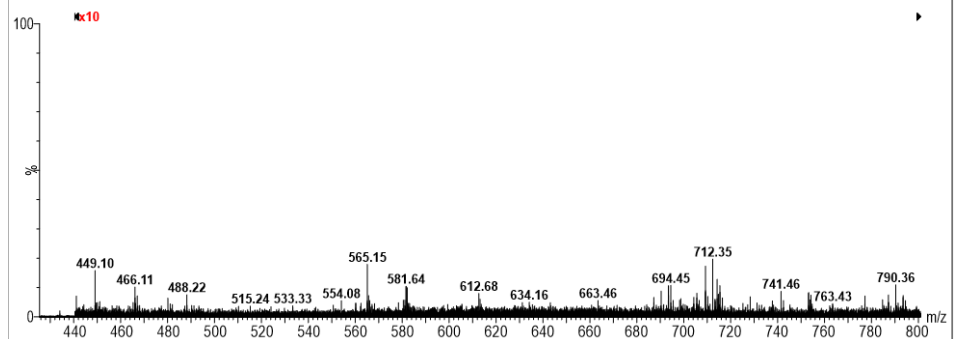
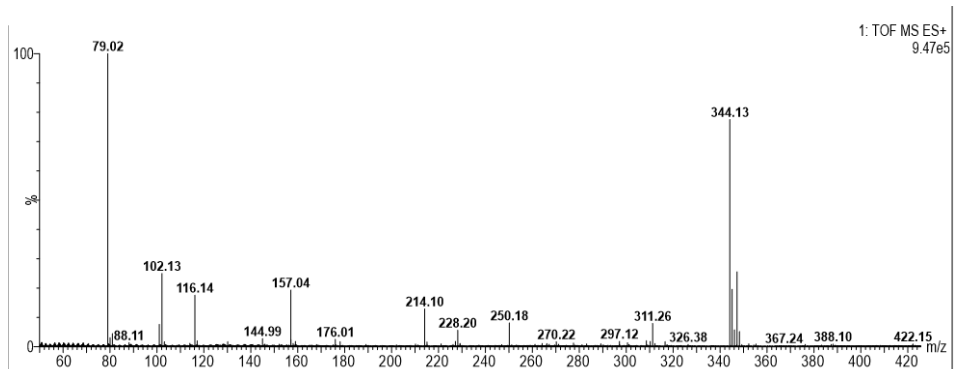
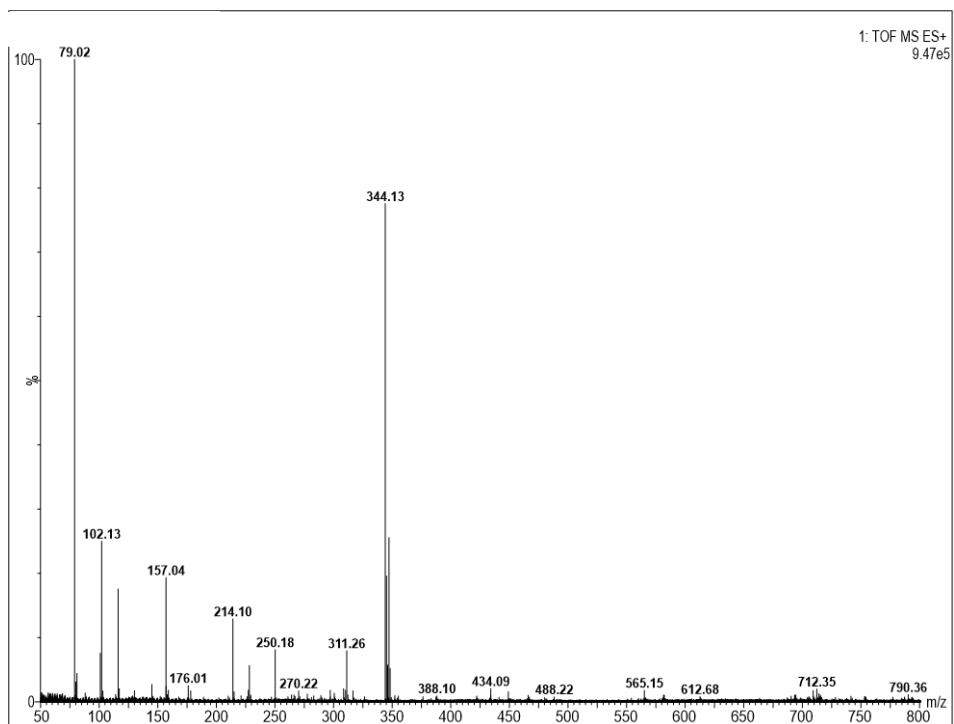


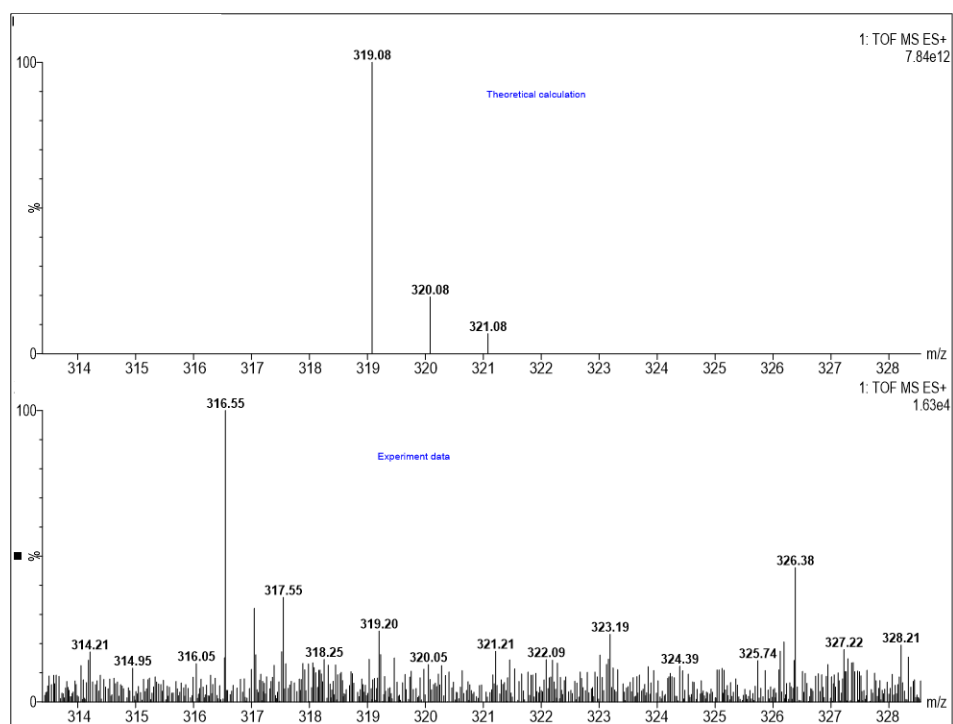
- SO₂NH₂ family – negative ionization
 - SO₂NH₂ CH₂CH₂CH₂CH₃



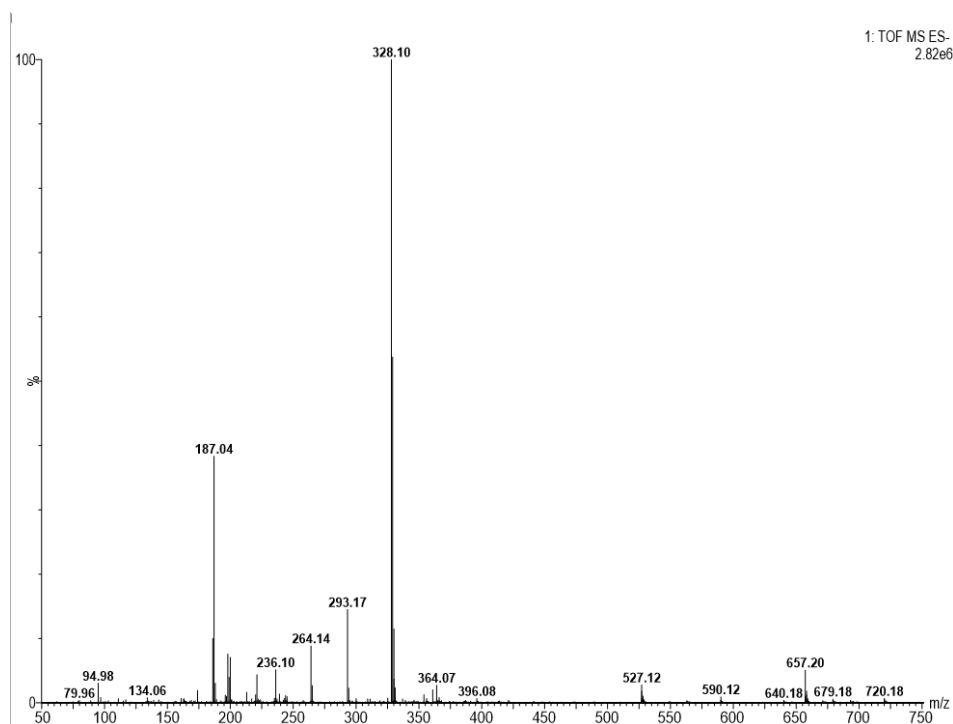


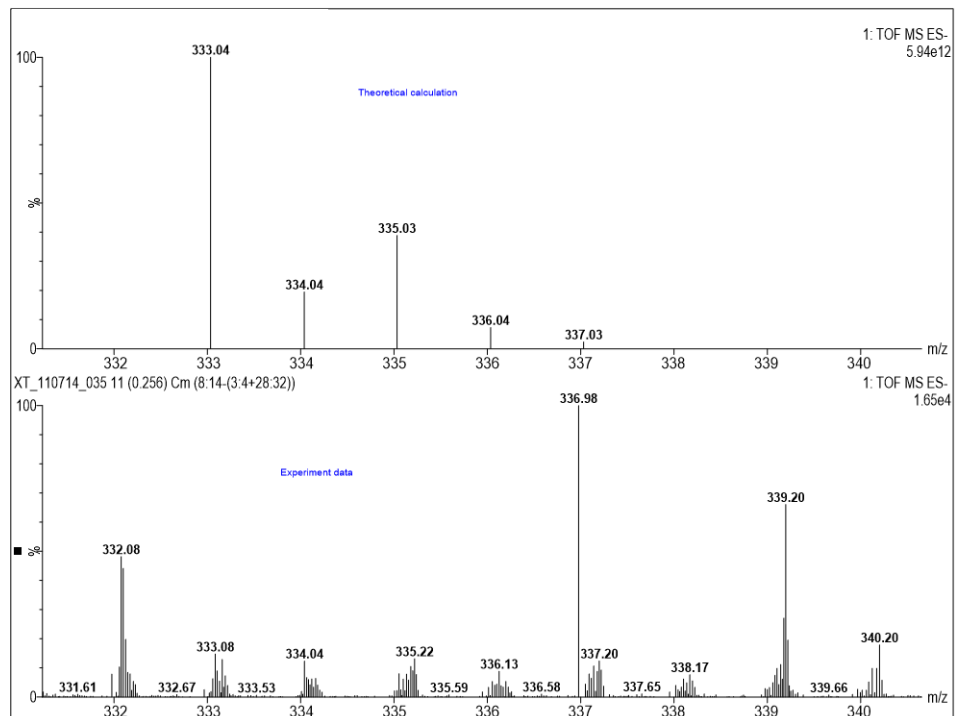
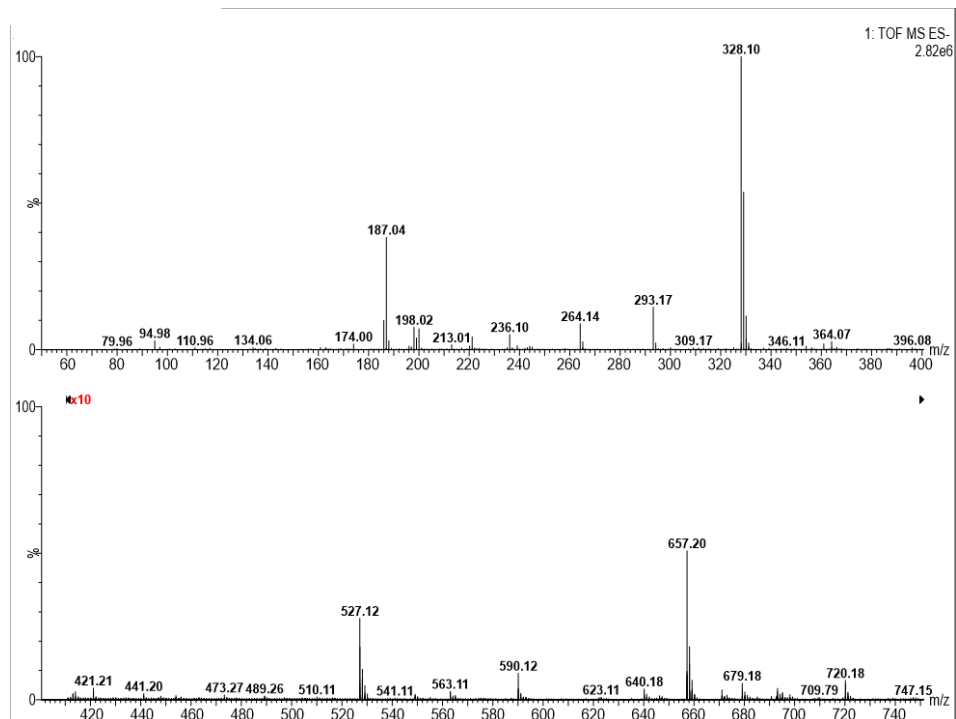
- SO₂NH₂ family – negative ionization
 - SO₂NH₂ CH₂CH₂CH₂CH₃



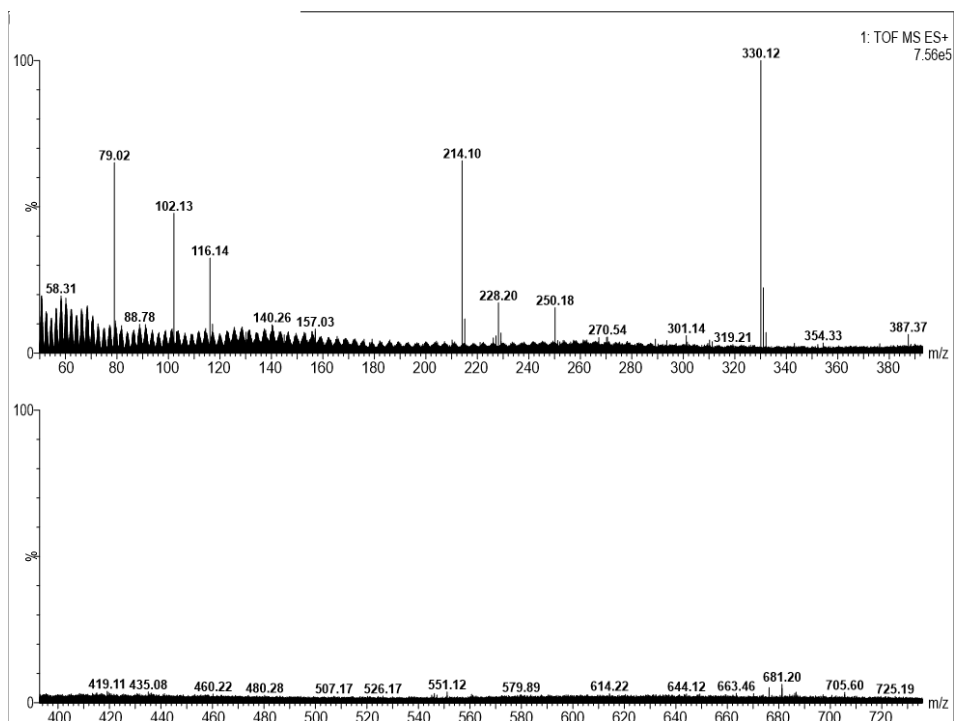
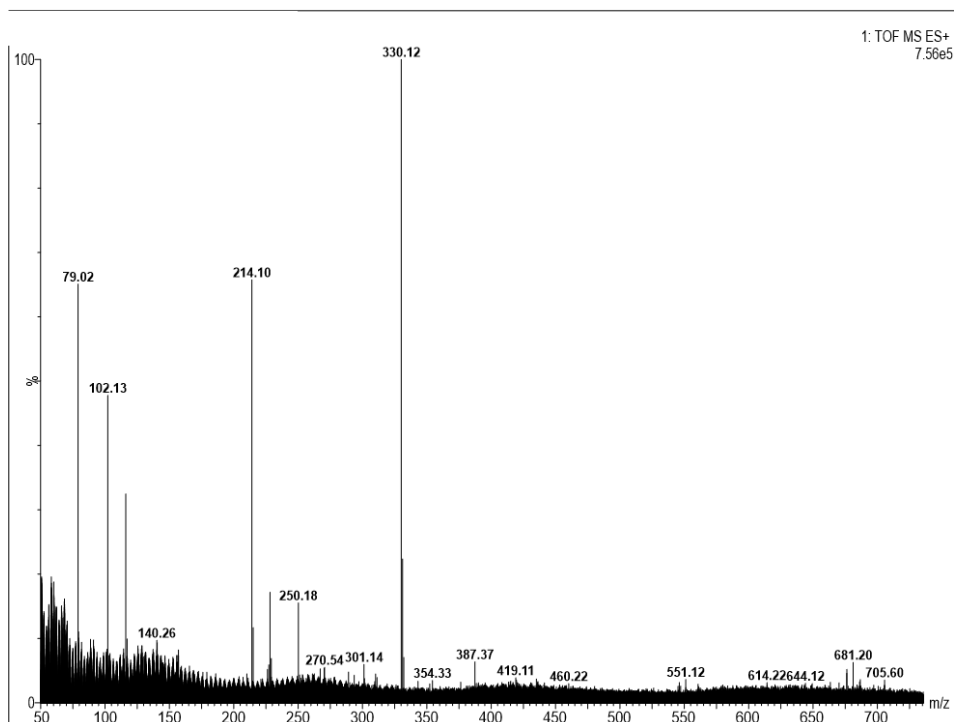


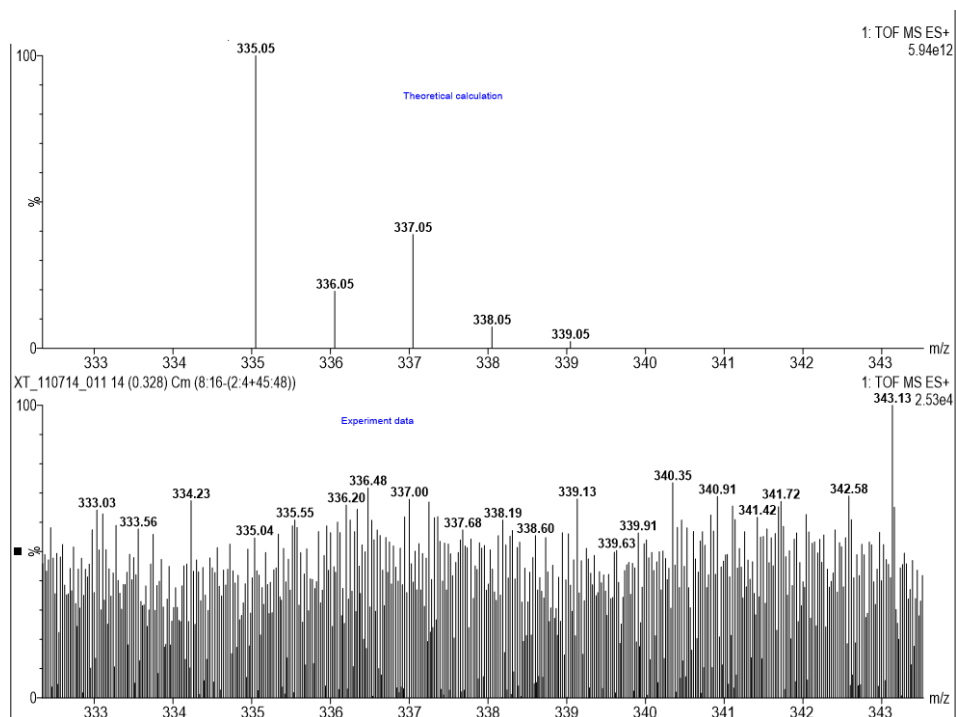
- SO_2NH_2 family – negative ionization
 - $\text{SO}_2\text{NH}_2\text{CH}_2\text{CH}_2\text{CH}_3$



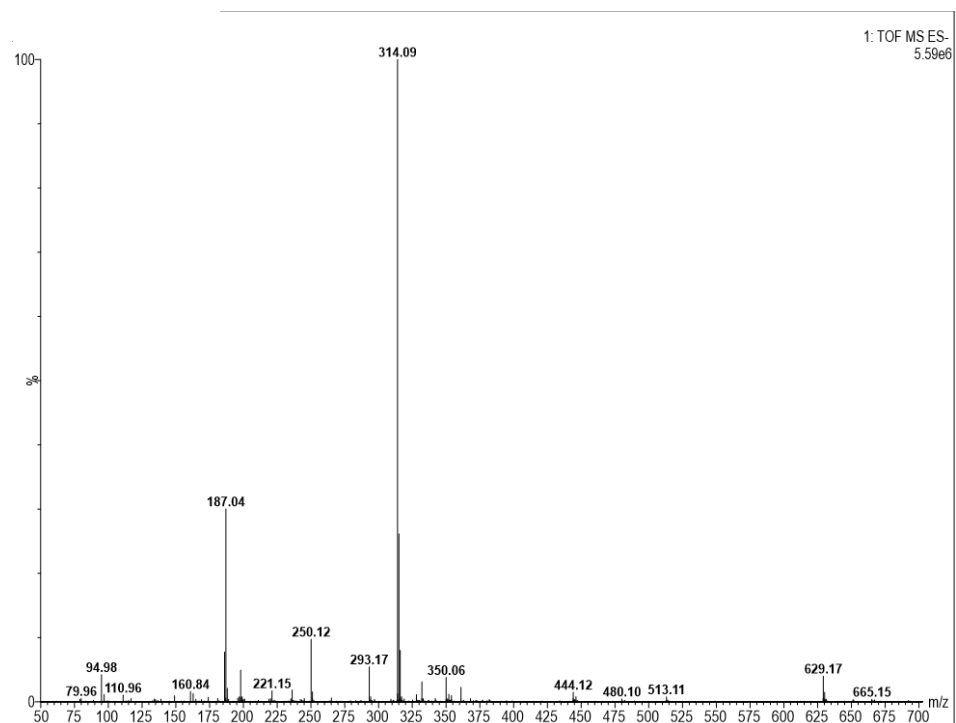


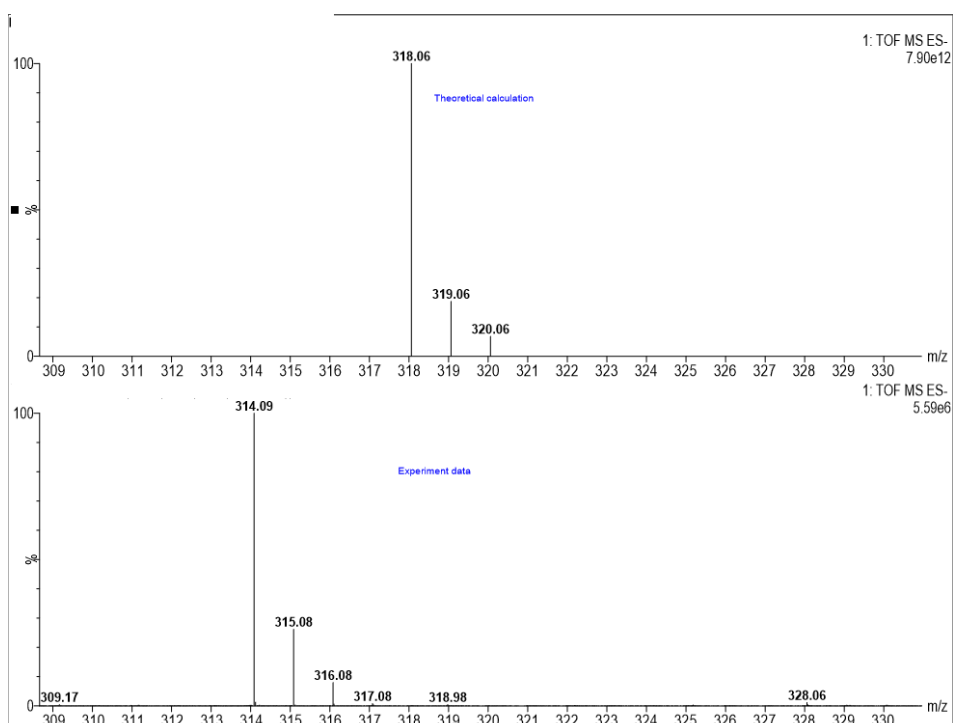
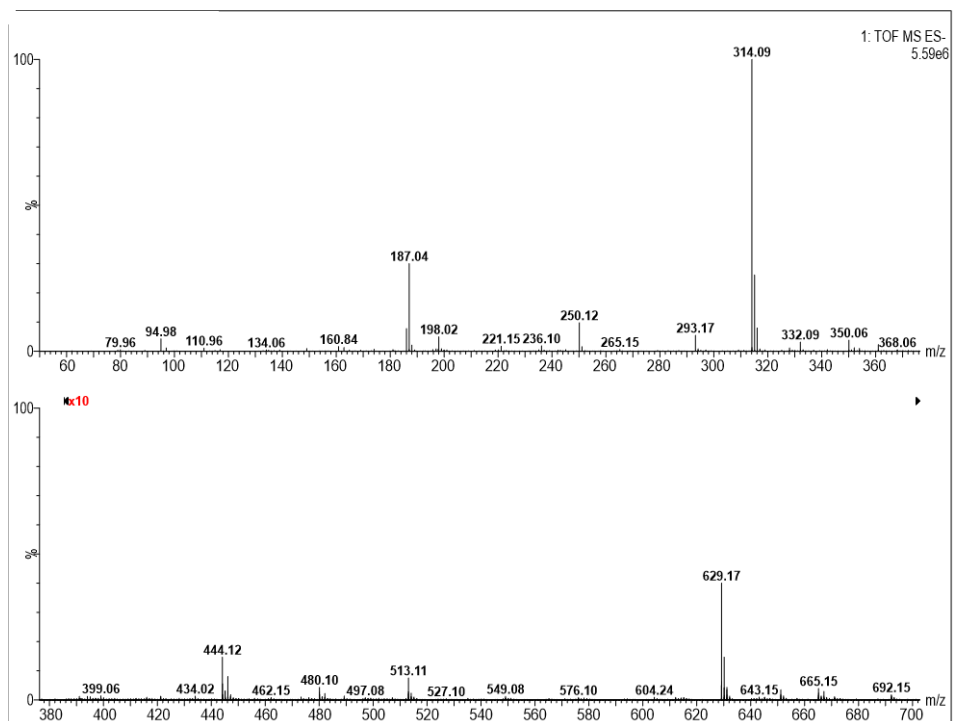
- SO_2NH_2 family – positive ionization
 - $\text{SO}_2\text{NH}_2\text{CH}_2\text{CH}_2\text{CH}_3$



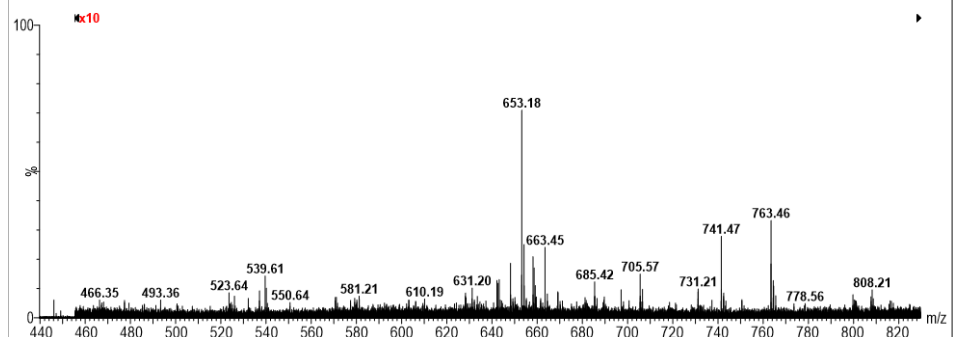
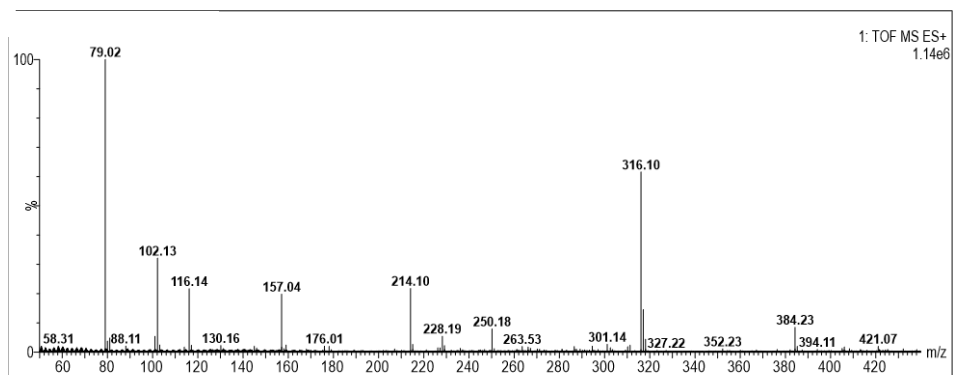
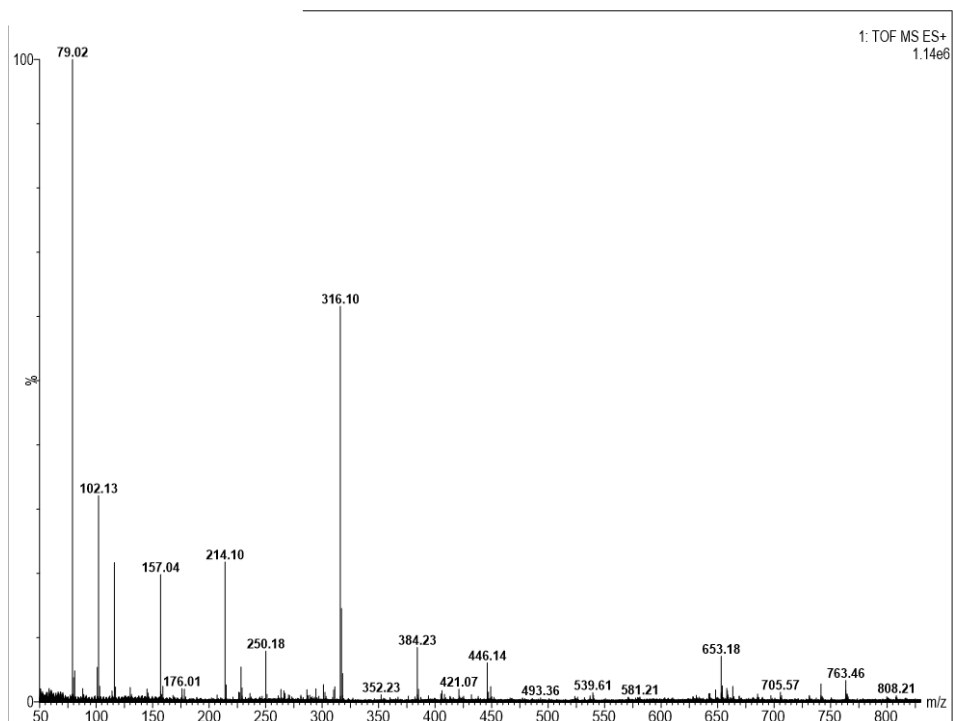


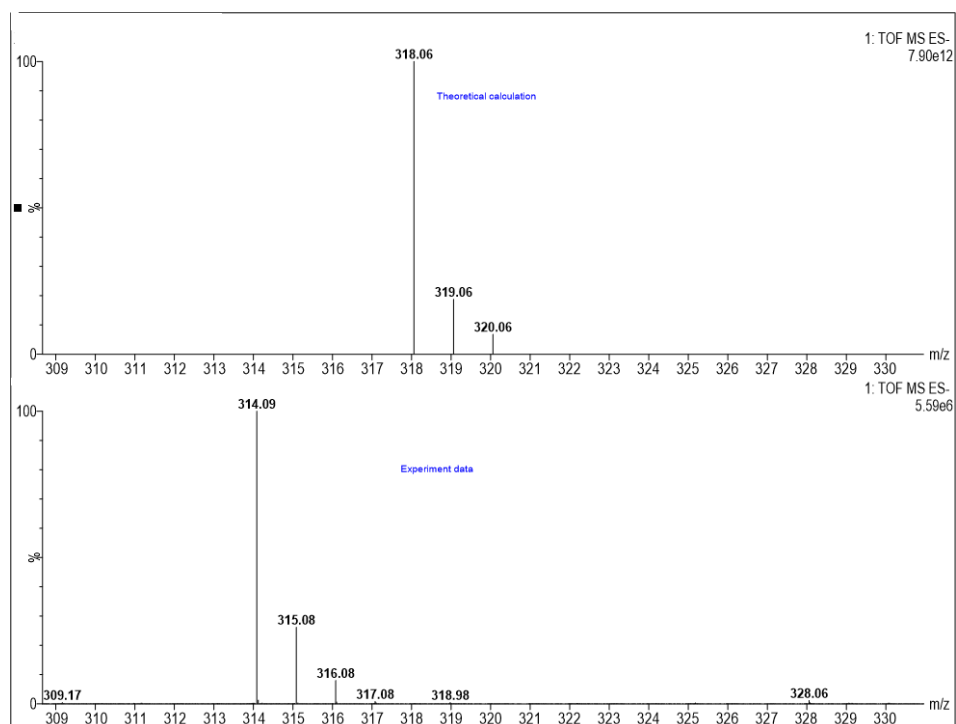
- SO_2NH_2 family – negative ionization
 - $\text{SO}_2\text{NH}_2\text{CH}_2\text{CH}_3$



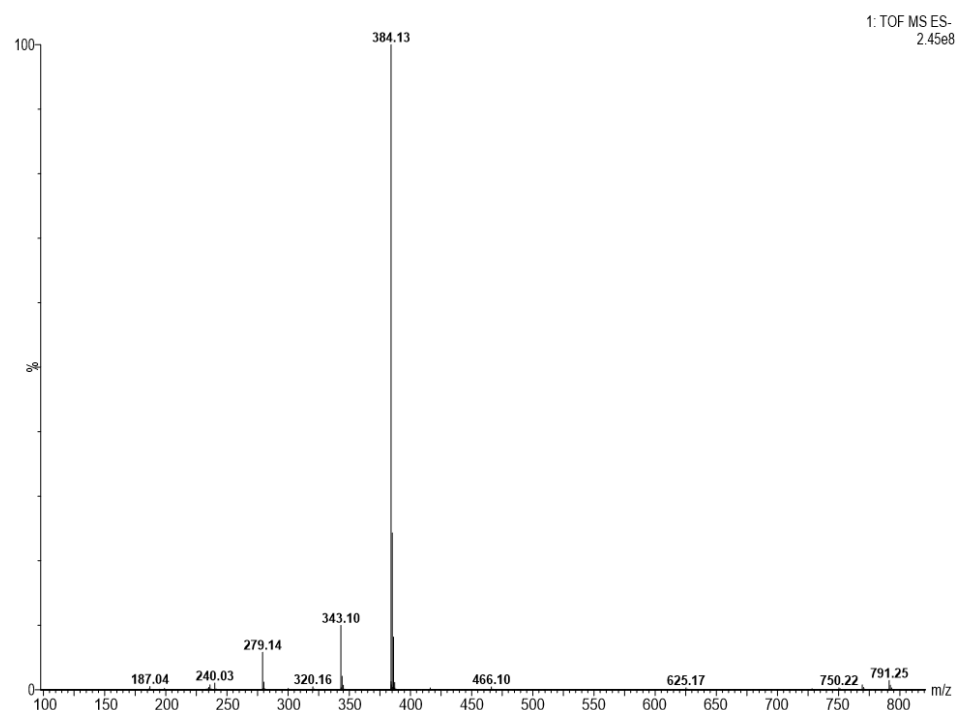


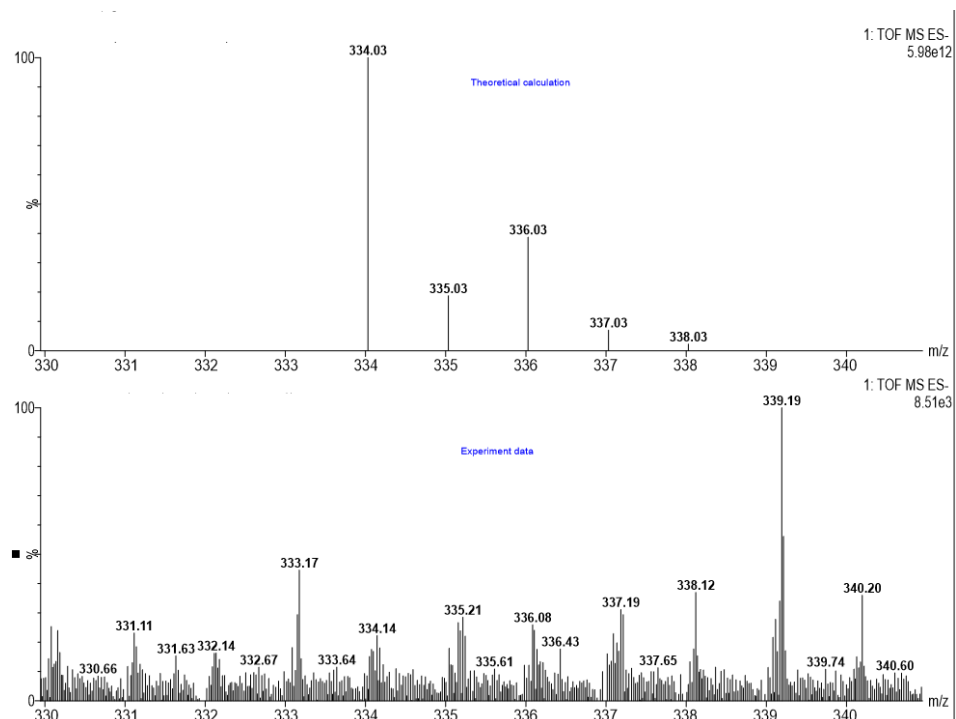
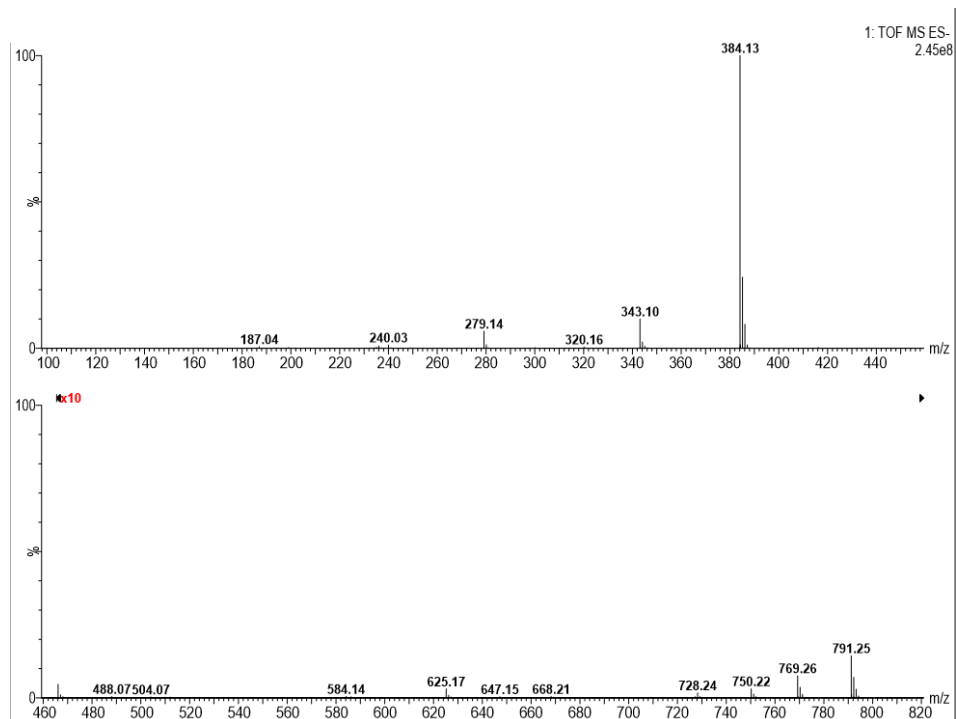
- SO_2NH_2 family – positive ionization
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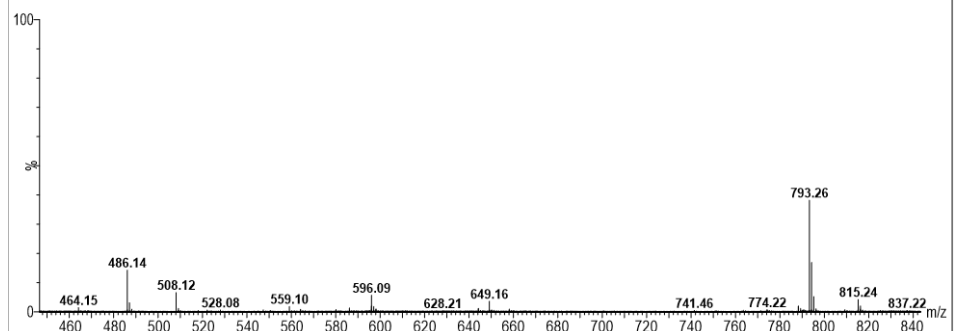
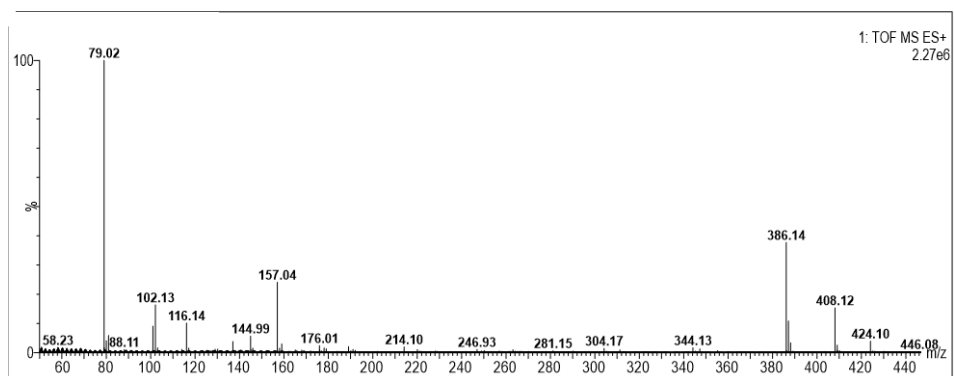
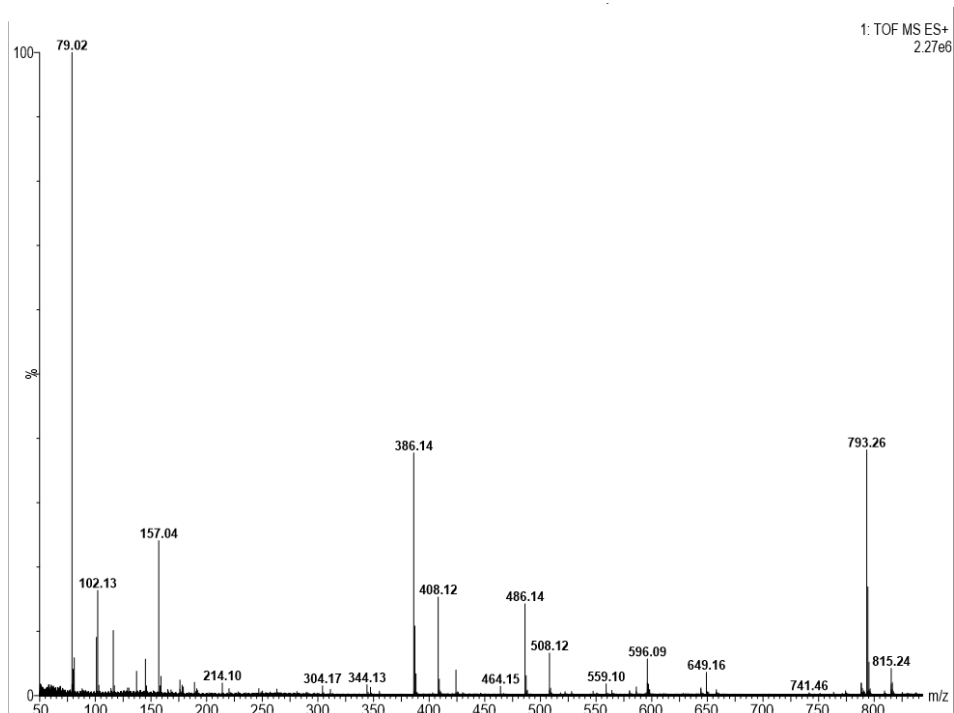


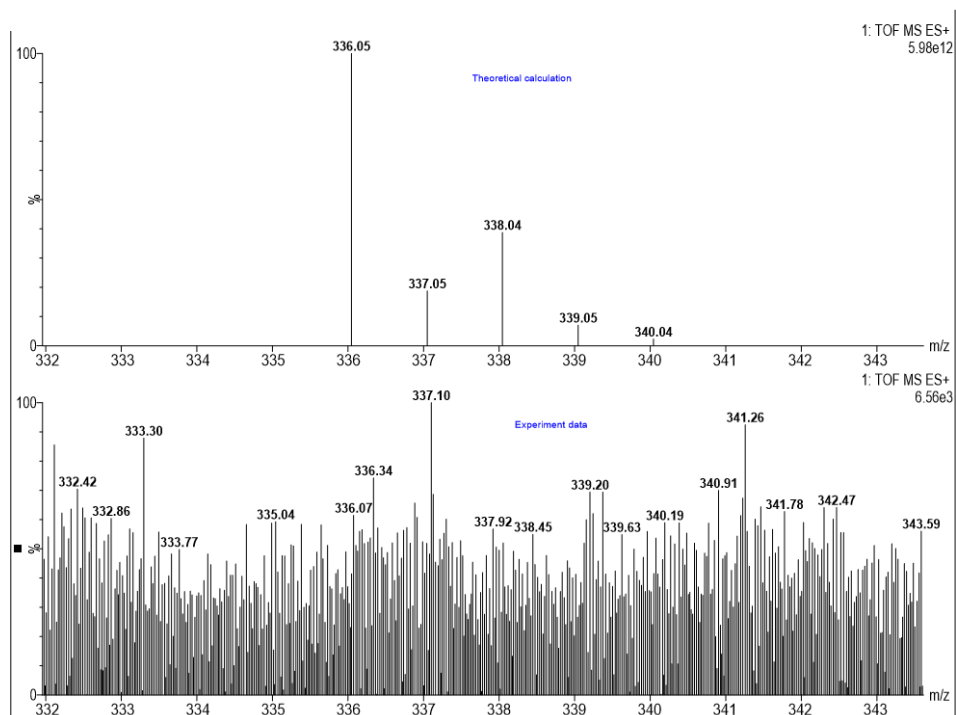
- $\text{SO}_2\text{NHCOCH}_3$ family – negative ionization
 - $\text{SO}_2\text{NHCOCH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$



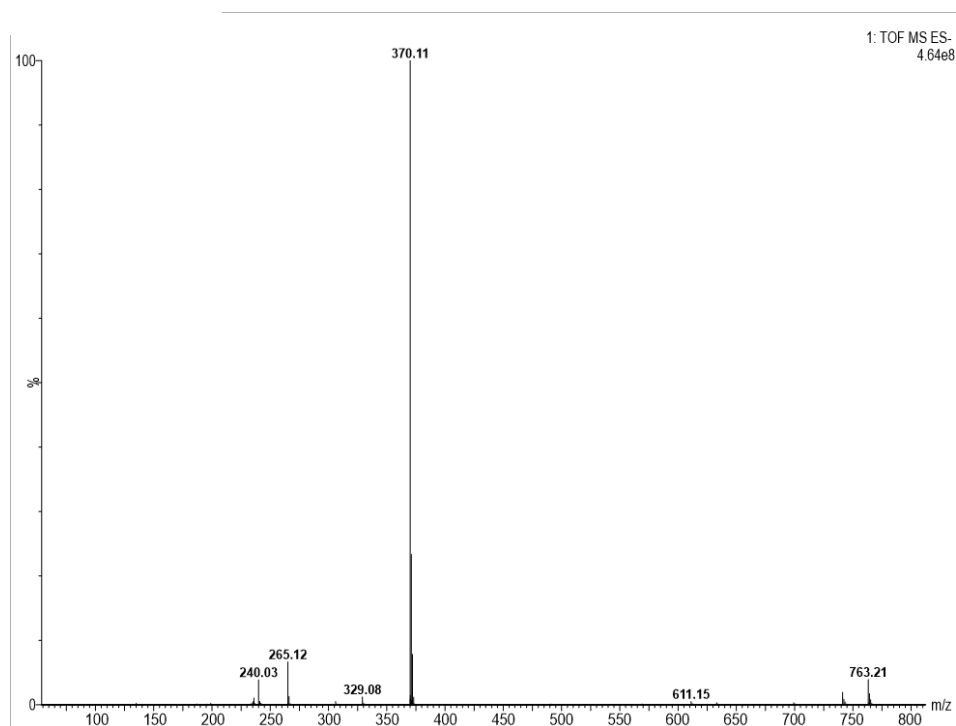


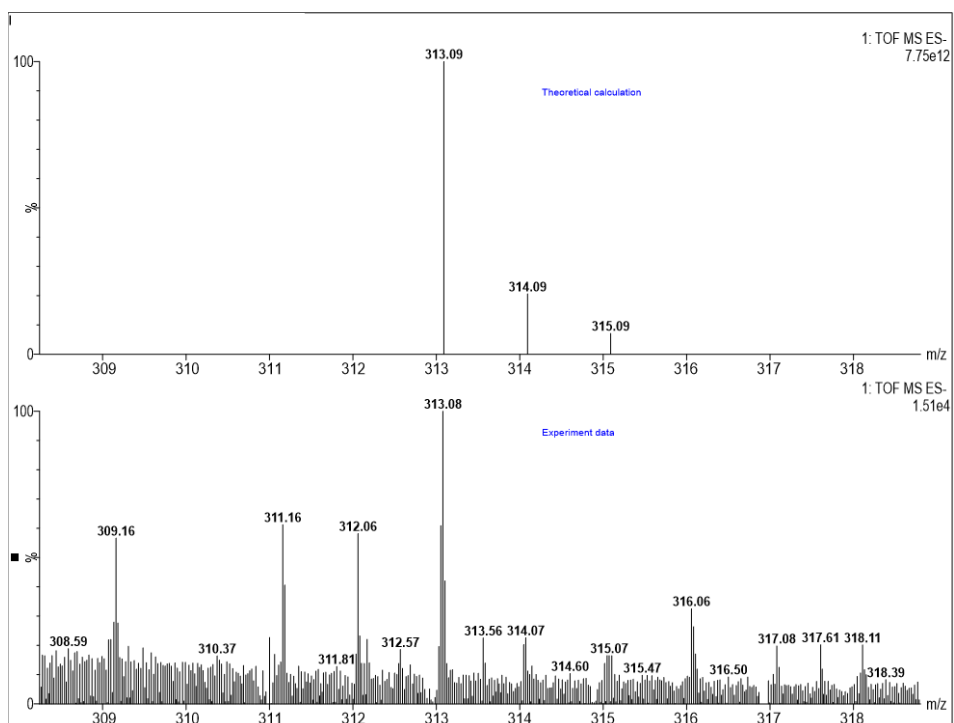
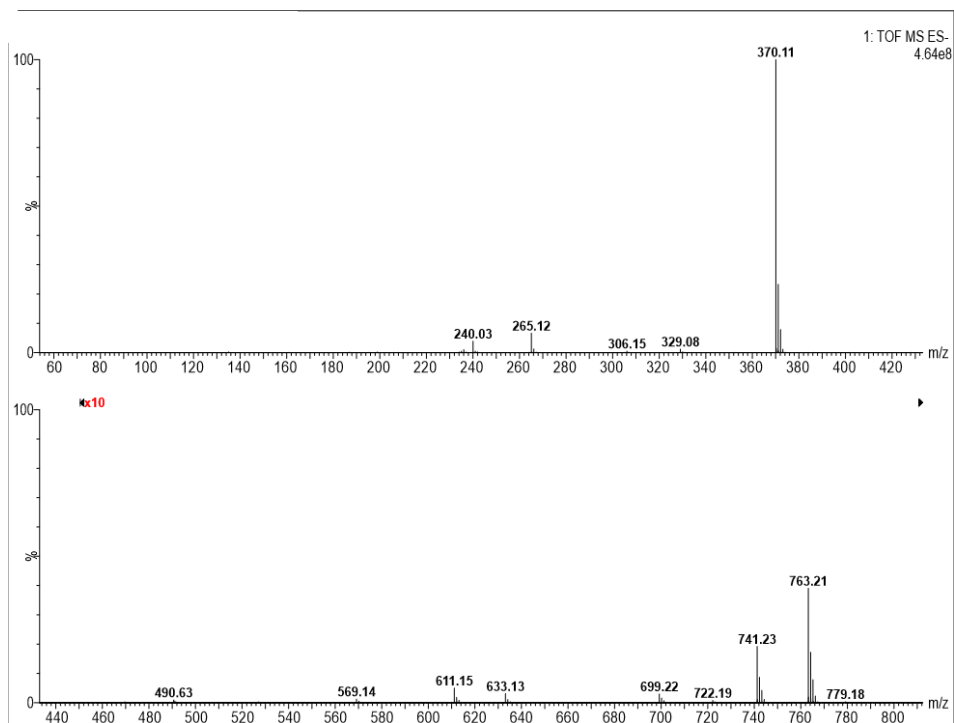
- $\text{SO}_2\text{NHCOCH}_3$ family – positive ionization
 - $\text{SO}_2\text{NHCOCH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$



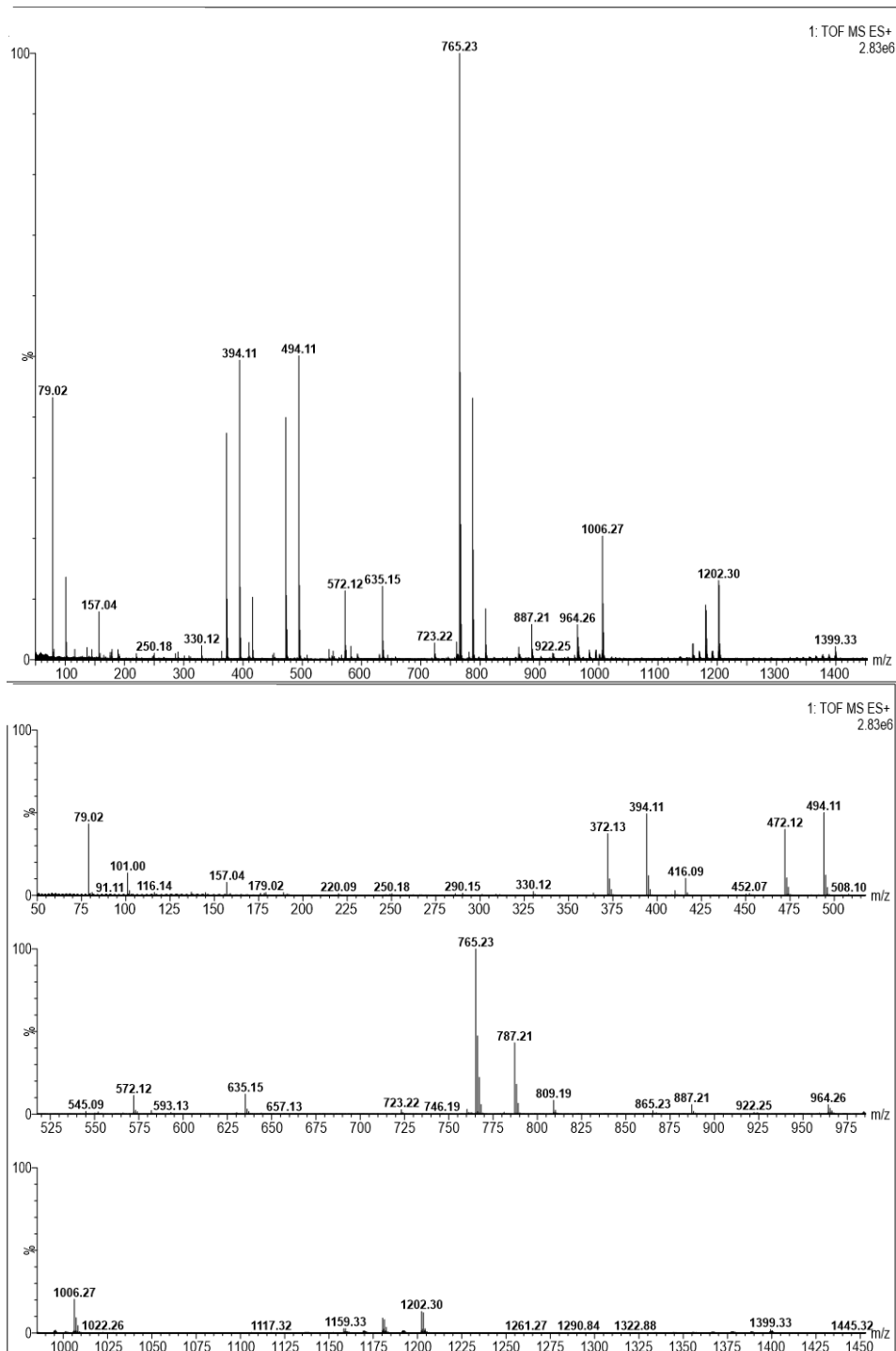


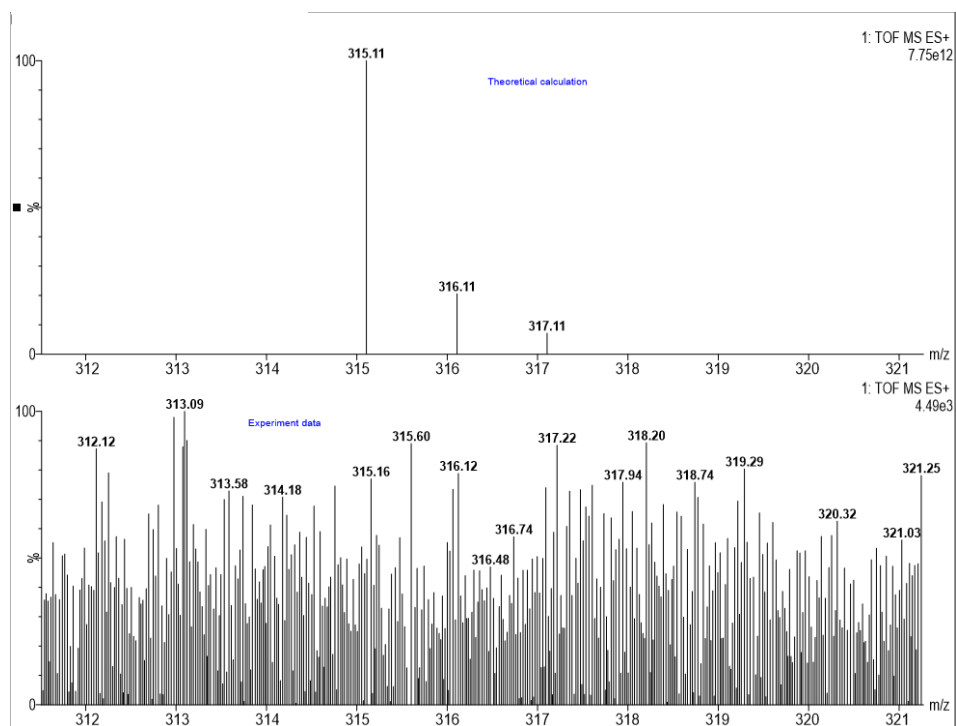
- $\text{SO}_2\text{NHCOCH}_3$ family – negative ionization
 - $\text{SO}_2\text{NHCOCH}_3\text{CH}_2\text{CH}_2\text{CH}_3$



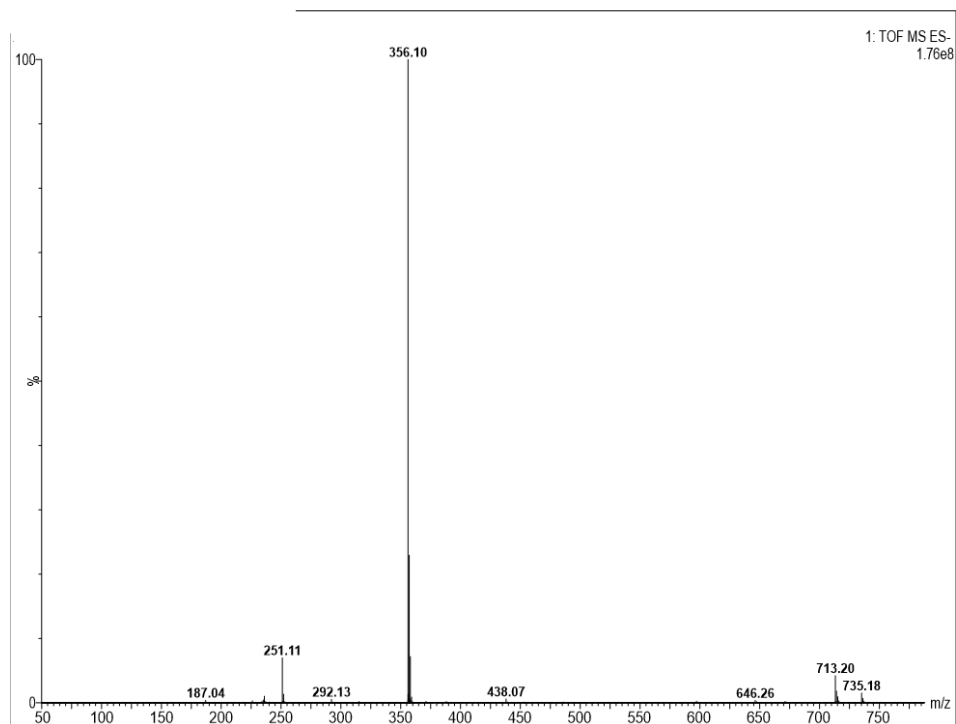


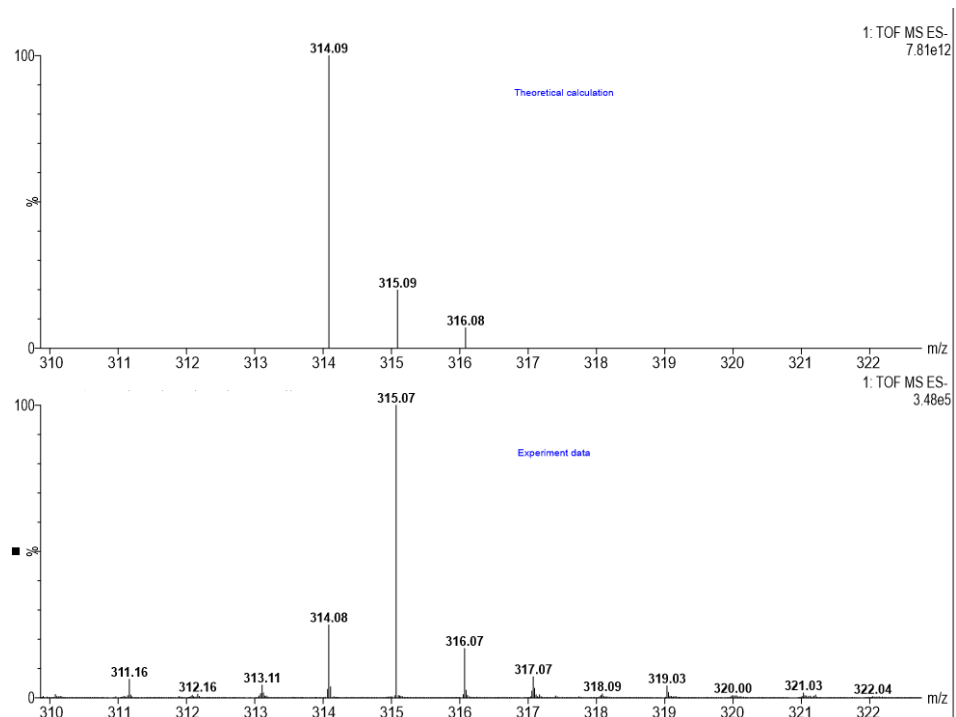
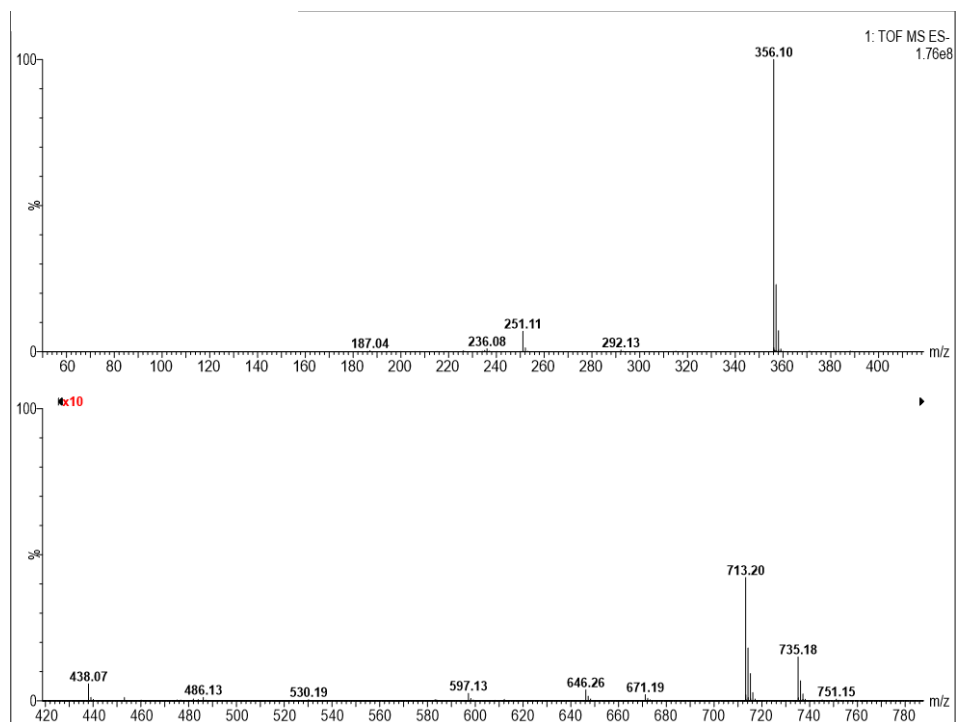
- $\text{SO}_2\text{NHCOCH}_3$ family – positive ionization
 - $\text{SO}_2\text{NHCOCH}_3\text{CH}_2\text{CH}_2\text{CH}_3$



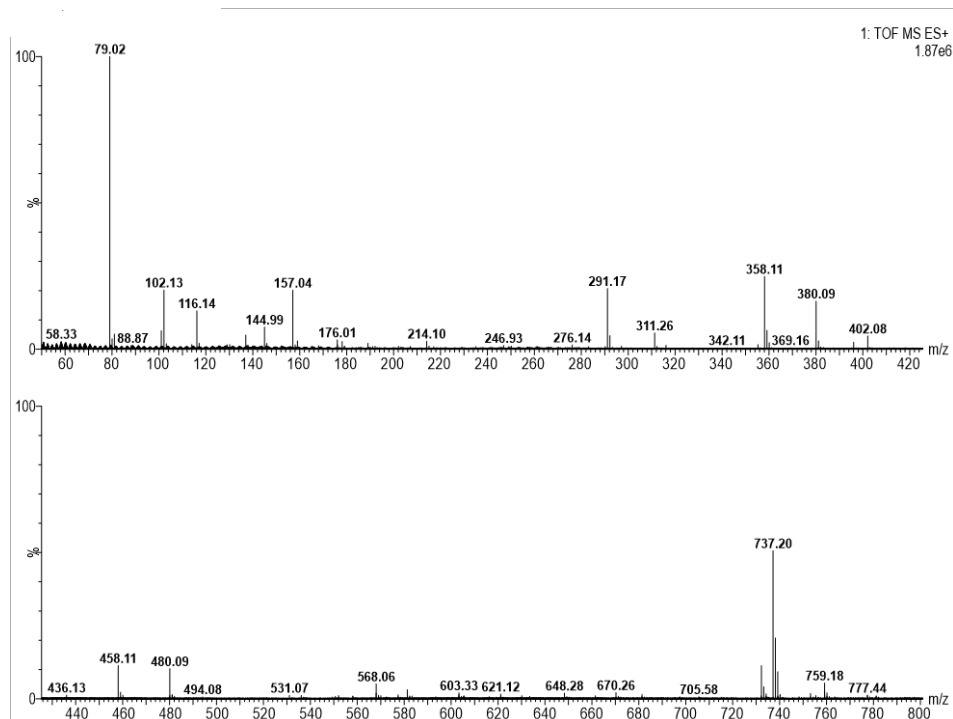
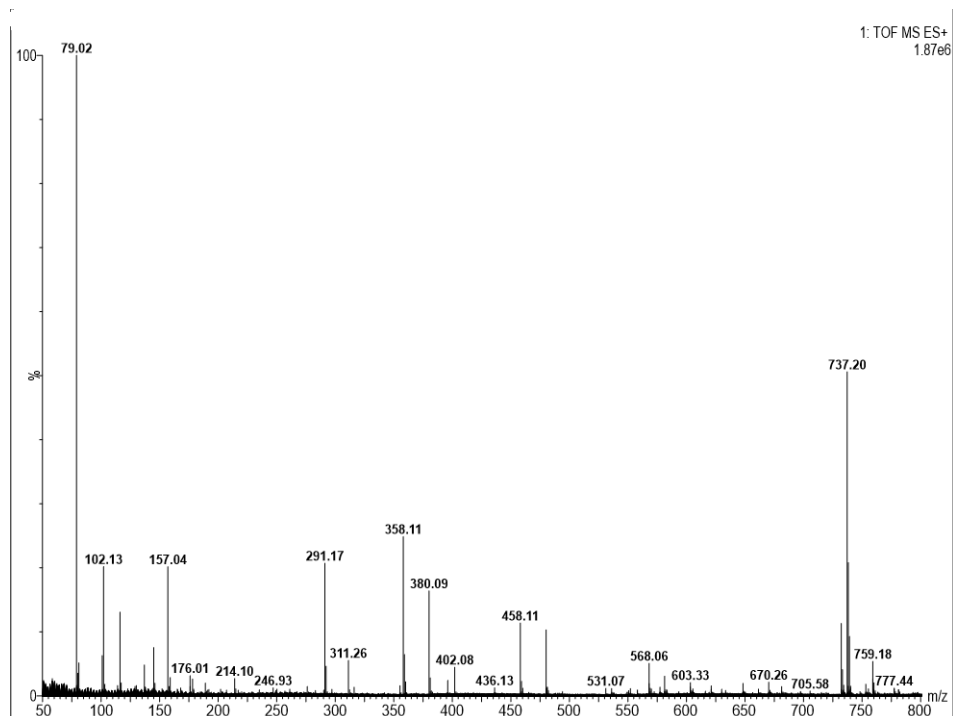


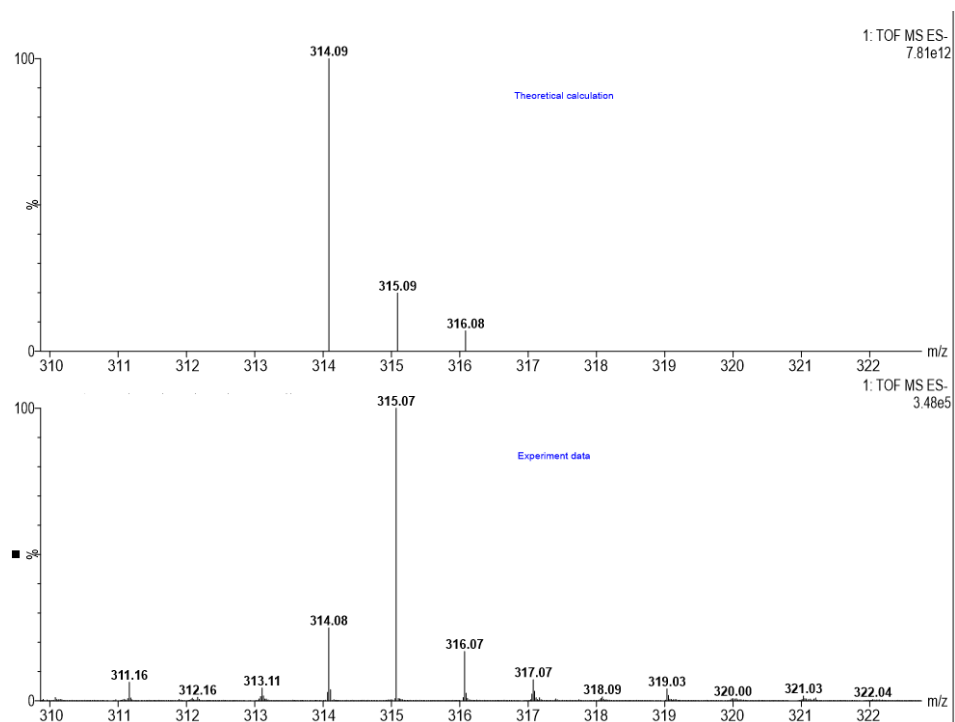
- $\text{SO}_2\text{NHCOCH}_3$ family – negative ionization
 - $\text{SO}_2\text{NHCOCH}_3\text{CH}_2\text{CH}_2\text{CH}_3$



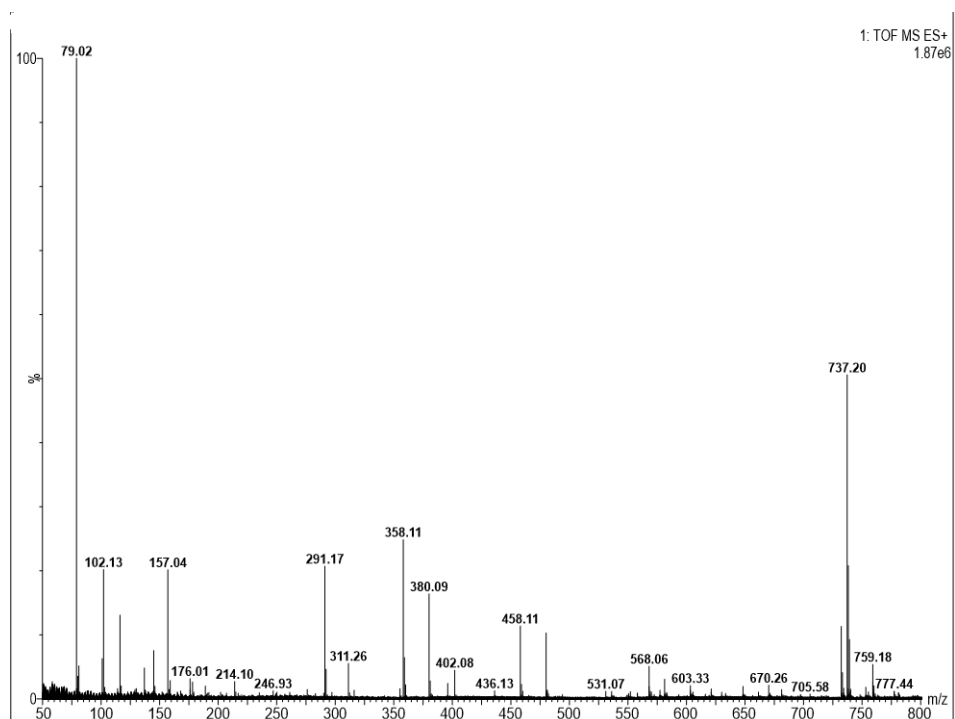


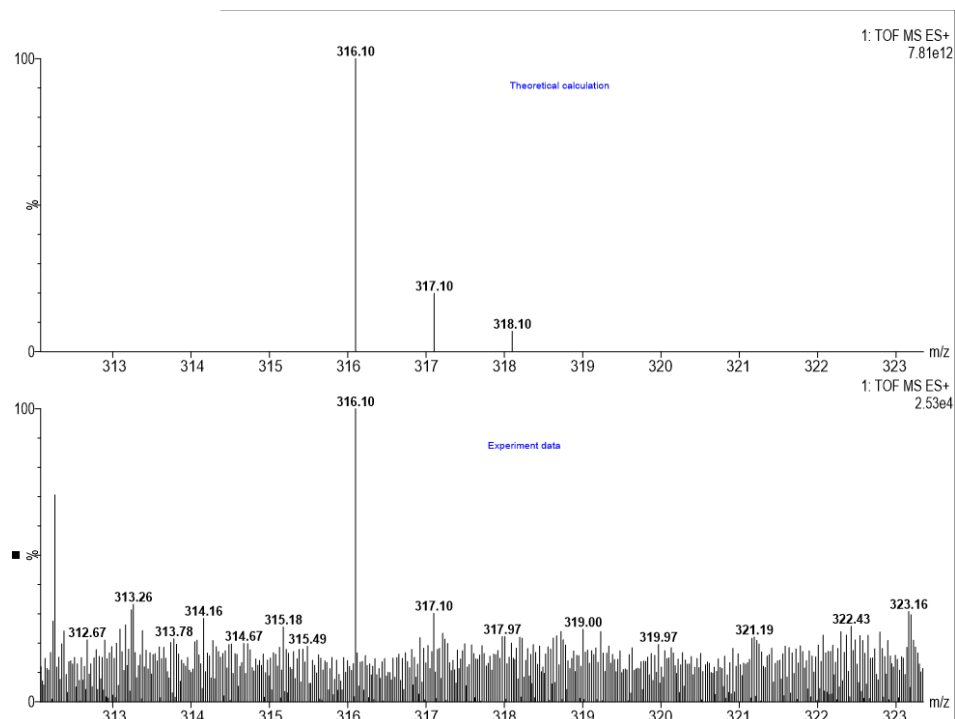
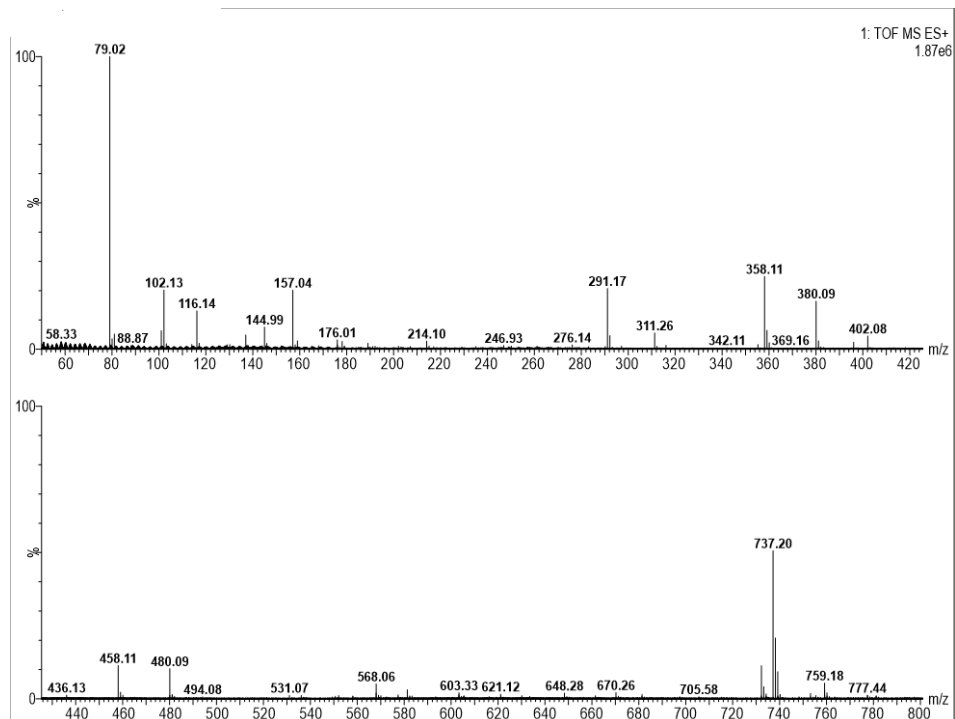
- $\text{SO}_2\text{NHCOCH}_3$ family – negative ionization
 - $\text{SO}_2\text{NHCOCH}_3\text{CH}_2\text{CH}_3$





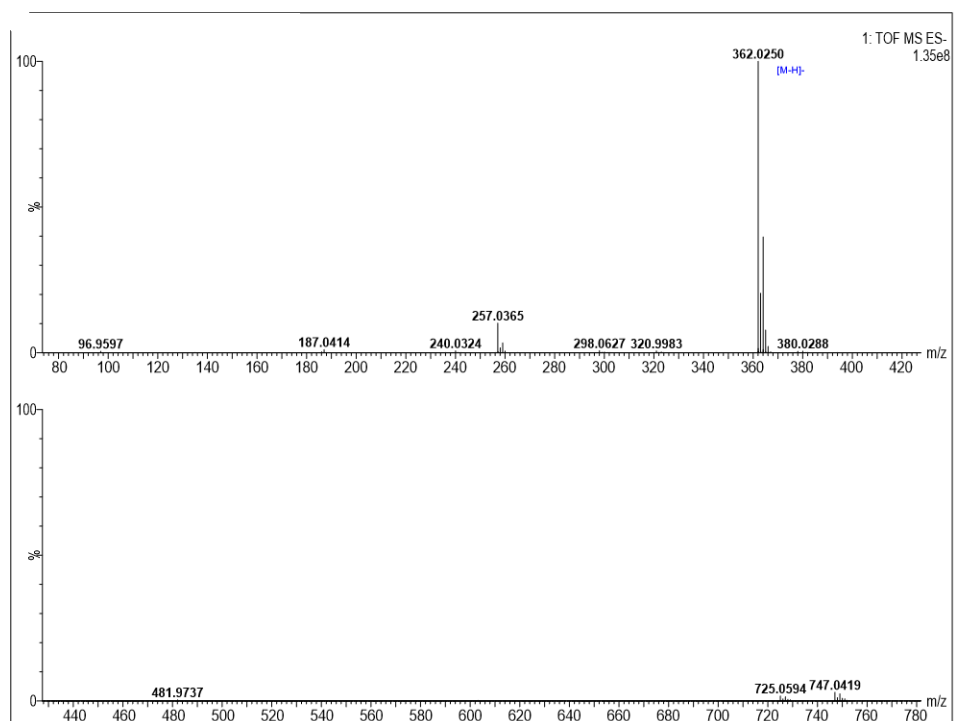
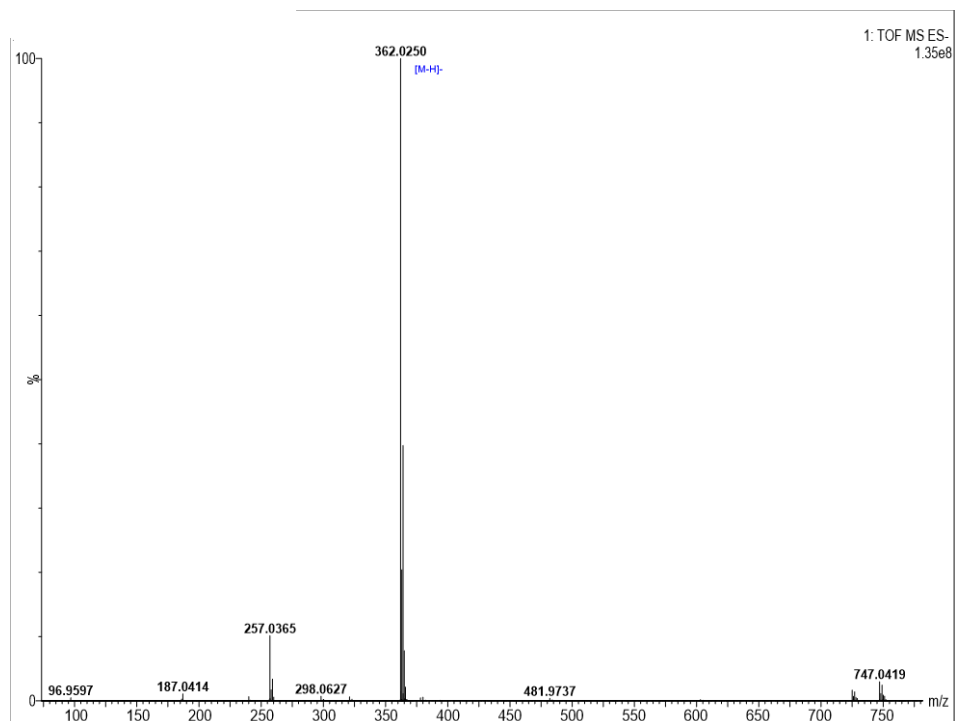
- $\text{SO}_2\text{NHCOCH}_3$ positive – negative ionization
 - $\text{SO}_2\text{NHCOCH}_3\text{CH}_2\text{CH}_3$

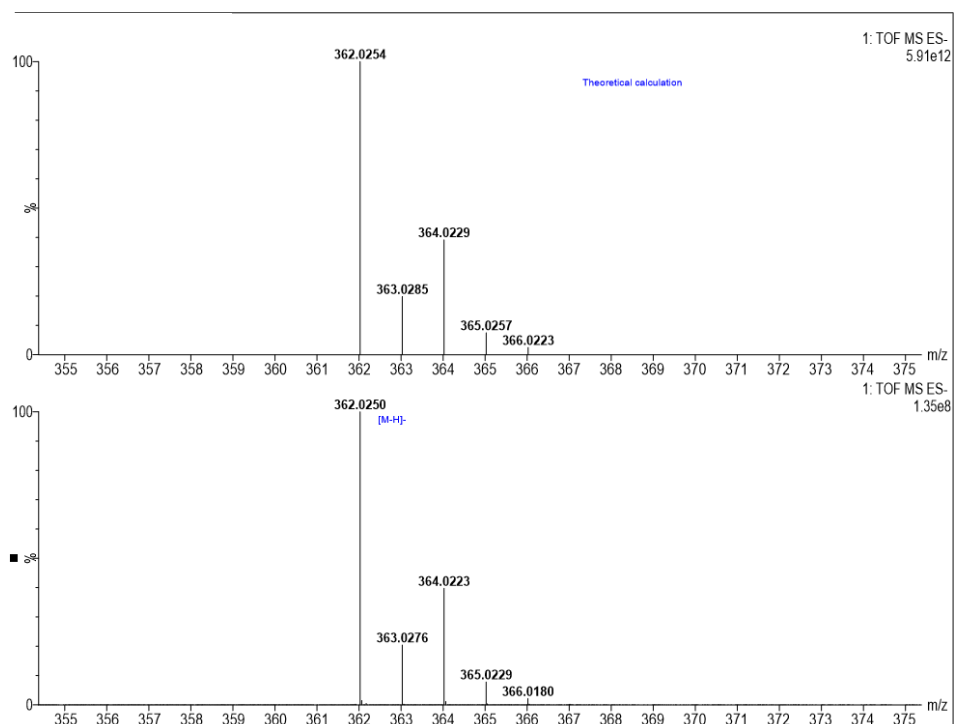




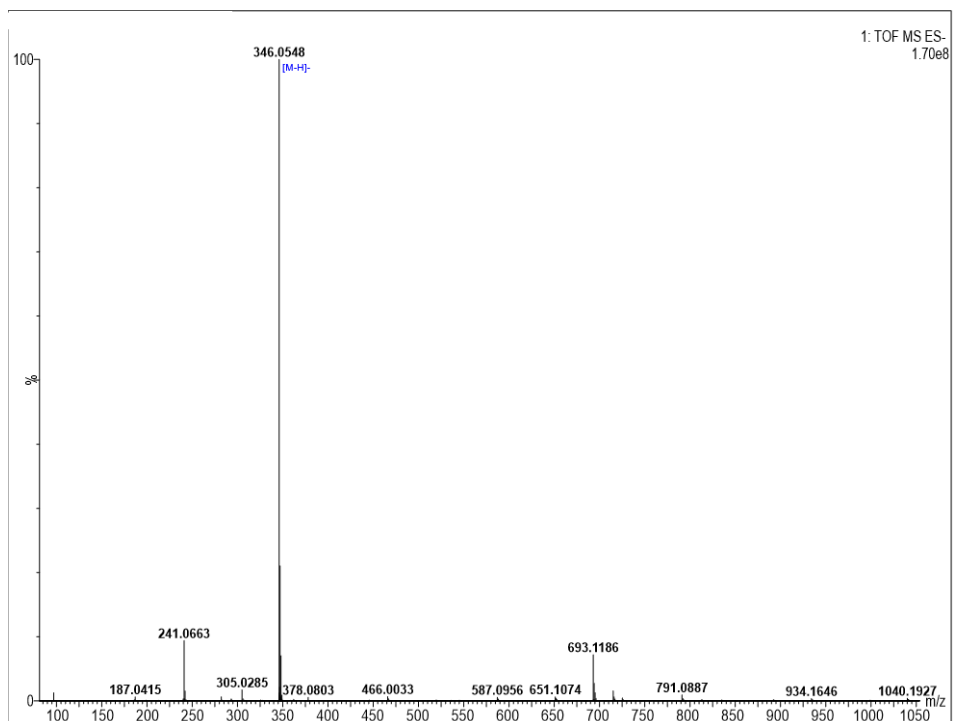
Study 2

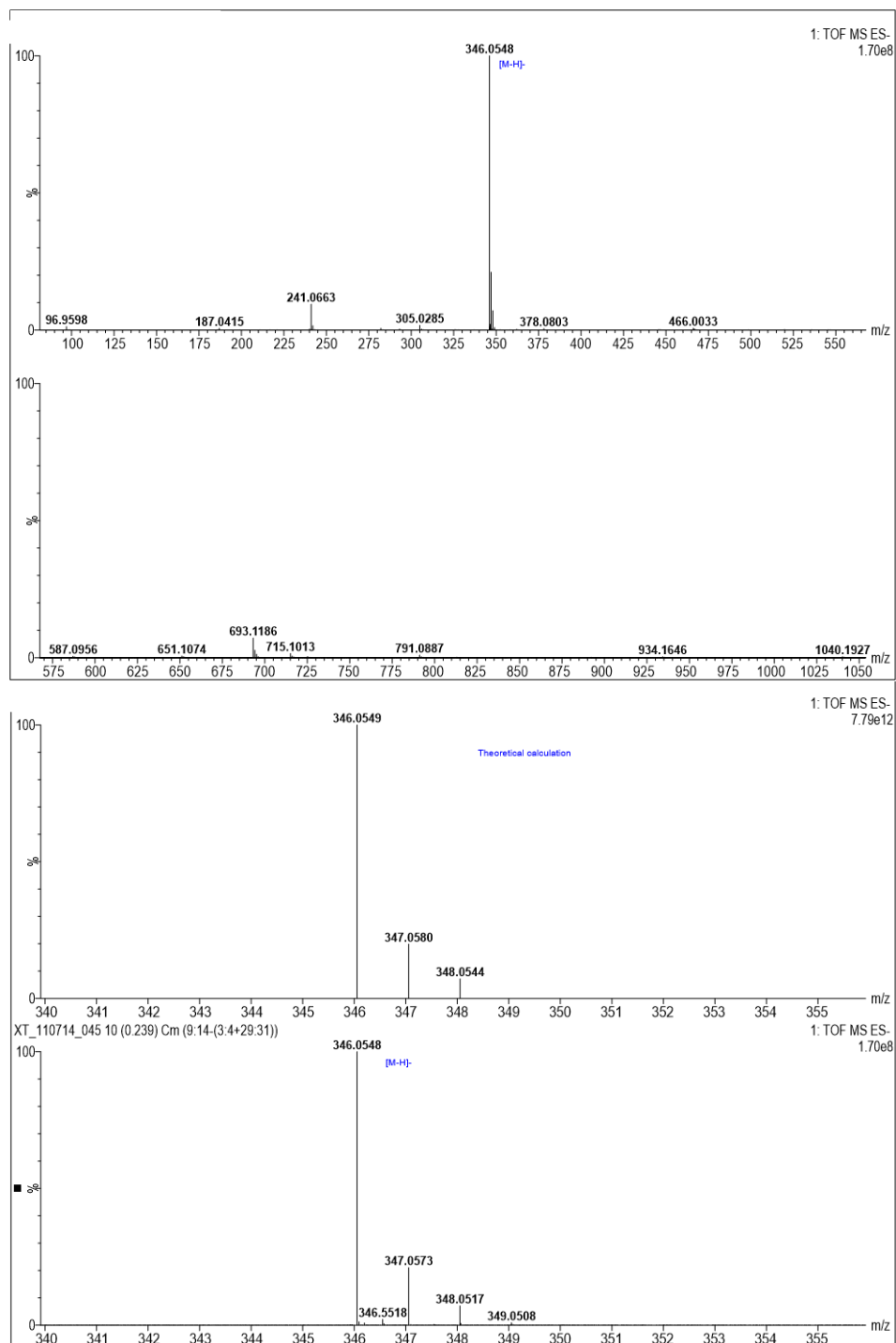
- $\text{SO}_2\text{NHCOCH}_3$ family- negative ionization
 - $\text{SO}_2\text{NHCOCH}_3\text{Cl}$



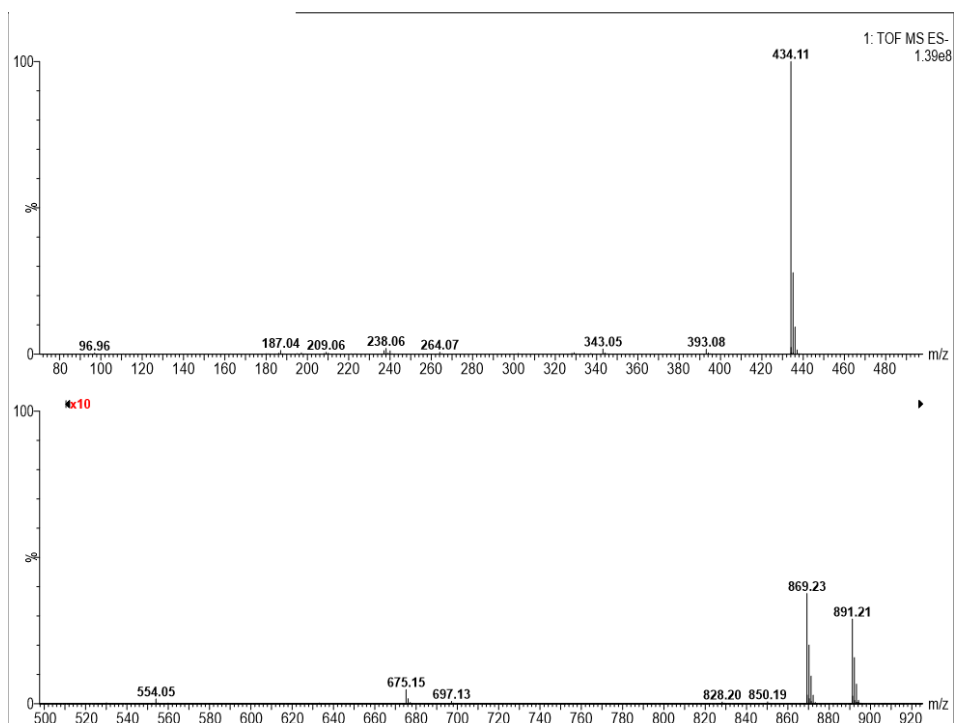
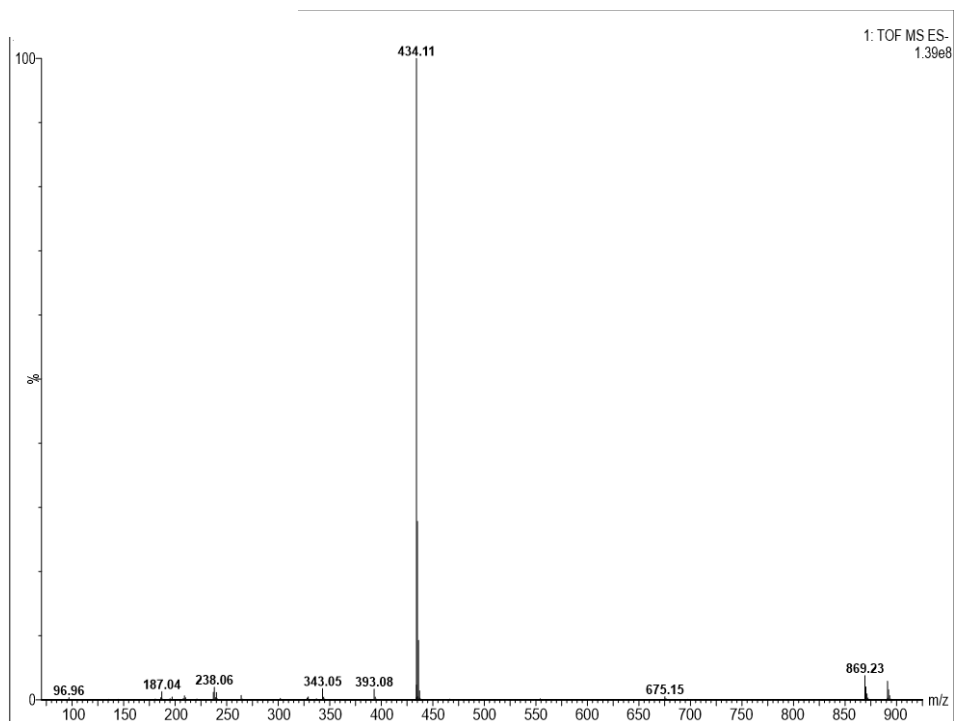


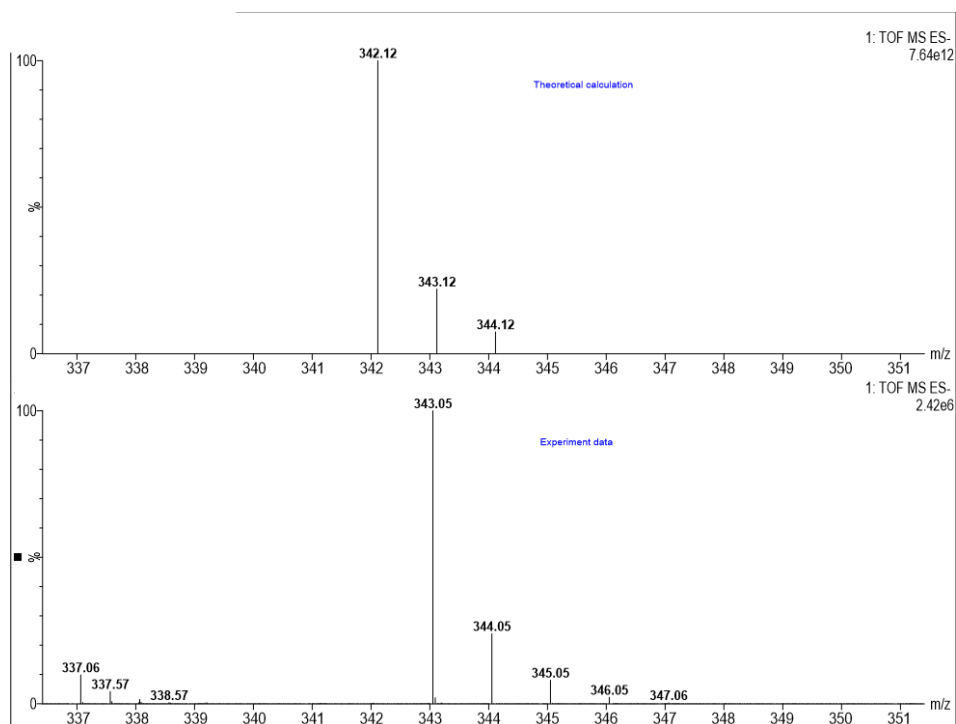
- $\text{SO}_2\text{NHCOCH}_3$ family- negative ionization
 - $\text{SO}_2\text{NHCOCH}_3\text{F}$



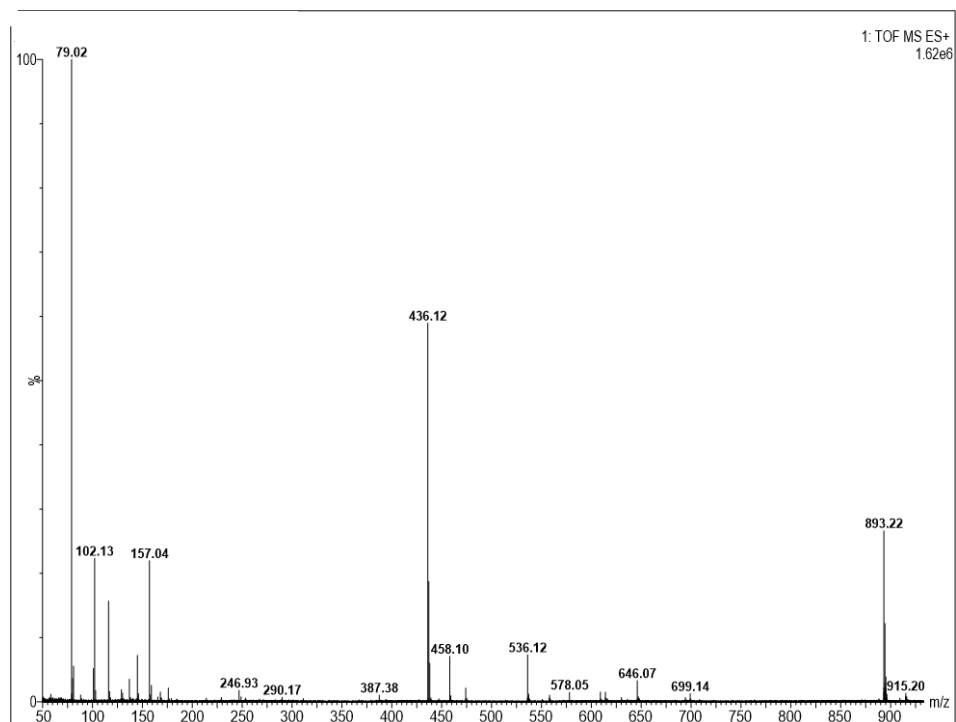


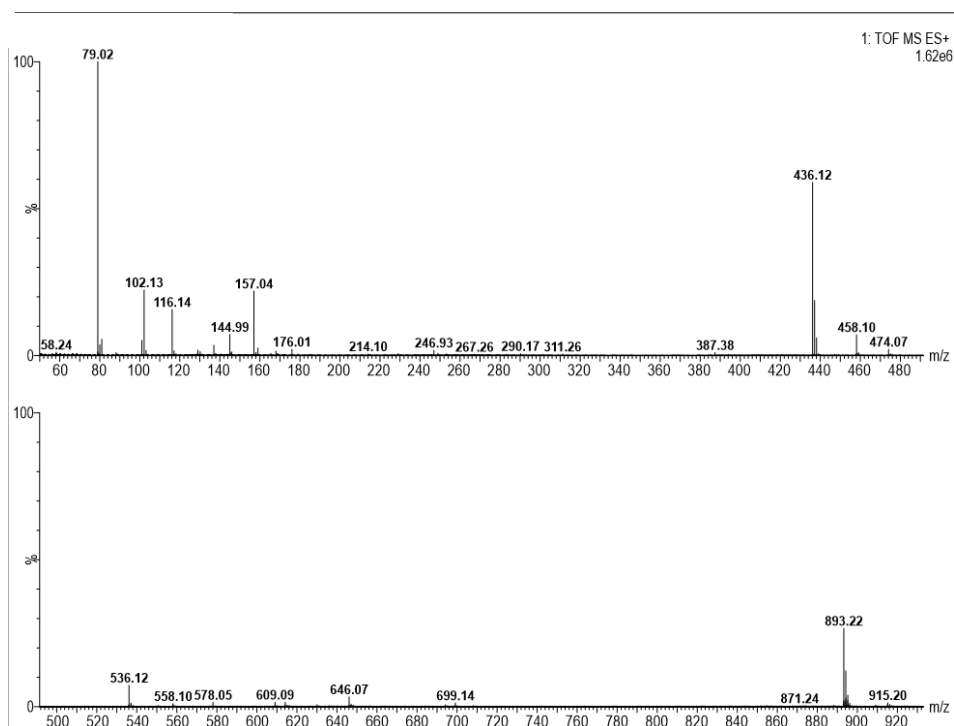
- $\text{SO}_2\text{NHCOCH}_3$ family- negative ionization
 - $\text{SO}_2\text{NHCOCH}_3\text{OBenzyl}$



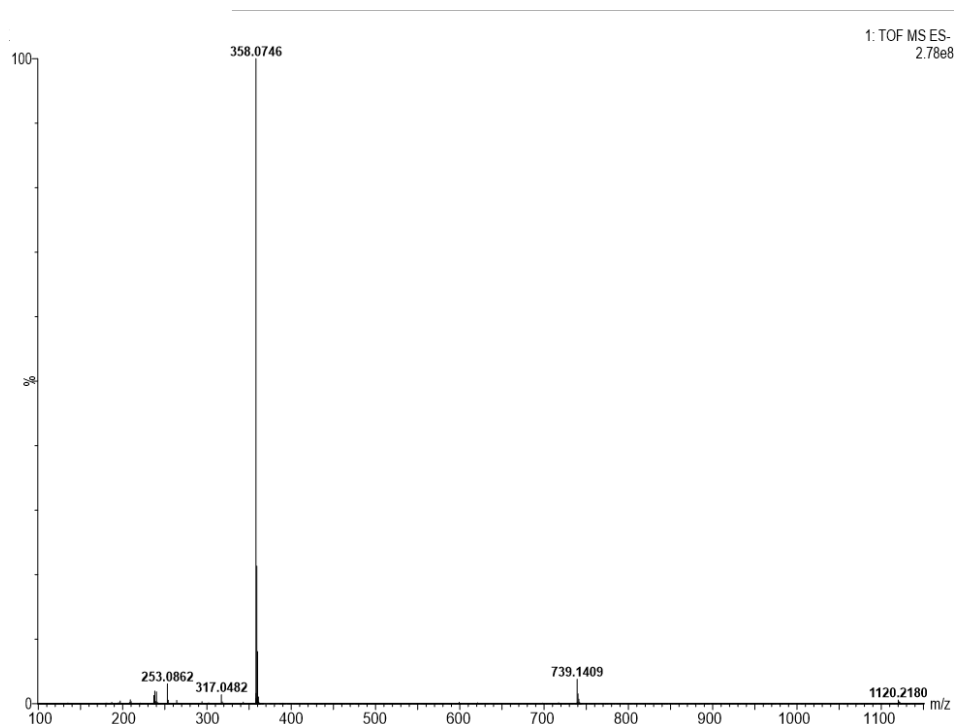


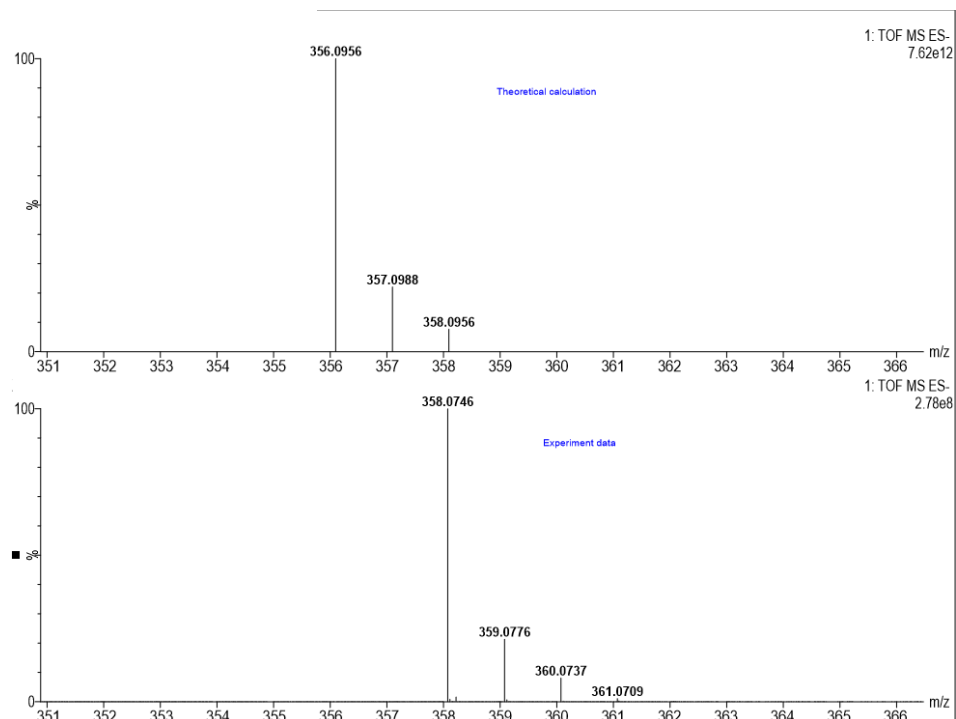
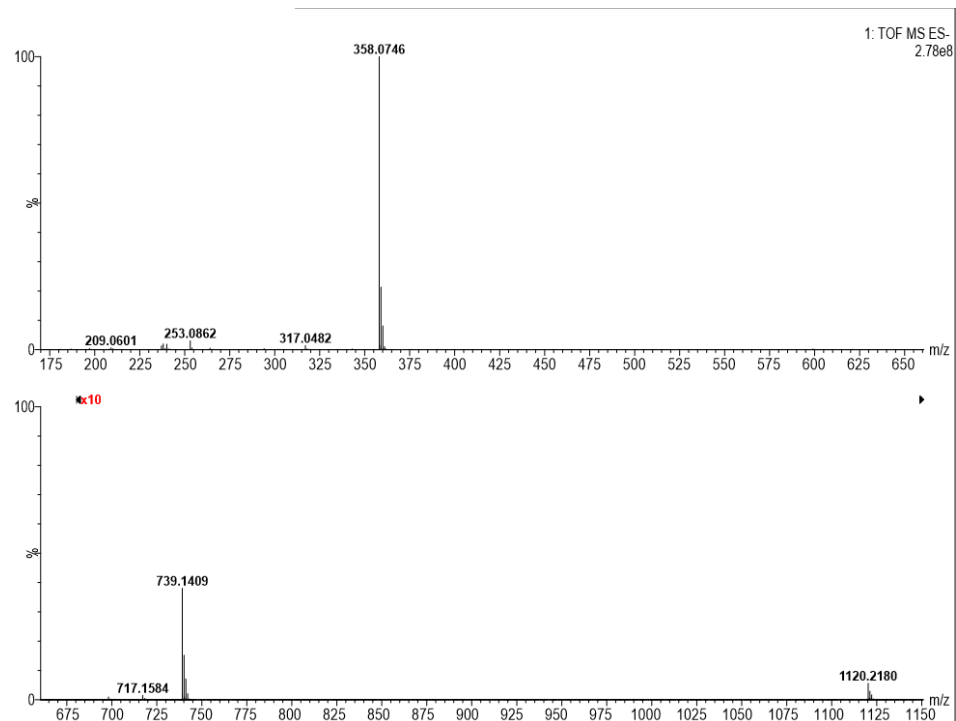
- $\text{SO}_2\text{NHCOCH}_3$ family- positive ionization
 - $\text{SO}_2\text{NHCOCH}_3\text{OBenzyl}$



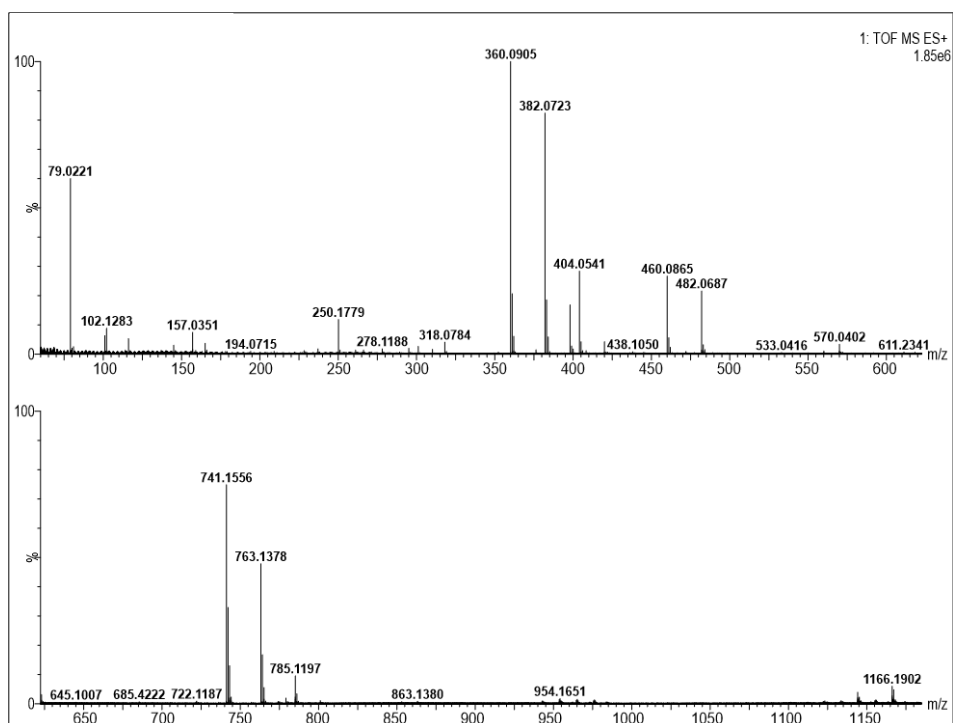
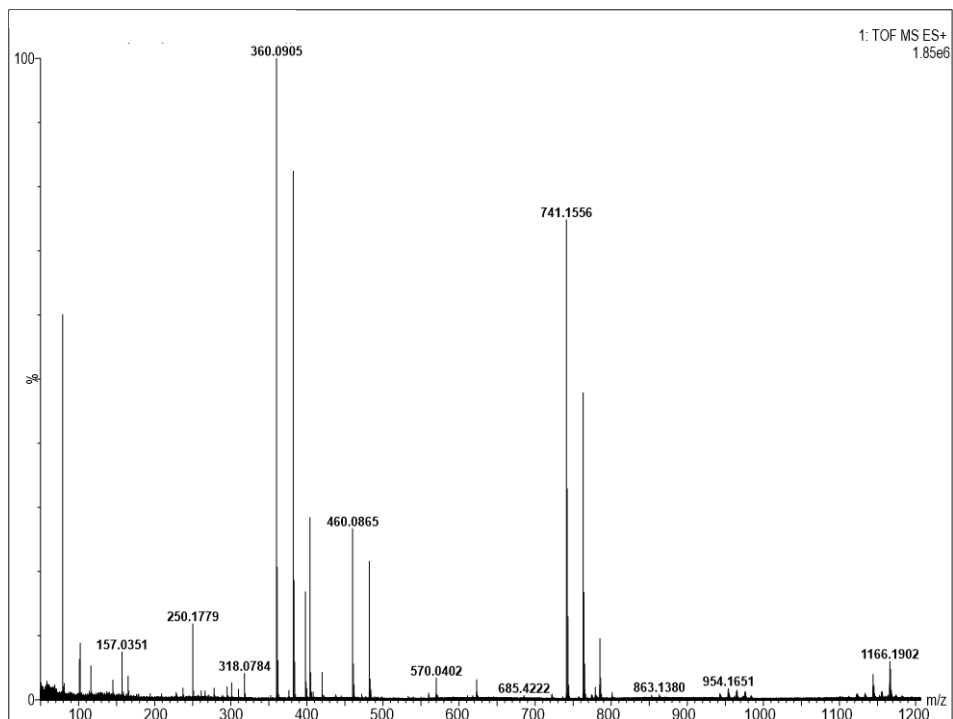


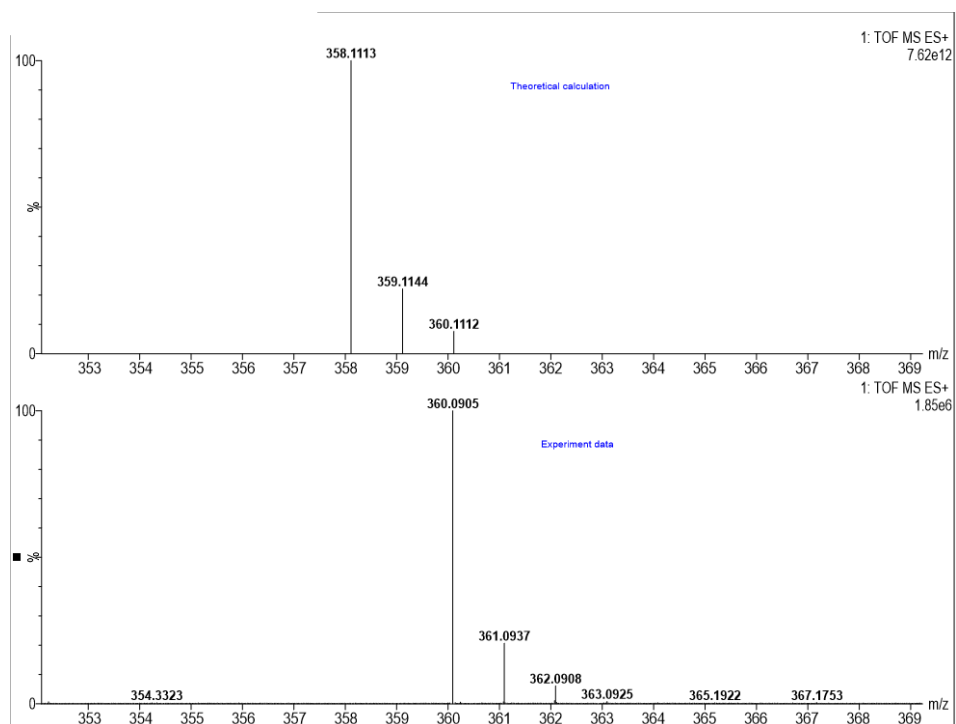
- $\text{SO}_2\text{NHCOCH}_3$ family- negative ionization
 - $\text{SO}_2\text{NHCOCH}_3\text{OCH}_3$





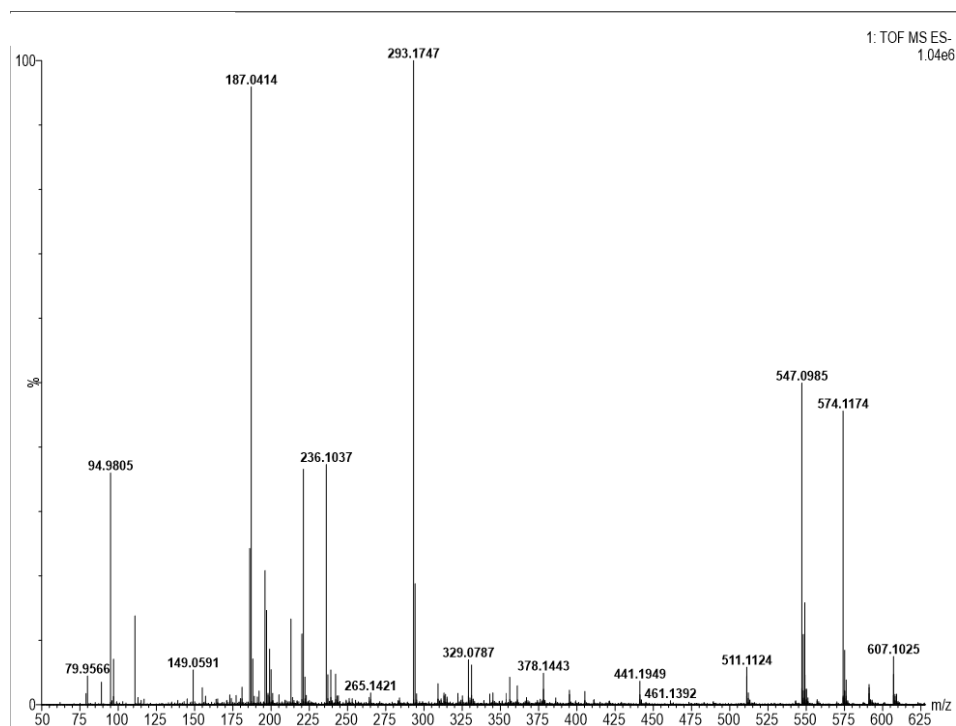
- $\text{SO}_2\text{NHCOCH}_3$ family- positive ionization
 - $\text{SO}_2\text{NHCOCH}_3\text{OCH}_3$

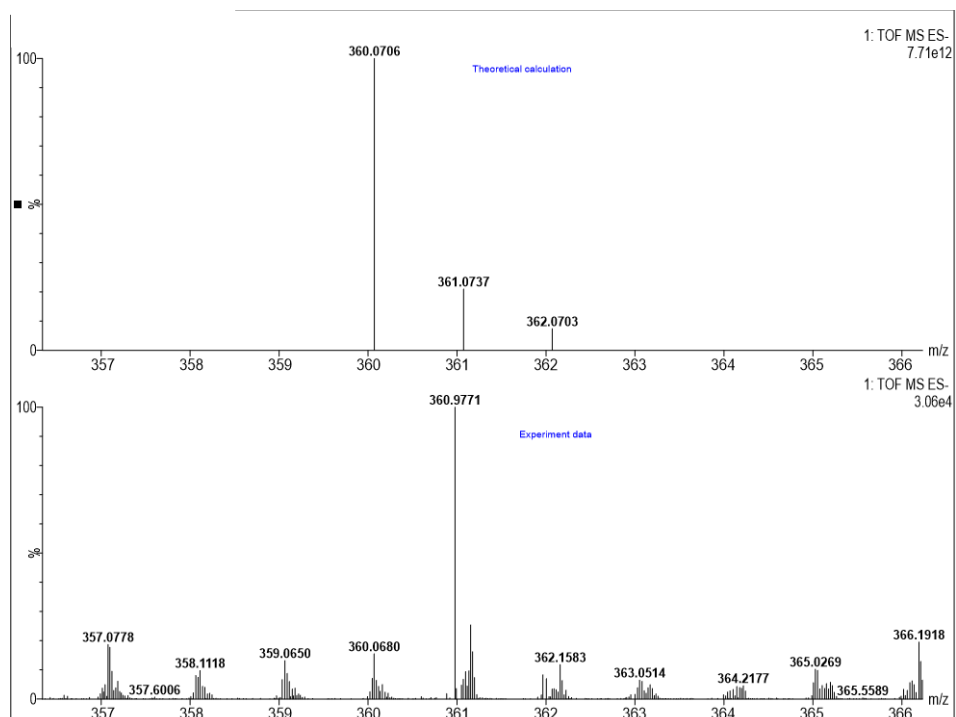
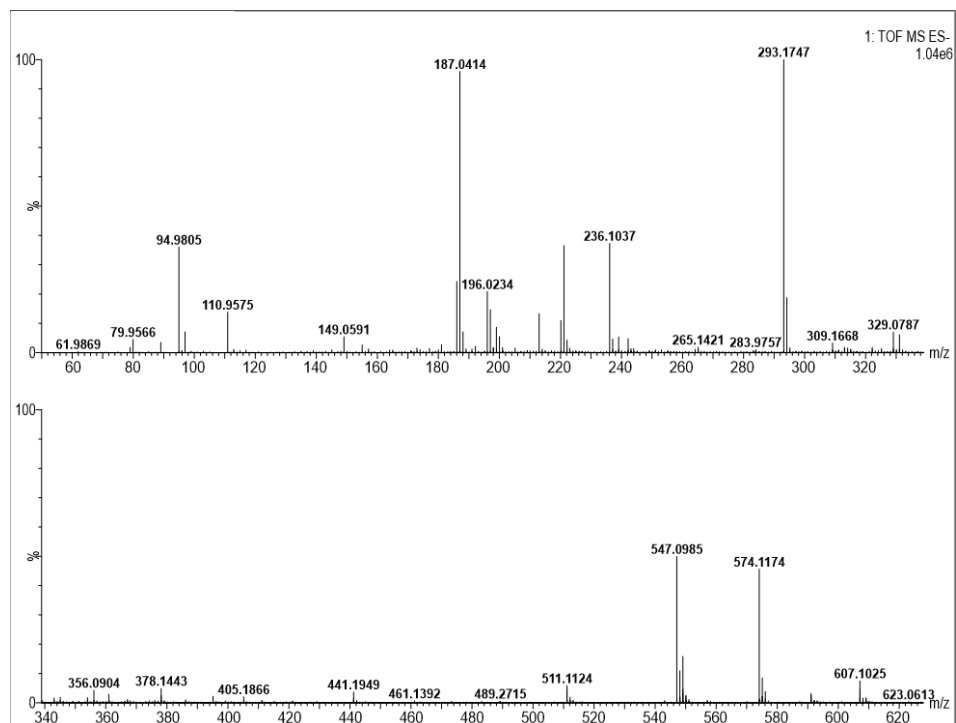




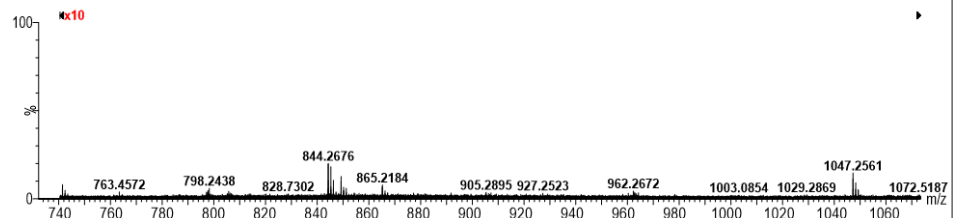
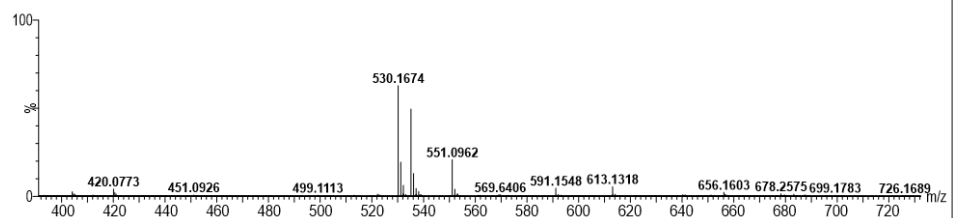
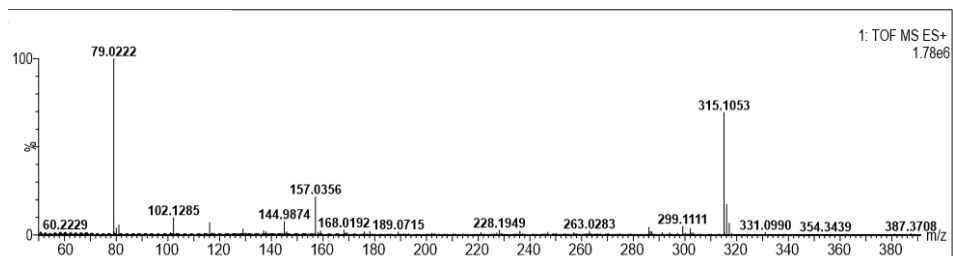
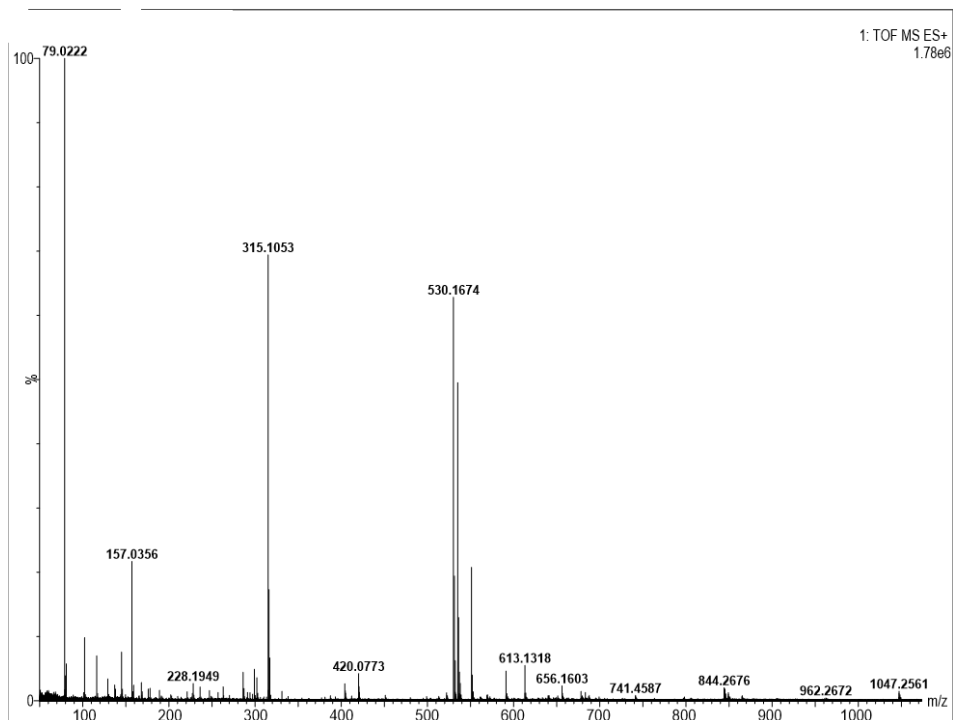
Study 3

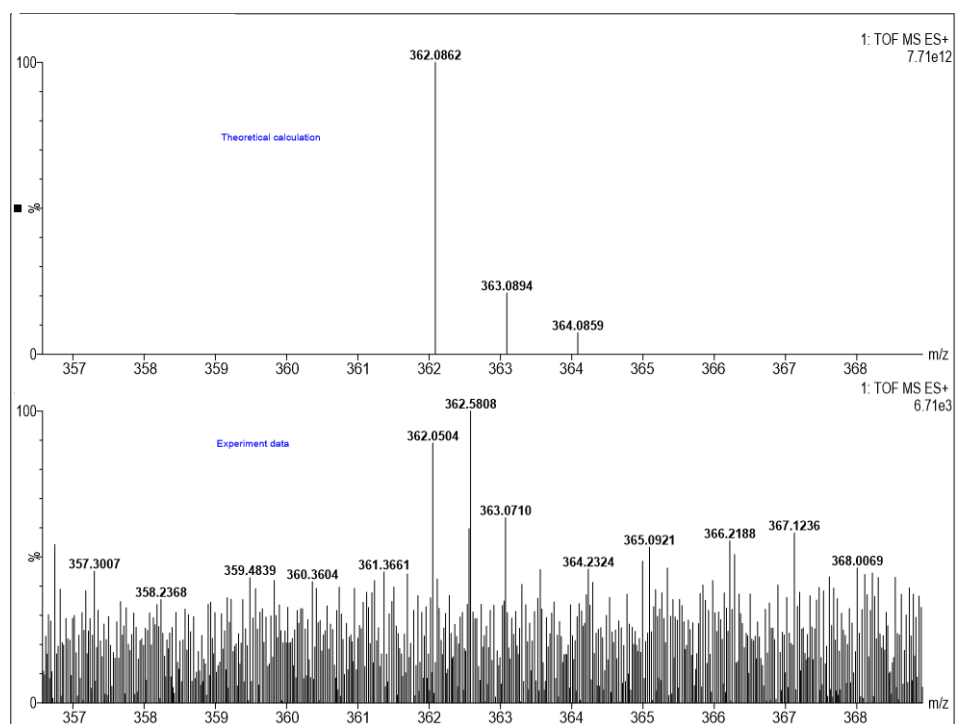
- $\text{SO}_2\text{CH}_3\text{CH}_3\text{CH}_3$ family- negative ionization



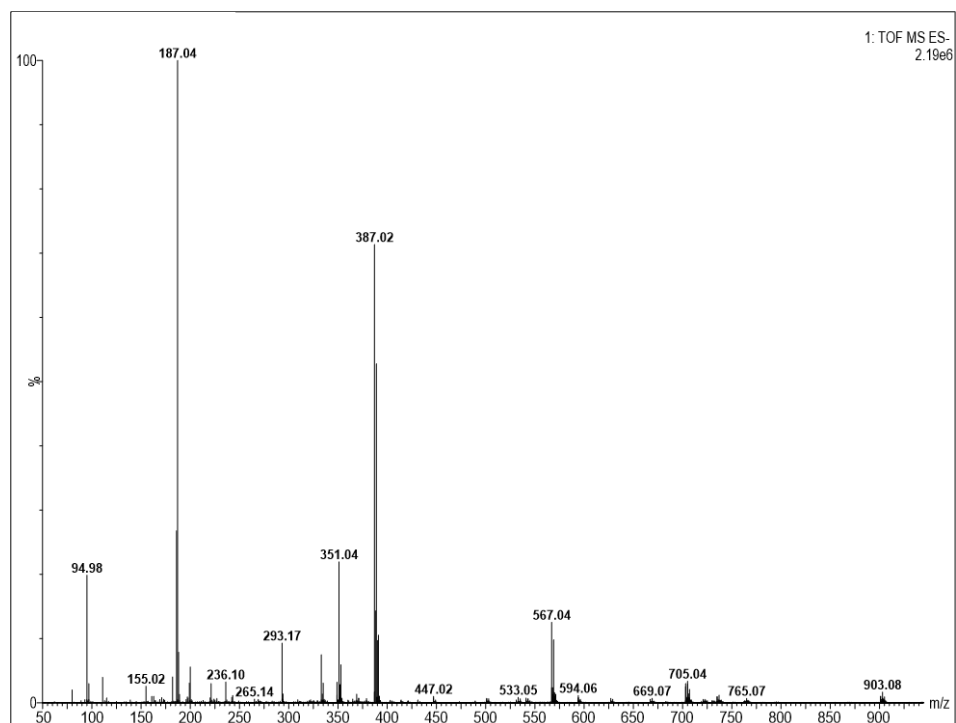


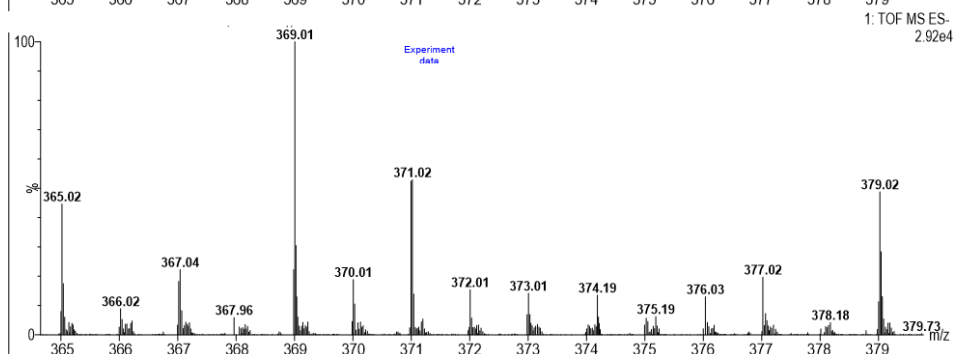
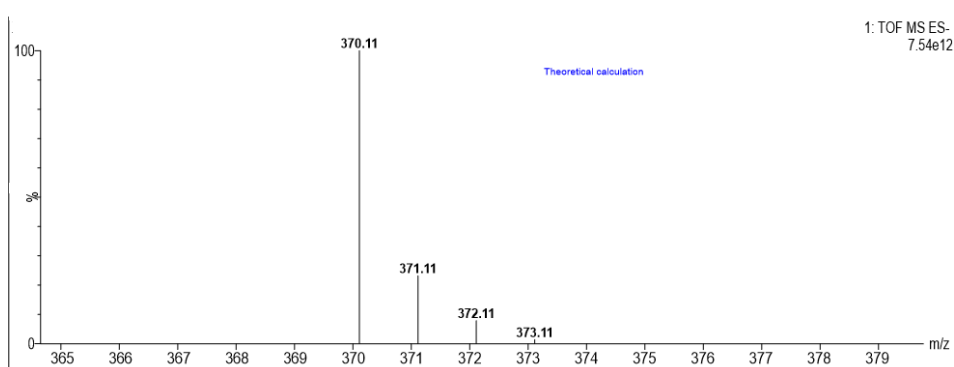
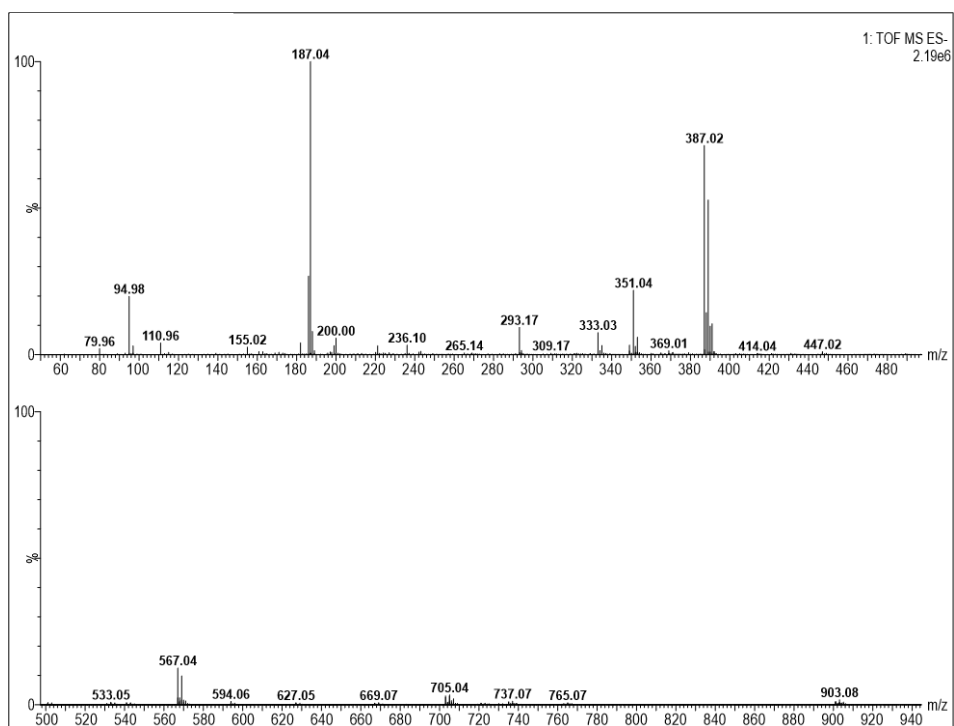
- $\text{SO}_2\text{CH}_3\text{CH}_3\text{CH}_3$ family- positive ionization



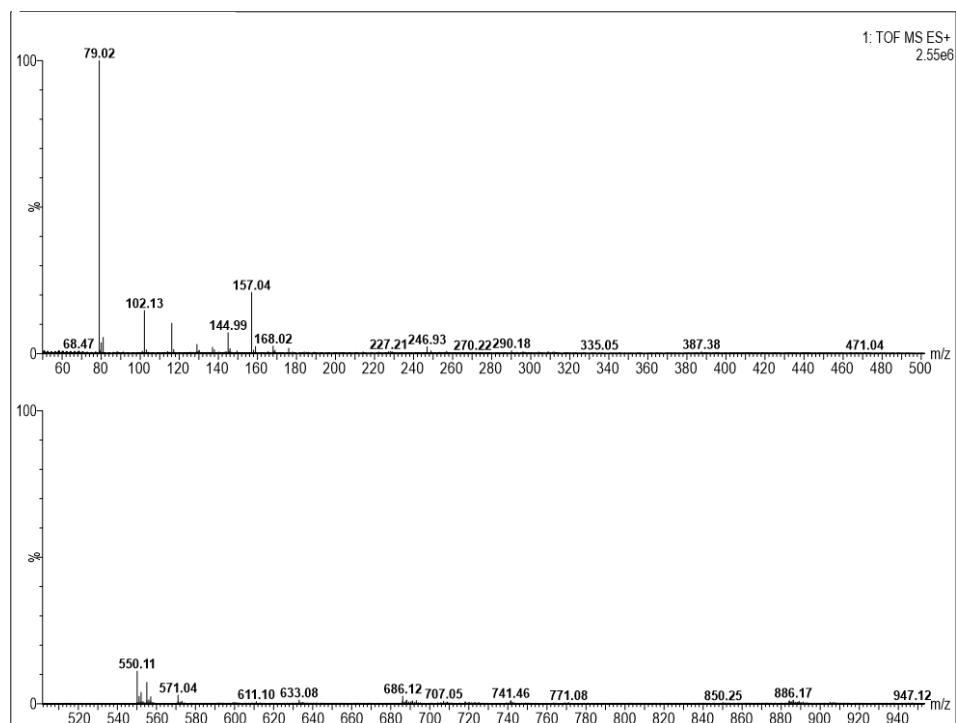
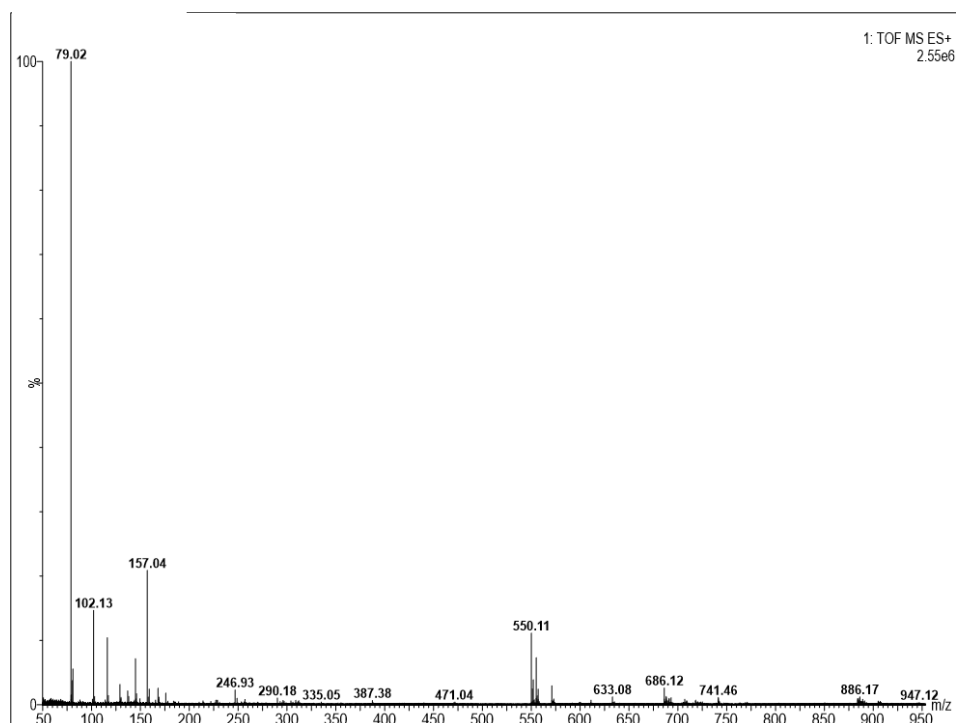


- $\text{SO}_2\text{CH}_3\text{CH}_3\text{Cl}$ family- negative ionization

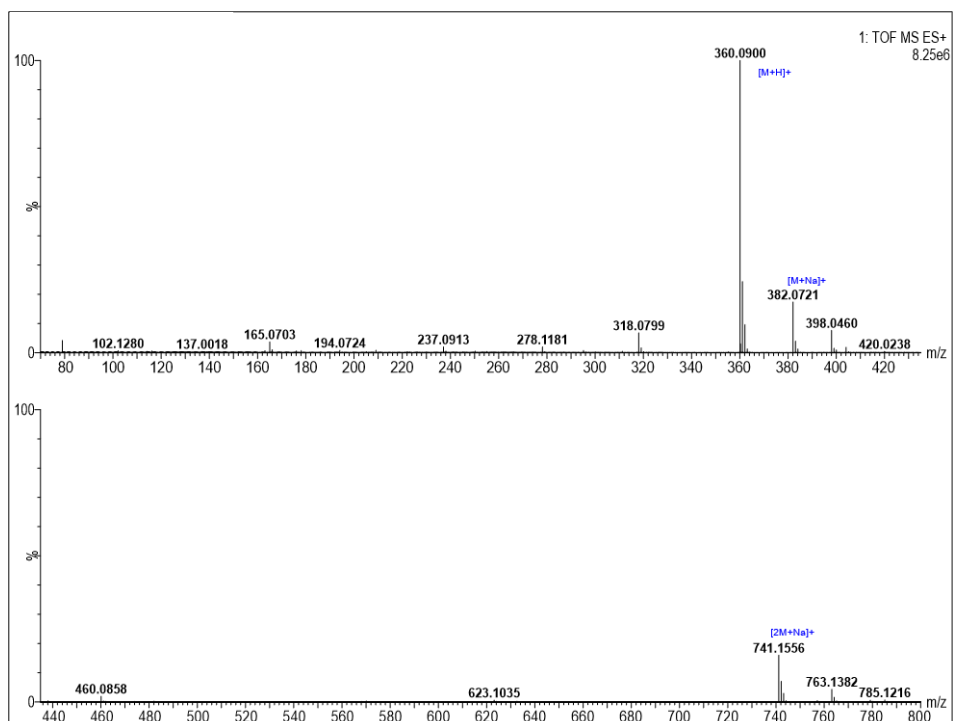
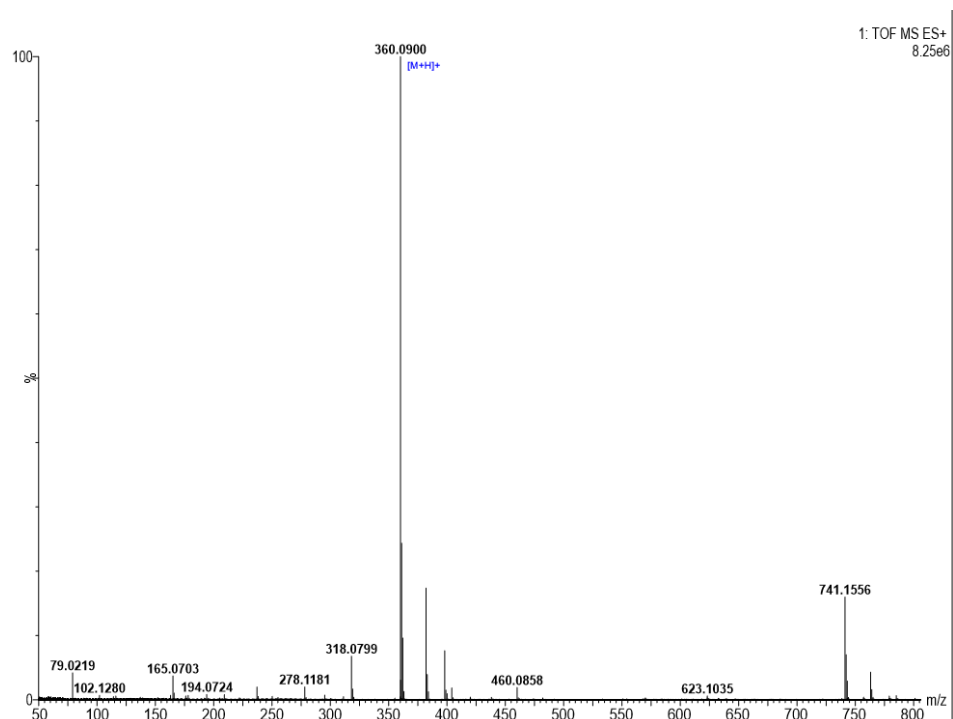


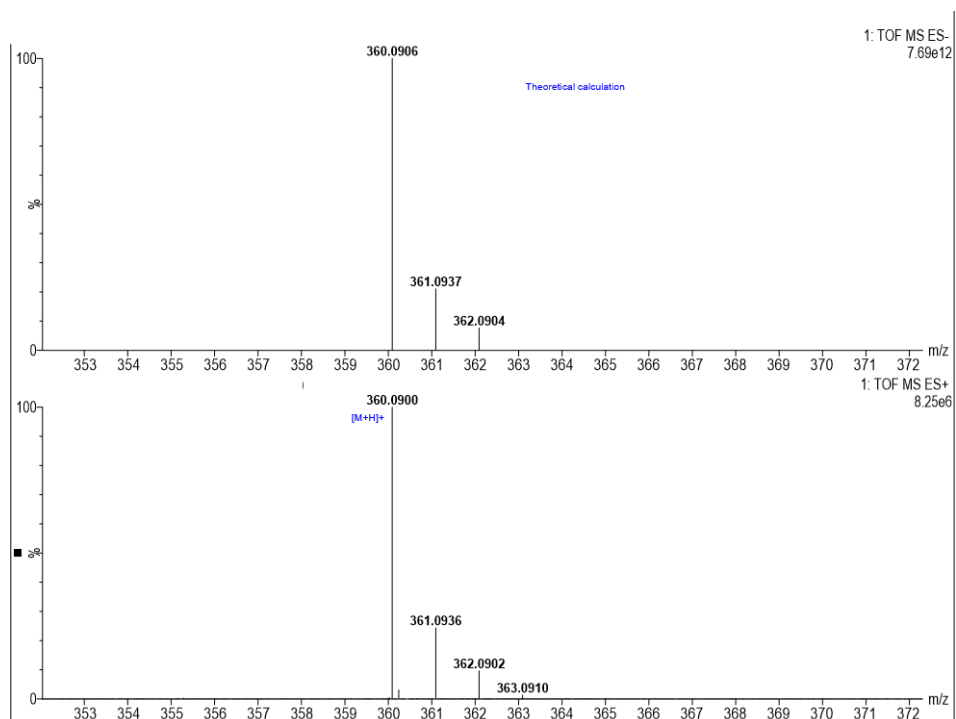


- $\text{SO}_2\text{CH}_3\text{CH}_3\text{Cl}$ family- positive ionization

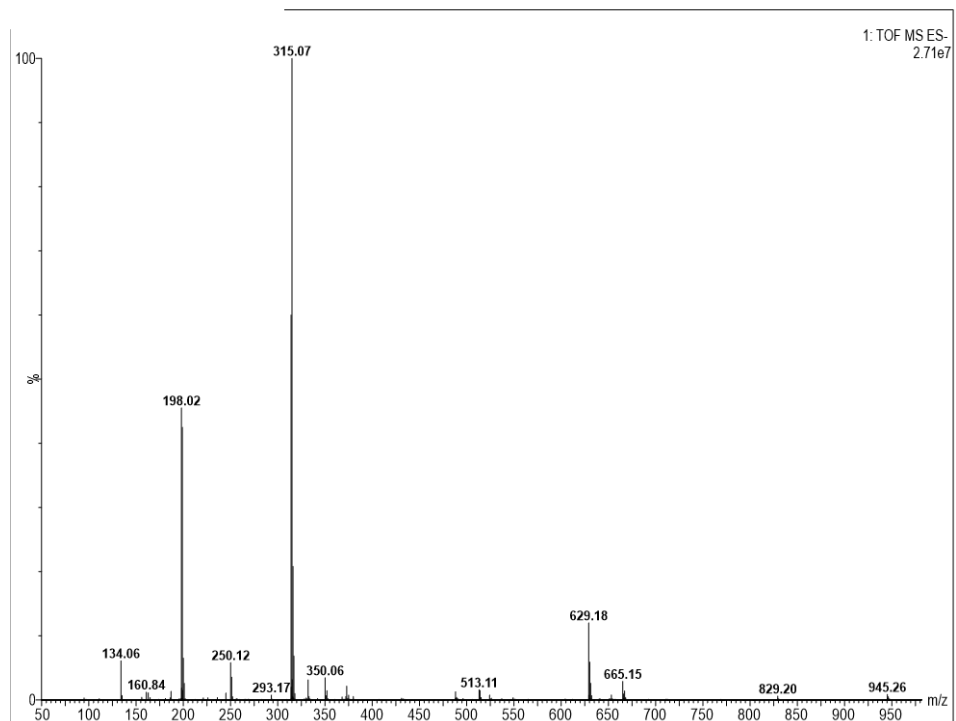


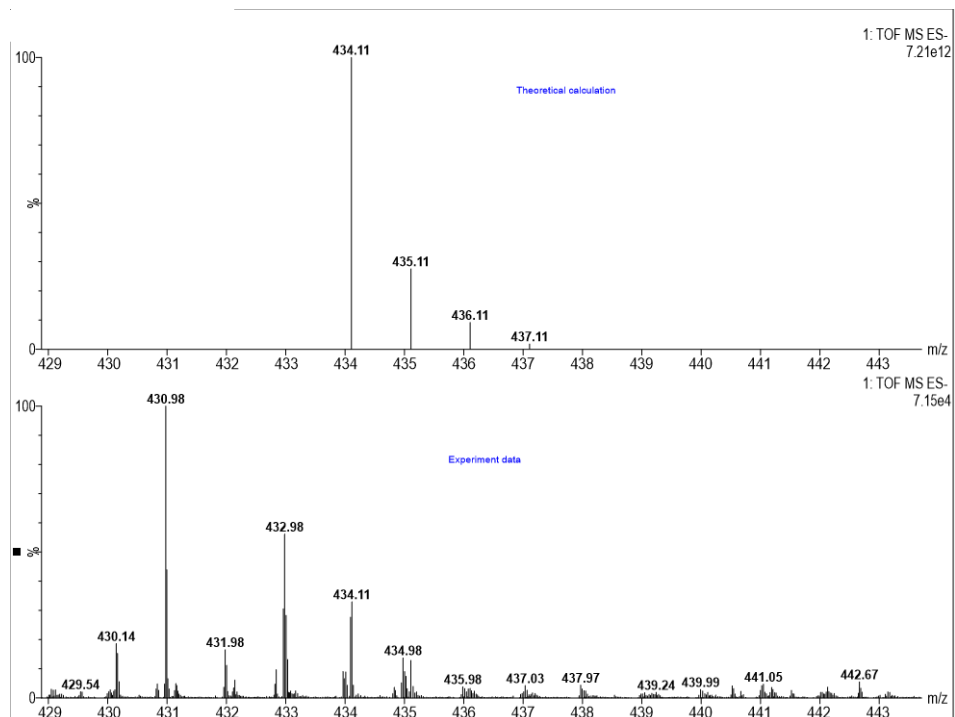
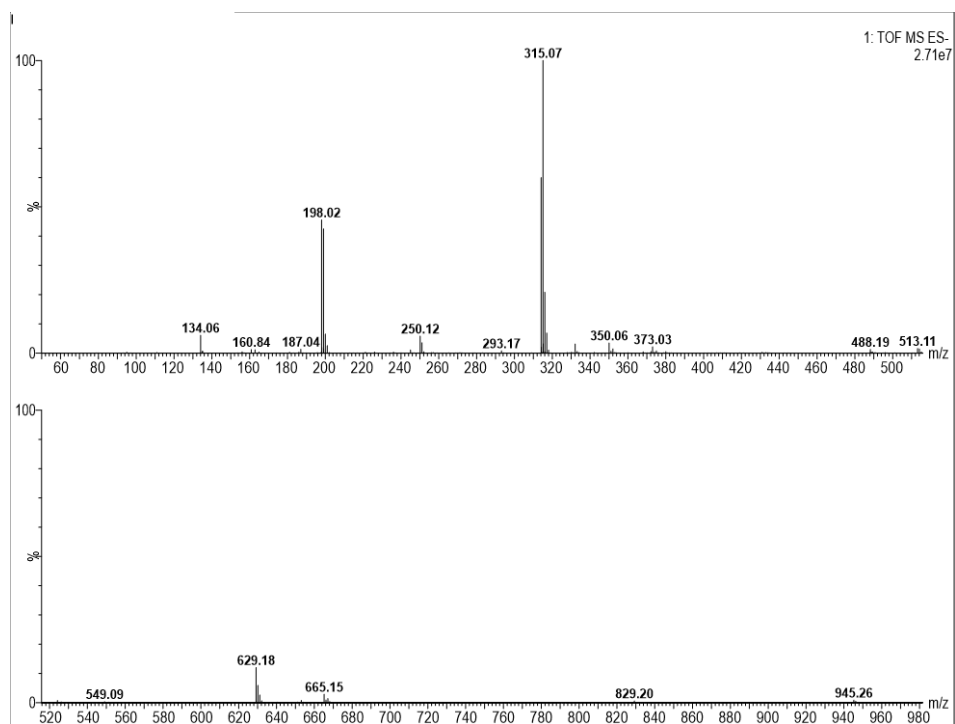
- $\text{SO}_2\text{CH}_3\text{CH}_3\text{F}$ family- positive ionization



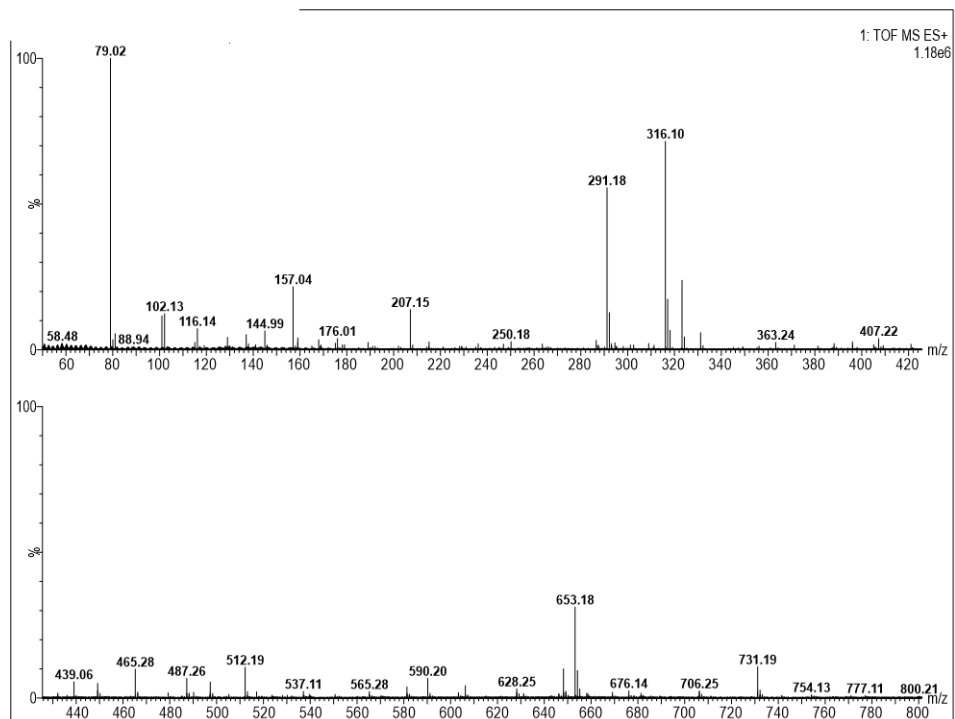
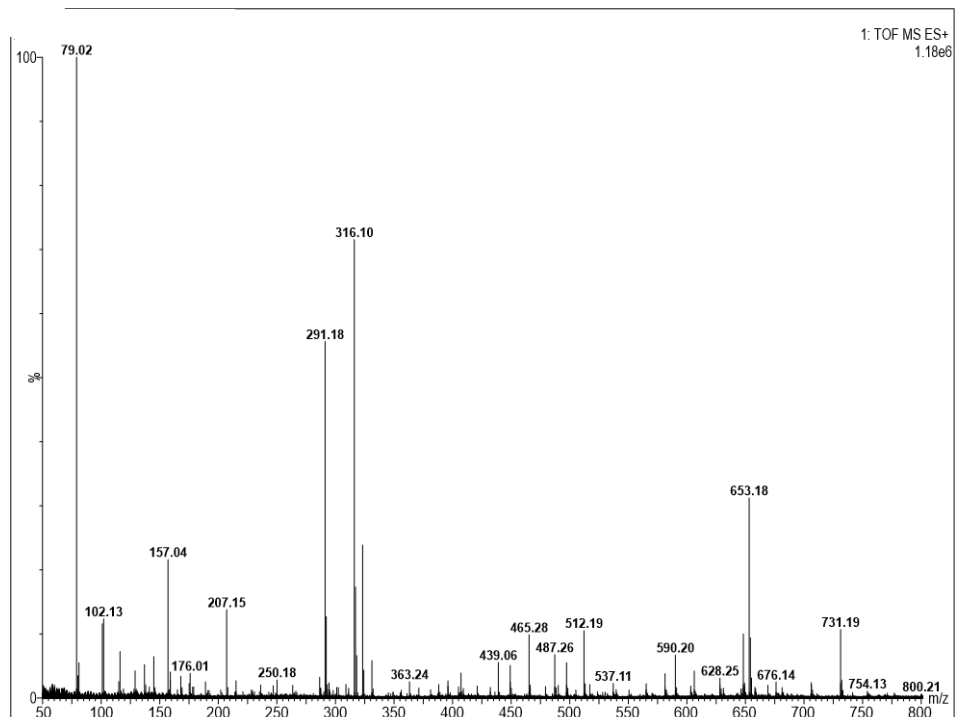


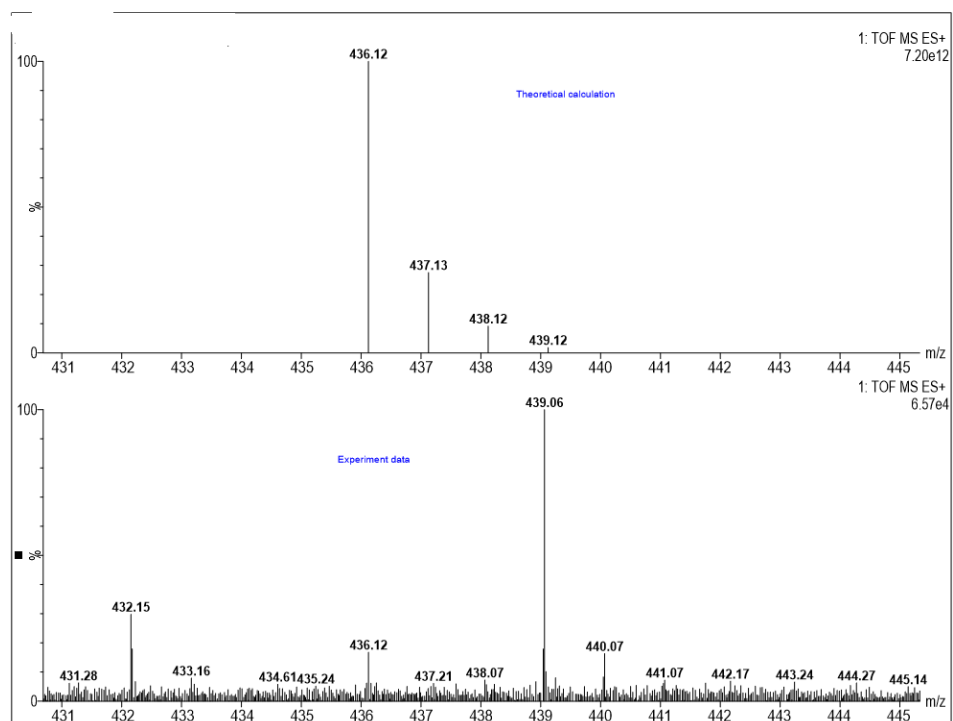
- $\text{SO}_2\text{CH}_3\text{OCH}_3\text{CH}_3$ family missing MS spectra
- $\text{SO}_2\text{NH}_2\text{CH}_3\text{CH}_3$ family –negative ionization



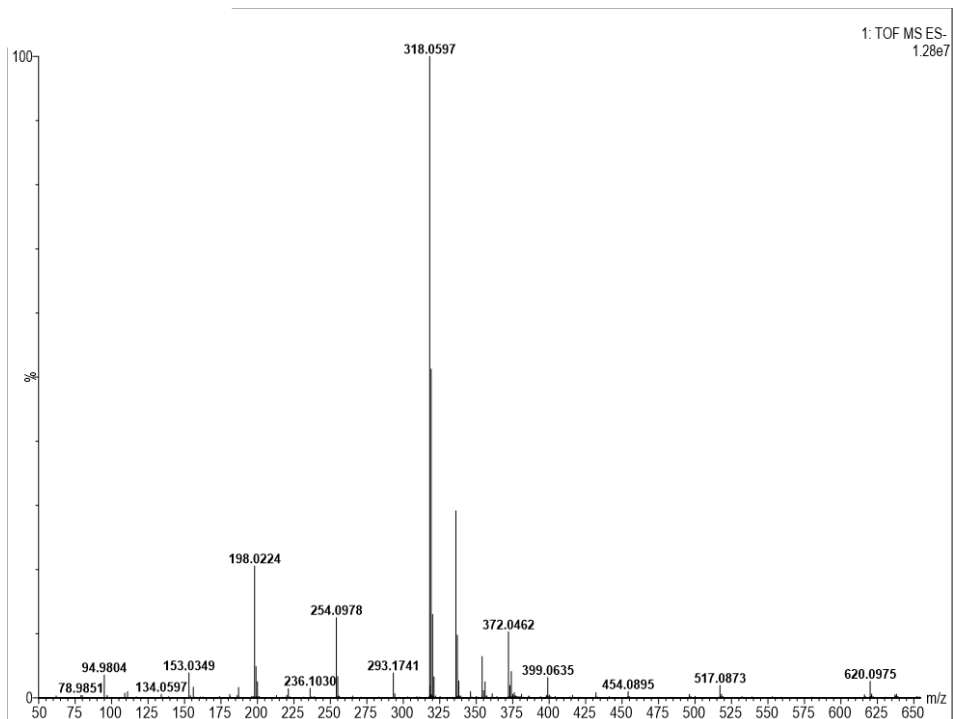


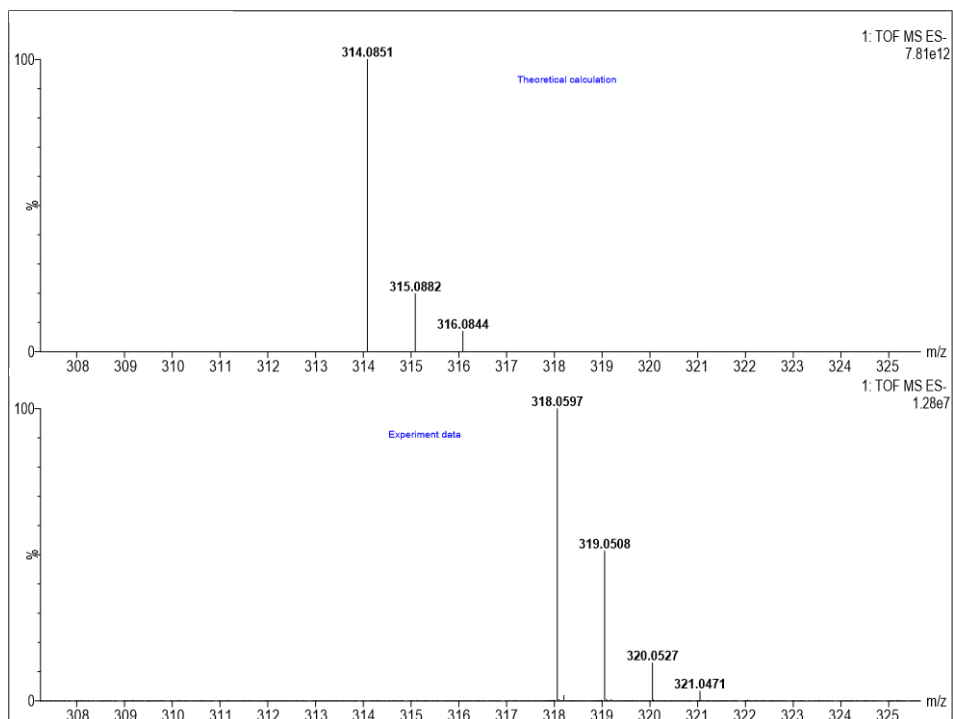
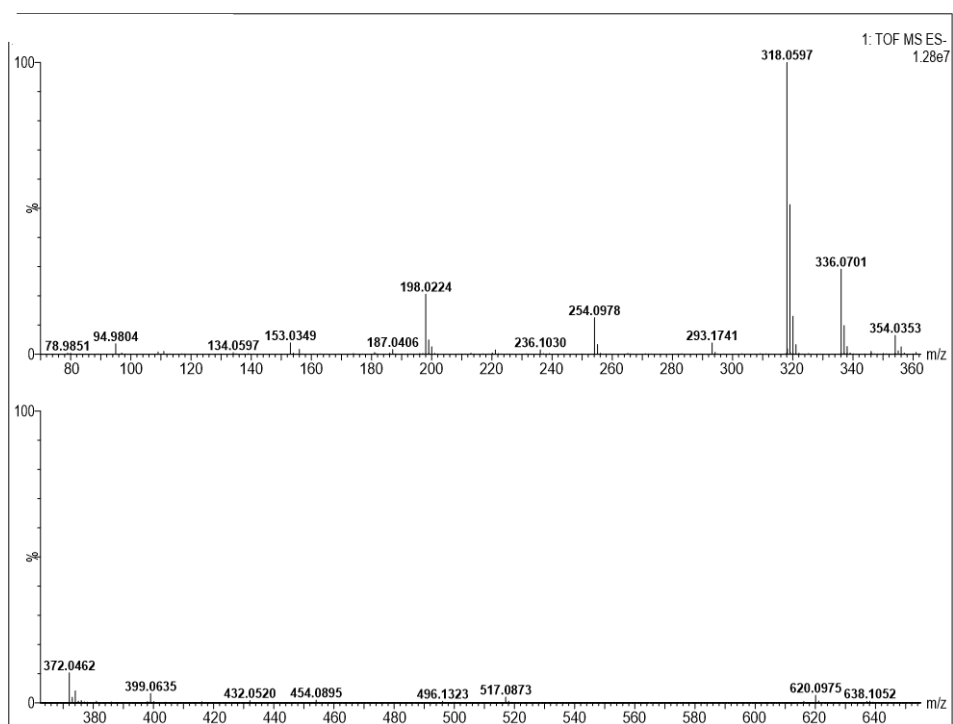
- $\text{SO}_2\text{NH}_2\text{CH}_3\text{CH}_3$ family –positive ionization



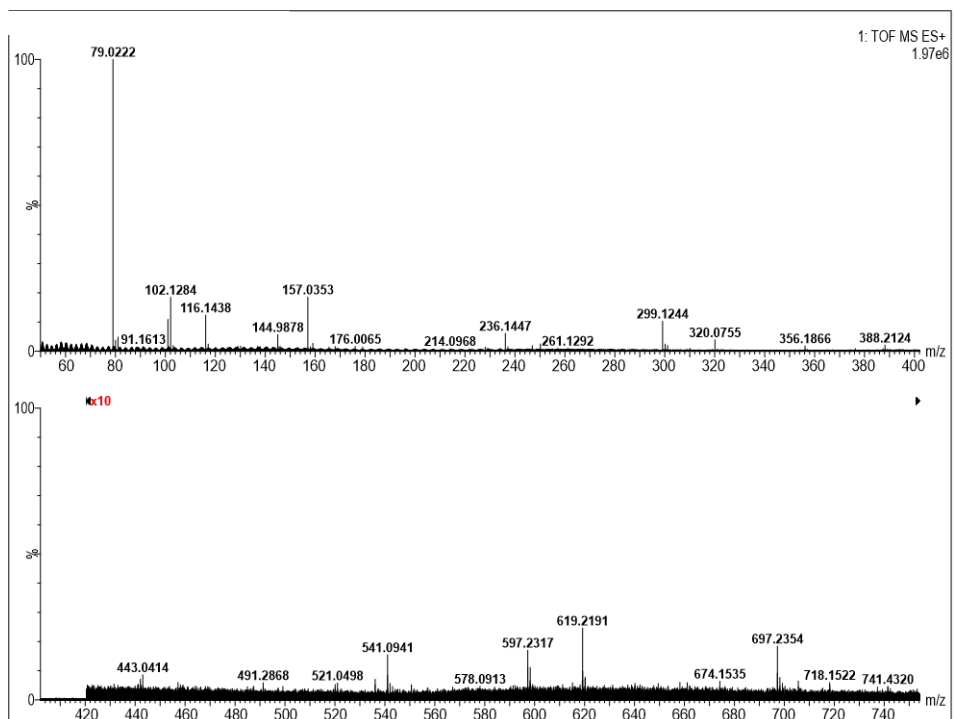
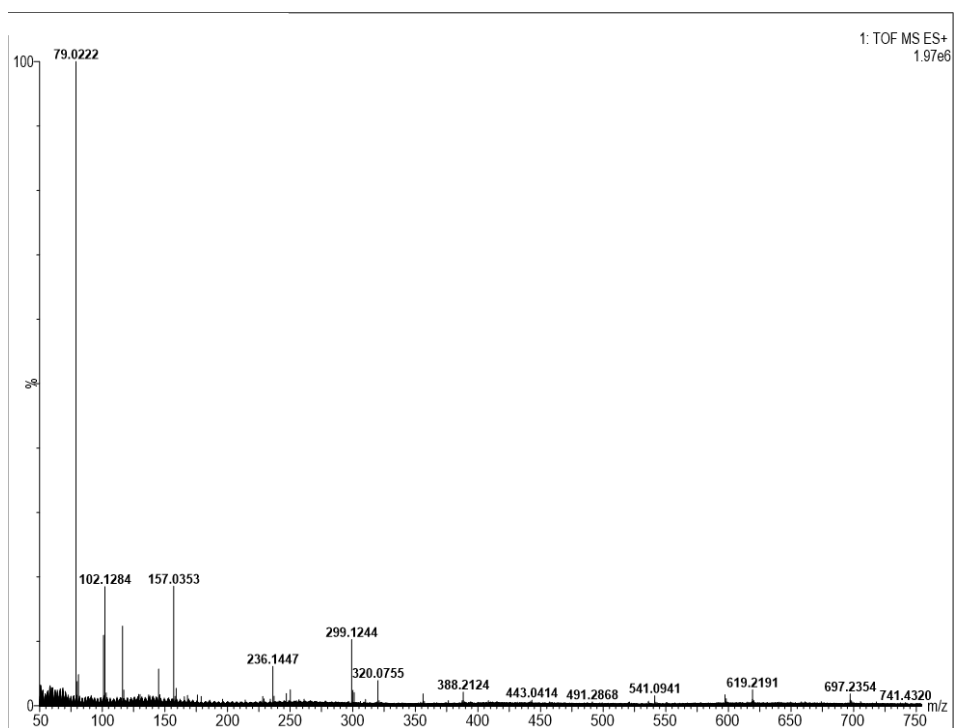


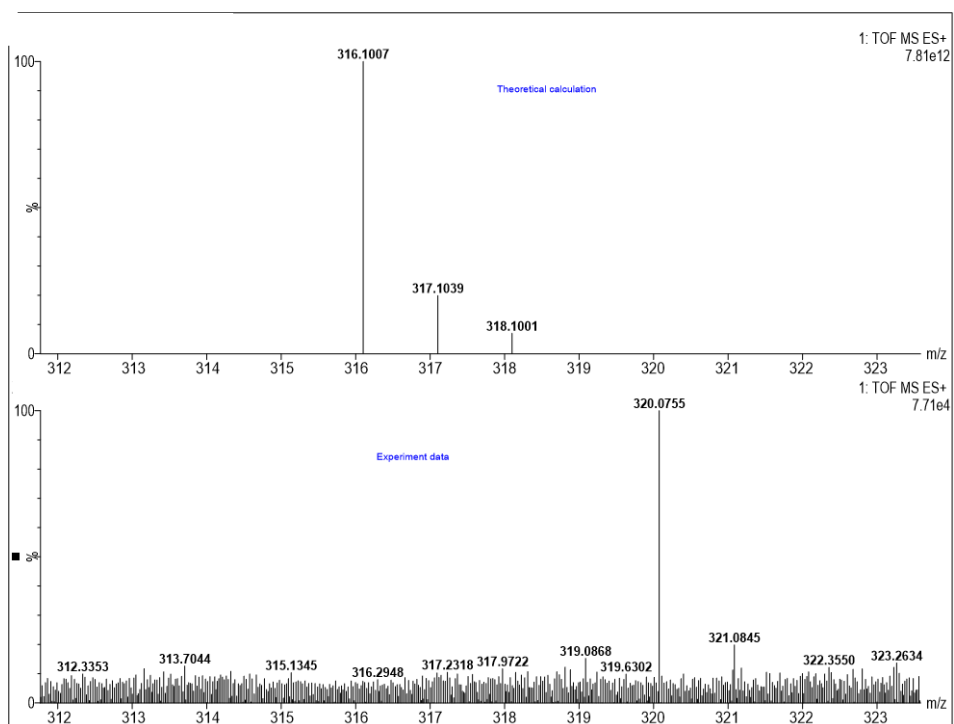
- $\text{SO}_2\text{NH}_2\text{CH}_3\text{F}$ family –negative ionization



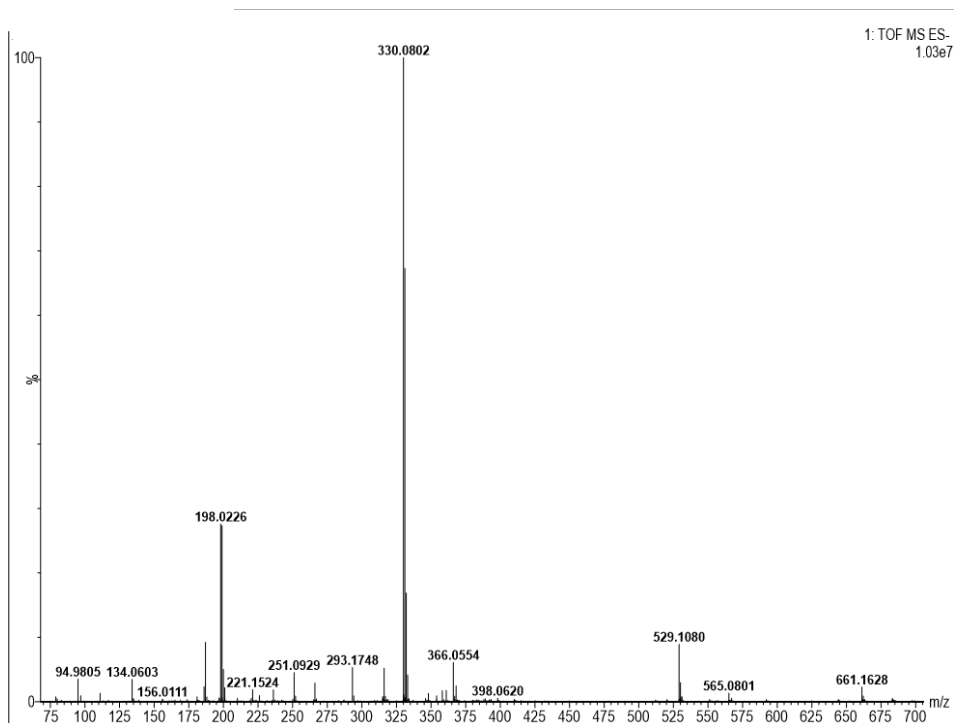


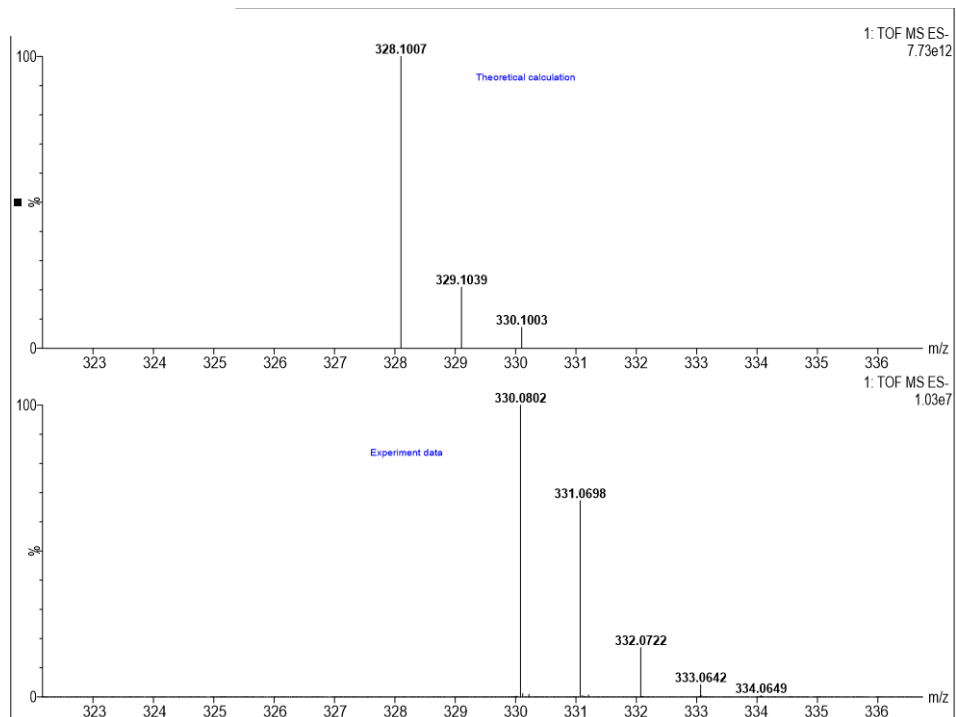
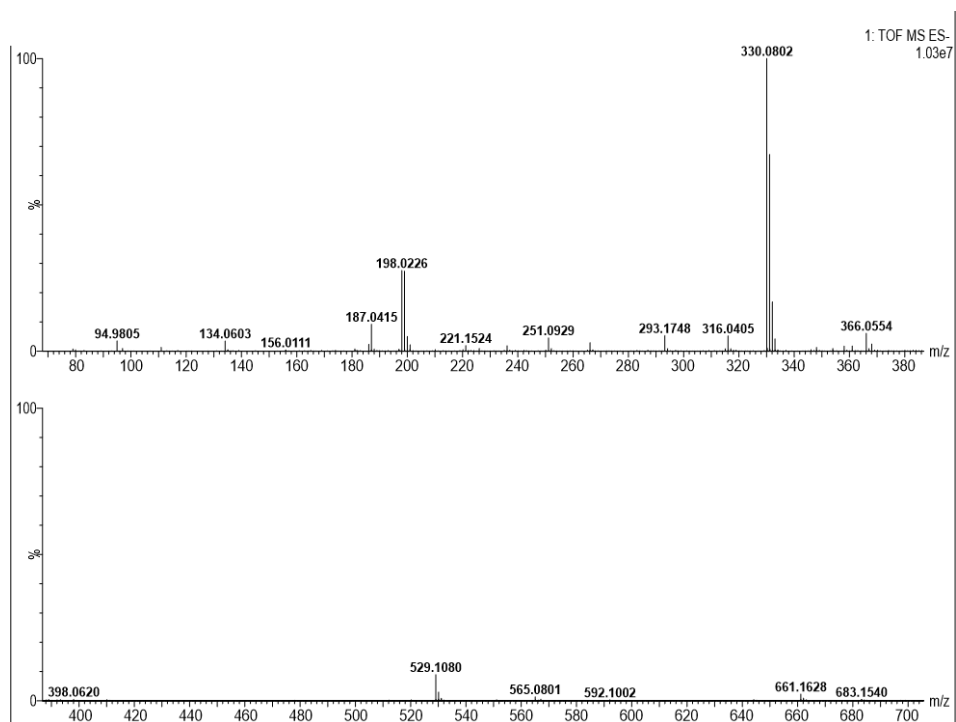
- $\text{SO}_2\text{NH}_2\text{CH}_3\text{F}$ family –positive ionization



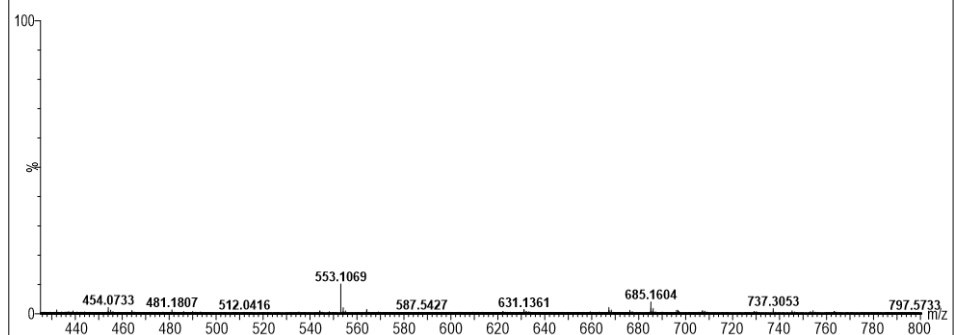
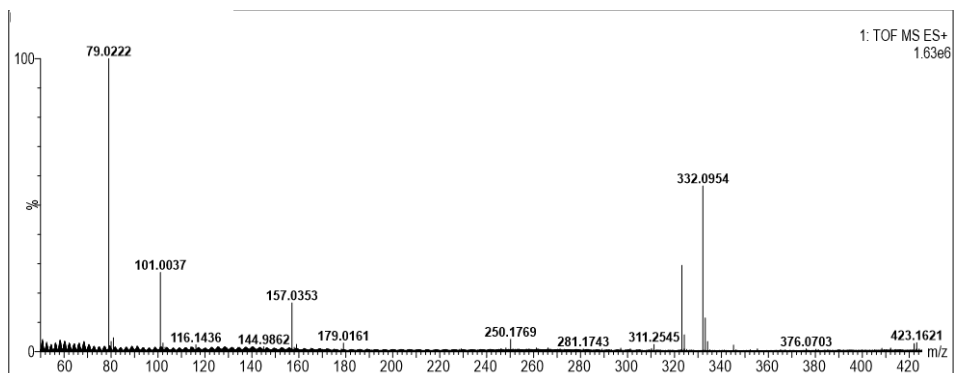
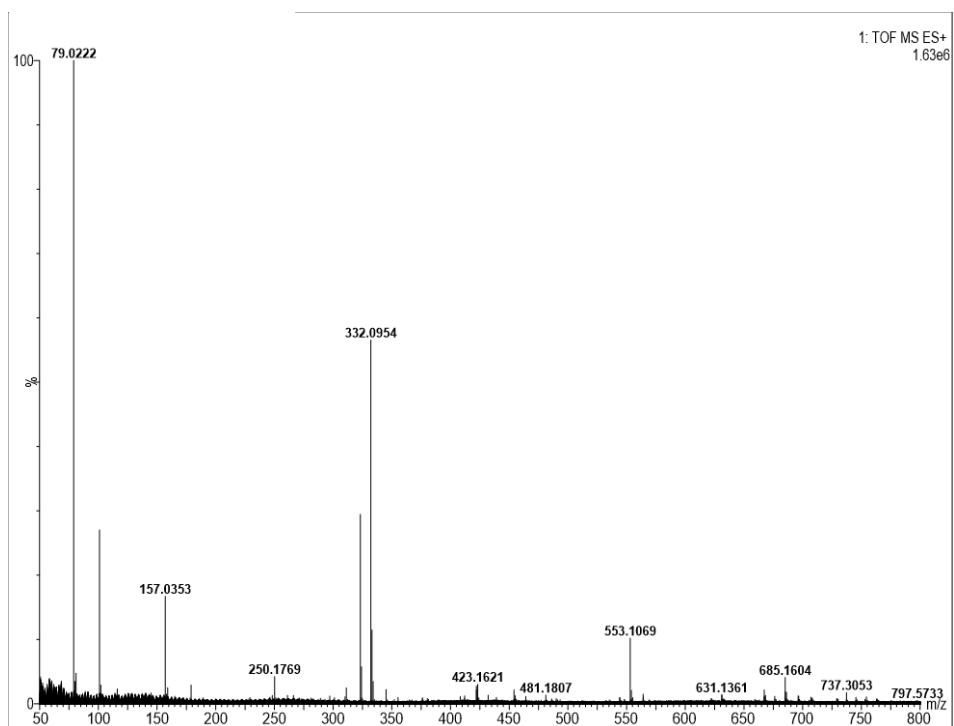


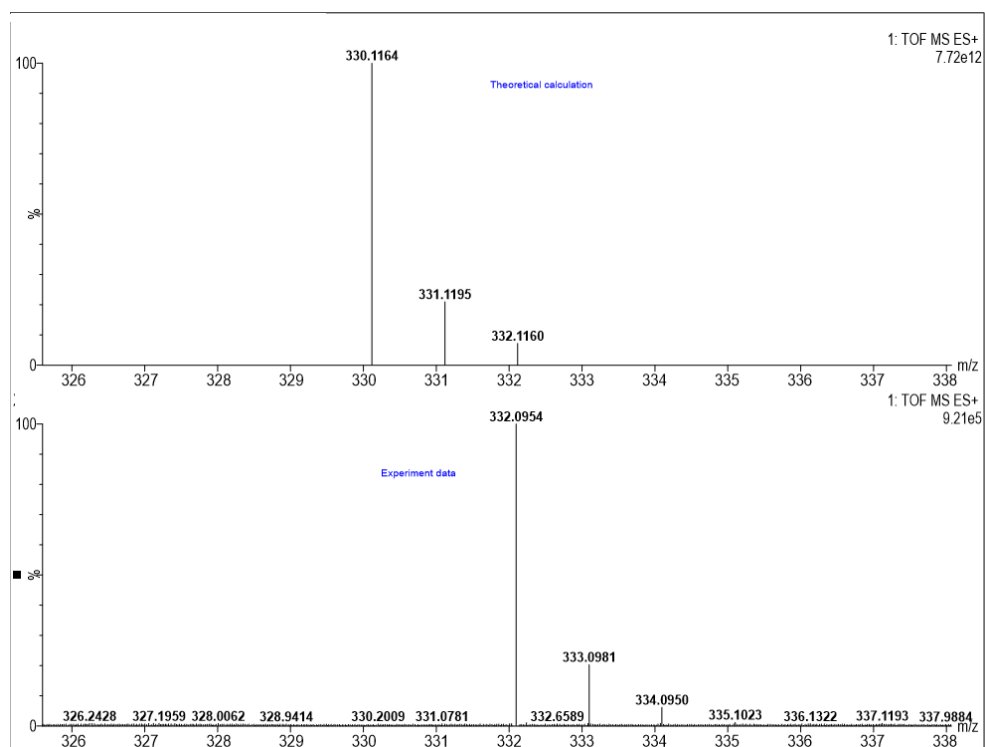
- $\text{SO}_2\text{NH}_2\text{CH}_3\text{OCH}_3$ family –negative ionization



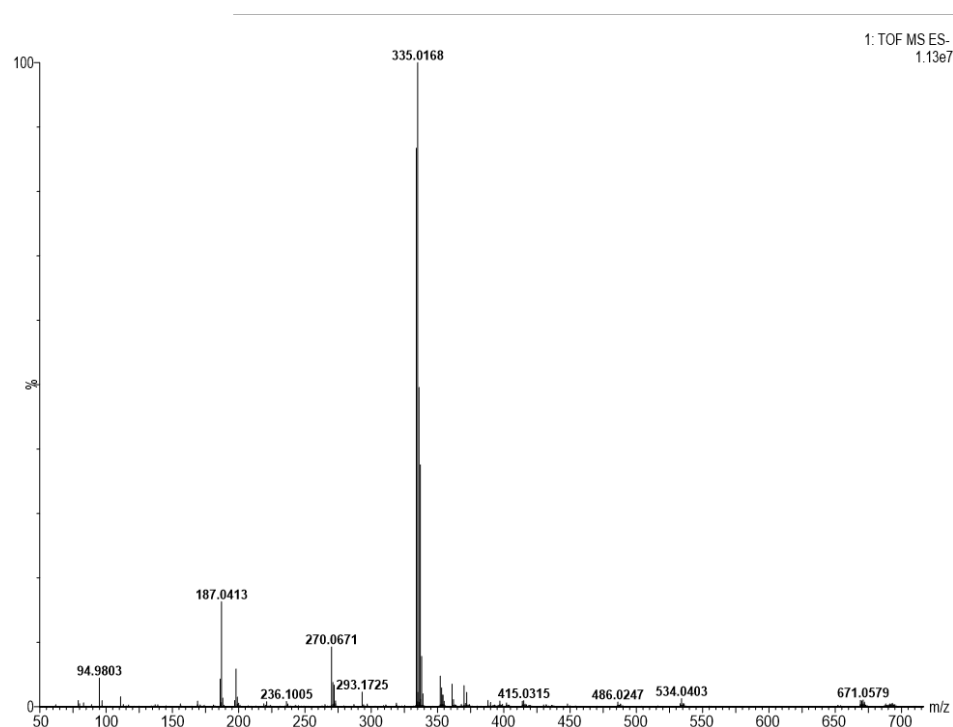


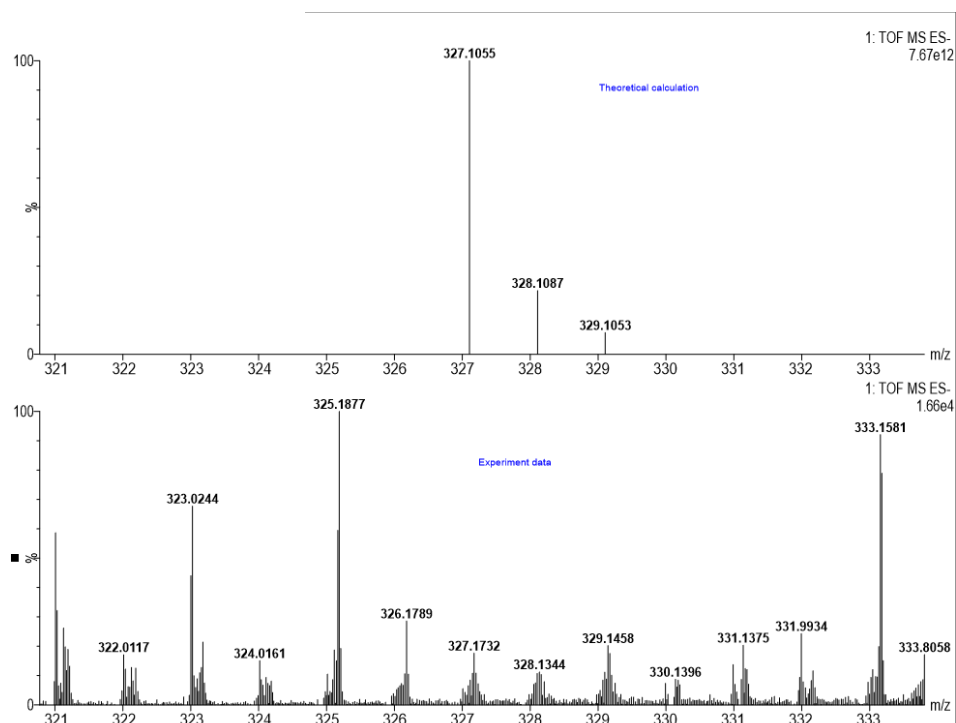
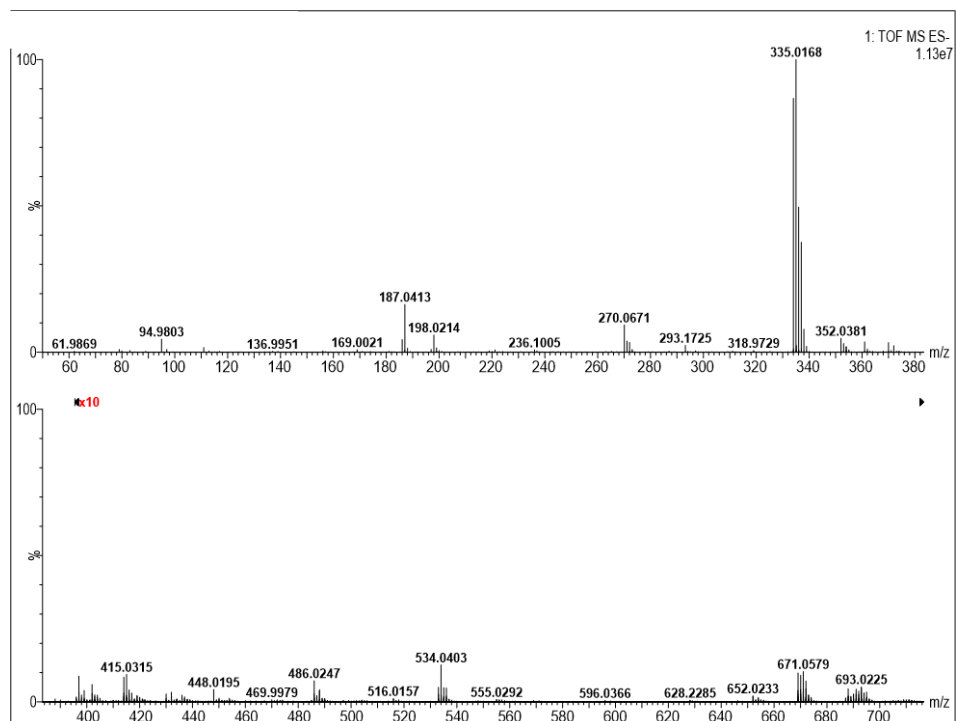
- $\text{SO}_2\text{NH}_2\text{CH}_3\text{OCH}_3$ family –negative ionization



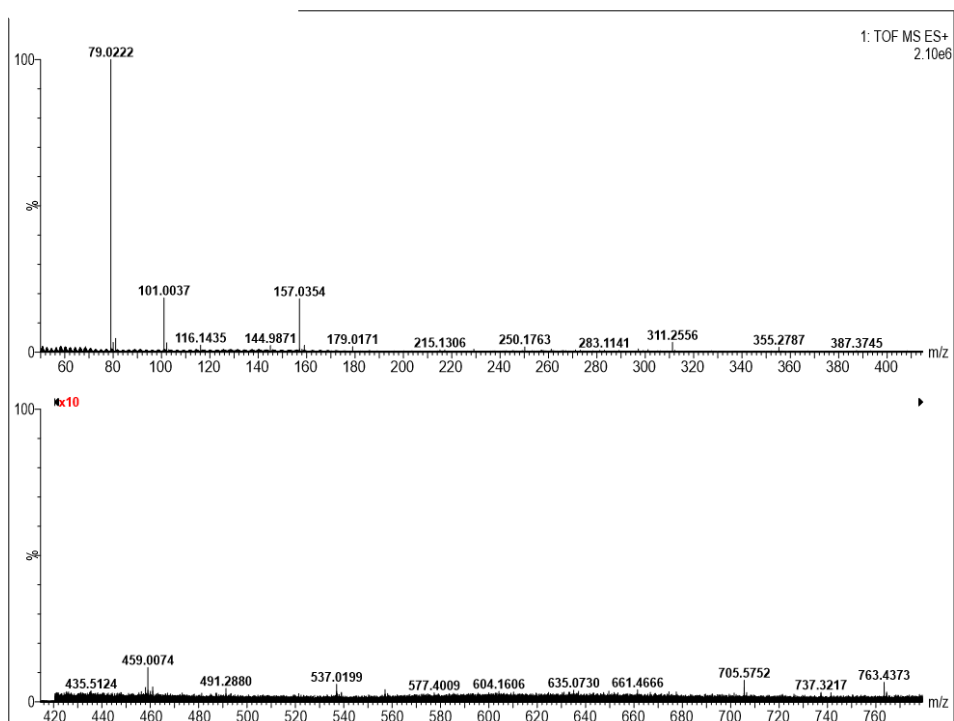
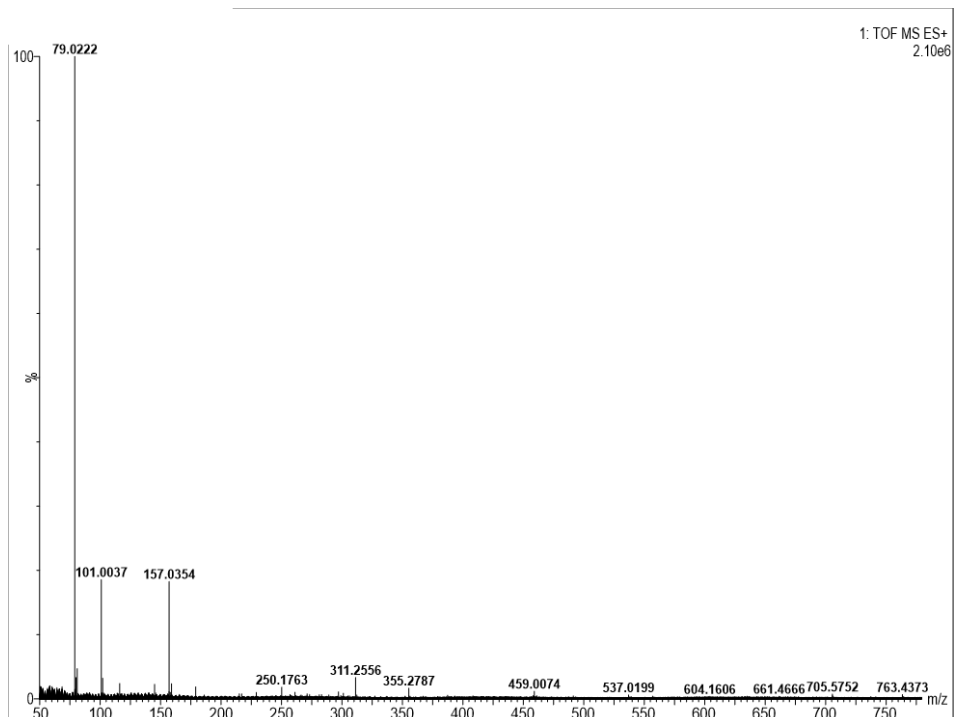


- $\text{SO}_2\text{NH}_2\text{CH}_3\text{Cl}$ family –negative ionization

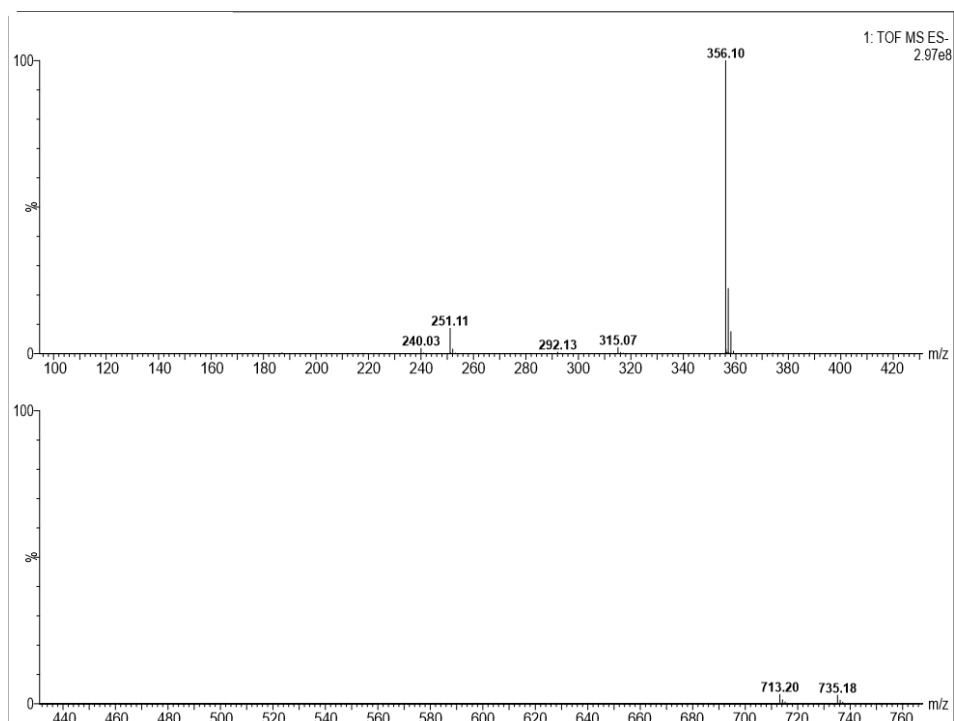
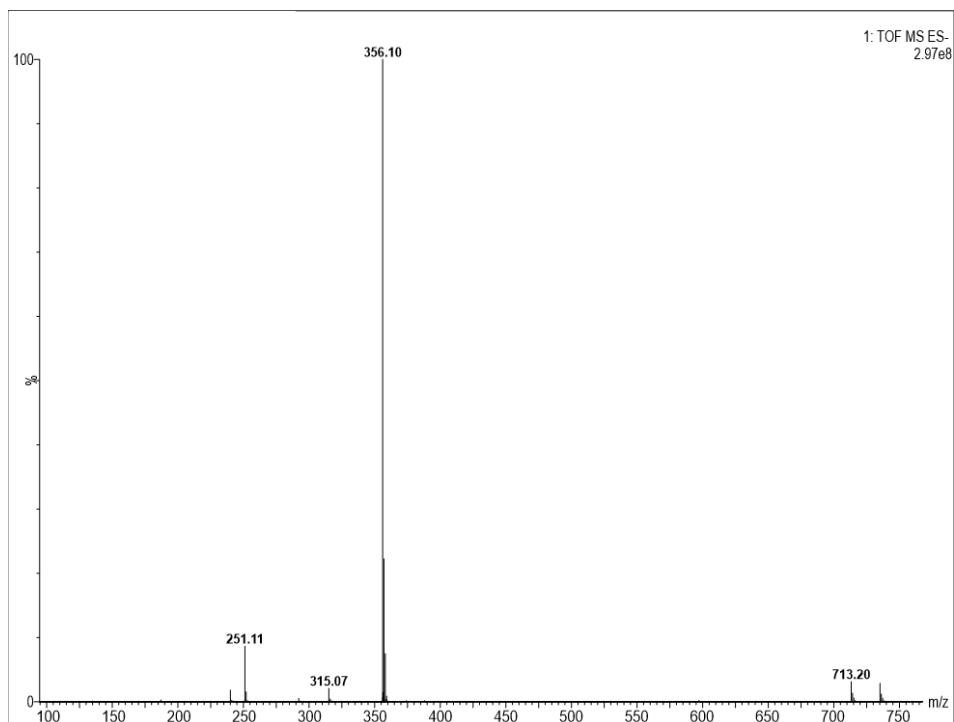


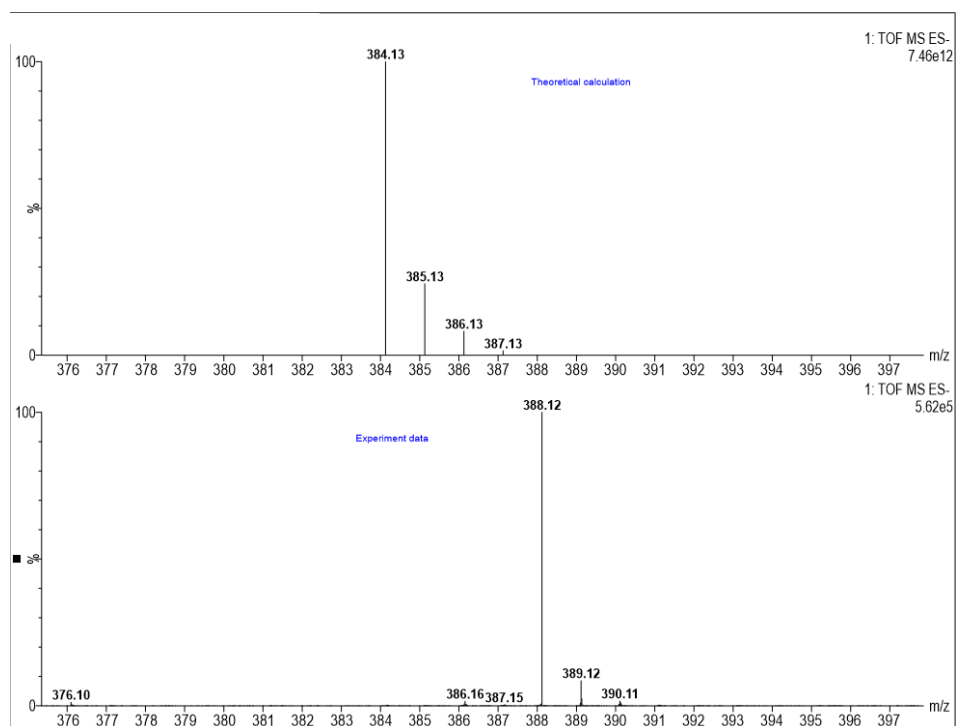


- $\text{SO}_2\text{NH}_2\text{CH}_3\text{Cl}$ family –positive ionization

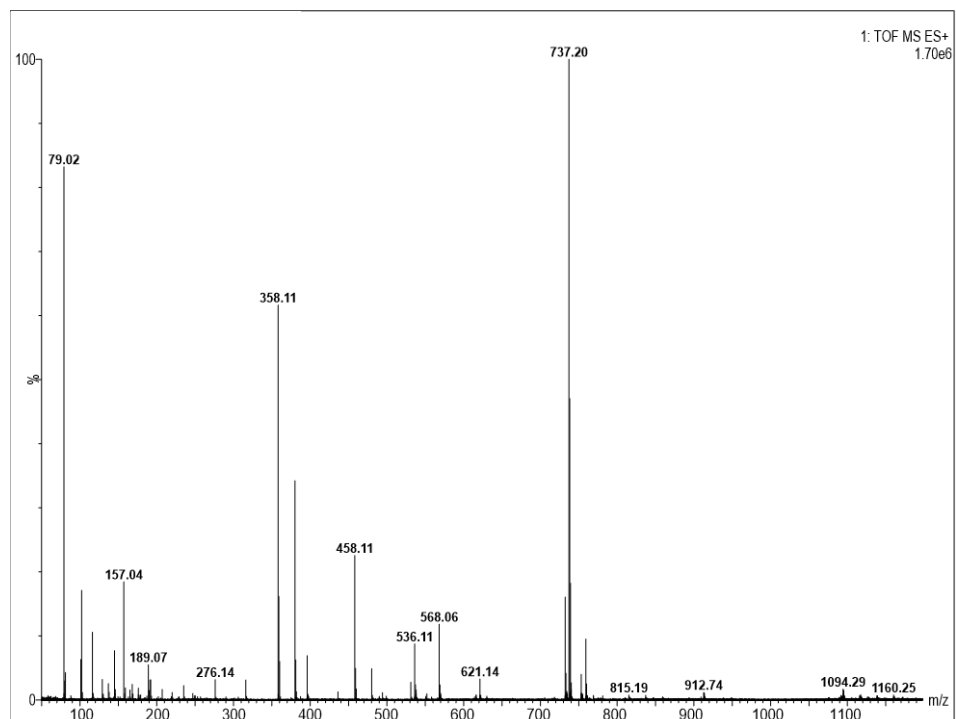


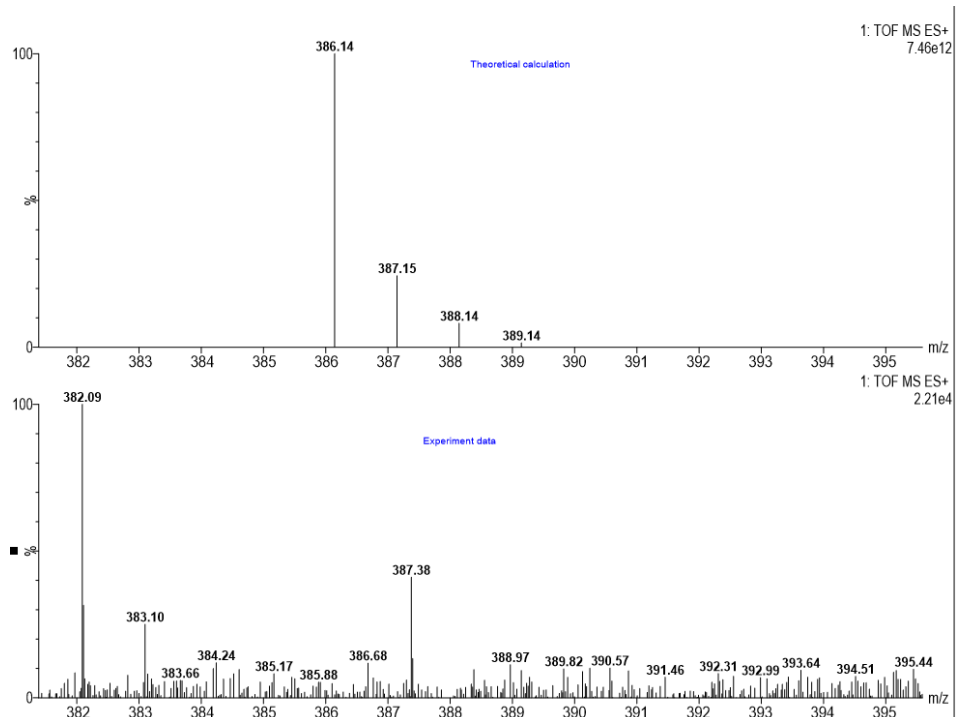
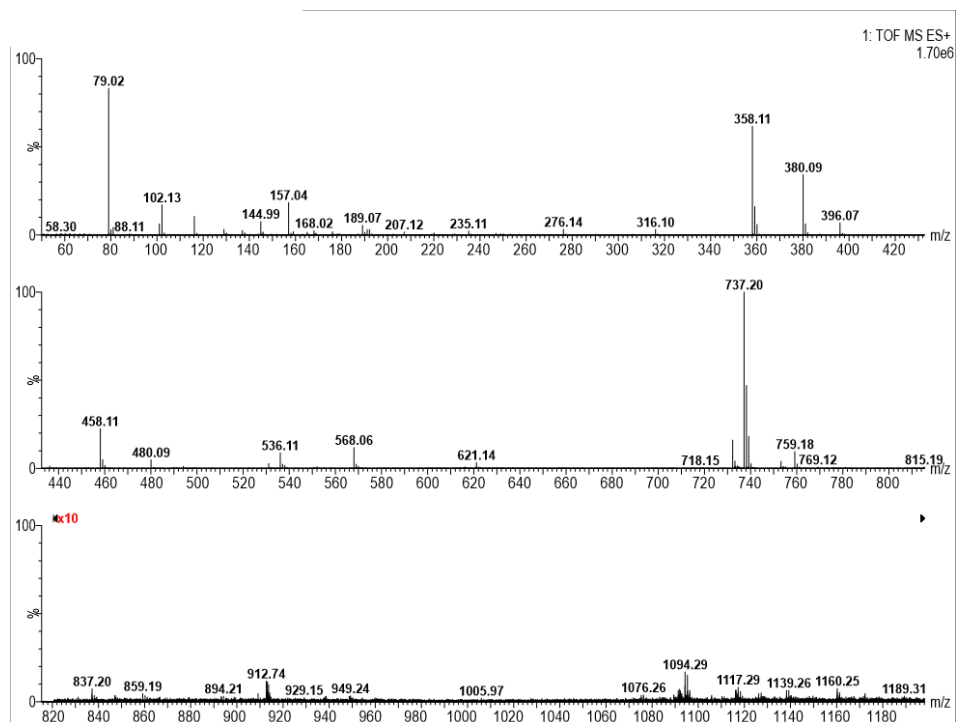
- $\text{SO}_2\text{NHCOCH}_3\text{CH}_3\text{CH}_3$ family –negative ionization



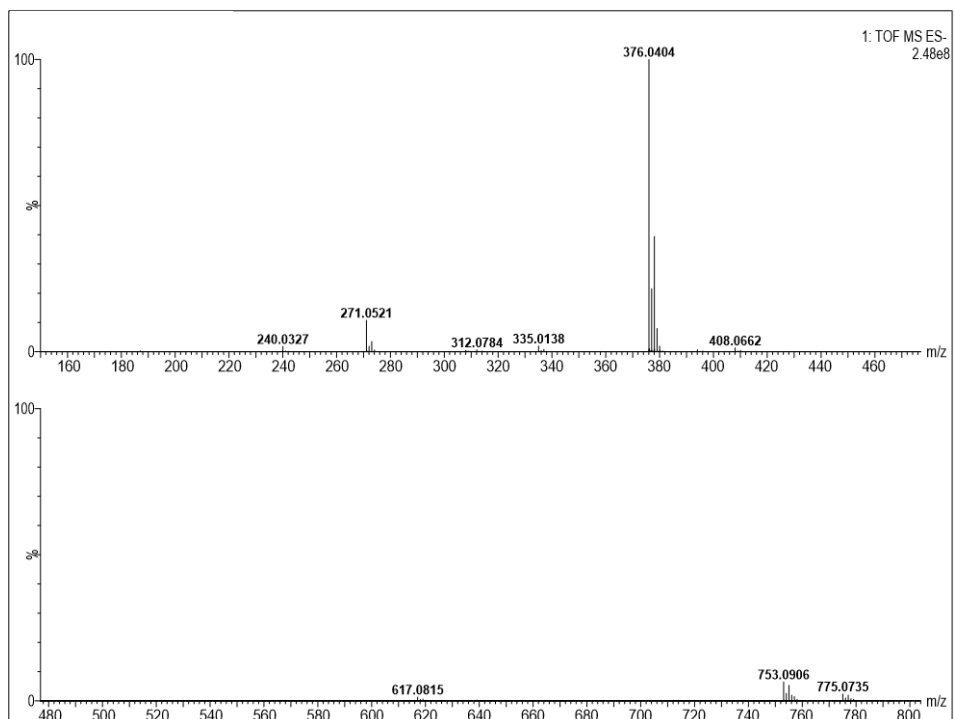
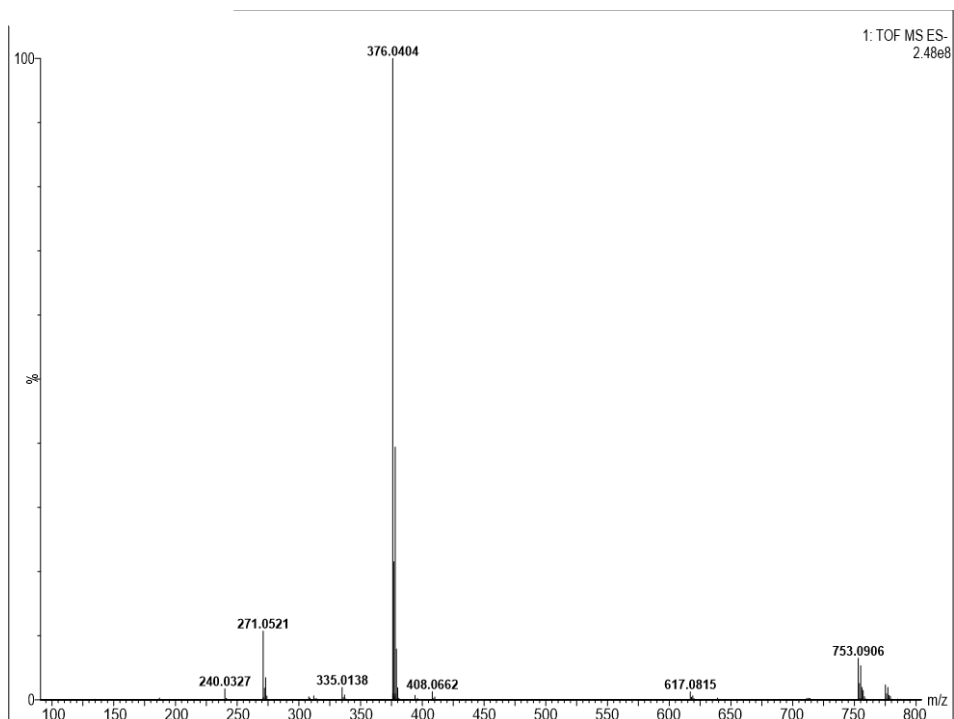


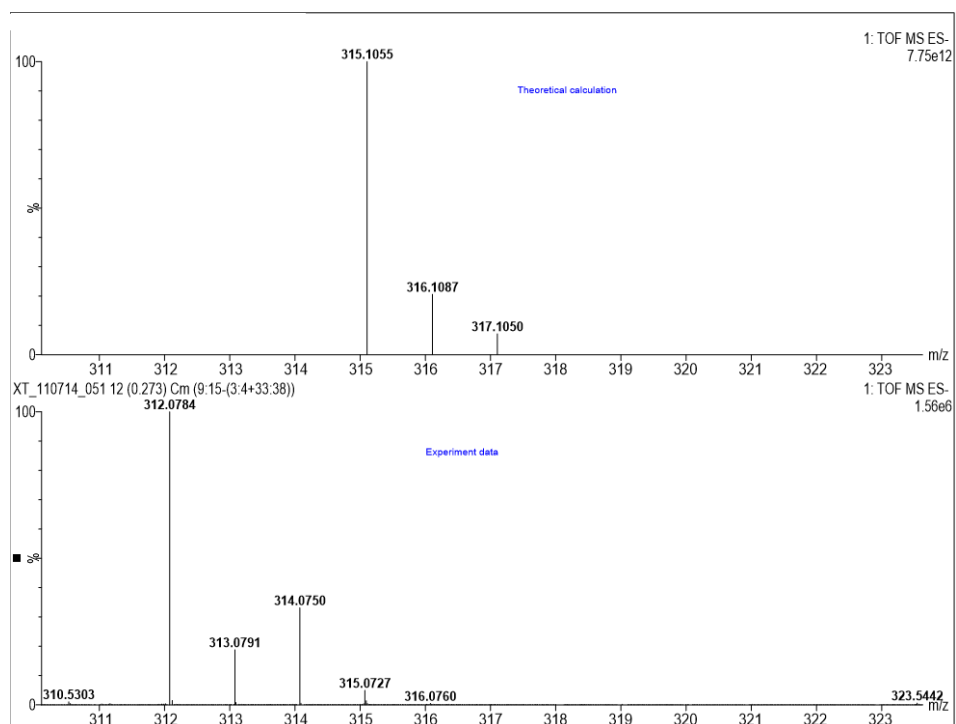
- $\text{SO}_2\text{NHCOCH}_3\text{CH}_3\text{CH}_3$ family –positive ionization



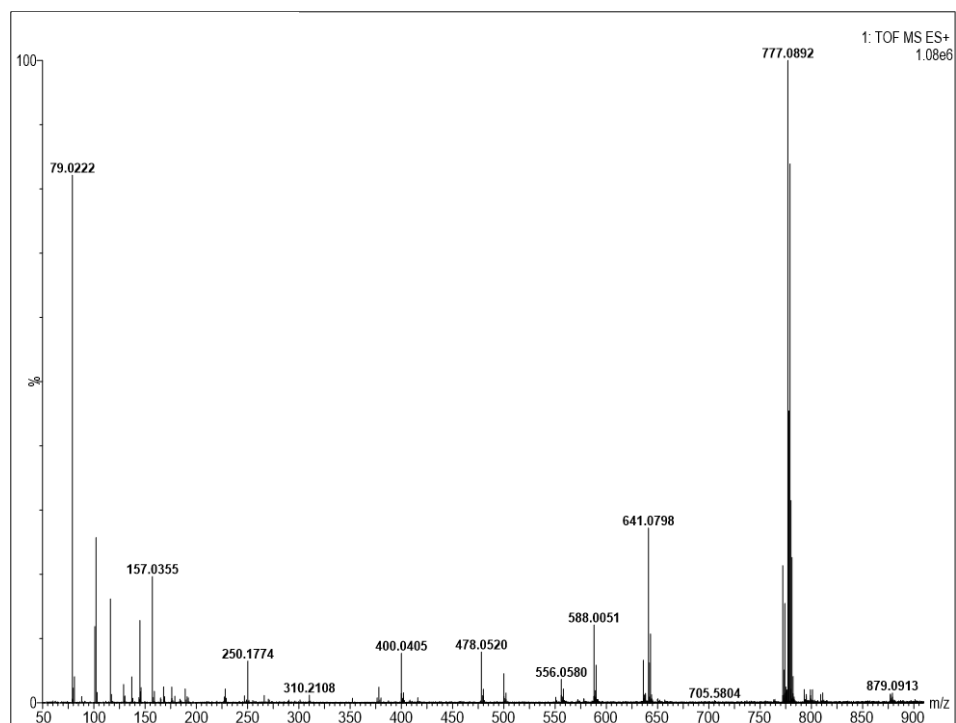


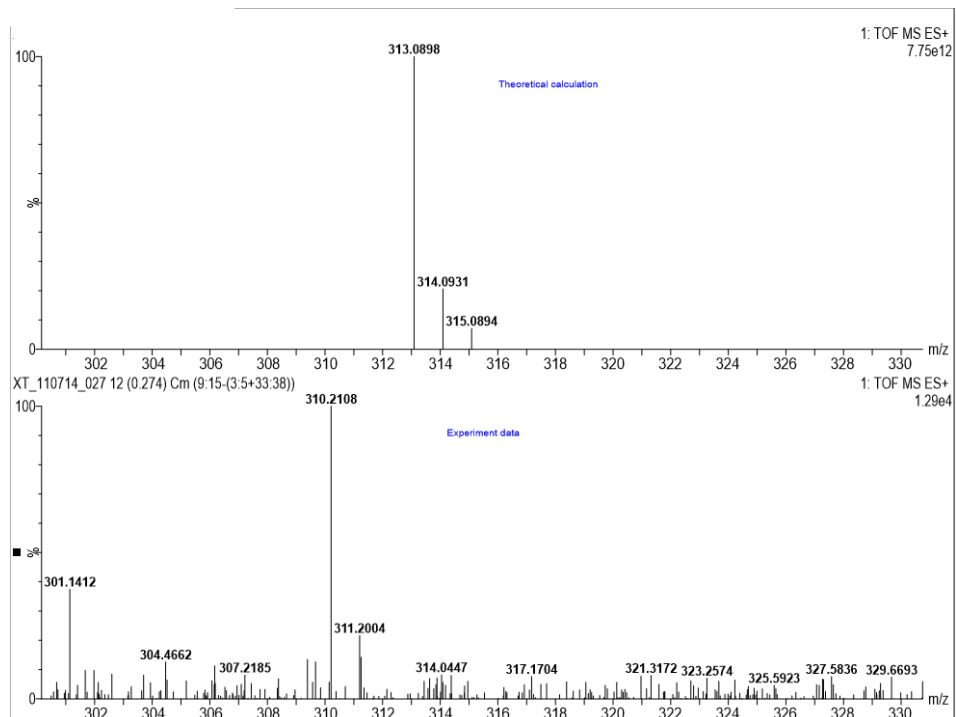
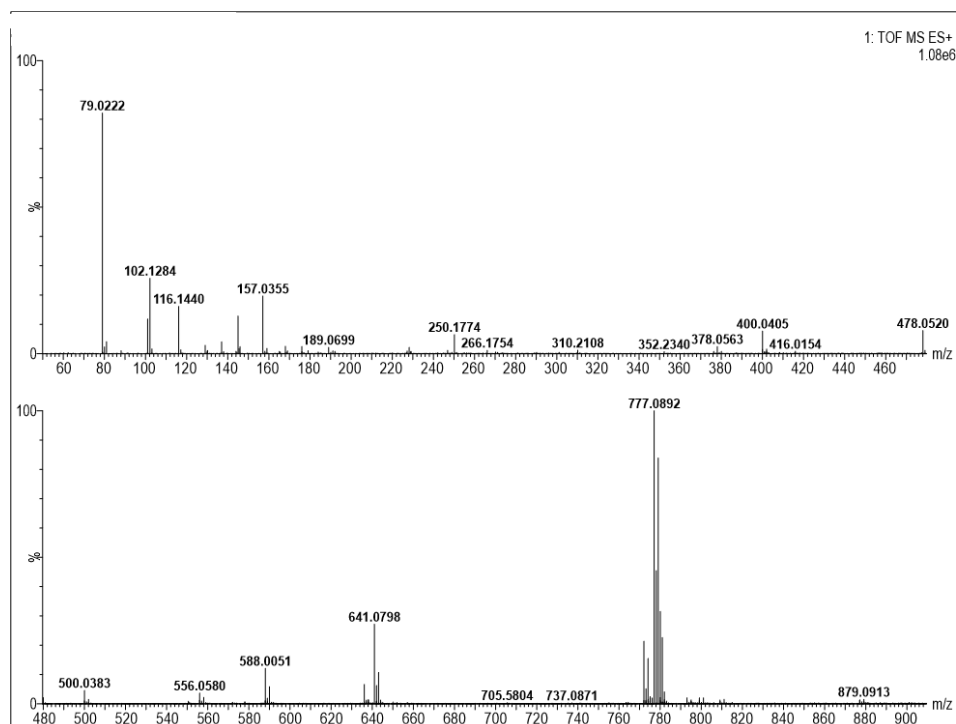
- $\text{SO}_2\text{NHCOCH}_3\text{CH}_3\text{Cl}$ family –negative ionization



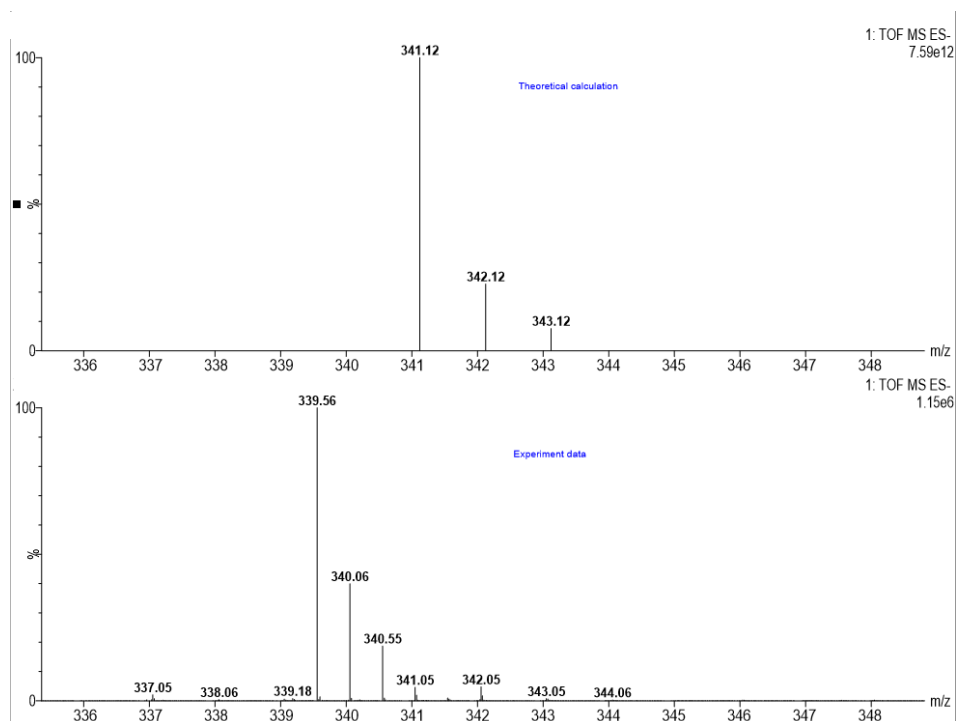
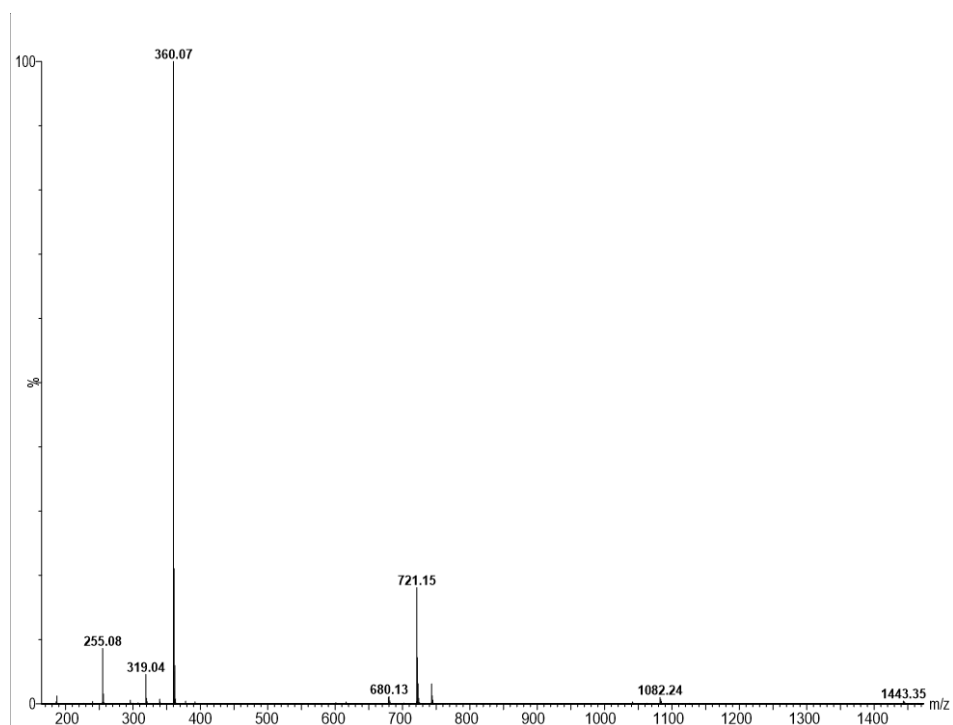


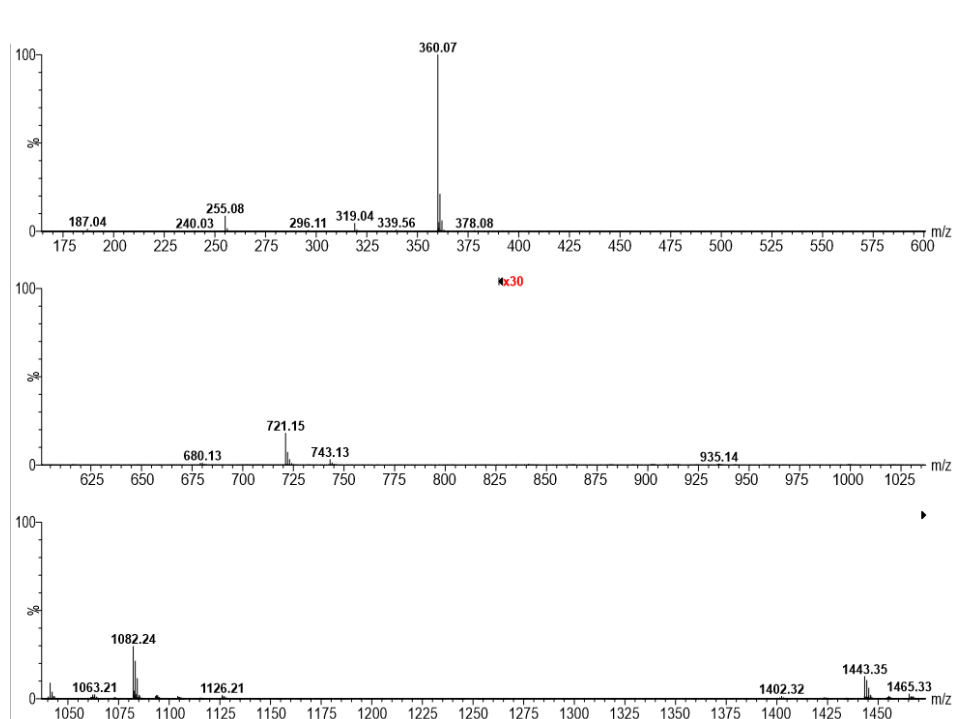
- SO2NHCOCH3CH3Cl family –positive ionization



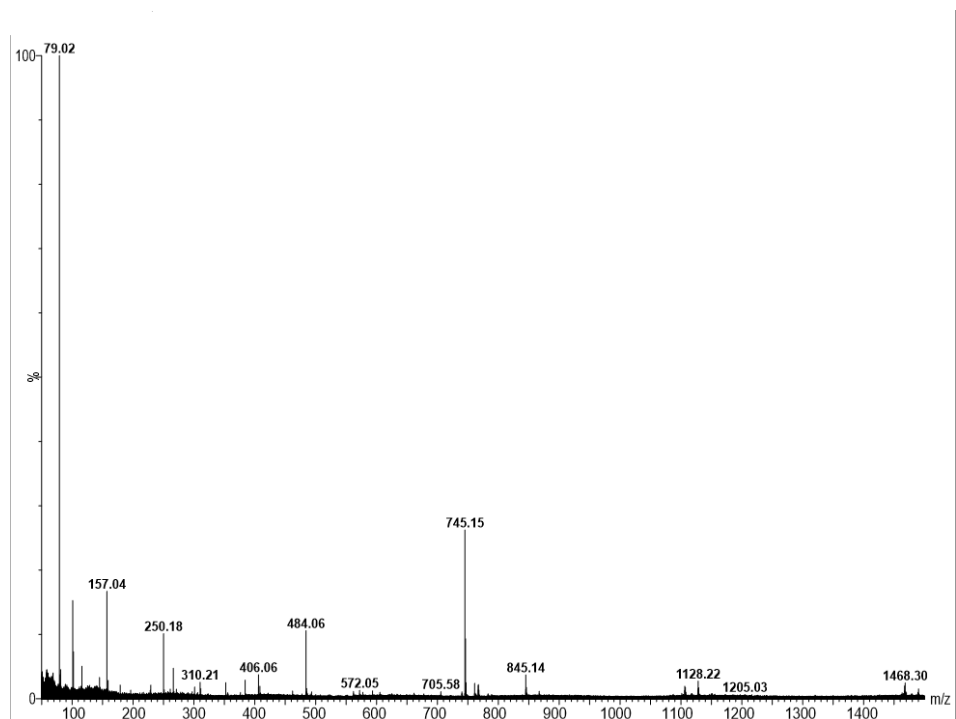


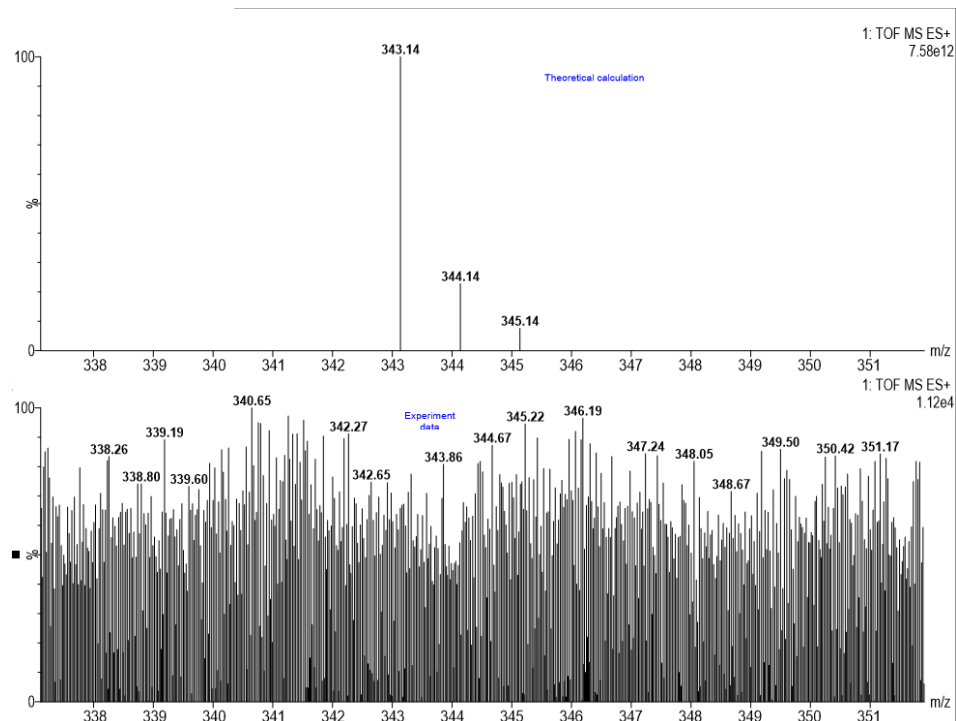
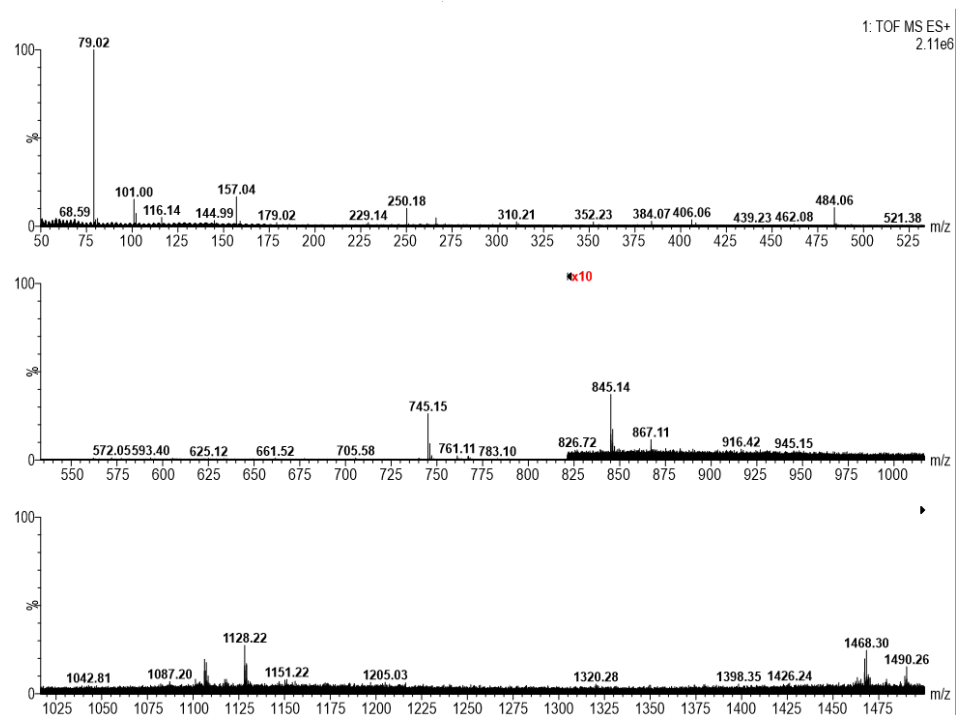
- $\text{SO}_2\text{NHCOCH}_3\text{CH}_3\text{F}$ family –negative ionization





- $\text{SO}_2\text{NHCOCH}_3\text{CH}_3\text{F}$ family –positive ionization



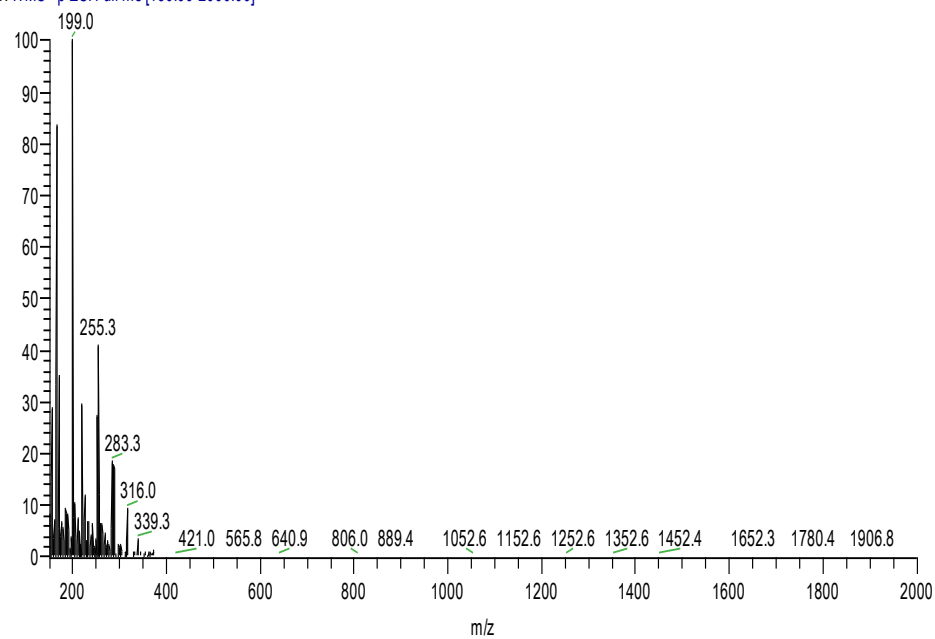


Study # 4

SO₂CH₃COOH – positive ionization

Wanda Sample_140411113803 #1-135 RT: 0.00-1.07 AV: 135 NL: 4.63E2

T: ITMS - p ESI Full ms [150.00-2000.00]

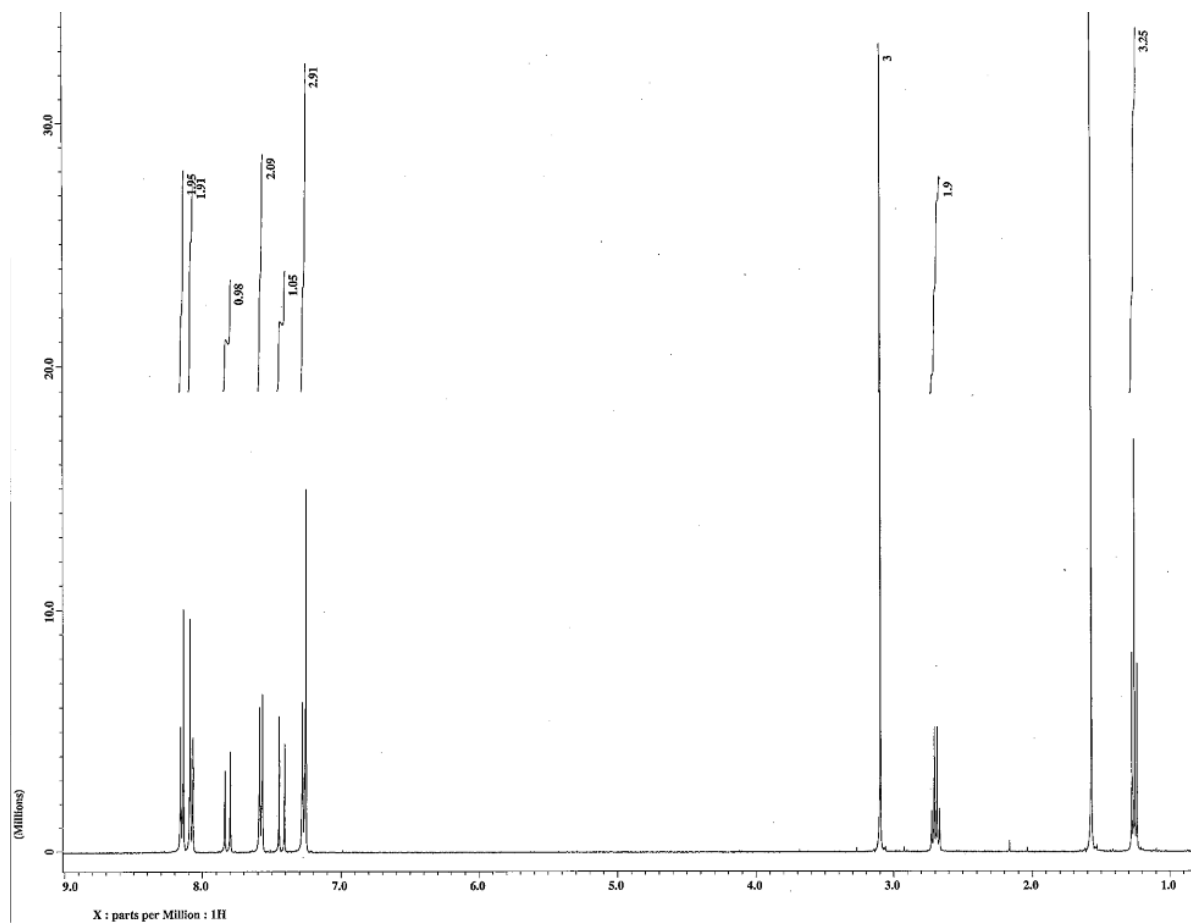


Appendix B

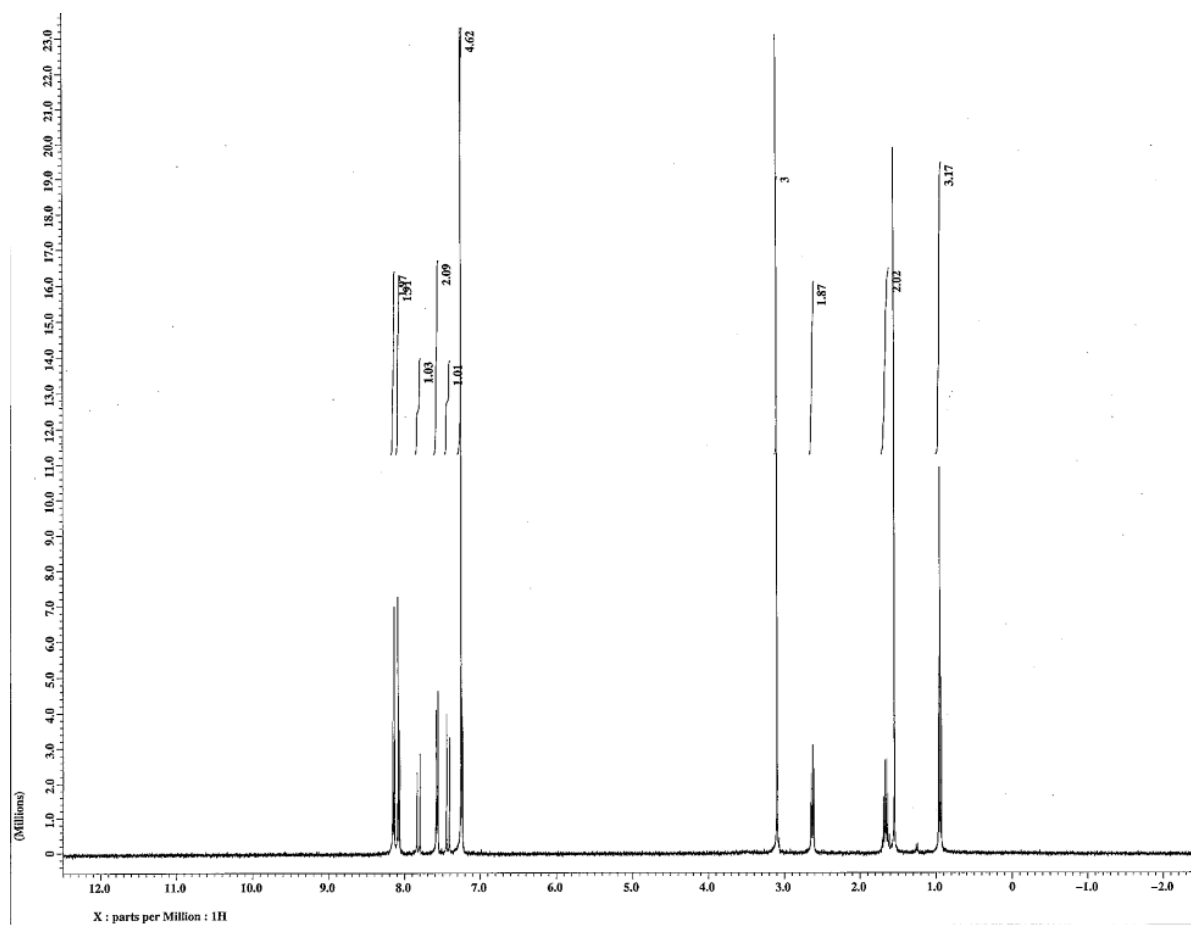
¹H NMR Spectra

Study # 1- SO₂CH₃ family

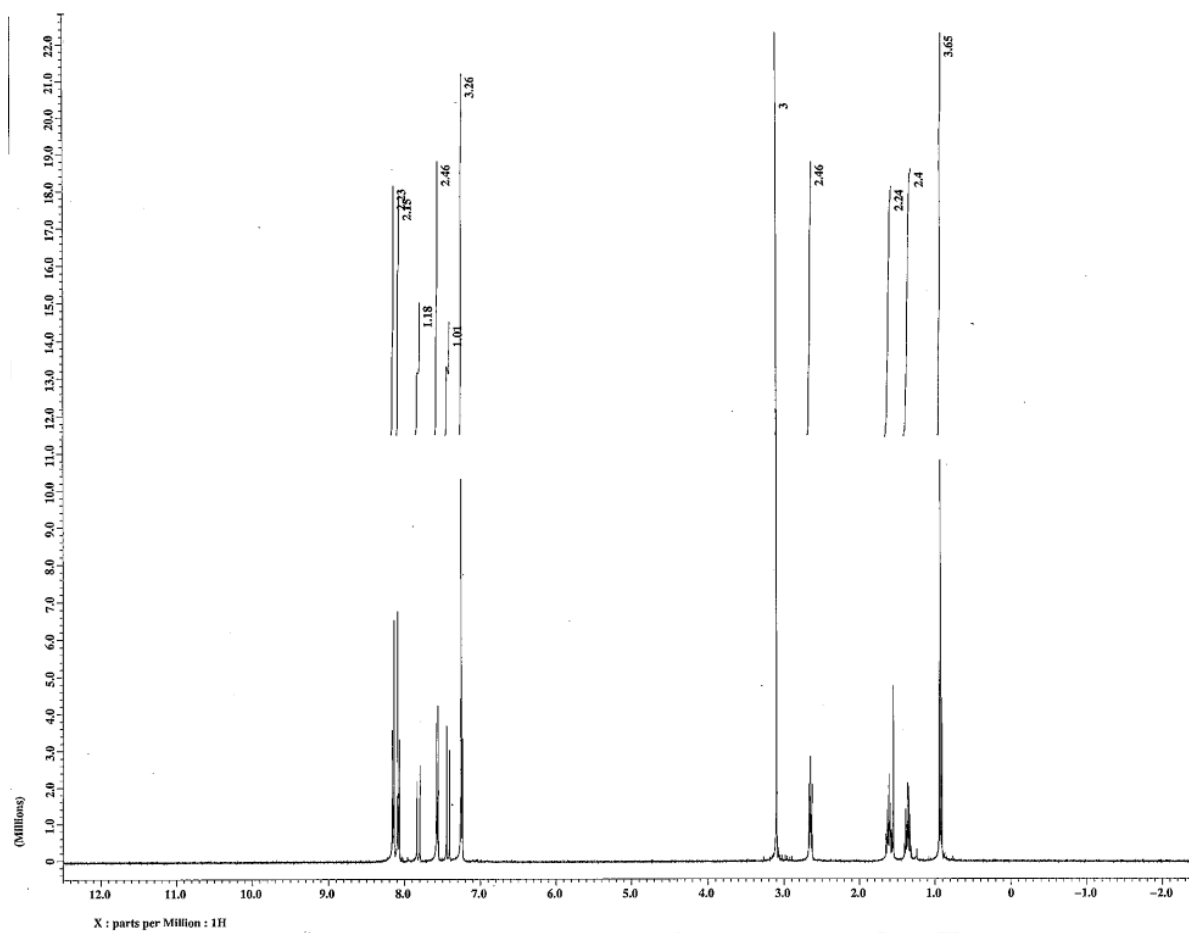
- SO₂CH₃CH₂CH₃



- $\text{SO}_2\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_3$

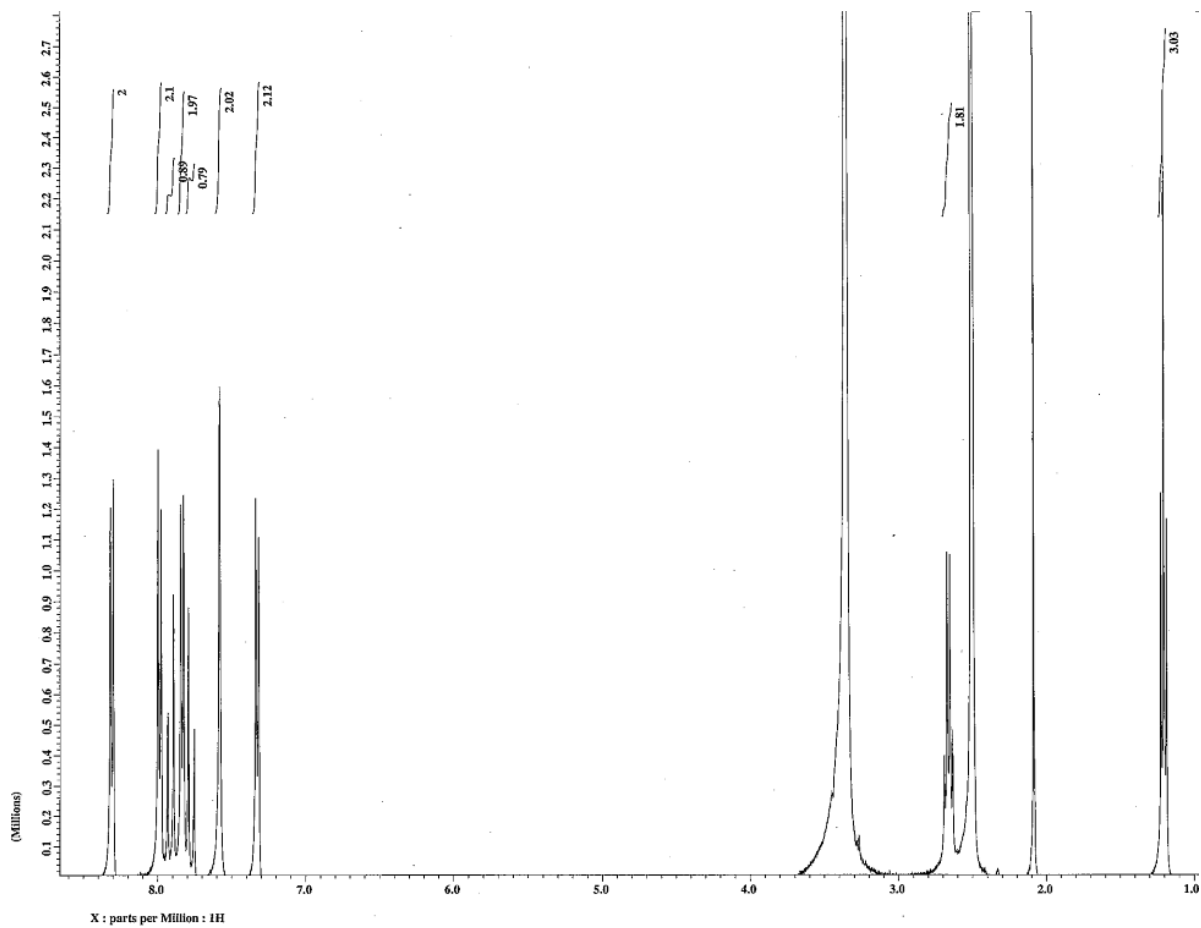


- $\text{SO}_2\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_3$

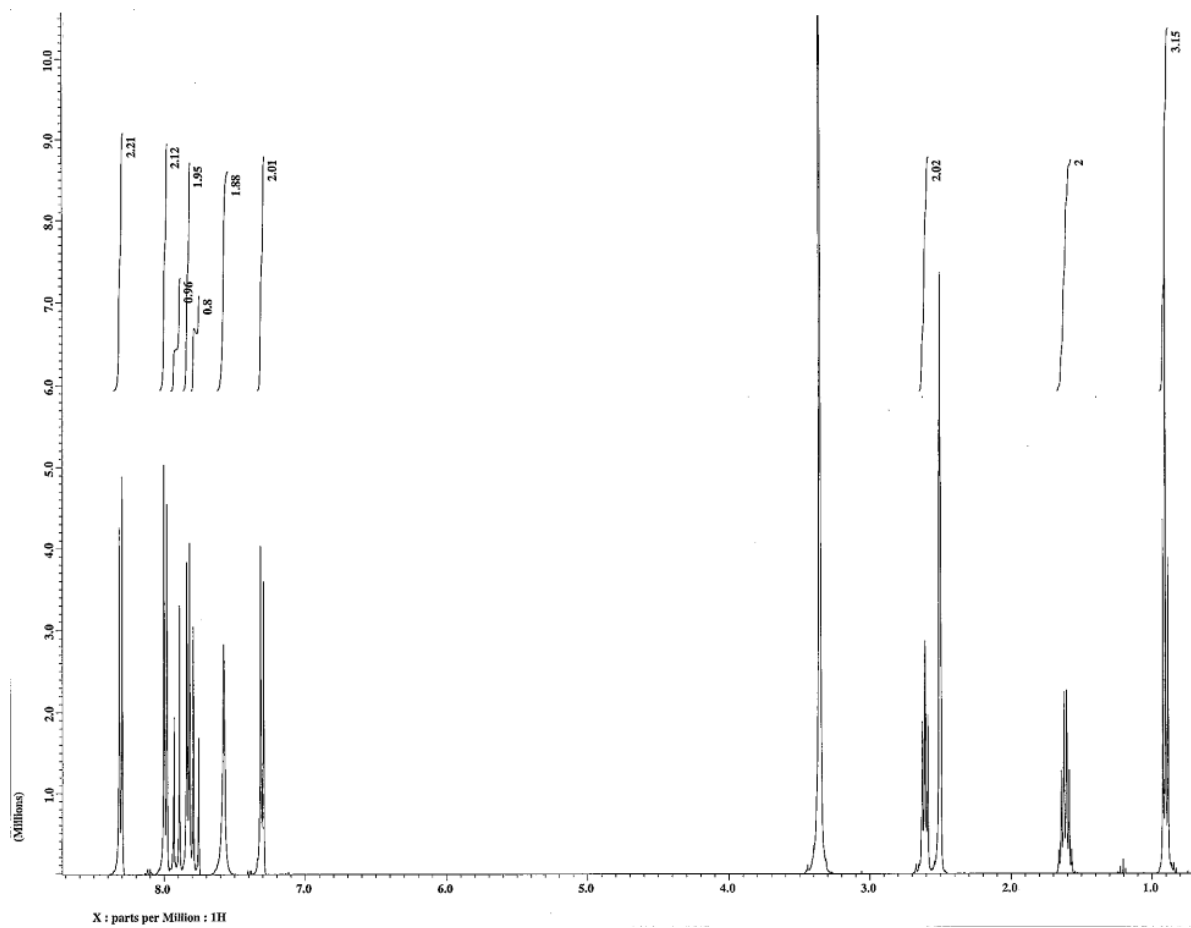


Study # 1- SO₂NH₂ family

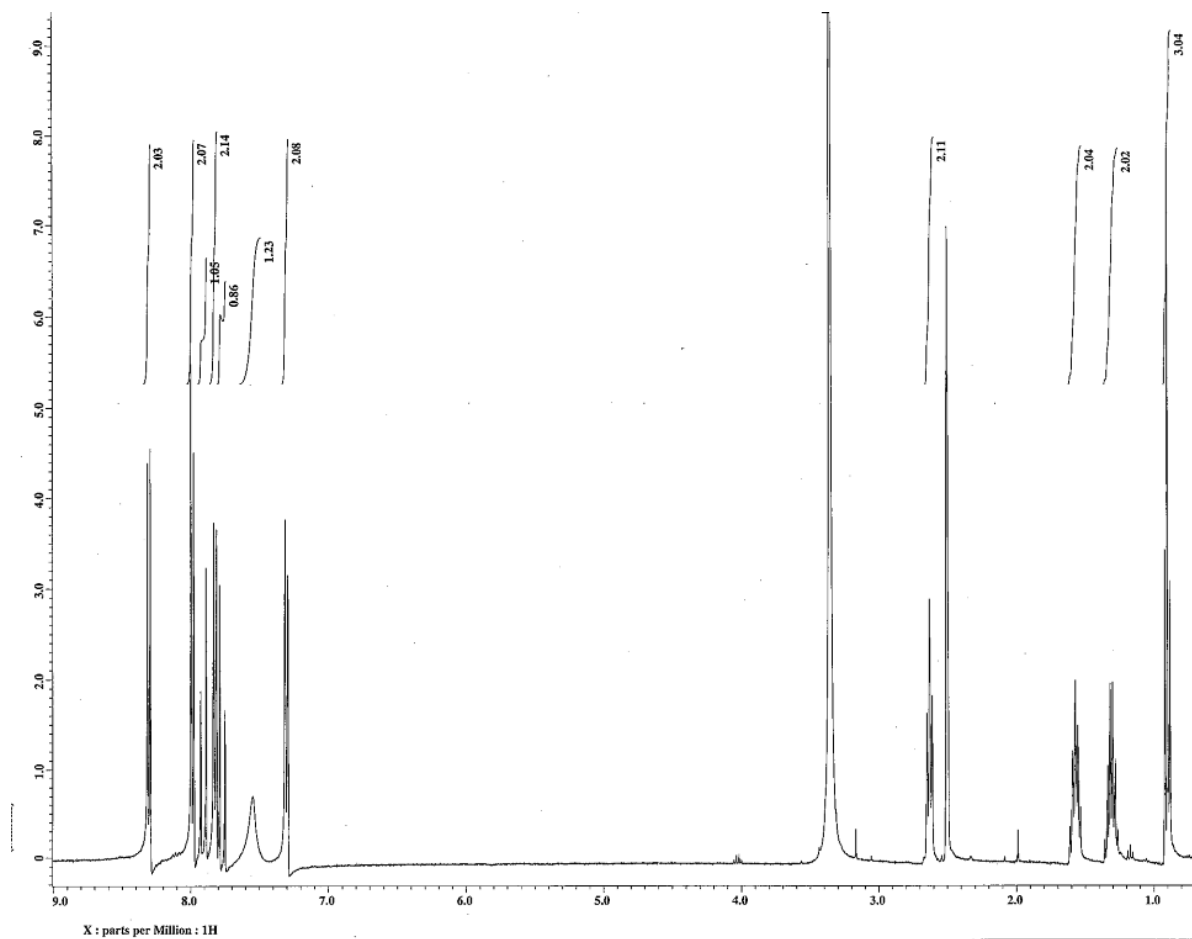
- SO₂NH₂CH₂CH₃



- $\text{SO}_2\text{NH}_2\text{CH}_2\text{CH}_2\text{CH}_3$

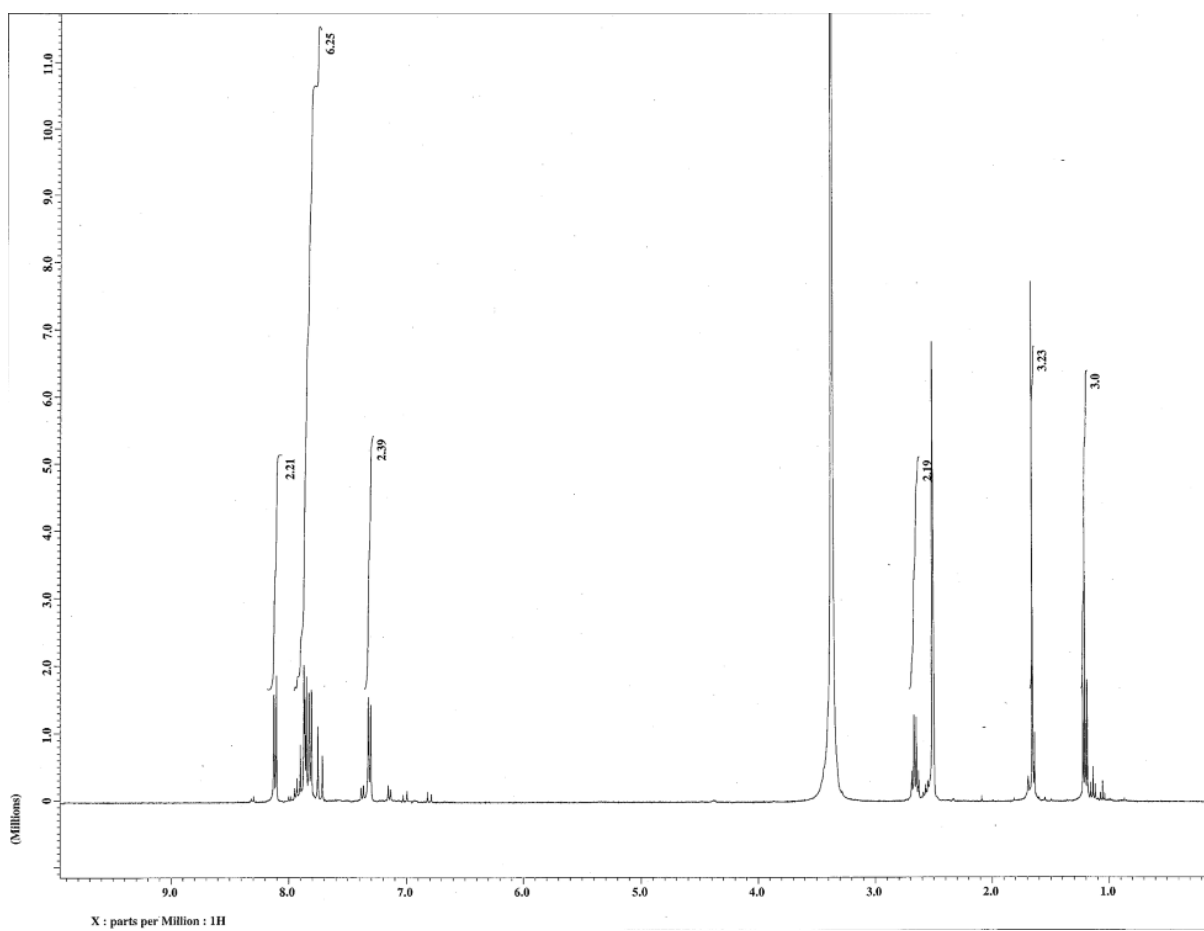


- $\text{SO}_2\text{NH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$

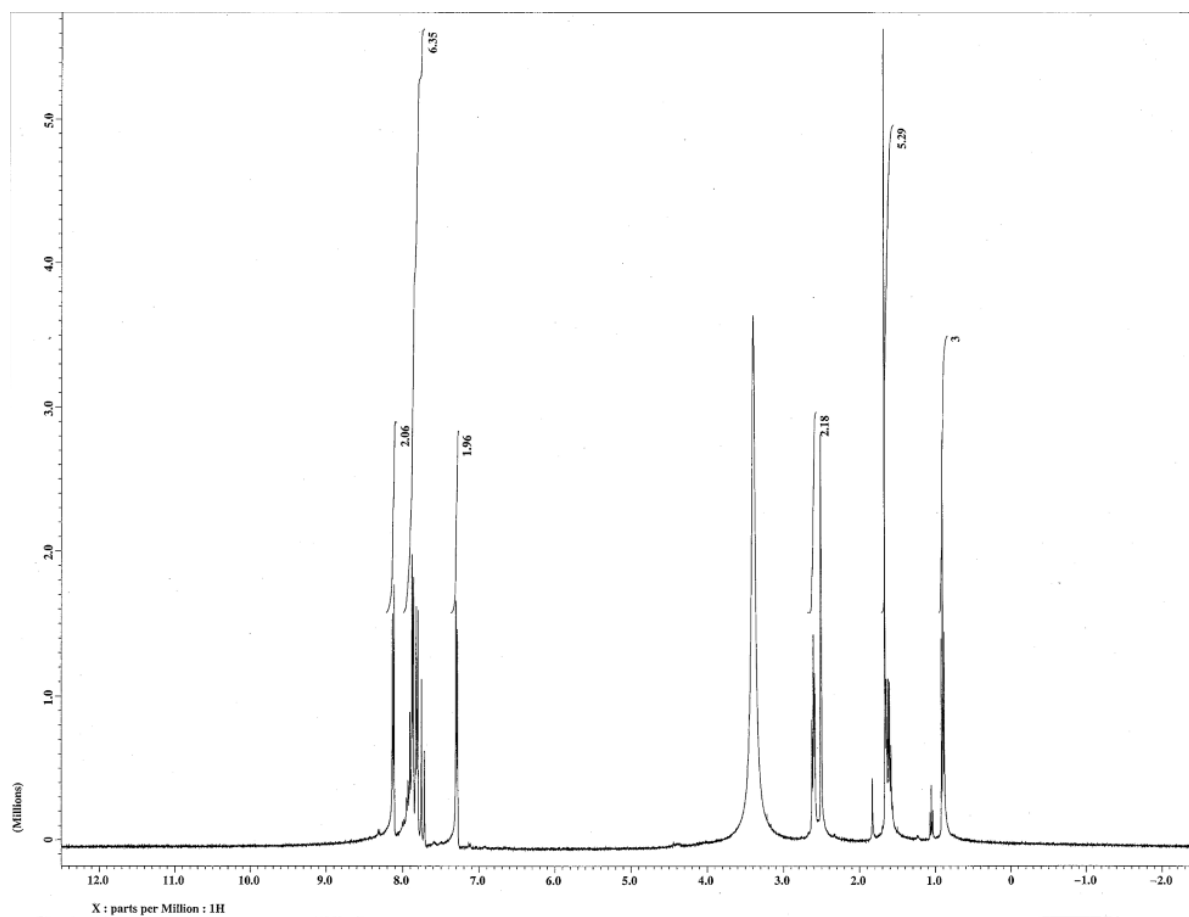


Study # 1- $\text{SO}_2\text{NHCOCH}_3$ family

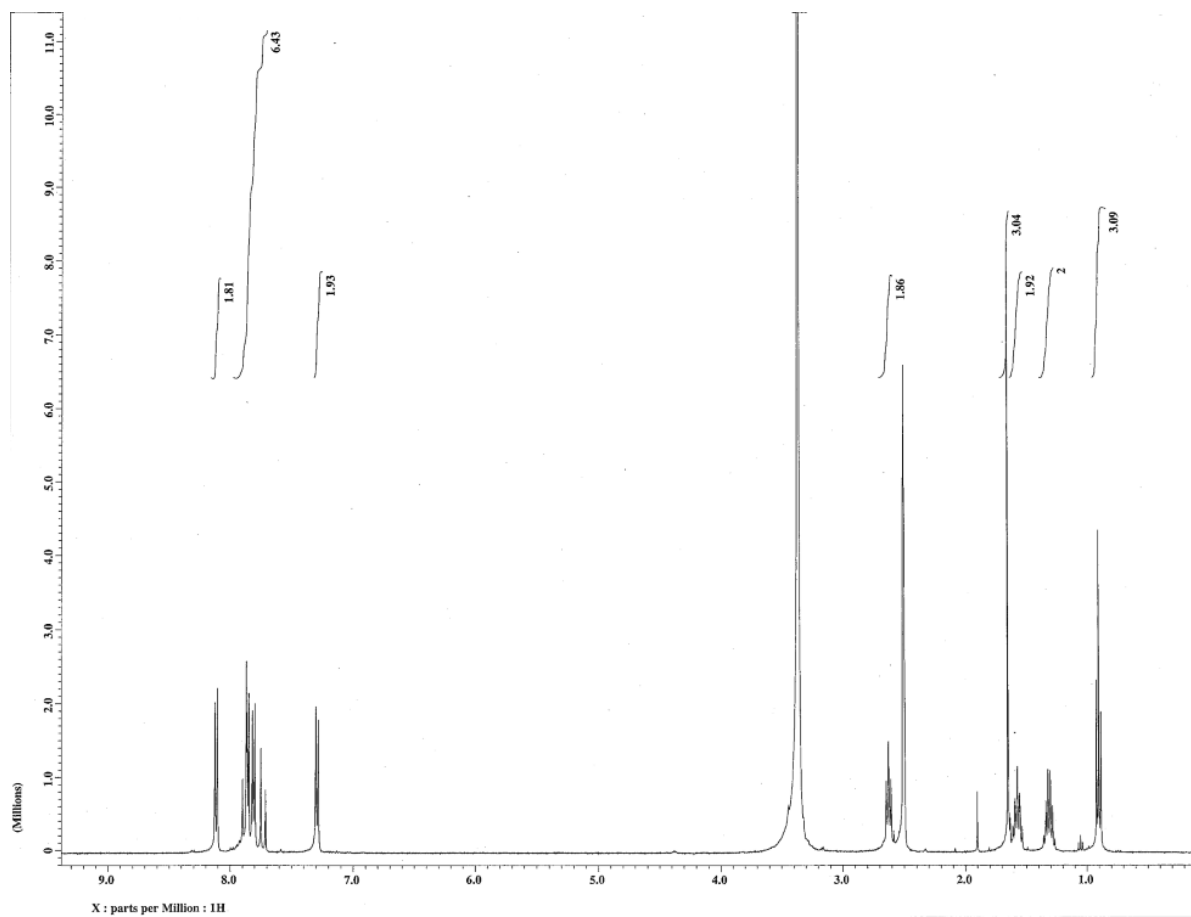
- $\text{SO}_2\text{NHCOCH}_2\text{CH}_3$



- $\text{SO}_2\text{NHCOCH}_2\text{CH}_2\text{CH}_3$

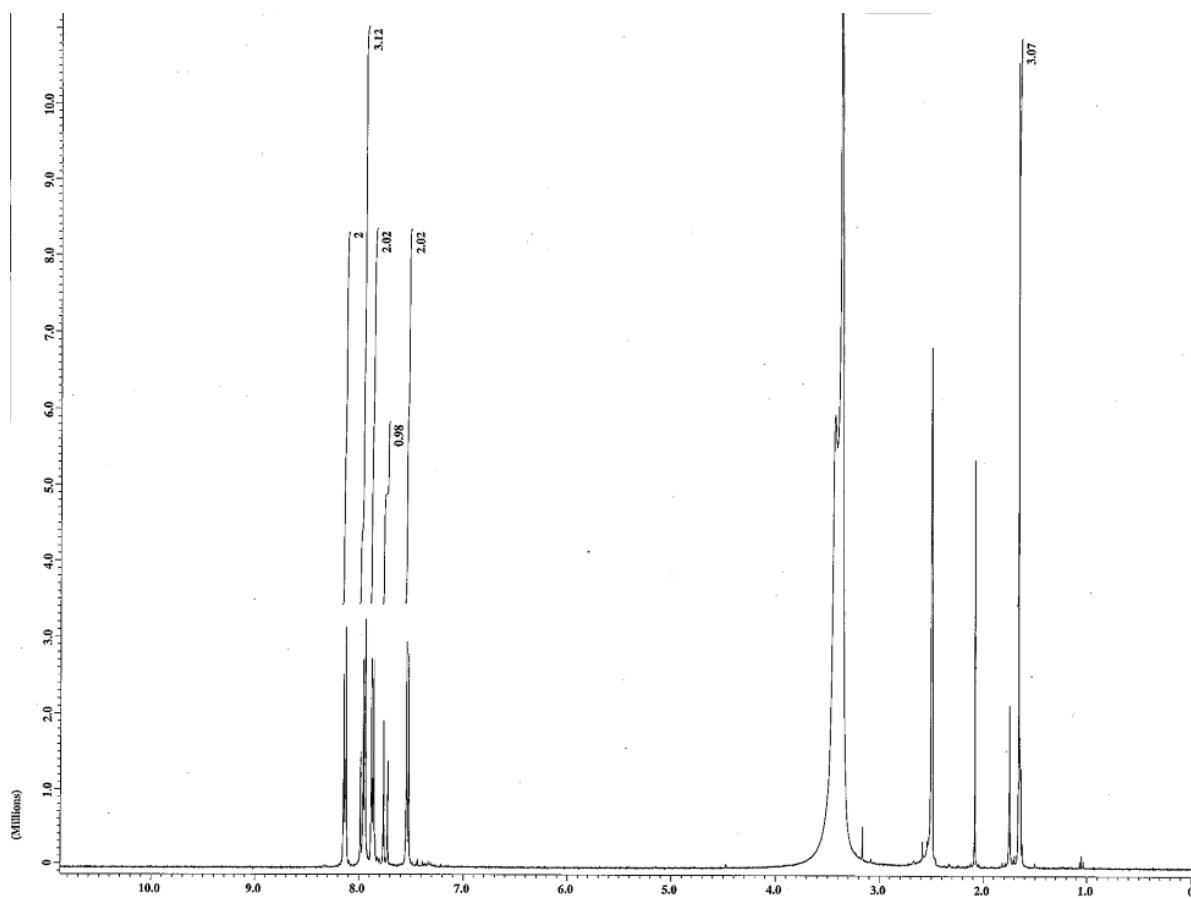


- $\text{SO}_2\text{NHCOCH}_2\text{CH}_2\text{CH}_2\text{CH}_3$

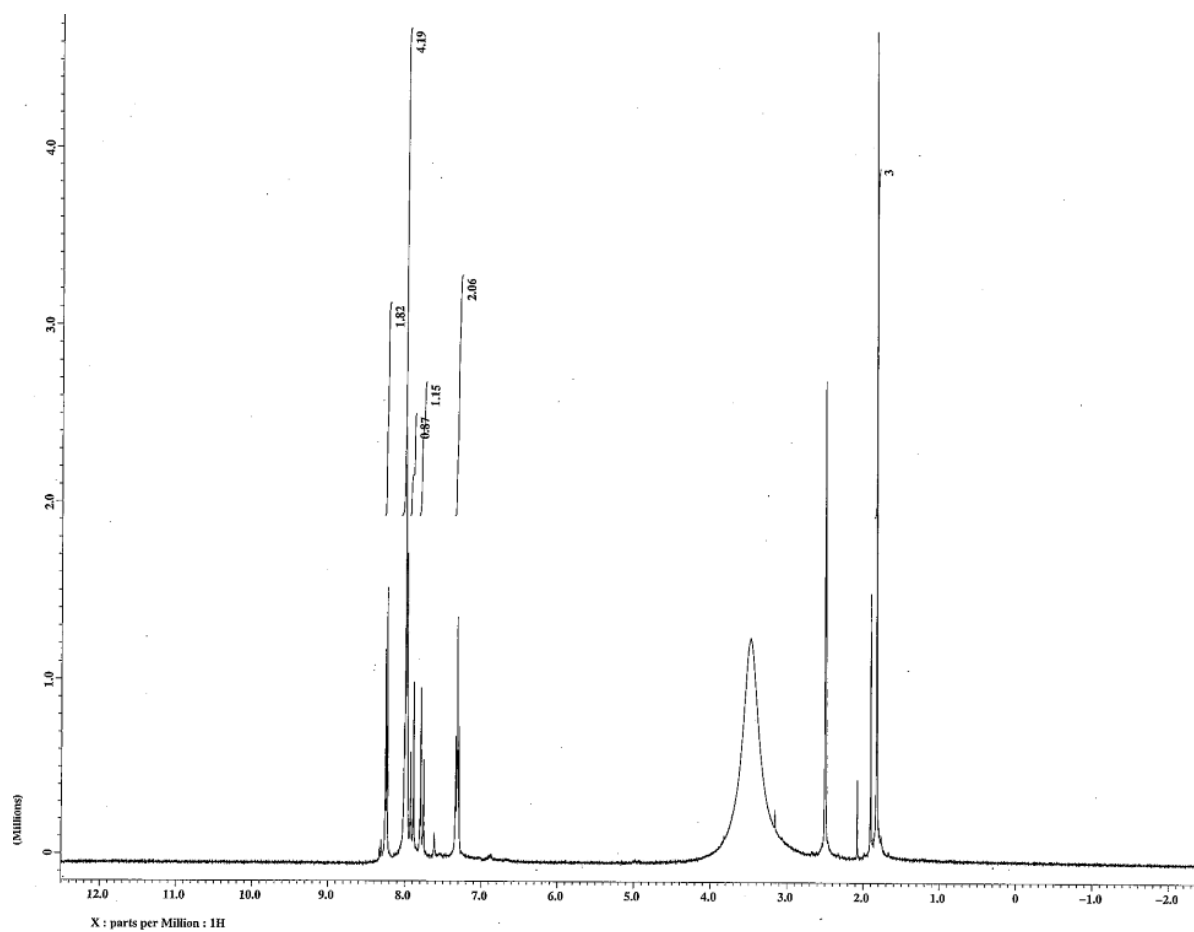


Study # 2- $\text{SO}_2\text{NHCOCH}_3$ family

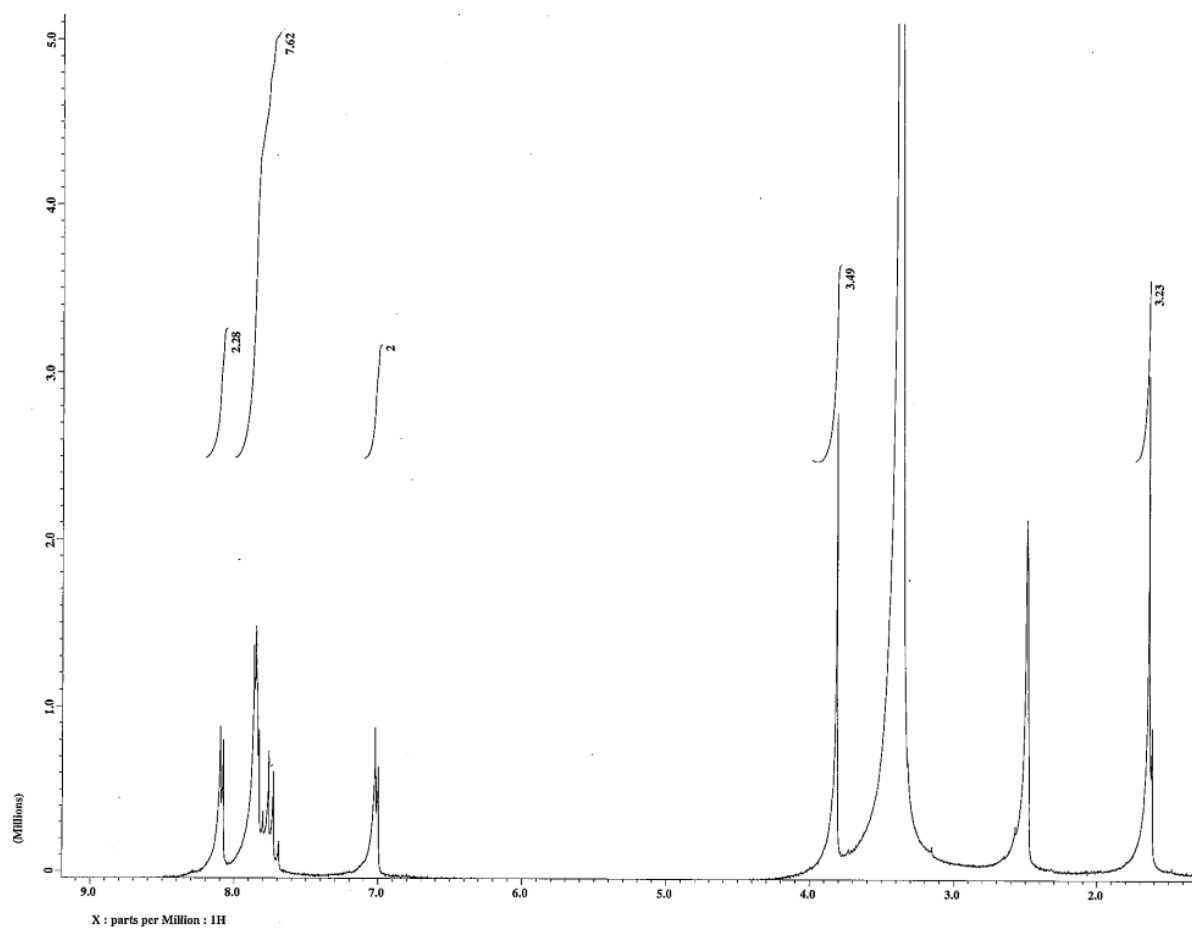
- $\text{SO}_2\text{NHCOCH}_3\text{Cl}$



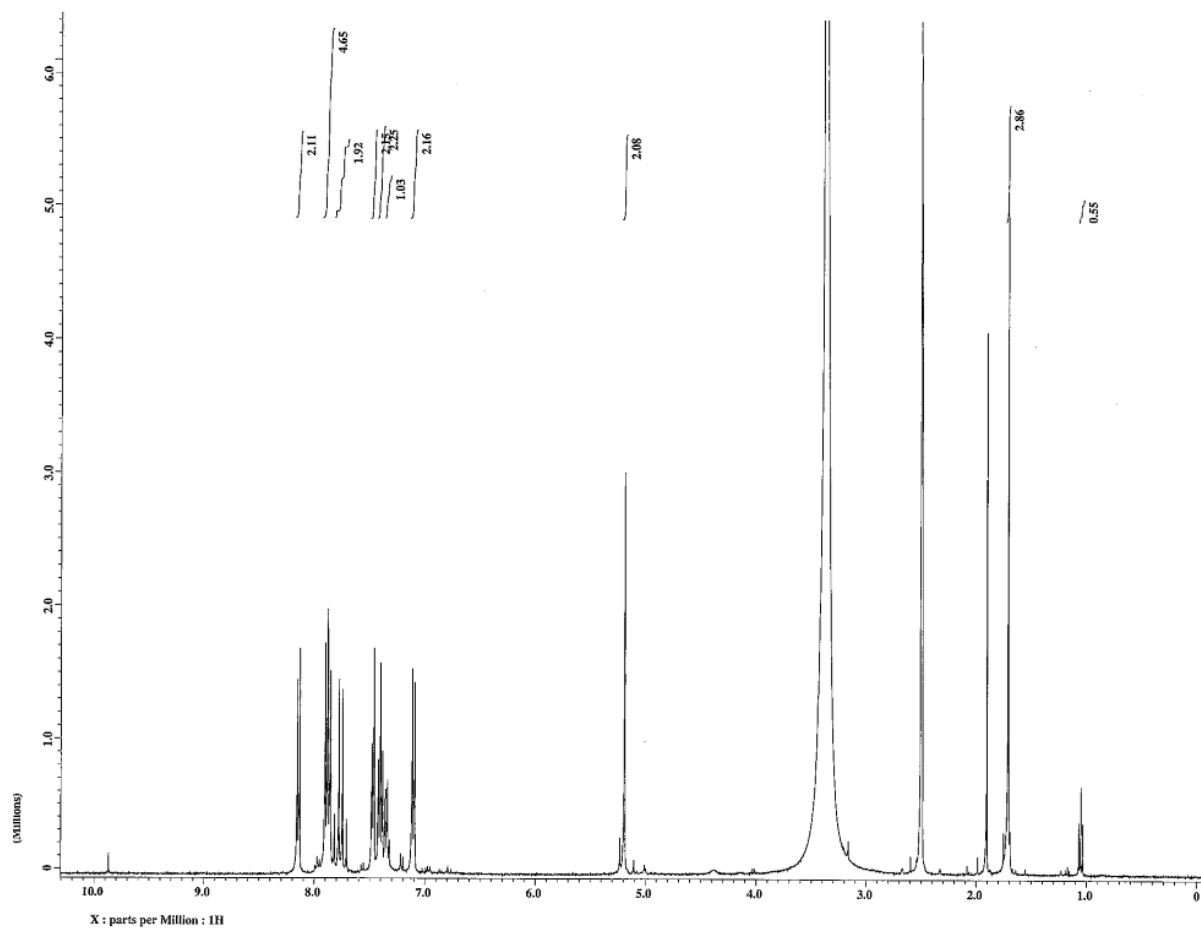
- $\text{SO}_2\text{NHCOCH}_3\text{F}$



- $\text{SO}_2\text{NHCOCH}_3\text{OCH}_3$

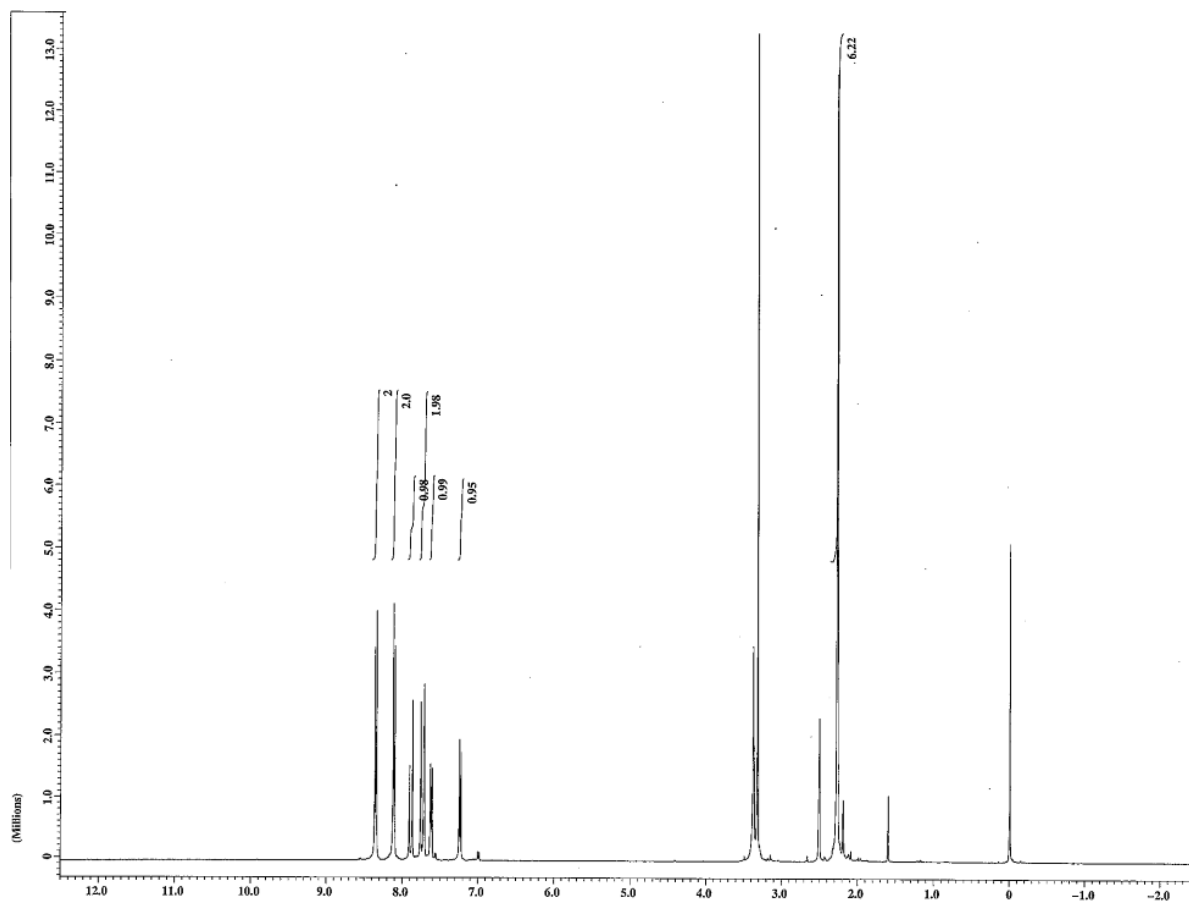


- $\text{SO}_2\text{NHCOCH}_3\text{OBenzyl}$

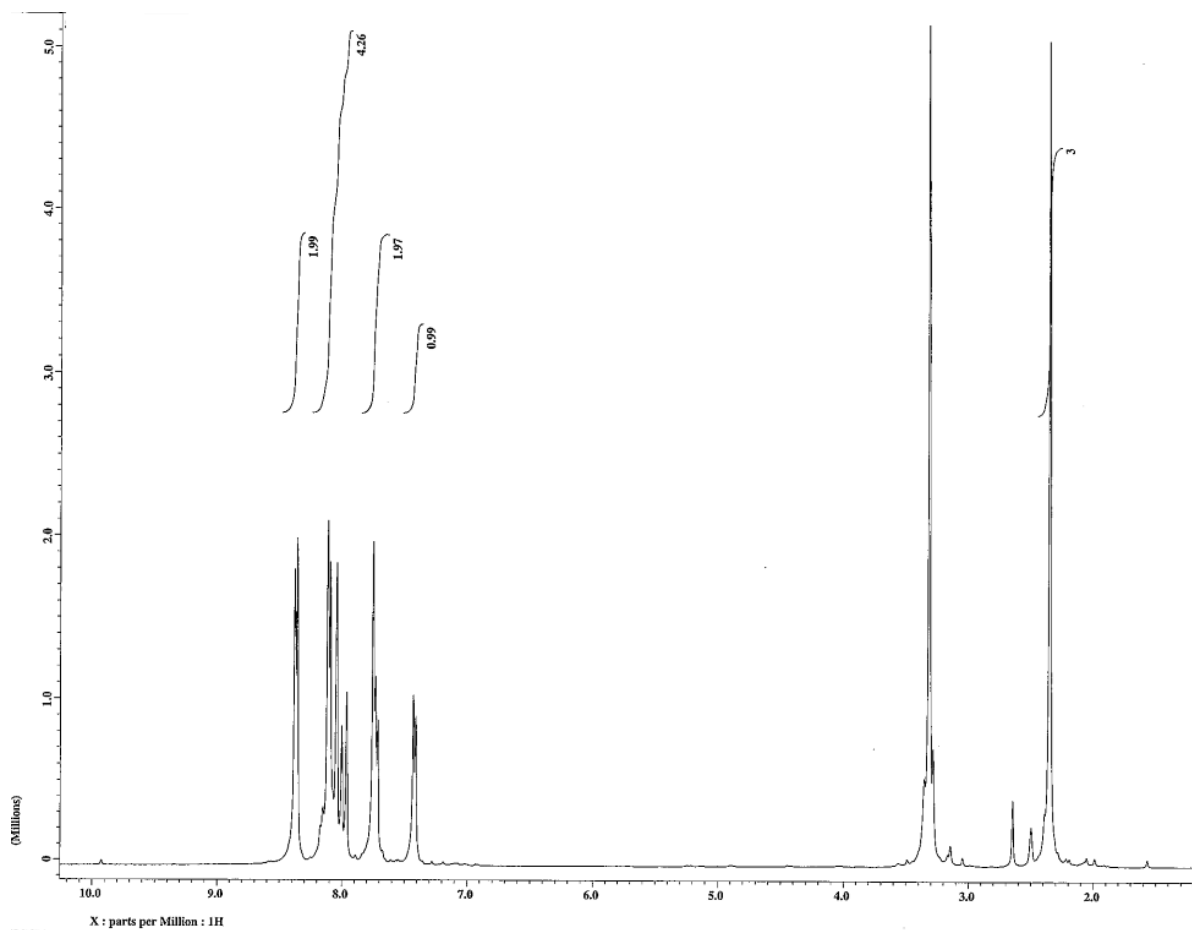


Study # 3- SO₂CH₃ family

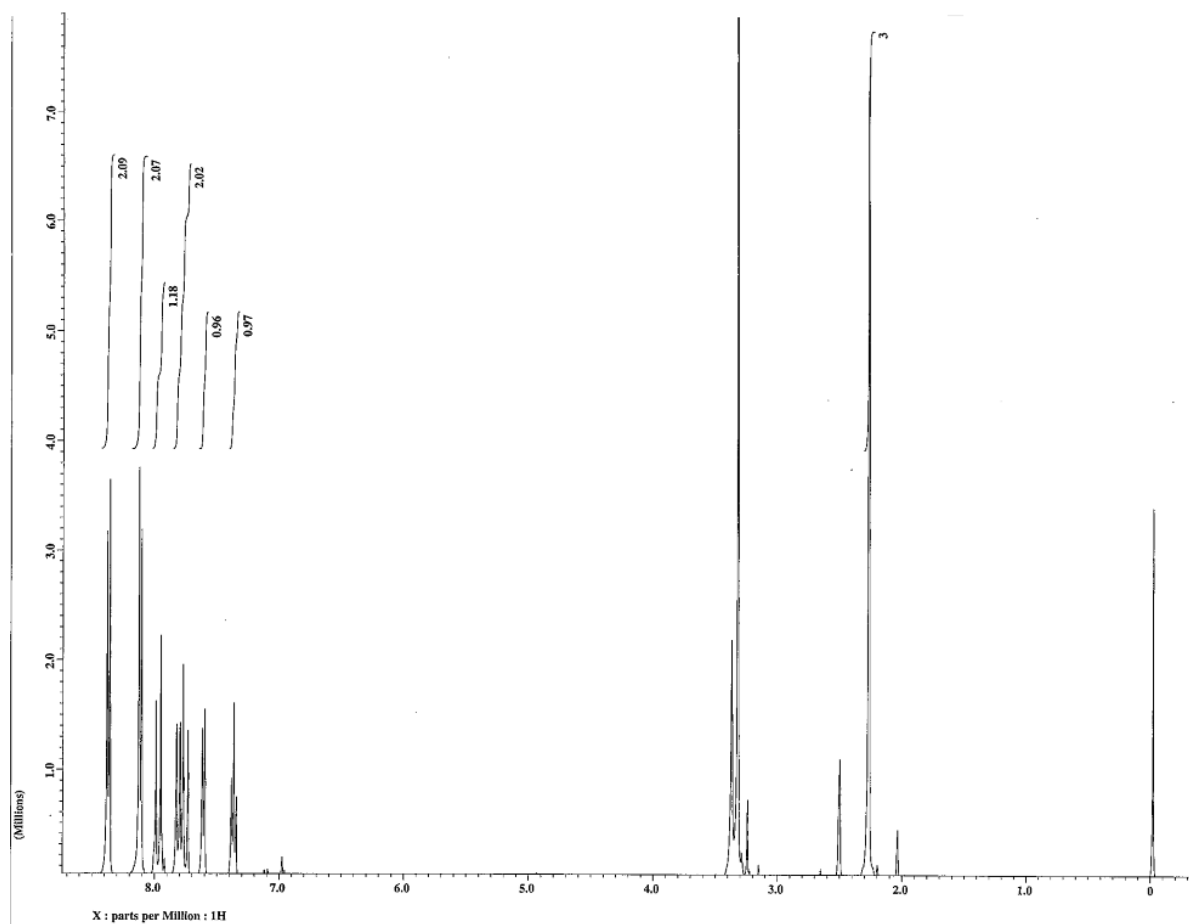
- SO₂CH₃CH₃CH₃



- $\text{SO}_2\text{CH}_3\text{CH}_3\text{Cl}$

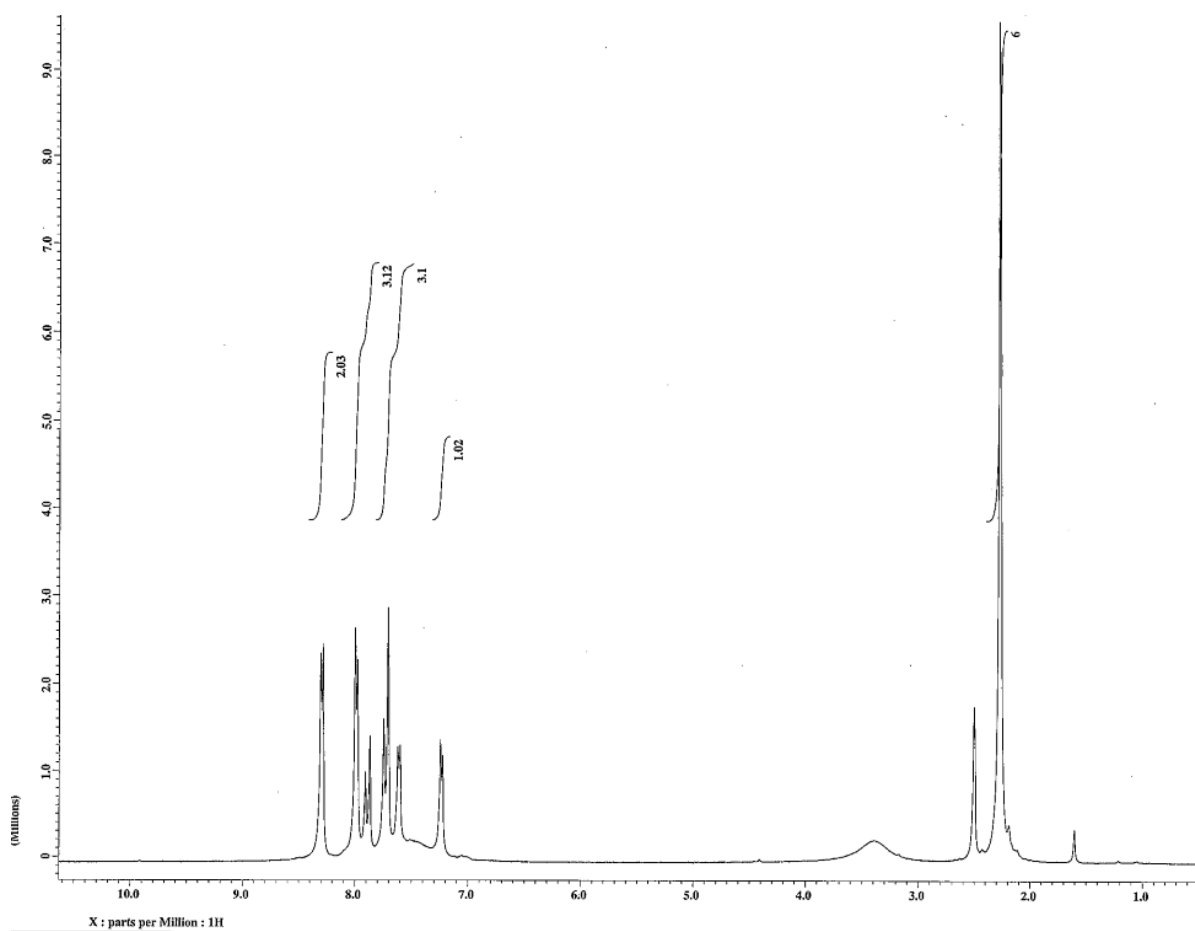


- $\text{SO}_2\text{CH}_3\text{CH}_3\text{F}$

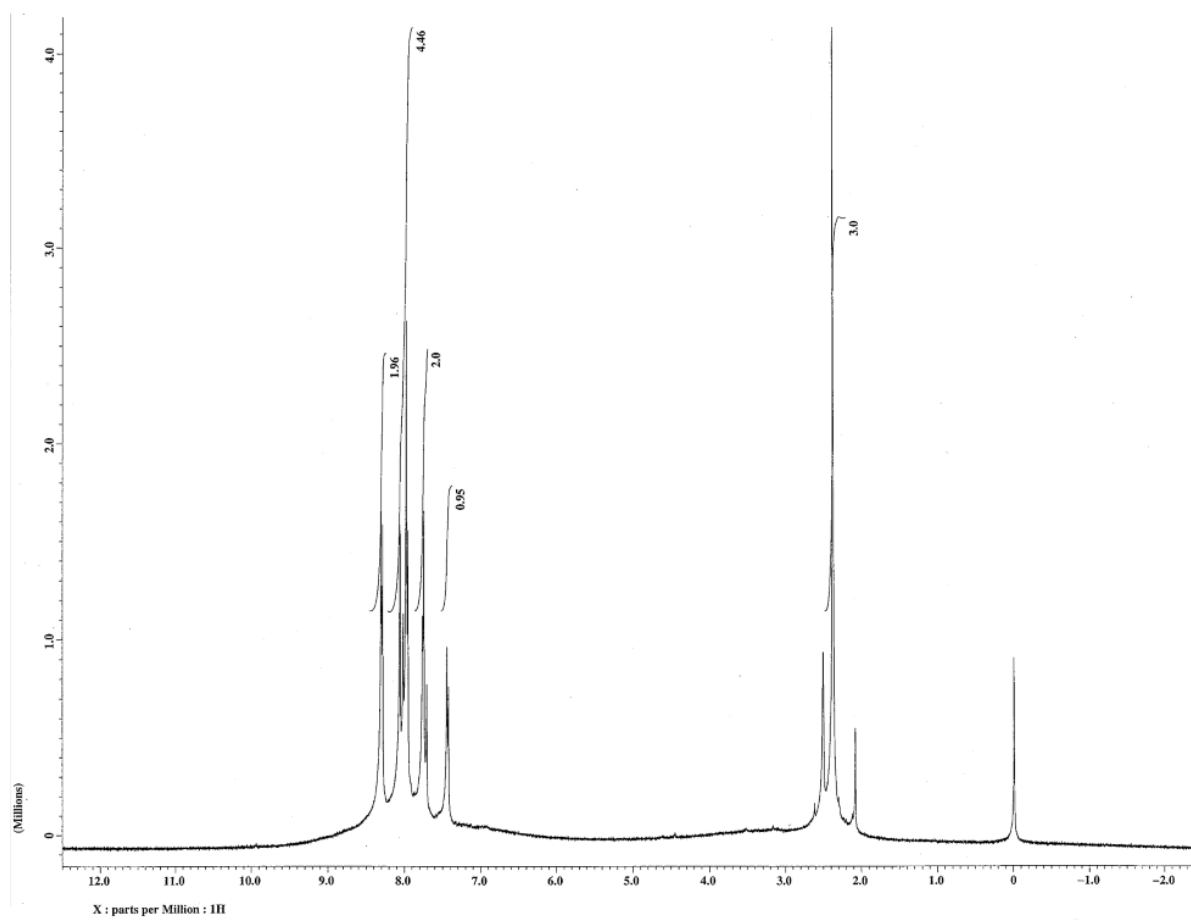


Study # 3- SO₂NH₂ family

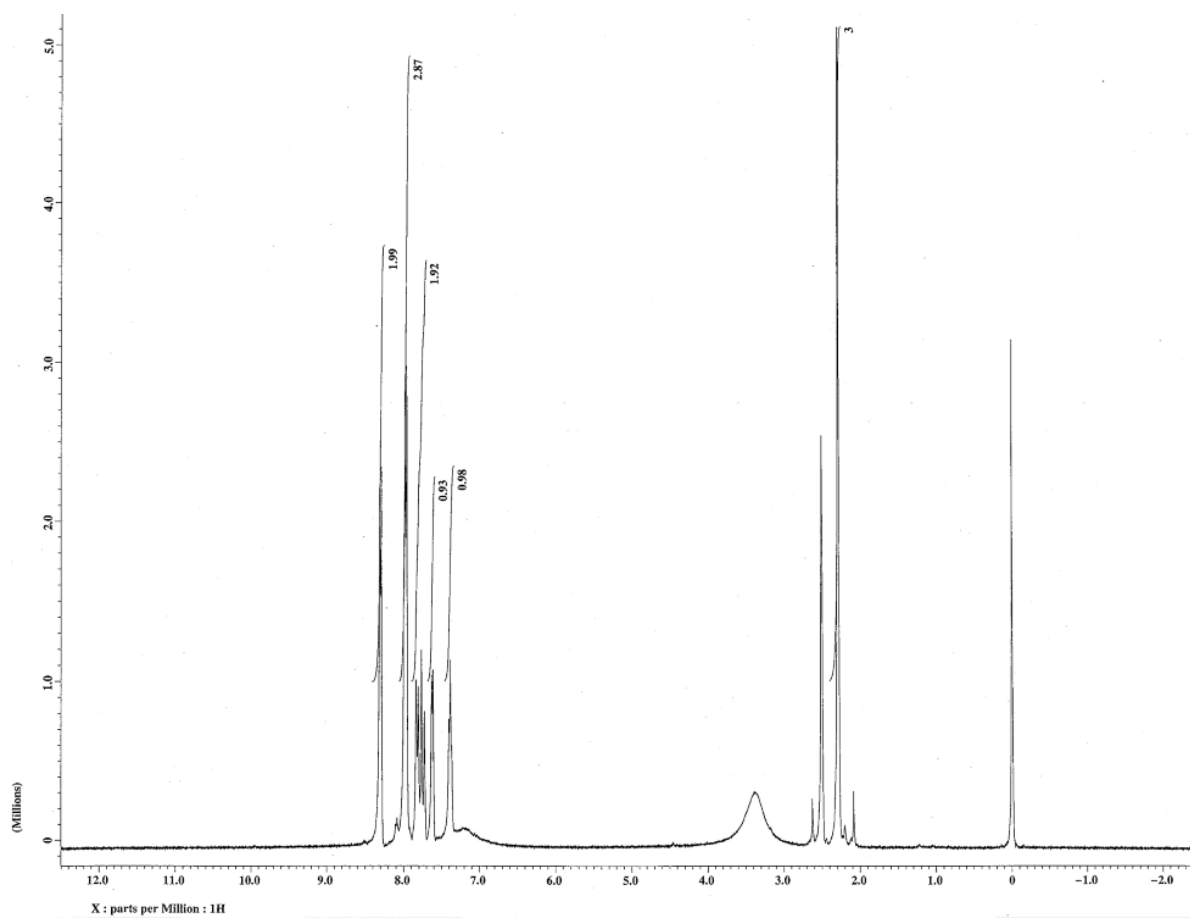
- SO₂NH₂CH₃CH₃



- $\text{SO}_2\text{NH}_2\text{CH}_3\text{Cl}$

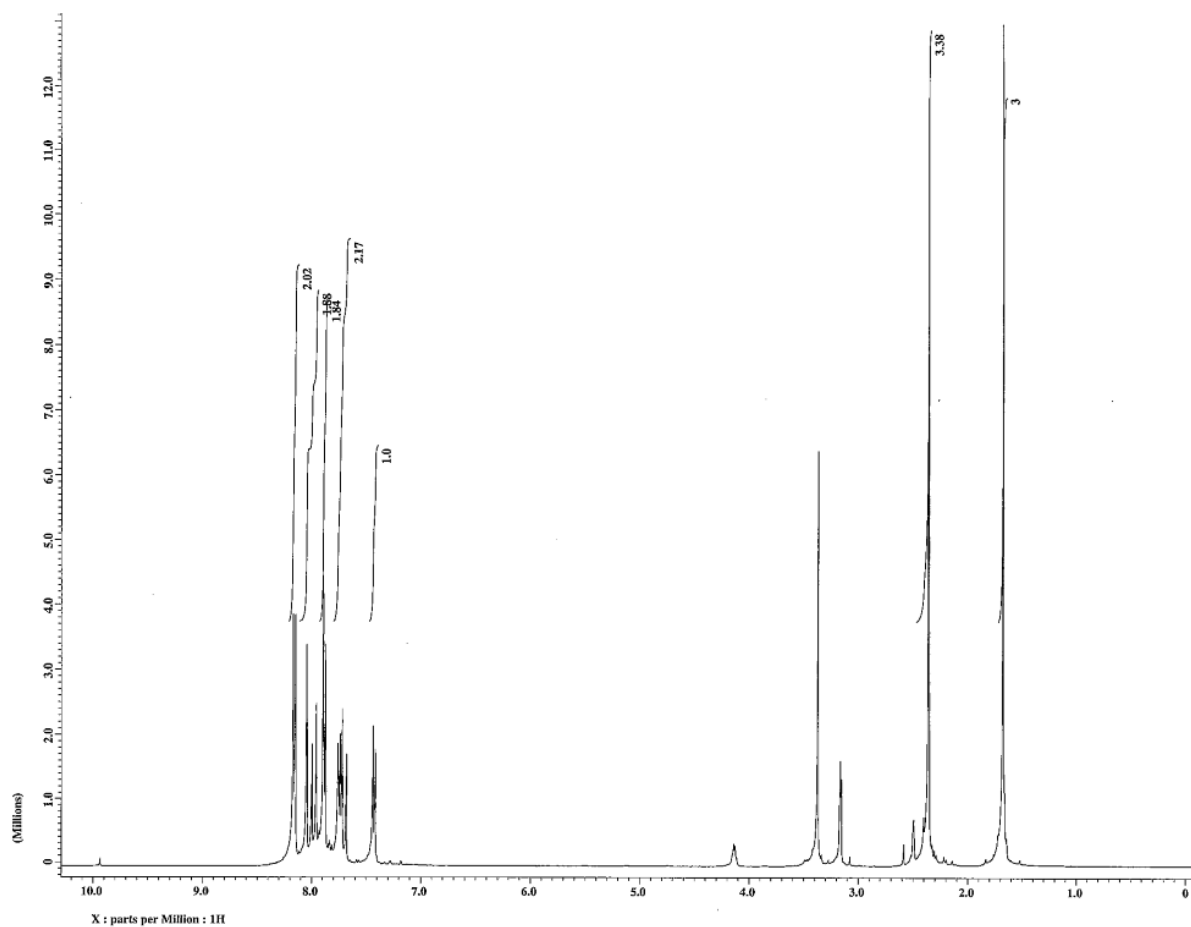


- $\text{SO}_2\text{NH}_2\text{CH}_3\text{F}$

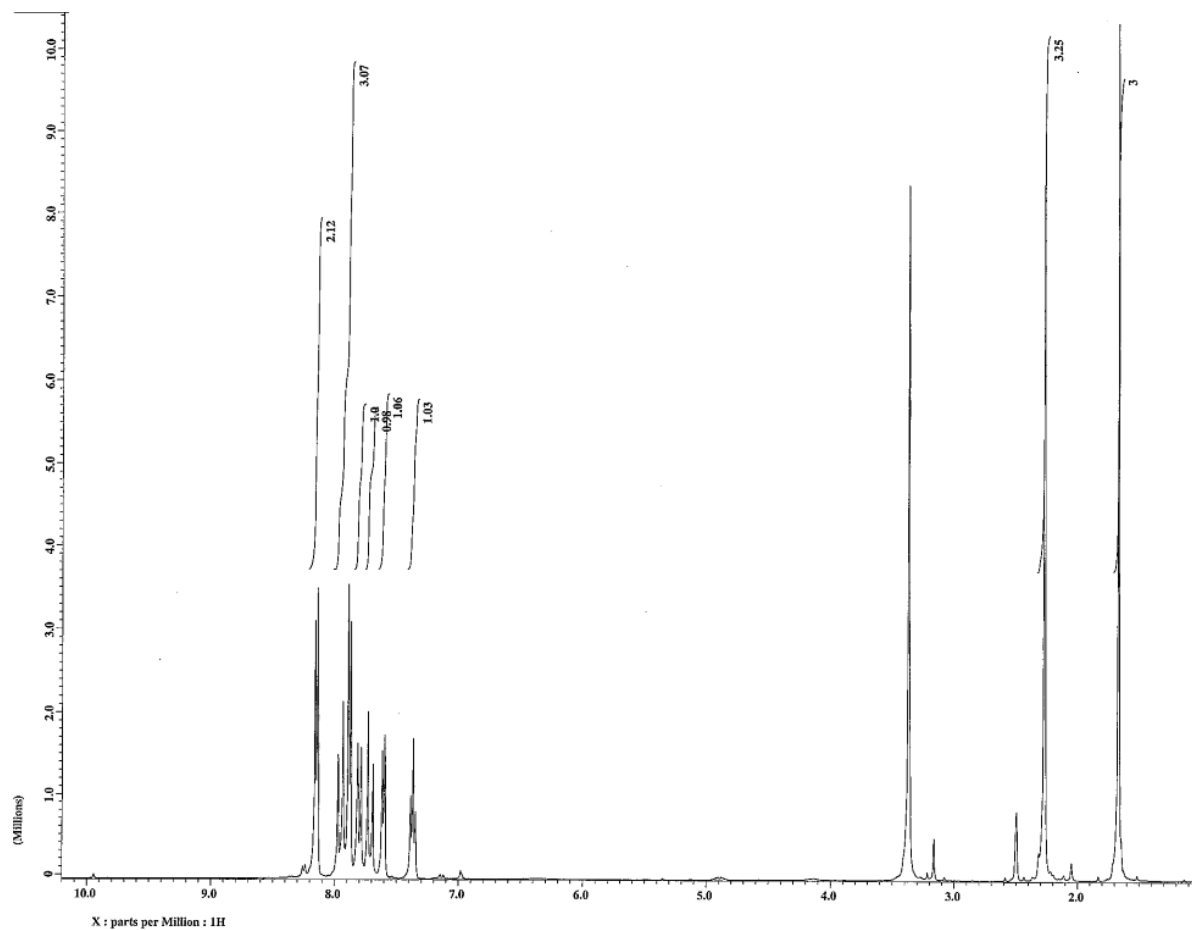


Study # 3- $\text{SO}_2\text{NHCOCH}_3$ family

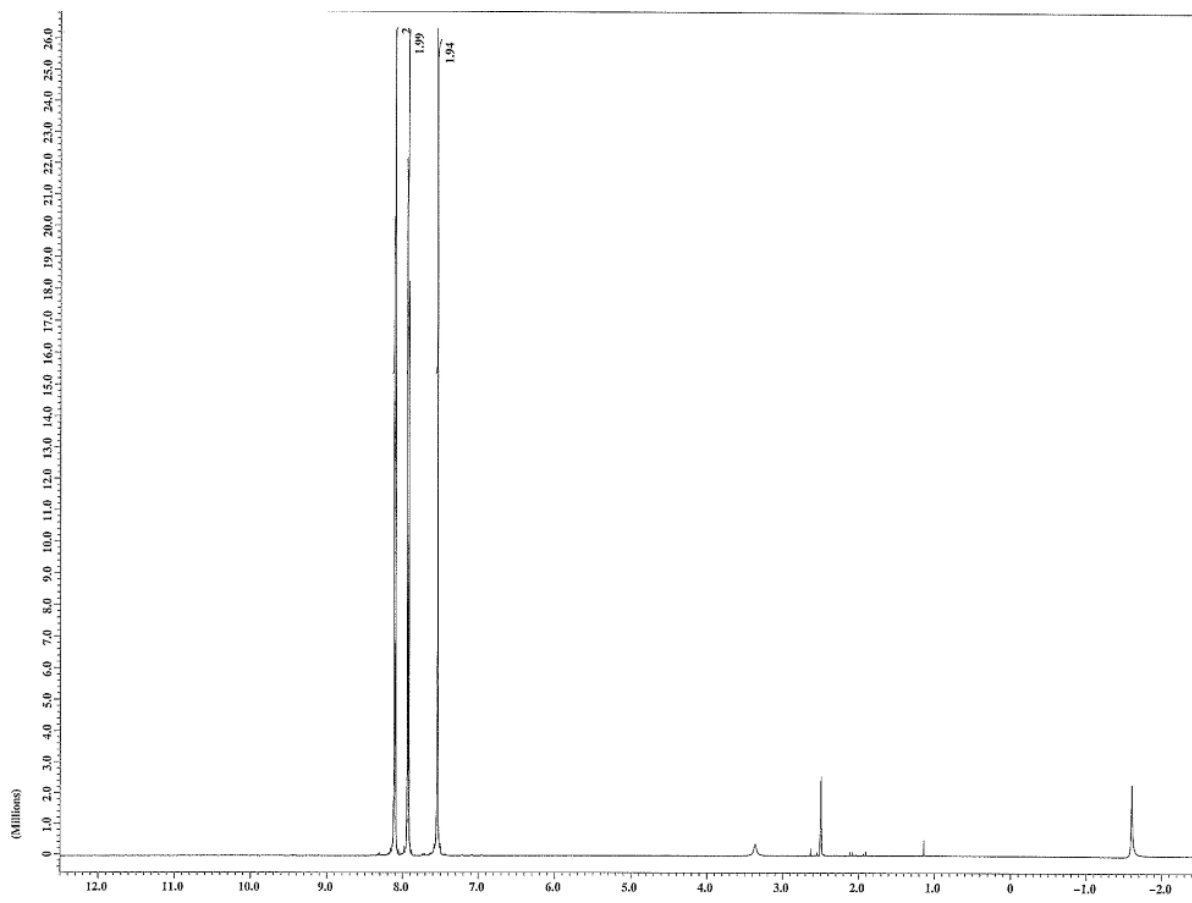
- $\text{SO}_2\text{NHCOCH}_3\text{CH}_3\text{Cl}$



- $\text{SO}_2\text{NHCOCH}_3\text{CH}_3\text{F}$



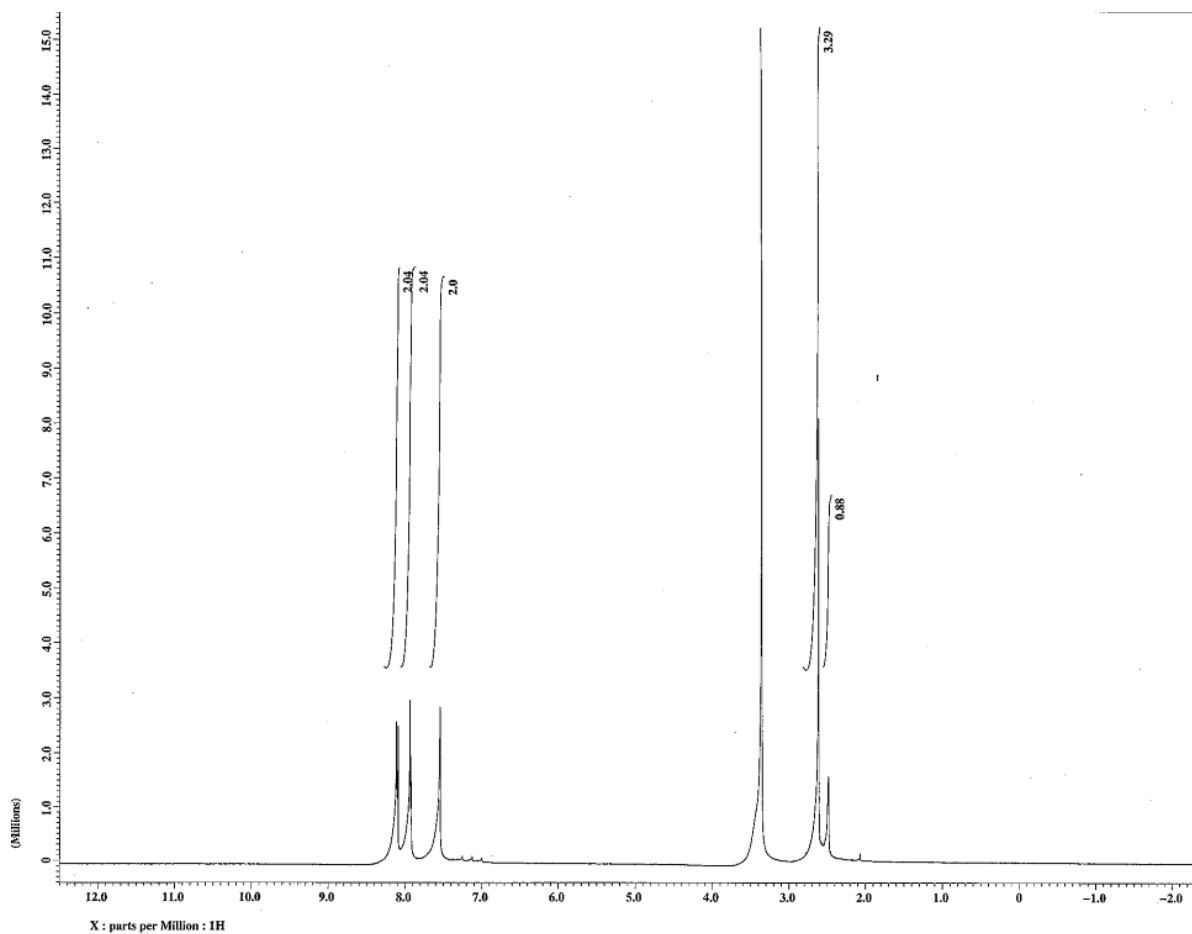
Study # 4- $\text{SO}_2\text{NH}_2\text{COOH}$ family



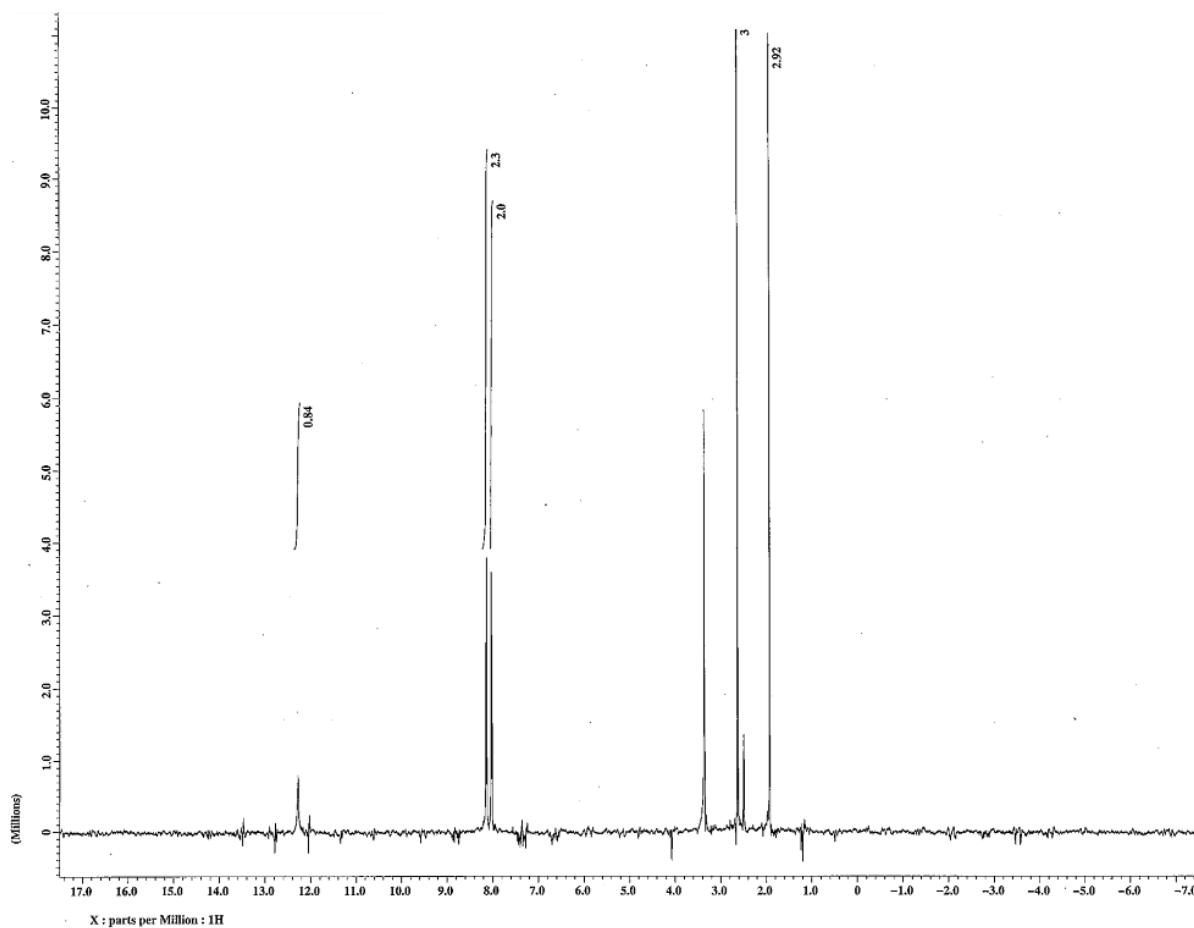
Study # 4- $\text{SO}_2\text{CH}_3\text{COOH}$ family

Intermediates Compounds

- SO_2NH_2



- $\text{SO}_2\text{NHCOCH}_3$

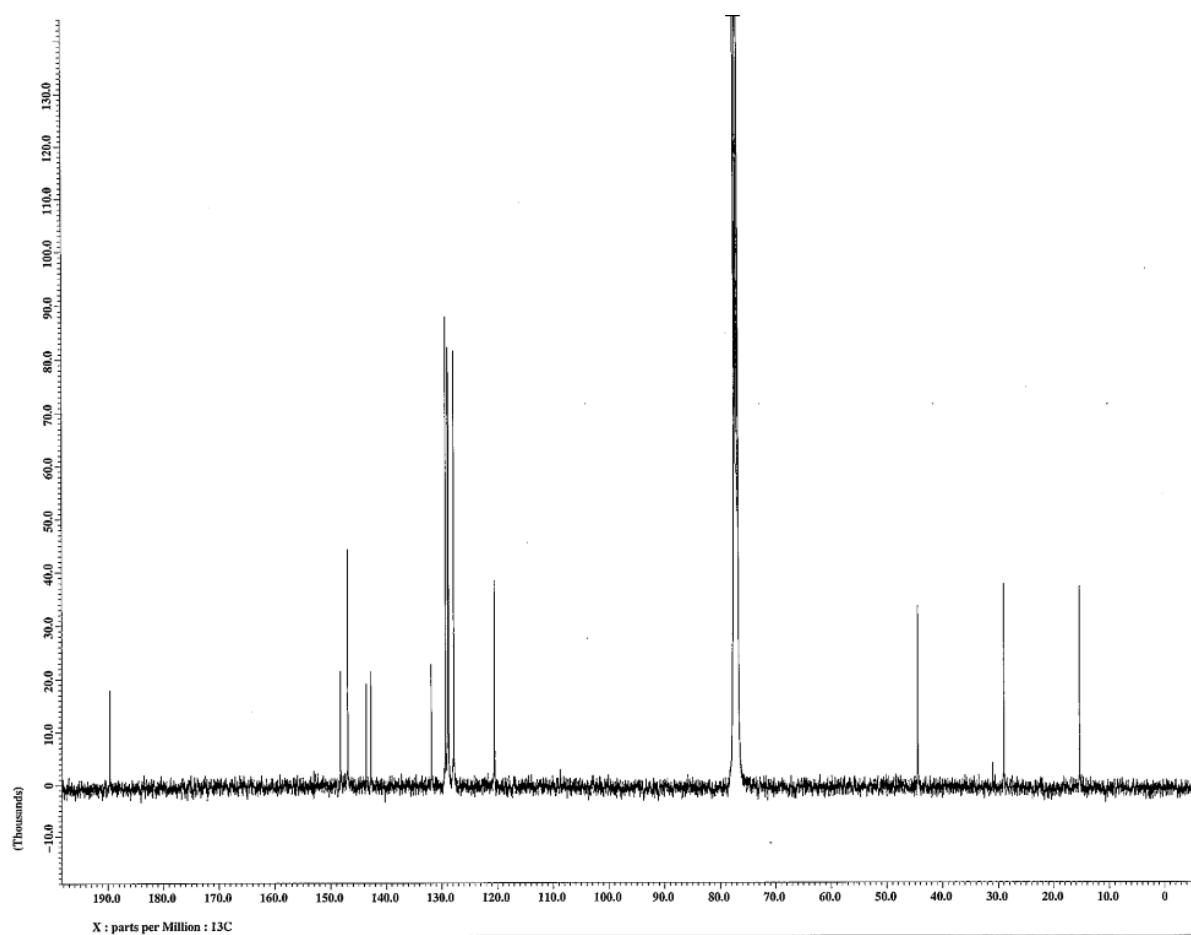


Appendix C

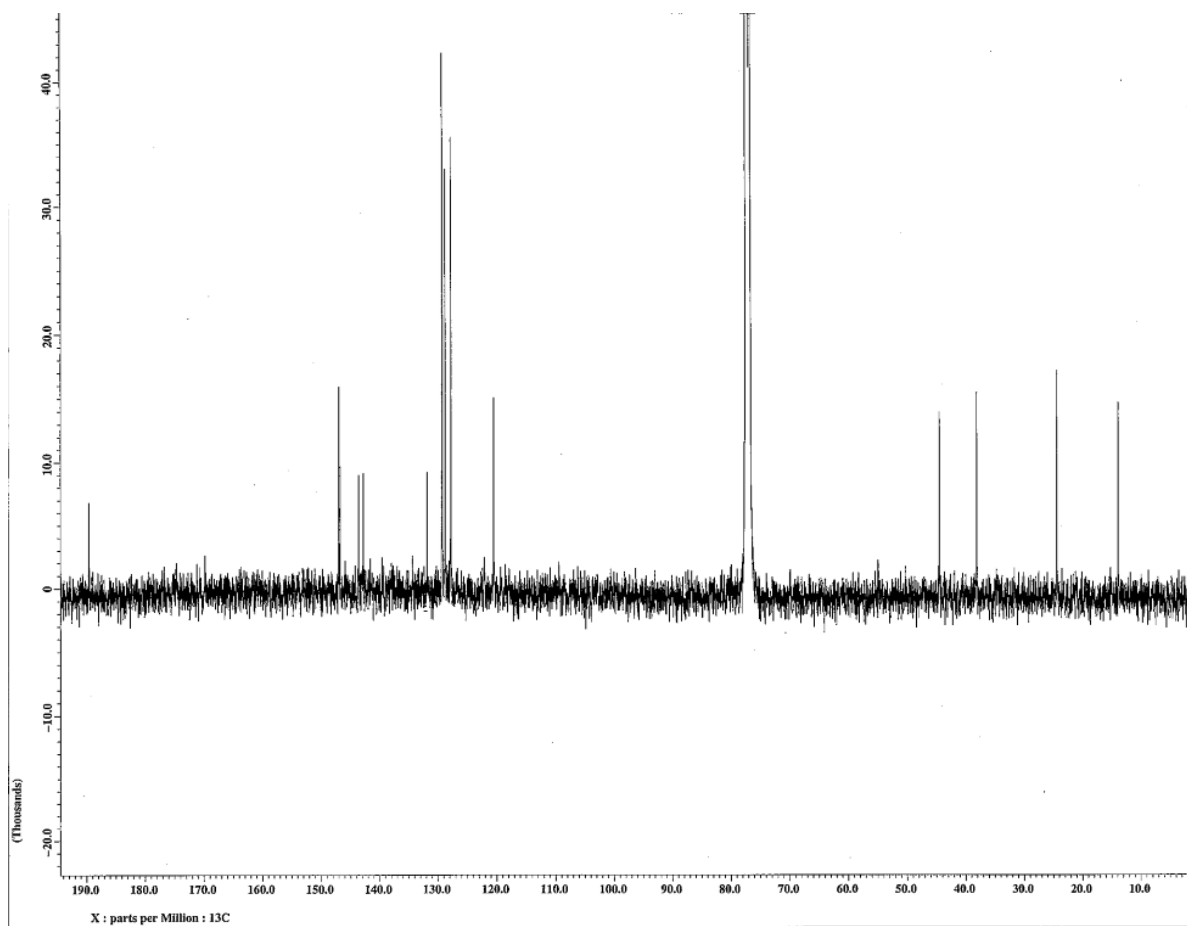
C13 NMR Spectra

Study # 1- SO₂CH₃ family

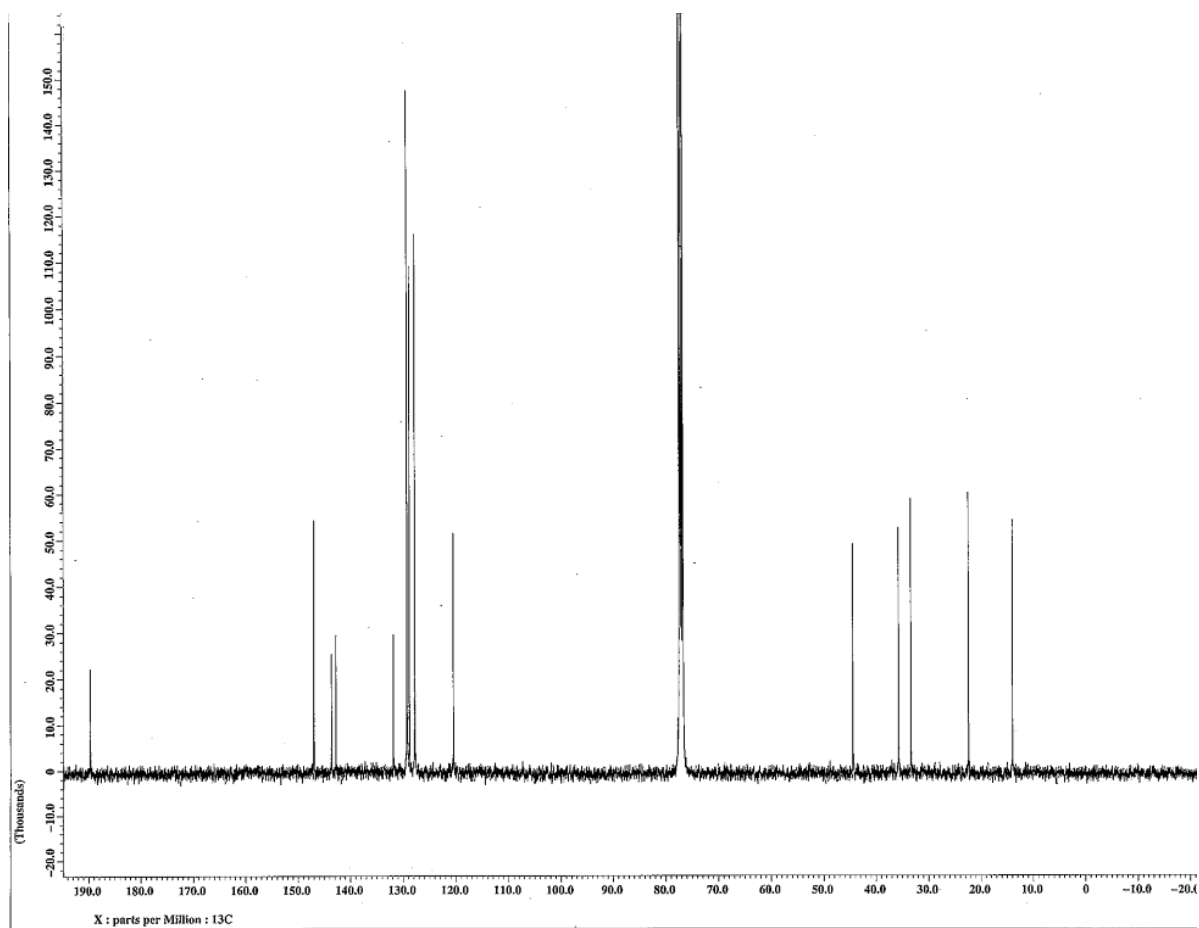
- SO₂CH₃CH₂CH₃



- $\text{SO}_2\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_3$

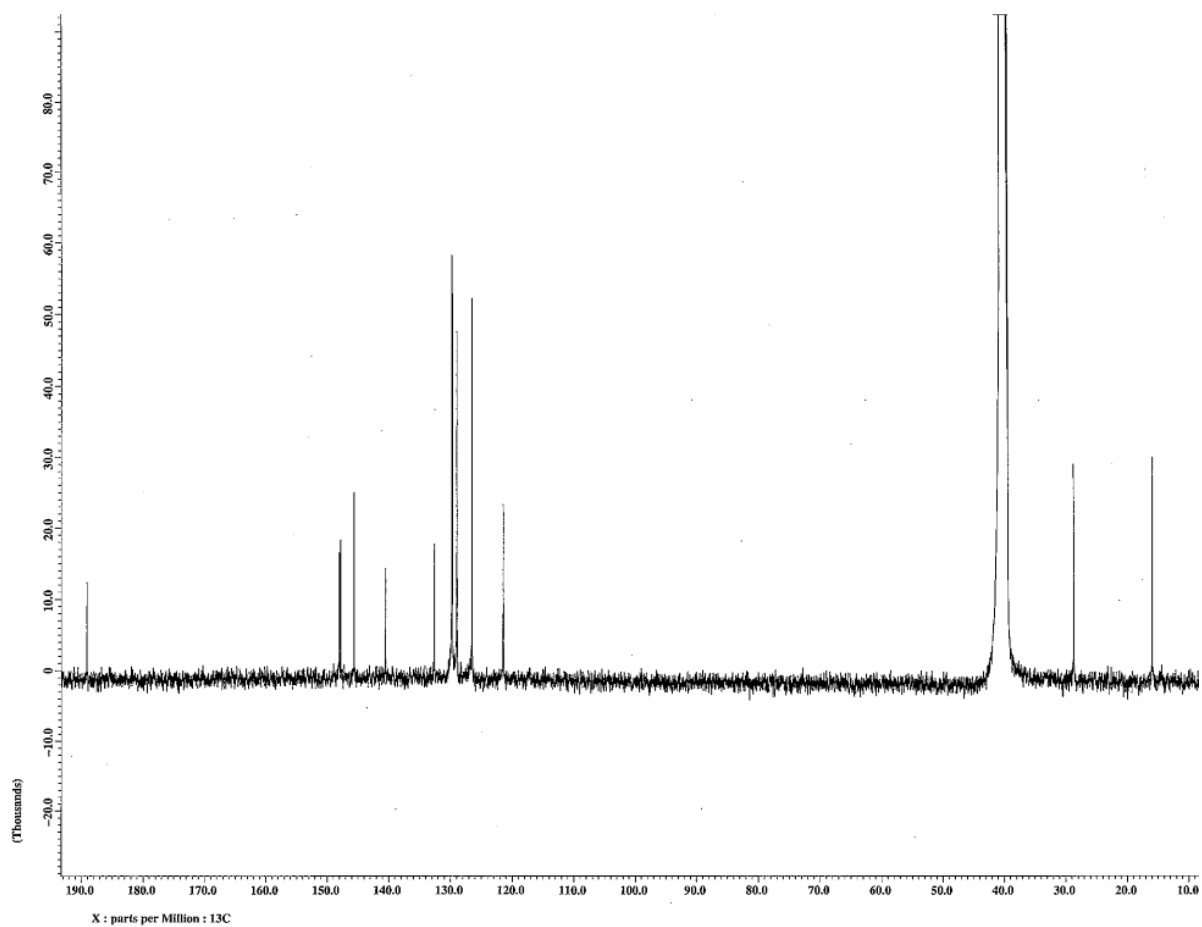


- $\text{SO}_2\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$

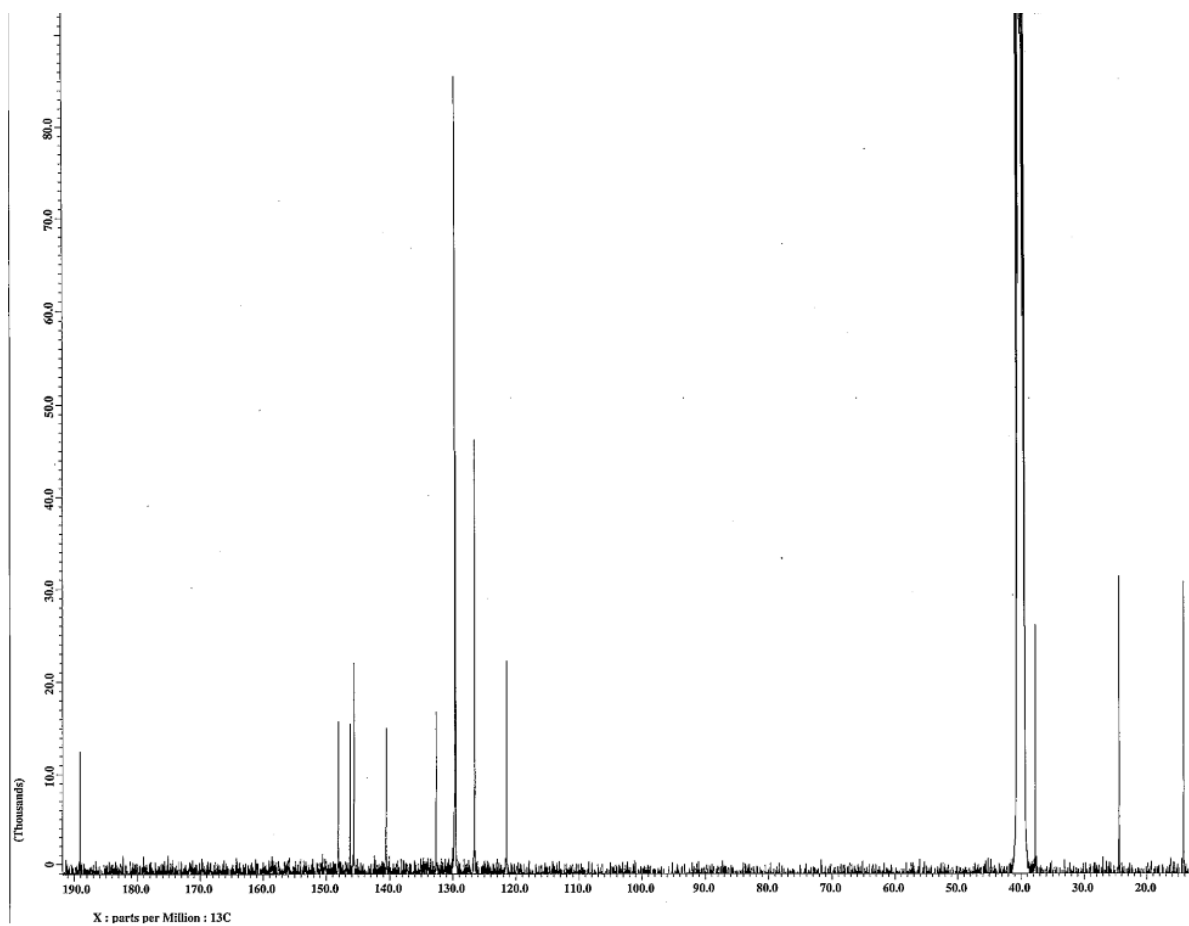


Study # 1- SO₂NH₂ family

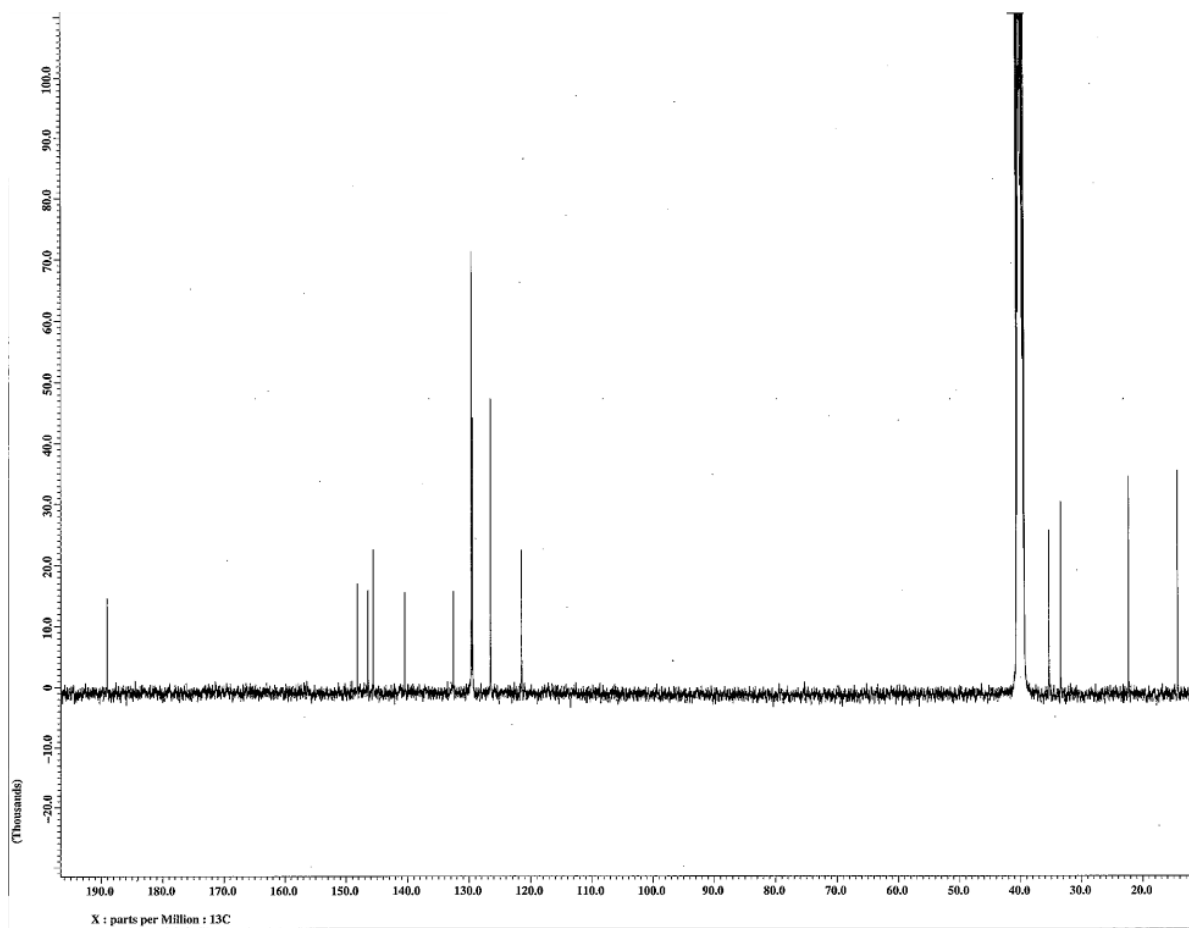
- SO₂NH₂CH₂CH₃



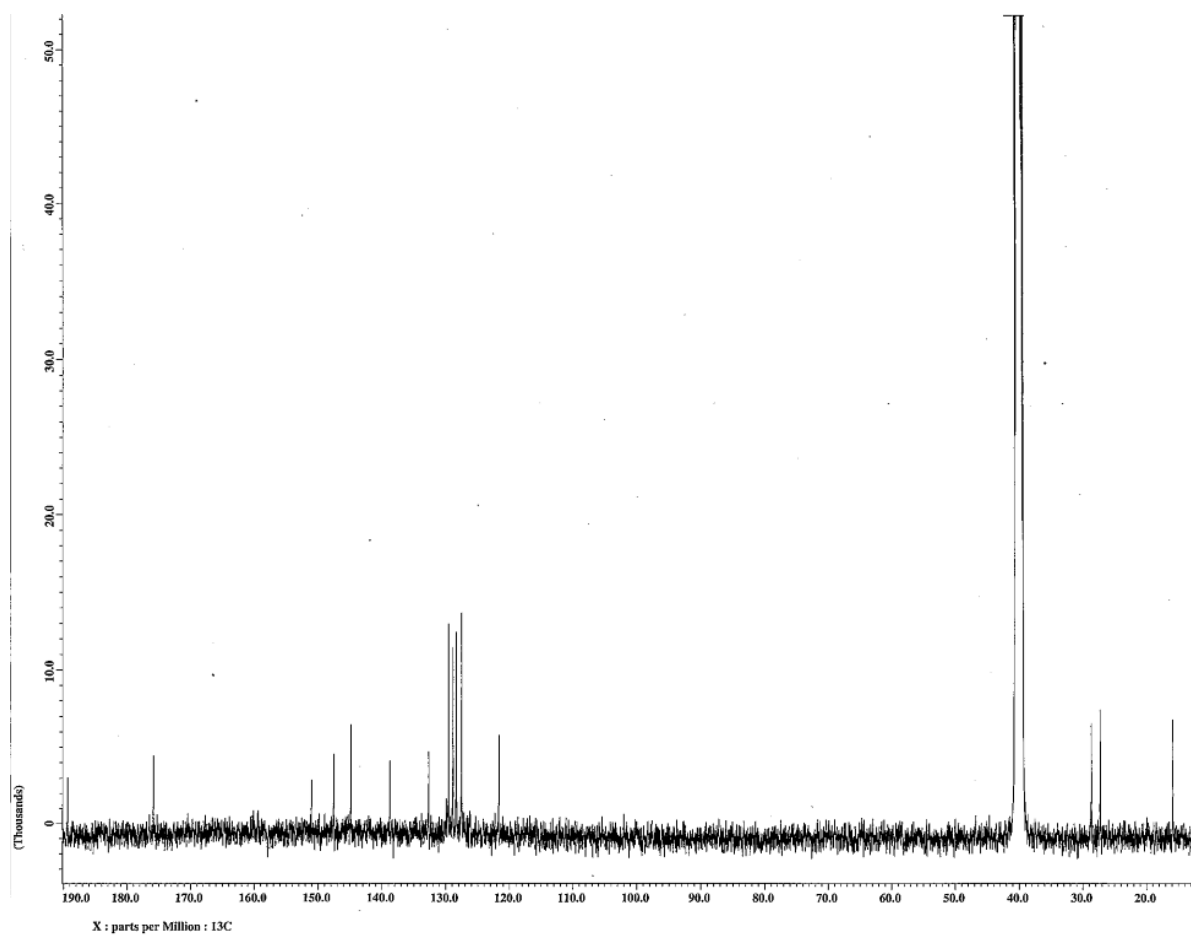
- $\text{SO}_2\text{NH}_2\text{CH}_2\text{CH}_2\text{CH}_3$



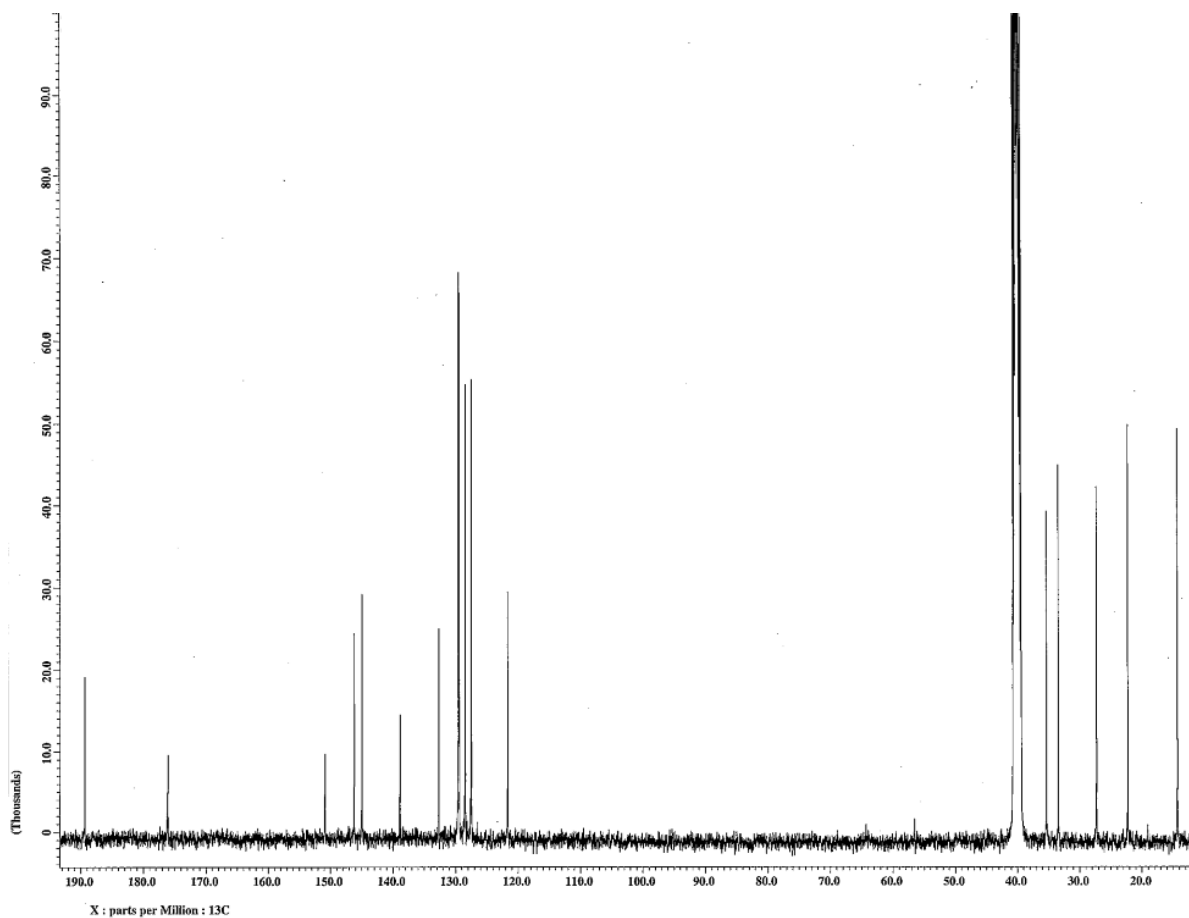
- $\text{SO}_2\text{NH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$



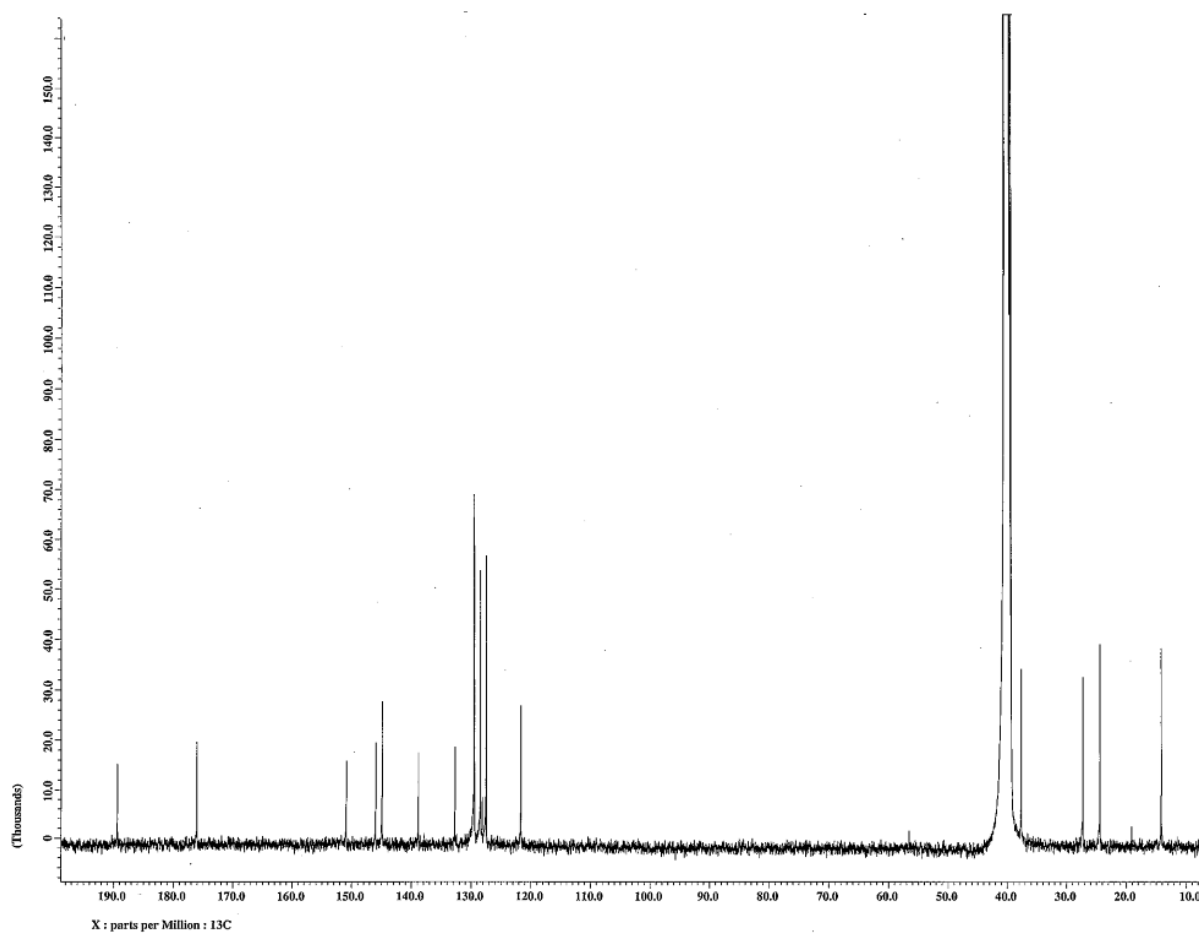
- $\text{SO}_2\text{NHCOCH}_3\text{CH}_2\text{CH}_3$



- $\text{SO}_2\text{NHCOCH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$



- $\text{SO}_2\text{NHCOCH}_3\text{CH}_2\text{CH}_2\text{CH}_3$



Appendix D

Phosphate Buffered Solution (PBS)

A 10X PBS solution was prepared by dissolving 80 grams of NaCl, 2 grams KCl, 21.7 grams of $\text{Na}_2\text{HPO}_4 \cdot 7 \text{H}_2\text{O}$, 2.0 grams KH_2PO_4 and 3.7 grams of EDTA in one liter of milli q water. The pH of the solution is adjusted to 7.4 using NH_4OH and HCl, then the solution was sterilized in an autoclave machine.

After the solution is sterilized, a new solution is prepared with a concentration of 1X PBS by diluting the 10X PBS solution. The pH of the resulting solution is adjusted to 7.4 and the solution is sterilized in an autoclave machine.

Appendix E

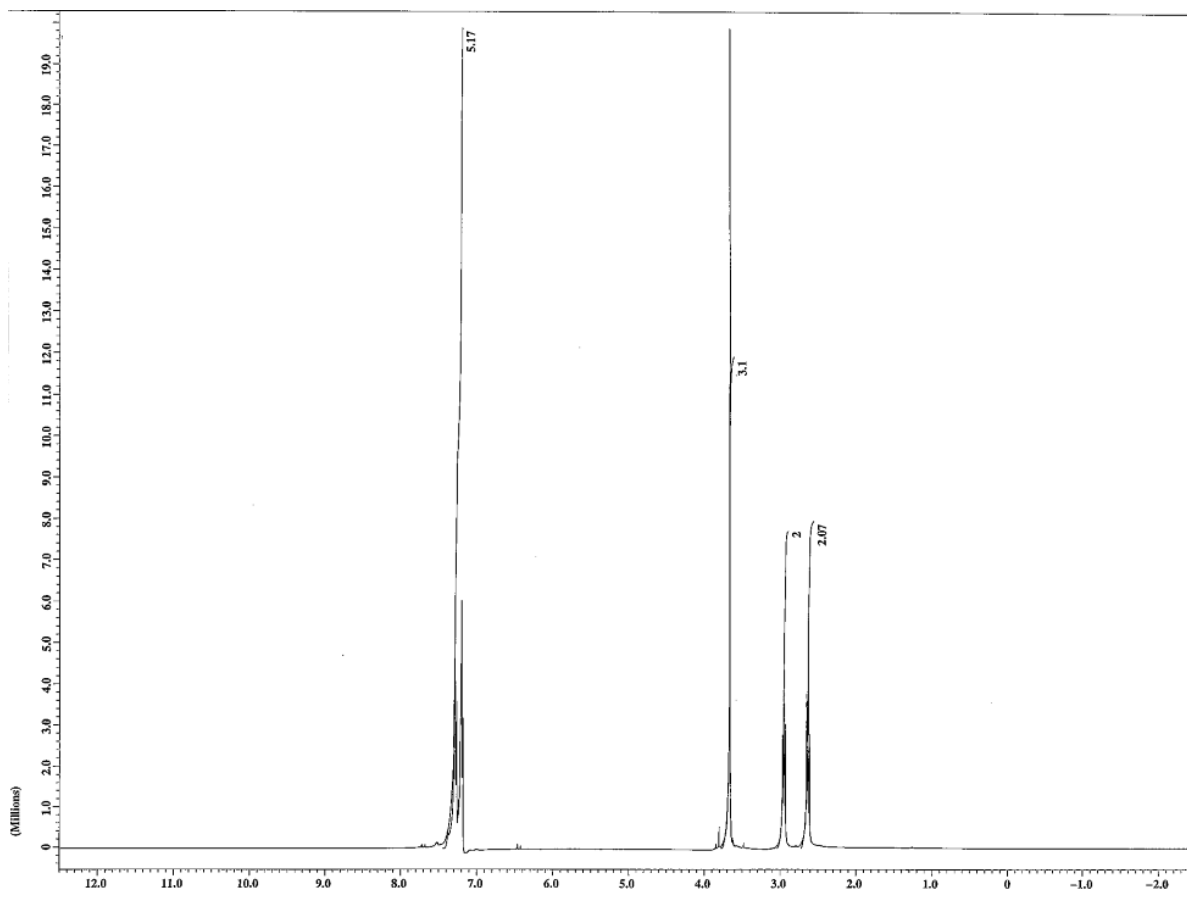
Melting Point Data

Compound	Identification	Study #	milligrams	MW (grams/mol)	Melting Point °C
Sobu	1	1	21.7	342	125
Sonpro	2	1	23.5	329	270
Sonet	3	1	26.4	315	188
Sonbu	4	1	21.8	343	295
Sopro	5	1	22	328	120
Soncet	6	1	20	357	236
Soncebu	7	1	23.7	385	221
Soncepro	8	1	21.3	371	248
Soet	9	1	24.4	314	133
Sonce-OCH3	10	2	22	359	ND
Sonce-Cl	11	2	25	363	>300
Sonce-Fl	12	2	22.5	347	273
Sonce-Ohprotected	13	2	22.5	435	250
SO2NH2-CH3CH3	14	3	20.7	315	>300
SO2NH2-CH3OCH3	15	3	24.7	331	>300
SO2NH2-CH3Cl	16	3	24.7	335	>300
SO2NH2-CH3F	17	3	26.3	319	>300
SO2NHCOCH3CH3Cl	18	3	27.7	377	233
SO2NHCOCH3CH3F	19	3	20.8	361	225
SO2CH3CH3Cl	20	3	25.7	334	158
SO2NHCOCH3CH3CH3	21	3	20.5	357	185
SO2CH3CH3OCH3	22	3	22.2	330	170
SO2CH3CH3CH3	23	3	20	314	155
SO2CH3CH3F	24	3	30	318	159

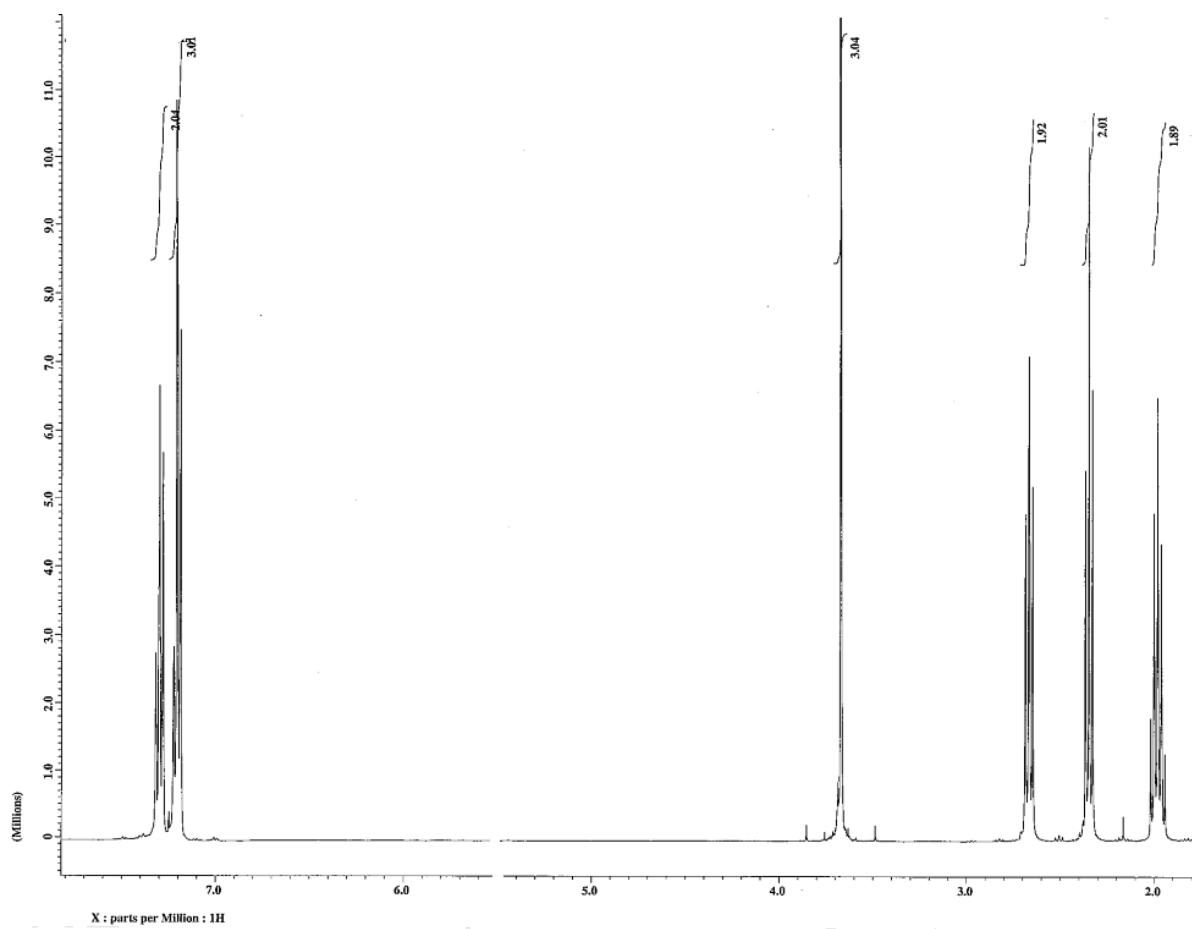
Appendix F

Additional compounds (^1H NMR)

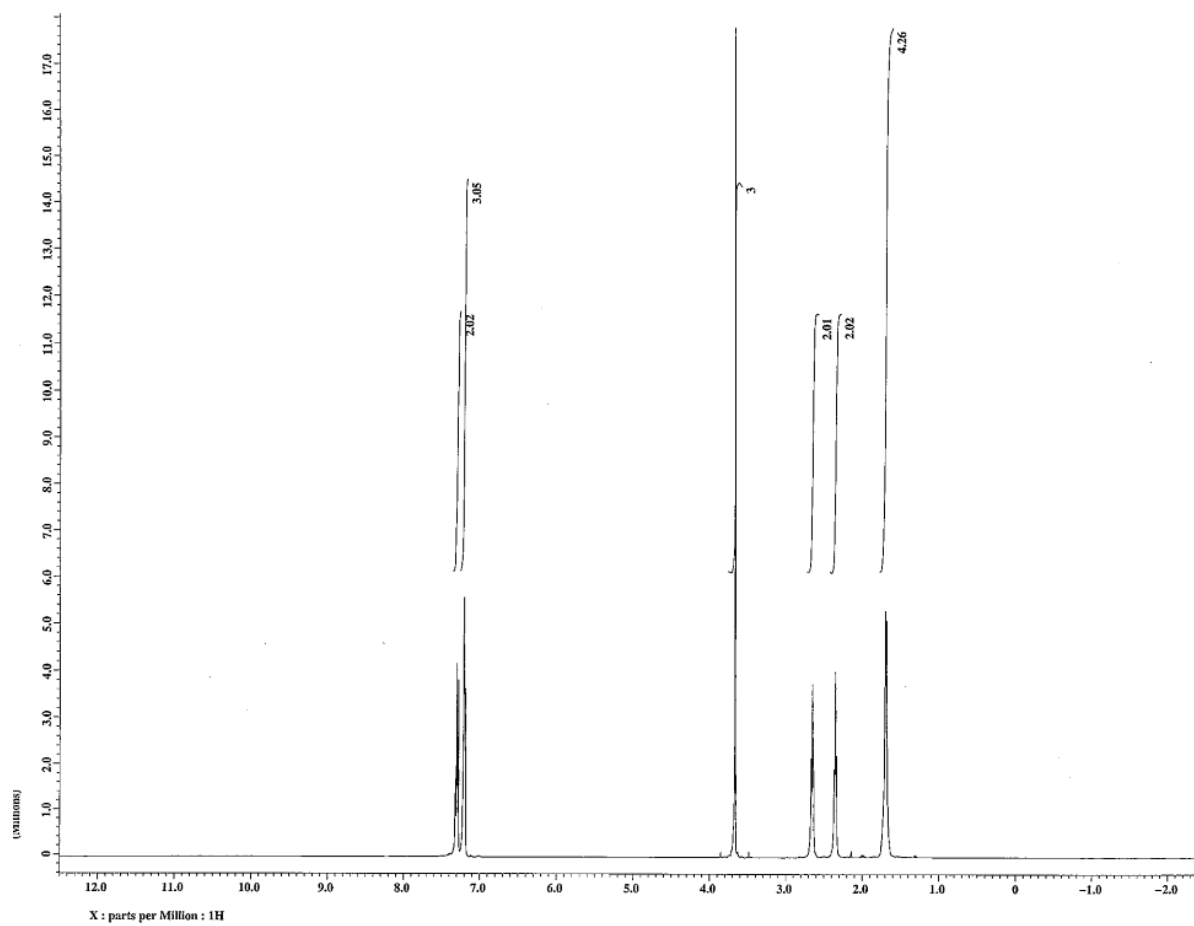
- Methyl phenyl propionate



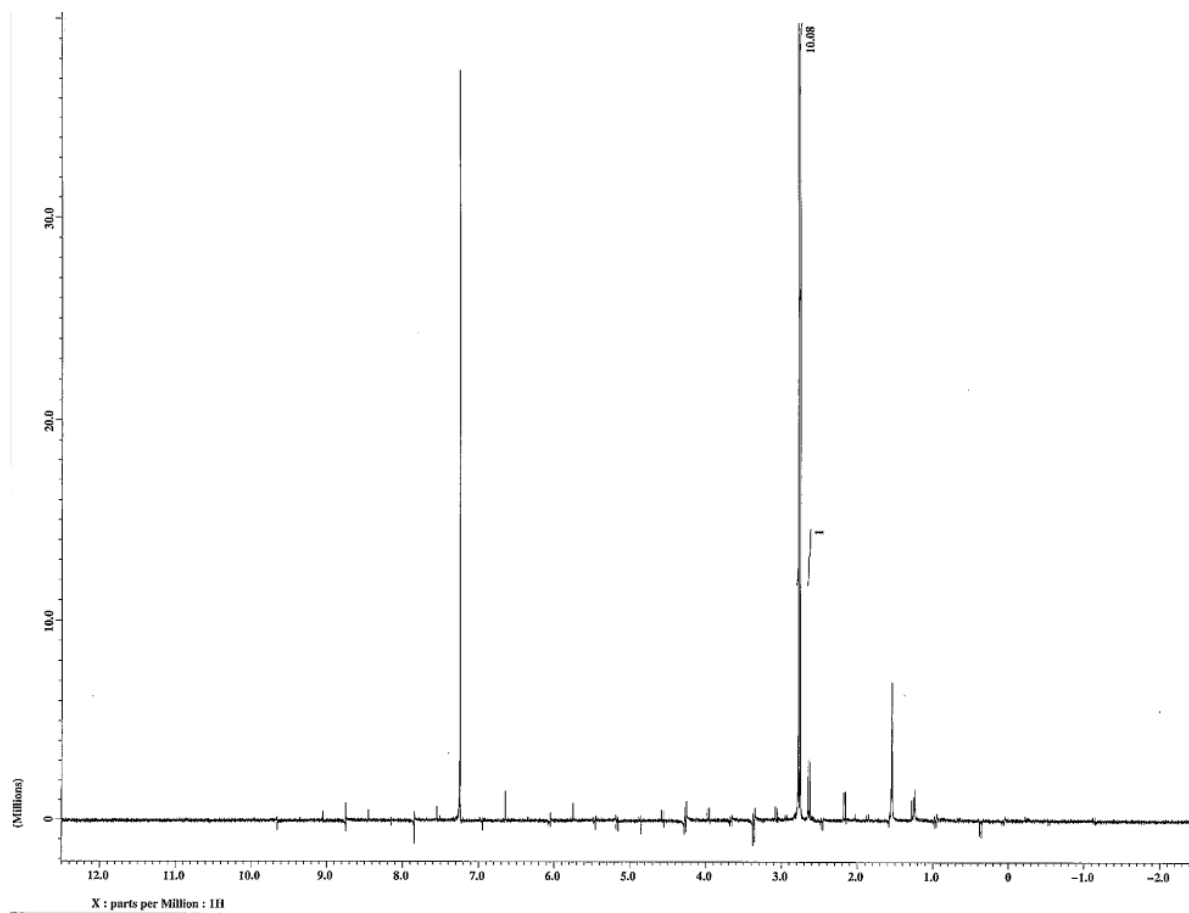
- Methyl phenyl butyrate



- Methyl phenyl valerate



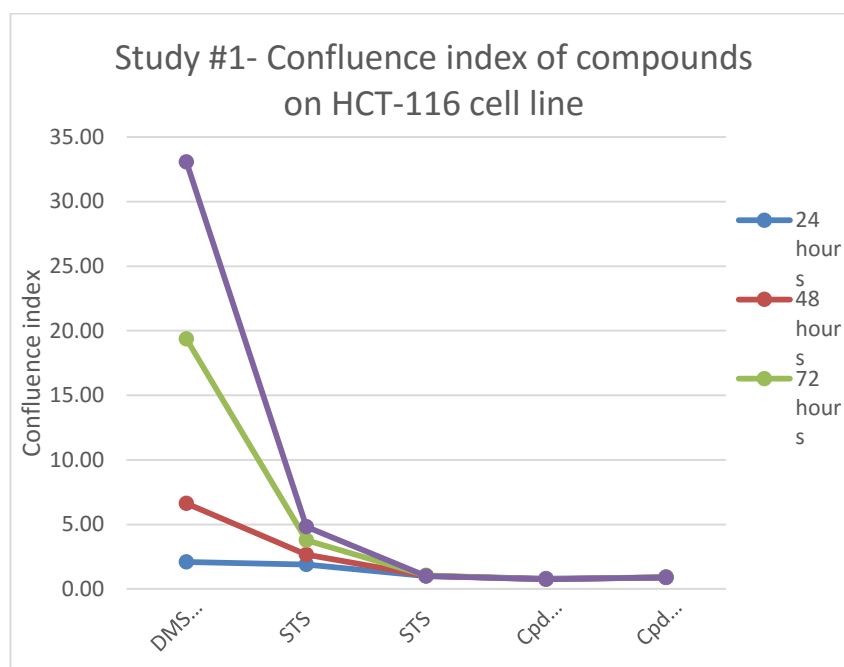
- MoO_5HMPT – Phosphorus NMR

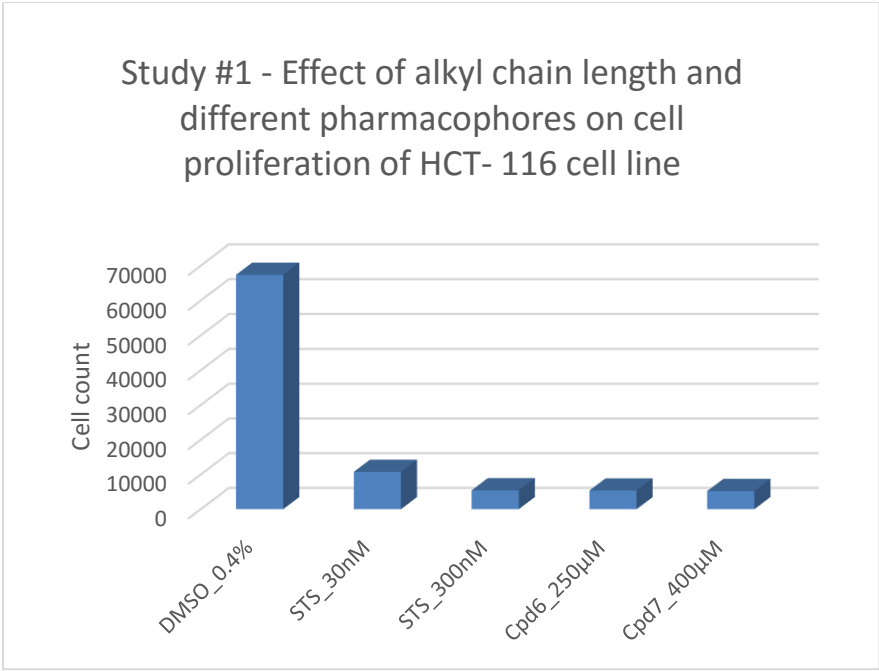


Appendix G

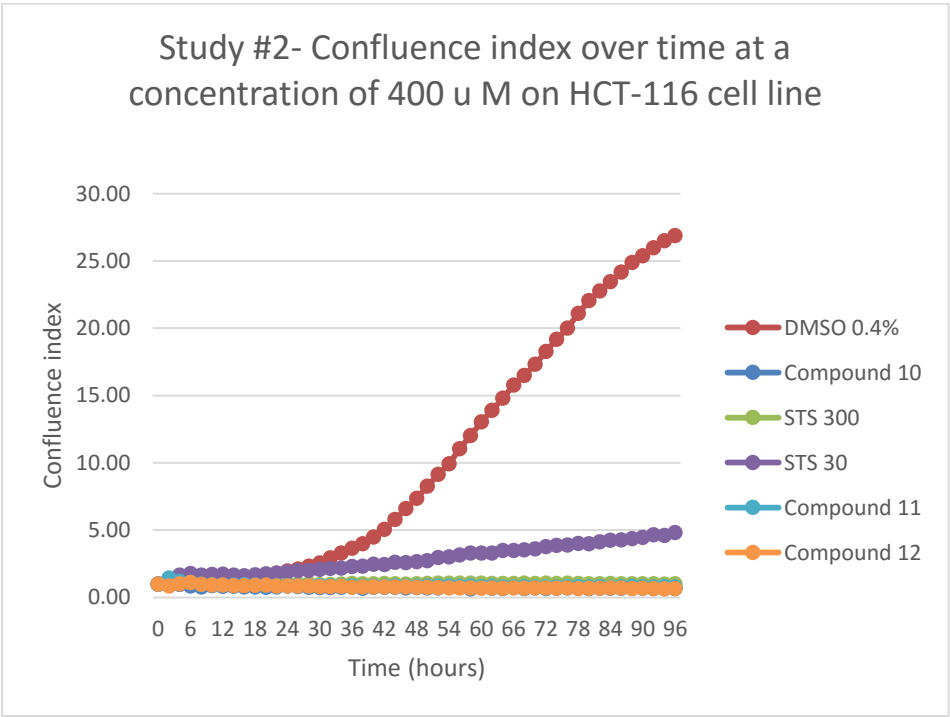
Cell Proliferation and Cytolysis Analysis at High Concentration

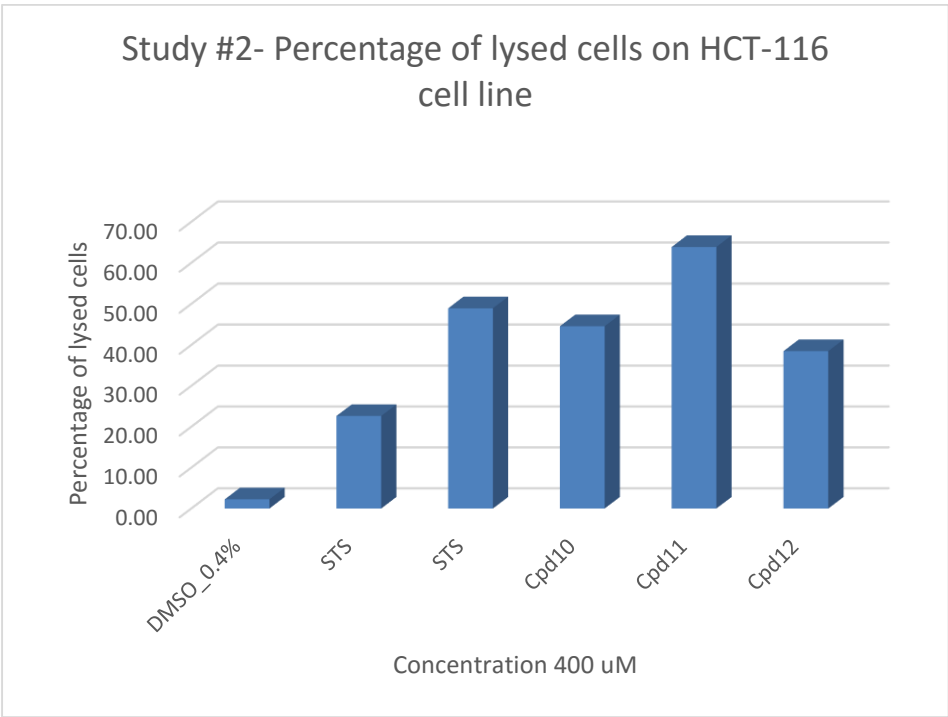
Study # 1



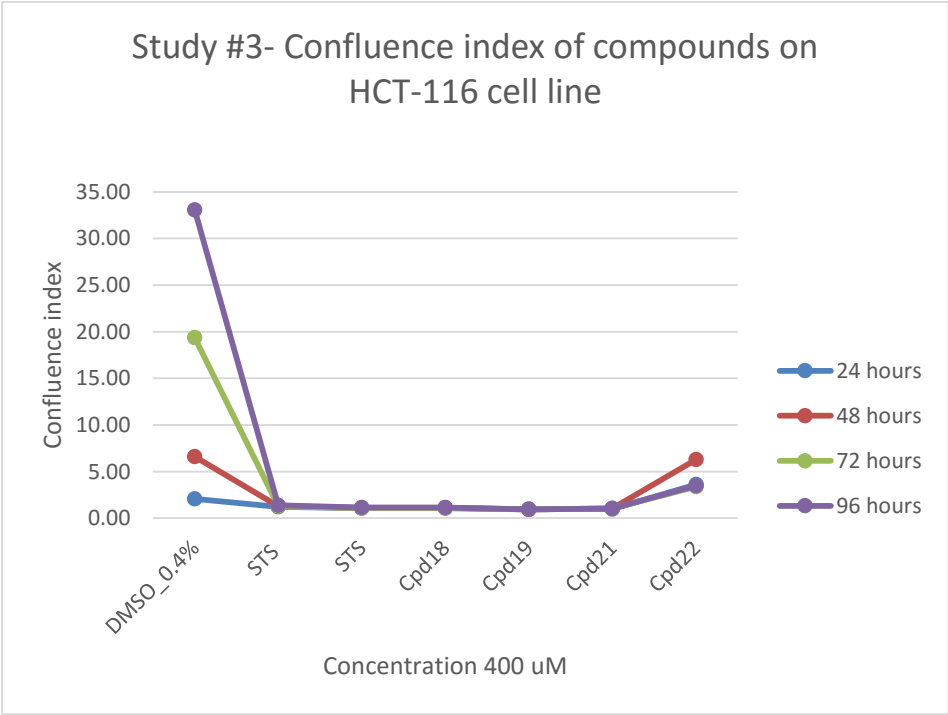


Study # 2

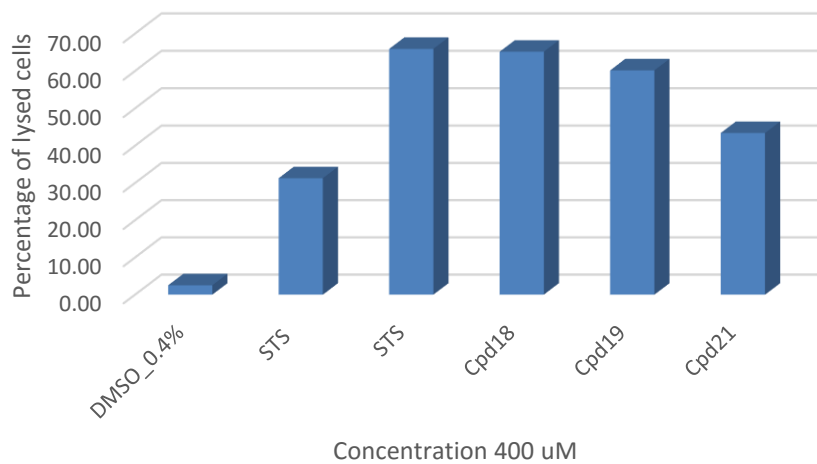




Study # 3



Study #3- Percentage of lysed cells on HCT-116 cell line

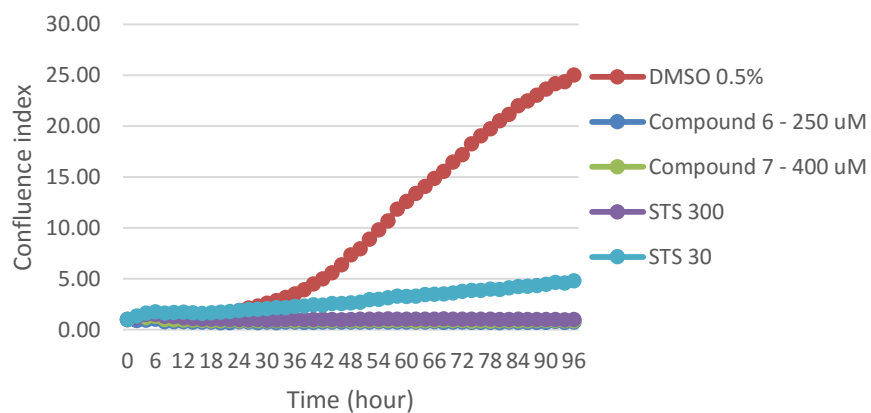


Appendix H

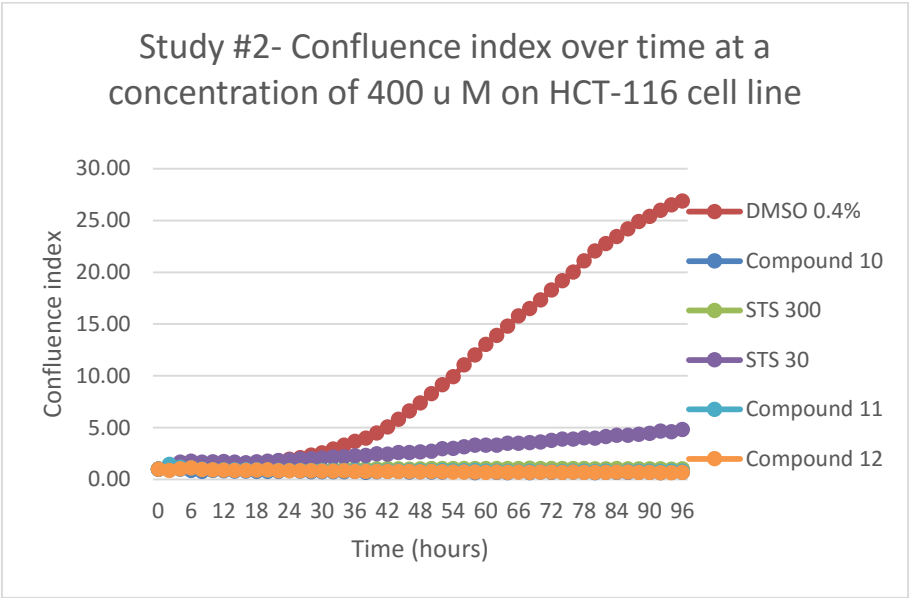
Kinetic Analysis on Cell Proliferation Analysis during 96 hr at High Concentration

Study # 1

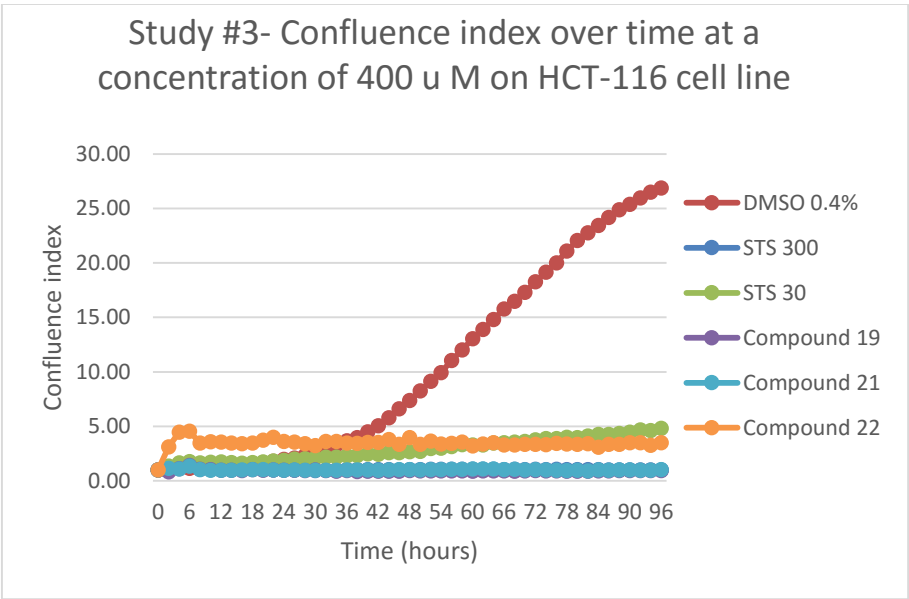
Study #1- Confluence index over time on HCT-116 cell line



Study # 2



Study # 3



Appendix I

Electrostatic Interactions after Docking Analysis of Ligand with COX-2 Enzyme

Study # 1 – X denotes no interaction

Compounds	Polar	Hydrophobic	HB	Halogen	Other
Study 1					
SO ₂ CH ₃ CH ₂ CH ₃	ARG120, GLN192, TYR355	ALA516, HIS90, PHE518, VAL523	X	X	ALA527, SER353, THR94, VAL349
SO ₂ CH ₃ CH ₂ CH ₂ CH ₃	ARG120, GLN192, TYR355	ALA516, HIS90, PHE518, VAL523	X	X	ALA527, SER353, THR94, VAL349
SO ₂ CH ₃ CH ₂ CH ₂ CH ₂ CH ₃	X	ALA527, ILE112, LEU352, LEU359, LEU531, LEU93, TRP100, TYR115, TYR355, VAL116, VAL349	X	X	ARG120, SER353, VAL523
SO ₂ NH ₂ CH ₂ CH ₃	X	ALA527, ILE112, LEU359, LEU93, TYR355, VAL116, VAL349	MET522, VAL523	X	ARG120, LEU352, LEU531, PHE518
SO ₂ NH ₂ CH ₂ CH ₂ CH ₃	X	ALA527, ILE112, LEU359, LEU531, TYR115, TYR355, VAL116, VAL349	MET522, VAL523	X	ARG120, PHE518
SO ₂ NH ₂ CH ₂ CH ₂ CH ₂ CH ₃	X	ALA527, ILE112, LEU359, LEU93, TYR355, VAL116, VAL349	MET522, VAL523	X	ARG120, LEU531, PHE518
SO ₂ NHCOCH ₃ CH ₂ CH ₃	ARG120, GLN192, TYR355	ALA516, HIS90, LEU359, PHE518, VAL349, VAL523	X	X	ALA527, LEU531, SER353
SO ₂ NHCOCH ₃ CH ₂ CH ₂ CH ₃	X	ALA527, ILE112, LEU352, LEU359, LEU531, LEU93, TYR355, TYR385, VAL116, VAL349	X	X	ARG120, SER353, VAL523
SO ₂ NHCOCH ₃ CH ₂ CH ₂ CH ₂ CH ₃	X	ALA527, ILE112, LEU352, LEU359, LEU531, LEU93, TRP100, TYR115, TYR355, VAL116, VAL349	X	X	ARG120, SER353, VAL523

Study # 2 – X denotes no interaction

Compounds	Polar	Hydrophobic	HB	Halogen	Other
Study 2					
SO2NHCOCH3Cl	X	TYR55	X	PRO35	GLU67, GLN54, LYS56
SO2NHCOCH3F	SER38	CYS37, TYR55	PRO35	GLU67	LYS56, VAL165
SO2NHCOCH3OCH3	SER38	PRO35, TYR55	X	X	GLU67, VAL165
SO2NHCOCH3OBENZYL	TYR55, ASN68, LYS468, GLN42, GLU465	PRO40, VAL165	X	X	SER38

Study # 3 – X denotes no interaction

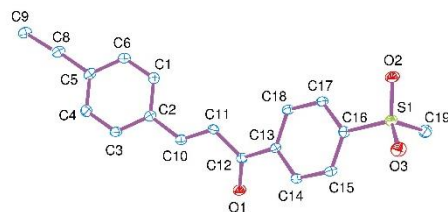
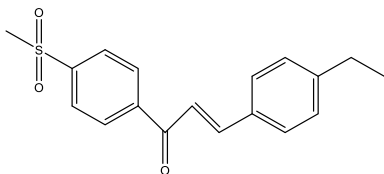
Compounds	Polar	Hydrophobic	HB	Halogen	Other
Study 3					
SO2CH3CH3CH3	X	PRO35, TYR55	X	X	GLN54, LYS56, GLU67, SER38
SO2CH3CH3Cl	X	TYR55, PRO35	X	ALA33	GLN54, LYS56, GLU67, SER38
SO2CH3CH3F	X	TYR55, PRO35	SER38	GLU67	GLN54, CYS57
SO2CH3OCH3CH3	X	PRO35, CYS37, TYR55	X	X	GLN54, LYS56, GLU67, SER38
SO2NH2CH3CH3	SER38		PRO35	X	TYR55, LYS56, GLN54, GLU67
SO2NH2CH3Cl	X	PRO35	TYR55	ALA33	GLN54, GLU67, SER38, LYS56
SO2NH2CH3F	TYR55, ASN68	PRO35	GLU67	X	GLN54, LYS56
SO2NH2OCH3CH3	X	X	PRO35, SER38	X	TYR55, GLU67
SO2NHCOCH3CH3CH3	X	PRO35, TYR55	X	X	LYS56, GLU67, GLN54, SER38
SO2NHCOCH3CH3Cl	X	TYR55	PRO35, SER38	X	GLU67, GLN54
SO2NHCOCH3CH3F	X	TYR55	PRO35, SER38	GLU67	LYS56, GLN54, CYS57
SO2NHCOCH3CH3OCH3	SER38	TYR55, PRO35	X	X	LYS56, GLU67, CYS37, VAL165

Appendix J

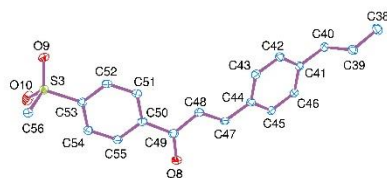
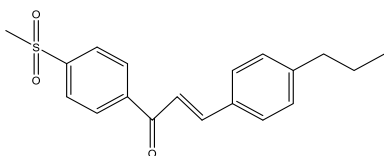
Crystallography

Study #1

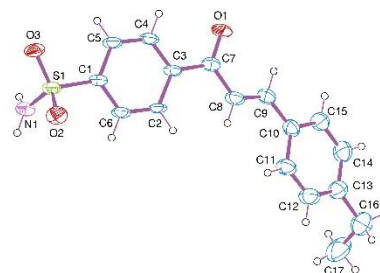
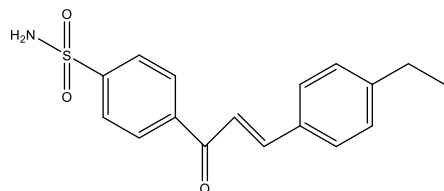
- $\text{SO}_2\text{CH}_3\text{CH}_2\text{CH}_3$



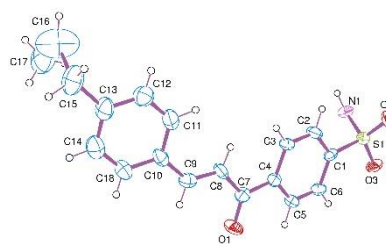
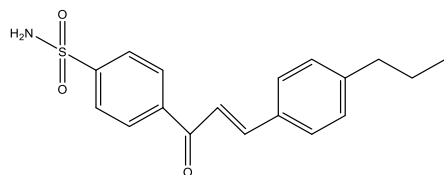
- $\text{SO}_2\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_3$



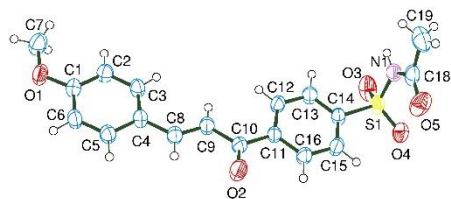
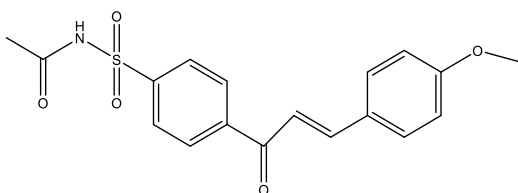
- $\text{SO}_2\text{NH}_2\text{CH}_2\text{CH}_3$



- $\text{SO}_2\text{NH}_2\text{CH}_2\text{CH}_2\text{CH}_3$

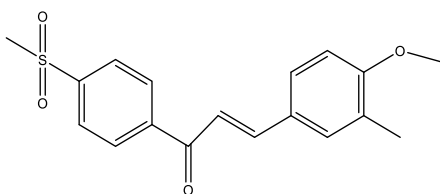
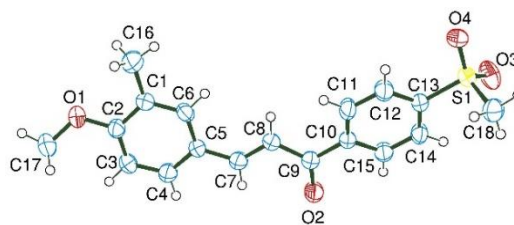


Study #2

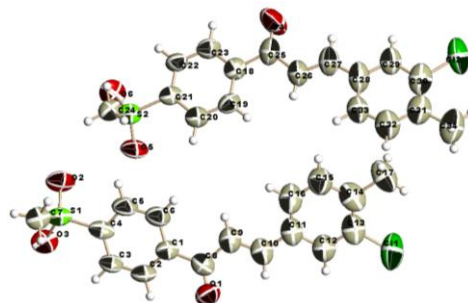
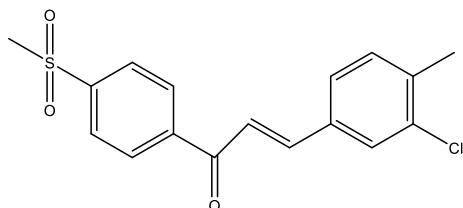


Study #3

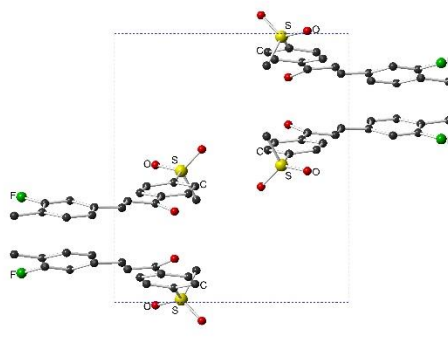
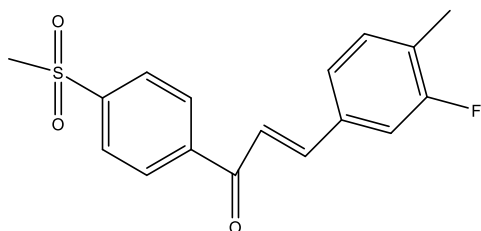
- $\text{SO}_2\text{CH}_3\text{CH}_3\text{OCH}_3$



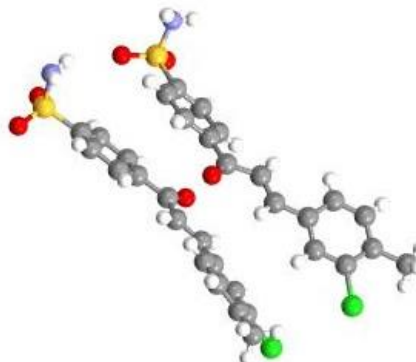
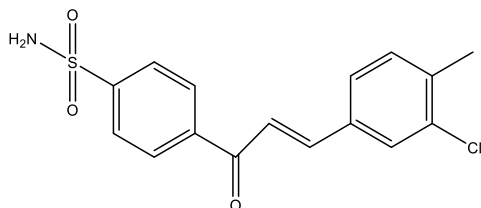
- $\text{SO}_2\text{CH}_3\text{CH}_3\text{Cl}$



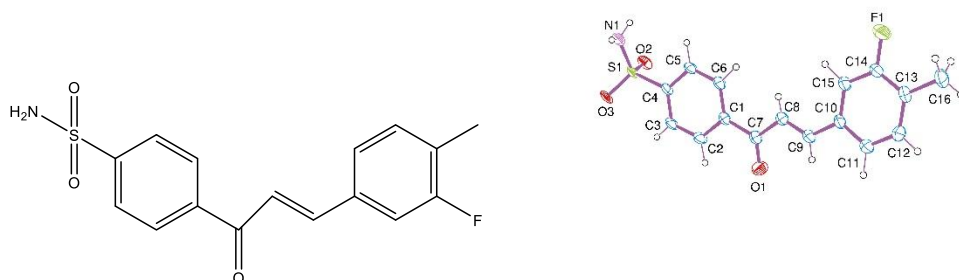
- $\text{SO}_2\text{CH}_3\text{CH}_3\text{F}$



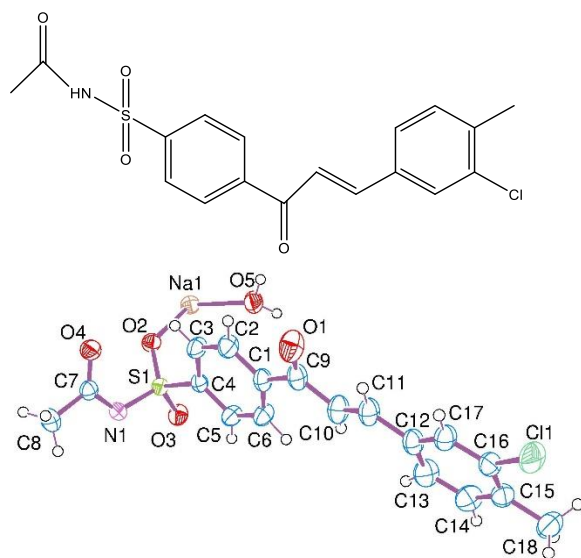
- $\text{SO}_2\text{NH}_2\text{CH}_3\text{Cl}$



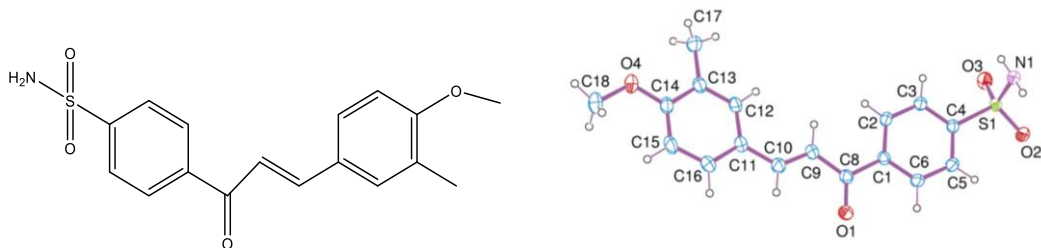
- $\text{SO}_2\text{NH}_2\text{CH}_3\text{F}$



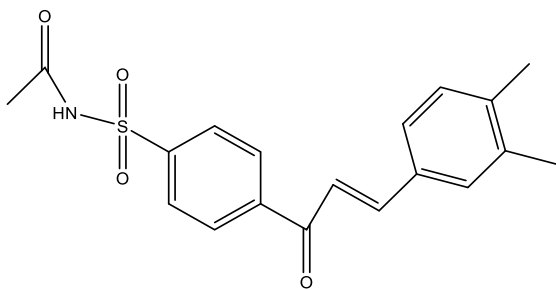
- $\text{SO}_2\text{NHCOCH}_3\text{CH}_3\text{Cl}$



- $\text{SO}_2\text{NH}_2\text{CH}_3\text{OCH}_3$

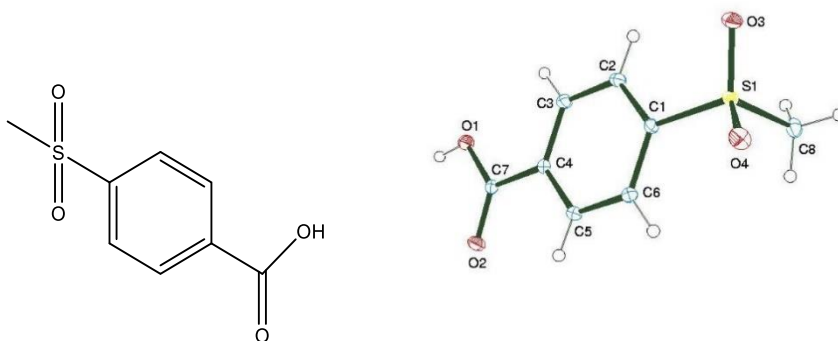


- $\text{SO}_2\text{NHCOCH}_3\text{CH}_3\text{CH}_3$

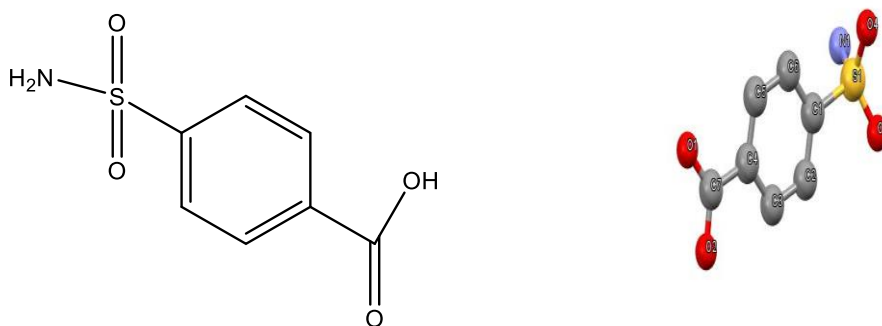


Study #4

- $\text{SO}_2\text{CH}_3\text{COOH}$

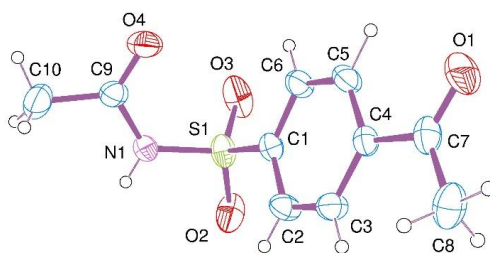
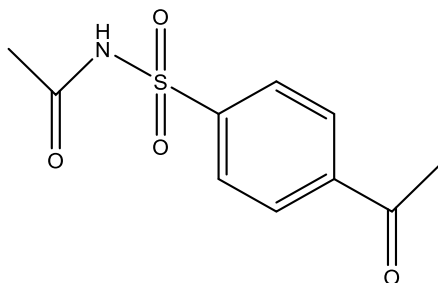


- $\text{SO}_2\text{NH}_2\text{COOH}$

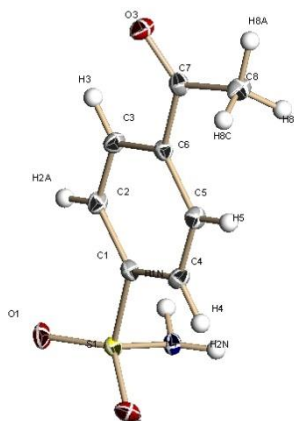
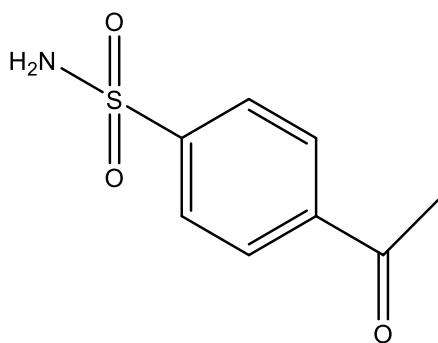


Intermediates

- $\text{SO}_2\text{NHCOCH}_3$



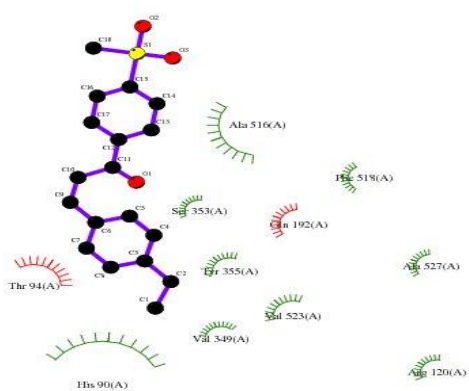
- SO_2NH_2



Appendix K
Docking Modeling Images

Study #1

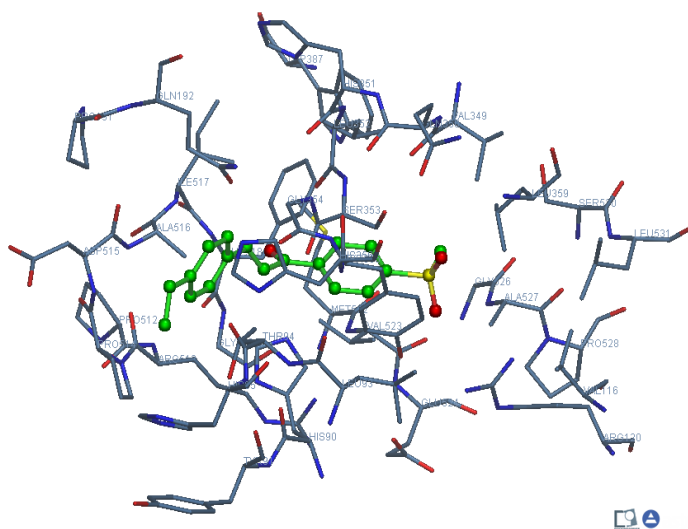
- $\text{SO}_2\text{CH}_3\text{CH}_2\text{CHS}_3$



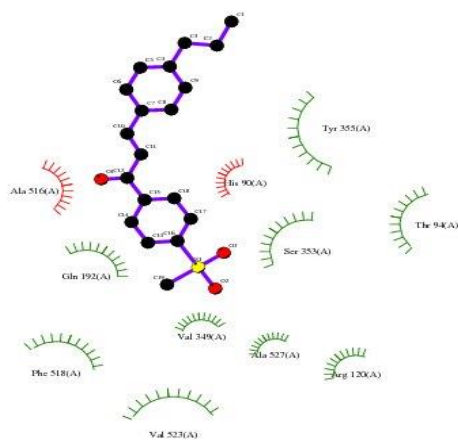
Key

- Ligand bond
- Non-ligand bond
- Hydrogen bond and its length
- (U.S.) Non-ligand residues involved in other contact(s)

docking



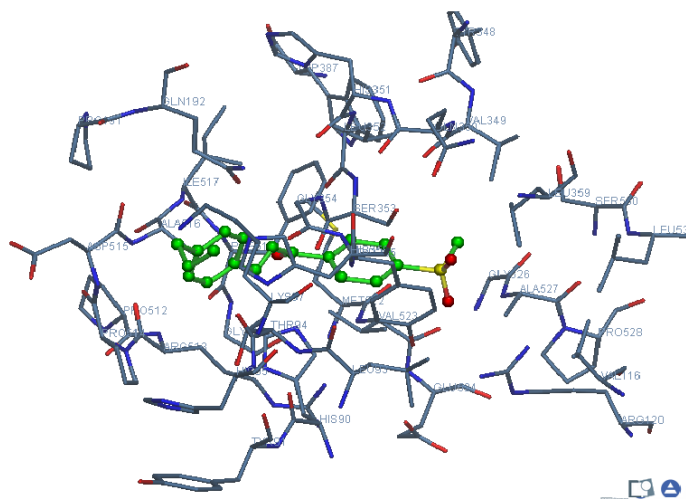
- $\text{SO}_2\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_3$



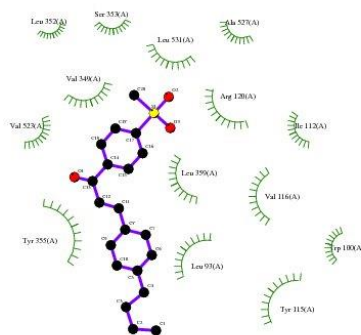
Key

- ● Ligand bond
- ● Non-ligand bond
- ● Hydrogen bond and its length
- ⌋ His 93 Non-ligand residues involved in other contact(s)

docking



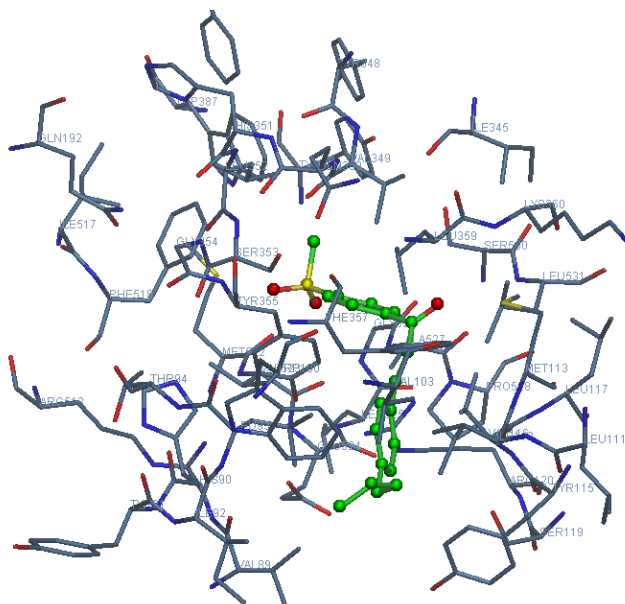
- $\text{SO}_2\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$



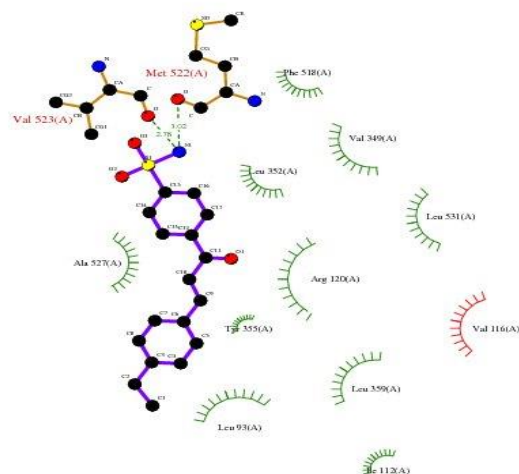
Key

-  Ligand bond
 Non-ligand bond
 Hydrogen bond and its length

docking



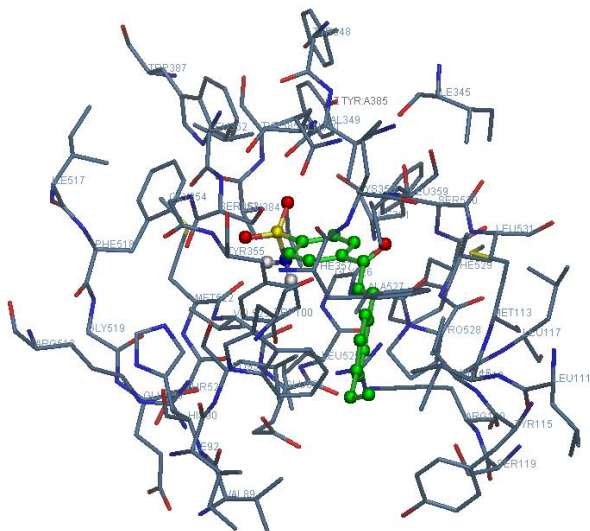
- $\text{SO}_2\text{NH}_2\text{CH}_2\text{CH}_3$



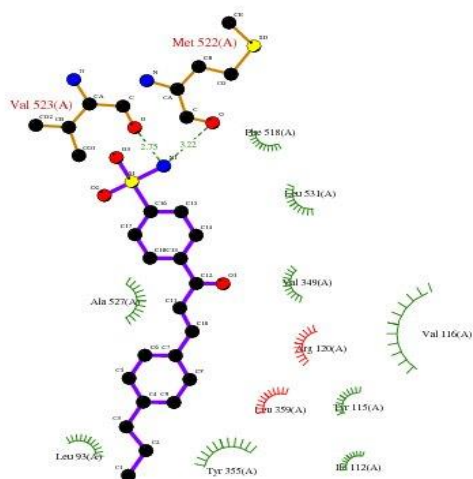
Key

- Ligand bond
- Non-ligand bond
- Hydrogen bond and its length
- It's 53 Non-ligand residues involved in other contact(s)

docking



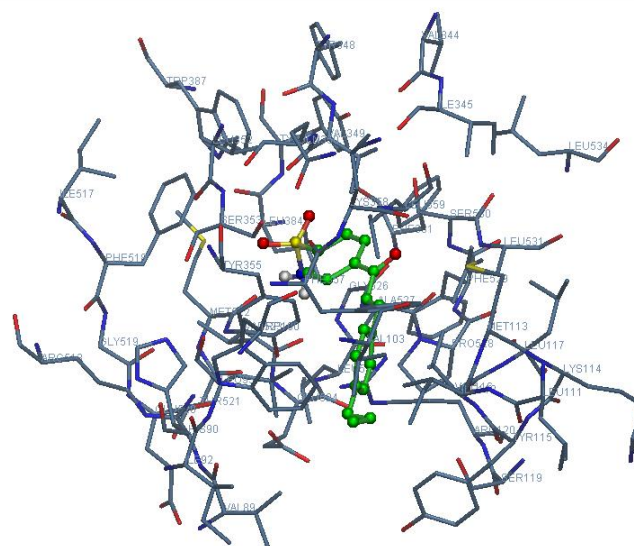
- $\text{SO}_2\text{NH}_2\text{CH}_2\text{CH}_2\text{CH}_3$



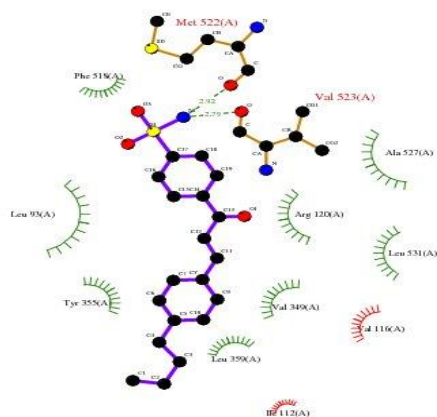
Key

- Ligand bond
- Non-ligand bond
- Hydrogen bond and its length
- His 53 Non-ligand residues involved in other contact(s)

docking



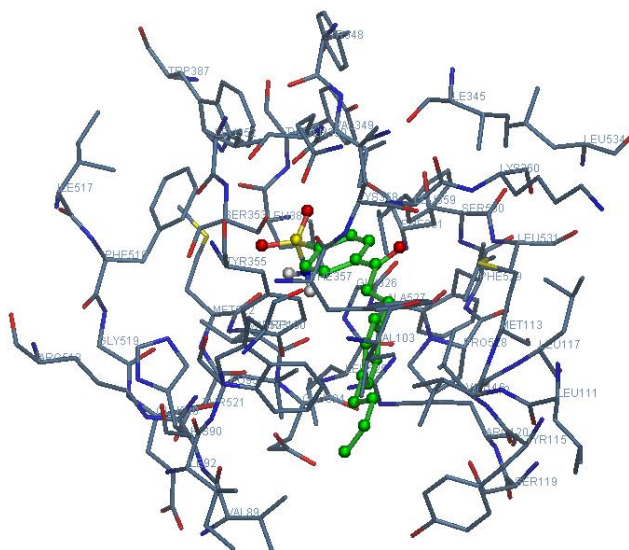
- $\text{SO}_2\text{NH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$



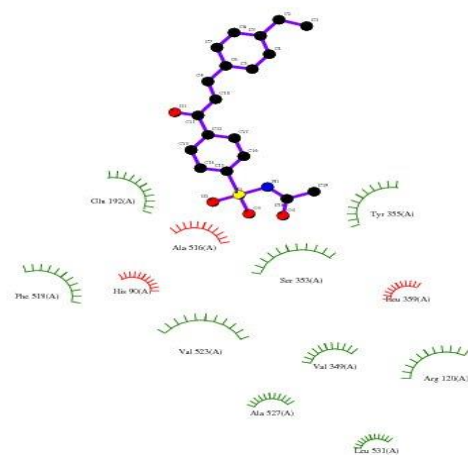
Key

- 
 Ligand bond
 Non-ligand bond
 Hydrogen bond and its length
- 
 His 53 Non-ligand residues involved in other contact(s)

docking



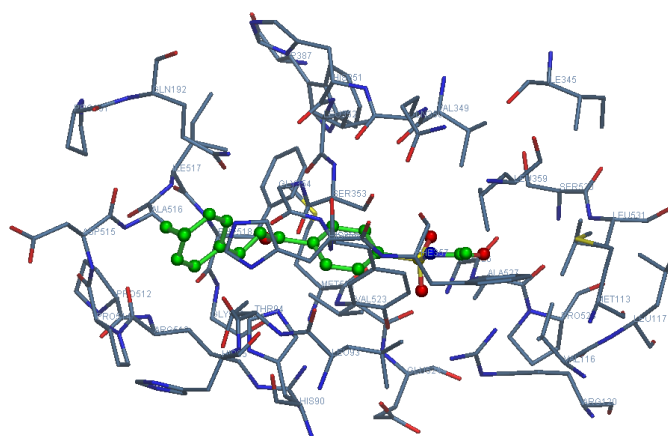
- $\text{SO}_2\text{NHCOCH}_3\text{CH}_2\text{CH}_3$



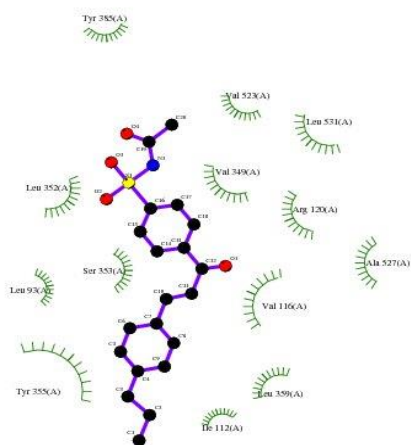
Key

- Ligand bond
- Non-ligand bond
- Hydrogen bond and its length
- 16.53 Non-ligand residues involved in other contact(s)

docking



- $\text{SO}_2\text{NHCOCH}_3\text{CH}_2\text{CH}_2\text{CH}_3$

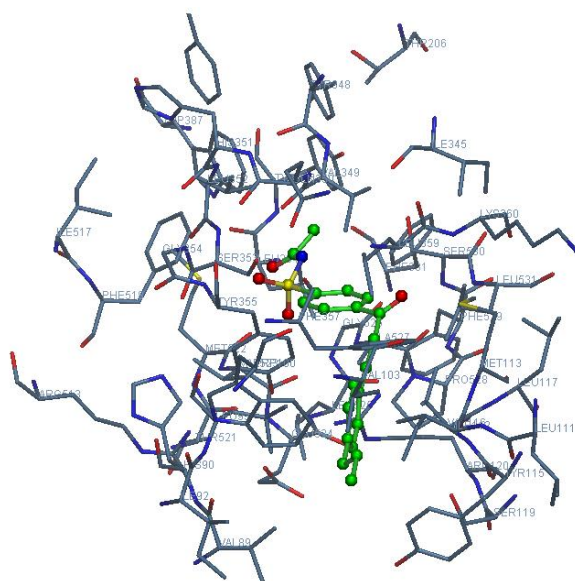


Key

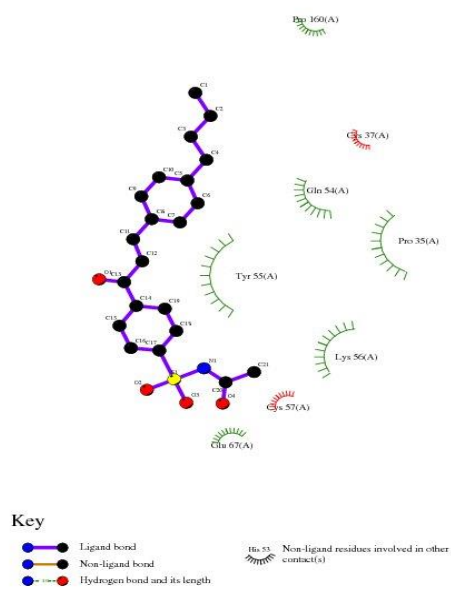
- Ligand bond
- Non-ligand bond
- Hydrogen bond and its length

the 53 Non-ligand residues involved in other contact(s)

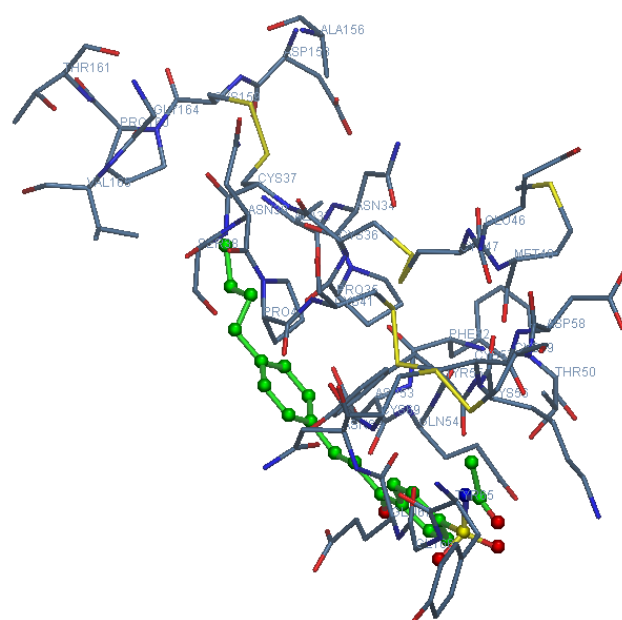
docking



- $\text{SO}_2\text{NHCOCH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$

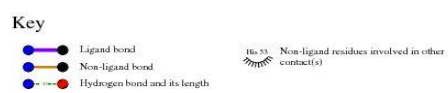


docking



Study #2

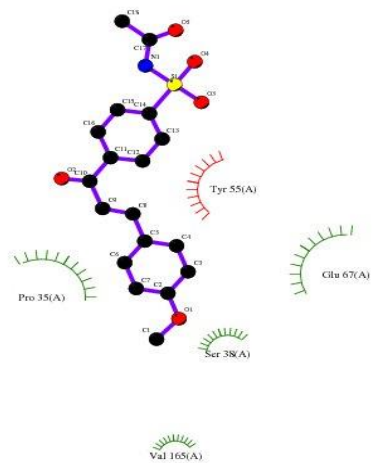
- $\text{SO}_2\text{NHCOCH}_3\text{Cl}$



- 231



- 232

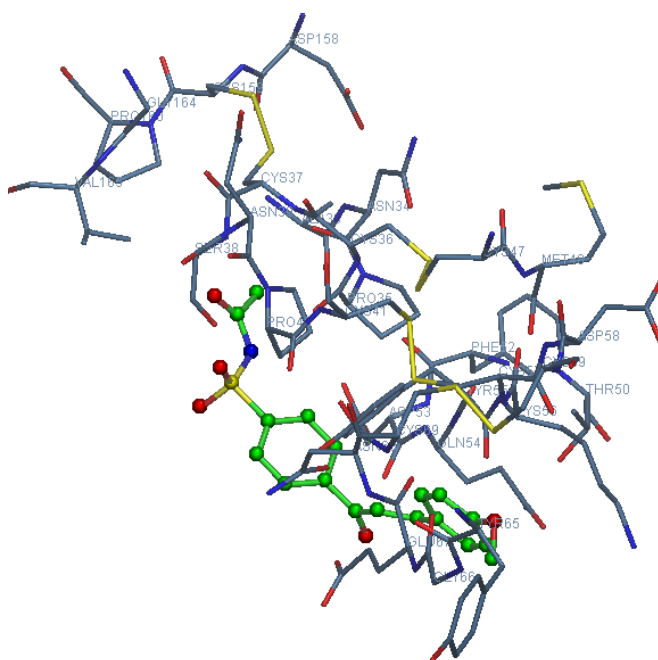


Key

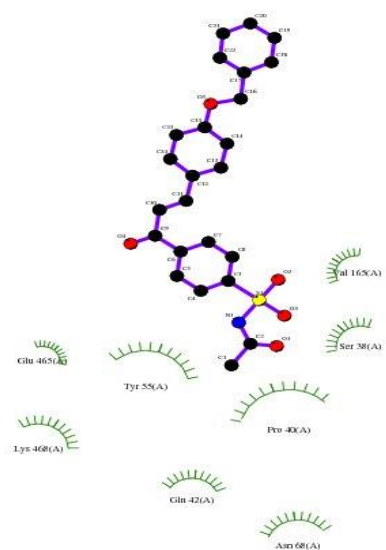
- Ligand bond
- Non-ligand bond
- —●— Hydrogen bond and its length

— Non-ligand residues involved in other contact(s)

docking



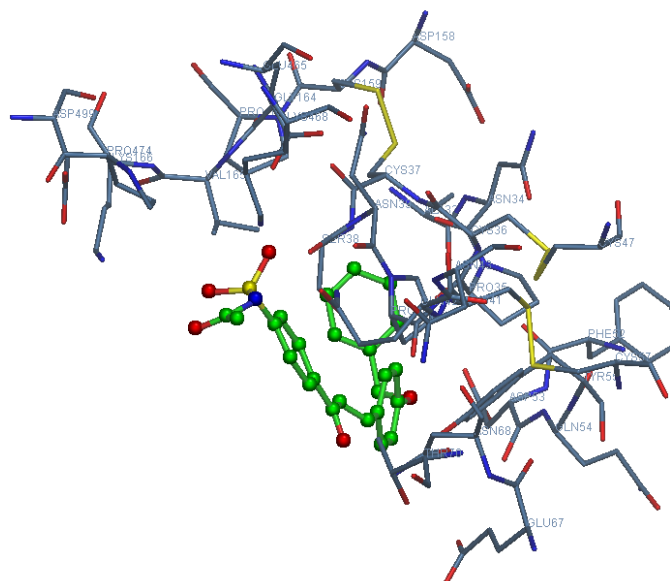
- SO2NHCOCH3OBenzyl



Key

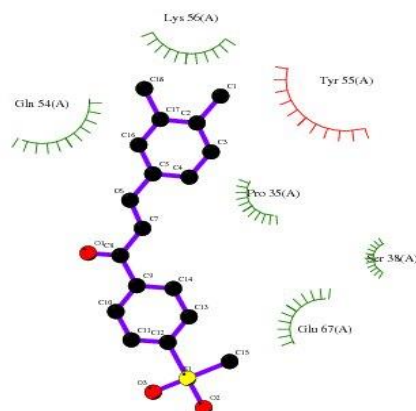
- ● Ligand bond
- ● Non-ligand bond
- ● Hydrogen bond and its length
- ● (6, 8, 9) Non-ligand residues involved in other contact(s)

docking



Study #3

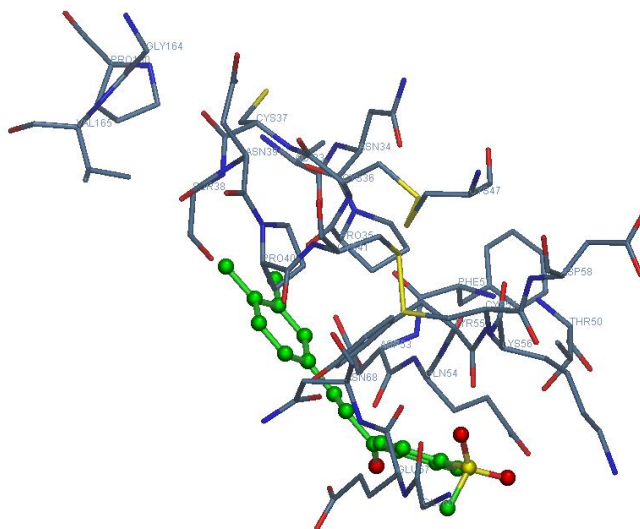
- $\text{SO}_2\text{CH}_3\text{CH}_3\text{CH}_3$



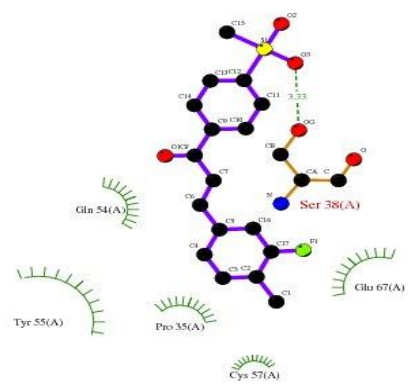
Key

- — Ligand bond
- — Non-ligand bond
- — 2.5 \AA Hydrogen bond and its length
- ⌋ 16s 53 Non-ligand residues involved in other contact(s)

docking



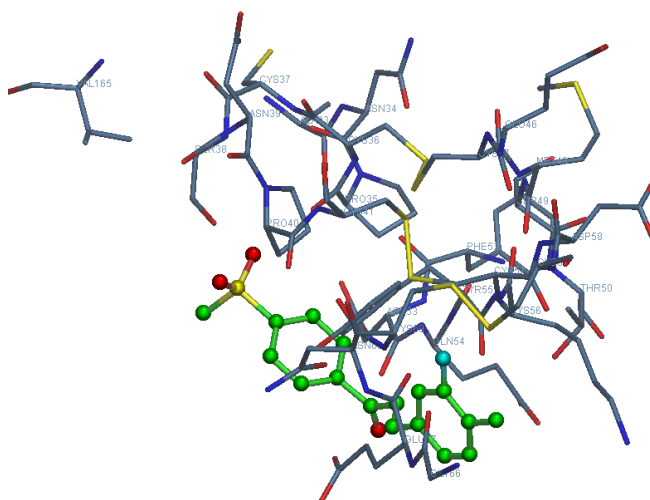
- $\text{SO}_2\text{CH}_3\text{CH}_3\text{F}$



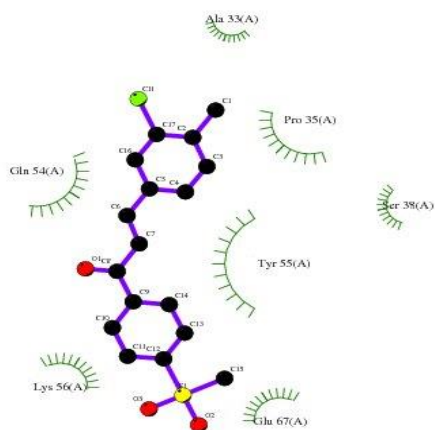
Key

- ● Ligand bond
- — Non-ligand bond
- — Hydrogen bond and its length
- ~ (Res 55) Non-ligand residues involved in other contact(s)

docking



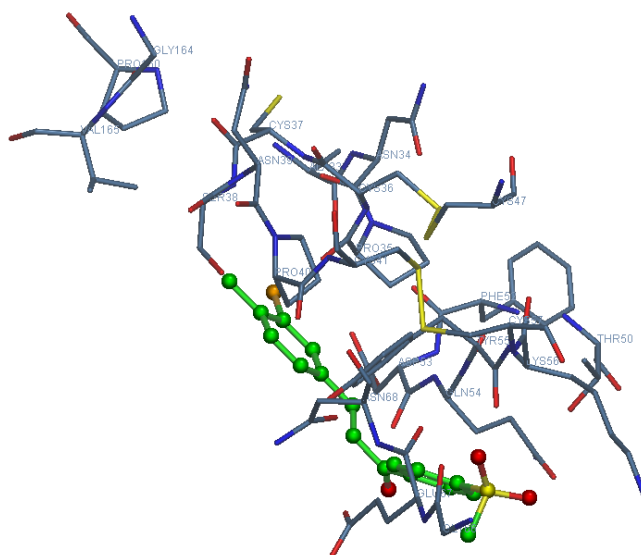
- $\text{SO}_2\text{CH}_3\text{CH}_3\text{Cl}$



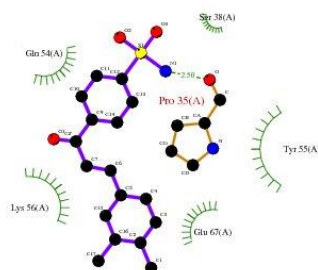
Key

- Ligand bond
- Non-ligand bond
- Hydrogen bond and its length
- (His 53) Non-ligand residues involved in other contact(s)

docking



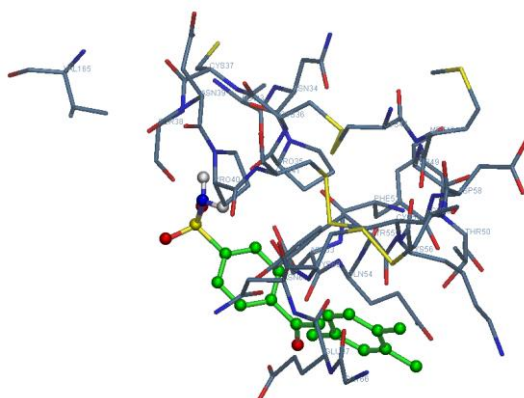
- $\text{SO}_2\text{NH}_2\text{CH}_3\text{CH}_3$



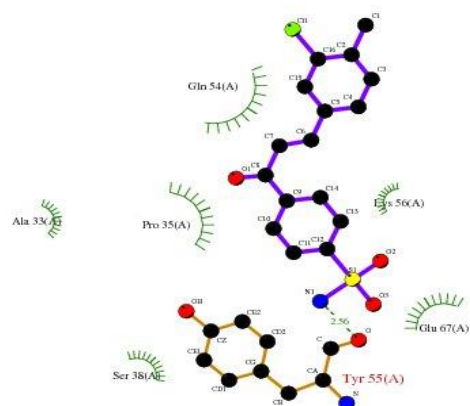
Key

- Ligand bond
- Non-ligand bond
- Hydrogen bond and its length
- 16, 55 Non-ligand residues involved in other contact(s)

docking



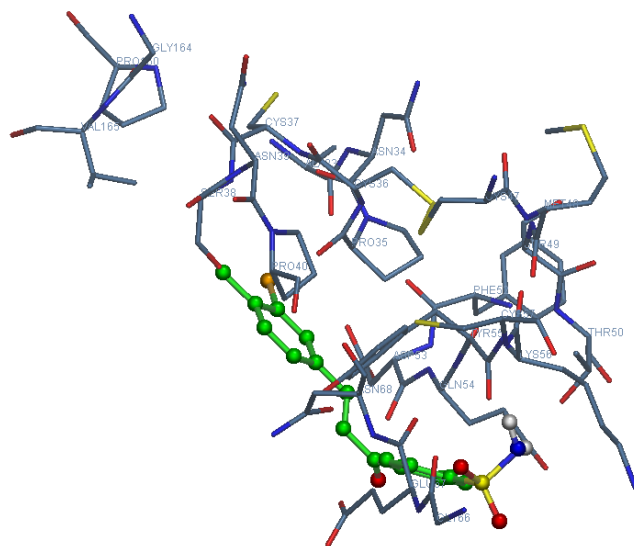
- $\text{SO}_2\text{NH}_2\text{CH}_3\text{Cl}$



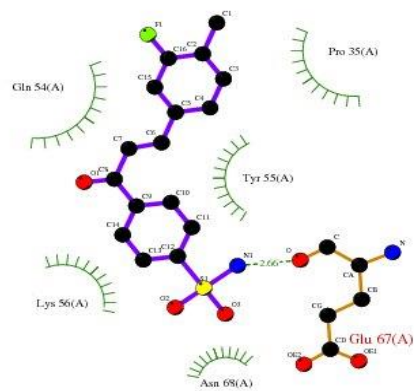
Key

- ● Ligand bond
- ● Non-ligand bond
- ● Hydrogen bond and its length
- ⌋ (As 53) Non-ligand residues involved in other contact(s)

docking



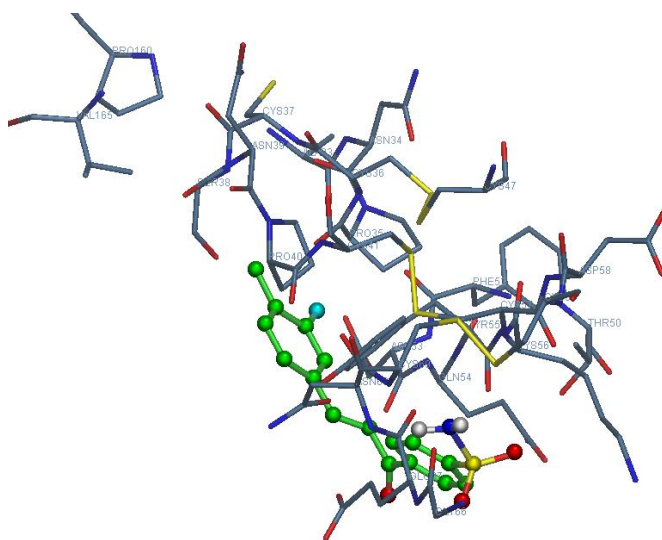
- $\text{SO}_2\text{NH}_2\text{CH}_3\text{F}$



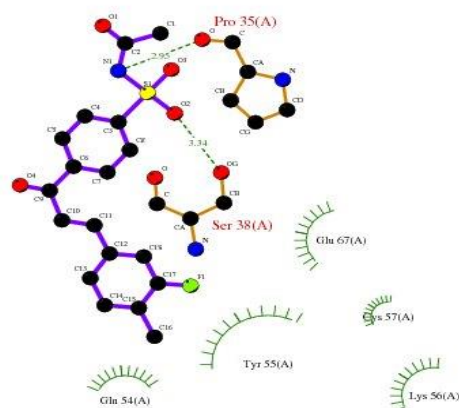
Key

- ● Ligand bond
- ● Non-ligand bond
- ● Hydrogen bond and its length
- 1fu5 Non-ligand residues involved in other contact(s)

docking



- SO2NHCOCH3CH3F

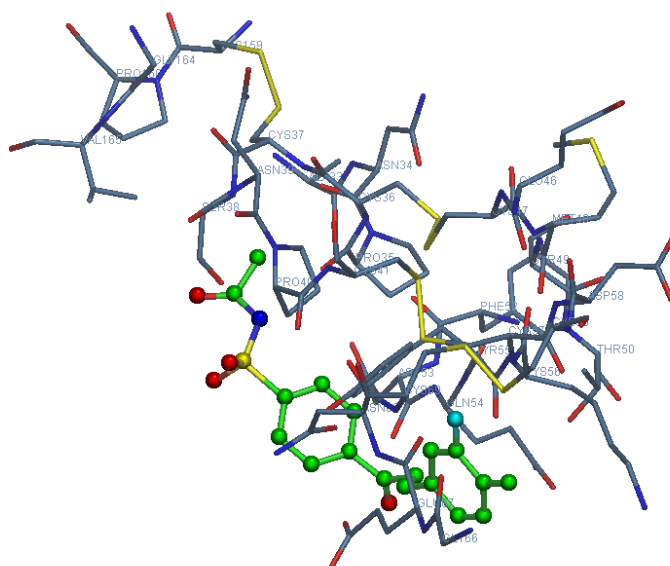


Key

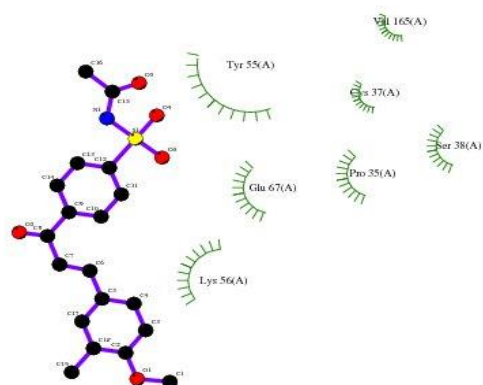
- Ligand bond
- Non-ligand bond
- Hydrogen bond and its length

His 53 Non-ligand residues involved in other contact(s)

docking



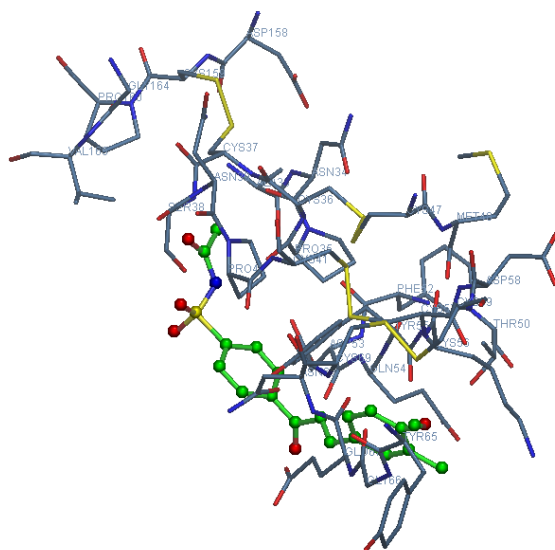
- $\text{SO}_2\text{NHCOCH}_3\text{OCH}_3\text{CH}_3$



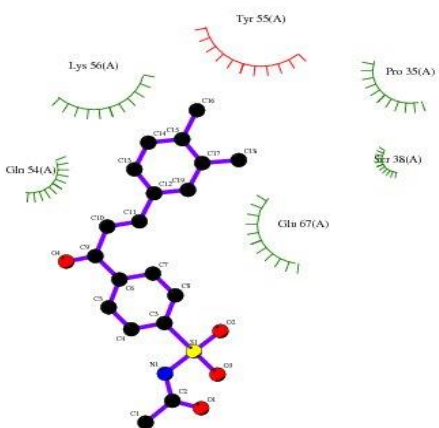
Key

- Ligand bond
- Non-ligand bond
- Hydrogen bond and its length
- (His 53) Non-ligand residues involved in other contact(s)

docking



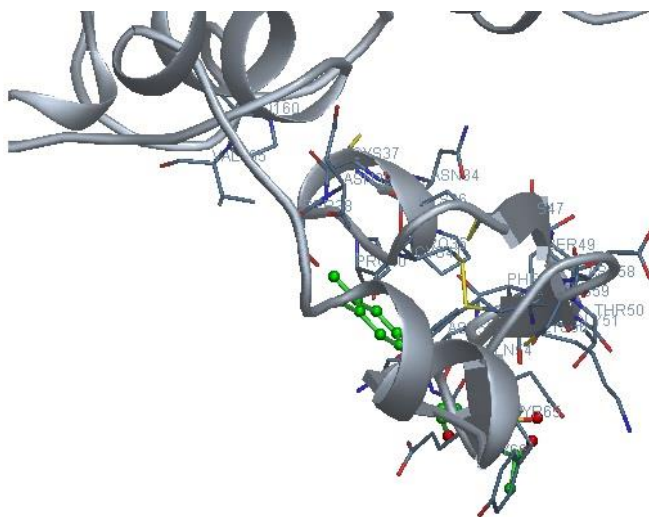
- $\text{SO}_2\text{NHCOCH}_3\text{CH}_3\text{CH}_3$



Key

- ● Ligand bond
- ● Non-ligand bond
- → Hydrogen bond and its length
- ⌋ (His 53) Non-ligand residues involved in other contact(s)

docking

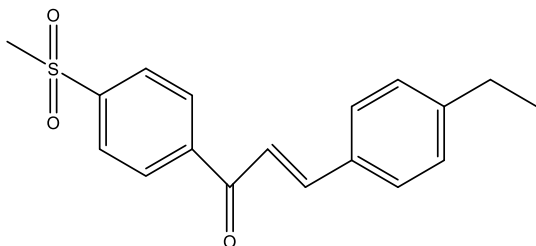


Appendix L

J Coupling Calculations – ^1H NMR

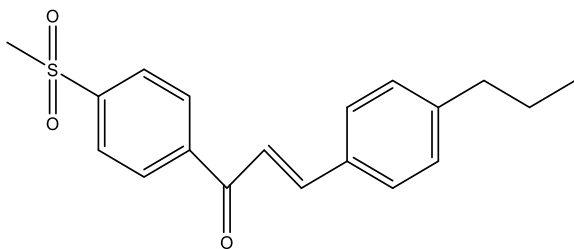
Study #1

- $\text{SO}_2\text{CH}_3\text{CH}_2\text{CH}_3$



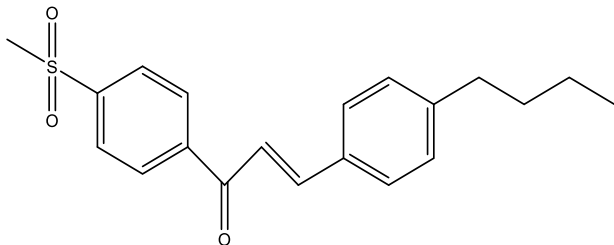
8. 17 ppm (2 hydrogen) doublet benzene (J- 12), 8.08 ppm (2 hydrogen) doublet benzene (J-8), 7.82 ppm (1 hydrogen) doublet (J-16), 7.58 ppm (2 hydrogen) doublet benzene (J-8), 7.43 ppm (1 hydrogen) doublet (J-12), 7.27 ppm (2 hydrogen) doublet benzene (J-12), 3.10 ppm (3 hydrogen) singlet, 2.70 ppm (2 hydrogen) triplet methyl (J-21.96), 1.26 ppm (3 hydrogen) triplet methyl (J-20).

- $\text{SO}_2\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_3$



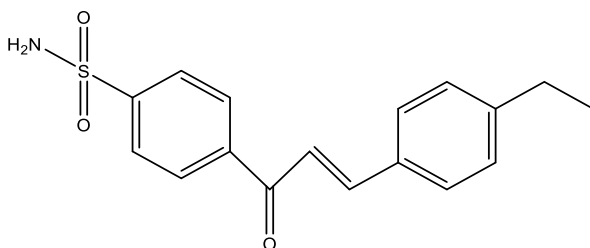
8.15 ppm (2 hydrogen) doublet benzene (J-10), 8.08 ppm (2 hydrogen) doublet benzene (J- 8), 7.82 ppm (1 hydrogen) doublet (J-16), 7.57 ppm (2 hydrogen) doublet benzene (J-8), 7.43 ppm (1 hydrogen) doublet (J-16), 7.26 ppm (2 hydrogen) doublet benzene (J-12), 3.12 ppm (3 hydrogen) singlet, 2.63 ppm (2 hydrogen) triplet methyl (J-28), 1.66 ppm (2 hydrogen) sextuplet (J-36), 0.95 ppm (3 hydrogen) methyl (J-16).

- $\text{SO}_2\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$



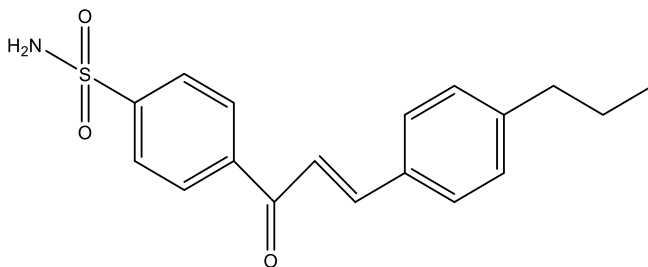
8.15 ppm (2 hydrogen) doublet benzene (J-12), 8.08 ppm (2 hydrogen) doublet benzene (J-10), 7.83 ppm (1 hydrogen) doublet (J-18), 7.56 ppm (2 hydrogen) doublet benzene (J-12), 7.43 ppm (1 hydrogen) doublet (J-8), 7.25 ppm (2 hydrogen) doublet benzene (J-12), 2.66 ppm (2 hydrogen) triplet methyl (J-20), 1.62 ppm (2 hydrogen) quintuplet methyl (J-36.7), 1.55 ppm (3 hydrogen) singlet methyl, 1.36 ppm (2 hydrogen) sextuplet methyl (J-36), 0.94 ppm (3 hydrogen) triplet methyl (J-24).

- $\text{SO}_2\text{NH}_2\text{CH}_2\text{CH}_3$



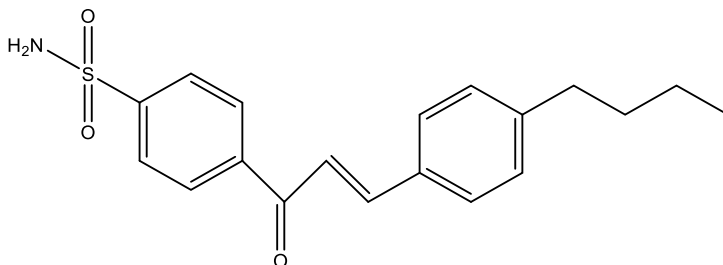
8.32 ppm (2 hydrogen) doublet benzene (J-8), 8.00 ppm (2 hydrogen) doublet benzene (J-12), 7.92 ppm (1 hydrogen) doublet (J-20), 7.84 ppm (2 hydrogen) doublet benzene (J-8), 7.78 ppm (1 hydrogen) doublet (J-16), 7.59 ppm (2 hydrogen) singlet, 7.34 ppm (2 hydrogen) doublet benzene (J-16), 2.66 ppm (2 hydrogen) quartet (J-25.9), 1.20 ppm (3 hydrogen) triplet (J-20.16)

- $\text{SO}_2\text{NH}_2\text{CH}_2\text{CH}_2\text{CH}_3$



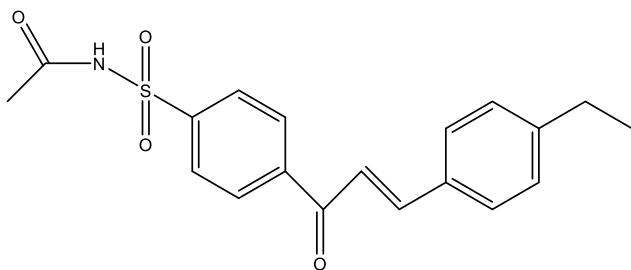
8.31 ppm (2 hydrogen) doublet benzene (J-12), 8.00 ppm (2 hydrogen) doublet benzene (J-24), 7.90 ppm (1 hydrogen) doublet (J-16), 7.82 ppm (2 hydrogen) doublet benzene (J-12), 7.77 ppm (1 hydrogen) doublet (J-16), 7.58 ppm (2 hydrogen) singlet, 7.30 ppm (2 hydrogen) doublet benzene (J-12), 2.62 ppm (2 hydrogen) triplet methyl (J-24), 1.62 ppm (2 hydrogen) sextuplet (J-37), 0.90 ppm (3 hydrogen) triplet methyl (J-24).

- $\text{SO}_2\text{NH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$



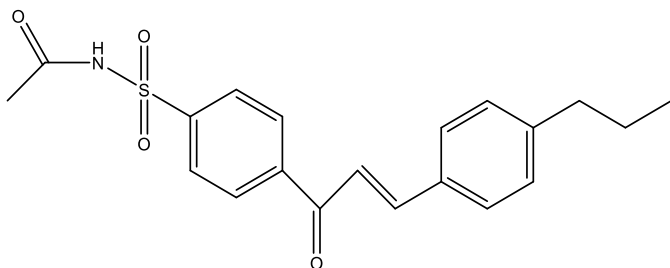
8.32 ppm (2 hydrogen) doublet benzene (J- 12), 7.99 ppm (2 hydrogen) doublet benzene (J-10), 7.92 ppm (1 hydrogen) doublet (J-16), 7.83 ppm (2 hydrogen) doublet benzene (J-12), 7.77 ppm (1 hydrogen) doublet (J-8), 7.56 ppm (2 hydrogen) singlet, 7.32 ppm (2 hydrogen) doublet benzene (J-12), 2.64 ppm (2 hydrogen) methyl (J-22.72), 1.57 ppm (2 hydrogen) quintuplet methyl (J-30.4), 1.30 ppm (2 hydrogen) sextuplet methyl (J-37), 0.90 ppm (3 hydrogen) triplet methyl (J-22.3).

- $\text{SO}_2\text{NHCOCH}_3\text{CH}_2\text{CH}_3$



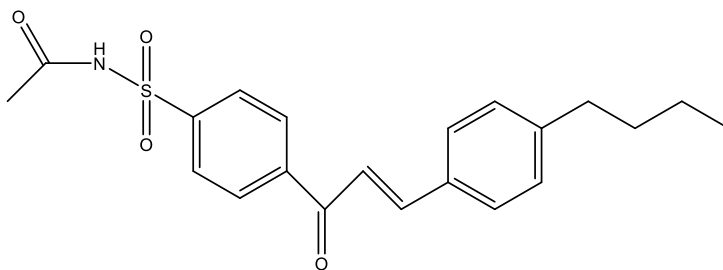
8.12 ppm (2 hydrogen) doublet benzene (J-10), 7.89 ppm (1 hydrogen) doublet (J-8), 7.86 ppm (2 hydrogen) doublet benzene (J-8), 7.82 ppm (2 hydrogen) doublet benzene (J-8), 7.74 ppm (1 hydrogen) doublet (J-12), 7.32 ppm (2 hydrogen) doublet benzene (J-12), 2.66 ppm (2 hydrogen) quadruple (J-28), 2.50 ppm (3 hydrogen) singlet, 1.20 ppm (3 hydrogen) triplet methyl (J-24). Hydrogen of nitrogen was not visible.

- $\text{SO}_2\text{NHCOCH}_3\text{CH}_2\text{CH}_2\text{CH}_3$



8.13 ppm (2 hydrogen) doublet benzene (J-12), 7.90 ppm (1 hydrogen) doublet (J-16), 7.88 ppm (2 hydrogen) doublet benzene (J-8), 7.83 ppm (2 hydrogen) doublet benzene (J-12), 7.74 ppm (1 hydrogen) doublet (J-20), 7.29 ppm (2 hydrogen) doublet benzene (J-16), 2.60 ppm (2 hydrogen) triplet methyl (J-8), 2.50 ppm (3 hydrogen) singlet methyl, 1.65 ppm (2 hydrogen) quintuplet methyl (J-32), 0.90 ppm (3 hydrogen) triplet methyl (J-28). Hydrogen of nitrogen was not visible.

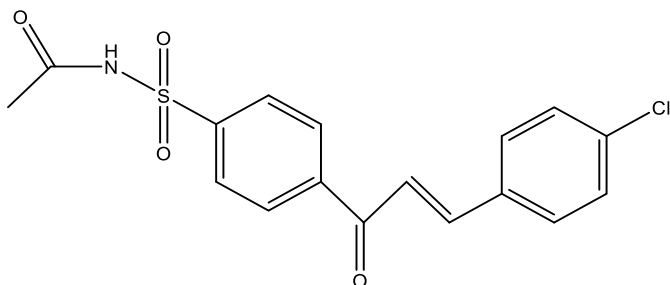
- $\text{SO}_2\text{NHCOCH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$



8.12 ppm (2 hydrogen) doublet benzene (J-12), 7.88 ppm (1 hydrogen) doublet (J-12), 7.86 ppm (2 hydrogen) doublet hydrogen (J-24), 7.81 ppm (2 hydrogen) doublet hydrogen (J-8), 7.73 ppm (1 hydrogen) doublet (J-16), 7.30 ppm (2 hydrogen) doublet benzene (J-12), 2.64 ppm (2 hydrogen) triplet methyl (J-16), 2.50 ppm (3 hydrogen) singlet methyl, 1.58 ppm (2 hydrogen) quintuplet (J-28), 1.32 ppm (2 hydrogen) sextuplet methyl (J-40), 0.91 ppm (3 hydrogen) triplet methyl (J-28). Hydrogen of nitrogen was not visible.

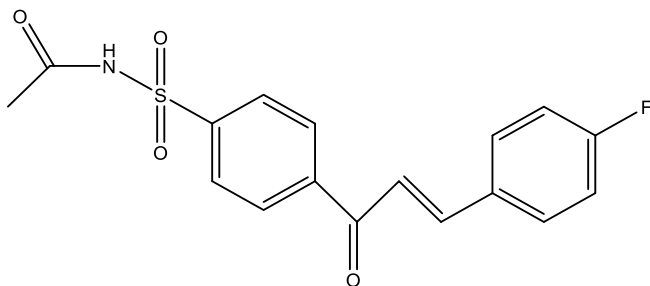
Study #2

- $\text{SO}_2\text{NHCOCH}_3\text{Cl}$



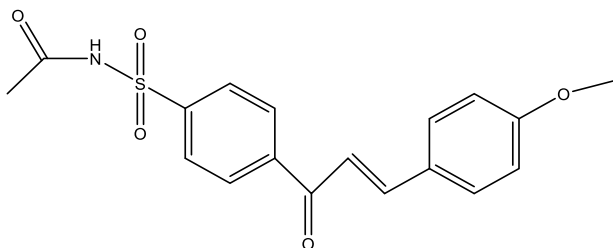
8.13 ppm (2 hydrogen) doublet benzene (J- 10), 7.98 ppm (1 hydrogen) doublet (J-8), 7.93 ppm (2 hydrogen) doublet benzene (J-8), 7.86 ppm (2 hydrogen) doublet benzene (J-20), 7.74 ppm (1 hydrogen) doublet (J-28), 7.53 ppm (2 hydrogen) doublet benzene (J-20), 2.50 ppm (3 hydrogen) singlet, 1.60 ppm (3 hydrogen) singlet, hydrogen of nitrogen not visible.

- $\text{SO}_2\text{NHCOCH}_3\text{F}$



8.24 ppm (2 hydrogen) doublet benzene (J-12), 8.00 ppm (3 hydrogen) doublet benzene (J-16), 8.01 ppm (1 hydrogen) doublet (J-12), 7.90 ppm (1 hydrogen) doublet (J-12), 7.76 ppm (1 hydrogen) doublet (J-20), 7.32 ppm (2 hydrogen) triplet benzene (J-24), 1.81 ppm (3 hydrogen) singlet, hydrogen of nitrogen not seen.

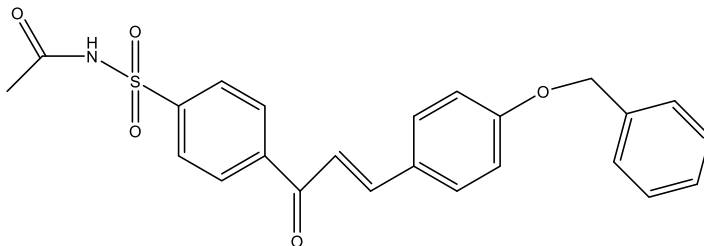
- $\text{SO}_2\text{NHCOCH}_3\text{OCH}_3$



8.10 ppm (2 hydrogen) doublet benzene (J-12), 7.82 ppm (6 hydrogen) multiplet (peaks are overlapping) (J-56), 7.20 ppm (2 hydrogen) doublet benzene (J-12), 3.80 ppm (3 hydrogen) singlet, 1.70 ppm (3 hydrogen) singlet, hydrogen of nitrogen not

visible.

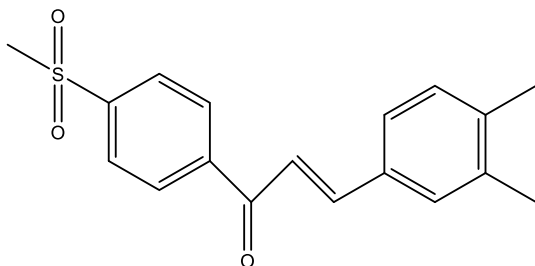
- $\text{SO}_2\text{NHCOCH}_3\text{OBenzyl}$



8.12 ppm (2 hydrogen) doublet benzene (J-23), 7.86 ppm (3 hydrogen) triplet (doublet benzene + benzene) (J-50), 7.76 ppm (3 hydrogen) multiplet (J-44), 7.45 ppm (2 hydrogen) doublet (J-8), 7.39 ppm (2 hydrogen) doublet (J-8), 7.35 ppm (1 hydrogen) doublet (J-24), 7.10 ppm (2 hydrogen) doublet benzene (J-18), 5.20 ppm (2 hydrogen) singlet, 1.90 ppm (3 hydrogen) singlet, hydrogen of nitrogen not visible.

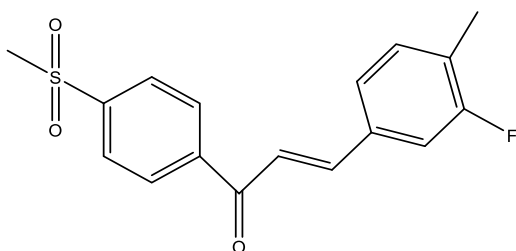
Study #3

- $\text{SO}_2\text{CH}_3\text{CH}_3\text{CH}_3$



8.35 ppm (2 hydrogen) doublet benzene (J- 8.08), 8.12 ppm (2 hydrogen) doublet benzene (J-8.4), 7.89 ppm (1 hydrogen) doublet (J-15.76), 7.75 ppm (1 hydrogen) doublet benzene (J-19.08), 7.62 ppm (1 hydrogen) doublet (J-8.04), 7.24ppm (2 hydrogen) doublet benzene (J-7.68), 3.38 ppm (3 hydrogen) singlet, 2.28 ppm (6 hydrogen) singlet.

- $\text{SO}_2\text{CH}_3\text{CH}_3\text{F}$



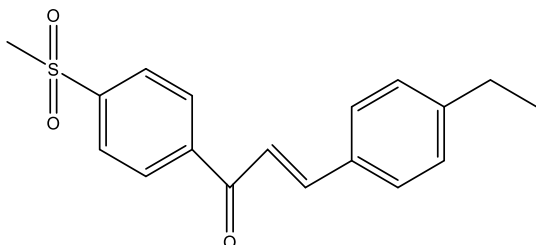
8.37 ppm (2 hydrogen) doublet benzene (J-8.44), 8.12 ppm (2 hydrogen) doublet benzene (J-21.6), 7.97 ppm (1 hydrogen) doublet (J-12), 7.97 ppm (1 hydrogen) doublet (J-28.6), 7.81 ppm (1 hydrogen) doublet (J-11), 7.75 ppm (1 hydrogen) doublet (J-8.04), 7.61 ppm (1 hydrogen) doublet (J-8.04), 7.38 ppm (2 hydrogen) triplet (J-15.76), 3.38 ppm (3 hydrogen) singlet, 2.28 ppm (6 hydrogen) singlet.

Appendix M

C13-NMR- Chemical Shift Identification

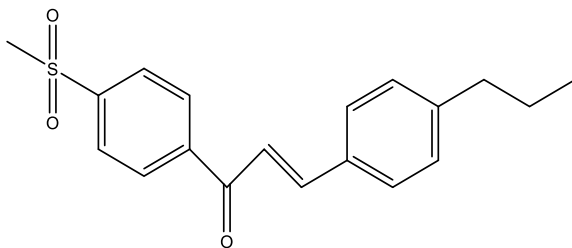
Study#1

- $\text{SO}_2\text{CH}_3\text{CH}_2\text{CH}_3$



44.5 ppm, methyl group attached to SO_2 ; 17.3 ppm, methyl group next to a CH_2 ; 29.5 ppm, CH_2 group next to benzyl and CH_3 ; 189.5 ppm, carbonyl group next to a benzyl group and double bond; 121 ppm, alkene next to carbonyl and double bond; 127.6 ppm, 2 carbons from right benzyl group; 128.8 ppm, 2 carbons from right benzyl group; 129 ppm, 2 carbons from left benzyl group, 130 ppm 2 carbons from left benzyl group; 132 ppm carbon from right benzyl next to double bond; 143 ppm carbon benzyl on left side next to carbonyl; 144 ppm carbon benzyl on right side next to CH_2 ; 147.6 ppm carbon double bond next to benzyl to the right and double bond to the left; 148 ppm carbon benzyl on left side, next to a sulfur and two carbons of benzyl

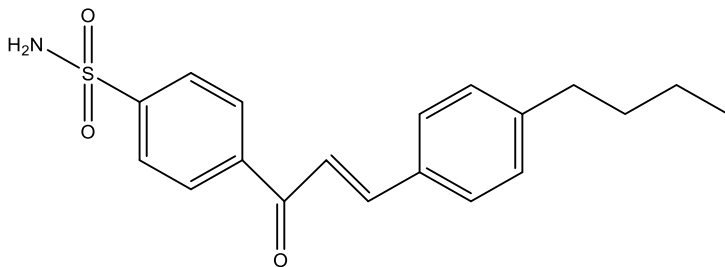
- $\text{SO}_2\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_3$



14 ppm, methyl group next to a CH_2 ; 24 ppm, CH_2 group next to a methyl group to the right and a CH_2 group to the left; 38 ppm, CH_2 group next to a CH_2 group to the right and a benzyl carbon to the left; 44 ppm, methyl group next to a sulfur with two oxygens; 120 ppm carbon double bond next to a carbon double bond to the right and a carbonyl group to the left; 128 ppm, two carbon benzyl group on the right; 129 ppm four carbon on benzyl groups, two on the left side benzyl group and two on the right side benzyl group; 130 ppm two carbon benzyl group on the left ring; 132 ppm,

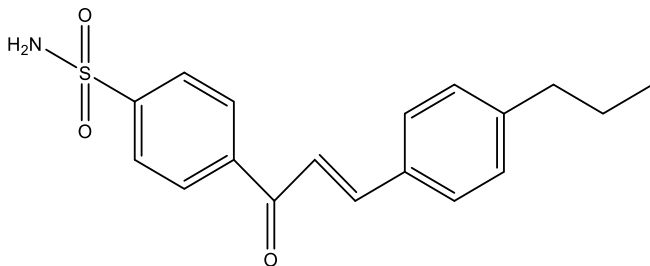
carbon benzyl group, next to a carbon double bond; 142 ppm carbon benzyl group to the right next to a CH₂ group; 144 ppm carbon benzyl group to the left next to a carbonyl group; 147 ppm carbon double bond next to double bond and benzyl group; 148 ppm carbon next to a SO₂ group next to a benzyl group; 189.6 ppm carbonyl next to a benzyl and double bond.

- SO₂NH₂CH₂CH₂CH₂CH₃



13 ppm, methyl group next to a CH₂; 20 ppm, CH₂ group next to a methyl group to the right and to a CH₂ group on the left; 32 ppm, CH₂ group next to two CH₂ groups on both sides; 35 ppm, CH₂ group next to a CH₂ group to the right and a benzyl group to the left; 122 ppm, carbon double bond next to carbonyl to the left and carbon double bond on the right; 127.5 ppm, four carbon on benzyl groups; 129 ppm, two carbon benzyl groups on the right ring; 130 ppm two carbons benzyl groups on the left ring; 132 ppm, carbon benzyl group right ring next to a double bond; 140 ppm, carbon benzyl group right ring, next to an alkane chain; 147 ppm, carbon double bond next to a carbon double bond to the left and a benzyl group to the right; 149 ppm, carbon benzyl group to the left next to a sulfur with two oxygen attach to it; 189 ppm, carbonyl next to a benzyl group and a carbon double bond.

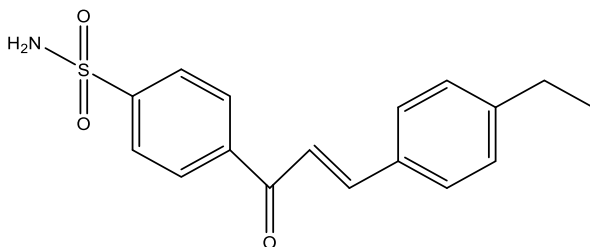
- SO₂NH₂CH₂CH₂CH₃



14.5 ppm, methyl group next to a CH₂ group; 24 ppm, CH₂ group next to a CH₂ group to the left and a methyl group to the right; 38 ppm CH₂ next to a CH₂ to the right and a benzyl group to the left; 121.5 ppm carbon double bond next to a carbon double bond to the right and a carbonyl to the left; 127 ppm four carbon on benzyl group, two on the right aryl and two on the benzyl on the left; 129 ppm two carbon on benzyl

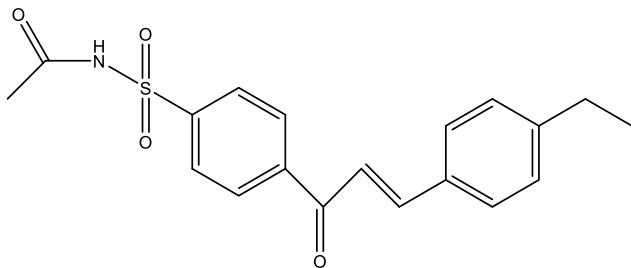
group on the right; 130 ppm two carbon on benzyl group to the left; 132 ppm carbon on benzyl group to the right next to a double bond; 140 ppm carbon on benzyl group to the left next to carbonyl group; 145 ppm carbon on benzyl group to the right next to a CH₂; 146 ppm double bond carbon next to a double bond to the left and a benzyl group to the right; 147 ppm carbon benzyl group left next to a SO₂ group; 189.6 ppm carbonyl group.

- SO₂NH₂CH₂CH₃



14.5 ppm methyl group next to a CH₂ group; 27.5 ppm, methyl group, methyl group to the right and benzyl of the right to the left; 121 ppm, double bond, carbonyl to the left and double bond to the right; 126.8 ppm, two carbon benzyl on the right; 189.6 ppm, carbonyl, benzyl to the left and double bond to the right; 150 ppm, carbon benzyl ring to the left, next to a SO₂ group to the left; 149 ppm, double bond, double bond to the left and carbon benzyl ring to the right; 148 ppm, carbon benzyl right, methyl to the right; 141 ppm, carbon benzyl to the left, carbonyl group to the right; 132 ppm, carbon benzyl to the right, double bond left; 130 ppm, two carbon benzyl ring to the left; 129 ppm, two carbon benzyl ring to the right; 126.8 ppm, four carbon, two carbon benzyl ring on the left and two other carbon benzyl ring on the right.

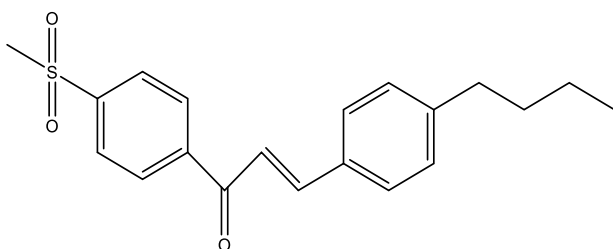
- SO₂NHCOCH₃CH₂CH₃ (check)



14.5 ppm, methyl group next to a methyl to the left; 32 ppm, methyl group, methyl group to the right and a benzyl group to the left; 30 ppm, methyl group next to a carbonyl group to the right; 190 ppm, carbonyl next to a double bond to the right and a benzyl group to the left; 176 ppm, carbonyl next to a methyl group on the left and a

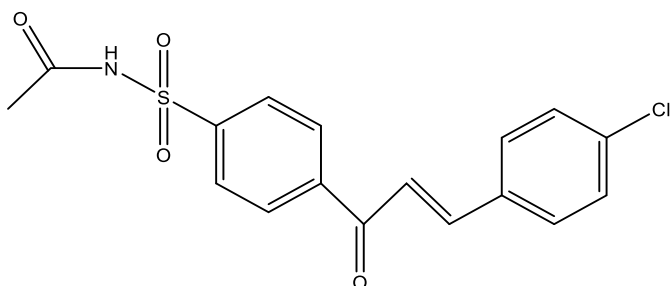
nitrogen to the right;

- $\text{SO}_2\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$



Study#2

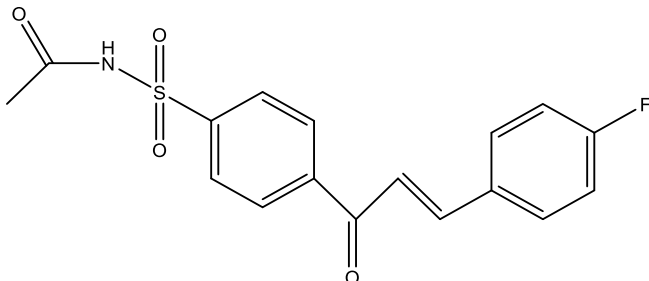
- $\text{SO}_2\text{NHCOCH}_3\text{Cl}$



26 ppm, methyl right next to a carbonyl group; double bond with a carbonyl group to the left and a double bond to the right; 123 ppm, double bond with a carbonyl group to the left and a double bond to the right; 128 ppm, two carbons group on the left; 131 ppm, two carbons benzyl group to the right; 132 ppm, two carbons benzyl group to the left; 135 ppm, carbon benzyl group to the right and double bond to the left; 135.6 ppm, carbon benzyl group on the left and carbonyl group to the right; 139 ppm, carbon double bond, double bond left and a benzyl group to the right; 143 ppm, carbon benzyl group to the left with a SO₂ group; 175 ppm carbonyl group, methyl to the left and a NH group to the right; 189.6 ppm, carbonyl, double bond to the right

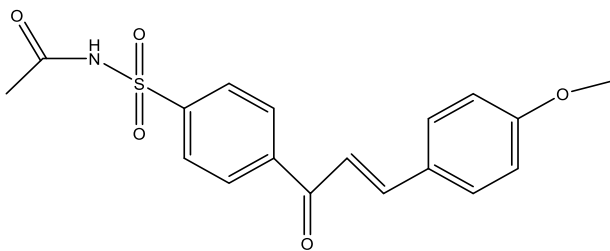
and benzyl group to the left.

- $\text{SO}_2\text{NHCOCH}_3\text{F}$



24 ppm methyl group next to a carbonyl; 115 ppm, two carbonyl benzyl group on the right; 122 ppm, double bond, carbonyl left, double bond on the right; 128 ppm, two carbon benzyl group on the left; 132 ppm, four carbons, two carbons benzyl on the right, 2 carbons benzyl on the left; 133 ppm carbon benzyl right, double bond to the left; 140 ppm, carbon benzyl left, carbonyl on the right; 143 ppm, carbon double bond, carbon double bond left, benzyl on the right; 146.5 ppm, carbon on benzyl group on the left, SO_2 group on the left side; 164 ppm carbon benzyl group to the right, F on the right; 172 ppm, carbonyl methyl left, NH on the right; 189.5 ppm, carbonyl next to a benzyl group on the left and a double bond on the right.

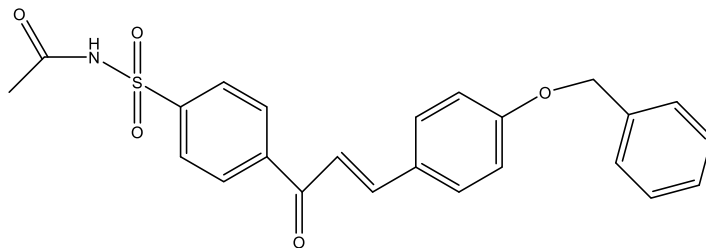
- $\text{SO}_2\text{NHCOCH}_3\text{OCH}_3$



27 ppm, methyl group, next to a carbonyl; 55 ppm, methyl group next to one oxygen of ether; 114 ppm, two carbon benzyl group on the right; 120 ppm, carbon double bond next to a carbonyl on the left and a double bond to the right; 127 ppm, carbon benzyl right next to a double bond; 128 ppm, two carbon benzyl left; 131.6 ppm, four carbons, two benzyl left, two benzyl right; 138.6 ppm, carbons of benzyl group, next to carbonyl on the right; 145 ppm, carbon double bond next to a carbon double bond to the left and a benzyl group to the right; 151.3 ppm, carbon benzyl group to the left next to a SO_2 group to the left; 162.5 ppm, carbon benzyl group, next to an oxygen to the right; 176 ppm, carbonyl next to methyl on the left and a nitrogen on the right; 189.6 ppm, carbonyl next to benzyl group on the left and a carbon double bond on the

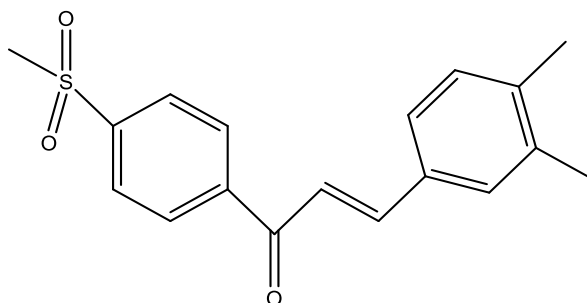
right.

- $\text{SO}_2\text{NHCOCH}_3\text{OBenzyl}$



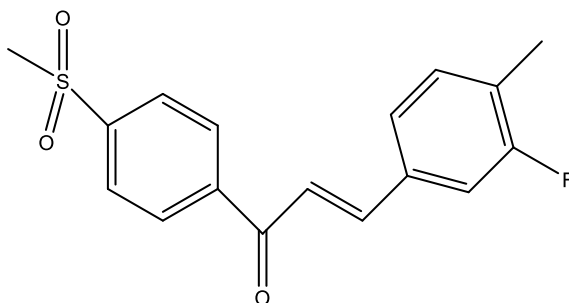
Study#3

- $\text{SO}_2\text{CH}_3\text{CH}_3\text{CH}_3$



18.8 ppm, methyl group next to a benzyl group; 19.2 ppm, methyl group next to a benzyl group; 47.7 ppm, methyl group next to sulfoxide; 128.8 ppm, two carbons of the benzyl group holding the sulfoxide; 130.9 ppm, two carbons of the benzyl group holding the sulfoxide; 146.8 ppm, carbon on benzyl group right next to sulfoxide; 142.9 ppm benzyl next to a carbonyl group; 189.7 ppm carbonyl; 121.3 ppm double bond next to carbonyl left and next to a double bond on the right; 145.1 ppm double bond next to double to the left and benzyl group to the right; 132.1 ppm, carbon benzyl next to double bond on the left; 131.3 ppm, carbon benzyl next to carbon benzyl attached to hydrocarbon chain on the left; 125.5 ppm, carbon benzyl next to carbon benzyl attached to hydrocarbon chain on the left and a carbon benzyl on the right; 132.0 ppm, carbon benzyl with a carbon attached to a methyl group to the right; 136.0 ppm, carbon benzyl with a benzyl group attached ; 136.7 ppm, carbon benzyl with a methyl group attached.

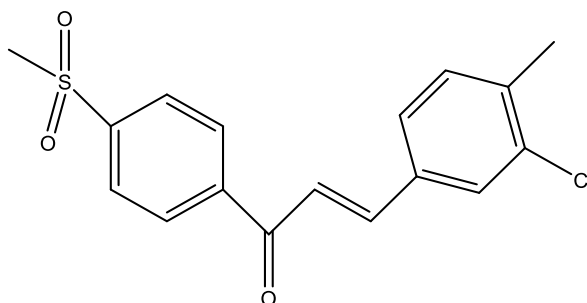
- $\text{SO}_2\text{CH}_3\text{CH}_3\text{F}$



14.5 ppm, methyl group next to a benzyl group; 47.7 ppm, methyl group next to sulfoxide; 128.8 ppm, two carbons of the benzyl group holding the sulfoxide; 130.9 ppm, two carbons of the benzyl group holding the sulfoxide; 146.8 ppm, carbon on benzyl group right next to sulfoxide; 142.9 ppm benzyl next to a carbonyl group; 189.7 ppm carbonyl; 121.3 ppm double bond next to carbonyl left and next to a

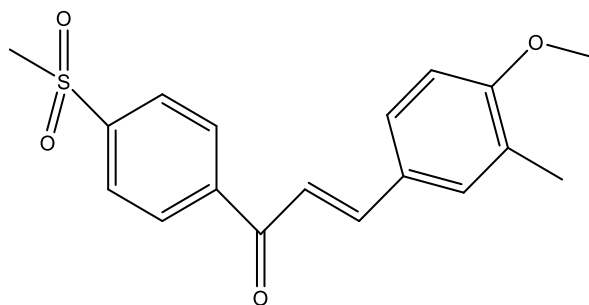
double bond on the right; 145.1 ppm double bond next to double to the left and benzyl group to the right; 133.8 ppm, carbon benzyl next to double bond on the left; 111.1 ppm, carbon benzyl next to carbon benzyl attached to hydrocarbon chain on the left; 124.1 ppm, carbon benzyl next to carbon benzyl attached to hydrocarbon chain on the left and a carbon benzyl on the right; 161.4 ppm, carbon benzyl with a fluorine attached to the right ; 130.5 ppm, carbon benzyl with a benzyl carbon attached to a methyl group to the right ; 123.1 ppm, carbon benzyl with a methyl group attached.

- $\text{SO}_2\text{CH}_3\text{CH}_3\text{Cl}$



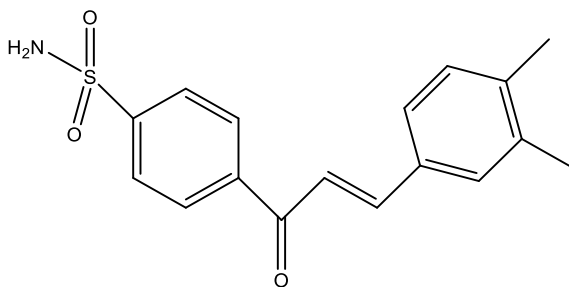
19.9 ppm, methyl group next to a benzyl group; 47.7 ppm, methyl group next to sulfoxide; 128.8 ppm, two carbons of the benzyl group holding the sulfoxide; 130.9 ppm, two carbons of the benzyl group holding the sulfoxide; 146.8 ppm, carbon on benzyl group right next to sulfoxide; 142.9 ppm benzyl next to a carbonyl group; 189.7 ppm carbonyl; 121.3 ppm double bond next to carbonyl left and next to a double bond on the right; 145.1 ppm double bond next to double to the left and benzyl group to the right; 133.6 ppm, carbon benzyl next to double bond on the left; 126.3 ppm, carbon benzyl next to carbon benzyl attached to hydrocarbon chain on the left; 126.6 ppm, carbon benzyl next to carbon benzyl attached to hydrocarbon chain on the left and a carbon benzyl on the right; 131.7 ppm, carbon benzyl with a chlorine attached to the right ; 121.1 ppm, carbon benzyl with a benzyl carbon attached to a methyl group to the right ; 135.7 ppm, carbon benzyl with a methyl group attached.

- $\text{SO}_2\text{CH}_3\text{CH}_3\text{OCH}_3$



15.8 ppm, methyl group attached to a benzyl group; 56.1 ppm, methyl group next to an oxygen that is attached to a benzyl group; 47.7 ppm, methyl group next to sulfoxide; 128.8 ppm, two carbons of the benzyl group holding the sulfoxide; 130.9 ppm, two carbons of the benzyl group holding the sulfoxide; 146.8 ppm, carbon on benzyl group right next to sulfoxide; 142.9 ppm benzyl next to a carbonyl group; 189.7 ppm carbonyl; 121.3 ppm double bond next to carbonyl left and next to a double bond on the right; 145.1 ppm double bond next to double to the left and benzyl group to the right; 127.4 ppm, carbon benzyl next to double bond on the left; 127.6 ppm, carbon benzyl next to carbon benzyl attached to hydrocarbon chain on the left; 132.3 ppm, carbon benzyl next to carbon benzyl attached to hydrocarbon chain on the left and a carbon benzyl on the right; 157.8 ppm, carbon benzyl with an oxygen attached to the right ; 114.1 ppm, carbon benzyl with a carbon attached to an oxygen group to the right ; 124.4 ppm, carbon benzyl with a methyl group attached.

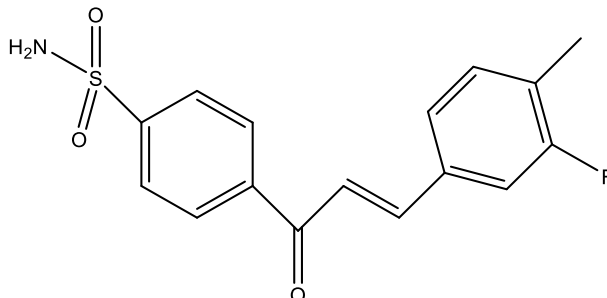
- $\text{SO}_2\text{NH}_2\text{CH}_3\text{CH}_3$



18.8 ppm, methyl group next to a benzyl group; 19.2 ppm, methyl group next to a benzyl group; 127.8 ppm, two carbons of the benzyl group holding the sulfoxide; 130.2 ppm, two carbons of the benzyl group holding the sulfoxide; 149.5 ppm, carbon on benzyl group right next to sulfoxide; 141.1 ppm benzyl next to a carbonyl group; 189.7 ppm carbonyl; 121.3 ppm double bond next to carbonyl left and next to a double bond on the right; 145.1 ppm double bond next to double to the left and benzyl group to the right; 132.1 ppm, carbon benzyl next to double bond on the left; 131.3 ppm, carbon benzyl next to carbon benzyl attached to hydrocarbon chain on the left; 125.5 ppm, carbon benzyl next to carbon benzyl attached to hydrocarbon chain on the left and a carbon benzyl on the right; 132.0 ppm, carbon benzyl with a carbon attached to a methyl group to the right; 136.0 ppm, carbon benzyl with a

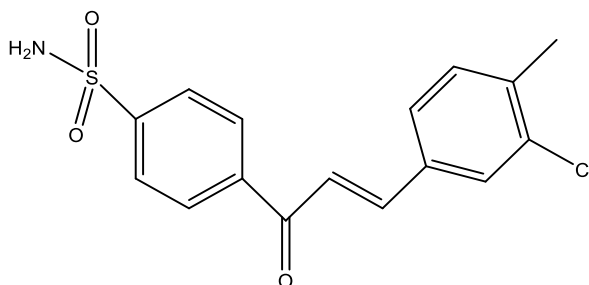
benzyl group attached ; 136.7 ppm, carbon benzyl with a methyl group attached.

- $\text{SO}_2\text{NH}_2\text{CH}_3\text{F}$



14.5 ppm, methyl group next to a benzyl group; 127.8 ppm, two carbons of the benzyl group holding the sulfoxide; 130.2 ppm, two carbons of the benzyl group holding the sulfoxide; 149.5 ppm, carbon on benzyl group right next to sulfoxide; 141.1 ppm benzyl next to a carbonyl group; 189.7 ppm carbonyl; 121.3 ppm double bond next to carbonyl left and next to a double bond on the right; 145.1 ppm double bond next to double to the left and benzyl group to the right; 133.8 ppm, carbon benzyl next to double bond on the left; 111.1 ppm, carbon benzyl next to carbon benzyl attached to hydrocarbon chain on the left; 124.1 ppm, carbon benzyl next to carbon benzyl attached to hydrocarbon chain on the left and a carbon benzyl on the right; 161.4 ppm, carbon benzyl with a fluorine attached to the right ; 130.5 ppm, carbon benzyl with a benzyl carbon attached to a methyl group to the right ; 123.1 ppm, carbon benzyl with a methyl group attached.

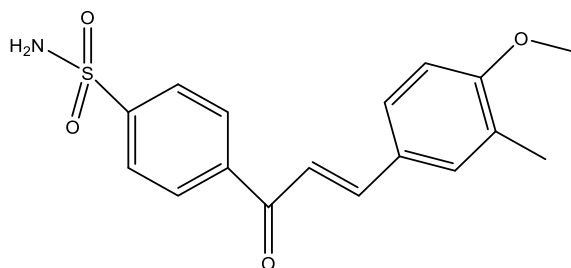
- $\text{SO}_2\text{NH}_2\text{CH}_3\text{Cl}$



19.9 ppm, methyl group next to a benzyl group; 127.8 ppm, two carbons of the benzyl group holding the sulfoxide; 130.2 ppm, two carbons of the benzyl group holding the sulfoxide; 149.5 ppm, carbon on benzyl group right next to sulfoxide; 141.1 ppm benzyl next to a carbonyl group; 189.7 ppm carbonyl; 121.3 ppm double bond next to carbonyl left and next to a double bond on the right; 145.1 ppm double bond next to double to the left and benzyl group to the right; 133.6 ppm, carbon benzyl next to

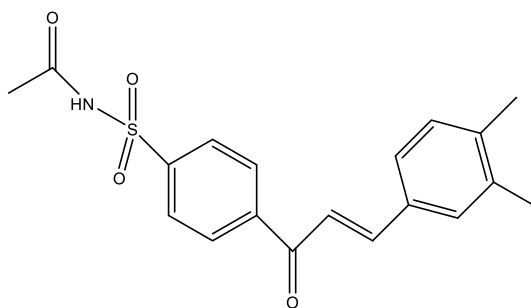
double bond on the left; 126.3 ppm, carbon benzyl next to carbon benzyl attached to hydrocarbon chain on the left; 126.6 ppm, carbon benzyl next to carbon benzyl attached to hydrocarbon chain on the left and a carbon benzyl on the right; 131.7 ppm, carbon benzyl with a chlorine attached to the right ; 121.1 ppm, carbon benzyl with a benzyl carbon attached to a methyl group to the right ; 135.1 ppm, carbon benzyl with a methyl group attached.

- $\text{SO}_2\text{NH}_2\text{CH}_3\text{OCH}_3$



15.8 ppm, methyl group attached to a benzyl group; 56.1 ppm, methyl group next to an oxygen that is attached to a benzyl group; 127.8 ppm, two carbons of the benzyl group holding the sulfoxide; 130.2 ppm, two carbons of the benzyl group holding the sulfoxide; 149.5 ppm, carbon on benzyl group right next to sulfoxide; 141.1 ppm benzyl next to a carbonyl group; 189.7 ppm carbonyl; 121.3 ppm double bond next to carbonyl left and next to a double bond on the right; 145.1 ppm double bond next to double to the left and benzyl group to the right; 127.4 ppm, carbon benzyl next to double bond on the left; 127.6 ppm, carbon benzyl next to carbon benzyl attached to hydrocarbon chain on the left; 132.3 ppm, carbon benzyl next to carbon benzyl attached to hydrocarbon chain on the left and a carbon benzyl on the right; 157.8 ppm, carbon benzyl with an oxygen attached to the right ; 114.1 ppm, carbon benzyl with a carbon attached to an oxygen group to the right ; 124.4 ppm, carbon benzyl with a methyl group attached.

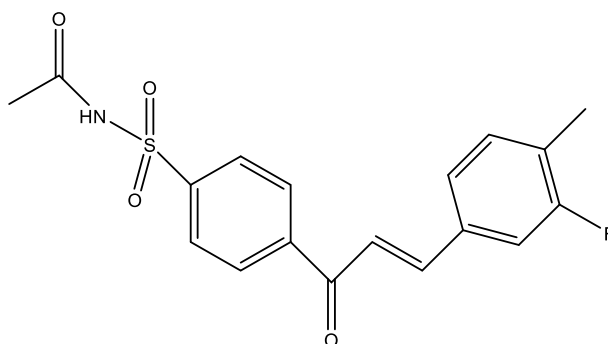
- $\text{SO}_2\text{NHCOCH}_3\text{CH}_3\text{CH}_3$



18.8 ppm, methyl group next to a benzyl group; 19.2 ppm, methyl group next to a

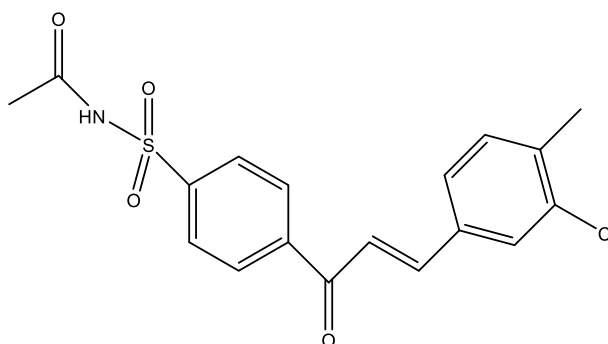
benzyl group; 21.9 ppm, methyl group next to a carbonyl; 127.8 ppm, two carbons of the benzyl group holding the sulfoxide; 130.2 ppm, two carbons of the benzyl group holding the sulfoxide; 146.6 ppm, carbon on benzyl group right next to sulfoxide; 141.1 ppm benzyl next to a carbonyl group; 171.0 ppm, carbonyl next to methyl group to the left and nitrogen to the right; 189.7 ppm carbonyl; 121.3 ppm double bond next to carbonyl left and next to a double bond on the right; 145.1 ppm double bond next to double to the left and benzyl group to the right; 132.1 ppm, carbon benzyl next to double bond on the left; 131.3 ppm, carbon benzyl next to carbon benzyl attached to hydrocarbon chain on the left; 125.5 ppm, carbon benzyl next to carbon benzyl attached to hydrocarbon chain on the left and a carbon benzyl on the right; 132.0 ppm, carbon benzyl with a carbon attached to a methyl group to the right; 136.0 ppm, carbon benzyl with a benzyl group attached ; 136.7 ppm, carbon benzyl with a methyl group attached.

- $\text{SO}_2\text{NHCOCH}_3\text{CH}_3\text{F}$



14.5 ppm, methyl group next to a benzyl group; 21.9 ppm, methyl group next to a carbonyl; 127.8 ppm, two carbons of the benzyl group holding the sulfoxide; 130.2 ppm, two carbons of the benzyl group holding the sulfoxide; 146.6 ppm, carbon on benzyl group right next to sulfoxide; 141.1 ppm benzyl next to a carbonyl group; 171.0 ppm, carbonyl next to methyl group to the left and nitrogen to the right; 189.7 ppm carbonyl; 121.3 ppm double bond next to carbonyl left and next to a double bond on the right; 145.1 ppm double bond next to double to the left and benzyl group to the right; 133.8 ppm, carbon benzyl next to double bond on the left; 111.1 ppm, carbon benzyl next to carbon benzyl attached to hydrocarbon chain on the left; 124.1 ppm, carbon benzyl next to carbon benzyl attached to hydrocarbon chain on the left and a carbon benzyl on the right; 161.4 ppm, carbon benzyl with a fluorine attached to the right ; 130.5 ppm, carbon benzyl with a benzyl carbon attached to a methyl group to the right ; 123.1 ppm, carbon benzyl with a methyl group attached.

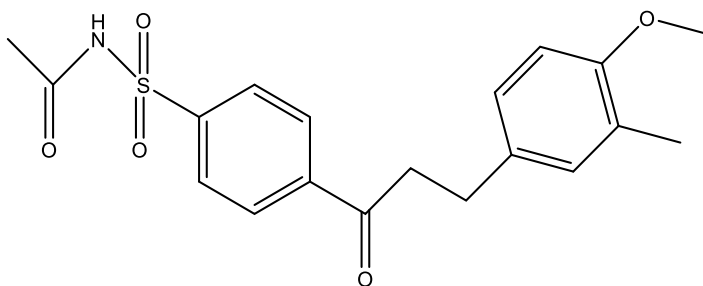
- $\text{SO}_2\text{NHCOCH}_3\text{CH}_3\text{Cl}$



19.9 ppm, methyl group next to a benzyl group; 21.9 ppm, methyl group next to a carbonyl group; 127.8 ppm, two carbons of the benzyl group holding the sulfoxide; 130.2 ppm, two carbons of the benzyl group holding the sulfoxide; 146.6 ppm, carbon on benzyl group right next to sulfoxide; 141.1 ppm benzyl next to a carbonyl group;

171.0 ppm, carbonyl next to a methyl group to the left and a nitrogen to the right; 189.7 ppm carbonyl; 121.3 ppm double bond next to carbonyl left and next to a double bond on the right; 145.1 ppm double bond next to double to the left and benzyl group to the right; 133.6 ppm, carbon benzyl next to double bond on the left; 126.3 ppm, carbon benzyl next to carbon benzyl attached to hydrocarbon chain on the left; 126.6 ppm, carbon benzyl next to carbon benzyl attached to hydrocarbon chain on the left and a carbon benzyl on the right; 131.7 ppm, carbon benzyl with a chlorine attached to the right ; 121.1 ppm, carbon benzyl with a benzyl carbon attached to a methyl group to the right ; 135.1 ppm, carbon benzyl with a methyl group attach

- $\text{SO}_2\text{NHCOCH}_3\text{CH}_3\text{OCH}_3$

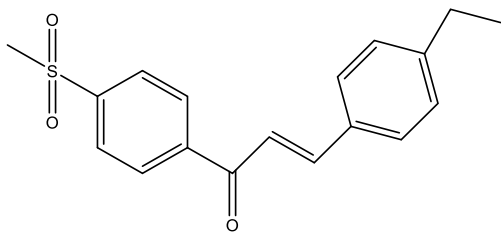


15.8 ppm, methyl group attached to a benzyl group; 21.9 ppm, methyl group next to a carbonyl; 56.1 ppm, methyl group next to an oxygen that is attached to a benzyl group; 127.8 ppm, two carbons of the benzyl group holding the sulfoxide; 130.2 ppm, two carbons of the benzyl group holding the sulfoxide; 146.6 ppm, carbon on benzyl group right next to sulfoxide; 141.1 ppm benzyl next to a carbonyl group; 171.0 ppm, carbonyl, next to a methyl group to the left and a nitrogen to the right; 189.7 ppm carbonyl; 121.3 ppm double bond next to carbonyl left and next to a double bond on the right; 145.1 ppm double bond next to double to the left and benzyl group to the right; 127.4 ppm, carbon benzyl next to double bond on the left; 127.6 ppm, carbon benzyl next to carbon benzyl attached to hydrocarbon chain on the left; 132.3 ppm, carbon benzyl next to carbon benzyl attached to hydrocarbon chain on the left and a carbon benzyl on the right; 157.8 ppm, carbon benzyl with an oxygen attached to the right ; 114.1 ppm, carbon benzyl with a carbon attached to an oxygen group to the right ; 124.4 ppm, carbon benzyl with a methyl group attached.

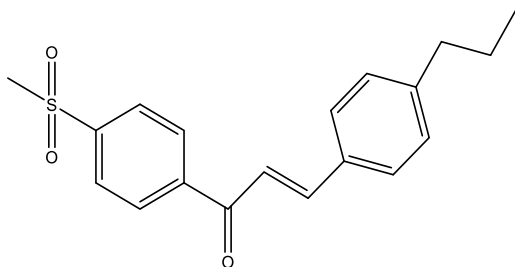
Appendix N

List of all Synthesized Compounds with their IUPAC Names

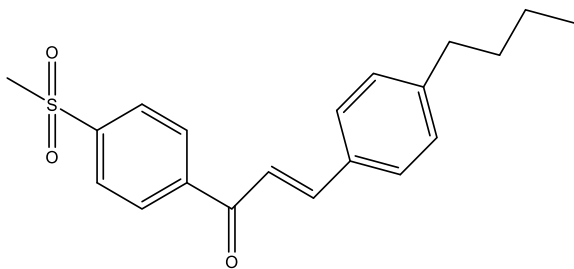
Study#1



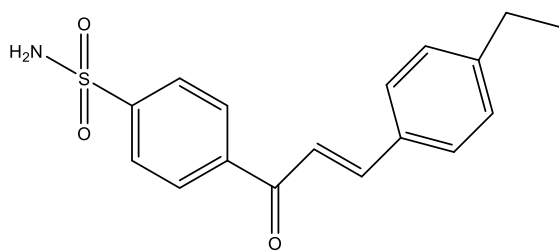
(*E*)-3-(4-ethylphenyl)-1-(4-(methylsulfonyl)phenyl)prop-2-en-1-one



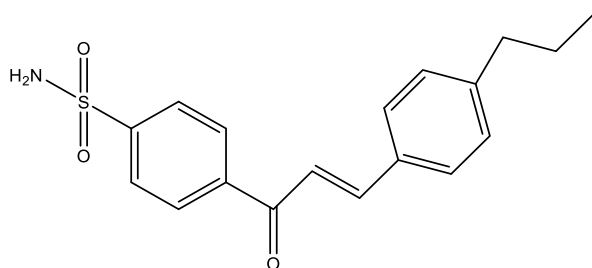
(*E*)-1-(4-(methylsulfonyl)phenyl)-3-(4-propylphenyl)prop-2-en-1-one



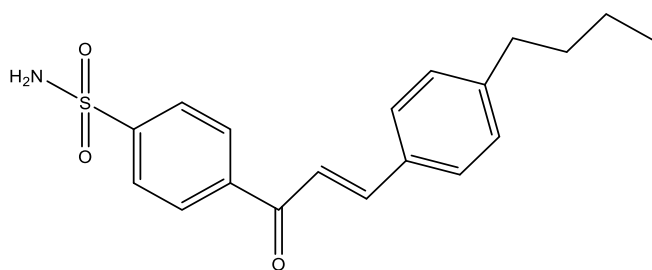
(*E*)-3-(4-butylphenyl)-1-(4-(methylsulfonyl)phenyl)prop-2-en-1-one



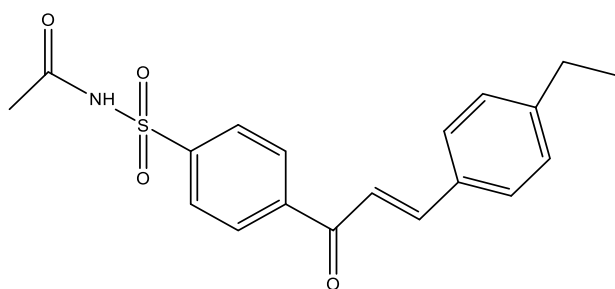
(*E*)-4-(3-(4-ethylphenyl)acryloyl)benzenesulfonamide



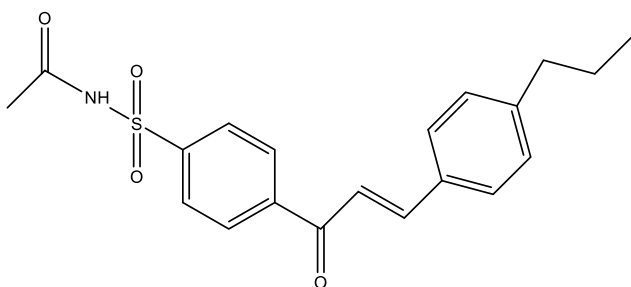
(*E*)-4-(3-(4-propylphenyl)acryloyl)benzenesulfonamide



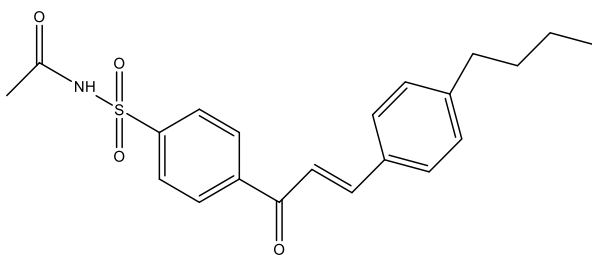
(*E*)-4-(3-(4-butylphenyl)acryloyl)benzenesulfonamide



(*E*)-*N*-((4-(3-(4-ethylphenyl)acryloyl)phenyl)sulfonyl)acetamide

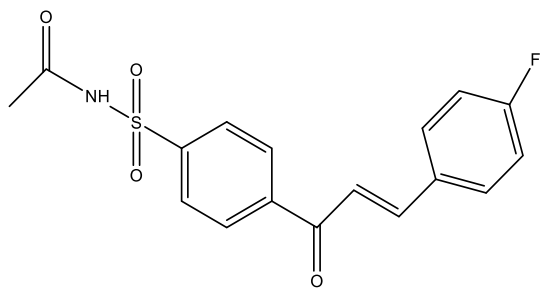


(E)-*N*-((4-(3-(4-propylphenyl)acryloyl)phenyl)sulfonyl)acetamide

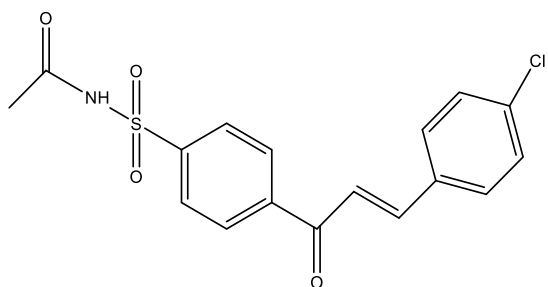


(E)-*N*-((4-(3-(4-butylphenyl)acryloyl)phenyl)sulfonyl)acetamide

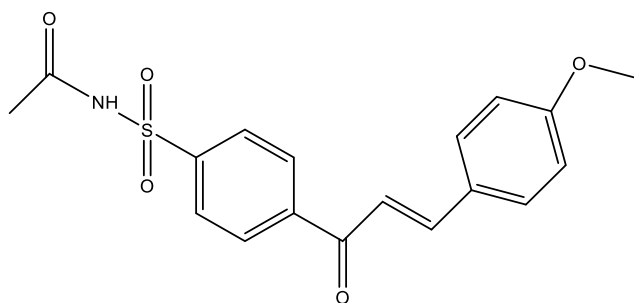
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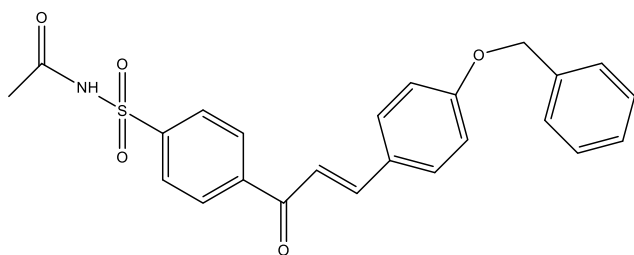
(E)-*N*-((4-(3-(4-fluorophenyl)acryloyl)phenyl)sulfonyl)acetamide



(E)-*N*-((4-(3-(4-chlorophenyl)acryloyl)phenyl)sulfonyl)acetamide

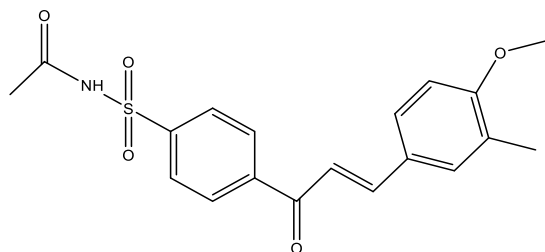


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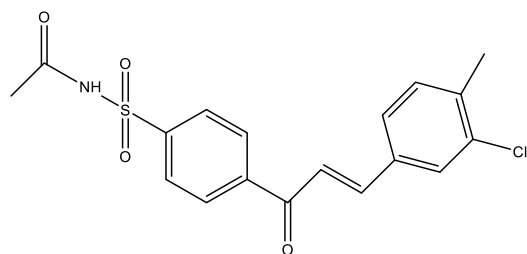


(E)-*N*-((4-(3-(4-(benzyloxy)phenyl)acryloyl)phenyl)sulfonyl)acetamide

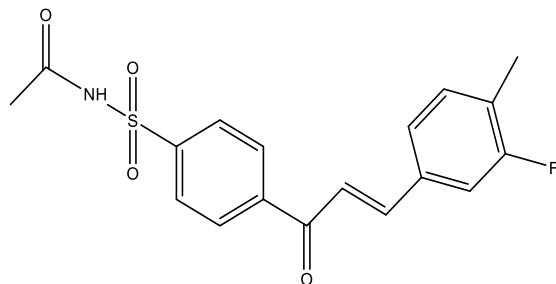
Study # 3



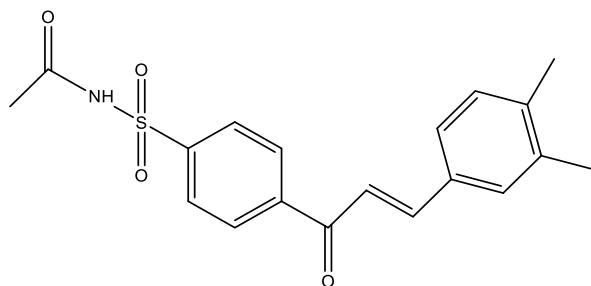
(E)-*N*-((4-(3-(4-methoxy-3-methylphenyl)acryloyl)phenyl)sulfonyl)acetamide



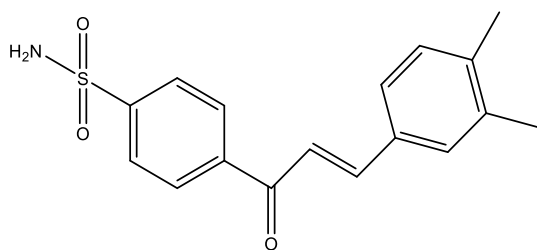
(*E*)-*N*-((4-(3-(3-chloro-4-methylphenyl)acryloyl)phenyl)sulfonyl)acetamide



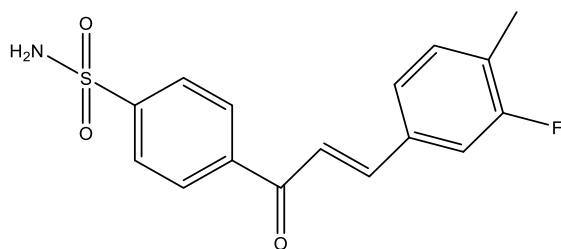
(*E*)-*N*-((4-(3-(3-fluoro-4-methylphenyl)acryloyl)phenyl)sulfonyl)acetamide



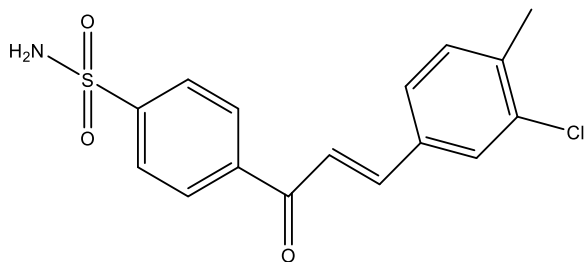
(*E*)-*N*-((4-(3-(3,4-dimethylphenyl)acryloyl)phenyl)sulfonyl)acetamide



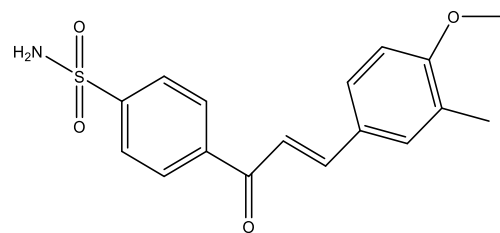
(*E*)-4-(3-(3,4-dimethylphenyl)acryloyl)benzenesulfonamide



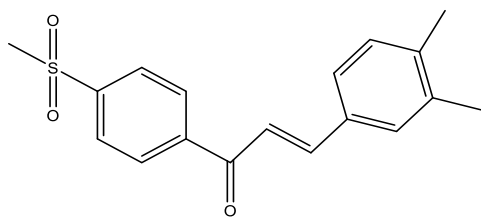
(*E*)-4-(3-(3-fluoro-4-methylphenyl)acryloyl)benzenesulfonamide



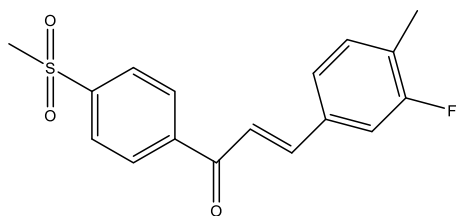
(*E*)-4-(3-(3-chloro-4-methylphenyl)acryloyl)benzenesulfonamide



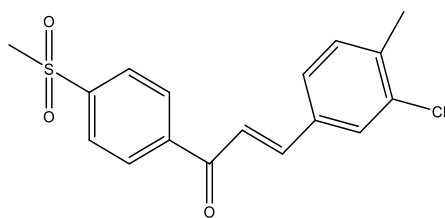
(*E*)-4-(3-(4-methoxy-3-methylphenyl)acryloyl)benzenesulfonamide



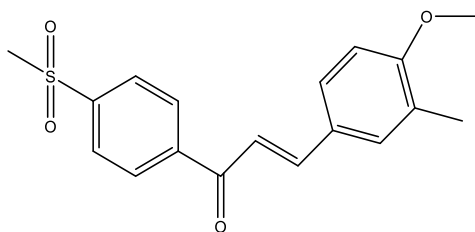
(*E*)-3-(3,4-dimethylphenyl)-1-(4-(methylsulfonyl)phenyl)prop-2-en-1-one



(*E*)-3-(3-fluoro-4-methylphenyl)-1-(4-(methylsulfonyl)phenyl)prop-2-en-1-one

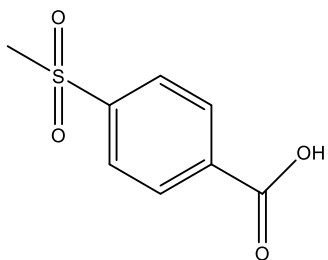


(*E*)-3-(3-chloro-4-methylphenyl)-1-(4-(methylsulfonyl)phenyl)prop-2-en-1-one

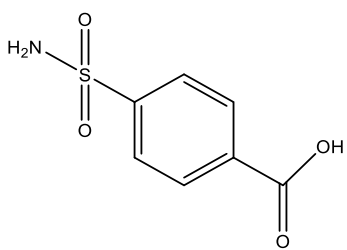


(*E*)-3-(4-methoxy-3-methylphenyl)-1-(4-(methylsulfonyl)phenyl)prop-2-en-1-one

Study # 4

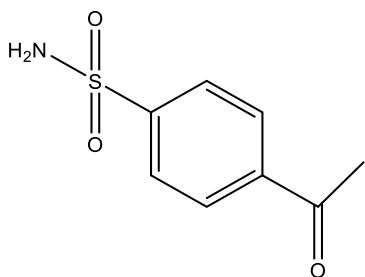


4-(methylsulfonyl)benzoic acid

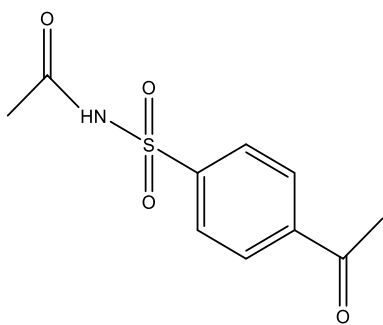


4-sulfamoylbenzoic acid

Intermediates



4-acetylbenzenesulfonamide



N-((4-acetylphenyl)sulfonyl)acetamide