universität wien

DIPLOMARBEIT / DIPLOMA THESIS

Titel der Diplomarbeit / Title of the Diploma Thesis

„Contributions to the Synthesis of Amides of Bumetanide“

verfasst von / submitted by

Xindi Wu

angestrebter akademischer Grad / in partial fulfilment of the requirements for the degree of

Magistra der Pharmazie (Mag. Pharm.)

Wien, 2019 / Vienna, 2019

Studienkennzahl lt. Studienblatt / degree programme code as it appears on the student record sheet:	A 449
Studienrichtung lt. Studienblatt / degree programme as it appears on the student record sheet:	Pharmazie
Betreut von / Supervisor:	Ao. Univ.-Prof. Mag. Dr. Thomas Erker
Mitbetreut von / Co-Supervisor:	

1 Acknowledgment

First and foremost, I would like to express my gratitude to my supervisor Ao. Univ. -Prof. Dr. Thomas Erker for enabling this diploma thesis and the excellent supervision.

On top of that, I owe a great debt of gratitude to Mag. pharm. Philipp Schreppel for patiently answering my questions.

I would like to thank Dipl.-Ing. (FH) Mario Gabriel and Mag. pharm. Michael Hintersteininger for all the support I received and the pleasant working atmosphere.

A special word of thanks to Saliha for her support during my studies and pushing me successfully through my exams.
Furthermore, I highly appreciate my fellow students Ceyhan, Medine and Thao My for their advices.
I would like to express my gratitude to Katja for her culinary supports while learning.

Especially, I would like to thank my best friend Vero for being there for me through my ups and downs.
Special recognition goes to my sisters Elisabeth and Jessica for their consistent support.
Moreover, I am highly grateful for my boyfriend Jeremy being by my side through all the hardships.

Last but not least, I would like to thank my parents for the tremendous and unconditional support throughout my studies.

I am deeply grateful and blessed for all the people in my life and I will not take each of you for granted.

2 Abstract

The aim of this diploma thesis was to synthesize derivatives of bumetanide to increase the lipophilicity of the structure. This allows the drug to diffuse better through the blood-brain barrier, resulting in greater biological activity due to increased inhibition of the sodium-potassium-chloride cotransporter (NKCC1) which is also expressed in the brain. The inhibition of NKCC1 lowers the intracellular chloride concentration. Therefore, the activation of the $GABA_A$-ionic channel now acts inhibitory instead of excitatory in immature brain cells, leading to a reduction of convulsions and epileptic seizures.

A total number of eleven compounds was synthesized. In case of the compounds **1**, **2** and **8** an amide was formed from the carboxyl group, whereas in compound **3** the carboxyl group was esterified, causing a higher lipophilicity. Compound **9** originates from compound **8** by thionation of the carbonyl group. Compound **5** was formed from compound **4**, that in turn used compound **3** as starting reagent. Compound **5** was used for the synthesis of following compounds: **6**, **7**, **10** and **11**.
Further *in-vitro* and *in-vivo* testing needs to be performed to verify that more drug molecules can cross the blood-brain barrier and block NKCC1.

Ziel dieser Diplomarbeit war es, Bumetanidderivate zu synthetisieren, um die Lipophilie der Struktur zu erhöhen. Durch die veränderte Struktur kann der Arzneistoff die Blut-Hirn-Schranke besser durchdringen und seinen Wirkort erreichen. Wirkort der Verbindungen ist der NKCC1-Transporter, der unter anderem auch im Gehirn exprimiert wird. Durch die erhöhte Lipophilie der Struktur soll dieser verstärkt blockiert werden. Auf diese Weise kann die intrazelluläre Chlorid-Konzentration gesenkt werden, wodurch die Aktivierung des $GABA_A$-Kanals eine inhibitorische statt einer exzitatorischen Wirkung im neonatalen Gehirn aufweist. Dies hat zur Folge, dass epileptische Anfälle und Krämpfe reduziert werden können.

Es wurden im Rahmen dieser Diplomarbeit insgesamt elf Derivate von Bumetanid synthetisiert. Bei den Derivaten **1**, **2** und **8** wurde ein Amid aus der Säuregruppe gebildet, während bei Derivat **3** die Carboxylgruppe verestert wurde, was zu einer erhöhten Lipophilie der Struktur führt. Derivat **9** wurde aus Derivat **8** durch Thionierung der Amidteilstruktur synthetisiert.

Derivat **5** wurde aus Derivat **4** gebildet, das sich wiederum von Derivat **3** ableitet. Derivat **5** dient als Ausgangsmaterial für die folgenden Derivate: **6**, **7**, **10** und **11**.

Es sind weitere *in-vitro* and *in-vivo* Testungen nötig, um signifikant zeigen zu können, dass durch erhöhte Lipophilie nun mehr Arzneistoffmoleküle die Blut-Hirn-Schranke passieren und NKCC1 blockieren können.

Inhaltsverzeichnis

3 Introduction

3.1 Epilepsy

Epilepsy is on the third rank of the most common neurological diseases and is characterized by persistent seizure susceptibility and by emotional and cognitive dysfunction. Approximately 50 million people suffer from this type of condition worldwide. (Vezzani, French, Bartfai, & Baram, 2010)

Epilepsy can be a consequence of brain-alterations, caused by head trauma, infections of the central nervous system, inflammations and tumors. (Vezzani, et al., 2015)

There are three major categories of epilepsy which can be differentiated on the basis of etiology: idiopathic, symptomatic/acquired and presumed symptomatic (or "cryptogenic"). While the idiopathic form is based on genetic abnormalities, symptomatic/acquired and cryptogenic epilepsies originate from epileptic lesions. These lesions can be focal, for instance a tumor, or a dysfunction in metabolism leading to major damage in the brain. (Löscher & Brandt, 2010)

There are many types of seizures which can be roughly classified as focal/partial, generalized (primary and secondary) and *status epilepticus*. (Cherian & Thomas, 2009)

Status Epilepticus (SE) may be caused by a failure of mechanisms which are responsible for seizure termination or by initiation of mechanisms which induce abnormally prolonged seizures. It can lead to neuronal injury, altered neuronal activity and neuronal death.

SE can be divided in two groups: convulsive and non-convulsive. Convulsive SE is described as abnormal muscle contractions that occur in episodes which are usually bilateral, whereas the non-convulsive type is characterized by coma and minor motor phenomena, resembling the so called subtle status. (Trinka, et al., 2015)

3.2 Neonatal Seizures

Neonatal seizures (also known as neonatal convulsions) are epileptic spasms, which may occur during the neonatal period. During this period, epileptic seizures are more frequently than at any other stages in life, mainly in the first days until the first week after birth. Approximately,

3 per 1000 live births are affected by neonatal seizures, while the incidence in preterm infants is even higher.

However, neonatal seizures often imply serious malfunction or damage to the infantile brain. 80% of all seizures in the neonatal period arise through hypoxic-ischemic encephalopathy. Other reasons for seizures in neonates are intracranial hemorrhage, infarction, stroke, prenatal and neonatal infections. (Panayiotopoulos, 2005, Chapter 5)

It has been proven that neonatal seizures can lead to permanent and severe cognitive and behavorial abnormalities and a higher epileptogenicity in adult age. (Khanna, Walcott, & Kahle, 2013)

3.3 GABA

It is known that γ-aminobutyric acid (GABA) is one of the major neurotransmitters. In the early development phase, it has an excitatory effect and is involved in many processes of neurogenesis. In adult age, the excitatory effect changes into an inhibitory one. At this stage, GABA is implicated in the development of interstitial neurons of the white matter and in oligodendrocyte development.

The GABA receptors are mainly divided in two types, the ionotropic $GABA_A$ receptor and the metabotropic $GABA_B$ receptor. (Wu & Sun, 2014) The binding of GABA to the $GABA_A$ receptor results in a Cl^- influx or outflow, depending on the Cl^--equilibrium potential of the cell. In addition to Cl^-, bicarbonate can also pass through the $GABA_A$ channel, whereby the effect increases. (Maa, Kahle, Walcott, Spitz, & Staley, 2011)

The intracellular concentration of chloride $[Cl^-]_i$ of immature neurons is high, therefore GABA leads to a Cl^- efflux and thus a membrane depolarization which implies excitation of the neuron. Due to a low $[Cl^-]_I$ of mature neurons, the activation of $GABA_A$-ionic channels results in an influx of Cl^- and thus a membrane hyperpolarization which indicates inhibition. (Kambli, Bhatt, Oza, & Prabhavalkar, 2017) Consequently, this explains why GABA is excitatory in the developing brain and inhibitory in adult age.

The seizure activity leads to an increase of $[Cl^-]_i$ which enhances the depolarizing effect of $GABA_A$-receptor activation. (Maa, Kahle, Walcott, Spitz, & Staley, 2011)

3.4 Chloride Homeostasis

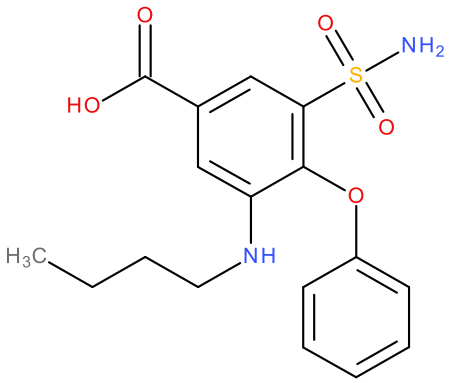
$[Cl^-]_i$ of neurons is mainly determined by the activities of two Cl^--cotransporters NKCC1 and KCC2 which belong to the family SLC12A. NKCC1 is considered as a symporter that imports sodium ions (Na^+), potassium ions (K^+) and Cl^- in a stoichiometry of 1Na:1K:2Cl. KCC2 is also a symporter which exports one K^+ along with one Cl^-. (Maa, Kahle, Walcott, Spitz, & Staley, 2011)
The higher expression of the NKCC1 transporter in neuronal membranes of neonates leads to a higher $[Cl^-]_i$, which explains the excitatory effect of GABA. In contrast, the higher KCC2 expression results in a low $[Cl^-]_i$, causing the inhibitory effect of GABA. (Maa, Kahle, Walcott, Spitz, & Staley, 2011)

3.5 Current Therapy of Epilepsy and Neonatal Seizures

Usually spasms can be terminated without medication. The use of antiepileptic drugs such as benzodiazepines, phenobarbital or phenytoin may shorten or even terminate extended seizures. (Panayiotopoulos, 2005)
Antiepileptic drugs (AEDs) mainly exert their effect through modulation of voltage-gated sodium or calcium ion channels, such as phenytoin. However, some of the AEDs, for example benzodiazepine and phenobarbital act by increasing the effect of GABA. Due to a higher $[Cl^-]_i$ of neonatal neurons, the response of the activation of $GABA_A$-receptor has an excitatory effect instead of an inhibitory one. Therefore, those AEDs that enhance the effect of GABA are rather ineffective in the neonatal period. However, phenobarbital is still used as first line AED in the treatment of neonatal seizures, although it has long-term effects on brain development. (Rennie & Boylan, 2007)
Therefore, there is a great demand for more efficient drugs.

3.6 Bumetanide

Scheme 1: Bumetanide



Bumetanide, belonging to the group called loop diuretics, is used in the treatment of congestive heart failure, pulmonary edema and essential hypertension. (Kelso, McDermott, Silke, & Spiers, 2000)

The target of bumetanide is the membrane transport protein NKCC which can be divided into two isoforms: NKCC1 which is ubiquitously expressed especially within the brain and NKCC2, which is to be found in the kidney, causing the diuretic effect. (Töllner , et al., 2014)

Due to the diuretic effect the chronic use of bumetanide can cause hypokalemic alkalosis. A further disadvantage for the potential treatment of neurological disorders is that bumetanide is highly dissociated at physiological pH. Therefore, it can barely penetrate the blood-brain barrier, while it is accumulated in the kidney, leading to the diuretic effect.

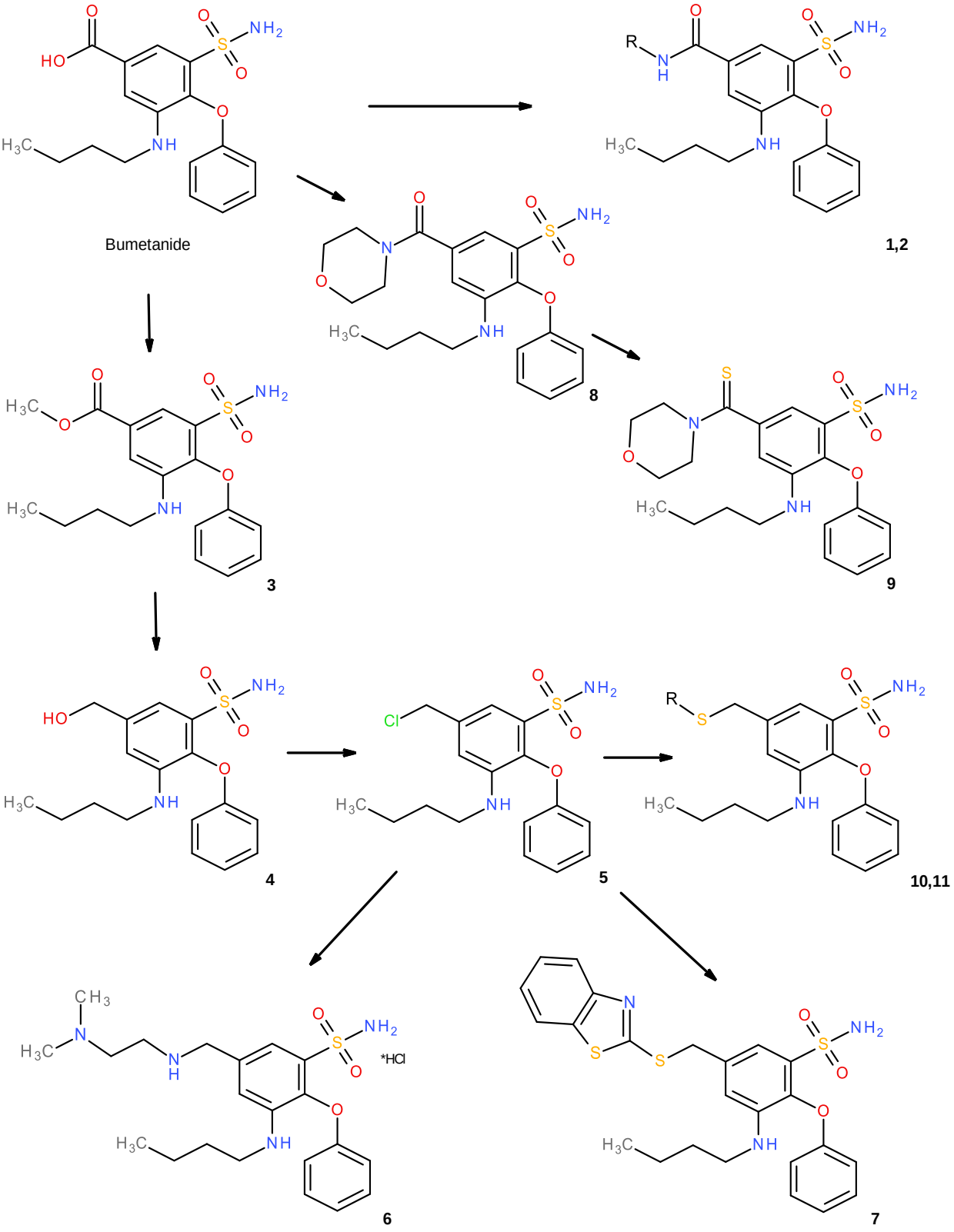
Hence, the development of more lipophilic derivatives of bumetanide is a promising approach to selectively inhibit NKCC1 in the brain. (Töllner , et al., 2014)

4 Objective

Bumetanide has great potential to be used in the treatment of neonatal seizures. However, due to its chemical properties, it achieves only very low concentrations in the brain. With the aim of increasing lipophilicity through synthesis of derivatives of bumetanide, more molecules are able to cross the blood-brain barrier. A higher concentration at the site of action results in increased reduction of $[Cl^-]_i$ in immature neurons and consequently in lower seizure activity. Therefore, bumetanide derivatives were synthesized.

5 Synthesis of Bumetanide Derivatives

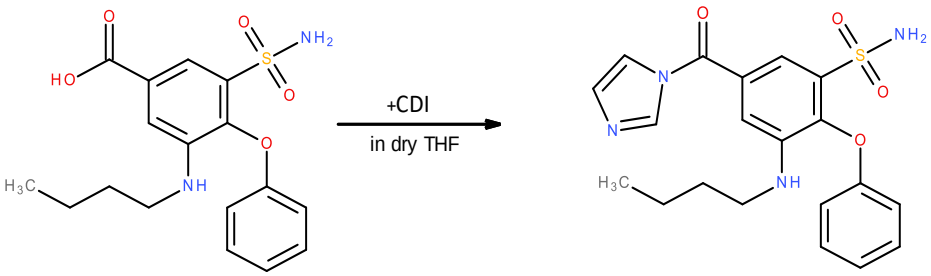
Scheme 2: Synthesis Overview for Compounds 1-11



5.1 Compound **1, 2** and **8**

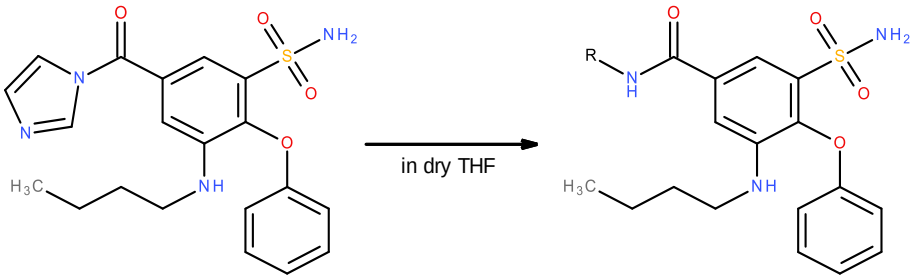
The aim in the first step of the synthesis was the activation of the carboxylic group of bumetanide which is dissolved in dry tetrahydrofuran (THF) by adding *N,N*-carbonyldiimidazole (CDI).

Scheme 3: Formation of Bumetanide Imidazolide



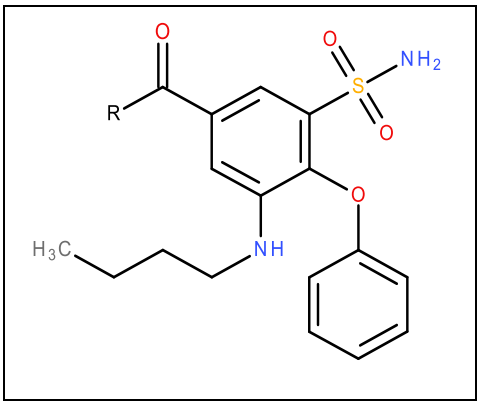
Bumetanide | Bumetanide Imidazolide

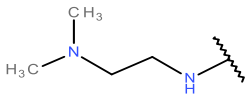
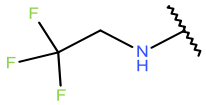
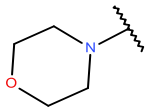
Scheme 4: Synthesis of Derivatives of Bumetanide



Bumetanide Imidazolide | Derivatives of Bumetanide

Compounds **1**, **2** and **8**



Compounds	**R**
Compound **1**	
Compound **2**	
Compound **8**	

After the formation of bumetanide imidazolide, which was promoted by CDI, *N,N*-dimethylethylenediamine (DMEDA) was added to form compound **1**.

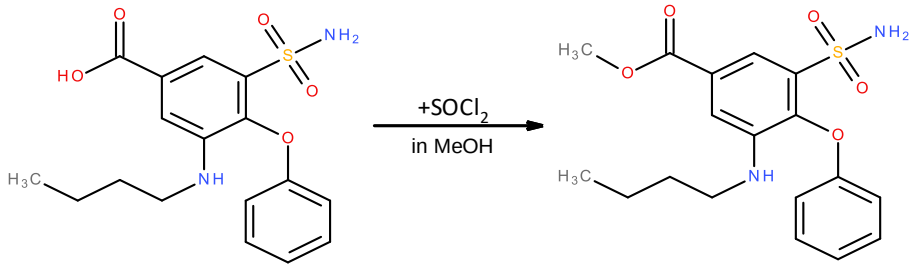
For the synthesis of compound **8,** morpholine was added to the mixture.

All these reactions were stirred overnight.

Compound **8** was used as starting reagent for the synthesis of compound **9**.

5.2 Compound **3**

Scheme 7: Esterification of the Carboxylic Group of Bumetanide (Compound 3)



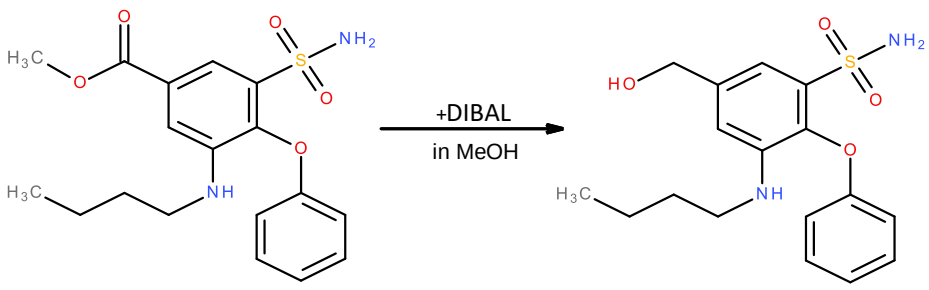
Bumetanide Compound **3**

Bumetanide was dissolved in MeOH and thionyl chloride was cautiously added for the esterification. The reaction was stirred overnight and delivered a yield of 96%. For the synthesis of compound **4**, compound **3** was used as a starting material.

5.3 Compound **4**

The main objective was to reduce the ester of compound **3** to the corresponding alcohol. Therefore, compound **3** was dissolved in dry THF and stirred overnight. Diisobutylaluminium hydride (DIBAL) was used for the reduction of the ester to the corresponding alcohol. Compound **4** was used for the synthesis of compound **5**.

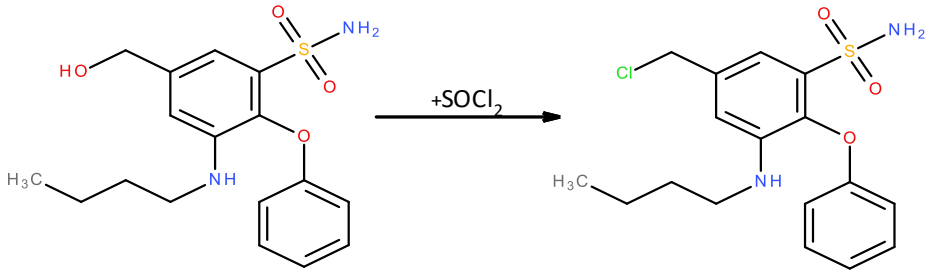
Scheme 8: Reduction of the Ester Group (Compound 4)



Compound **3** Compound **4**

5.4 Compound **5**

Scheme 9: Conversion of the Alcohol to the Corresponding Alkyl Chloride (Compound 5)

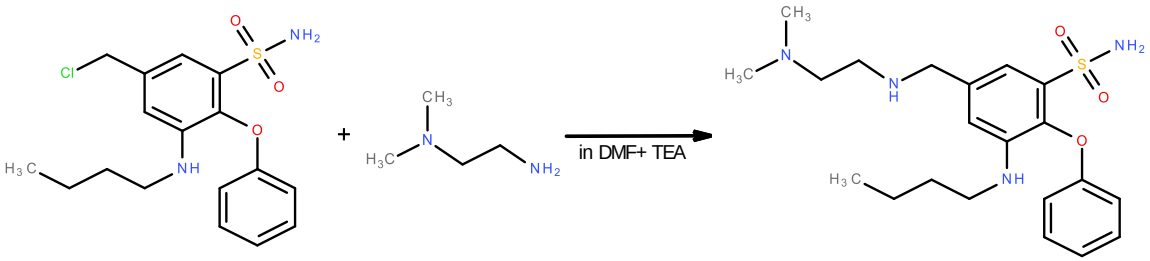


Compound **4** Compound **5**

Alcohols have their limits as reactants because they interfere with many of the reactions with their hydroxyl groups, such as nucleophilic acyl substitution. The conversion of alcohol into alkyl chloride gives rise to a starting material for the synthesis of compounds **6**, **7**, **10** and **11**. Therefore, compound **4** was dissolved in thionyl chloride to form compound **5**.

5.5 Compound **6**

Scheme 10: Synthesis of Compound 6

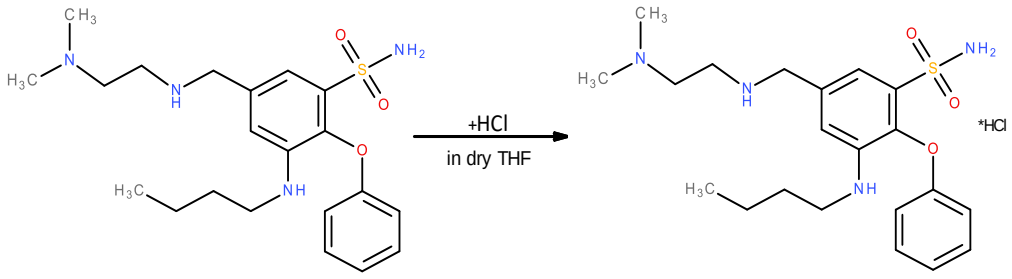


Compound **5** DMEDA Amine of Compound **6**

The aim was to increase the basicity of the structure. Therefore, compound **5** was dissolved in a solution consisting of *N,N*-dimethylformamide (DMF) and triethylamine (TEA) in the ratio of 5:1 and *N,N*-dimethylethylenediamine was added to the mixture. The reaction was stirred overnight.

Converting the insoluble, basic structure of the product into hydrochloride will increase its solubility in water and the bioavailability. After the conversion the product was purified by recrystallization.

Scheme 11: Conversion of Amine 6 into a Hydrochloride

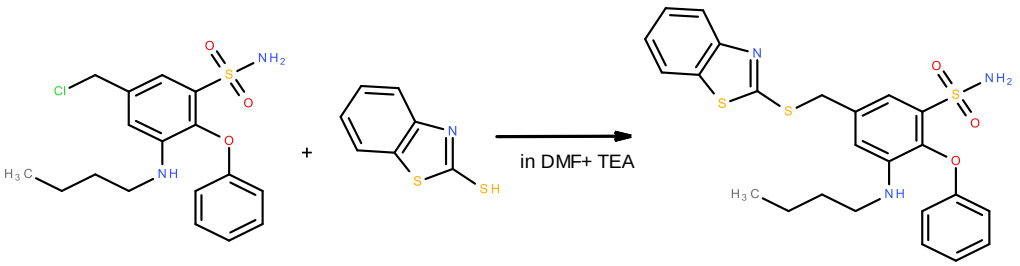


Amine of Compound **6** Compound **6**

5.6 Compound **7**

For the synthesis of compound **7,** compound **5** was dissolved in a solution consisting of DMF and triethylamine (TEA) in the ratio of 3:1 and 2-mercaptobenzoimidazole was added to the mixture. It was stirred overnight, and, in addition, column chromatography was performed to separate compound **7** from other components.

Scheme 12: Synthesis of Compound 7

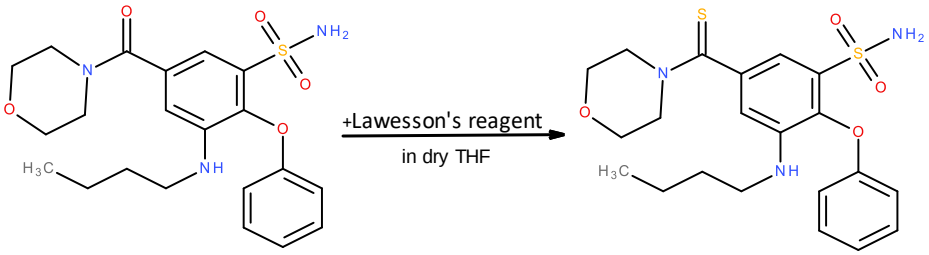


Compound **5** 2-Mercaptobenzimidazole Compound **7**

5.7 Compound **9**

For the synthesis of compound **9,** compound **8** was dissolved in dry THF and Lawesson's reagent was added to the mixture with the aim to convert the carbonyl group of compound **8** into a thiocarbonyl group. In addition, flash column chromatography was performed to accomplish a higher purity of the product.

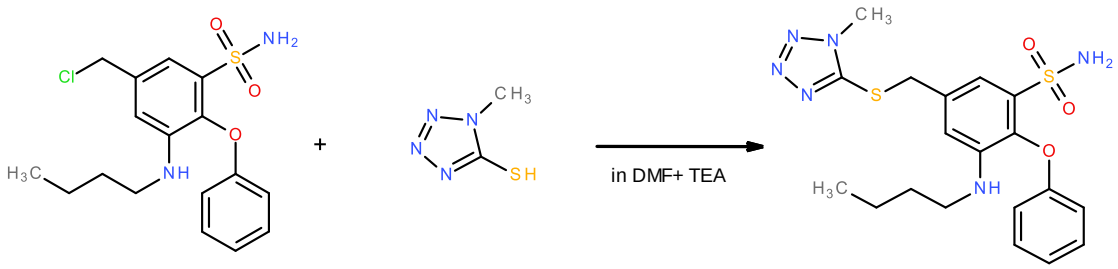
Scheme 13: Thionation of the Carbonyl Group (Compound 9)



Compound **8** Compound **9**

5.8 Compound **10, 11**

Scheme 14: Synthesis of Compound 10

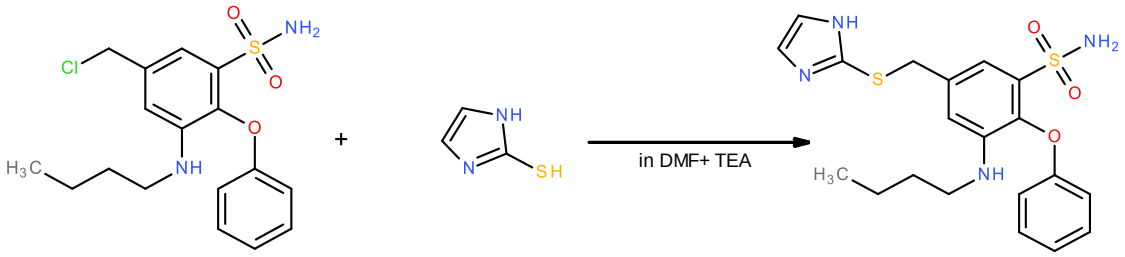


Compound **5** 5-Mercapto-1-methyltetrazole Compound **10**

Compound **5** was dissolved in a solution consisting of DMF and TEA in the ratio of 3:1 and 5-mercapto-1-methyltetrazole was added to form compound **10**. Tetrazoles have a higher stability to the metabolism and they can also act as bioisosteres for carboxylate groups because they have similar pKa and are deprotonated at physiological pH.

For the synthesis of Compound **11** 2-mercaptoimidazole was added to the mixture.

Scheme 15: Synthesis of Compound 11



Compound **5** 2-Mercaptoimidazole Compound **11**

6 Discussion

The aim of this diploma thesis was to synthesize derivatives of bumetanide that are able penetrate the blood-brain barrier better than bumetanide itself and thus can inhibit NKCC 1 more intensively. Bumetanide can barely penetrate die blood-brain barrier because it has a strong protein binding and it is also highly ionized at physiological pH. Hence, we decided to synthesize lipophilic prodrugs, which are able to penetrate the blood-brain barrier faster than bumetanide itself. (Töllner , et al., 2014)

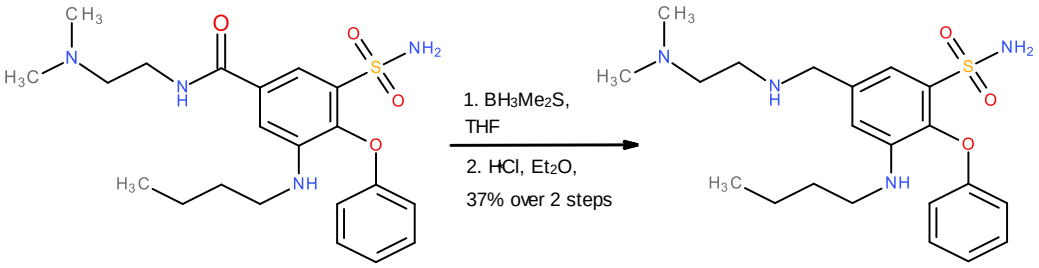
Studies showed that ester prodrugs of bumetanide resulted in a significantly higher drug concentration in the brain than the parent drug. However, the methyl ester of bumetanide (BUM 6, prodrug) has a short half-life, because it was quickly metabolized in the periphery. The *N,N'*-dimethylaminoester prodrug of bumetanide (BUM 5, prodrug) was more effective than bumetanide in enhancing the anticonvulsive effect of phenobarbital. Compound BUM 5 is more alkaline than that of BUM 6. (Töllner , et al., 2014).

An increased basicity of the structure, especially the dimethylamino-group seems to enhance the biological activity. Hence, we have synthesized amide and amine derivatives of bumetanide. Bumetanide ester prodrugs such as BUM 5 are rapidly metabolized and therefore less effective. Whereas amine derivatives of bumetanide such as compound **6** should be more stable.

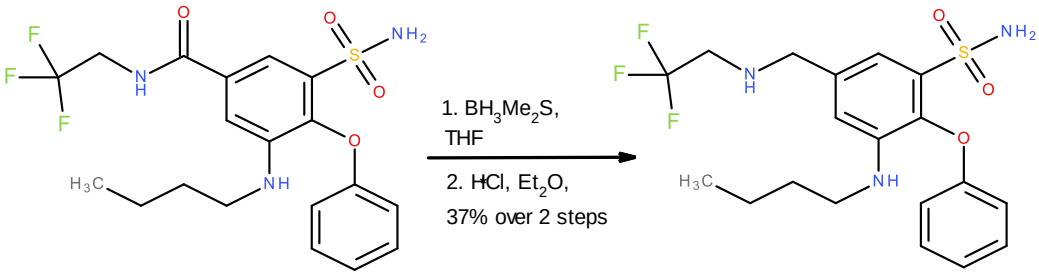
Therefore, the main objective was the conversion of amide groups of compound **1**, **2** and **8** into amines. The reduction could most likely occur via the dimethylsulfide complex. In this way, the basicity of the structures could be increased, and we can compare the effect of amide and amine derivatives of bumetanide.

Unfortunately, the timeframe was too tight and therefore the experiments could not be carried out.

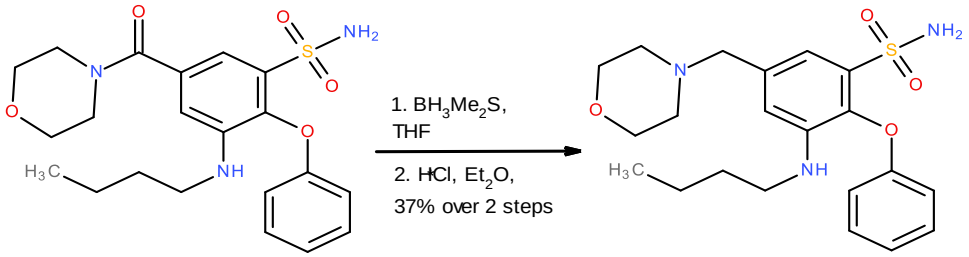
Scheme 15: Reduction of Amide into Amine



Compound **1** — Amine of Compound **6**



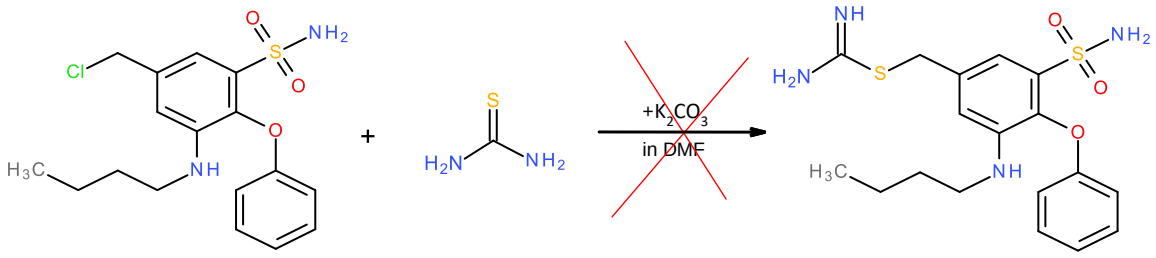
Compound **2** — BUM 66



Compound **8** — Amine of Compound **8**

We found other ways which used compound **5** as starting reagent to synthesize amine derivatives of bumetanide. Further research needs to be performed to synthesize the other compounds.

Scheme 15: Failed Attempt to react with Thiourea



Compound **5** thiourea 2-[[3-(butylamino)-4-phenoxy-5-sulfamoyl-phenyl]methyl]isothiourea

Furthermore, we tried to attach a thiourea to bumetanide to alter the basicity and thus the biological activity. Therefore, compound **5** was dissolved in DMF which was charged with potassium carbonate (K_2CO_3), generating an alkaline reaction environment. To this solution, thiourea was added to form 2-[[3-(butylamino)-4-phenoxy-5-sulfamoyl-phenyl]-methyl]-isothiourea. The mixture was stirred overnight at room temperature. The NMR showed that the thiourea was not converted.

Even after several attempts with higher temperatures of 60°C and 80°C, the reactions still failed.

Further research needs to be performed to synthesize these compounds, however, this would go beyond the scope of this diploma thesis.

7 Experimental section

7.1 General methods

All chemicals and solvents were acquired from commercial manufacturers (Apollo Scientific, Merck, Sigma Aldrich and TCI Europe) at analytical grade.

Chemical reactions were observed *via* thin layer chromatography, silica gel F254 coated aluminum sheets from Merck were used for it.

Silica gel 60 70 – 230 mesh ASTM from Merck was used as a stationary phase for column chromatography.

The measurements of melting points were performed on a Thermo Galen Kofler hot stage microscope.

^{1}H- and ^{13}C-NMR spectra were generated on a Bruker Advance (200 and 50 MHz respectively) and chemical shifts are specified in ppm relatively to the solvent residual line or tetramethylsilane as internal standard.

High-resolution mass spectra were performed on a MALDI-q/q-TOF-spectrometer (Bruker maxis HD) by Dr. Judith Wackerlig at the University of Vienna.

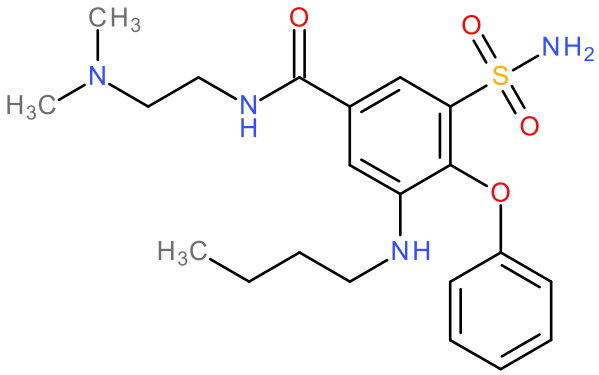
Mass spectra were recorded on a Shimadzu (GC-17A; MS-QP5050A) spectrometer. The peak intensity is stated in per cent relatively to the biggest signal in the spectrum.

Elemental analyses were performed by Mag. Johannes Theiner at the University of Vienna and all reported values are within +/- 0.4% of the calculated values.

7.2 Compound **1**

3-(Butylamino)-N-[2-(dimethylamino)-ethyl]-4-phenoxy-5-sulfamoyl-benzamide

Internal code: BUM 10



Molecular formula	$C_{21}H_{30}N_4O_4S$
Molecular weight	434.55 g/mol
Melting point	73°C-78°C

(Töllner , et al., 2014)

A novel prodrug-based strategy to increase effects of bumetanide in epilepsy

Annals of Neurology

2.4 mmol (392 mg) CDI were dissolved in a solution consisting of 2 mmol (729 mg) of bumetanide and 10 mL dry THF. The mixture was stirred at room temperature overnight. After the thin layer chromatography did not show any bumetanide remaining, 2.4 mmol (435 µL) of *N,N*-dimethylethylenediamine were added and the mixture was stirred at room temperature overnight. Once the reaction was completed, it was poured into 20 mL of ethyl acetate and washed with 15 mL water and 5 mL 2N hydrochloric acid. The aqueous phase was alkalinized to pH 8 with 2N sodium hydroxide solution and extracted with ethyl acetate. The organic phase was dried over Na_2SO_4 and the solvent was removed under reduced pressure (yield: 631 mg, 73% of compound **1**).

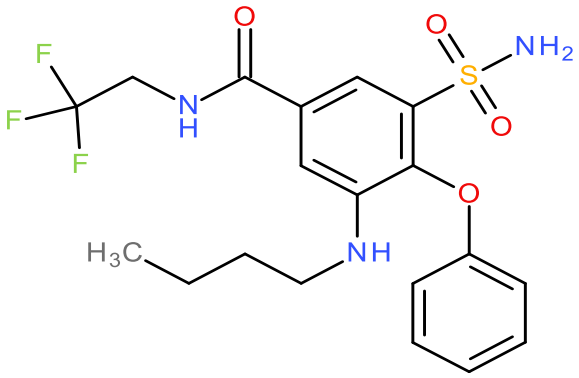
^{1}H NMR (200 MHz, DMSO-d_6) δ 7.71 (d, *J* = 2.2 Hz, 1H), 7.44 (d, *J* = 2.2 Hz, 1H), 7.36 – 7.23 (m, 2H), 7.14 – 7.00 (m, 1H), 6.97 – 6.82 (m, 2H), 3.57 (t, *J* = 6.6 Hz, 2H), 3.13 (t, *J* = 6.7 Hz, 2H), 2.67 (t, *J* = 6.6 Hz, 2H), 2.39 (s, 6H), 1.49 – 1.32 (m, 2H), 1.25 – 1.05 (m, 2H), 0.97 – 0.67 (m, 3H).

^{13}C NMR (50 MHz, DMSO-d_6) δ 166.9, 155.6, 148.7, 141.7, 138.3, 136.1, 130.9, 128.4, 121.8, 114.4, 112.8, 112.4, 57.0, 43.2, 41.5, 36.3, 29.8, 18.6, 11.8.

7.3 Compound **2**

3-(Butylamino)-4-phenoxy-5-sulfamoyl-N-(2,2,2-trifluoroethyl)-benzamide

Internal code: BUM 95



Molecular formula	$C_{19}H_{22}F_3N_3O_4S$
Molecular weight	445.46 g/mol
Melting point	193.5°C-197°C

(Schreppel, 2015)

1.2 mmol (194 mg) CDI were dissolved in a solution consisting of 1 mmol (368 mg) of bumetanide in 5 mL dry THF. Thereafter, the mixture was stirred for three hours. The thin layer chromatography showed bumetanide remaining, therefore 0.3 mmol (61 mg) CDI were added to the mixture additionally. After the thin layer chromatography did not show any bumetanide remaining, 2 mmol (157 µL) of trifluoroethylamine were added and the mixture was stirred at room temperature overnight. As soon as the reaction was completed, it was poured into 20 mL of 5% $NaHCO_3$ and extracted with ethyl acetate. The organic phase was then dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was then purified by recrystallization from EtOH to yield 138 mg of white powder (yield: 138 mg, 31% of compound **2**).

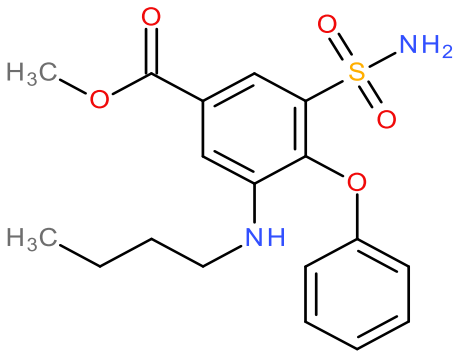
^{1}H NMR (200 MHz, MeOD) δ 7.73 (d, *J* = 2.1 Hz, 1H), 7.44 (d, *J* = 2.1 Hz, 1H), 7.38 – 7.18 (m, 2H), 7.11 – 6.99 (m, 1H), 6.99 – 6.84 (m, 2H), 4.10 (q, *J* = 9.3 Hz, 2H), 3.13 (t, *J* = 6.8 Hz, 2H), 1.52 – 1.33 (m, 2H), 1.25 – 1.06 (m, 2H), 0.82 (t, *J* = 7.2 Hz, 3H).

^{13}C NMR (50 MHz, MeOD) δ 167.9, 156.4, 142.7, 139.5, 137.1, 132.9, 130.9, 129.2, 124.5 (q *J* = 278.2 Hz), 122.6, 115.2, 113.7, 113.3, 42.3, 40.5 (q, *J* = 34.7 Hz), 30.6, 19.4, 12.6.

7.4 Compound **3**

Methyl 3-(butylamino)-4-phenoxy-5-sulfamoyl-benzoate

Internal code: BUM 6



Molecular formula	$C_{18}H_{22}N_2O_5S$
Molecular weight	378.44 g/mol
Melting point	135°C-142°C

(Germany Patent No. DE 1964503, 1970)

Sulfamylbenzoic acid derivatives

Ger. Offen

(Schreppel, 2015)

10.4 mmol (3.79 g) of bumetanide were added to 15 mL of MeOH to form a suspension. 22 mmol (1.6 mL) of thionyl chloride were added to this mixture resulting in a clear, yellow solution. Thereafter, it was stirred at room temperature under argon atmosphere overnight resulting in a brown solution. After the reaction was completed, the mixture was poured into 80 mL of 5% aqueous $NaHCO_3$-solution and extracted three times with ethyl acetate. The combined organic phase was washed twice with water and after that once with brine. The organic phase was dried over Na_2SO_4 and the vacuum dried to yield 3.786 g of a white solid (yield: 3 786 mg, 96% of compound **3**).

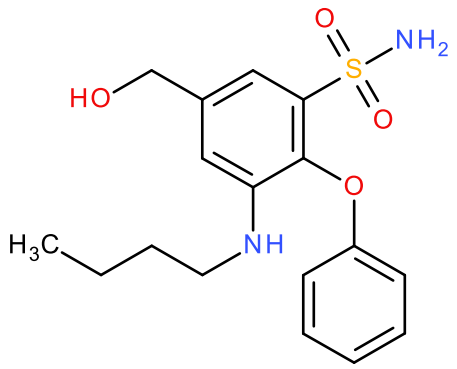
^{1}H NMR (200 MHz, $CDCl_3$) δ 7.94 (d, *J* = 2.0 Hz, 1H), 7.57 (d, *J* = 2.0 Hz, 1H), 7.40 – 7.21 (m, 2H), 7.19 – 7.01 (m, 1H), 6.99 - 6.84 (m, 2H), 5.01 (s, 2H), 3.93 (s, 3H), 3.09 (t, *J* = 6.9 Hz, 2H), 1.55 – 1.27 (m, 2H), 1.26 – 1.04 (m, 2H), 0.82 (t, *J* = 7.2 Hz, 3H).

^{13}C NMR (50 MHz, $CDCl_3$) δ 166.1, 155.8, 142.3, 139.9, 136.2, 130.2, 128.1, 123.9, 116.7, 116.5, 115.4, 52.6, 43.1, 31.0, 19.9, 13.8.

7.5 Compound **4**

3-(Butylamino) -5-(hydroxymethyl) -2-phenoxy-benzenesulfonamide

Internal code: BUM 7



Molecular formula	$C_{17}H_{22}N_2O_4S$
Molecular weight	350.43 g/mol
Melting point	158°C-159°C

(Germany Patent No. DE 2630637, 1977)

Sulfamoylbenzyl compounds in drug preparations

Ger. Offen

(Schreppel, 2015)

To 15 mL anhydrous THF 10.8 mmol (4.08 g) methyl 3-(butylamino)-4-phenoxy-5-sulfamoyl-benzoate (compound **3**, internal code: BUM 6) were added and the mixture was stirred at room temperature. Thereafter 20 mL of a 1 M di-isobutyl aluminum hydride (DIBAL-H) solution in toluene were added. After one, two, three and four hours, respectively, another 10 mL of the 1 M DIBAL -H solution in toluene were added each time. After six hours the mixture was cooled to 0°C and quenched with 5% aqueous $NHCl_4$-solution causing a gel-like substance to precipitate. The precipitate was then dissolved in 2N HCl and extracted three times with ethyl acetate. The combined organic layers were washed three times with water,

once with saline and dried over with Na_2SO_4. The fluids were evaporated under reduced pressure to obtain 2.83 g of a light brown solid (yield: 2 832 mg, 75% of compound **4**).

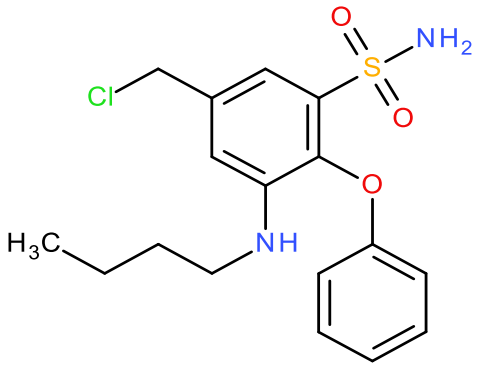
^{1}H NMR (200 MHz, DMSO-d_6) δ 7.38 – 7.17 (m, 2H), 7.14 – 6.67 (m, 7H), 5.31 (t, *J* = 5.7 Hz, 1H), 4.69 (t, *J* = 5.7 Hz, 1H), 4.50 (d, *J* = 5.7 Hz, 2H), 3.02 (q, *J* = 6.5 Hz, 2H), 1.51 – 1.22 (m, 2H), 1.25 – 0.98 (m, 2H), 0.77 (t, *J* = 7.1 Hz, 3H).

^{13}C NMR (50 MHz, DMSO-d_6) δ 156.9, 141.9, 140.2, 136.9, 134.9, 129.0, 121.8, 115.5, 112.7, 111.8, 62.7, 42.1, 30.4, 19.3, 13.6.

7.6 Compound **5**

3-(Butylamino) -5-(chloromethyl) -2-phenoxy-benzenesulfonamide

Internal code: BUM 63



Molecular formula	$C_{17}H_{21}ClN_2O_3S$
Molecular weight	368.88 g/mol
Melting point	158°C-159°C

3.66 mmol (1.28 g) of 3-(butylamino)-5-(hydroxymethyl)-2-phenoxy-benzene-sulfonamide (compound **4**, internal code: BUM 7) were dissolved in 10 mL thionyl chloride and heated to 80°C for three hours. The thionyl chloride was removed under reduced pressure and the substance was vacuum-dried overnight. It was then purified through column chromatography with the mobile phase ethyl acetate and petroleum ether (3+7). Those fractions which contained the eluted sample were united and the solvent was evaporated under reduced pressure (yield: 645 mg, 48% of compound **5**).

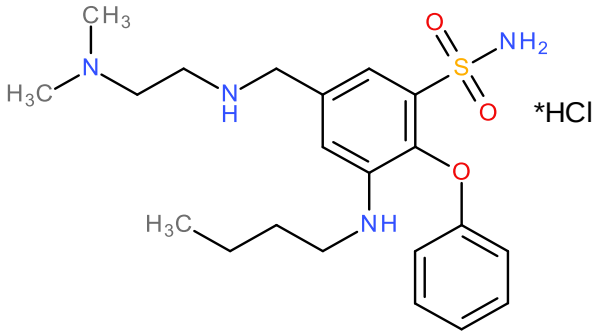
^{1}H NMR (200 MHz, $CDCl_3$) δ 7.49 – 7.21 (m, 3H), 7.17 – 6.79 (m, 4H), 4.93 (s, 2H), 4.57 (s, 2H), 3.05 (t, *J* = 7.0 Hz, 2H), 1.57 – 1.32 (m, 2H), 1.30 – 1.03 (m, 2H), 0.95 – 0.70 (m, 3H).

^{13}C NMR (50 MHz, $CDCl_3$) δ 156.1, 142.6, 136.5, 136.0, 135.9, 130.2, 123.7, 115.9, 115.4, 115.0, 45.8, 43.2, 31.1, 19.9, 13.8.

7.7 Compound **6**

3-(Butylamino)-5-[[2-(dimethylamino)- ethylamino]- methyl]-2-phenoxy-benzenesulfonamide Hydrochloride

Internal code: BUM 640 HCl



Molecular formula	$C_{21}H_{32}N_4O_3S.HCl$
Molecular weight	457.03 g/mol
Melting point	189°C-196°C

2 mmol (740 mg) of 3-(butylamino)-5-(chloromethyl)-2-phenoxy-benzenesulfonamide (compound **5**, internal code: BUM 63) were dissolved in a solution consisting of 5 mL DMF and 1 mL TEA. To this 2 mmol (440 µL) of *N,N'*- dimethylethylendiamine 98% were added and the mixture was stirred at room temperature overnight. After the reaction was accomplished, which was verified by the thin layer chromatography, it was purified through column chromatography with the mobile phase consisting of ethyl acetate, triethylamine and EtOH (6+3+1). Those fractions which contained the sample were united and the mobile phase was removed under reduced pressure. The crude product was dissolved in dry THF and 1 mL of a hydrogen chloride solution 1.0 M in diethyl ether was added. The resulting precipitate was filtered off to yield 123 mg of yellow-orange powder (yield: 123 mg, 13% of compound **6**).

^{1}H NMR (200 MHz, MeOD) δ 7.42 (d, *J* = 2.0 Hz, 1H), 7.37 – 7.18 (m, 3H), 7.05 (t, *J* = 7.3 Hz, 1H), 6.98 – 6.82 (m, 2H), 4.34 (s, 2H), 3.71 – 3.51 (m, 4H), 3.15 (t, *J* = 6.8 Hz, 2H), 3.00 (s, 6H), 1.57 – 1.27 (m, 2H), 1.30 – 1.00 (m, 2H), 0.81 (t, *J* = 7.2 Hz, 3H).

^{13}C NMR (50 MHz, MeOD) δ 156.6, 142.7, 137.9, 137.4, 129.2, 128.7, 122.6, 116.7, 115.8, 115.2, 52.9, 51.2, 42.7, 42.6, 41.9, 30.5, 19.5, 12.6.

CHN Analysis

Formula: $C_{21}H_{32}N_4O_3S.2HCl.\ H_2O$	C%	H%	N%
Calculated	49.31%	7.09%	10.95%
Found	49.57%	6.87%	10.97%

HRMS Analysis of free base

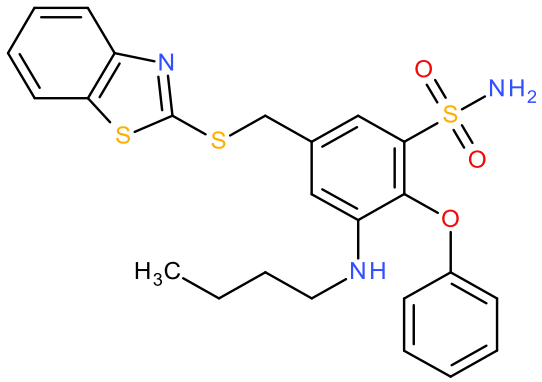
Meas. m/z	**Formula**	**m/z**	**Err. [ppm]**
421.2266	$C_{21}H_{33}N_4O_3S$	421.2268	0.5

Mass spectrum (EI) analysis of Compound **6** and also mass spectrum (EI) analysis of the free base are not possible.

7.8 Compound **7**

5-(1,3-Benzothiazolyl-2-sulfanylmethyl)-3-(butylamino)-2-phenoxy-benzenesulfonamide

Internal code: BUM 650



Molecular formula	$C_{24}H_{25}N_3O_3S_3$
Molecular weight	499.67 g/mol
Melting point	149.5 °C-153 °C

1 mmol (368 mg) of 3-(butylamino)-5-(chloromethyl)-2-phenoxy-benzenesulfonamide (compound **5**, internal code: BUM 63) was dissolved in a solution consisting of 3 mL DMF and 1 mL TEA. Thereafter, 2 mmol (338 mg) 2- mercaptobenzothiazole were added and the mixture was stirred at room temperature overnight. After the reaction was accomplished, which was verified by thin layer chromatography, it was purified through column chromatography with the mobile phase ethyl acetate and petroleum ether (3+7). Those fractions which contained the sample were united and the mobile phase was evaporated under reduced pressure. The crude product was then purified by recrystallization from EtOH to yield 104 mg of white crystal (yield: 104 mg, 21% of compound **7**).

^{1}H NMR (200 MHz, DMSO-d_6) δ 8.01 (d, J = 7.8 Hz, 1H), 7.89 (d, J = 7.8 Hz, 1H), 7.54 – 7.32 (m, 2H), 7.30 – 7.07 (m, 6H), 7.03 – 6.87 (m, 1H), 6.88 - 6.62 (m, 2H), 4.83 (t, J = 5.9 Hz, 1H), 4.67 (s, 2H), 3.10 - 2.79 (m, 2H), 1.41 – 1.15 (m, 2H), 1.13 – 0.87 (m, 2H), 0.83 – 0.53 (m, 3H).

^{13}C NMR (50 MHz, DMSO-d_6) δ 166.5, 157.1, 153.0, 142.6, 137.8, 136.0, 136.0, 135.2, 135.0, 129.5, 126.9, 125.1, 122.4, 122.3, 121.6, 115.9, 114.7, 42.5, 36.9, 30.7, 19.7, 14.0.

Mass Analysis

m/z	499	100%, M+
	466	33%
	333	67%
	196	37%
	77	31%

CHN Analysis

Formula: $C_{24}H_{25}N_3O_3S_3$	C%	H%	N%
Calculated	57.69%	5.04%	8.41%
Found	57.60%	5.05%	8.34%

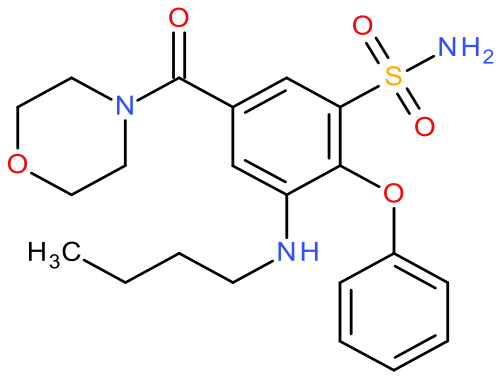
HRMS Analysis

Meas. m/z	**Formula**	**m/z**	**Err. [ppm]**
522.0949	$C_{24}H_{25}N_3NaO_3S_3$	522.0950	0.2

Compound **8**

3-(Butylamino)-5-(morpholine-4-carbonyl)-2-phenoxy-benzenesulfonamide

Internal code: BUM 641



Molecular formula	$C_{21}H_{27}N_3O_5S$
Molecular weight	433.52 g/mol
Melting point	204.5 °C- 208 °C

(United States Patent No. WO 2008052190, 2008)

Aquaporin modulators and methods of using them for the treatment of edema and fluid imbalance

PCT Int. Appl.

To a solution of 1 mmol (365 mg) of bumetanide in 5 mL dry THF 1.2 mmol (197 mg) 1,1-CDI were added and the mixture was stirred for two hours. Once the thin layer chromatography did not show any bumetanide remaining, 2 mmol (175 µL) of morpholine were added and the mixture was stirred at room temperature overnight. After the reaction was completed, it was poured into 20 mL of 5% $NaHCO_3$ and extracted with ethyl acetate. The combined organic phase was dried over Na_sSO_4 and the solvent was evaporated under reduced pressure. Thereafter the crude product was purified by recrystallization from EtOH to yield 333 mg of white crystal (yield: 333 mg, 77% of compound **8**).

^{1}H NMR (200 MHz, $CDCl_3$) δ 7.55 – 7.20 (m, 3H), 7.16 – 7.05 (m, 1H), 7.02 – 6.84 (m, 3H), 5.11 (s, 2H), 4.02 - 3.27 (m, 8H), 3.04 (t, *J* = 6.9 Hz, 2H), 1.57 – 1.34 (m, 2H), 1.30 – 1.00 (m, 2H), 0.81 (t, *J* = 7.2 Hz, 3H).

^{13}C NMR (50 MHz, $CDCl_3$) δ 169.0, 155.9, 142.1, 137.9, 136.1, 130.1, 123.8, 115.4, 115.1, 113.9, 66.8, 43.5, 30.8, 19.8, 13.6.

Mass Analysis

m/z	433	100%, M+
	390	66%
	303	53%
	286	46%
	70	49%

CHN Analysis

Formula: $C_{21}H_{27}N_3O_5S_3$	C%	H%	N%
Calculated	58.18%	6.28%	9.69%
Found	57.90%	6.16%	9.65%

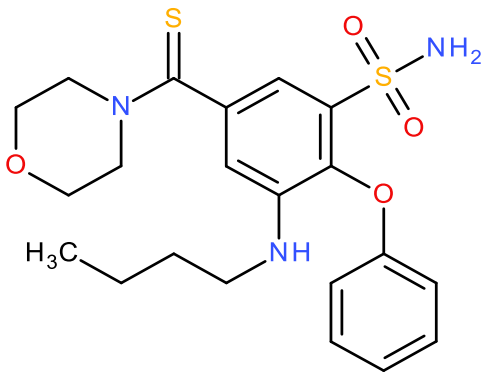
HRMS Analysis

Meas. m/z	**Formula**	**m/z**	**Err. [ppm]**
456.1563	$C_{21}H_{27}N_3NaO_5S$	456.1564	0.1

7.9 Compound **9**

3-(Butylamino)-5-(morpholine-4-carbothioyl)-2-phenoxy-benzenesulfonamide

Internal code: BUM 647



Molecular formula	$C_{21}H_{27}N_3O_4S_2$
Molecular weight	449.59 g/mol
Melting point	180°C-188°C

2.2 mmol (773 mg) of Lawesson's reagent were dissolved in a solution consisting of 1 mmol (433 mg) of 3-(butylamino)-5-(morpholine-4-carbonyl)-2-phenoxy-benzenesulfonamide (compound **8**, internal code: BUM 641) and 9 mL dry THF. Thereafter the mixture was stirred for 2.5 hours. The thin layer chromatography showed that there was still 3-(butylamino)-5-(morpholine-4-carbonyl)-2-phenoxy-benzenesulfonamide (compound **8**, internal code: BUM 641) remaining. Therefore, it was purified through Flash Column Chromatography with the mobile phase ethyl acetate and petroleum ether (6+4). The crude product was then purified by recrystallization from EtOH to yield 241 mg of light green crystal (yield: 241 mg, 54% of compound **9**).

^{1}H NMR (200 MHz, $CDCl_3$) δ 7.39 – 7.25 (m, 2H), 7.25 - 7.19 (m, 1H), 7.17 – 7.05 (m, 1H), 7.02 – 6.92 (m, 3H), 5.02 (brs, 1H), 4.42 (t, *J* = 4.8 Hz, 2H), 3.89 (t, *J* = 4.8 Hz, 2H), 3.79 – 3.50 (m, 4H), 3.02 (t, *J* = 7.0 Hz, 2H), 1.61 – 1.31 (m, 2H), 1.30 – 0.98 (m, 2H), 0.80 (t, *J* = 7.2 Hz, 3H).

^{13}C NMR (50 MHz, $CDCl_3$) δ 198.8, 155.8, 142.3, 140.3, 136.7, 135.8, 130.1, 123.7, 115.3, 113.7, 111.8, 66.8, 66.4, 52.8, 49.6, 43.1, 30.9, 19.8, 13.7.

Mass Analysis

m/z	449	100%, M+
	448	27%
	416	14%
	364	52%
	71	22%

CHN Analysis

Formula: $C_{21}H_{27}N_3O_4S_2$	C%	H%	N%
Calculated	56.10%	6.05%	9.35%
Found	55.89%	5.99%	9.04%

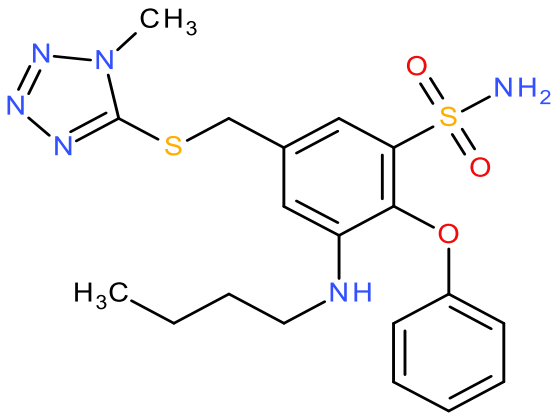
HRMS Analysis

Meas. m/z	**Formula**	**m/z**	**Err. [ppm]**
472.1334	$C_{21}H_{27}N_3NaO_4S_2$	472.1335	0.2

7.10 Compound **10**

3-(Butylamino)-5-[(1-methyltetrazol-5-yl)-sulfanylmethyl]-2-phenoxy-benzenesulfonamide

Internal code: BUM 648



Molecular formula	$C_{19}H_{24}N_6O_3S_2$
Molecular weight	448.56 g/mol
Melting point	156°C-162°C

0.7 mmol (260 mg) of 3-(butylamino)-5-(chloromethyl)-2-phenoxy-benzenesulfonamide (compound **5**, internal code: BUM 63) were dissolved in a solution consisting of 3 mL DMF and 1 mL TEA. To this 2 mmol (164 mg) 5-mercapto-1-methyltetrazole were added and the mixture was stirred at room temperature overnight. After the reaction was accomplished, which was verified by thin layer chromatography, the fluid was evaporated under reduced pressure. Thereafter the crude product was purified by recrystallization from EtOH 70% to yield 243 mg of light brown powder (yield: 243 mg, 77% of compound **10**).

^{1}H NMR (200 MHz, DMSO-d_6) δ 7.34 – 7.08 (m, 5H), 7.06 – 6.89 (m, 2H), 6.89 - 6.55 (m, 2H), 4.85 (t, *J* = 5.8 Hz, 1H), 4.53 (s, 2H), 3.89 (s, 3H), 2.96 (q, *J* = 6.5 Hz, 2H), 1.42 – 1.21 (m, 2H), 1.26 – 0.95 (m, 2H), 0.77 (t, *J* = 7.2 Hz, 3H).

^{13}C NMR (50 MHz, DMSO-d_6) δ 157.1, 153.6, 142.6, 137.8, 136.1, 134.8, 129.5, 122.4, 115.9, 115.7, 114.5, 42.5, 42.5, 37.1, 37.1, 34.1, 30.7, 30.7, 19.8, 19.8, 14.1, 9.1.

Mass Analysis

m/z	448	80%, M+
	333	95%
	196	94%
	128	91%
	77	100%

CHN Analysis

Formula: $C_{19}H_{24}N_6O_3S_2$	C%	H%	N%
Calculated	50.88%	5.39%	18.74%
Found	50.66%	5.13%	18.50%

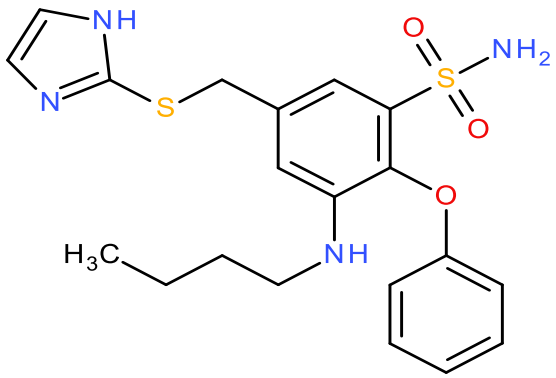
HRMS Analysis

Meas. m/z	Formula	m/z	Err. [ppm]
471.1243	$C_{19}H_{24}N_6NaO_3S_2$	471.1244	0.1

7.11 Compound **11**

3-(Butylamino)-5-(1H-imidazol-2-ylsulfanylmethyl)-2-phenoxy-benzenesulfonamide

Internal code: BUM 651



Molecular formula	$C_{20}H_{24}N_4O_3S_2$
Molecular weight	432.56 g/mol
Melting point	159°C-165°C

1 mmol (369 mg) of 3-(butylamino)-5-(chloromethyl)-2-phenoxy-benzenesulfonamide (compound **5**, internal code: BUM 63) was dissolved in 3 mL DMF and 1 mL TEA. To this 2 mmol (205 mg) of 2-mercaptoimidazole were added and the mixture was stirred at room temperature overnight. After the reaction was accomplished, which was verified by thin layer chromatography, it was purified through column chromatography with the mobile phase consisting of ethyl acetate and petroleum ether (9+1). Those fractions which contained the sample were united and the mobile phase was evaporated under reduced pressure, yielding 308 mg of white brown powder (yield: 308 mg, 71% of compound **11**).

^{1}H NMR (200 MHz, MeOD) δ 7.35 – 7.17 (m, 2H), 7.14 – 6.93 (m, 4H), 6.93 – 6.77 (m, 2H), 6.62 – 6.59(m, 1H), 4.14 (s, 1H), 2.94 (t, *J* = 6.7 Hz, 2H), 1.46 – 1.22 (m, 2H), 1.23 – 0.98 (m, 2H), 0.80 (t, *J* = 7.1 Hz, 3H).

^{13}C NMR (50 MHz, MeOD) δ 156.8, 142.2, 138.4, 136.5, 136.0, 135.8, 129.1, 122.3, 115.3, 115.1, 114.4, 42.3, 39.1, 30.6, 19.5, 12.6.

One CH group could not be detected.

Mass Analysis

m/z	432	42%, M+
	399	39%
	333	100%
	196	63%
	41	45%

CHN Analysis

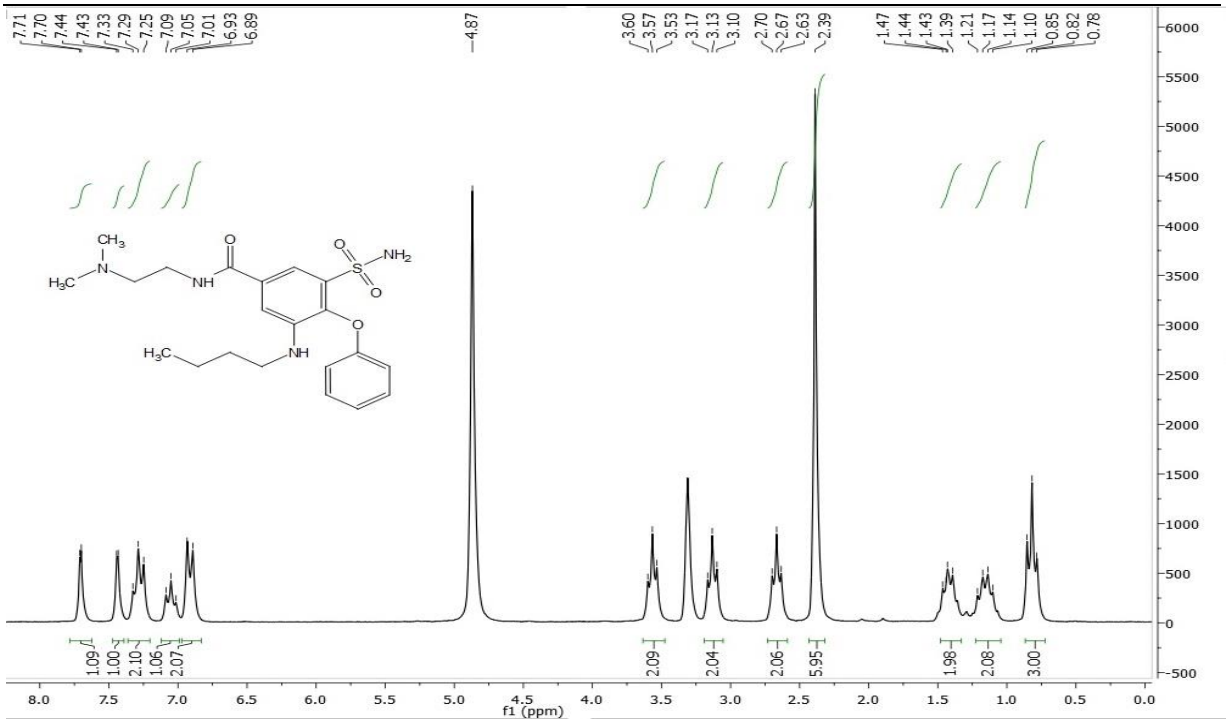
Formula: $C_{20}H_{24}N_4O_3S_2$	C%	H%	N%
Calculated	55.54%	5.59%	12.95%
Found	55.01%	5.38%	12.79%

HRMS Analysis

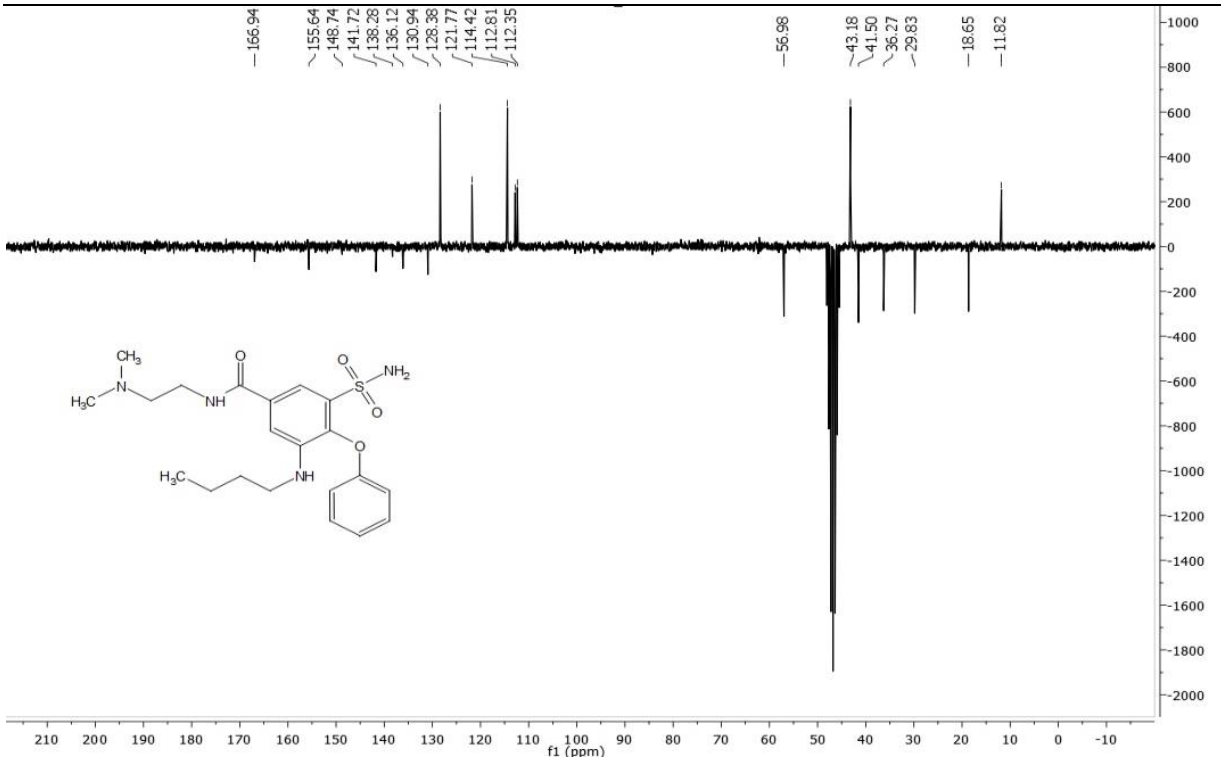
Meas. m/z	**Formula**	**m/z**	**Err. [ppm]**
455.1180	$C_{20}H_{24}N_4NaO_3S_2$	455.1182	0.4

8 Analytics

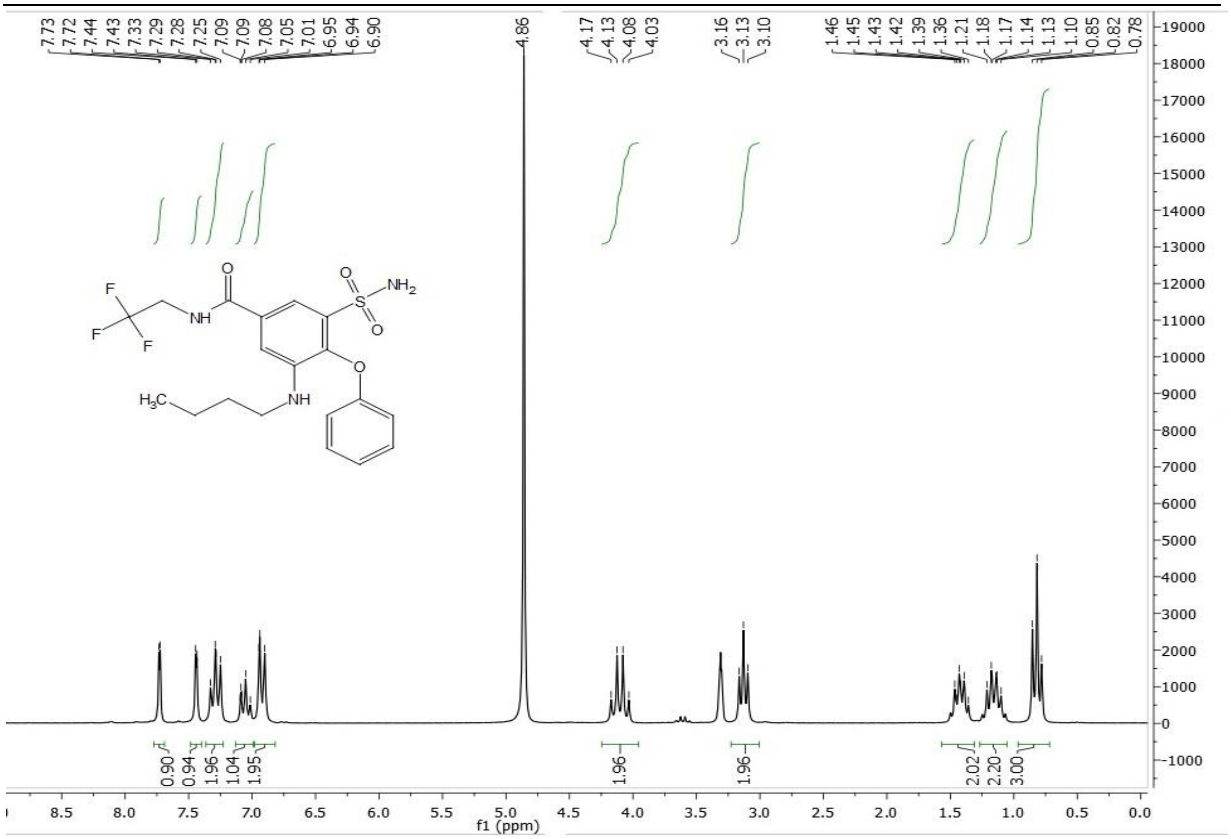
Compound **1** ^{1}H-NMR (DMSO-d_6)



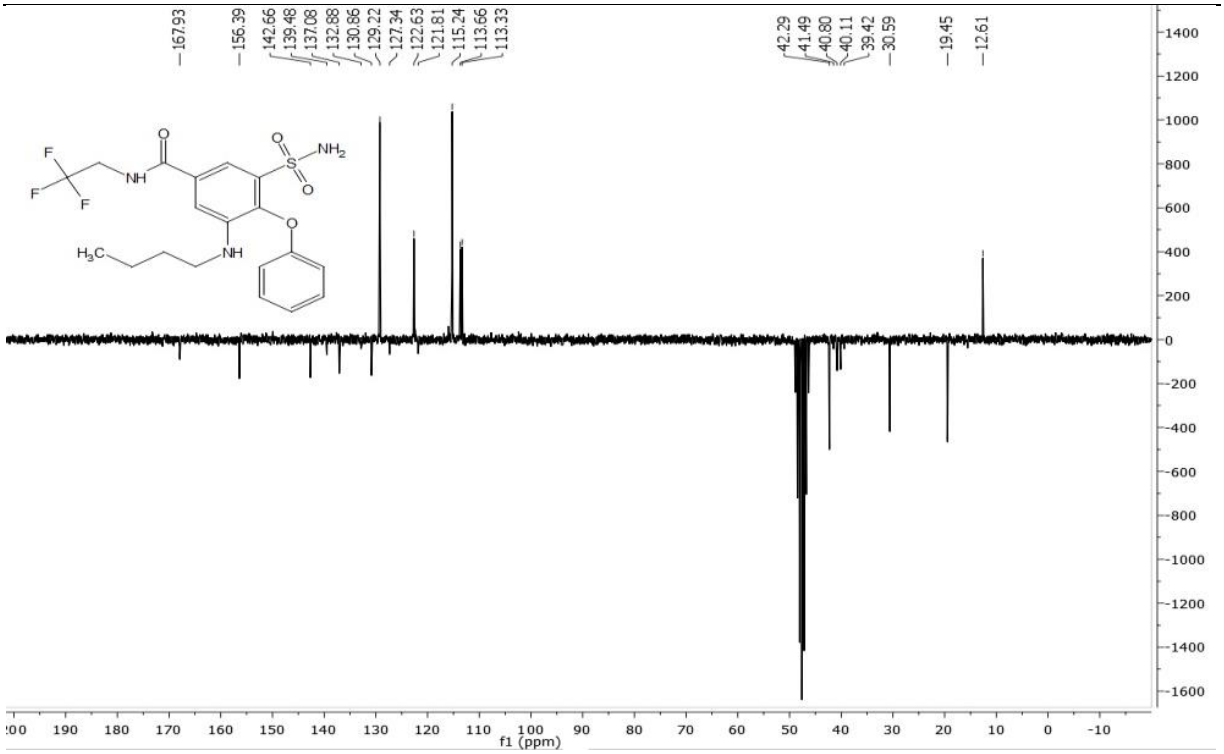
Compound **1** ^{13}C-NMR (DMSO-d_6)



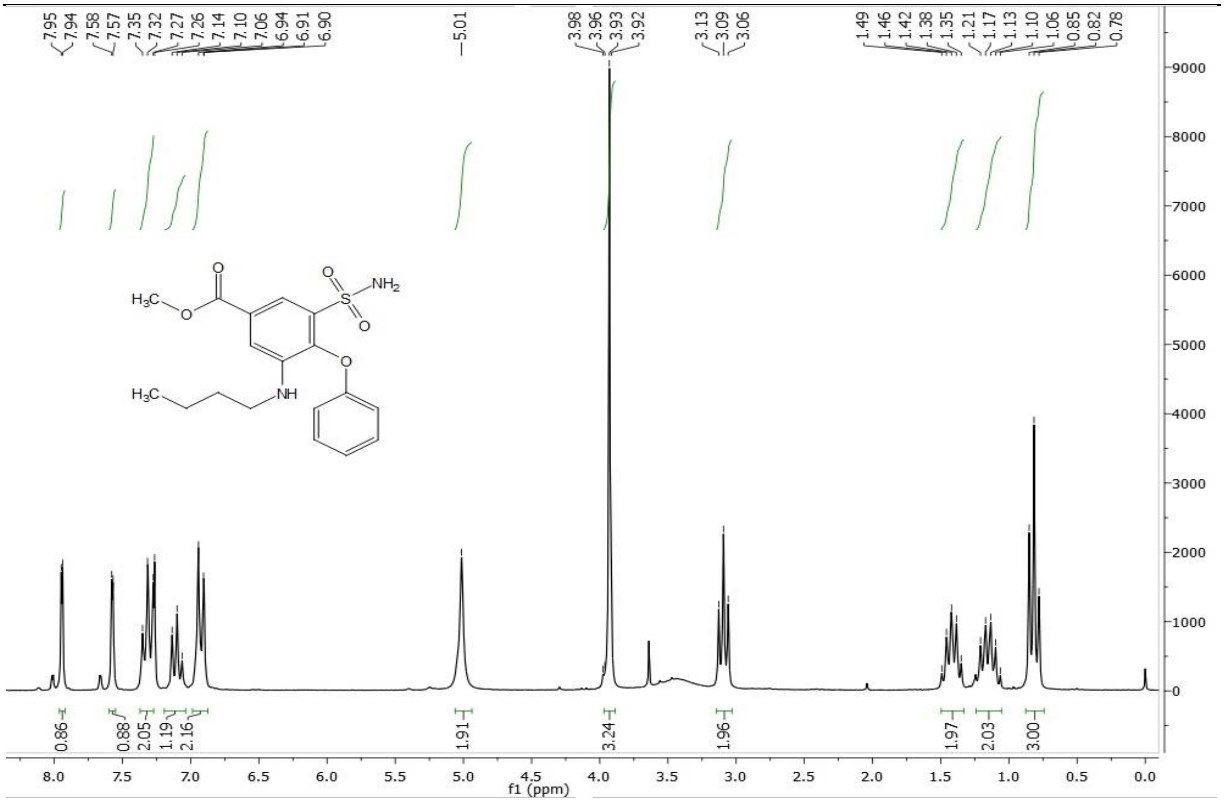
Compound **2** ^{1}H-NMR (MeOD)



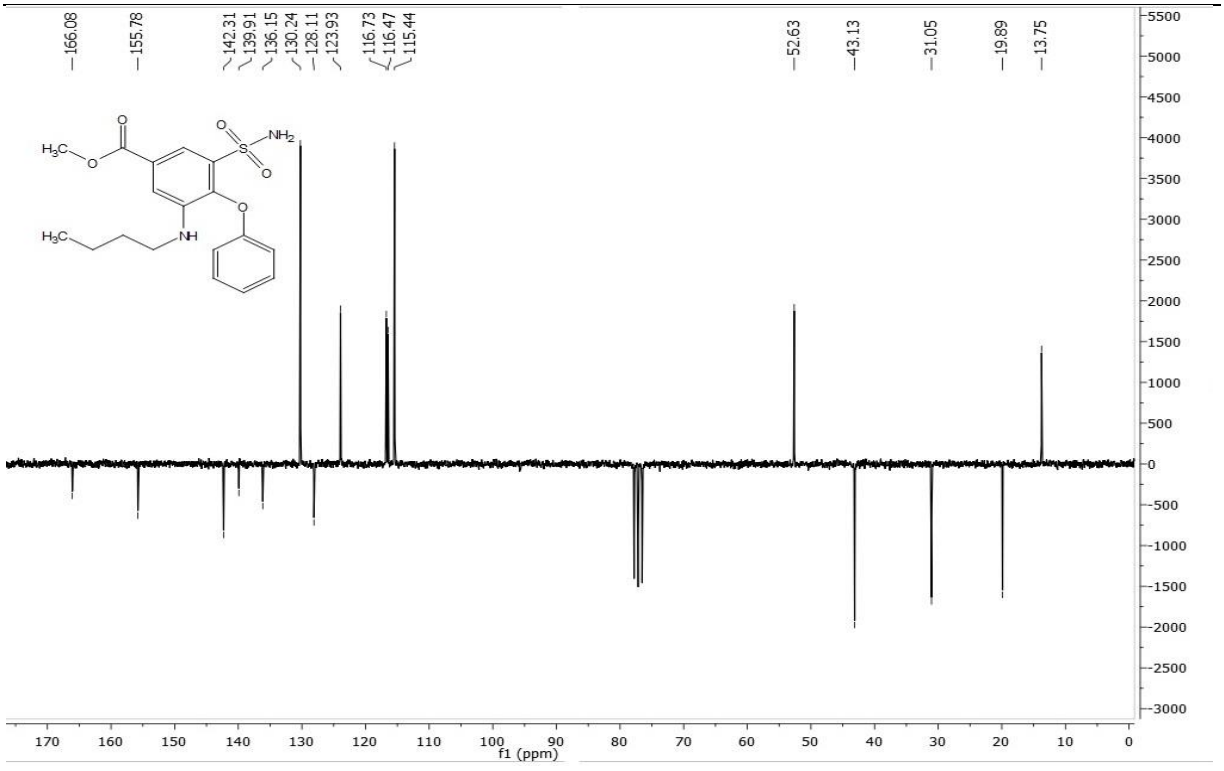
Compound **2** ^{13}C-NMR (MeOD)



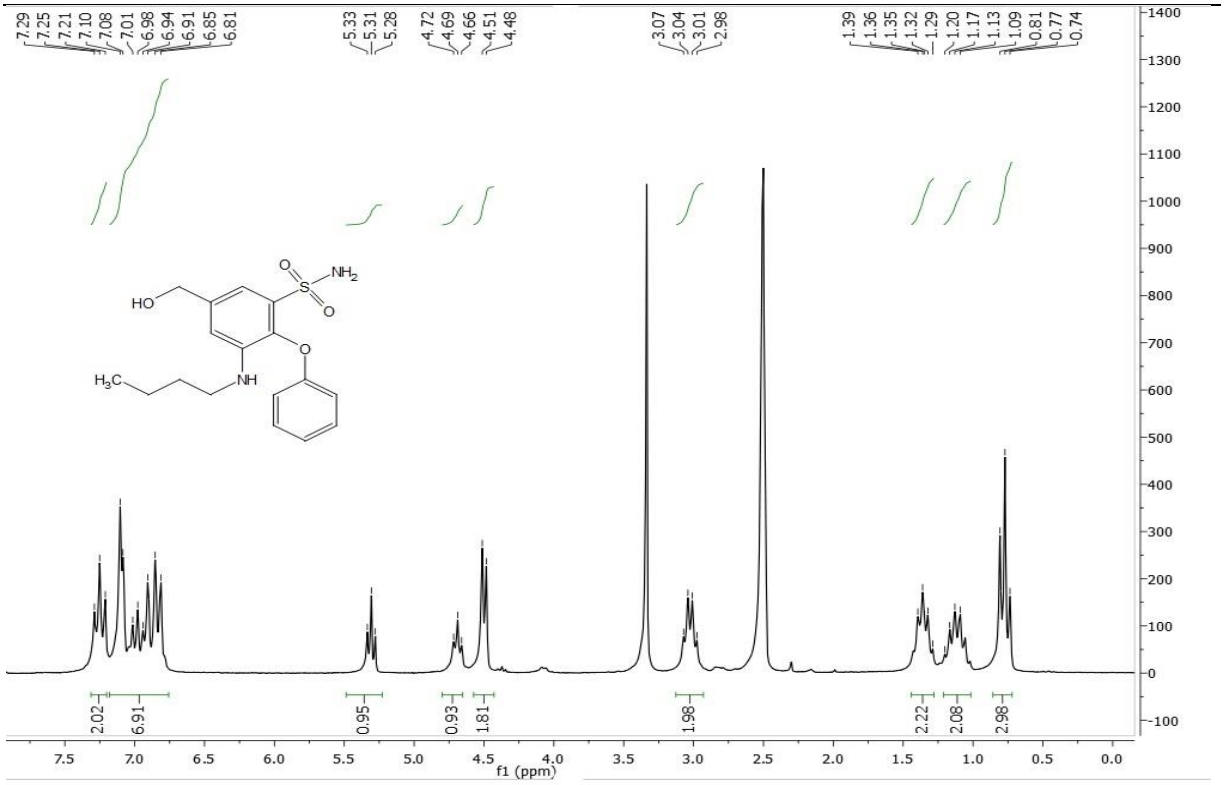
Compound **3** ^{1}H-NMR ($CDCl_3$)



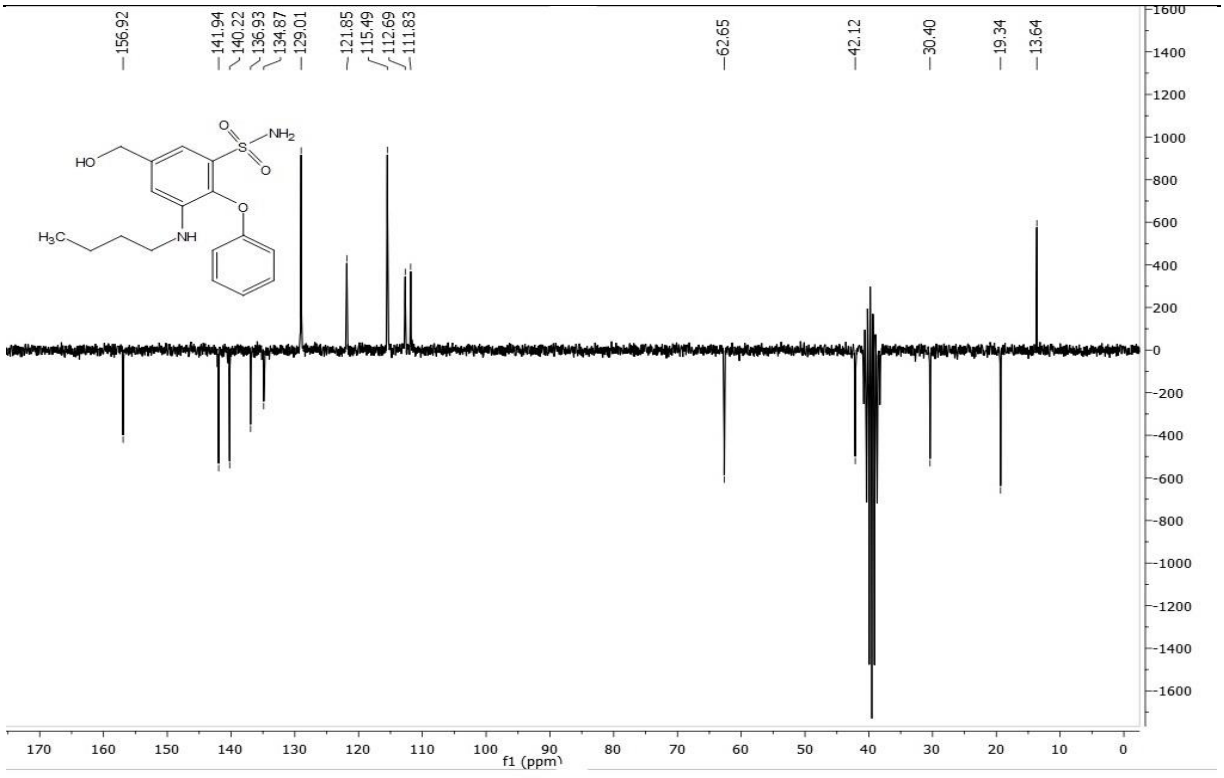
Compound **3** ^{13}C-NMR ($CDCl_3$)



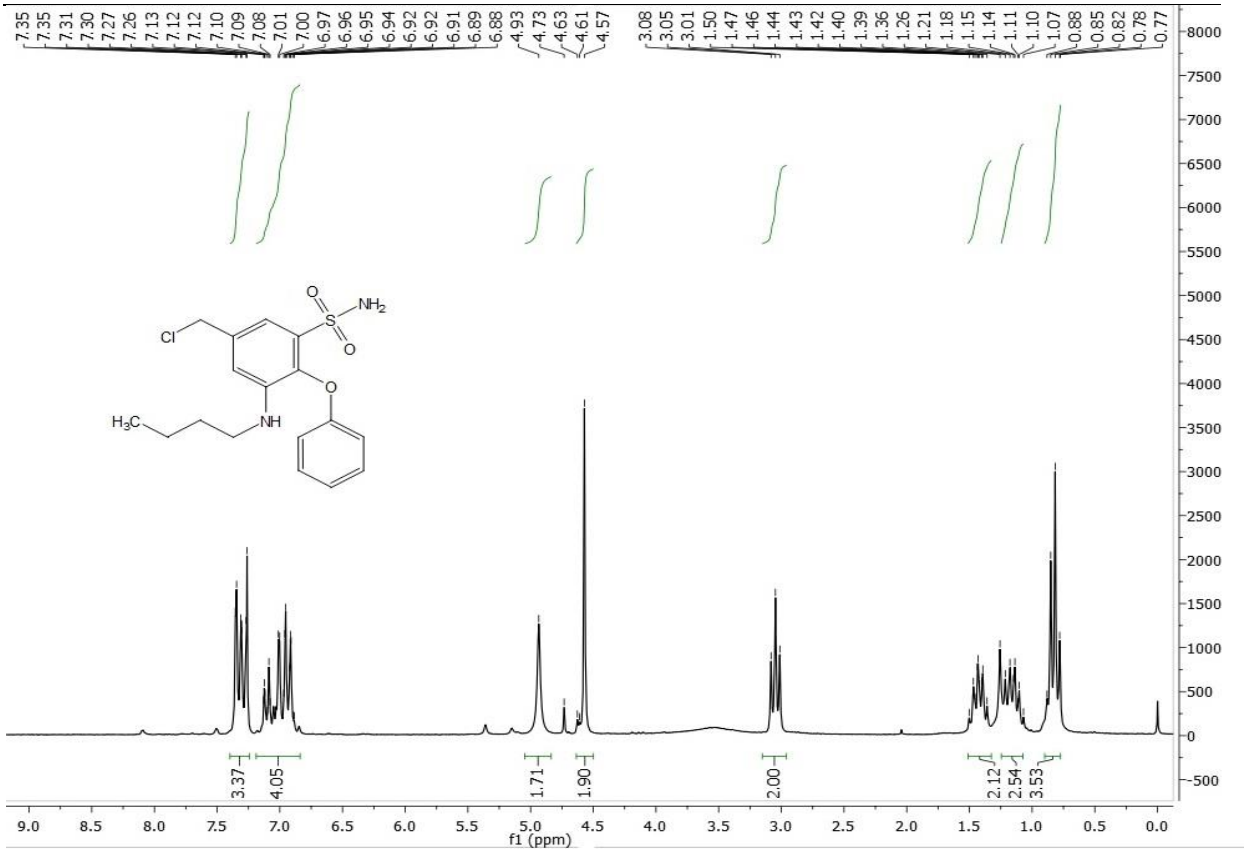
Compound **4** ^{1}H-NMR (DMSO-d_6)



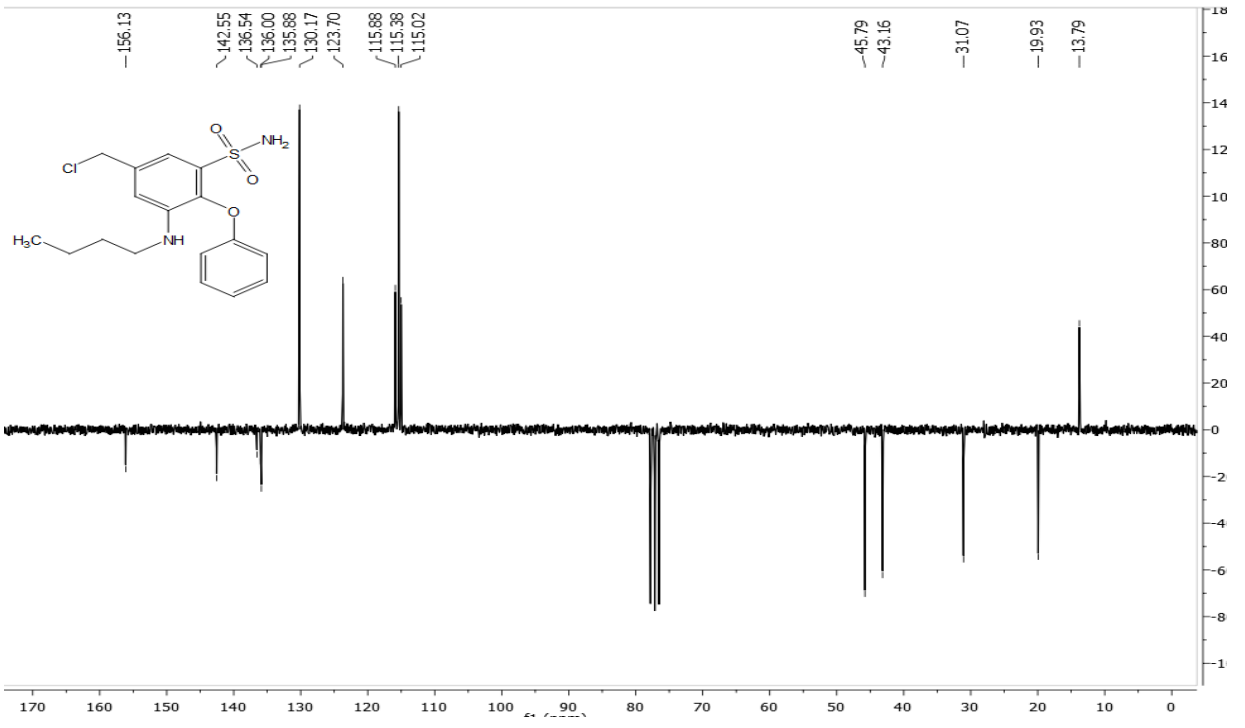
Compound **4** ^{13}C-NMR (DMSO-d_6)



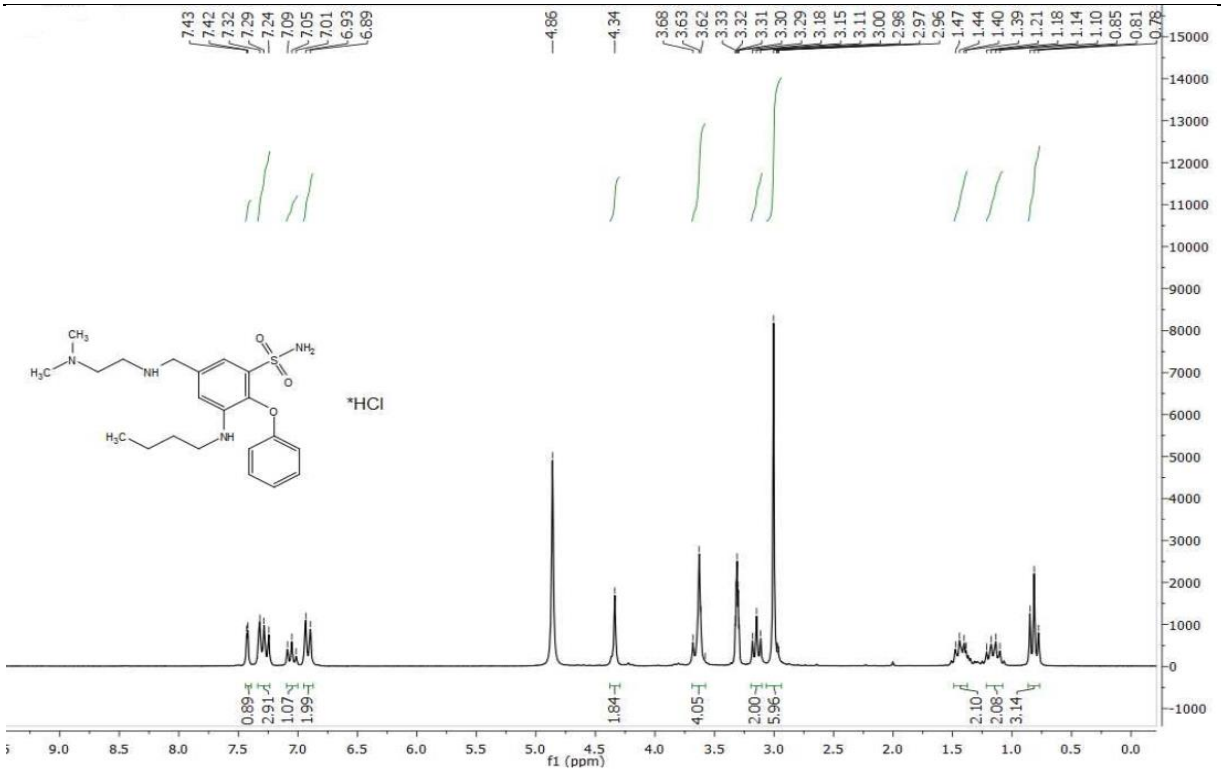
Compound **5** ^{1}H-NMR ($CDCl_3$)



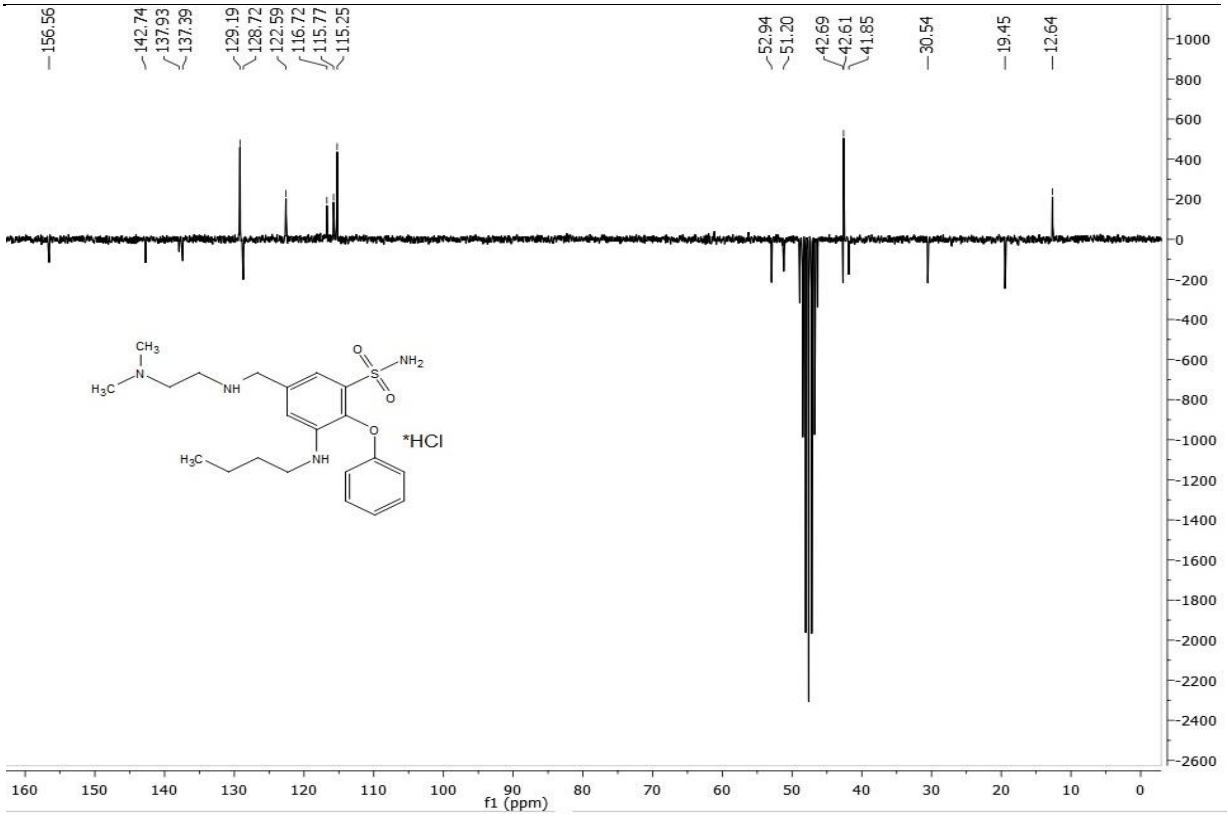
Compound **5** ^{13}C-NMR ($CDCl_3$)



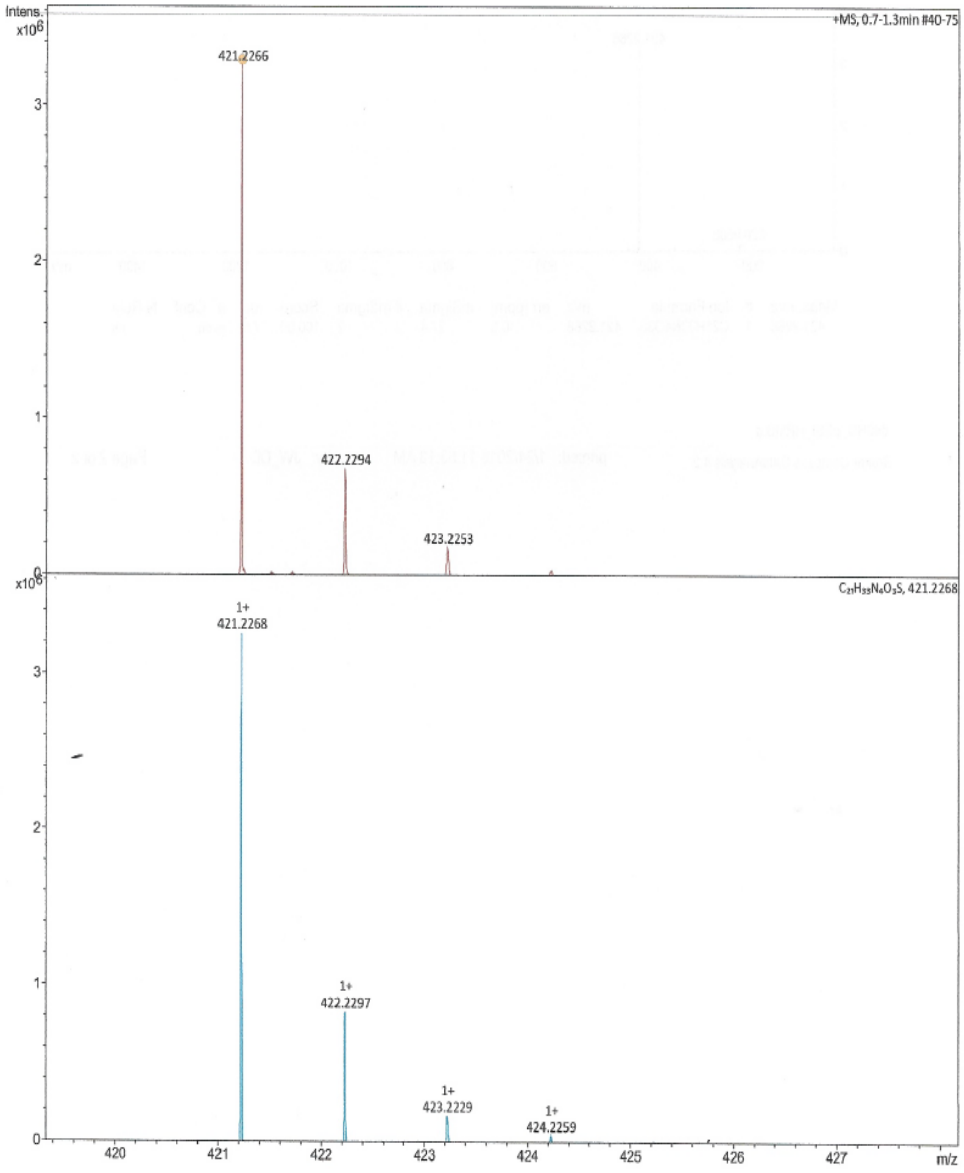
Compound **6** ^{1}H-NMR (MeOD)



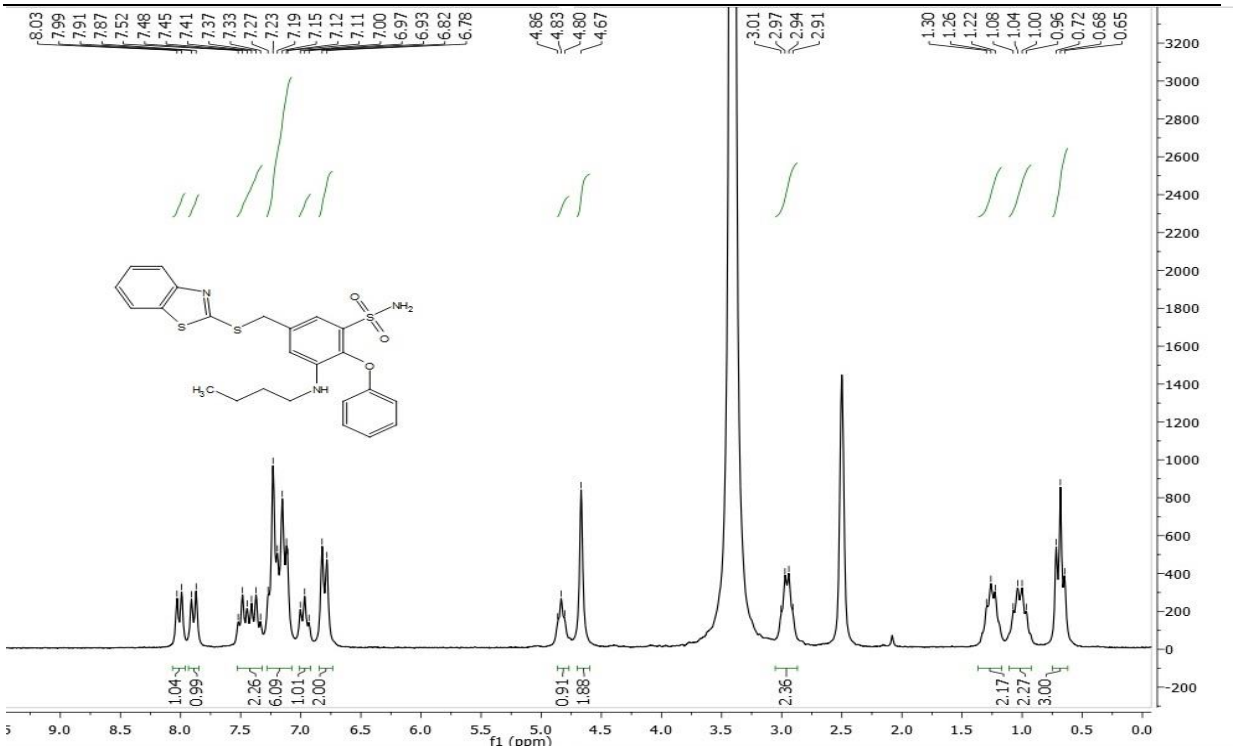
Compound **6** ^{13}C-NMR (MeOD)



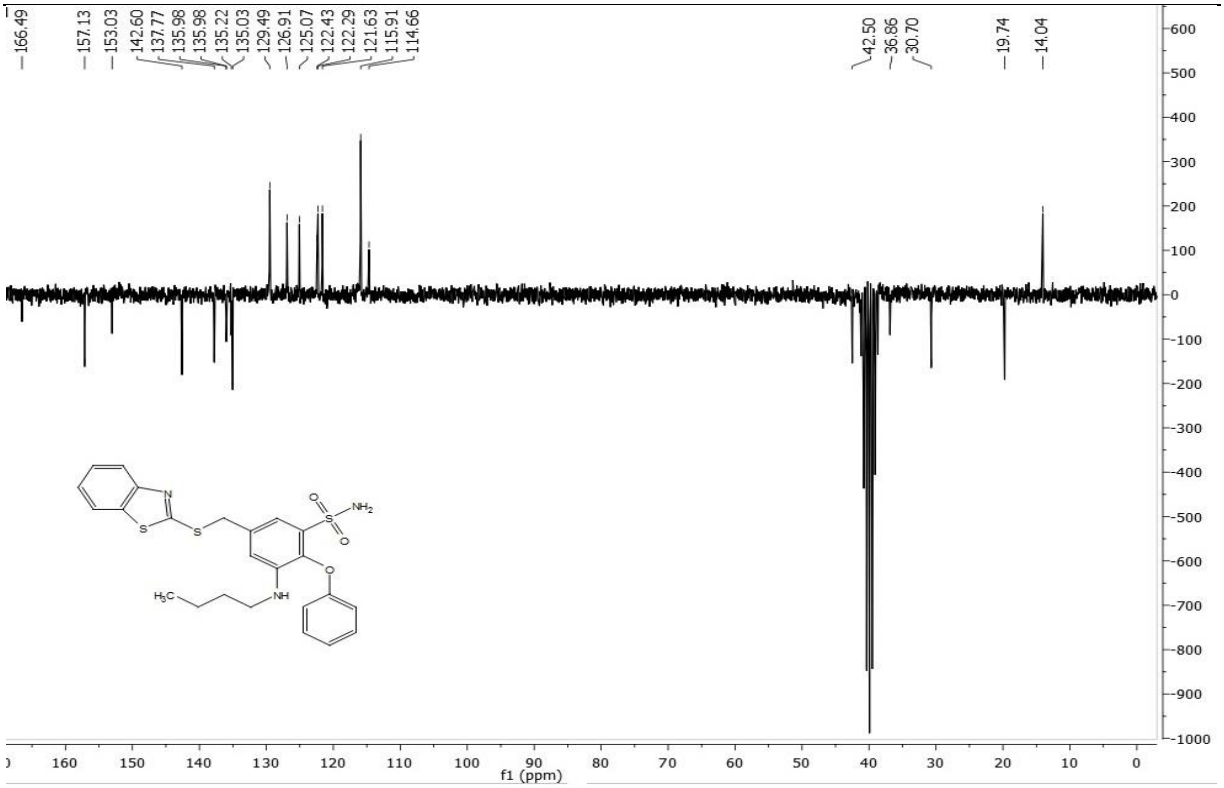
Compound **6** HRMS



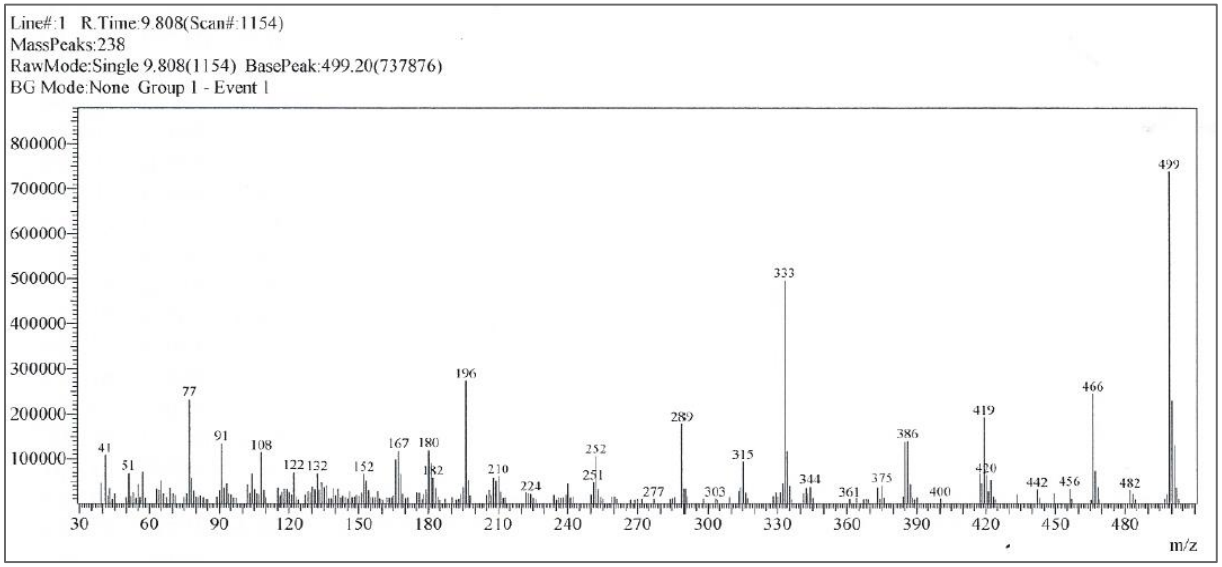
Compound **7** ^{1}H-NMR (DMSO-d_6)



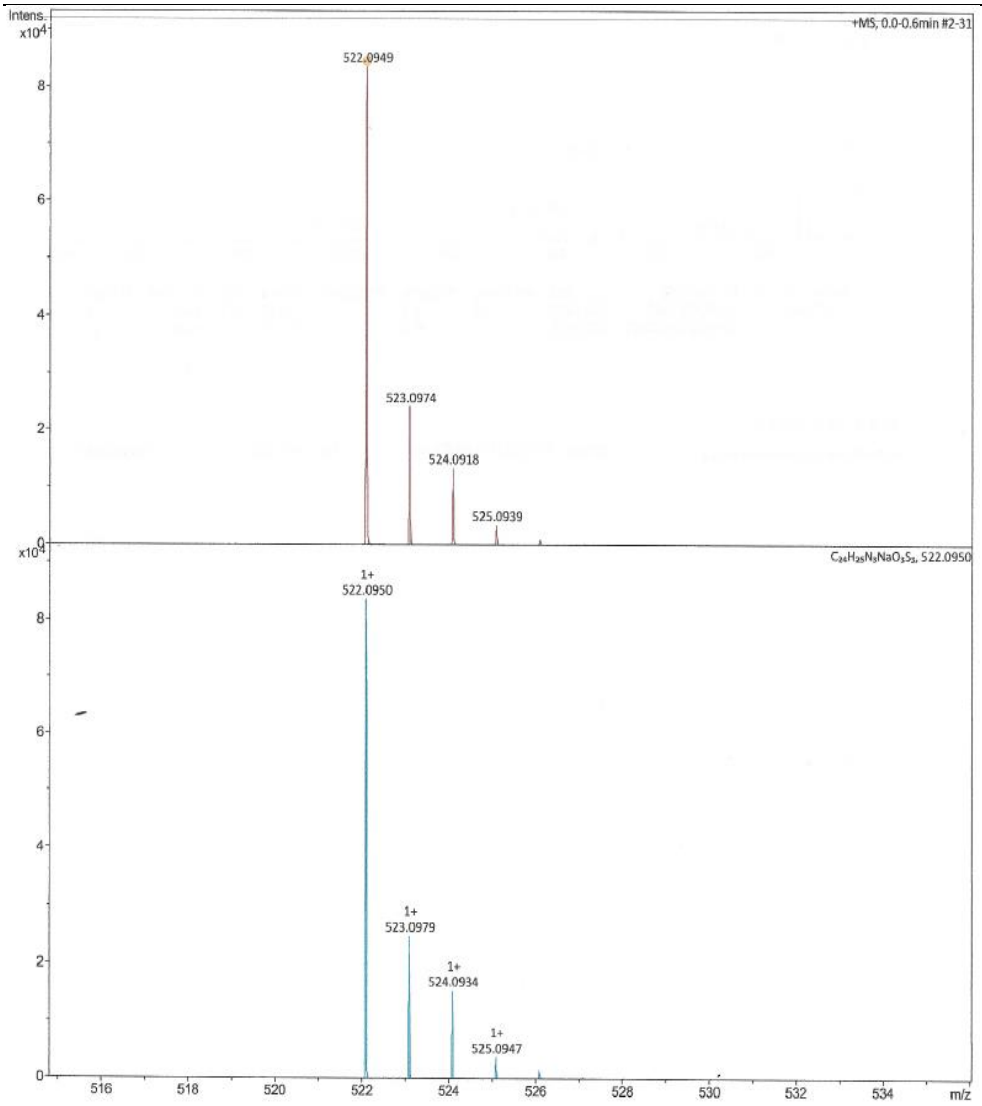
Compound **7** ^{13}C-NMR (DMSO-d_6)



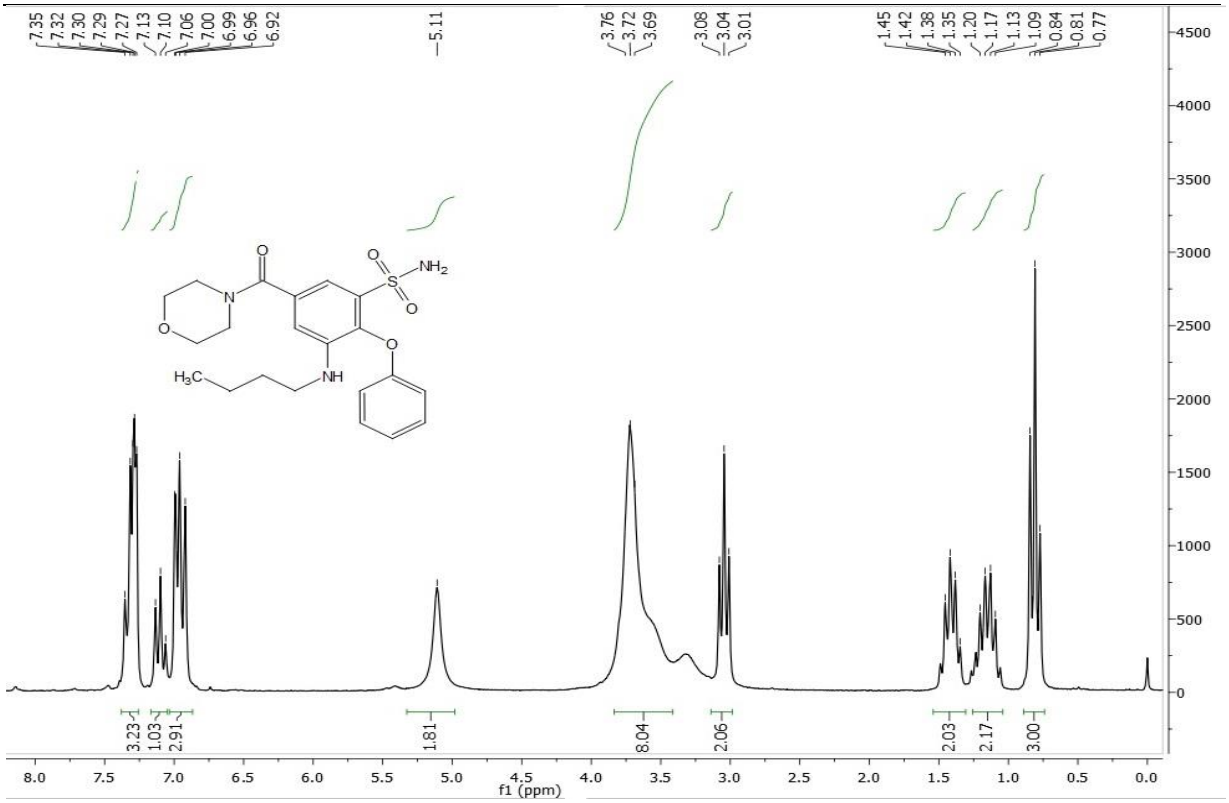
Compound **7** mass spectrum



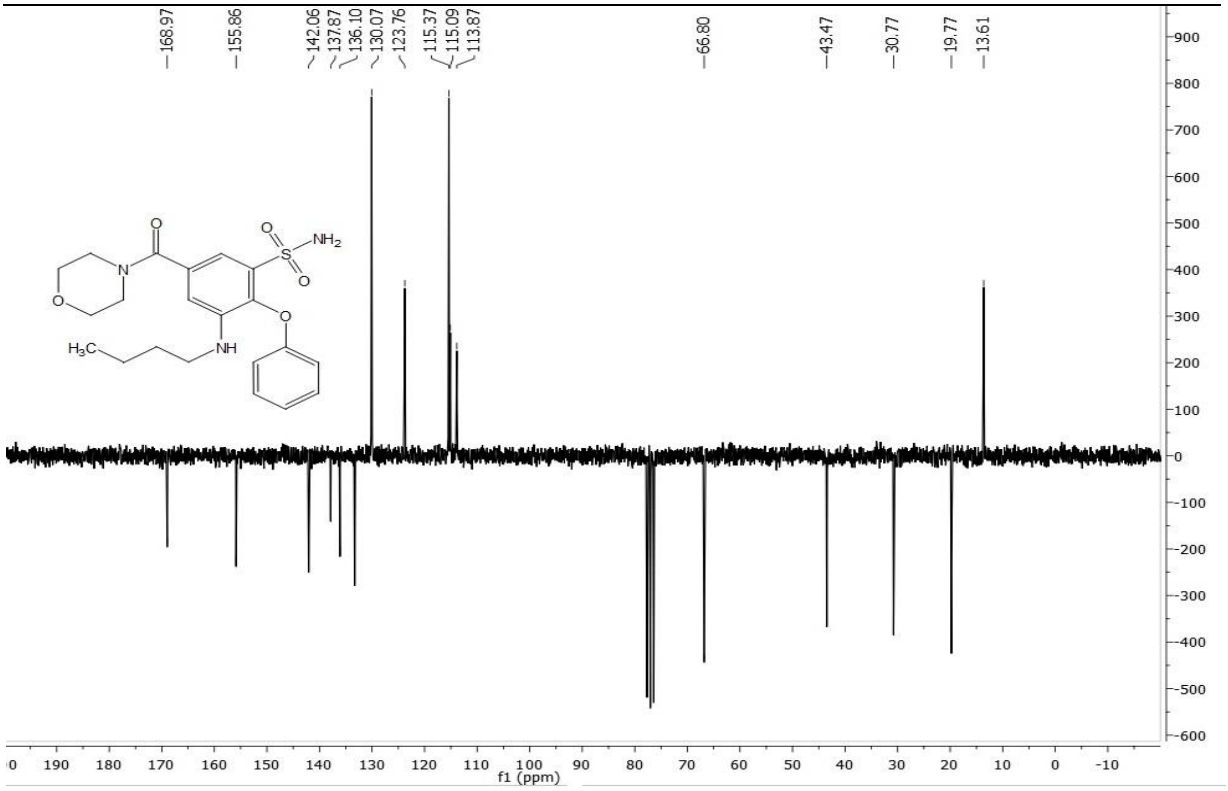
Compound **7** HRMS



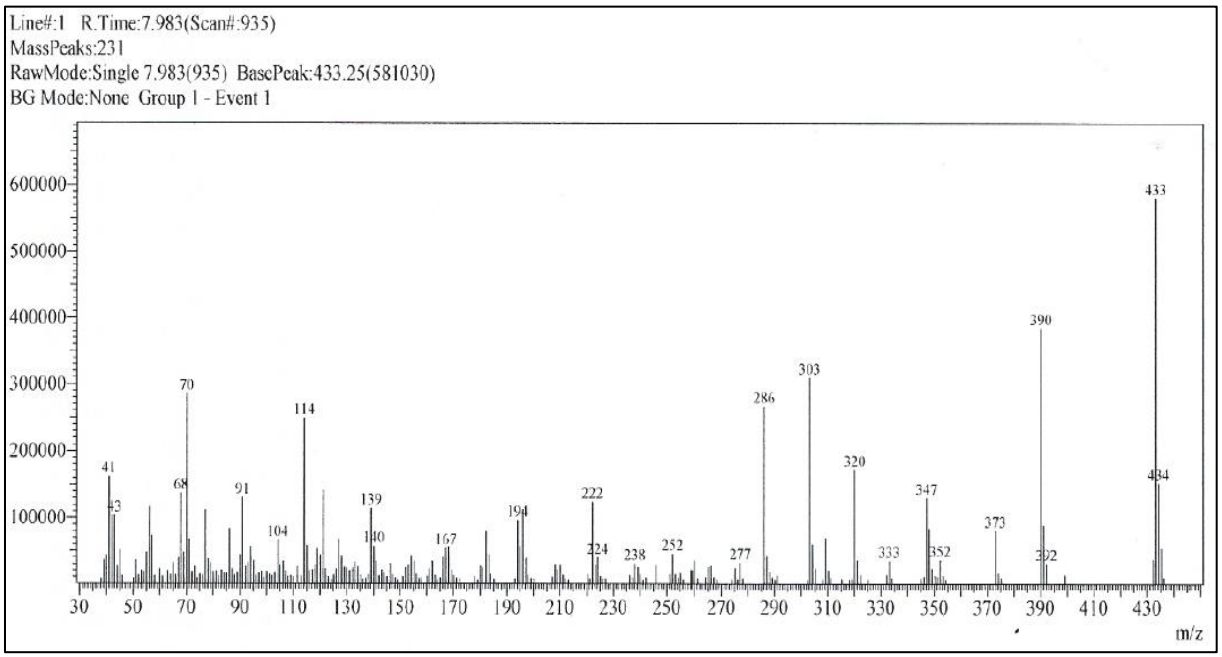
Compound **8** ^{1}H-NMR ($CDCl_3$)



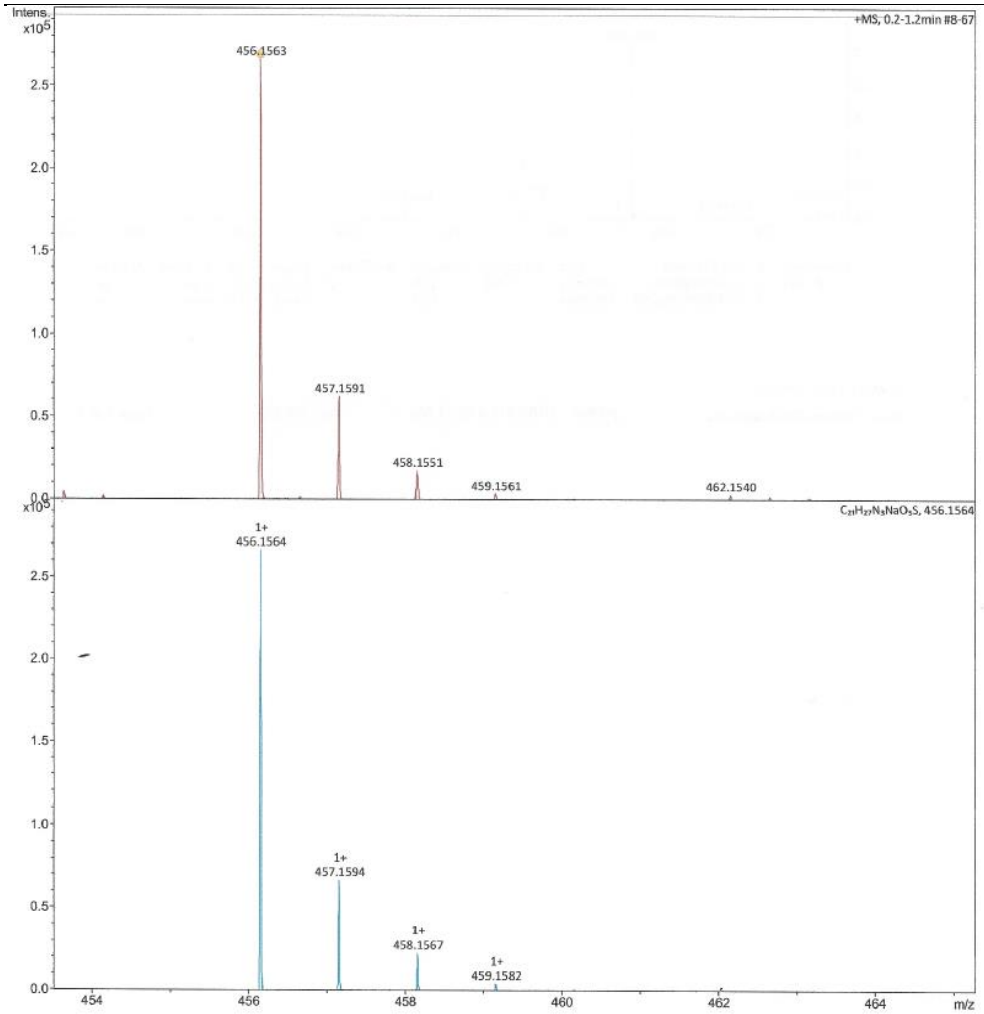
Compound **8** ^{13}C-NMR ($CDCl_3$)



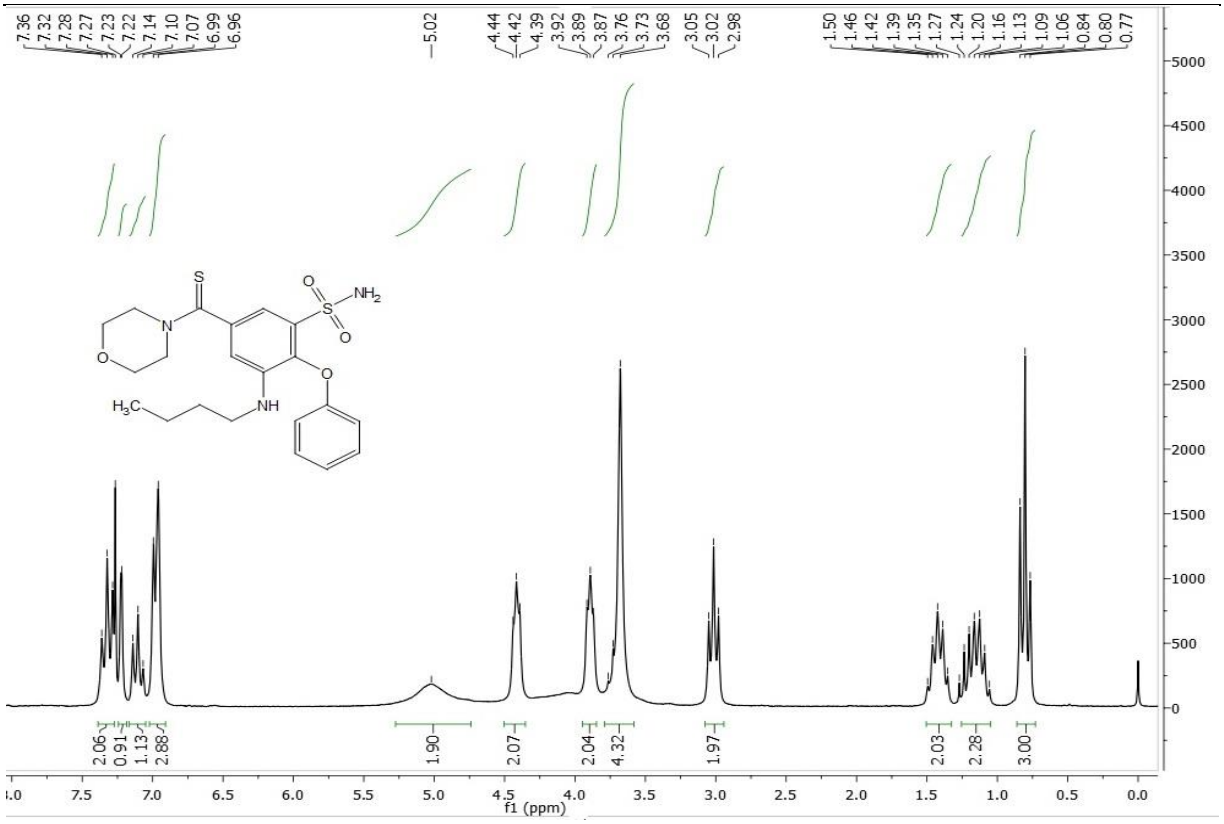
Compound **8** mass spectrum



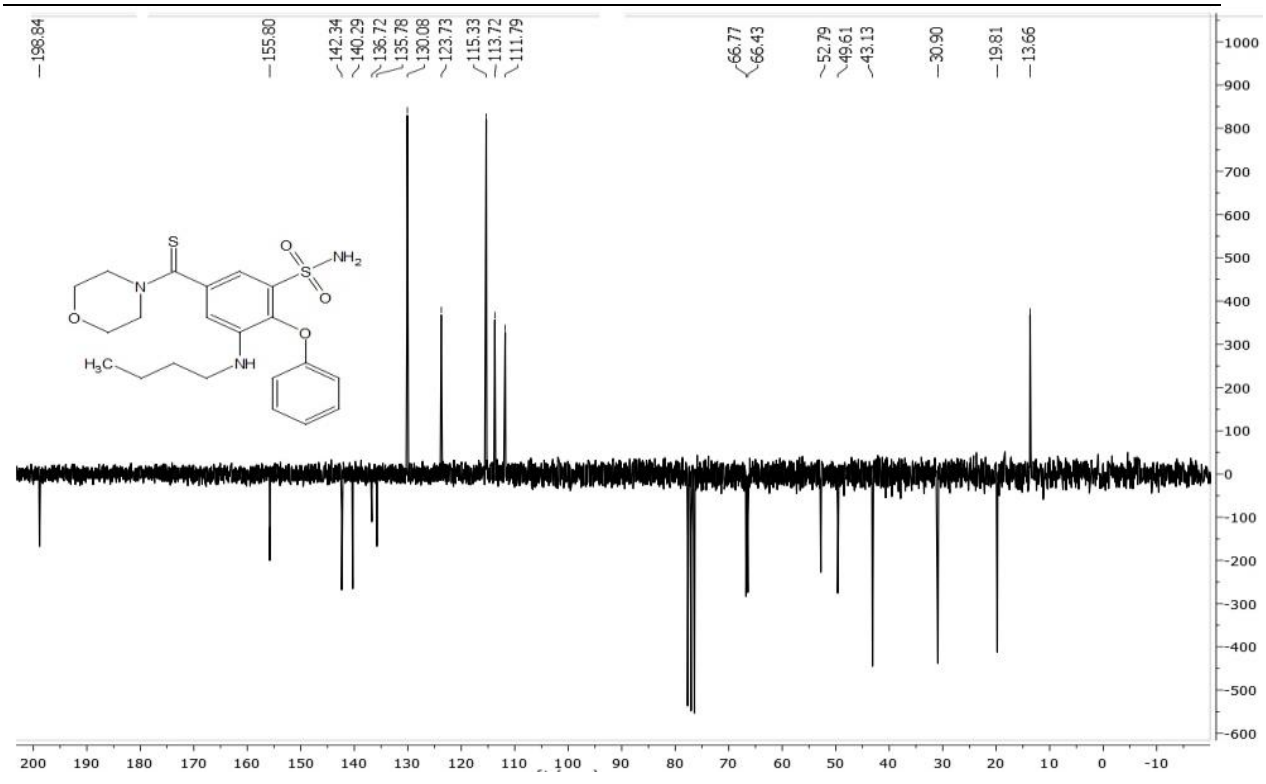
Compound **8** HRMS



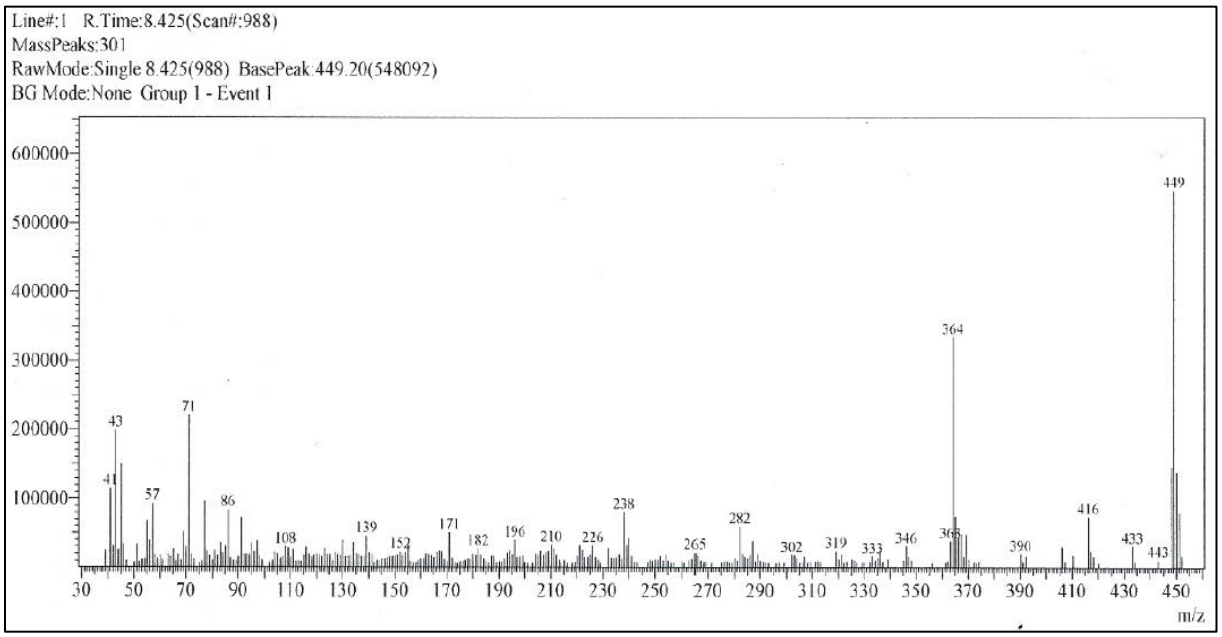
Compound **9** ^{1}H-NMR ($CDCl_3$)



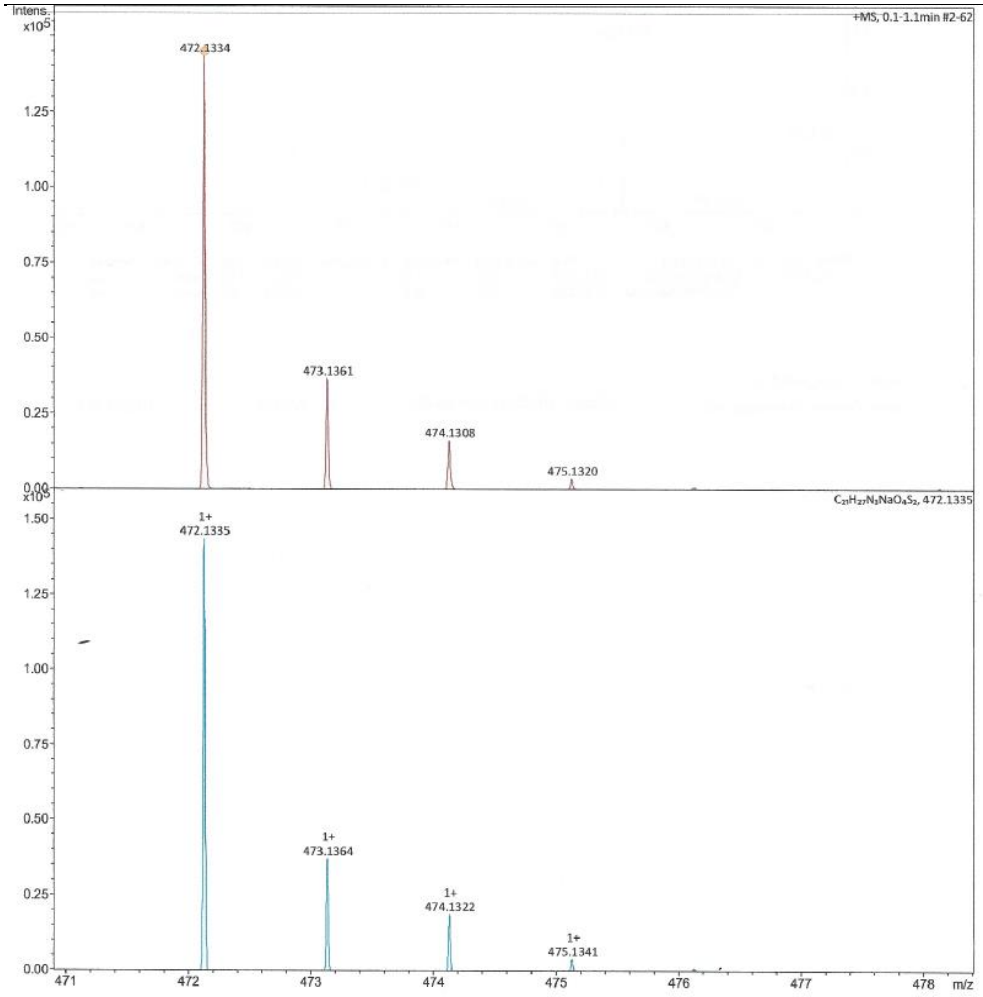
Compound **9** ^{13}C-NMR ($CDCl_3$)



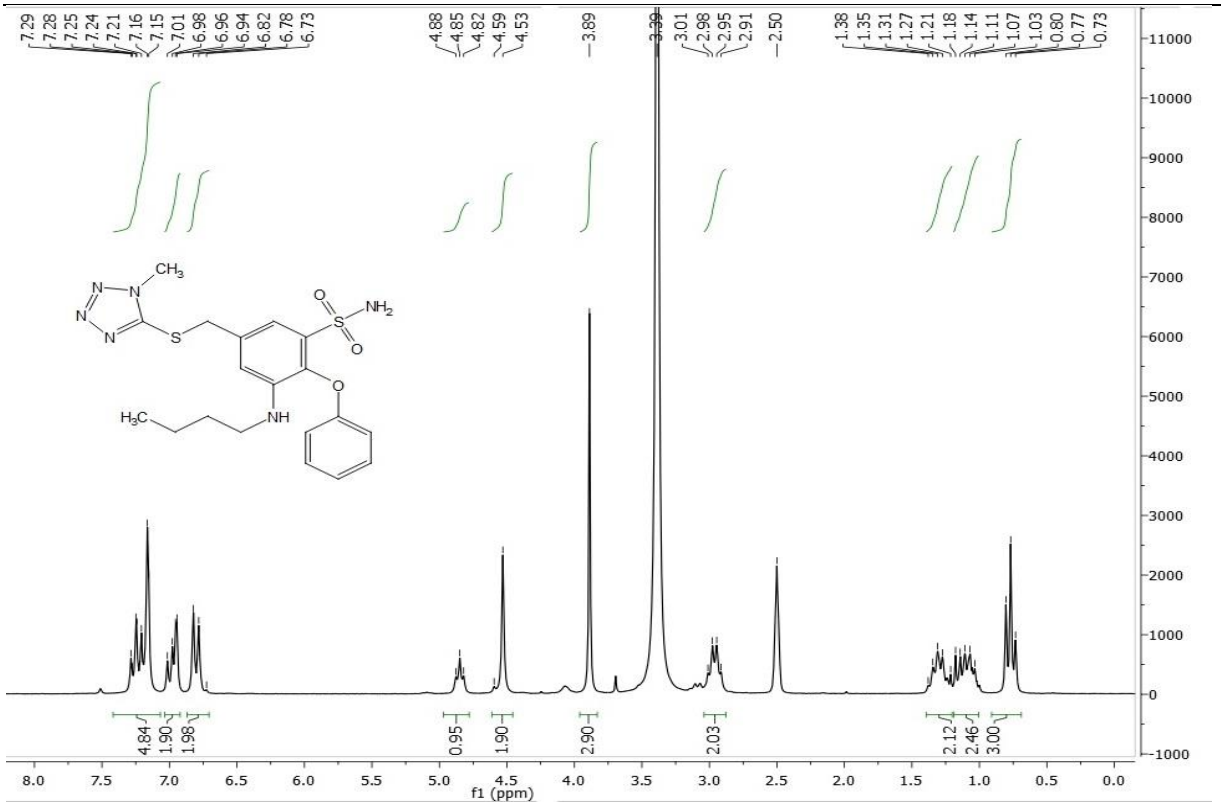
Compound **9** mass spectrum



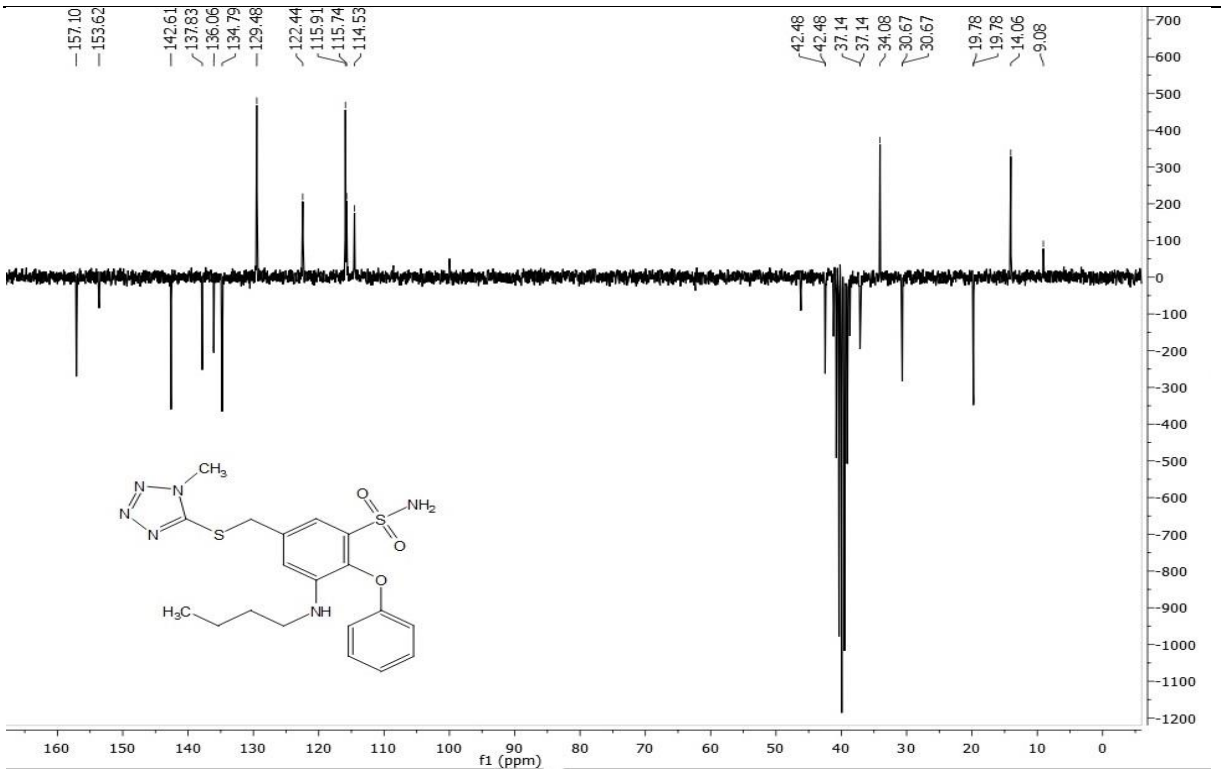
Compound **9** HRMS



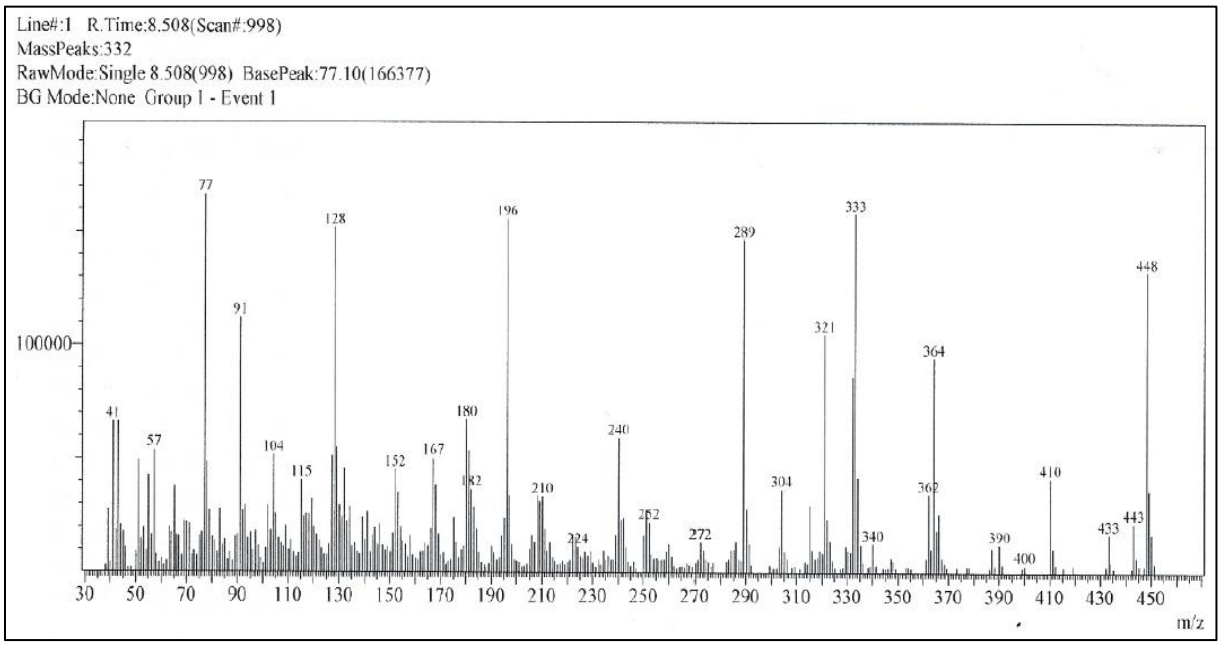
Compound **10** ^{1}H-NMR (DMSO-d_6)



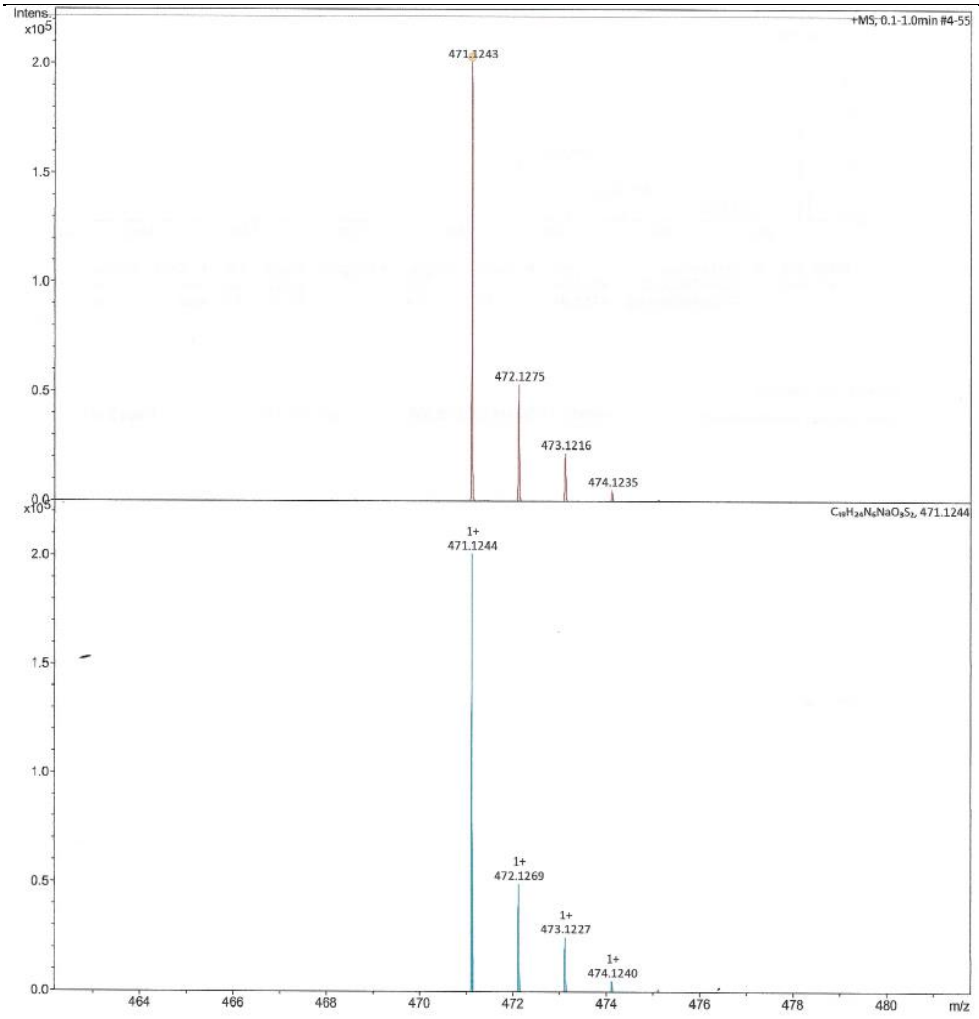
Compound **10** ^{13}C-NMR (DMSO-d_6)



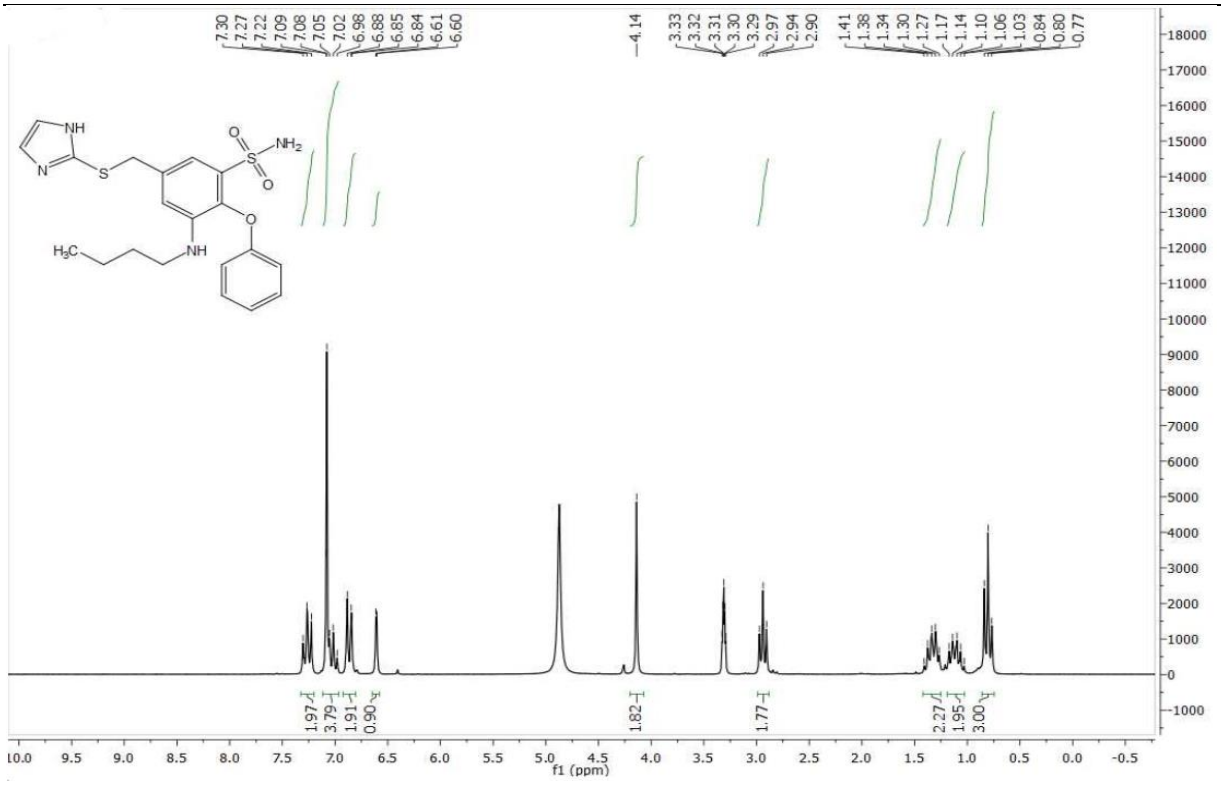
Compound **10** mass spectrum



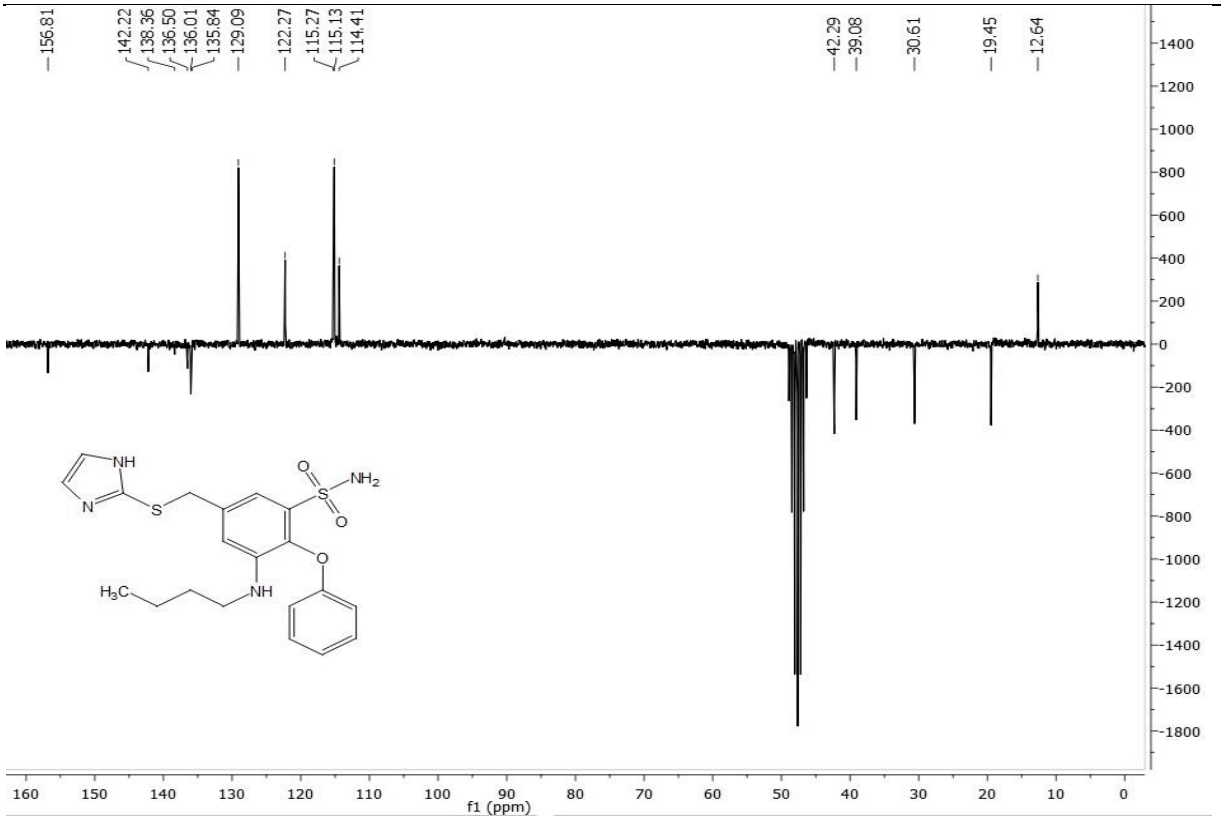
Compound **10** HRMS



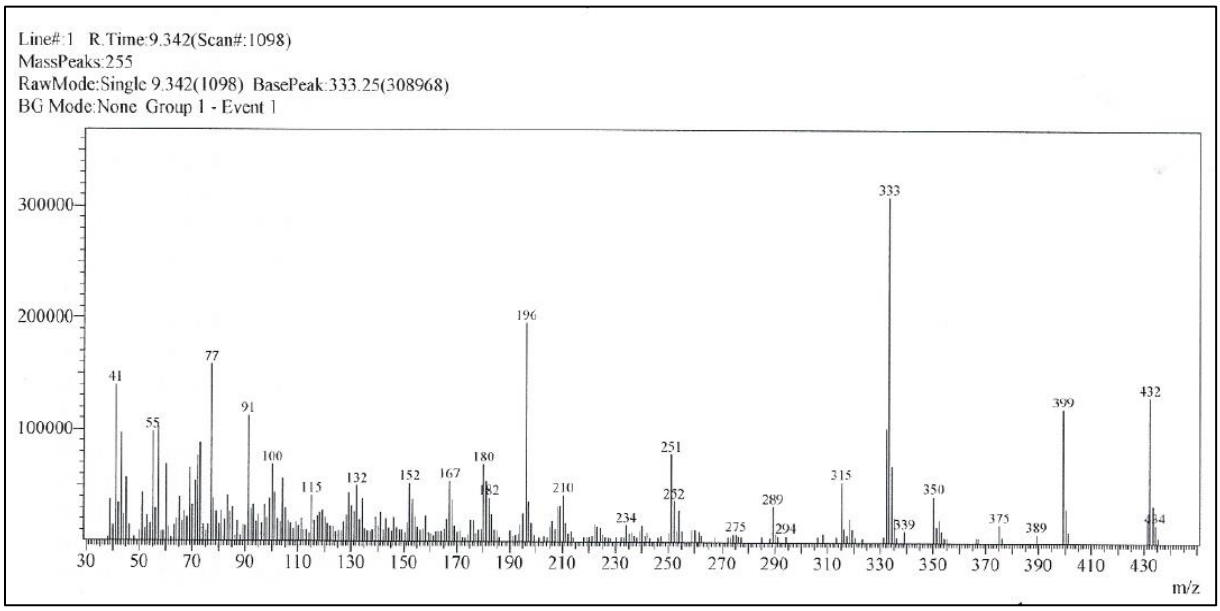
Compound **11** ^{1}H-NMR (MeOD)



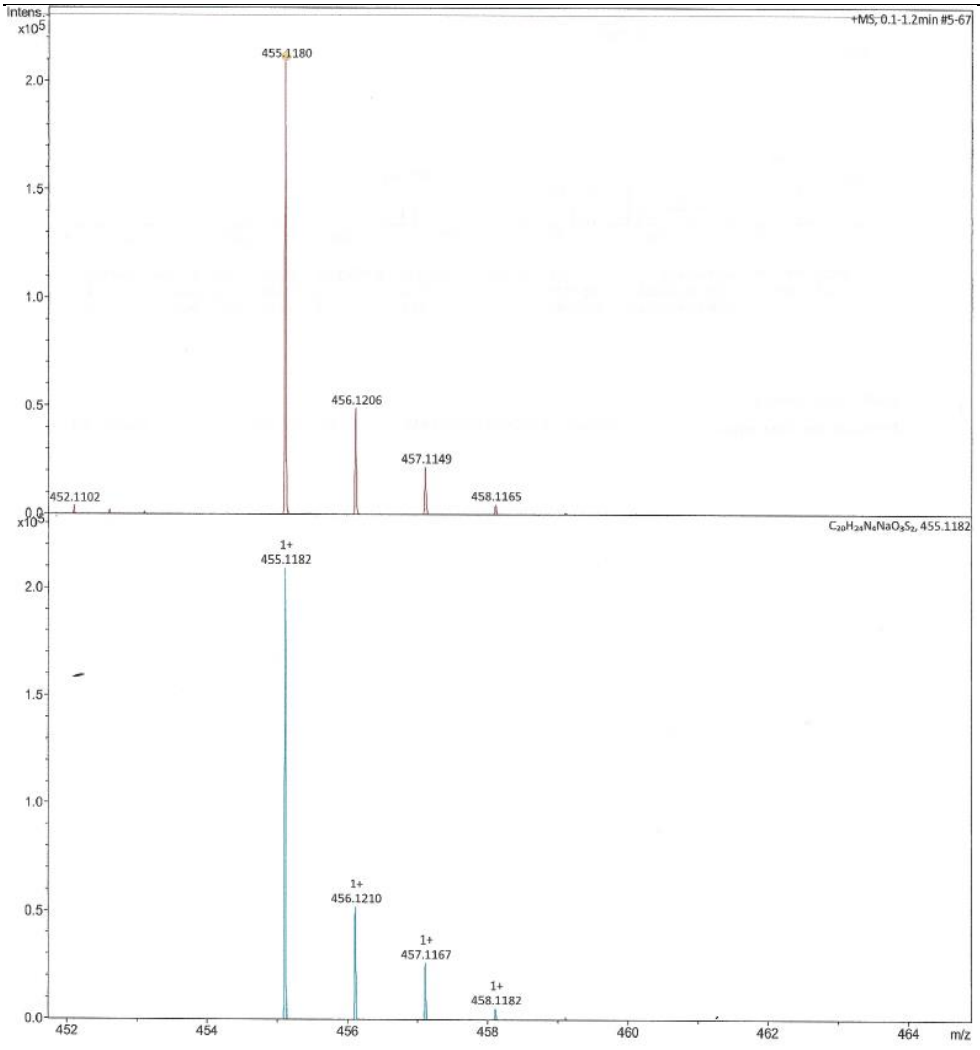
Compound **11** ^{13}C-NMR (MeOD)



Compound **11** mass spectrum



Compound **11** HRMS



8.1 Internal codes for Compound **1-11**

Compound **1** = BUM 10

Compound **2** = BUM 95

Compound **3** = BUM 6

Compound **4** = BUM 7

Compound **5** = BUM 63

Compound **6** = BUM 640 HCl

Compound **7** = BUM 650

Compound **8** = BUM 641

Compound **9** = BUM 647

Compound **10** = BUM 648

Compound **11** = BUM 651

9 References

Cherian, A., & Thomas, S. V. (2009). Status epilepticus. *Annals of Indian Academy of Neurology; 12 (3)*, pp. 140-153.

Feit, P. W. (1970). *Germany Patent No. DE 1964503.*

Feit, P. W., Bruun, H., Nielsen, O. T., & Bretting, C. A. (1977). *Germany Patent No. DE 2630637.*

Flynn, G. A., Yool, A. J., Migliati, E. R., & Ritter, L. S. (2008). *United States Patent No. WO 2008052190.*

Kambli, L., Bhatt, L. K., Oza, M., & Prabhavalkar, K. (2017, July). Novel therapeutic targets for epilepsy intervention. *Seizure; 51*, pp. 27-34.

Kelso, E., McDermott, B., Silke, B., & Spiers, P. (2000, July 14). Positive effect of bumetanide on contractile activity of ventricular cardiomyocytes. *European Journal of Pharmacology; 400 (1)*, pp. 43-50.

Khanna, A., Walcott, B. P., & Kahle, K. T. (2013, June 25). Limitations of current GABA agonists in neonatal seizures: toward GABA modulation via the targeting of neuronal chloride transport. *Frontiers in neurology; 4 (78)*.

Löscher, W., & Brandt, C. (2010). Prevention or modification of epileptogenesis after brain insults: experimental approaches and translational research. *Pharmacological reviews; 62 (4)*, pp. 668-700.

Maa, E. H., Kahle, K. T., Walcott, B. P., Spitz, M. C., & Staley, K. J. (2011, August 12). Diuretics and epilepsy: Will the past and present meet? *Epilepsia; 52 (9)*, pp. 1559-1569.

Panayiotopoulos, C. P. (2005). *The Epilepsies: Seizures, Syndromes and Management.* Oxfordshire (UK): Bladon Medical Publishing.

Rennie, J., & Boylan, G. (2007, March). Treatment of neonatal seizures. *Archives of disease in childhood. Fetal and neonatal edition; 92 (2)*, pp. 148-150.

Schreppel, P. (2015). Diploma Thesis "Synthese von Bumetanid-Derivaten mit Wirkung auf das zentrale Nervensystem". University of Vienna, Austria.

Töllner , K., Brandt, C., Töpfer, M., Brunhofer, G., Erker, T., Gabriel, M., . . . Löscher, W. (2014). A Novel Prodrug-Based Strategy to Increase Effects of Bumetanide in Epilepsy. *Annals of Neurology; 75*, pp. 550-562.

Trinka, E., Cock, H., Hesdorffer, D., Rossetti, A. O., Scheffer, I. E., Shinnar, S., . . . Lowenstein, D. H. (2015, September). A definition and classification of status epilepticus-Report of

the ILAE Task Force on Classification of Status Epilepticus. *Epilepsia; 56 (10)*, pp. 1515-1523.

Vezzani, A., French, J., Bartfai, T., & Baram, T. Z. (2010, January). The role of inflammation in epilepsy. *Nature reviews. Neurology; 7(1)*, pp. 31-40.

Vezzani, A., Fujinami, R. S., White, H. S., Preux, P.-M., Blümcke, I., Sander, J. W., & Löscher, W. (2015, February). Infections, inflammation and epilepsy. *Acta neuropathologica; 131 (2)*, pp. 211-234.

Wu, C., & Sun, D. (2014). GABA receptors in brain development, function and injury. *Metabolic brain disease; 30 (2)*, pp. 367-379.