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NKCC1”

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Table of Content

1. Abstract.....	1
2. Introduction	3
2.1 Epilepsy	3
2.2 Autism Spectrum Disorders (ASD)	4
2.3 Neonatal Seizures.....	5
2.4 GABA	6
2.5 Chloride homeostasis	7
2.6 Current Therapy	9
2.7 Bumetanide	10
3. Objective	11
4. Synthesis of Bumetanide derivatives.....	12
4.1 Compound 1	15
4.2 Compounds 2-6	16
4.3 Compound 7	17
4.4 Compound 8	17
4.5 Compounds 9, 10.....	18
4.6 Compound 11	19
4.7 Compound 12	20
5. Discussion	20
6. Experimental Section.....	23
6.1 General Methods.....	23
6.2 Compound 1	24
6.3 Compound 2	25
6.4 Compound 3	27
6.5 Compound 4	29
6.6 Compound 5	31
6.7 Compound 6	33
6.8 Compound 7	35
6.9 Compound 8	37
6.10 Compound 9	38
6.11 Compound 10	39
6.12 Compound 11	40
6.13 Compound 12	41
7. Analytics.....	43
7.1 Internal Codes for compounds 1-12	62
8. References	63

1. Abstract

The main objective of this diploma thesis was to synthesize bumetanide (BTN) derivatives. The new drugs should penetrate the blood-brain-barrier (BBB) better than bumetanide itself. It is known that due to its pharmacokinetics, BTN only penetrates the BBB by 1-2%. Up to 98% of the drug is plasma-bound.

A higher concentration of BTN in brain cells would be a novel treatment option of epilepsy, especially neonatal seizures. If a sufficient concentration in the brain cells is present, BTN blocks the sodium-potassium-chloride cotransporter (NKCC1). Since the GABA_A receptor in newborns has an inverse direction compared to the GABA_A receptor in adults, a block of NKCC1 might counterbalance this switch of the ion flow in GABA_A. The excitatory effect of GABA_A in neonates would then become inhibitory, which is essential for the treatment of epilepsy. Blocking NKCC1 would decrease $[Cl^-]_i$, resulting in an activation of GABA_A leading to an Cl^- ion influx and thus to a hyperpolarization of the neurons. This inhibits temporarily brain cell signaling and should reduce neonatal seizures.

During this thesis 12 compounds were synthesized. Compounds **1-6** are amides. Compound **1** is a primary amide. Compounds **2-6** are secondary amides. These amides were synthesized by modification at position 1 of BTN, where a benzoic acid remnant is located. In addition, compound **6**, a 4-fluorophenyl benzamide, was reduced to an amine (compound **7**).

Furthermore, the lipophilicity of BTN was increased by replacing the benzoic acid at position 1 with a carbon chain. This was succeeded with compound **12**, which carries a nitroethylene remnant. To synthesize compound **12**, a methyl ester was formed out of the benzoic acid (compound **8**), then reduced to an alcohol (compound **9**) and the alcohol finally oxidized to an aldehyde (compound **11**). With compound **10**, a derivative was synthesized which is a precursor for the other derivatives.

Whether the formed derivatives penetrate the BBB better and inhibit NKCC1, must be explored in further *in vitro* / *in vivo* tests.

Das Hauptziel dieser Diplomarbeit war es, Bumetanid (BTN)-Derivate herzustellen. Die neu gewonnenen Derivate sollten die Blut-Hirn-Schranke besser durchdringen als Bumetanid. Es ist bekannt, dass BTN aufgrund seiner Pharmakokinetik die Bluthirnschranke nur zu 1-2% passiert. Bis zu 98% von BTN sind im Plasma gebunden.

Eine höhere Konzentration von BTN im Gehirn wäre ein möglicher Ansatz zur Therapie von Epilepsie, speziell von Epilepsie bei Neugeborenen. Man geht davon aus, dass bei ausreichender Konzentration in den Gehirnzellen BTN den Natrium-Kalium-Chlorid Transporter (NKCC1) blockiert. Da der GABA_A Rezeptor bei Neugeborenen einen entgegengesetzten Ionendurchfluss hat im Vergleich zum GABA_A Rezeptor bei Erwachsenen, könnte ein Block von NKCC1 diese Richtungsänderung wieder ausgleichen. Durch die Blockade von NKCC1 würde die intrazelluläre Chloridkonzentration [Cl⁻]_i sinken und eine Aktivierung von GABA_A hätte eine inhibierende Wirkung zur Folge. Dies geschieht dadurch, dass es zu einem Cl⁻ Ionen Einstrom kommt und somit zu einer Hyperpolarisation in den Neuronen. Dadurch wäre die Signalübertragung der Gehirnzellen vorübergehend gehemmt und epileptische Anfälle bei Neugeborenen verringert.

Im Zuge dieser Diplomarbeit wurden 12 Derivate synthetisiert. Die Derivate **1-6** sind Amide. Verbindung **1** ist ein primäres Amid. Die Verbindungen **2-6** sind sekundäre Amide. Darüber hinaus wurde die Verbindung **6**, ein *N*-4-Fluorophenyl-benzamid-Derivat, zu einem Amin reduziert (Verbindung **7**).

Des Weiteren wurde die Lipophilie von BTN erhöht, indem wir an Position **1** die Carbonsäure-Funktionalität durch eine weniger polare Seitenkette ersetzt haben. Dies ist uns mit Verbindung **12** gelungen, welche einen Nitroethylen-Rest trägt. Um Verbindung **12** herzustellen, wurde anfangs ein Methylester hergestellt (Verbindung **8**), dieser dann zu einem Alkohol reduziert (Verbindung **9**) und schließlich der Alkohol zu einem Aldehyd oxidiert (Verbindung **11**). Mit Verbindung **10** haben wir auch ein Derivat synthetisiert, welches eine Ausgangssubstanz für die weiteren Syntheseschritte darstellt.

Ob schlussendlich die gebildeten Derivate die Blut-Hirn-Schranke besser passieren und folglich eine bessere Hemmung von NKCC1 bewirken, muss in weiteren *in-vitro/in-vivo* Versuchen erforscht werden.

2. Introduction

2.1 Epilepsy

Epilepsy is one of the most common neurological diseases nowadays, affecting more than 70 million people worldwide. It is defined by the predisposition to recurrent unprovoked (two or more) seizures due to brain alteration. [1]

Epilepsy is divided into three major categories: the idiopathic, the symptomatic and the presumed symptomatic (“cryptogenic”) form. The main type (affecting 6 out of 10) is called idiopathic epilepsy, meaning there is no recognizable cause. It is discussed that this type arises from genetical abnormalities which lead to alteration of the brain. Whereas the symptomatic (acquired) form arises from pathophysiological processes and lesions in the brain caused by an insult, such as traumatic brain injuries (TBI), prenatal/perinatal injuries, CNS malformations and tumors, infection, intracerebral hemorrhage and stroke. These causes, alone or in combination, can increase the risk of developing epilepsy. That is what scientists describe as “epileptogenesis”. Cryptogenic epilepsy is presumably due to a lesion although the evaluation therefore is not clear. Epilepsy is one of the few brain diseases in which people at risk can be diagnosed with and yet there is no therapy approach to prevent it from developing. [2] [3]

An epileptic seizure is the result of abnormal electrical discharges in the cerebrum. Depending on which cerebral area is affected consciousness, motor activities/skills, sensibility, vegetative nervous system, thinking, memory, perception and emotions can be disturbed. [4]

A seizure lasts for a few seconds to minutes except the status epilepticus (SE) – the most severe form of an epileptic seizure. Traditionally a SE was defined as a seizure lasting 30 minutes without recovery in between. The International League Against Epilepsy (ILAE) redefined SE in 2015. They distinguished between a bilateral tonic-clonic seizure lasting longer than 5 minutes and absence SE and focal SE lasting longer than 10 minutes. [5]

Regarding the types of seizures, it is necessary to know that different causes of epilepsy lead to different seizures. A genetic predisposition leads to primary generalized seizures in which both brain hemispheres are affected. An acquired epilepsy leads to focal seizures in which (at least in the beginning) only one brain hemisphere is affected. The resulting seizures occur in the opposite body region of the affected hemisphere. Both seizure types can lead to unconsciousness. [4]

2.2 Autism Spectrum Disorders (ASD)

According to the World Health Organization (WHO) 1 out of 160 children is affected by autism spectrum disorder (ASD), a neuronal developmental disorder. [6] It appears 5 times more often in males than in females. [7] Most commonly ASD appears within the first 5 years of life and can hold on until adolescence and adulthood. ASD is associated with impaired social behavior, restricted communication skills as well as one-sided repetitive behaviors and restricted interests. Further, ASD as a neuronal disorder can be a cause of epilepsy, depression, anxiety and attention deficit hyperactivity disorder (ADHD). [8]

The GABA signaling of patients with ASD is different to that of a healthy person. Therefore, reducing the intracellular Cl^- concentration can benefit the treatment of ASD. [7] The efficacy of bumetanide treatment against autism was reported by *Lemonnier et al.* [9] for the first time – in a double-blinded controlled study including 60 children. The therapeutic effects of applied behavior analysis (ABA) treatment can be increased when adding bumetanide to the therapy as shown in a recent pilot study. [8]

Bumetanide is an NKCC1 antagonist and has been used since 1986 in children as a loop diuretic to treat hypertension, bronchopulmonary dysplasia, nephritic syndromes or heart congestions. When blocking NKCC1 the intracellular Cl^- concentration decreases. This therapeutic approach regarding the GABA_A signaling is also used to treat neonatal seizures and described in detail later. [7]

2.3 Neonatal Seizures

In the neonatal period seizures are much more often than at any other age. The incidence of epileptic seizures with 2-3.5 per 1.000 live births is higher compared to adults. [10] Regarding epileptogenesis, infants suffering from epileptic seizures are more likely to develop epilepsy and significant cognitive and motor disabilities later in life. To be more precise a 7-year-follow-up study [11] shows that 17 – 56% patients are likely to develop epilepsy as a result of neonatal seizures. The most common causes are hypoxic-ischemic encephalopathy (HIE), hemorrhage or cerebral infarction. [10] [12]

The conventional treatment of epilepsy using antiepileptic drugs (AED) is described later. In the treatment of neonatal seizures, the molecular and cellular signals need to be examined more closely. The main inhibitory neurotransmitter is γ -aminobutyric acid (GABA). [4] However, this is only partially right when discussing the GABA-receptors of infants. These young patients have a significantly lower expression of the classical GABA-receptors. It is proven that there is an age-dependent difference in neuronal signaling regarding this receptor. An activation of GABA_A in a normal adult brain results in membrane hyperpolarization due to a Cl⁻ influx, causing inhibitory effects, whereas an activation in an immature brain causes Cl⁻ ions to leave the cell, resulting in a depolarization which leads to neuronal excitation. Depending on the patient's age the GABA_A receptor can cause inhibitory or excitatory effects. [13]

2.4 GABA

γ -Aminobutyric acid (GABA) is synthesized from glutamate by catalysis of glutamate decarboxylase. In one single step the most important excitatory neurotransmitter, glutamate, turns into the main inhibitory neurotransmitter GABA. Glutamate decarboxylase, which is necessary for the transformation, is expressed in the central nervous system exclusively in GABA neurons. There are 2 main types of receptors: the GABA_A and the GABA_B receptor. The GABA_A receptor is a ligand-gated ion channel carrying primarily Cl⁻ ions, but also HCO₃⁻. The GABA_B receptor belongs to the G protein-coupled receptors. [4]

When antiepileptic drugs like benzodiazepines or barbiturates, bind at their binding site at

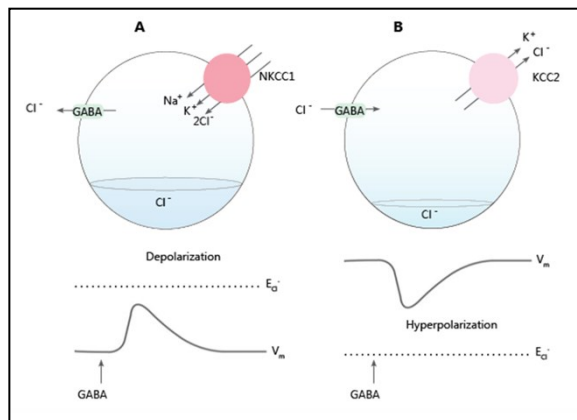


Figure 1 GABA_A Hyperpolarization/Depolarization
[own picture]

the GABA_A receptor, an allosteric conformational change occurs. The result of both drugs is a higher permeation of Cl⁻ ions leading to a hyperpolarization and therefore inhibiting the excitability of the cell. Regarding the neuronal membrane an electrochemical gradient determines whether Cl⁻ flux is directed outwardly or inwardly (Fig. 1). The adult brain has a low Cl⁻ concentration [Cl⁻]_i, meaning an activation of GABA_A leads to an

influx of Cl⁻ ions, whereas immature neurons are depolarized due to a high intracellular concentration of chloride [Cl⁻]_i. It was shown that the depolarizing and excitatory effect of GABA in neonates can result in seizures, neuronal excitation and epilepsy later on. The more glutamate and GABA synapses are developed, the more likely it is that the GABA_A receptor is starting to exert its inhibitory function. [14]

2.5 Chloride homeostasis

In the progress of maturation of the neuronal cells, starting from the very first postnatal day, there is a switch in the electrophysiological function of GABA. Responsible therefore are the cation-chloride co-transporters (CCC): the inwardly-directed $\text{Na}^+\text{-K}^+\text{-2Cl}^-$ Cotransporter (NKCC) and outwardly-directed $\text{K}^+\text{-Cl}^-$ Cotransporter (KCC). In the neonatal period the NKCC1 cotransporter is highly expressed compared to KCC2. The resulting high influx of Cl^- ions and low efflux causes a higher Cl^- concentration. Therefore, GABA_A has a depolarizing effect as Cl^- leaves the cell – seizures occur. Due to osmotic changes during maturation, as the expression of NKCC1 decreases and KCC2 increases, the inwardly directed Cl^- flow turns into an outward flow. GABA_A now has a hyperpolarizing effect when activated.

These cotransporters are encoded by the SLC12A gene. [15] The intrinsic membrane proteins are responsible for the regulation of the chloride concentration $[\text{Cl}^-]_i$ within a cell. (Figure 2) For the transport of Cl^- through the plasma membrane, they make use of the electrical gradient provided by the counterions sodium (Na^+) and potassium (K^+). The intracellular substrate concentration (i.e., cations) is determined by an active Na^+ / K^+ pump.

NKCC1 makes use of the electrical gradient with Na^+ and increases the intracellular Cl^- concentration, whereas KCC2 lowers the intracellular concentration due to the gradient mediated by K^+ . [14] Although the cotransporters are considered electrically neutral, they indirectly cause a change of the membrane potential of the cell. [15] [16]

KCC is subdivided KCC1, KCC2, KCC3, KCC4. Relevant for epileptic seizures is KCC2, which occurs exclusively in mature neurons. [14]

NKCC is subdivided into subtype 1 and 2. These phosphoproteins are active when phosphorylated and inactive when dephosphorylated being regulated by WNK-STE20/SPS1-related proline/alanine-rich kinase (SPAK) and WNK-SPAK/Oxidative stress response 1 (OSR1) kinase. (Figure 2) [17] NKCC1 occurs in neurons, glial cells, choroid plexus, and in the vascular endothelium, whereas NKCC2 occurs mainly in the kidney respectively in the thick ascending limb of Henle (TAL). Bumetanide inhibits both forms, especially NKCC2 as a loop diuretic to reduce blood volume and consequently blood pressure. The effect on NKCC1 is being discussed and bumetanide is currently in phase 1 clinical trials as an add-on therapy in neonatal seizures in HIE infants. [13] [18]

According to *Delpire and Gagnon 2008*: “NKCC function depends on three basic factors: how many cotransporter molecules are expressed at the cell surface, the concentration of the ionic substrates on each side of the plasma membrane, and the intrinsic rate of transmembrane cotransport.” [15]

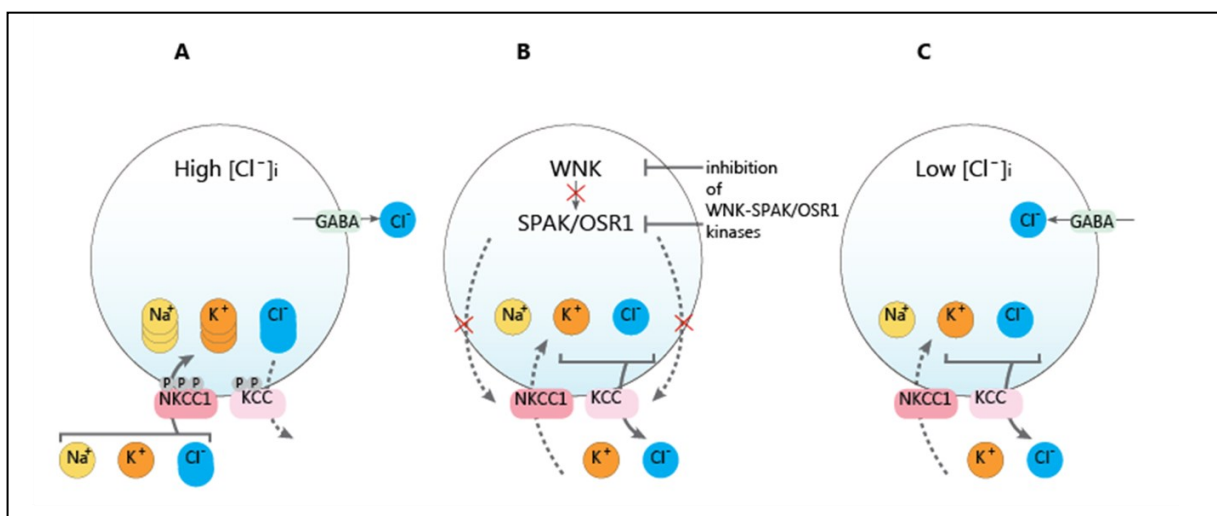


Figure 2 Mechanism of GABAA/NKCC1 [own picture]: (A) Showing a pathological increase in activity of NKCC1 and/or decrease of KCC2 resulting in several diseases like temporal lobe epilepsy, neonatal seizures, neuropathic pain, schizophrenia and motor disabilities. These diseases result from an influx of Cl^- . Since the $[Cl^-]_i$ is raised, the GABAA mediated hyperpolarization does not work. (B) NKCC1 is stimulated and KCC2 is inhibited by the WNK-SPAK/Oxidative stress response 1 (OSR1) kinases. Therapeutic inhibition of WNK-SPAK/OSR1 kinases would hold back the phosphorylation of both NKCC1 and KCC2, resulting in reciprocal inhibition of NKCC1 and stimulating KCC2. Thus, a net efflux of Cl^- and K^+ is promoted. (C) Due to reciprocal activation of KCC and inhibition of NKCC1 $[Cl^-]_i$ is lower compared to (A). This signaling is used as a strategy for treating neurological disorders by normalizing the hyperpolarization ability of GABAA. [17]

2.6 Current Therapy

Since neonatal and adult seizures are different in etiology, one might assume that a different treatment exists. Currently, adult seizures are treated with a variety of antiepileptic drugs (AED) (Tab. 1) including benzodiazepines and barbiturates – exerting their effects *via* modulation of GABA_A or by antagonism of NMDA receptors. Due to developmental changes in the expression of neurotransmitters and receptors (as mentioned earlier) these therapeutic strategy does not fit for neonates accurately. Therefore, benzodiazepines and barbiturates may have limited efficacy in controlling neonatal seizures as they modulate GABA_A receptor resulting in paradoxically excitatory effects. [19]

First Generation	Second Generation	Third Generation
Phenobarbital	Felbamate	Clobazam
Phenytoin	Gabapentin	Lacosamide
Valproic acid	Lamotrigine	Perampnenel
Carbamazepine	Levetiracetam	Rufinamide
Ethosuximide	Oxcarbazepine	Vigabatrin
	Topiramate	
	Zonisamide	

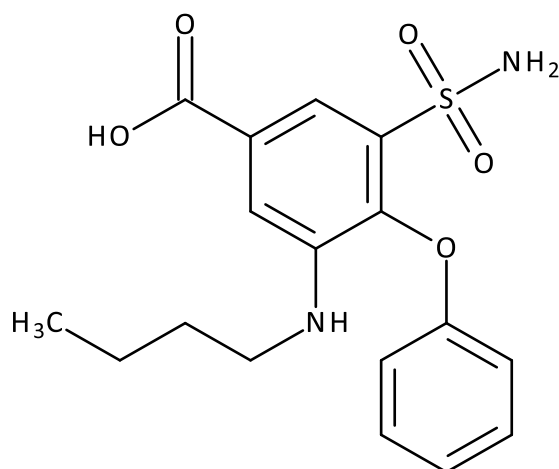
Table 1: Antiepileptic Drugs

And yet, phenobarbital (PB) is the most common treatment of neonatal seizures. A study, performed 20 years ago, pointed out that PB as well as phenytoin stopped seizures in less than 50% of cases. [20] The AED levetiracetam is currently discussed whether or not it should be used as first-line therapy in neonates. An actual clinical trial is comparing the efficacy of PB and levetiracetam as first- line therapy for neonatal seizures. [12] [21]

The imbalance of NKCC1 and KCC2 in immature brains put the focus on bumetanide as a treatment option for epilepsy, especially against neonatal seizures. Regarding the high $[Cl^-]_i$ in neonates, bumetanide could be a novel treatment as it inhibits NKCC1 and causes a decrease of the $[Cl^-]_i$ and therefore GABA_A to transport Cl^- ions into the cell resulting in a hyperpolarization. Bumetanide is currently in the first phase of a clinical trial as an add-on therapy for neonatal seizures. [13] [18]

2.7 Bumetanide

Scheme 1: Bumetanide



Bumetanide (BTN) is an FDA approved fast-acting loop diuretic. It inhibits the $\text{Na}^+\text{-K}^+\text{-2Cl}^-$ cotransporters NKCC1 and NKCC2. The isoform NKCC1 is expressed ubiquitously in many tissues including the brain whereas NKCC2 is exclusively expressed in the renal tubule cells in the loop of Henle. To be more precise BTN and other loop diuretics act at the ascending limb of Henle's Loop (TALH) in the kidney. Regarding the treatment against epilepsy it stabilizes the membrane potential. [22]

This makes NKCC1 an interesting target for developing new treatments against neonatal seizures, epilepsy, schizophrenia and autism. Pharmacokinetics make it more difficult to establish BTN as a novel treatment option. Basically, its ability to penetrate the blood-brain-barrier (BBB) is highly limited particularly because of the plasma protein binding (up to 98%) and extensive ionization (>99%) at physiological pH. Furthermore, the brain concentration of BTN is reduced by organic anion transporters. [23]

Systemic administration of BTN means that approximately 1-2% of the concentration found in plasma predominates in the brain. This amount in the brain is below the required dose for a therapeutic effect and in the course of dose increasing there are numerous side effects of bumetanide like increased diuresis, hypokalemic alkalosis and loss of hearing that can occur. [23]

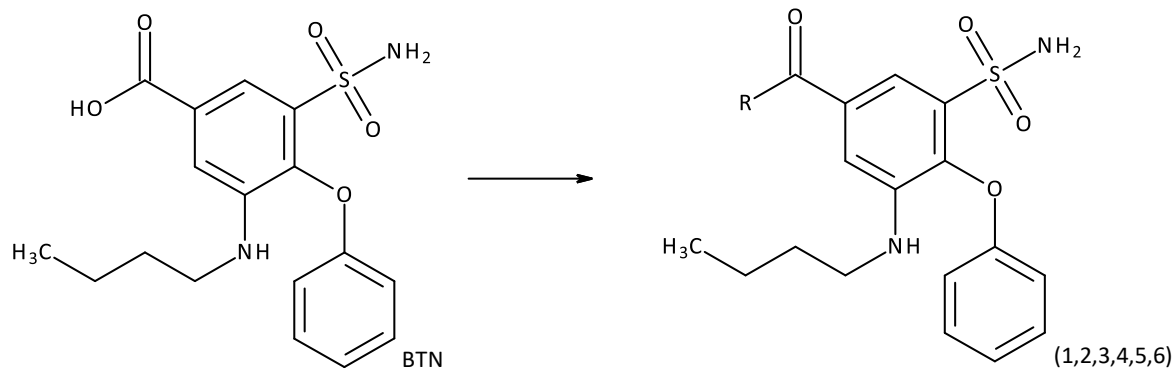
After all, bumetanide derivatives with better pharmacokinetics need to be developed and potentially become a novel therapy against epilepsy, especially neonatal seizures.

3. Objective

Bumetanide is gaining interest in the therapy of neuronal diseases, as it is considered to inhibit NKCC1 in immature neurons. However, its pharmacokinetics limit the potential effect against e.g. neonatal seizures. Its poor ability to pass through the blood-brain-barrier (BBB) makes it difficult to reach an effective concentration. Therefore, the objective of this thesis is to synthesize bumetanide derivatives with a higher lipophilicity making them penetrate the BBB better than bumetanide. Consequently, these derivatives should have a better efficacy, inhibit NKCC1 at a lower concentration and be able to lessen seizure activity. By blocking NKCC1 the paradoxical, depolarizing effect of GABA_A in immature brains should be inhibited and antiepileptic drugs like phenobarbital should cause a hyperpolarization in neonates as they do in adults. Clinical trials are required to determine whether these derivatives can act as monotherapy or add-on therapy for common AEDs that affect the GABA_A receptor.

4. Synthesis of Bumetanide derivatives

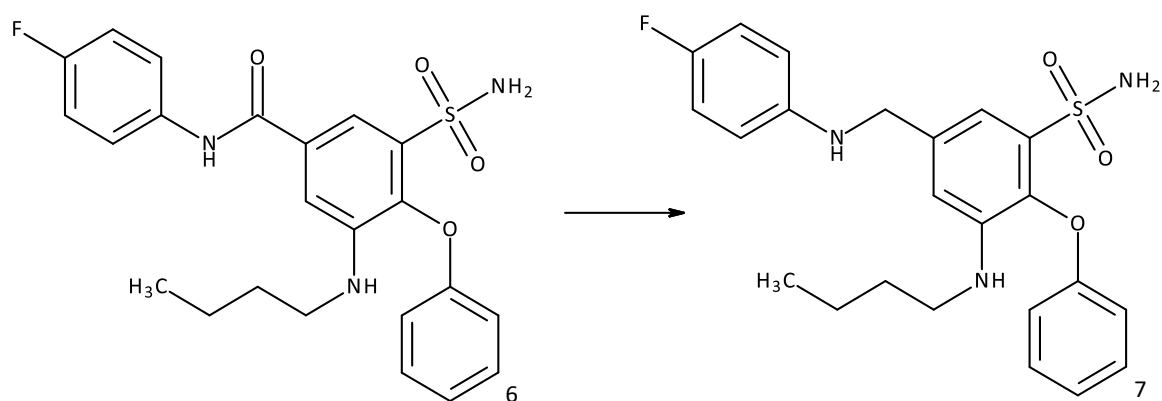
Scheme 2: Synthesis overview for compounds **1-6**



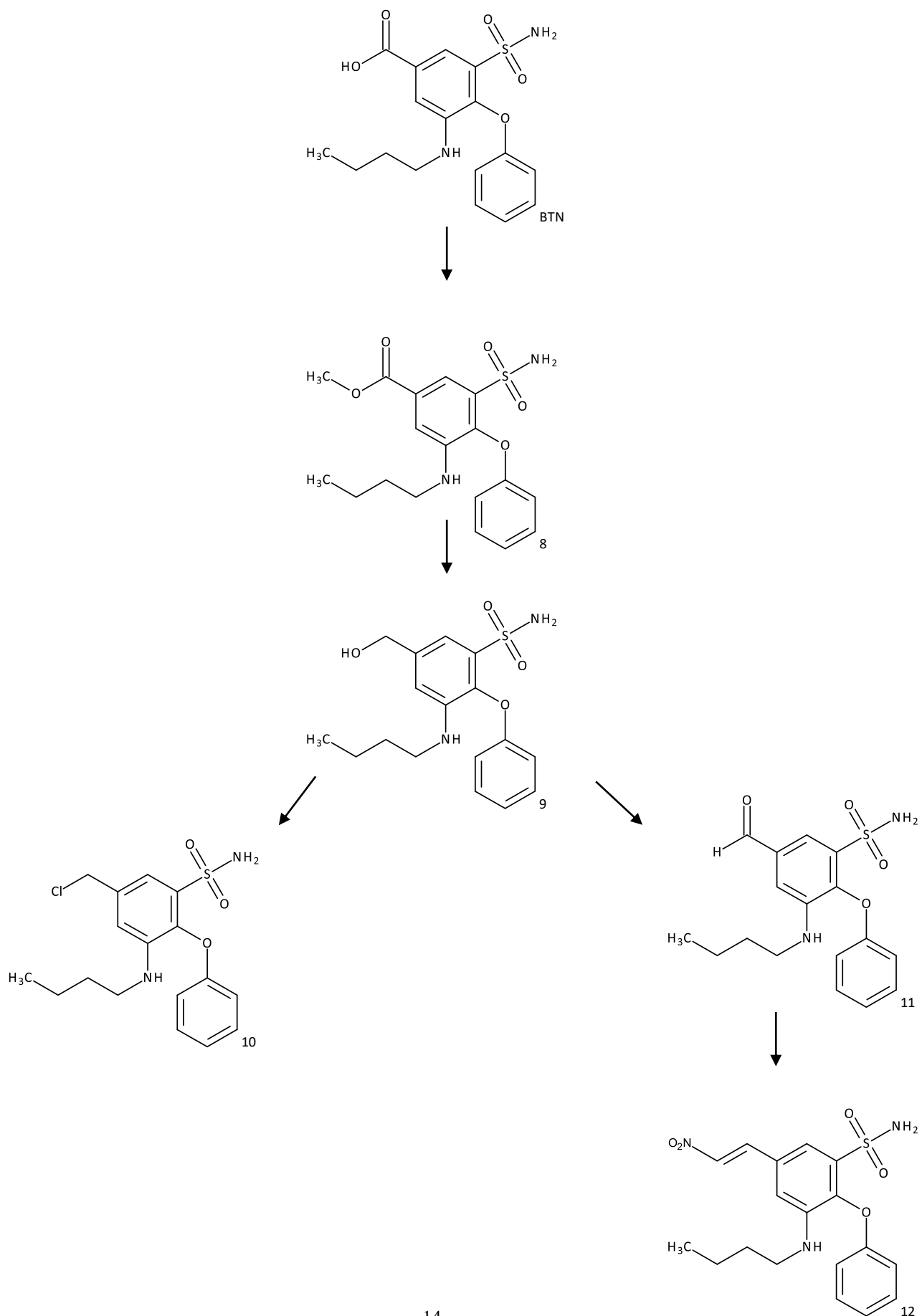
Compounds	R
Compound 1	
Compound 2	
Compound 3	
Compound 4	
Compound 5	
Compound 6	

Compound 7

Scheme 3: Synthesis of compound **7**

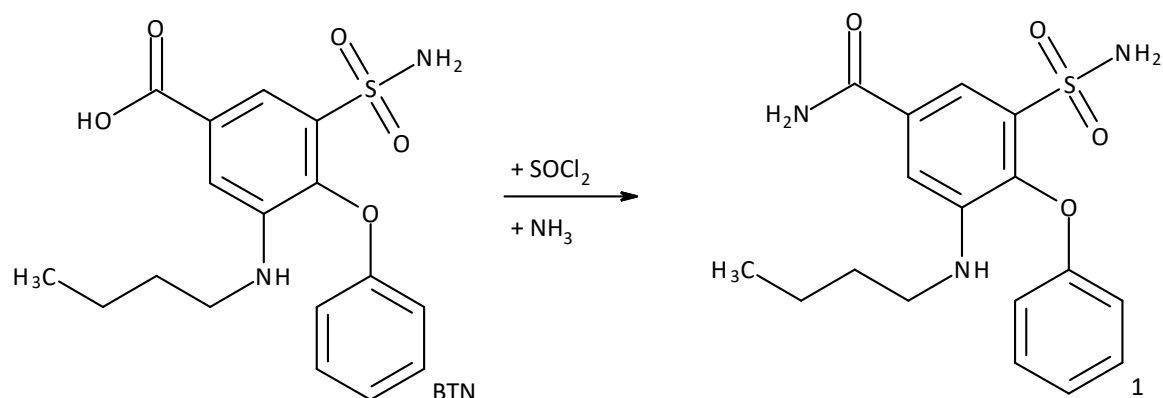


Synthesis Overview for compounds 8-12



4.1 Compound 1

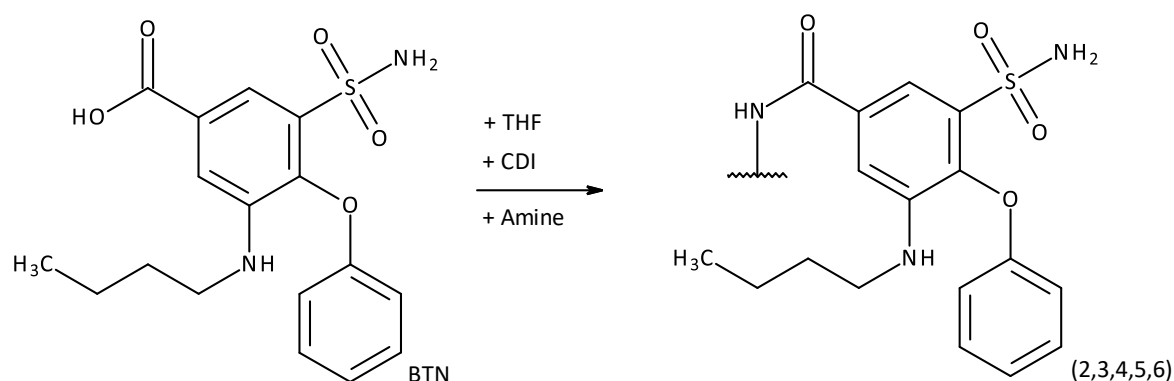
Scheme 4: Synthesis of compound 1



In this synthesis, benzoic acid reacts with thionyl chloride (SOCl_2) to form an acyl chloride. After the residual thionyl chloride was removed with an aspirator (pump) ammonia solution was added. Nucleophilic substitution afforded the desired product, a benzamide with a yield of 34%.

4.2 Compounds 2-6

Scheme 5: Synthesis of compounds 2-6



Originating from bumetanide which contains a benzoic acid as a functional group, the following amides were synthesized *via* nucleophilic substitution. Bumetanide was mixed with 1,1'-carbonyldiimidazole (CDI), which activates the carboxylic acid. Afterwards, different amines were added to synthesize the desired amides:

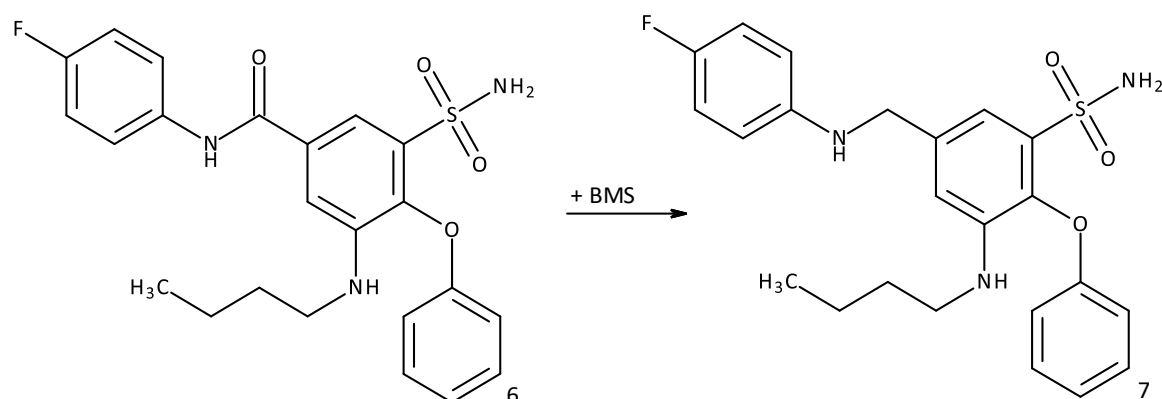
Compounds	Amines added
Compound 2	Cyclohexylamine
Compound 3	2-Picolylamine
Compound 4	3-Picolylamine
Compound 5	4-Picolylamine
Compound 6	4-Fluoroaniline

The reactions went smoothly. The yields were between 68 and 90% for all compounds except compound 6 (44%).

The only successful amide reduction resulted in compound 7. There would still be the possibility to perform a reductive amination. Although first attempts were promising, there was not enough time in the course of this diploma thesis to start a new attempt here.

4.3 Compound 7

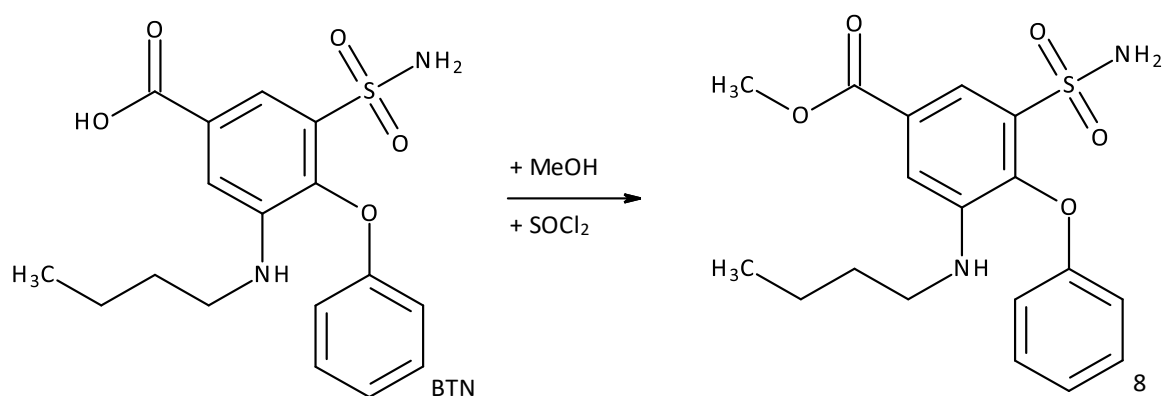
Scheme 6: Synthesis of compound 7



Compound **6** has a higher lipophilicity compared to bumetanide. The aim of this synthesis was to reduce the amide group. Originating from compound **6** borane dimethyl sulfide complex (BMS) was added as a reduction agent (4 equivalents). This resulted in a yield of only 38%.

4.4 Compound 8

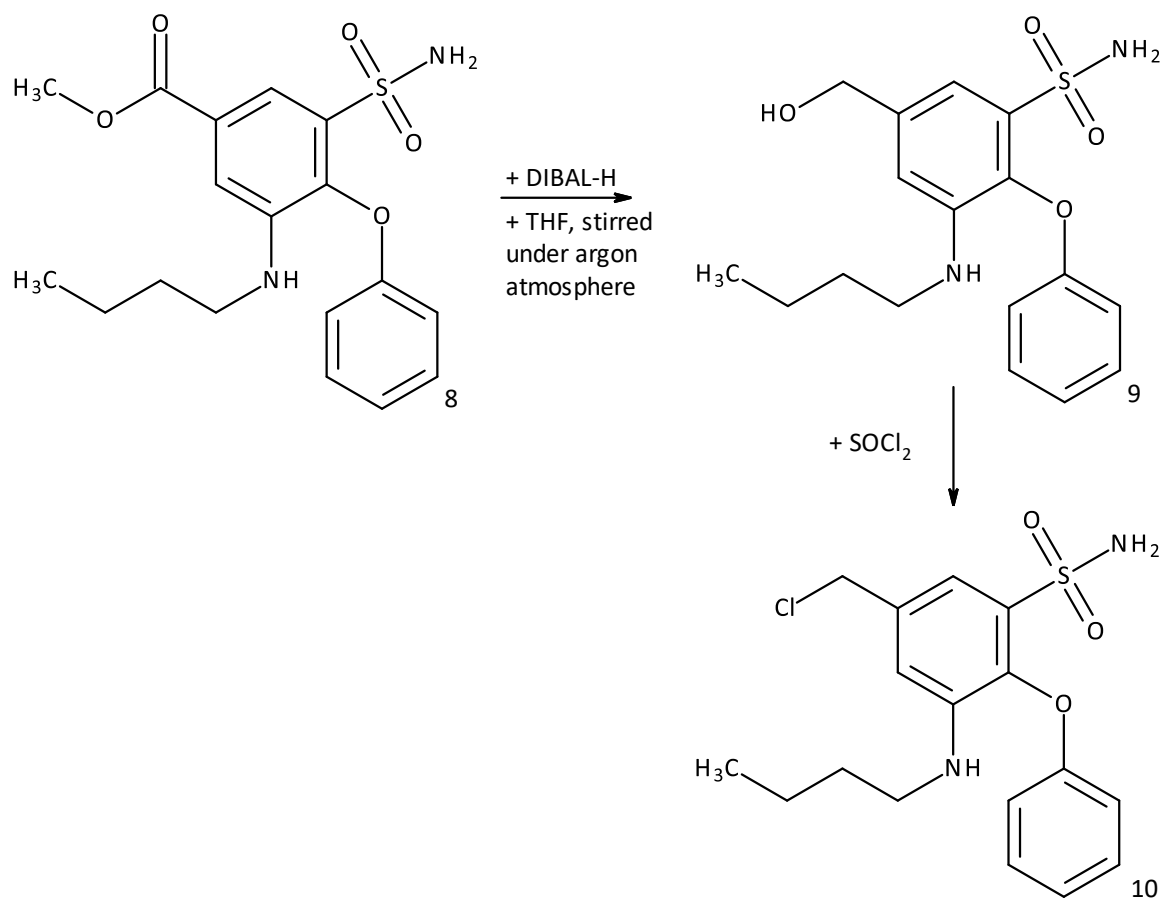
Scheme 7: Synthesis of compound 8



The aim of this synthesis was to increase the lipophilicity and to get a product which could serve as a prodrug of bumetanide. Therefore, SOCl₂ was mixed with a suspension of bumetanide and methanol. The benzoic acid was esterified *via* nucleophilic substitution. The reaction worked well with a yield of 94%.

4.5 Compounds 9, 10

Scheme 8: Synthesis of compound 9, 10

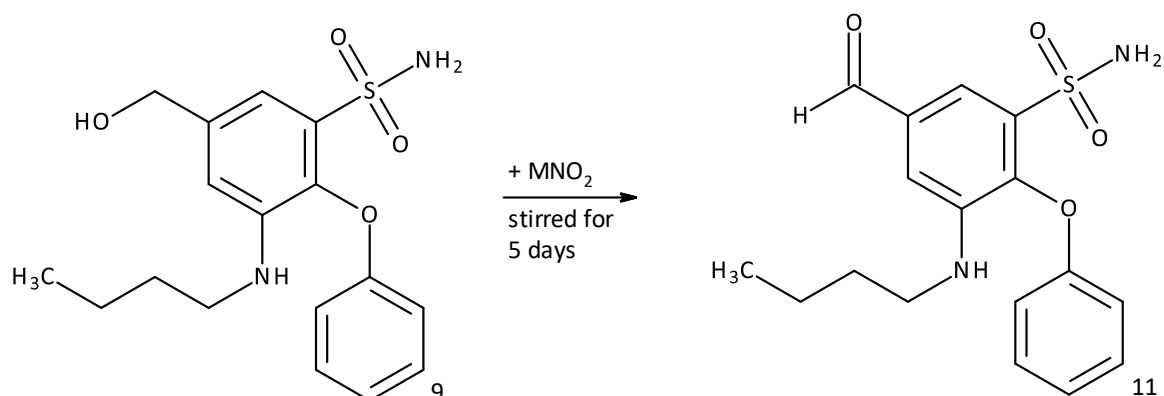


Originating from compound **8** the carboxylic ester was reduced to an alcohol (compound **9**). Diisobutylaluminium hydride (DIBAL-H) was used as a reducing agent. Since synthesis with DIBAL-H required an inert atmosphere, it was carried out under argon atmosphere.

Further, the alcohol was substituted with chloride (compound **10**) using SOCl_2 . Although this synthesis was performed several times the yield was always below 30%.

4.6 Compound 11

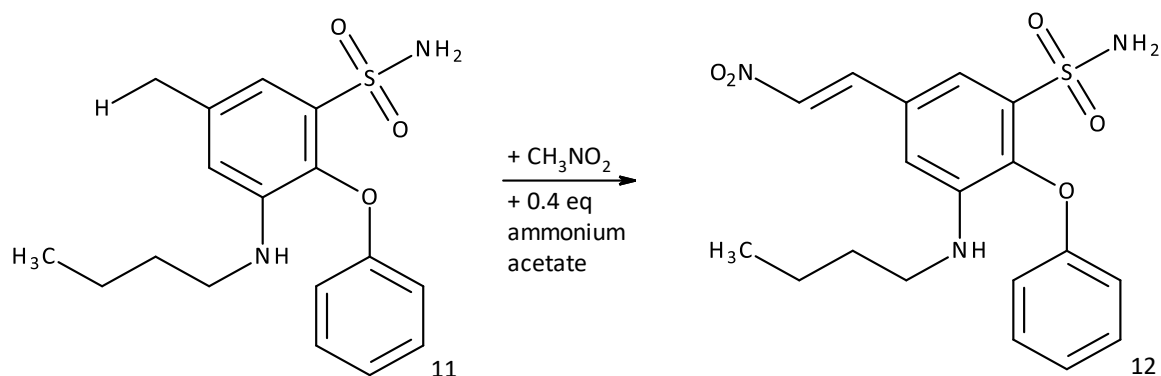
Scheme 9: Synthesis of compound 11



This synthesis took almost 5 days. A benzylic alcohol substructure was oxidized to an aldehyde. Compound 9 was dissolved in toluene/ethyl acetate and MnO₂ was added. The mixture was heated to 40°C and stirred for three days. Afterwards, the TLC showed that there was still reactant present. Therefore, MnO₂ was added again and the mixture was stirred for two more days. After 5 days the yellow-greenish crystals yielded 34%.

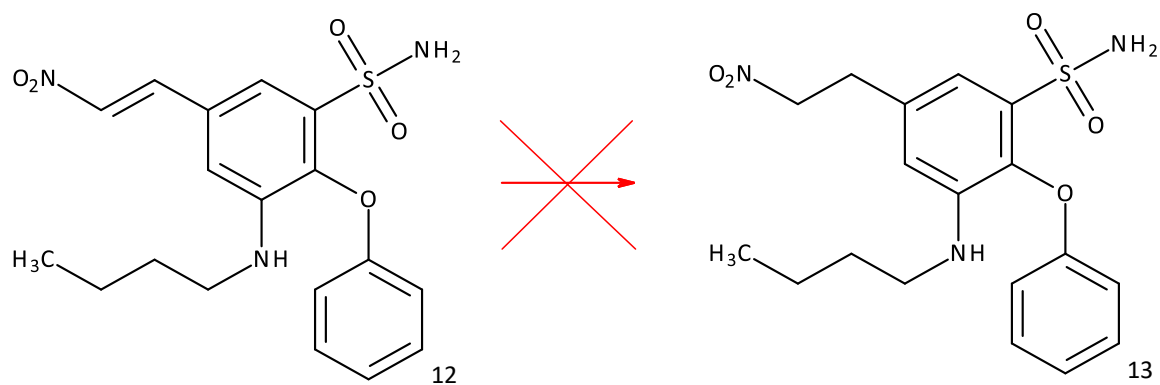
4.7 Compound 12

Scheme 10: Synthesis of compound **12**



For this product compound **11** was dissolved in nitromethane and ammonium acetate was added. The mixture was heated to 100°C under reflux. The TLC showed that there was still reactant present. Therefore, ammonium acetate was added again and stirred overnight. Unfortunately, we were not able to synthesize compound **13**. We tried several times to hydrogenate the ethylene group – without success.

Scheme 11: Failed attempt to synthesize compound **13**



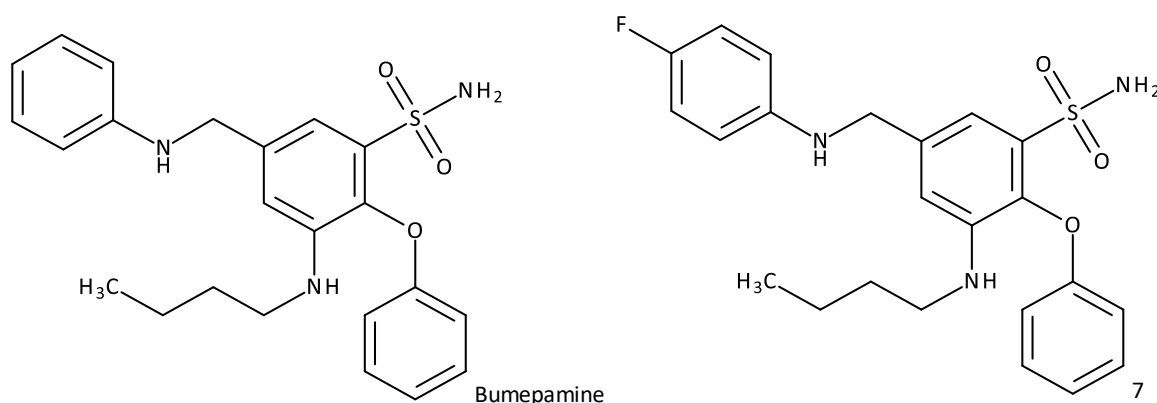
5. Discussion

The main objective of this thesis was to design derivatives of bumetanide with a higher lipophilicity. Since bumetanide is able to block NKCC1, it is gaining interest as a novel drug therapy against neonatal seizures. In fact, bumetanide hardly passes the blood-brain barrier (as described in more details above). For this reason, we have taken the goal to increase the lipophilicity of bumetanide.

Since the carboxylic group is significant for diuresis, substructures were synthesized at this position. The required dose of bumetanide to form a sufficient concentration in the brain would lead to serious side effects such as severe diuresis and ototoxicity. [24]

Brand et al. synthesized bumepamine and described its enhancing effect on phenobarbital's antiseizure efficacy. The synthesized amides during this thesis are structurally similar to bumepamine. Consequently, similar effects regarding the BBB can be expected.

Scheme 12: Comparison between bumepamine and compound **7**



In total, 5 amides were synthesized at position 1. Basically, it can be said that it is easily possible to synthesize amides from the benzoic acid of bumetanide (compound **2-6**). In addition, compound **6** was the only amide being successfully reduced to an amine (compound **7**). During the whole thesis the most challenging part was to reduce these amides. Unfortunately, at the end we ran out of time to perform a reductive amination. Bumepamine has an 80% lower diuretic activity in dogs when compared to bumetanide, a higher lipophilicity and a lower ionization at physiological pH.[24] Since compound **7** and bumepamine are structurally very similar, one can assume that the behavior concerning the BBB and pharmacodynamics are similar as well.

Further, the previously formed alcohol was oxidized to an aldehyde (compound **11**). The oxidizing agent used in this synthesis was MnO_2 .

Originating from compound **11**, compound **12** was formed by adding nitromethane and ammonium acetate. Despite several attempts, it was not possible to hydrogenate the ethylene group.

6. Experimental Section

6.1 General Methods

All chemicals and solvents were purchased from commercial suppliers (Sigma Aldrich, Merck, Apollo Scientific and TCI Europe) at analytical grade. Bumetanide was obtained from OChem Inc., Des Plaines, IL, US.

To monitor reactions *via* thin layer chromatography, silica gel F254 coated aluminum sheets from Merck were used.

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

Melting points were measured on a ThermoGalen Kofler hot stage microscope.

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

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

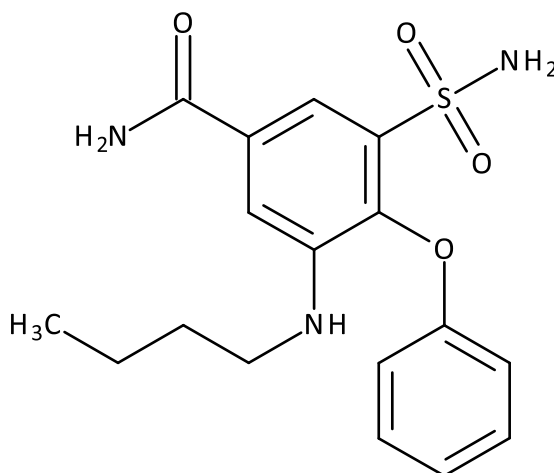
High-resolution mass spectra were performed on a MALDI-q/q-TOF-spectrometer (Bruker maxis HD) by Daniel Dobusch, Msc. at the University of Vienna.

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

6.2 Compound 1

3-(Butylamino)-4-phenoxy-5-sulfamoyl-benzamide

BUM 698



Feit P.W: Sulfamoylbenzoic acid derivatives (1970) [25]

Molecular weight: 363.43 g/mol	Yield: 1.7 g (34%)
Molecular formula: C ₁₇ H ₂₁ N ₃ O ₄	Physical form: solid
Melting point: 223-224°C	Colour: white

Synthesis:

13.74 mmol (5 g) of bumetanide were dissolved in 50 mL thionyl chloride, stirred and refluxed for five hours. After the thionyl chloride was removed under reduced pressure the resulting 3-(butylamino)-4-phenoxy-5-sulfamoyl-benzoyl chloride was slowly added to ammonia solution (50 mL) and stirred overnight. After the reaction was completed, excess ammonia was removed under reduced pressure. The crude product was purified *via* column chromatography using ethyl acetate/petroleum ether (9+1) as eluent and recrystallization from EtOH yielding 1.7 g (34%) of a white powder.

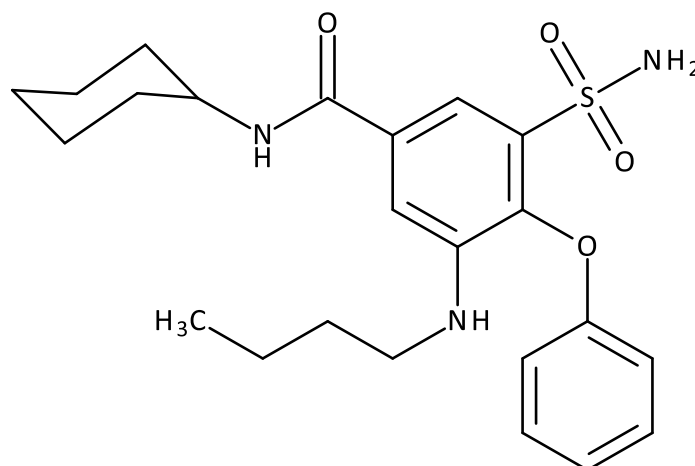
¹H NMR (200 MHz, DMSO-*d*₆) δ 8.10 (s, 1H), 7.64 (d, *J* = 1.9 Hz, 1H), 7.42 (s, 2H), 7.35 – 7.14 (m, 4H), 7.00 (t, *J* = 7.3 Hz, 1H), 6.93 – 6.77 (m, 2H), 4.86 (t, *J* = 5.7 Hz, 1H), 3.08 (q, *J* = 6.5 Hz, 2H), 1.51 – 1.22 (m, 2H), 1.22 – 1.00 (m, 2H), 0.77 (t, *J* = 7.1 Hz, 3H).

¹³C NMR (50 MHz, DMSO-*d*₆) δ 167.0, 156.5, 142.1, 138.2, 137.3, 131.6, 129.1, 122.1, 115.6, 113.7, 113.5, 42.1, 30.4, 19.3, 13.6.

6.3 Compound 2

3-(Butylamino)-*N*-cyclohexyl-4-phenoxy-5-sulfamoyl-benzamide

BUM 675



Molecular weight: 445.57 g/mol	Yield: 605 mg (68%)
Molecular formula: C ₂₃ H ₃₁ N ₃ O ₄ S	Physical form: solid
Melting point: 120-122°C	Colour: white

Synthesis:

2 mmol (728 mg) of bumetanide were dissolved in 10 mL tetrahydrofuran. 2.4 mmol (389 mg) of 1,1-carbonyldiimidazole were added and the mixture was stirred for four hours. The end of the reaction was controlled *via* thin-layer chromatography. Afterwards 4 mmol (397 mg) of cyclohexylamine were added and the mixture was stirred at room temperature overnight. After the reaction was completed, 40 mL of 5% sodium bicarbonate solution were added and the mixture extracted three times with ethyl acetate. The collected organic phases were washed with saturated sodium chloride solution, dried over sodium sulfate and evaporated under reduced pressure. The crude product was purified *via* recrystallization from EtOH yielding 605 mg (68%) of a white powder.

¹H NMR (200 MHz, CDCl₃) δ 7.51 – 7.39 (m, 2H), 7.37 – 7.19 (m, 2H), 7.07 (t, *J* = 7.3 Hz, 1H), 6.96 – 6.84 (m, 2H), 6.36 (d, *J* = 8.0 Hz, 1H), 5.28 (s, 2H), 4.01 – 3.80 (m, 2H), 3.18 – 3.00 (m, 2H), 2.10 – 1.91 (m, 2H), 1.87 – 1.54 (m, 3H), 1.54 – 1.00 (m, 9H), 0.80 (t, *J* = 7.2 Hz, 3H).

¹³C NMR (50 MHz, CDCl₃) δ 165.6, 155.9, 142.5, 138.6, 135.7, 133.0, 130.1, 123.7, 115.5, 115.4, 112.2, 77.2, 49.4, 43.1, 33.1, 31.1, 25.6, 25.2, 19.9, 13.7.

Mass analysis

m/z		
	445	100%, M ⁺
	402	60%
	320	39%
	303	29%
	41	30%

HRMS analysis

Measured m/z	Ion Formula	Calculated m/z	Err [ppm]
468.1932	C ₂₃ H ₃₁ N ₃ NaO ₄ S	468.1927	-1.0

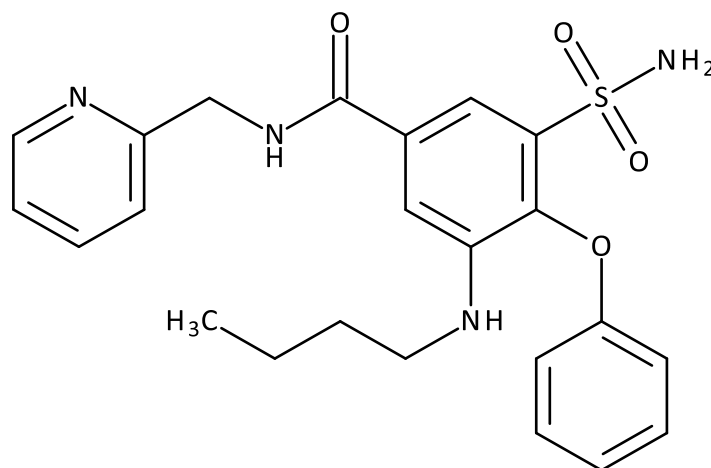
Elemental analysis

Molecular formula: C ₂₃ H ₃₁ N ₃ O ₄ S · 0.1 C ₂ H ₆ O	C%	H%	N%	S%
Calculated	61.90%	7.07%	9.33%	7.12%
Found	61.77%	7.11%	9.56%	6.86%

6.4 Compound 3

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

BUM 771



Wanaksi, Stephen; Partridge, John J.; Collins, Stephen: Preparation of bumetanide, furosemide, piretanide, azosemide, and torasemide analogs and therapeutic compositions containing same for treating disorders involving the Na⁺/K⁺/Cl⁻ cotransporter and GABAA receptors (2010) [26]

Molecular weight: 454.54 g/mol	Yield: 672 mg (74%)
Molecular formula: C ₂₃ H ₂₇ N ₄ O ₄ S	Physical form: solid
Melting point: 57-59°C	Colour: yellow

Synthesis:

2 mmol (728 mg) of bumetanide were dissolved in 10 mL tetrahydrofuran. 2.4 mmol (389 mg) of 1,1-carbonyldiimidazole were added and the mixture was stirred for three hours. The end of the reaction was controlled *via* thin-layer chromatography. Afterwards 4 mmol (433 mg) of 2-picolylamine were added and the mixture was stirred at room temperature overnight.

After the reaction was completed, 40 mL of 5% sodium bicarbonate solution were added and the mixture was extracted three times with ethyl acetate. The collected organic phases were washed with saturated sodium chloride solution, dried over sodium sulfate and evaporated under reduced pressure. The crude product was purified *via* recrystallization from EtOH yielding 672 mg (74%) of a yellow powder.

¹H NMR (200 MHz, CDCl₃) δ 8.48 – 8.31 (m, 2H), 7.72 – 7.59 (m, 2H), 7.43 – 7.12 (m, 5H), 7.09 – 6.99 (m, 1H), 6.91 – 6.82 (m, 2H), 5.62 (s-br, 2H), 4.78 – 4.68 (m, 2H), 3.89 – 3.77 (m, 1H), 3.13 – 2.94 (m, 2H), 1.47 – 1.28 (m, 2H), 1.22 – 1.00 (m, 2H), 0.79 (t, *J* = 7.1 Hz, 3H).

¹³C NMR (50 MHz, CDCl₃) δ 166.3, 156.9, 156.0, 148.8, 142.4, 138.8, 137.2, 136.0, 131.9, 130.1, 123.6, 122.6, 115.4, 115.3, 113.0, 45.1, 43.0, 31.1, 19.9, 13.8.

Mass analysis

m/z	454	100%, M ⁺
	303	90%
	107	89%
	43	97%
	41	64%

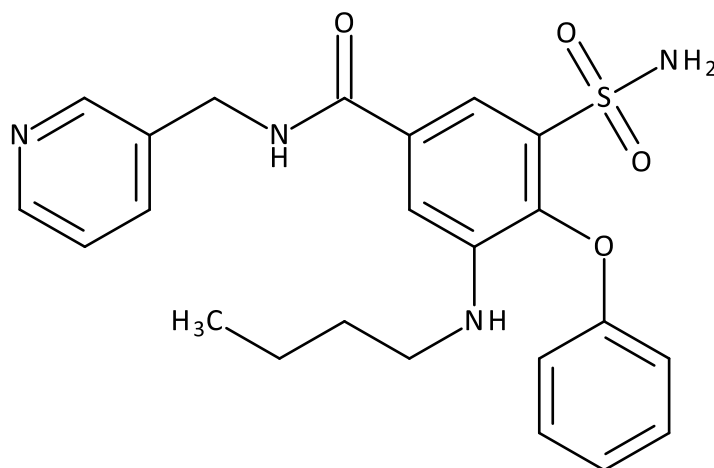
HRMS analysis

Measured m/z	Ion Formula	Calculated m/z	Err [ppm]
455.1748	C ₂₃ H ₂₇ N ₄ NaO ₄ S	455.1748	-0.1

6.5 Compound 4

3-(Butylamino)-4-phenoxy-N-(3-pyridylmethyl)-5-sulfamoyl-benzamide

BUM 772



Wanaksi, Stephen; Partridge, John J.; Collins, Stephen: Preparation of bumetanide, furosemide, piretanide, azosemide, and torasemide analogs and therapeutic compositions containing same for treating disorders involving the Na⁺/K⁺/Cl⁻ cotransporter and GABAA receptors (2010) [26]

Molecular weight: 454.54 g/mol	Yield: 820 mg (90%)
Molecular formula: C ₂₃ H ₂₇ N ₄ O ₄ S	Physical form: solid
Melting point: 172-173°C	Colour: white

Synthesis:

2 mmol (728 mg) of bumetanide were dissolved in 10 mL tetrahydrofuran. 2.4 mmol (389 mg) of 1,1-carbonyldiimidazole were added and the mixture was stirred for three hours. The reaction was controlled via thin-layer chromatography. Afterwards 4 mmol (433 mg) of 3-picolylamine were added and the mixture was stirred at room temperature overnight.

After the reaction was completed, 40 mL of 5% sodium bicarbonate solution were added and the mixture was extracted three times with ethyl acetate. The collected organic phases were washed with saturated sodium chloride solution, dried over sodium sulfate and evaporated under reduced pressure. The crude product was purified *via* recrystallization from EtOH yielding 820 mg (90%) of a white powder.

¹H NMR (200 MHz, MeOD) δ 8.57 (d, J = 1.6 Hz, 1H), 8.46 – 8.41 (m, 1H), 7.90 – 7.82 (m, 1H), 7.75 – 7.67 (m, 1H), 7.46 – 7.37 (m, 2H), 7.33 – 7.22 (m, 2H), 7.09 – 6.99 (m, 1H), 6.97 – 6.86 (m, 2H), 4.61 (s, 2H), 3.11 (t, J = 6.8 Hz, 2H), 1.49 – 1.33 (m, 2H), 1.24 – 1.03 (m, 2H), 0.80 (t, J = 7.2 Hz, 3H).

¹³C NMR (50 MHz, MeOD) δ 169.0, 157.8, 149.5, 148.8, 144.0, 140.6, 138.4, 137.7, 136.8, 132.8, 130.6, 125.2, 124.0, 116.6, 115.0, 114.6, 43.7, 42.2, 32.0, 20.8, 14.0.

Mass analysis

m/z	454	100%, M ⁺
	92	38%
	71	29%
	57	42%
	43	42%

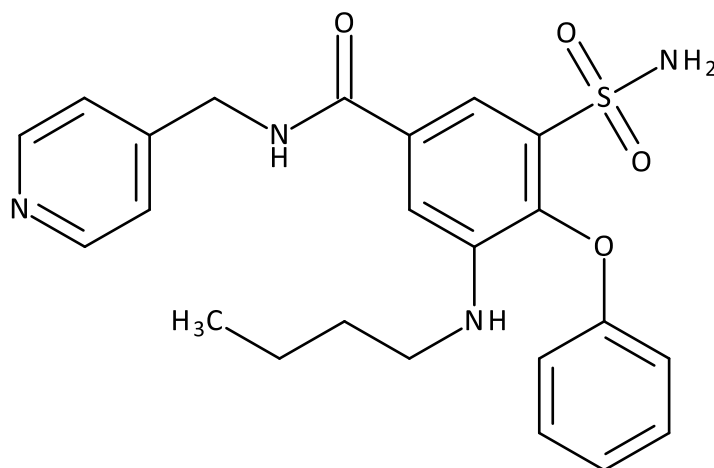
HRMS analysis

Measured m/z	Ion Formula	Calculated m/z	Err [ppm]
455.1743	C ₂₃ H ₂₇ N ₄ NaO ₄ S	455.1748	1.0

6.6 Compound 5

3-(Butylamino)-4-phenoxy-N-(4-pyridylmethyl)-5-sulfamoyl-benzamide

BUM 774



Wanaksi, Stephen; Partridge, John J.; Collins, Stephen: Preparation of bumetanide, furosemide, piretanide, azosemide, and torasemide analogs and therapeutic compositions containing same for treating disorders involving the Na+K+Cl⁻ cotransporter and GABAA receptors (2010) [26]

Molecular weight: 454.54 g/mol	Yield: 685 mg (75%)
Molecular formula: C ₂₃ H ₂₇ N ₄ O ₄ S	Physical form: solid
Melting point: 207-209°C	Colour: white

Synthesis:

2 mmol (728 mg) of bumetanide were dissolved in 10 mL tetrahydrofuran. 2.4 mmol (389 mg) of 1,1-carbonyldiimidazole were added and the mixture was stirred for three hours. The end of the reaction was controlled via thin-layer chromatography. Afterwards 4 mmol

(433 mg) of 4-(aminomethyl)pyridine were added and the mixture was stirred at room temperature overnight. After the reaction was completed, 40 mL of 5% sodium bicarbonate solution were added and the mixture was extracted three times with ethyl acetate. The collected organic phases were washed with saturated sodium chloride solution, dried over sodium sulfate and evaporated under reduced pressure. The crude product was purified *via* recrystallization from EtOH yielding 685 mg (75%) of a white powder.

¹H NMR (200 MHz, MeOD) δ 8.48 (dd, J = 4.7, 1.5 Hz, 2H), 7.77 (d, J = 2.0 Hz, 1H), 7.52 – 7.35 (m, 3H), 7.38 – 7.20 (m, 2H), 7.05 (t, J = 7.4 Hz, 1H), 6.99 – 6.85 (m, 2H), 4.63 (s, 2H), 3.12 (t, J = 6.8 Hz, 2H), 1.54 – 1.30 (m, 2H), 1.29 – 1.01 (m, 2H), 0.81 (t, J = 7.2 Hz, 3H).

¹³C NMR (50 MHz, MeOD) δ 169.2, 157.8, 150.1, 140.7, 138.5, 132.7, 130.6, 124.0, 123.9, 116.6, 115.0, 114.6, 49.0, 43.7, 43.6, 32.0, 20.8, 14.0.

Mass analysis

m/z		
	454	19%, M ⁺
	412	25%
	411	100%
	394	25%
	57	19%

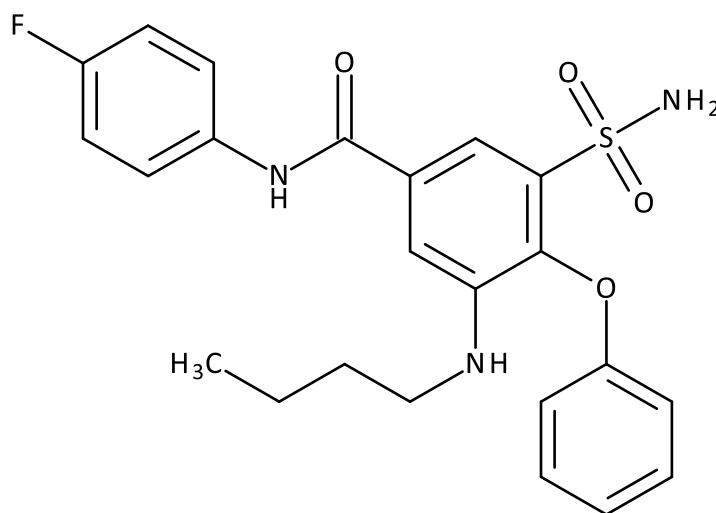
HRMS analysis

Measured m/z	Ion Formula	Calculated m/z	Err [ppm]
455.1749	C ₂₃ H ₂₇ N ₄ NaO ₄ S	455.1748	-0.4

6.7 Compound 6

3-(Butylamino)-N-(4-fluorophenyl)-4-phenoxy-5-sulfamoyl-benzamide

BUM745



Molecular weight: 457.52 g/mol	Yield: 200 mg (44%)
Molecular formula: C ₂₃ H ₂₄ FN ₃ O ₄ S	Physical form: solid
Melting point: 192-194 °C	Colour: white/yellow

Synthesis:

1 mmol (364 mg) of bumetanide was dissolved in 5 mL tetrahydrofuran. 1.2 mmol (194 mg) 1,1-carbonyldiimidazole were added and the mixture was stirred for three hours. The end of the reaction was controlled *via* thin-layer chromatography. Afterwards 2 mmol (222 mg) of 4-fluoroaniline were added and the mixture was stirred at room temperature overnight. After the reaction was completed, 20 mL of 5% sodium bicarbonate solution were added and the mixture was extracted three times with ethyl acetate. The collected organic phases were washed with saturated sodium chloride solution, dried over sodium sulfate and evaporated under reduced pressure. The crude product was purified *via* recrystallization from EtOH yielding 200 mg (44%) of a white/yellowish powder.

¹H NMR (200 MHz, DMSO-*d*₆) δ 10.40 (s, 1H), 7.86 – 7.69 (m, 2H), 7.68 (d, *J* = 1.9 Hz, 1H), 7.45 (d, *J* = 1.9 Hz, 1H), 7.41 – 7.10 (m, 6H), 7.02 (t, *J* = 7.3 Hz, 1H), 6.96 – 6.77 (m, 2H), 4.97 (t, *J* = 5.6 Hz, 1H), 3.22 – 3.02 (m, 2H), 1.49 – 1.28 (m, 2H), 1.23 – 0.97 (m, 2H), 0.78 (t, *J* = 7.2 Hz, 3H).

¹³C NMR (50 MHz, DMSO-*d*₆) δ 164.7, 158.4 (d, *J* = 240.4 Hz), 156.4, 142.2, 138.5, 137.5, 135.3 (d, *J* = 2.5 Hz), 132.2, 129.1, 122.5 (d, *J* = 7.9 Hz), 122.2, 115.6, 115.2 (d, *J* = 22.2 Hz), 113.6, 42.1, 30.3, 19.3, 13.6.

Mass analysis

m/z	457	75%, M ⁺
	414	39%
	348	22%
	347	100%
	333	21%

HRMS analysis

Measured m/z	Ion Formula	Calculated m/z	Err [ppm]
480.1367	C ₂₃ H ₂₄ FN ₃ NaO ₄ S	480.1364	-0.8

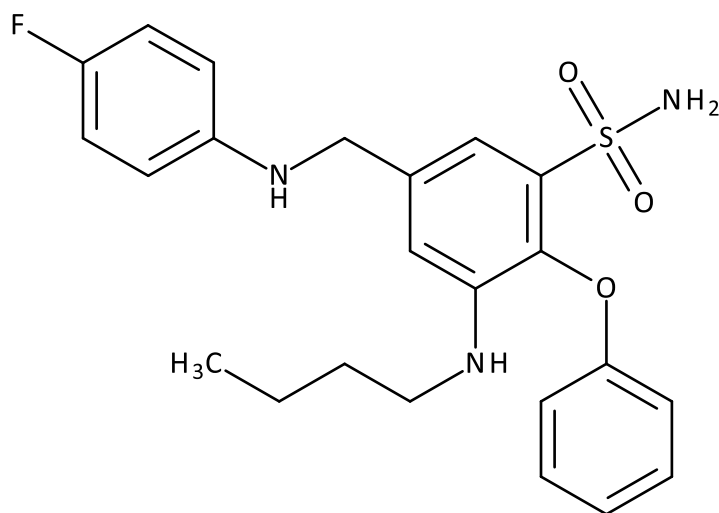
Elemental analysis

Molecular formula: C ₂₃ H ₂₄ FN ₃ O ₄ S · 0.4 C ₂ H ₆ O	C%	H%	N%	S%
Calculated	60.06%	5.59%	8.83%	6.74%
Found	59.81%	5.59%	9.04%	6.87%

6.8 Compound 7

3-(Butylamino)-5-[(4-fluoroanilino) methyl]-2-phenoxy-benzenesulfonamide

BUM746



Molecular weight: 443.53 g/mol	Yield: 56 mg (38%)
Molecular formula: C ₂₃ H ₂₆ FN ₃ O ₃ S	Physical form: solid
Melting point: 140-142°C	Colour: white/yellow

Synthesis:

0.33 mmol (150 mg) of compound **6** was dissolved in 20 mL tetrahydrofuran. 1.32 mmol (4 equivalent, 100 mg) of borane dimethyl sulfide complex were added. The mixture was stirred at 86°C overnight under reflux. After TLC showed that no starting material was present anymore, the mixture was cooled to room temperature and quenched with 20 mL of half-saturated aqueous sodium bicarbonate. Afterwards the mixture was extracted three times with ethyl acetate, washed with saturated sodium chloride solution and dried over sodium sulfate.

The solvent was removed under reduced pressure and the crude product was purified by recrystallization from EtOH yielding 56 mg (38%) of a white/yellowish powder.

¹H NMR (200 MHz, DMSO-*d*₆) δ 7.25 (t, *J* = 7.7 Hz, 2H), 7.15 – 7.04 (m, 3H), 7.06 – 6.76 (m, 6H), 6.67 – 6.50 (m, 2H), 6.24 (t, *J* = 6.0 Hz, 1H), 4.72 (t, *J* = 5.7 Hz, 1H), 4.24 (d, *J* = 5.9 Hz, 2H), 3.09 – 2.89 (m, 2H), 1.40 – 1.18 (m, 2H), 1.21 – 0.93 (m, 2H), 0.74 (t, *J* = 7.1 Hz, 3H).

¹³C NMR (50 MHz, DMSO-*d*₆) δ 156.8, 154.3 (d, *J* = 241.5 Hz), 145.3, 145.3 (d, *J* = 1.4 Hz), 137.9, 137.1, 134.9, 129.0, 121.8, 115.5, 115.2 (d, *J* = 21.9 Hz), 113.5, 113.0 (d, *J* = 7.3 Hz), 112.4, 46.8, 42.1, 30.3, 19.3, 13.6.

Mass analysis

m/z	443	61%, M ⁺
	333	100%
	208	27%
	196	40%
	77	38%

HRMS analysis

Measured m/z	Ion Formula	Calculated m/z	Err [ppm]
466.1578	C ₂₃ H ₂₆ FN ₃ NaO ₃ S	466.1571	-1.4

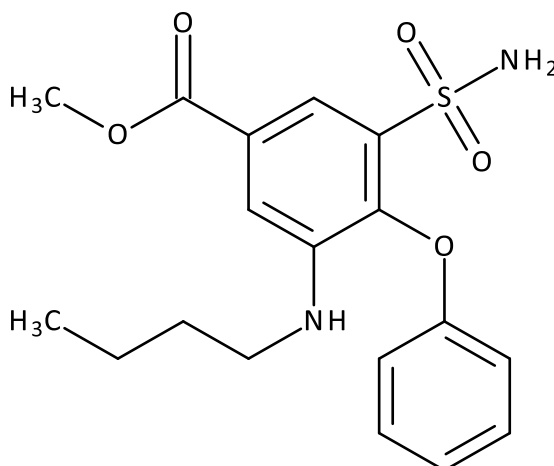
Elemental analysis

Molecular formula: C ₂₃ H ₂₆ FN ₃ O ₃ S · 0.25 C ₂ H ₆ O	C%	H%	N%	S%
Calculated	62.03%	6.09%	9.23%	7.05%
Found	61.87%	5.96%	9.39%	7.18%

6.9 Compound 8

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

BUM 6



Rivera, Claudio; Tuominen, Raimo; Pellegrino, Christophe; Kourdougli, Nazim; Crepel, Valerie: *Methods and pharmaceutical composition for the treatment of post-traumatic epilepsies* (2017) [27]

Molecular weight: 378.44 g/mol	Yield: 3.46 g (91%)
Molecular formula: C ₁₈ H ₂₂ N ₂ O ₅ S	Physical form: solid
Melting point: 149-150°C	Colour: white

Synthesis:

To 15 mL of MeOH 10 mmol of bumetanide (3.64 g) were added to form a suspension. To this mixture 22 mmol (1.6 mL) of SOCl₂ were added resulting in a clear, yellow solution. The mixture was stirred at room temperature overnight. After the reaction was completed, the mixture was washed with 5 % aqueous sodium bicarbonate solution and the mixture was extracted three times with ethyl acetate. The combined organic phases were washed with brine, dried over Na₂SO₄ and then vacuum-dried. Yield: 3.46 g (94 %) of a white solid.

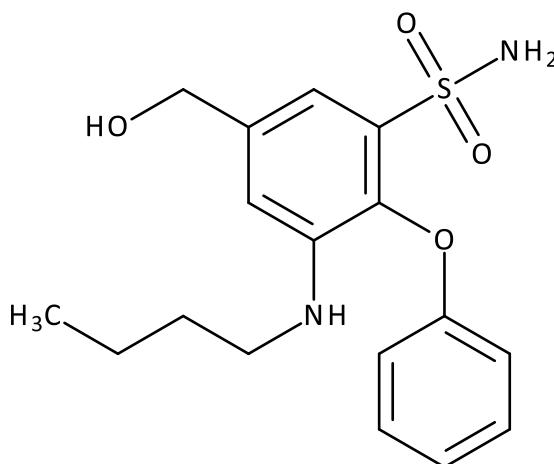
¹H NMR (200 MHz, CDCl₃) δ 7.93 (d, *J* = 1.9 Hz, 1H), 7.56 (d, *J* = 1.9 Hz, 1H), 7.40 – 7.22 (m, 2H), 7.10 (t, *J* = 7.3 Hz, 1H), 6.99 – 6.85 (m, 2H), 4.98 (s, 2H), 3.93 (s, 3H), 3.91 – 3.79 (m, 1H), 3.20 – 3.01 (m, 2H), 1.54 – 1.30 (m, 2H), 1.30 – 1.02 (m, 2H), 0.82 (t, *J* = 7.1 Hz, 3H).

¹³C NMR (50 MHz, CDCl₃) 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.

6.10 Compound 9

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

BUM 7



Toellner, Kathrin et al.: A novel prodrug-based strategy to increase effects of bumetanide in epilepsy (2014) [28]

Molecular weight: 350.43 g/mol	Yield: 3.23 g (96%)
Molecular formula: C ₁₇ H ₂₂ N ₂ O ₄ S	Physical form: solid
Melting point: 158-159°C	Colour: white/yellow

Synthesis:

9.62 mmol of Methyl-3-(butylamino)-4-phenoxy-5-sulfamoyl-benzoate were dissolved in 10 mL anhydrous THF and stirred at room temperature under argon atmosphere. Then 20 mL of a 1 M DIBAL-H solution in toluene were added every 30 minutes for three times. The reaction was stirred overnight and afterwards cooled to 0-4°C and quenched with 5 % aqueous NHCl₄ solution causing a gel-like substance to precipitate. The precipitate was then dissolved in 2 N HCl and the mixture was extracted three times with ethyl acetate. The combined organic phases were washed three times with water, once with brine and dried over Na₂SO₄. The fluids were evaporated under reduced pressure to obtain 3.23 g (96%) of a slightly yellow solid.

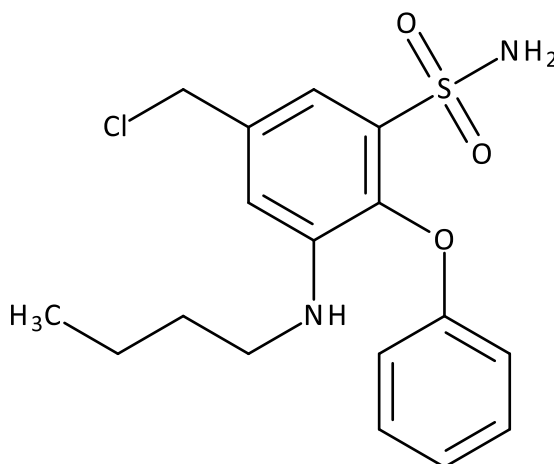
¹H NMR (200 MHz, DMSO-*d*₆) δ 7.25 (t, *J* = 7.8 Hz, 2H), 7.18 – 6.74 (m, 7H), 5.31 (t, *J* = 5.7 Hz, 1H), 4.69 (t, *J* = 5.7 Hz, 1H), 4.50 (d, *J* = 5.8 Hz, 2H), 3.02 (q, *J* = 6.5 Hz, 2H), 1.51 – 1.23 (m, 2H), 1.22 – 0.97 (m, 2H), 0.77 (t, *J* = 7.1 Hz, 3H).

¹³C 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.

6.11 Compound 10

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

BUM 63



Schreppel, P. (2015). *Synthese von Bumetanid-Derivaten mit Wirkung auf das zentrale Nervensystem. Diploma thesis, University of Vienna.* [29]

Molecular weight: 368.88 g/mol	Yield: 1.04 g (30%)
Molecular formula: C ₁₇ H ₂₁ ClN ₂ O ₃ S	Physical form: solid
Melting point: 138-140°C	Colour: white

Synthesis:

9.22 mmol of compound **9** were dissolved 20 mL SOCl₂. The mixture was stirred at room temperature and the end of the reaction was controlled *via* TLC. SOCl₂ was removed under reduced pressure. The crude product was purified by column chromatography (ethyl acetate/petroleum ether 3/7). The product-containing fractions were collected and the solvent was removed under reduced pressure. Yield: 1.04 g (30%).

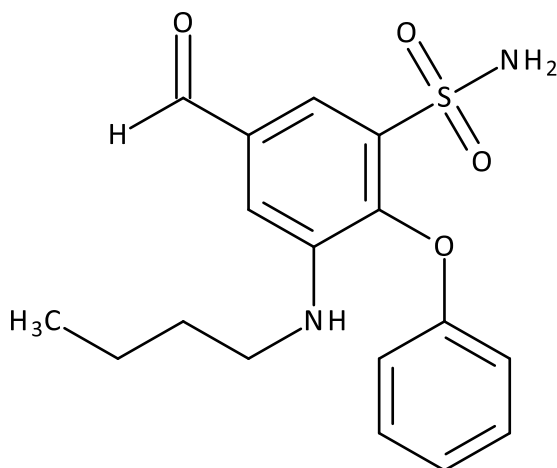
¹H NMR (200 MHz, CDCl₃) δ 7.38 – 7.24 (m, 3H), 7.08 (t, *J* = 7.3 Hz, 1H), 6.99 – 6.87 (m, 3H), 4.88 (s, 2H), 4.57 (s, 2H), 3.07 (t, *J* = 6.9 Hz, 2H), 1.51 – 1.34 (m, 2H), 1.28 – 1.05 (m, 2H), 0.83 (t, *J* = 7.1 Hz, 3H).

¹³C NMR (50 MHz, CDCl₃) 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.

6.12 Compound 11

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

BUM 8



Rivera, Claudio; Tuominen, Raimo; Pellegrino, Christophe; Kourdougli, Nazim; Crepel, Valerie: *Methods and pharmaceutical composition for the treatment of post-traumatic epilepsies* (2017) [27]

Molecular weight: 348.42 g/mol	Yield: 460 mg (34%)
Molecular formula: C ₁₇ H ₂₀ N ₂ O ₄ S	Physical form: solid
Melting point: 134-136°C	Colour: yellow/green

Synthesis:

3.83 mmol (1.34 g) of compound **9** were dissolved in 61 mL of toluene/ethyl acetate (1+1). 11.5 mmol (1 g) of MnO₂ were added. The mixture was heated to 40°C under reflux for three days. The TLC showed that there was still reactant present. Therefore, the same amount of MnO₂ was added and again stirred for two more days. After the reaction was finished, which was controlled *via* thin layer chromatography, the mixture was filtered. The filtrate was evaporated under reduced pressure and the resulting crude product was purified by column chromatography (ethyl acetate/petroleum ether 1+1) and recrystallization from EtOH yielding 460 mg (34%) of yellow-greenish crystals.

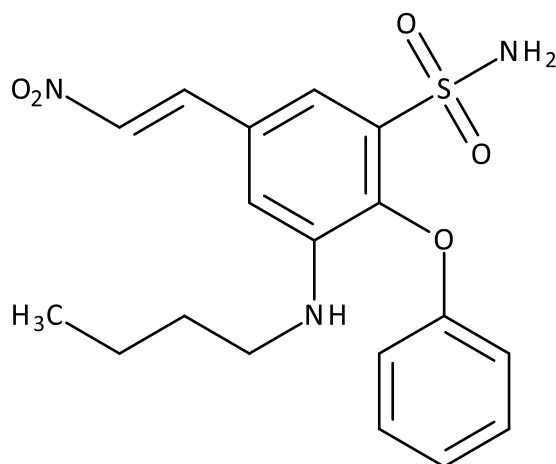
¹H NMR (200 MHz, CDCl₃) δ 9.94 (s, 1H), 7.76 (d, *J* = 1.9 Hz, 1H), 7.44 – 7.23 (m, 4H), 7.12 (t, *J* = 7.4 Hz, 1H), 7.03 – 6.86 (m, 3H), 5.01 (s, 2H), 3.96 (t, *J* = 5.3 Hz, 1H), 3.11 (q, *J* = 6.7 Hz, 3H), 1.55 – 1.30 (m, 3H), 1.31 – 1.01 (m, 3H), 0.82 (t, *J* = 7.2 Hz, 3H).

¹³C NMR (50 MHz, CDCl₃) δ 190.8, 155.6, 143.0, 141.4, 136.8, 134.0, 130.3, 124.2, 118.6, 115.5, 113.6, 43.1, 30.9, 19.9, 13.7.

6.13 Compound 12

3-(Butylamino)-5-[(E)-2-nitrovinyl]-2-phenoxy-benzenesulfonamide

BUM 751



Molecular weight: 391.44 g/mol	Yield: 150 mg (38%)
Molecular formula: C ₁₈ H ₂₁ N ₃ O ₅ S	Physical form: solid
Melting point: 188-190°C	Colour: yellow

Synthesis:

1 mmol (348 mg) of compound **11** was dissolved in 10 mL nitromethane and ammonium acetate was added (28 mg, 0.4 mmol, 0.4 eq.) The mixture was heated under reflux. The TLC showed that the starting substance was still present, therefore the same amount of ammonium acetate was added and again stirred under reflux overnight at room temperature. Water was added and the mixture was extracted three times with ethyl acetate. The collected organic phases were washed with saturated sodium chloride solution, dried over sodium sulfate and evaporated under reduced pressure. The crude product was passed through a silica column using ethyl acetate/petroleum ether (1:1) as eluent. The product-containing fractions (controlled *via* TLC) were evaporated under reduced pressure. The yellow powder was recrystallized using EtOH (80%). The yellow spherical crystals were dried *in vacuo*. Yield: 150 mg (38%).

¹H NMR (200 MHz, DMSO-*d*₆) δ 8.34 – 8.05 (m, 2H), 7.54 – 7.41 (m, 2H), 7.33 – 7.21 (m, 4H), 7.01 (t, *J* = 7.2 Hz, 1H), 6.93 – 6.78 (m, 2H), 4.98 (t, *J* = 5.8 Hz, 1H), 3.10 (q, *J* = 6.5 Hz, 2H), 1.48 – 1.24 (m, 2H), 1.25 – 0.97 (m, 2H), 0.78 (t, *J* = 7.2 Hz, 3H).

¹³C NMR (50 MHz, DMSO-*d*₆) δ 156.4, 142.9, 139.0, 138.7, 138.5, 138.1, 129.1, 127.8, 122.3, 116.1, 115.6, 114.2, 41.9, 30.3, 19.3, 13.6.

Mass analysis

m/z		
	391	100%, M+
	348	74%
	331	76%
	267	75%
	91	54%

HRMS analysis

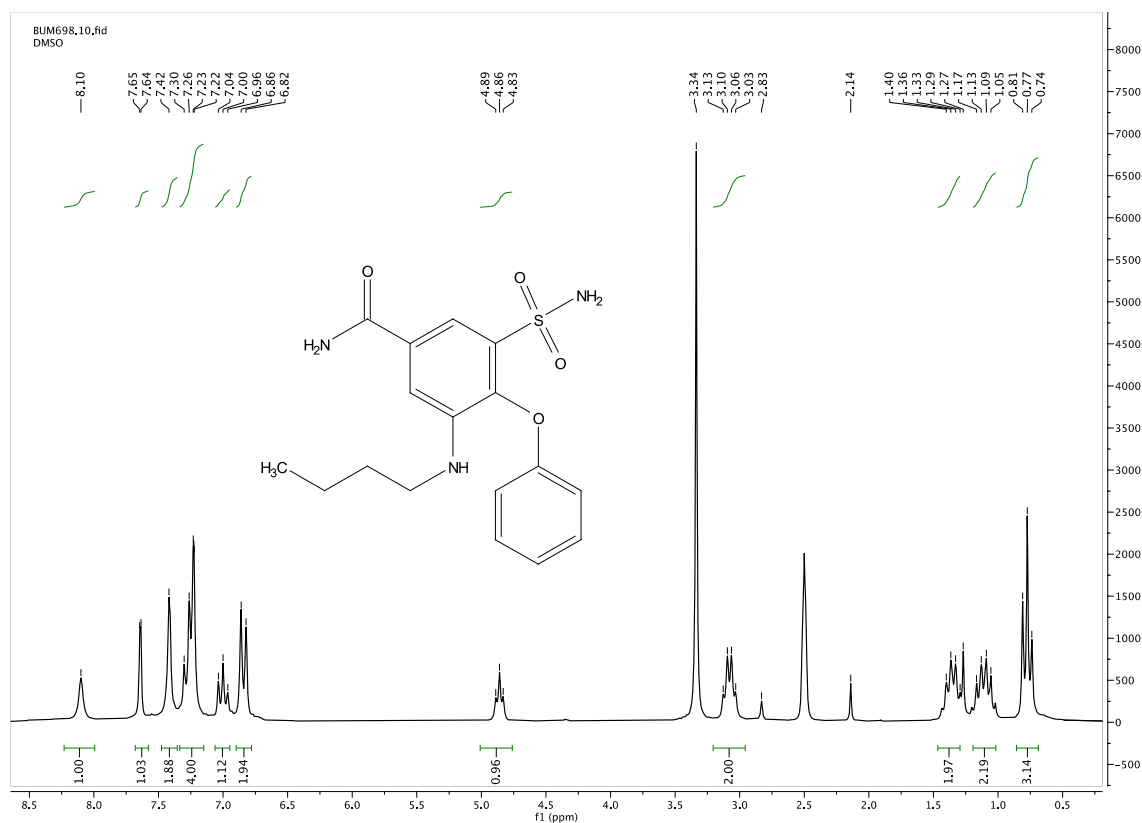
Measured m/z	Ion Formula	Calculated m/z	Err [ppm]
414.1091	C ₁₈ H ₂₁ N ₃ NaO ₅ S	414.1094	0.8

Elemental analysis

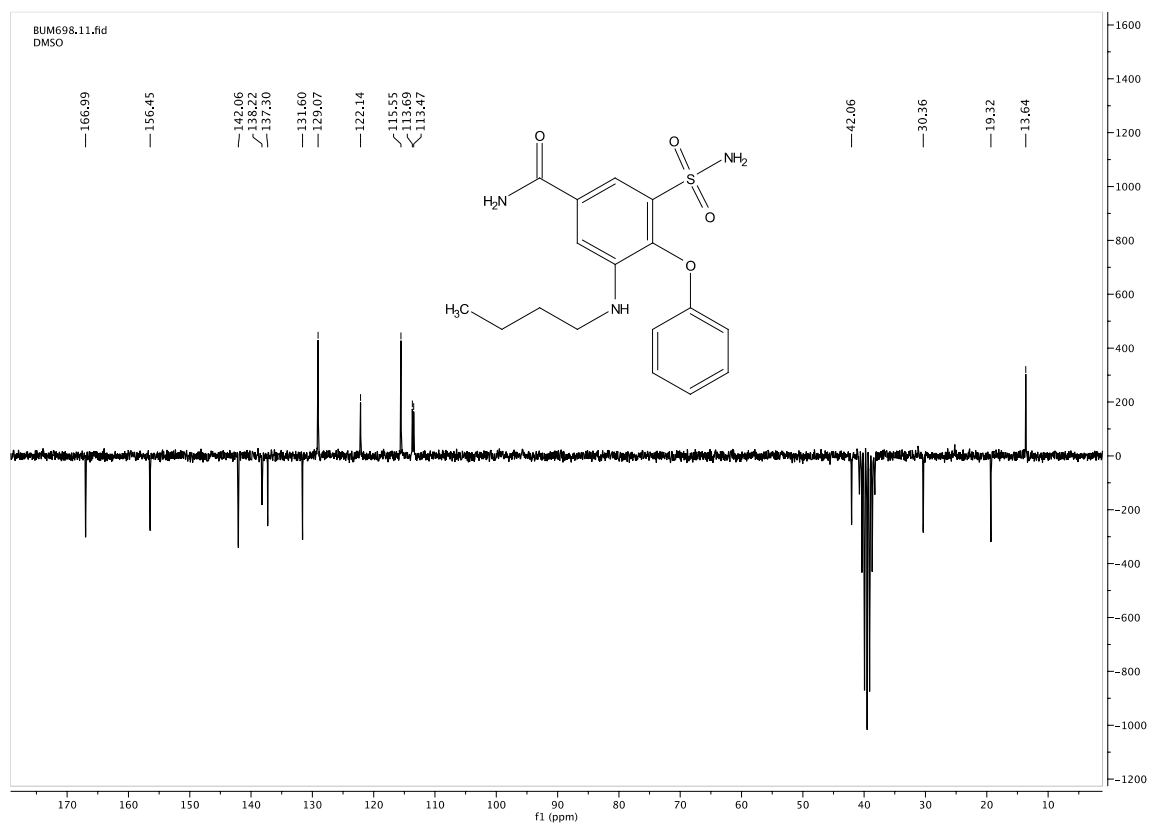
Molecular formula: C ₁₈ H ₂₁ N ₃ O ₅ S · 0.2 H ₂ O	C%	H%	N%	S%
Calculated	54.73%	5.46%	10.64%	8.12%
Found	54.64%	5.38%	10.56%	8.08%

7. Analytics

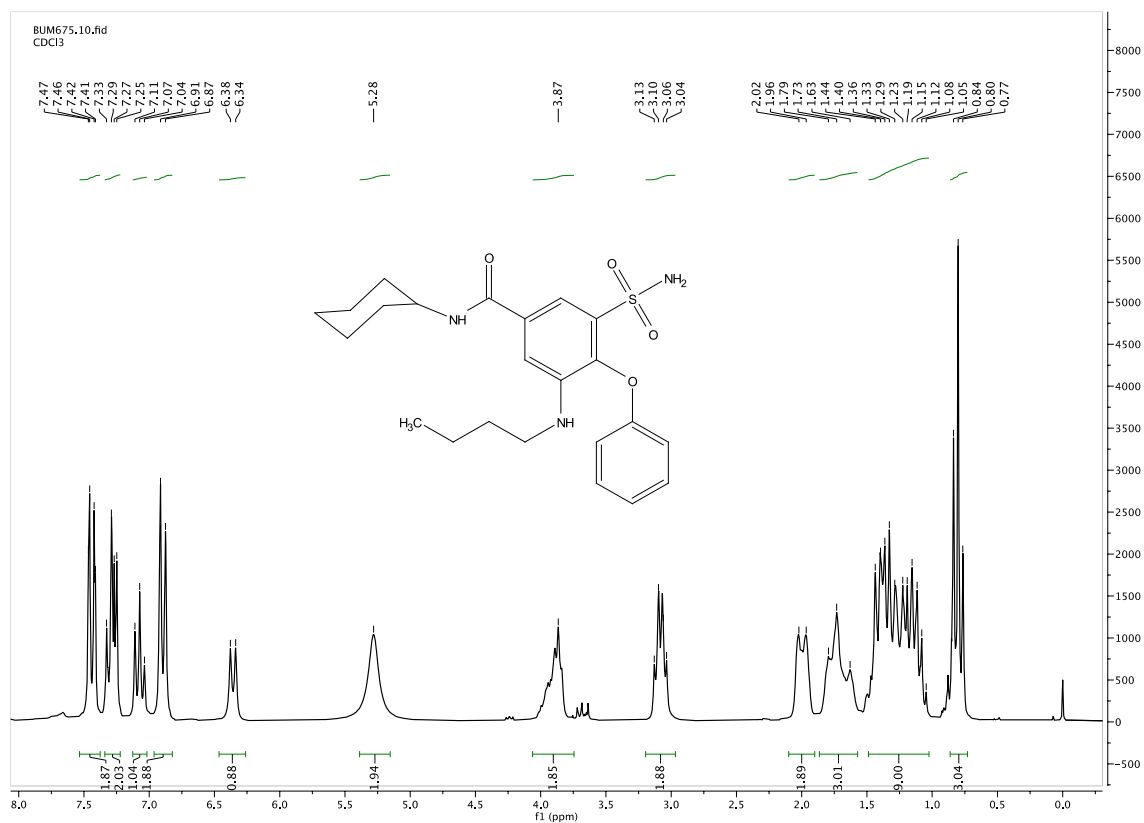
Compound 1 $^1\text{H-NMR}$ ($\text{DMSO-}d_6$)



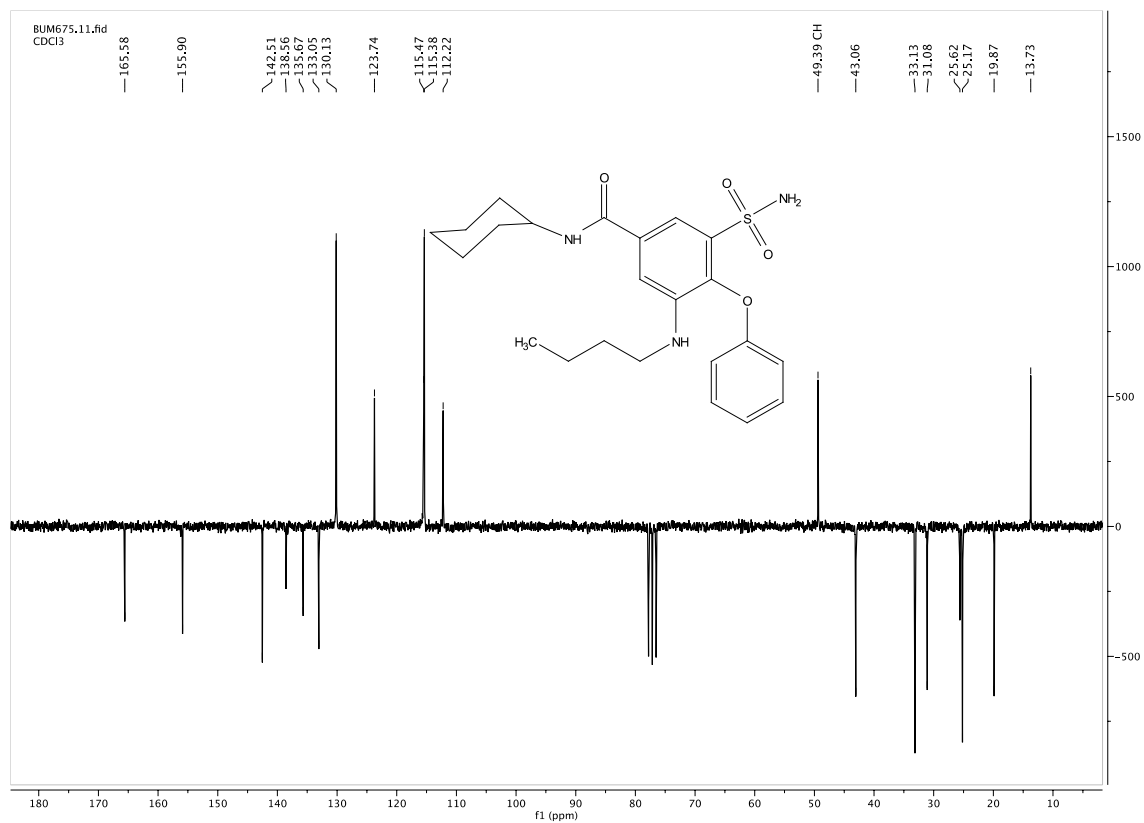
Compound 1 $^{13}\text{C-NMR}$ ($\text{DMSO-}d_6$)



Compound 2 ¹H-NMR (CDCl₃)



Compound 2 ¹³C-NMR (CDCl₃)



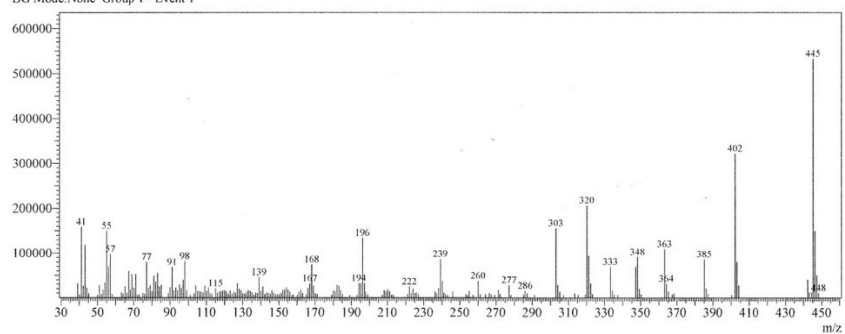
Compound 2 mass spectrum

Line#:1 R.Time:9.125(Scan#:1072)

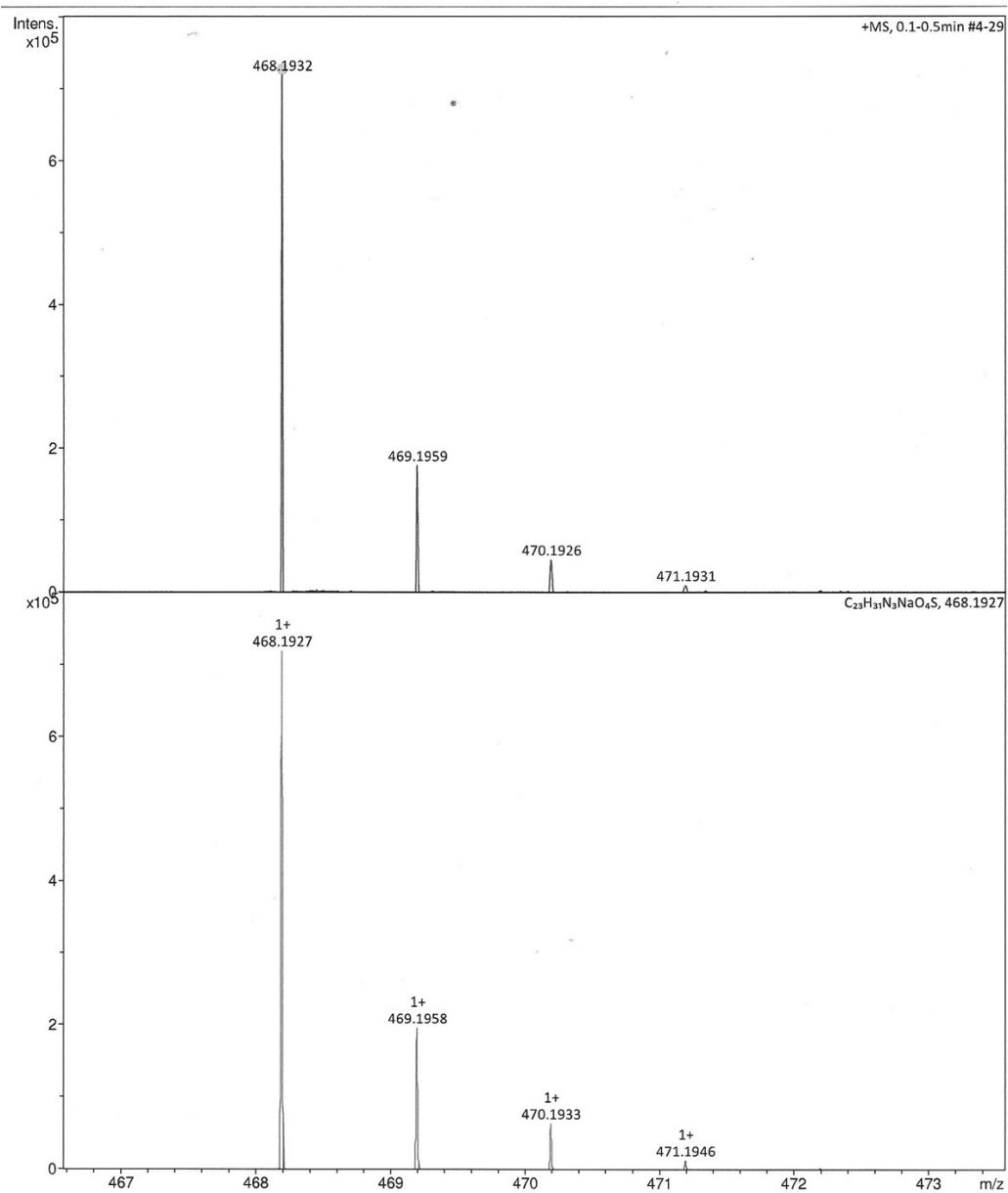
MassPeaks:210

RawMode:Single 9.125(1072) BasePeak:445.30(532228)

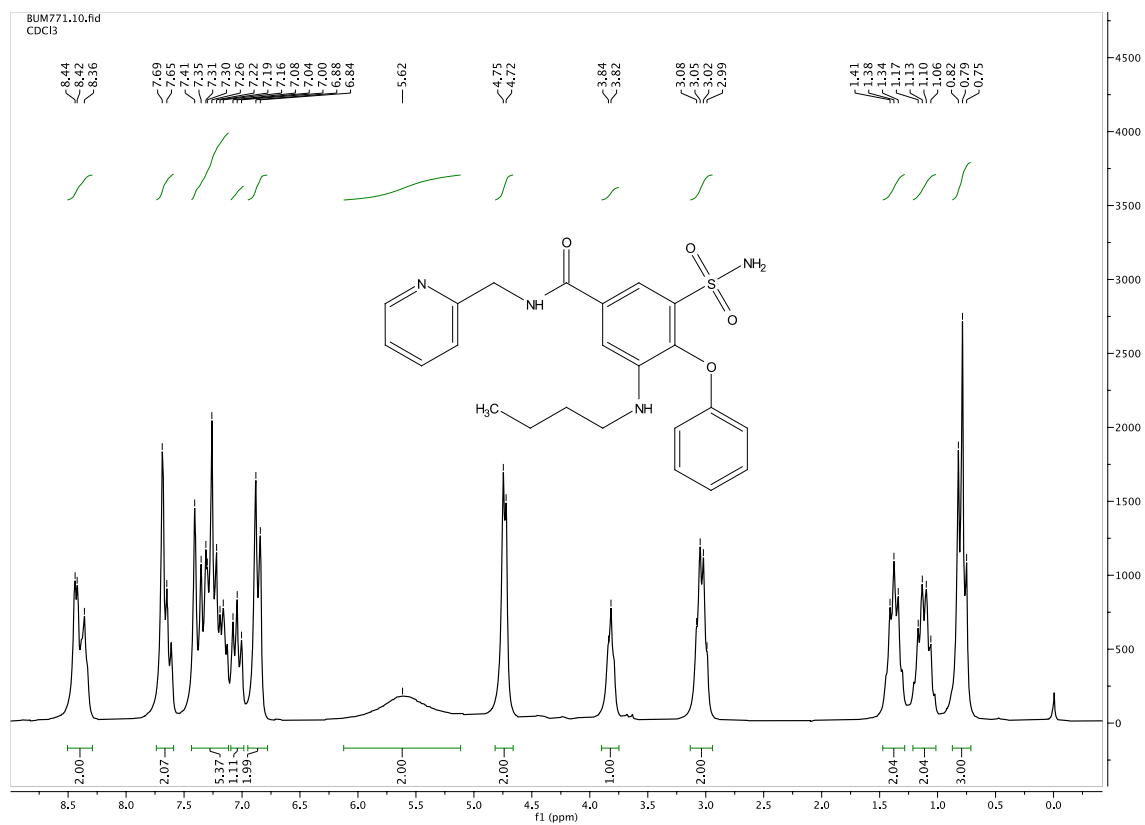
BG Mode:None Group 1 - Event 1



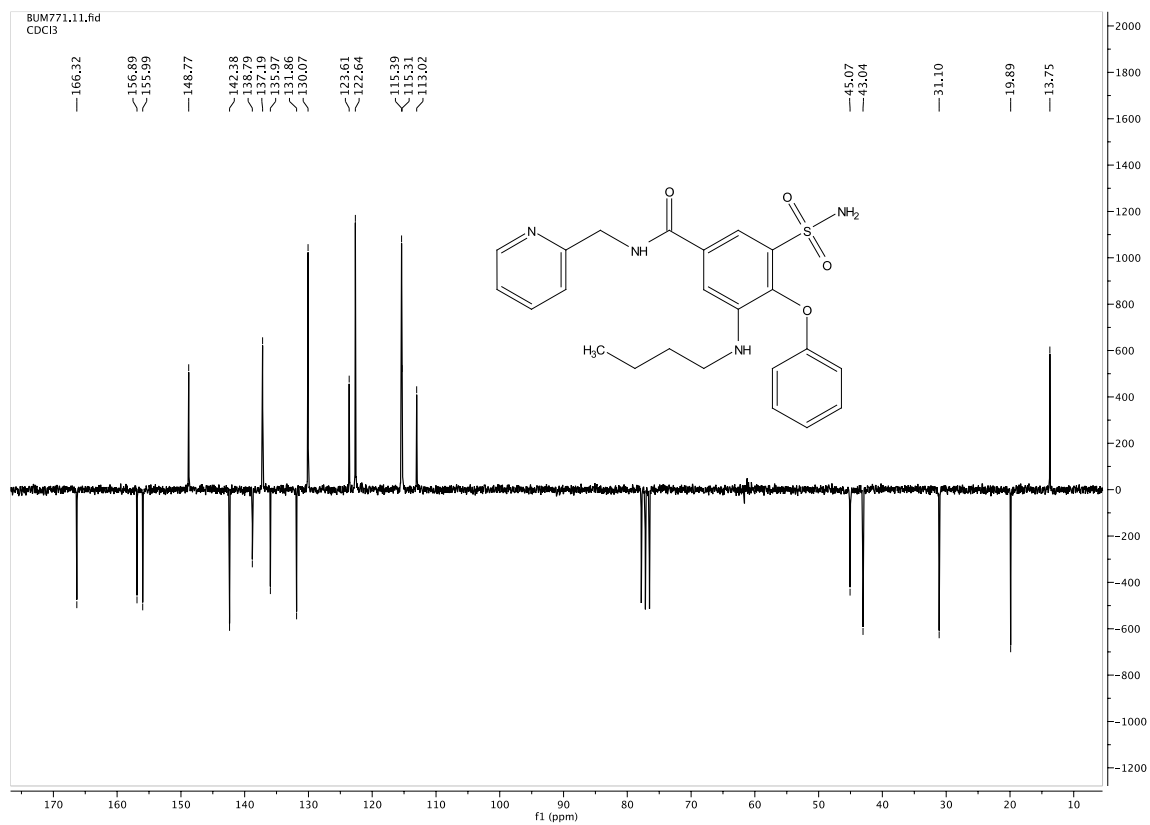
Compound 2 HRMS



Compound 3 ¹H-NMR (CDCl₃)



Compound 3 ¹³C-NMR (CDCl₃)



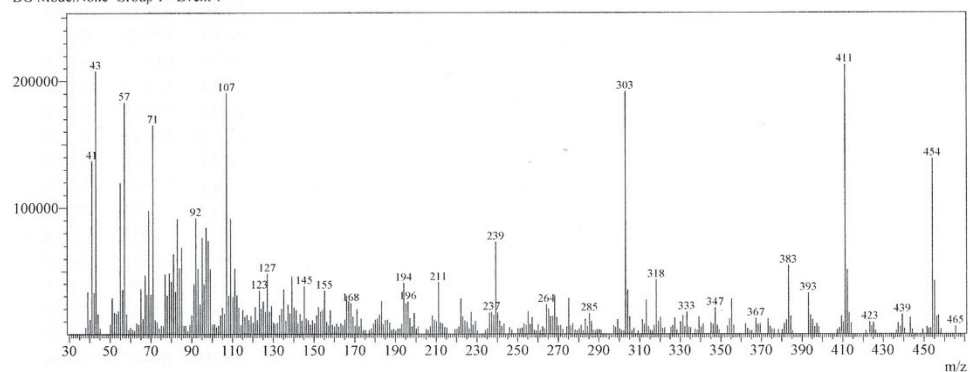
Compound 3 mass spectrum

Line#:1 R.Time:10.050(Scan#:1183)

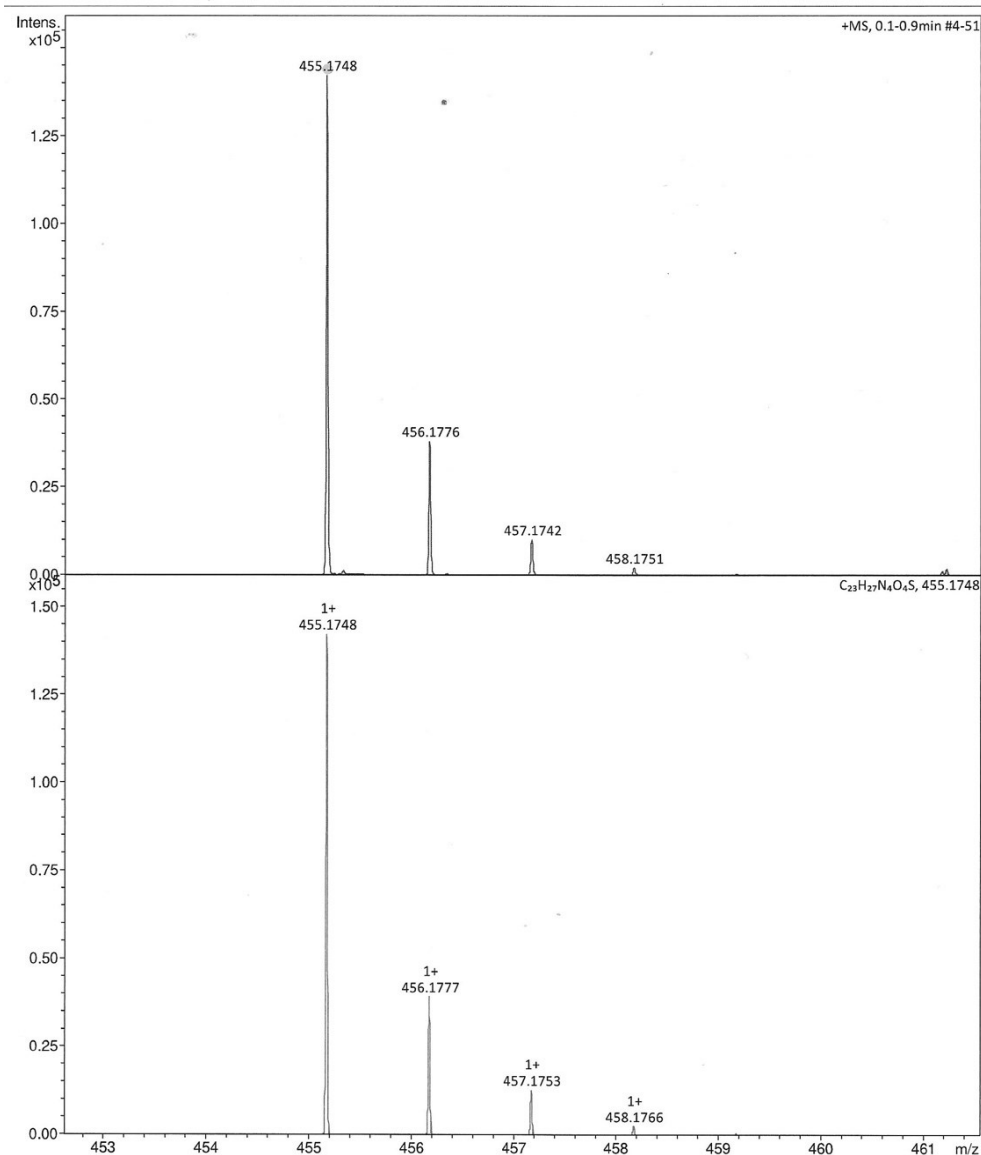
MassPeaks:337

RawMode:Single 10.050(1183) BasePeak:411.20(212366)

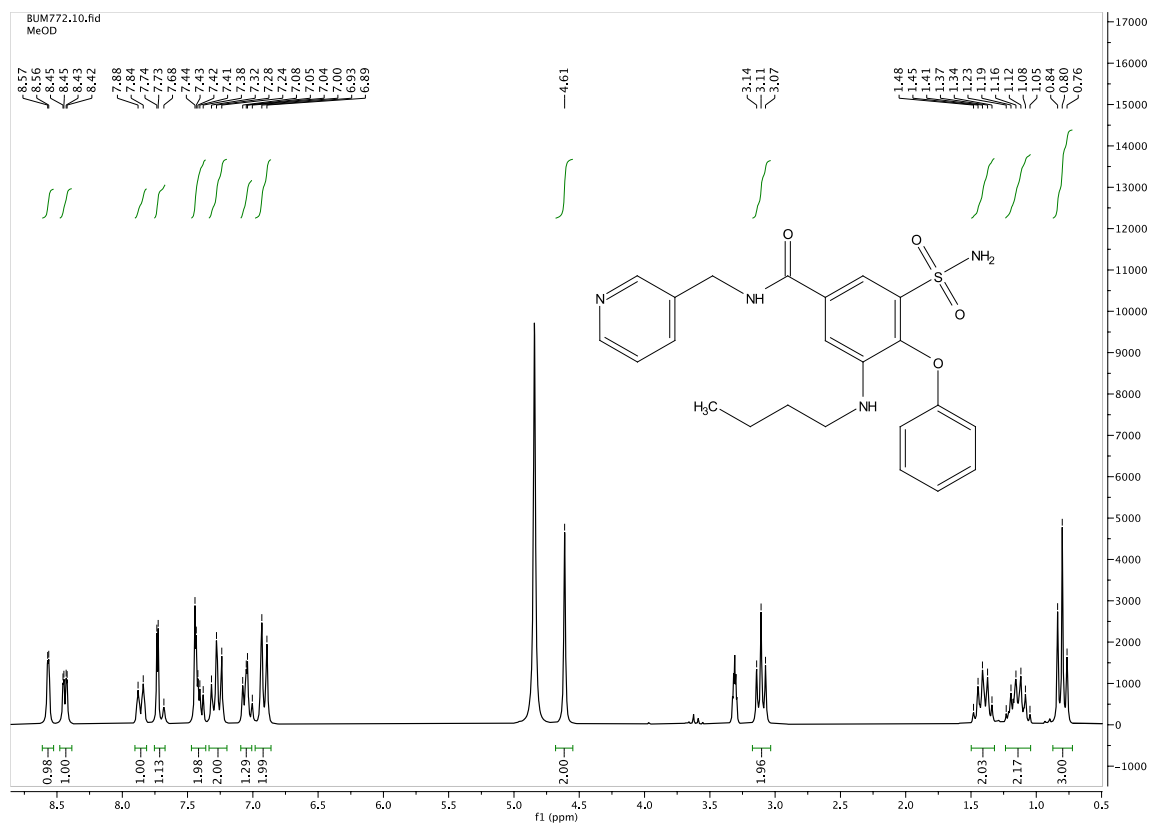
BG Mode:None Group 1 - Event 1



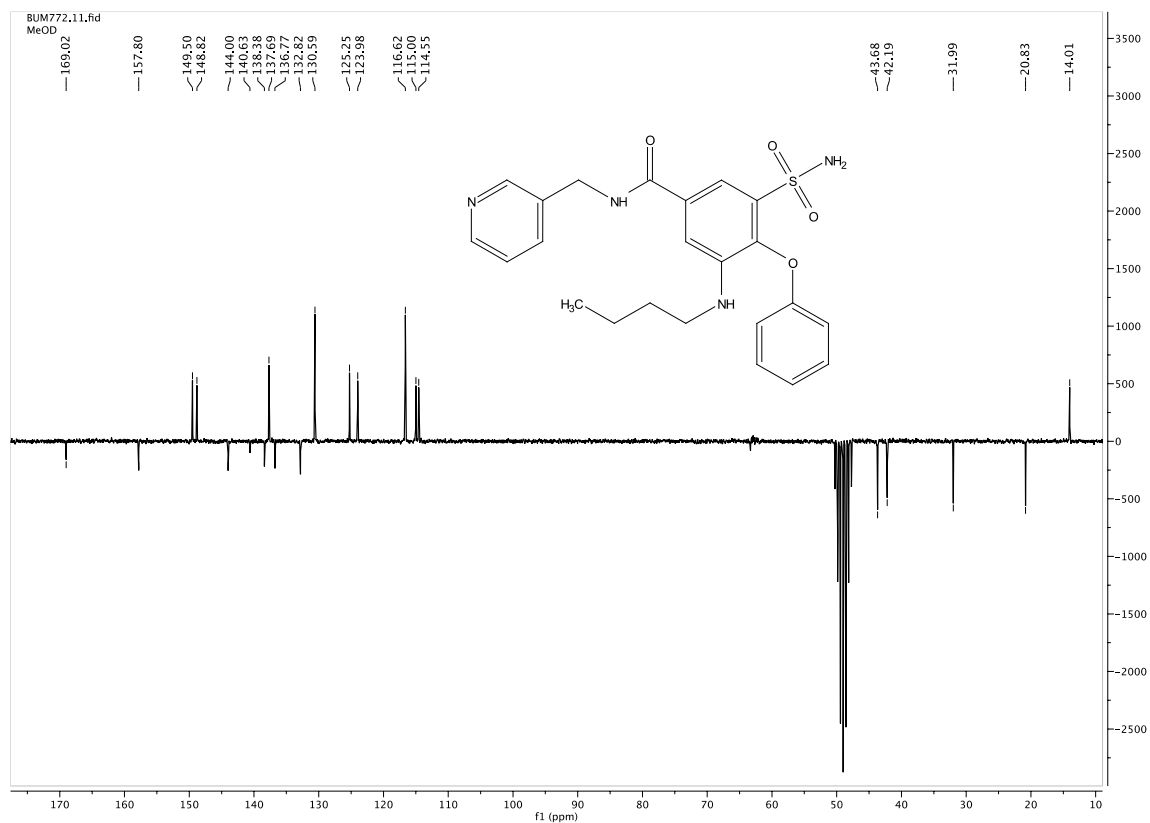
Compound 3 HRMS



Compound 4 ¹H-NMR (MeOD)



Compound 4 ¹³C-NMR (MeOD)



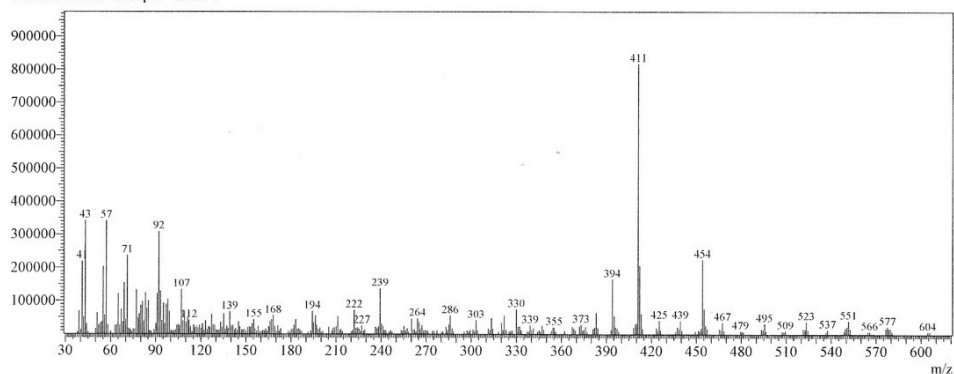
Compound 4 mass spectrum

Line#:1 R.Time:11.125(Scan#:1312)

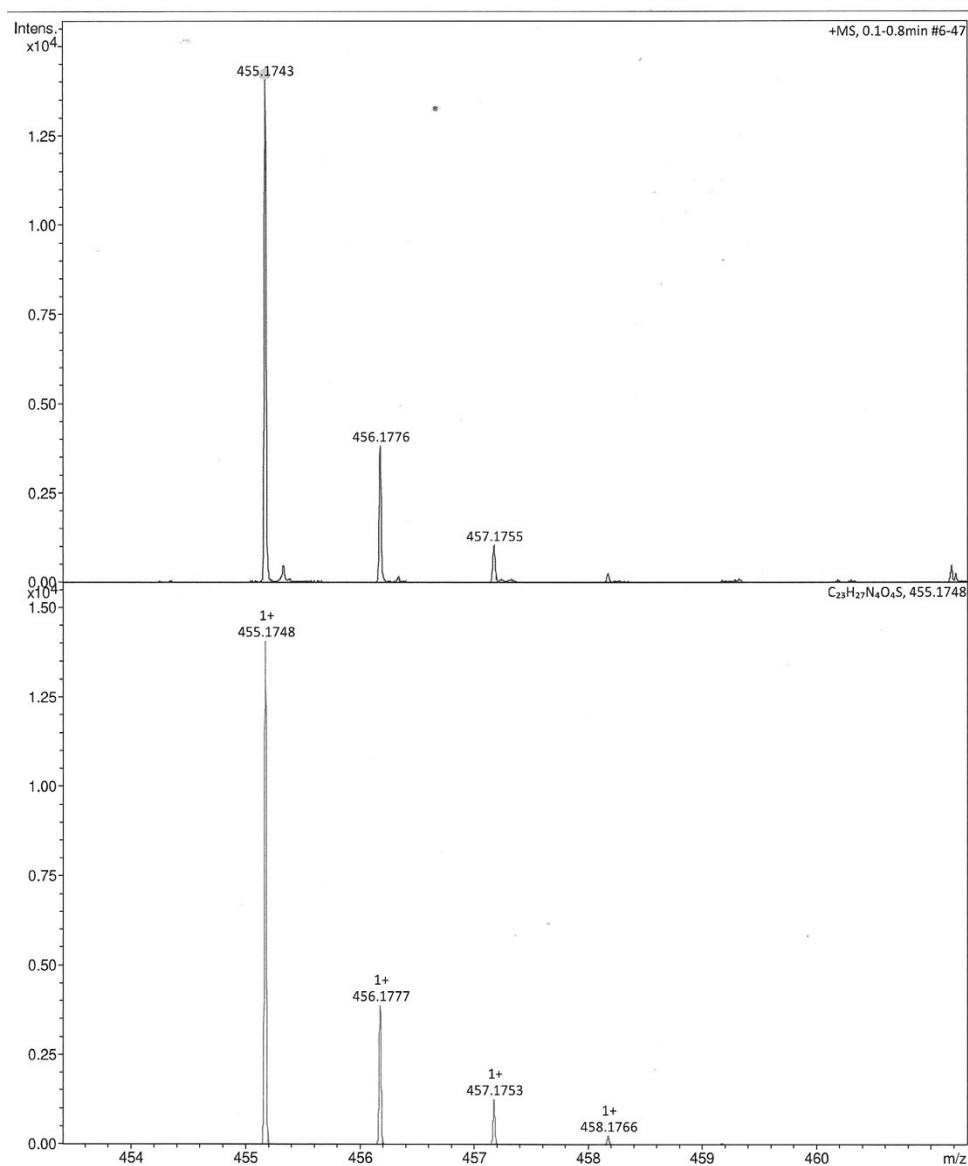
MassPeaks:323

RawMode:Single 11.125(1312) BasePeak:411.15(816802)

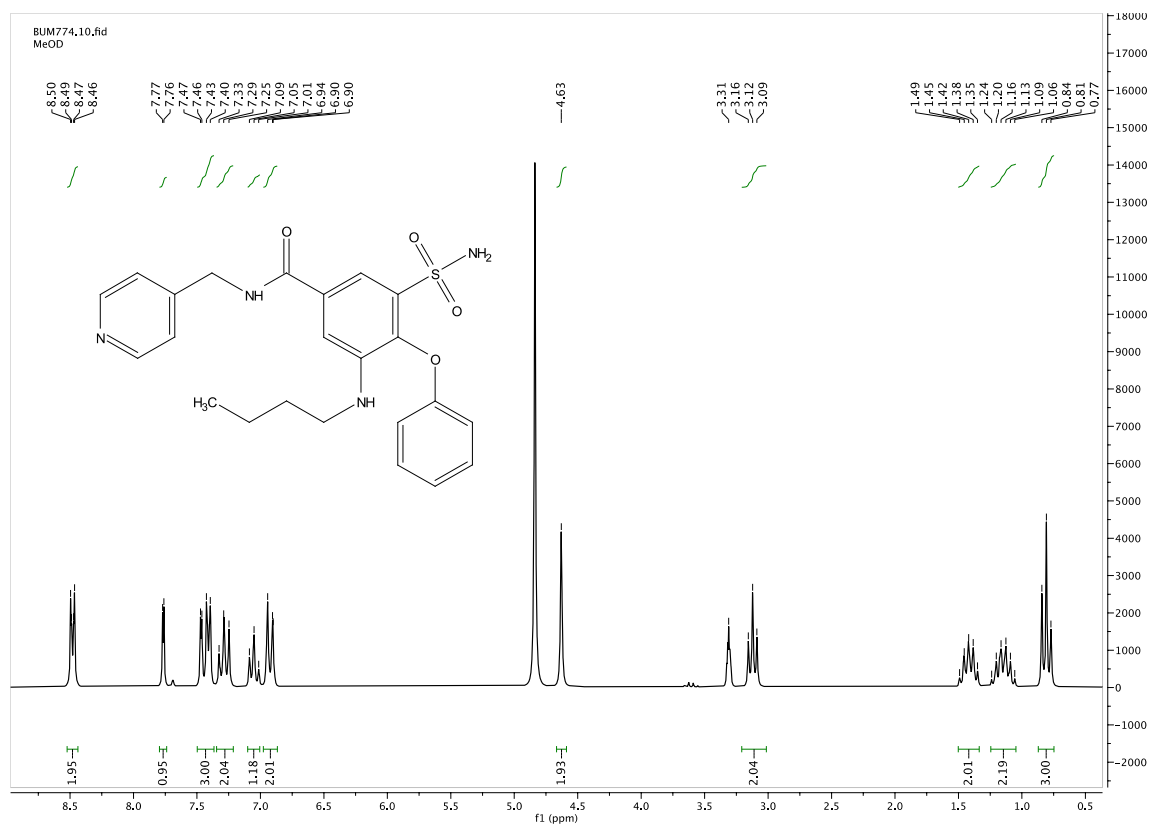
BG Mode:None Group 1 - Event 1



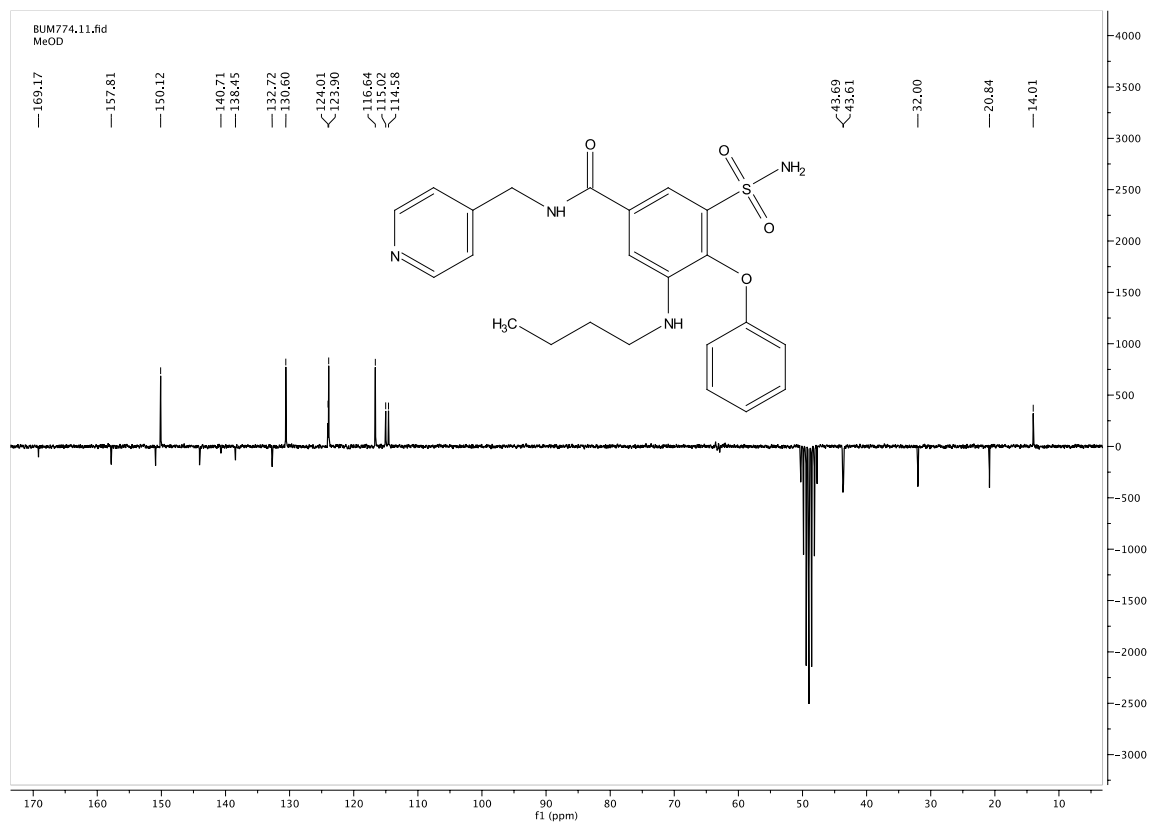
Compound 4 HRMS



Compound 5 ¹H-NMR (MeOD)



Compound 5 ¹³C-NMR (MeOD)



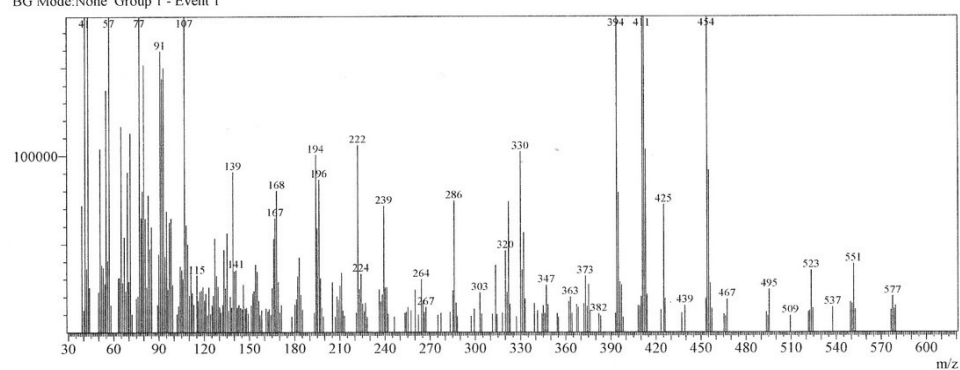
Compound 5 mass spectrum

Line#:1 R.Time:11.808(Scan#:1394)

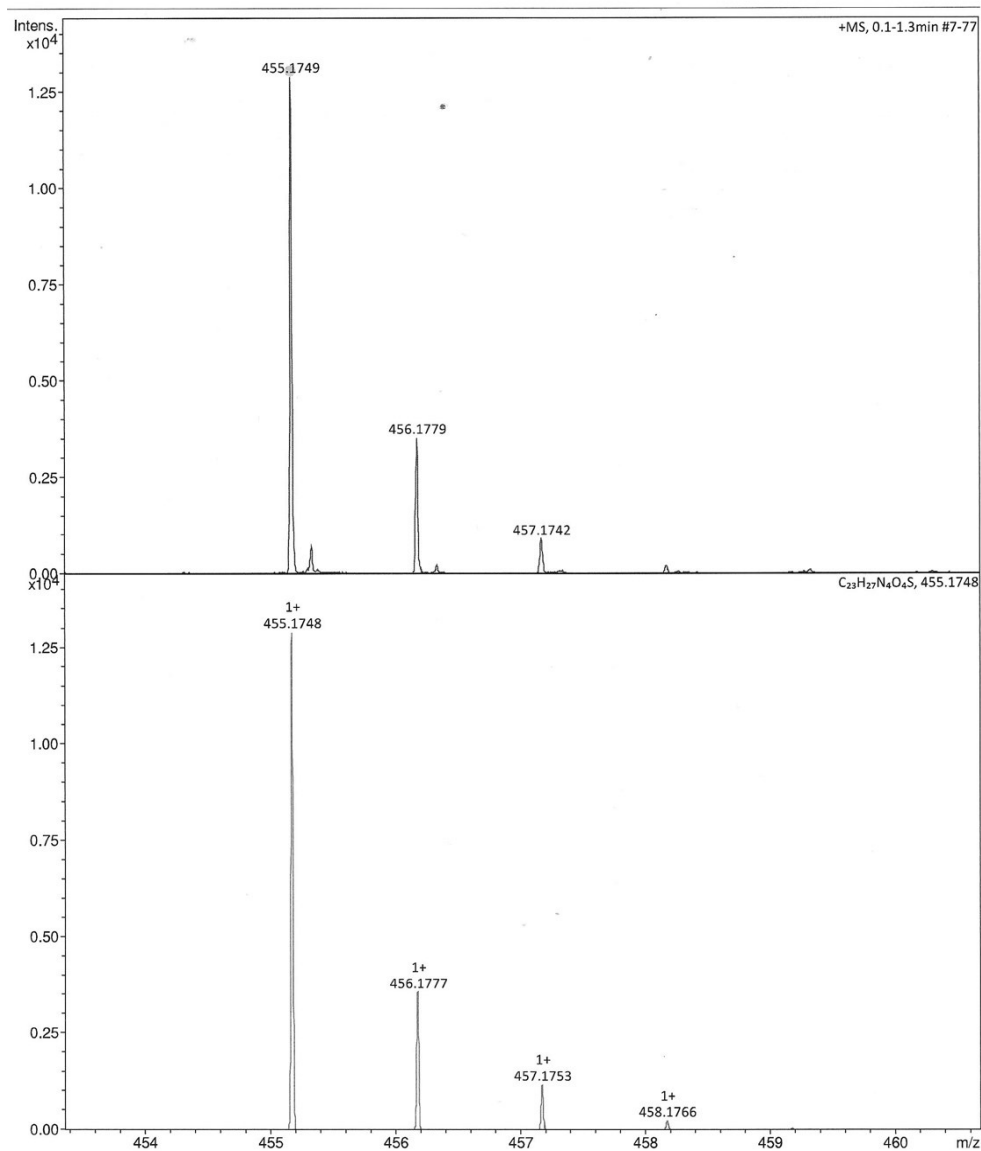
MassPeaks:249

RawMode:Single 11.808(1394) BasePeak:411.10(1199281)

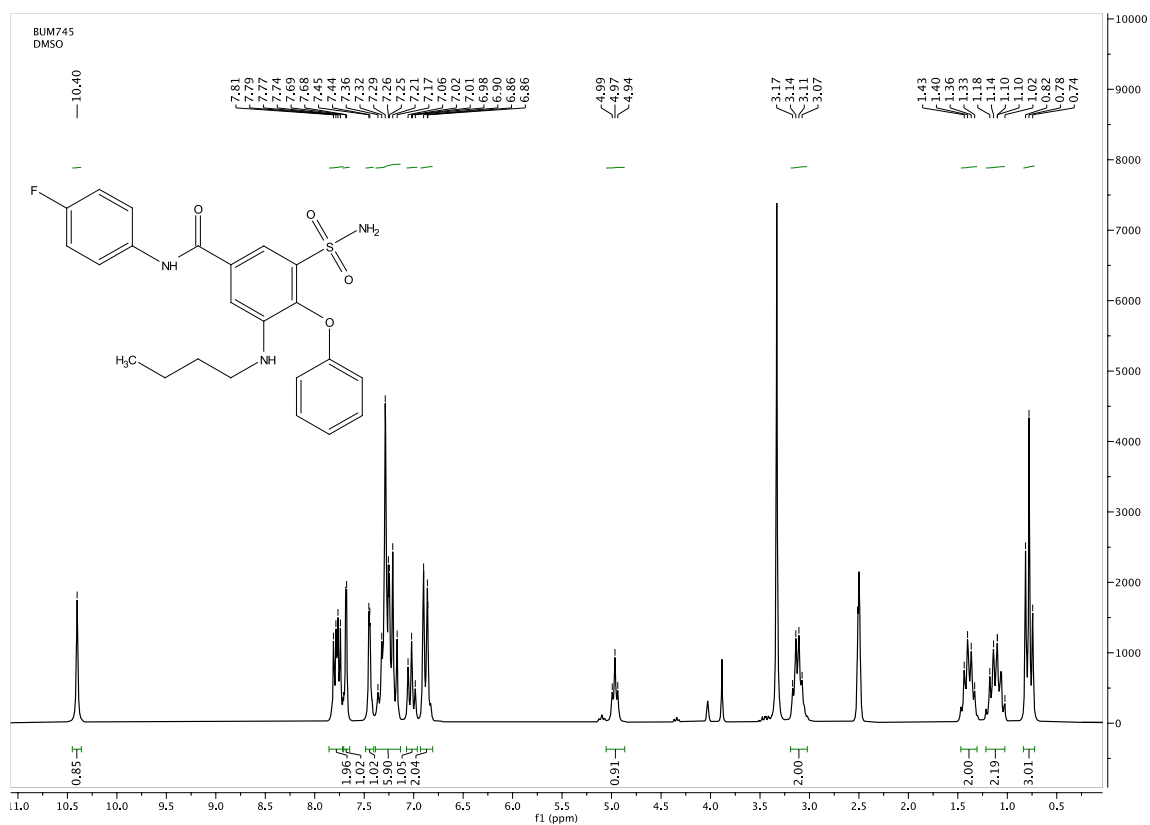
BG Mode:None Group 1 - Event 1



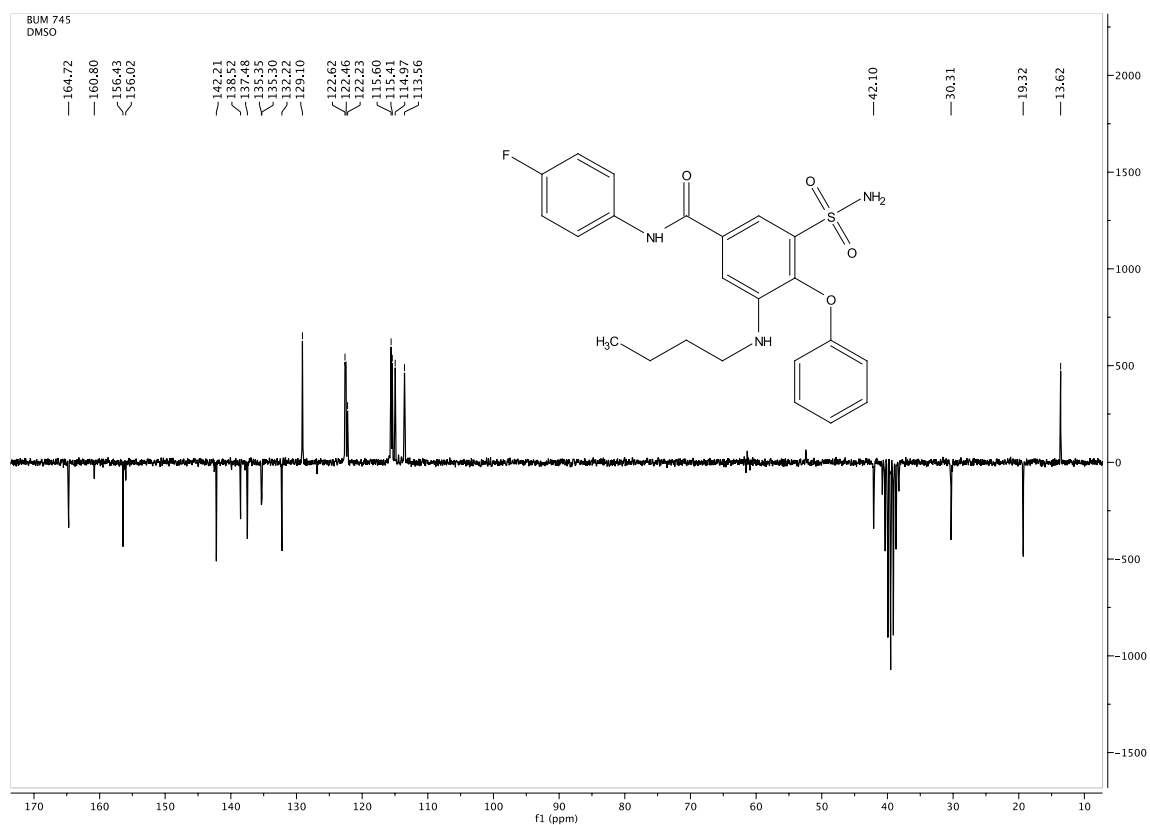
Compound 5 HRMS



Compound 6 ¹H-NMR (DMSO-*d*₆)



Compound 6 ¹³C-NMR (DMSO-*d*₆)



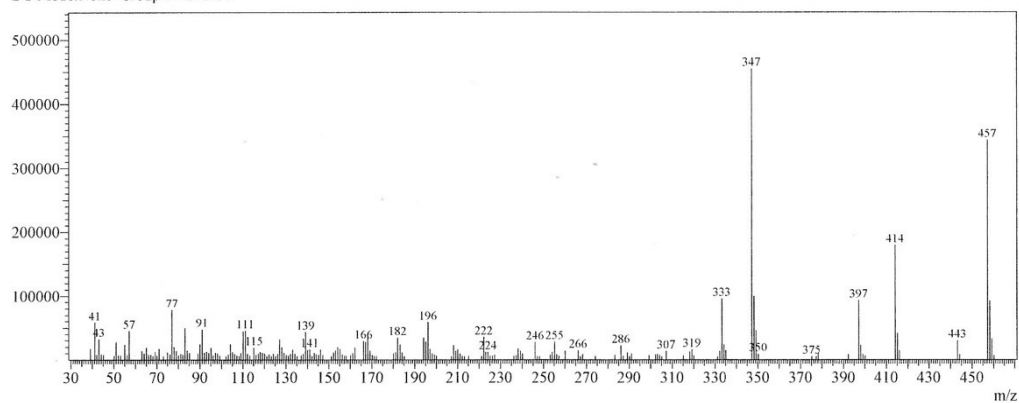
Compound 6 mass spectrum

Line#:1 R.Time:9.750(Scan#:1147)

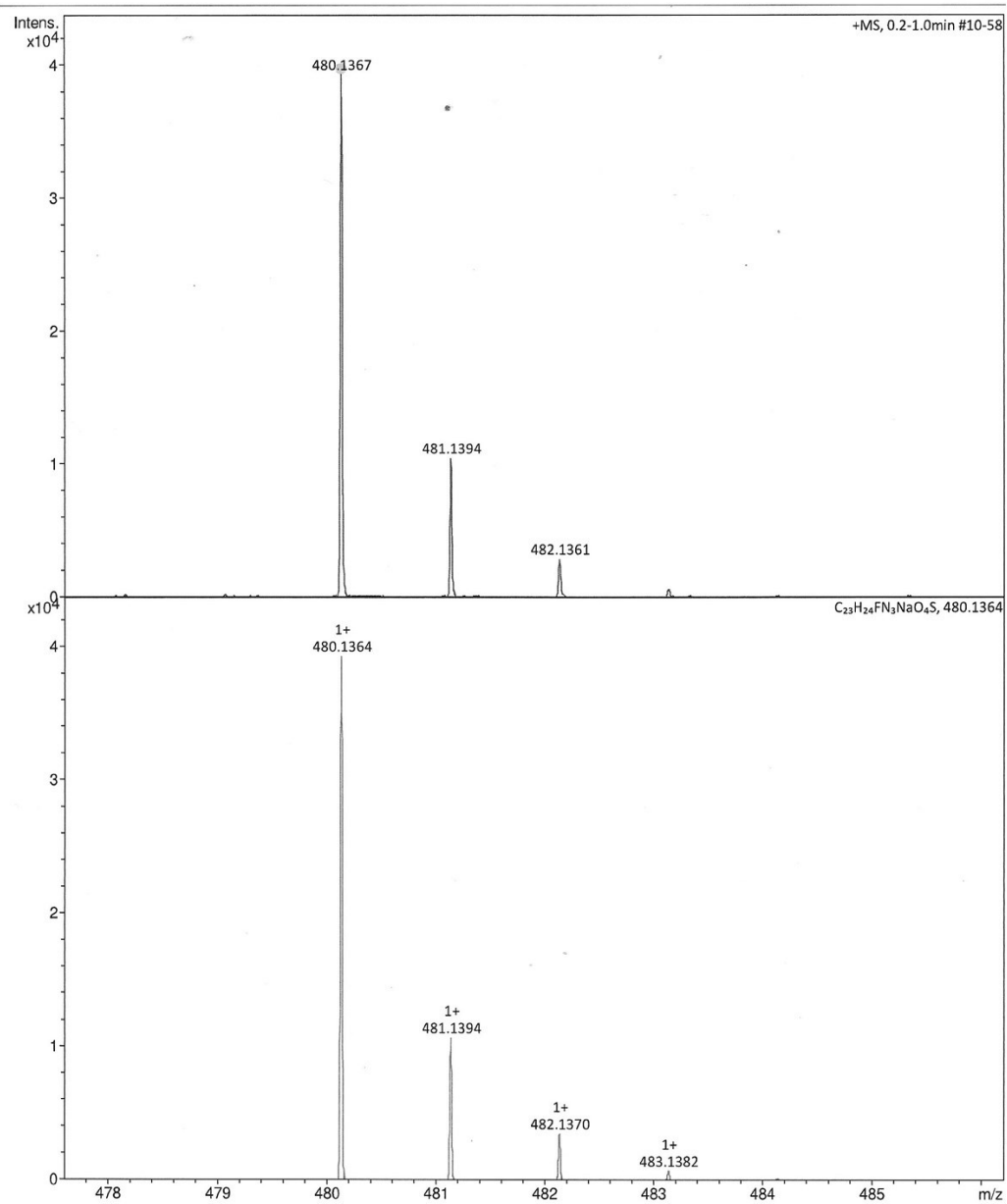
MassPeaks:195

RawMode:Single 9.750(1147) BasePeak:347.15(454466)

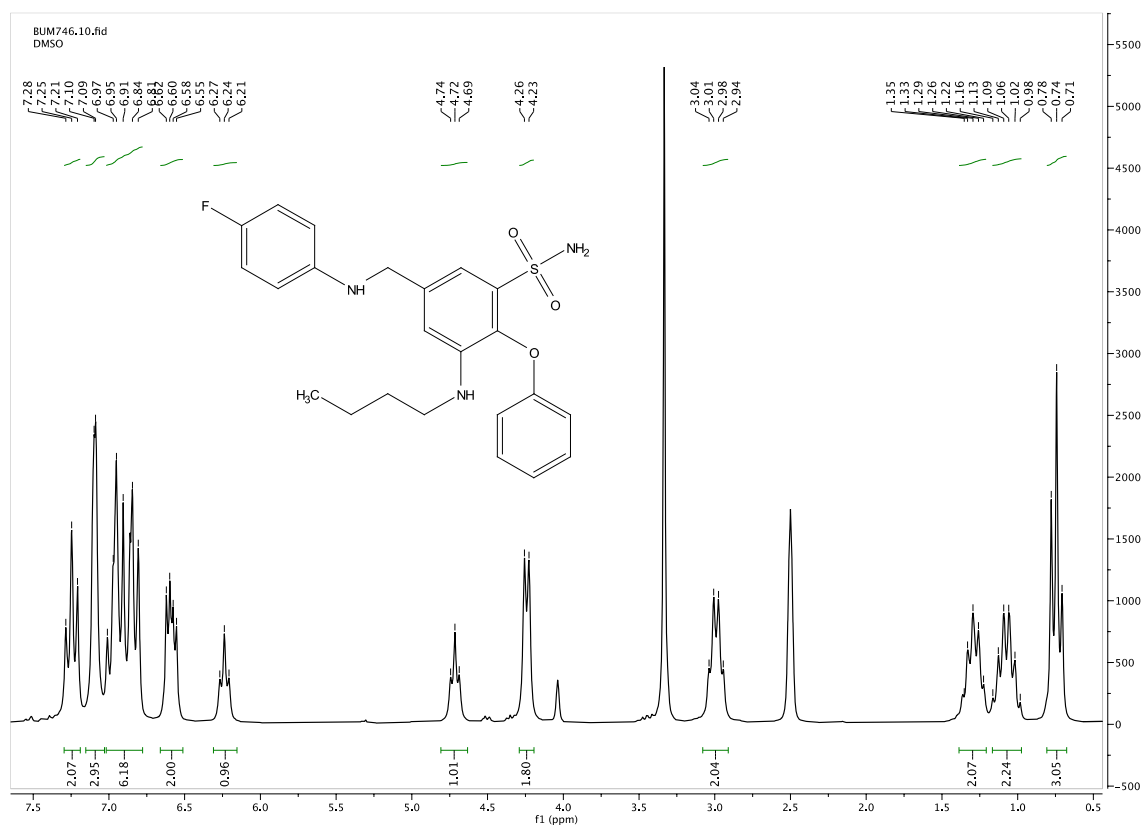
BG Mode:None Group 1 - Event 1



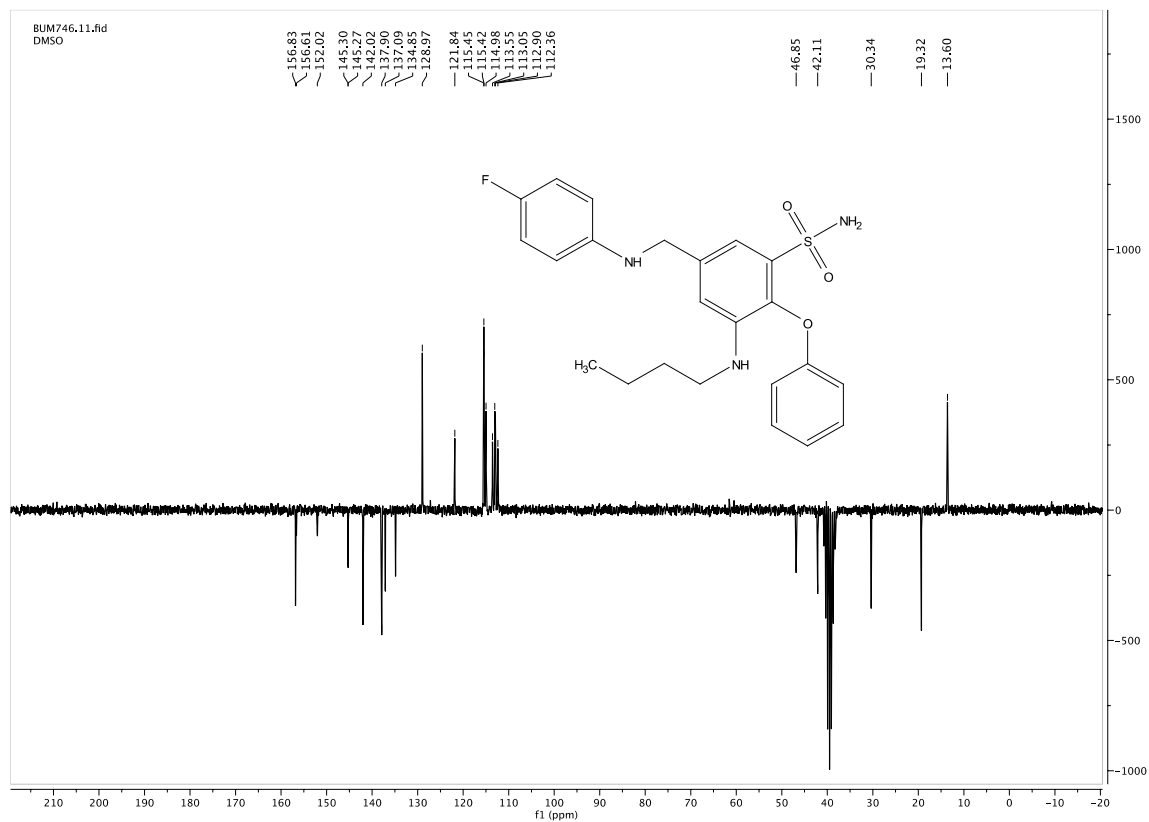
Compound 6 HRMS



Compound 7 ¹H-NMR (DMSO-*d*₆)



Compound 7 ¹³C-NMR (DMSO-*d*₆)



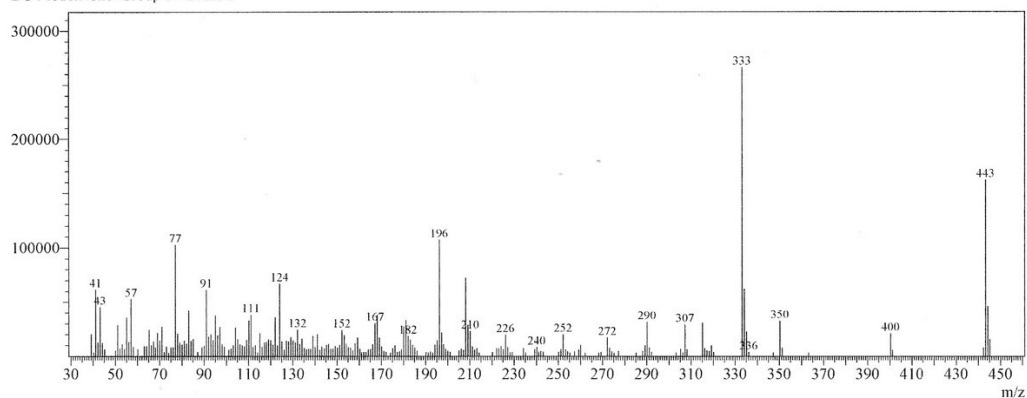
Compound 7 mass spectrum

Line#:1 R.Time:7.725(Scan#:904)

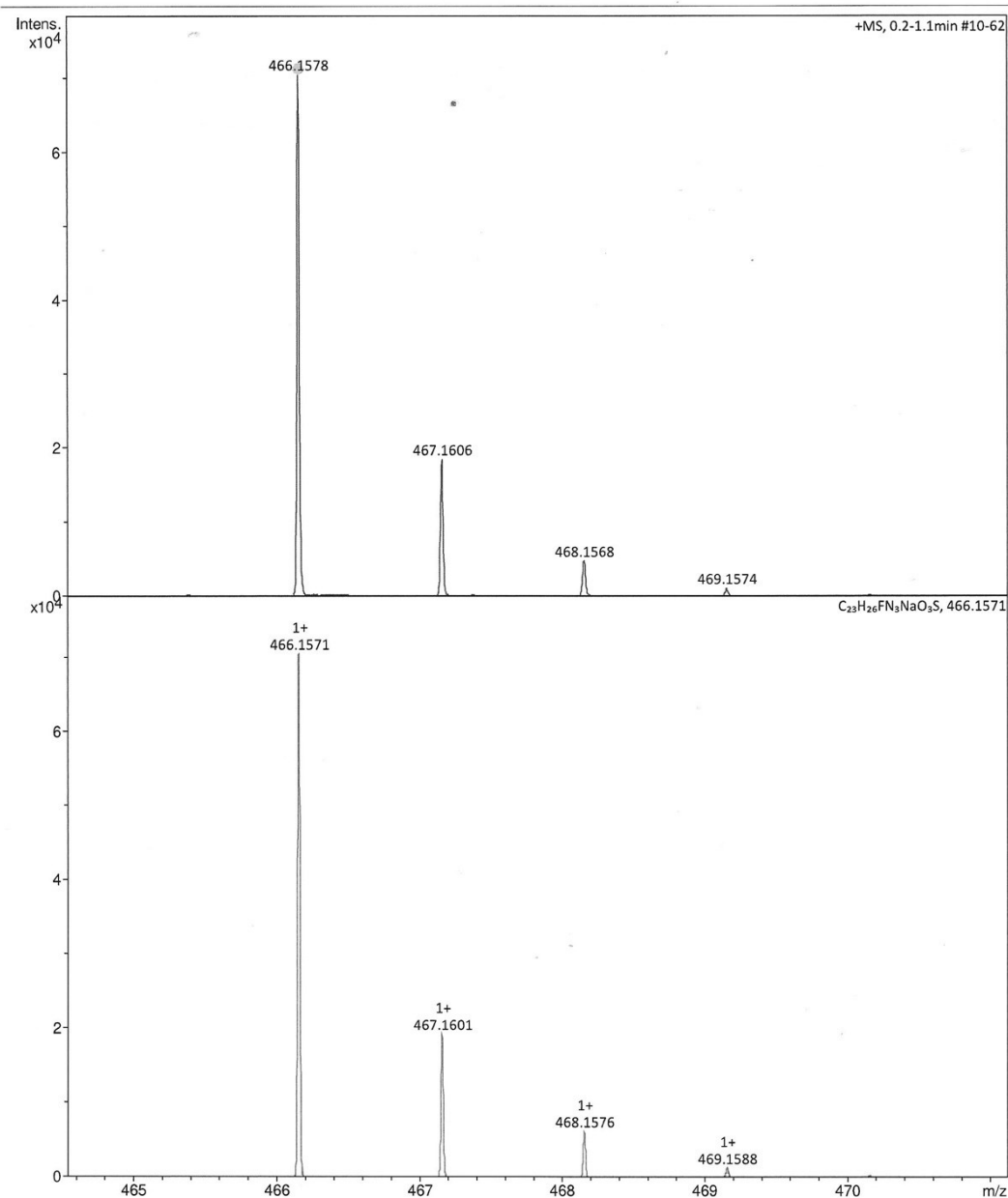
MassPeaks:223

RawMode:Single 7.725(904) BasePeak:333.15(265946)

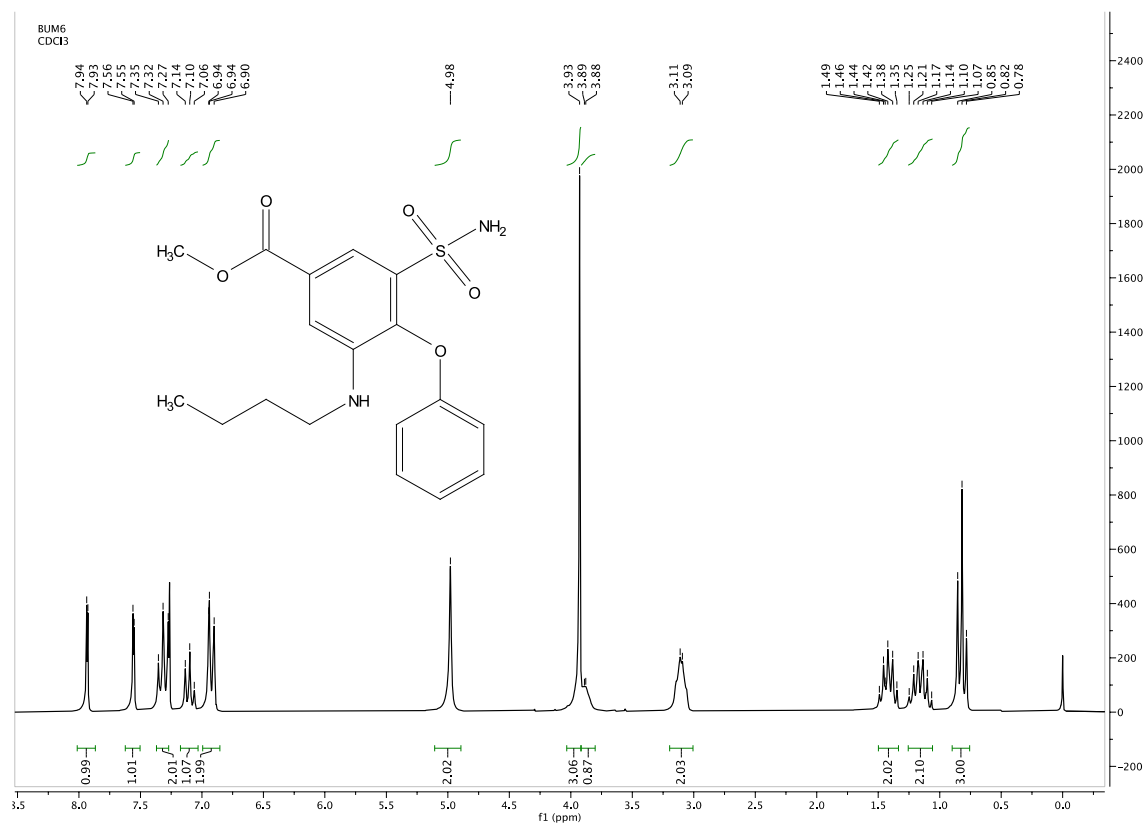
BG Mode:None Group 1 - Event 1



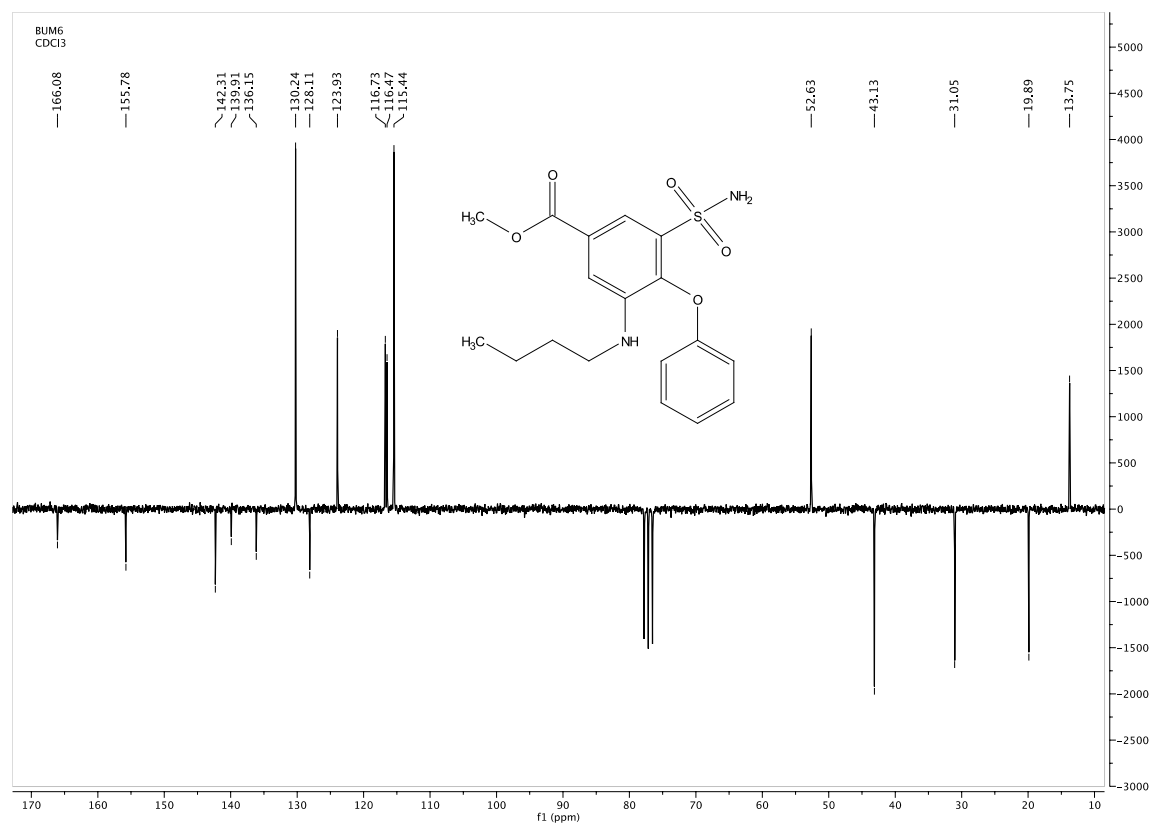
Compound 7 HRMS



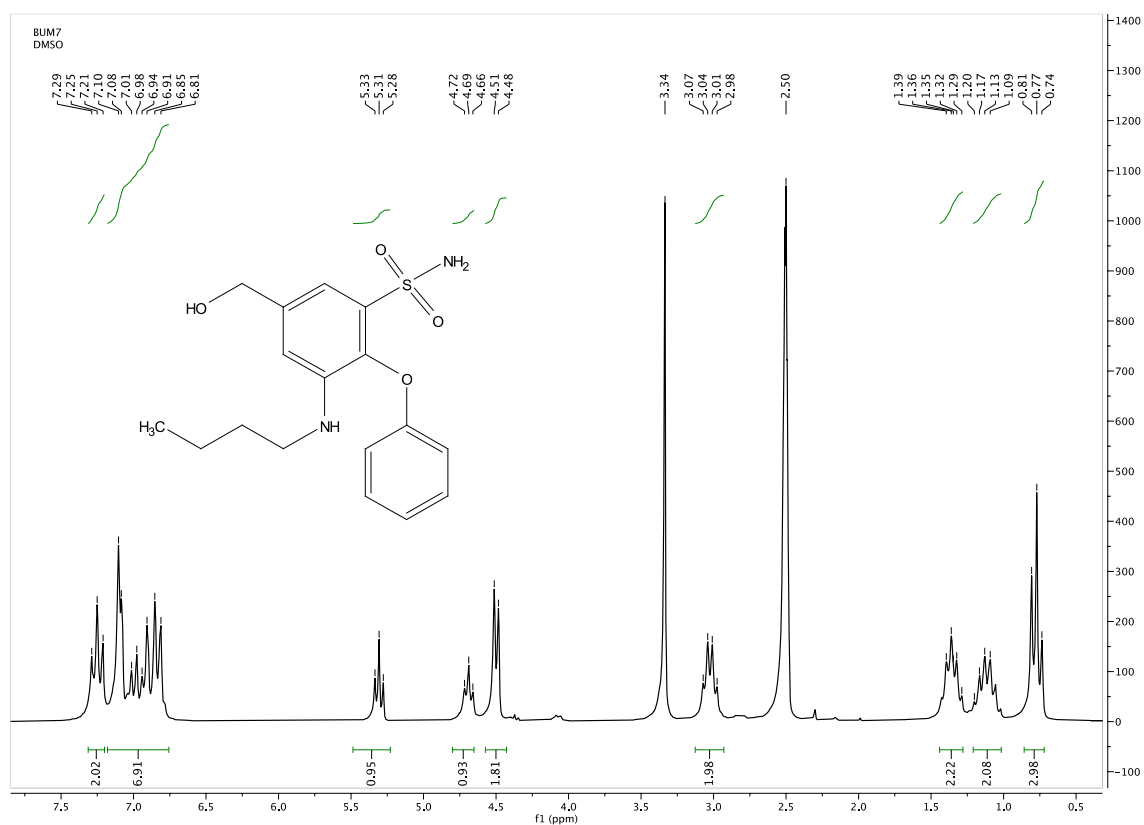
Compound 8 ¹H-NMR (CDCl₃)



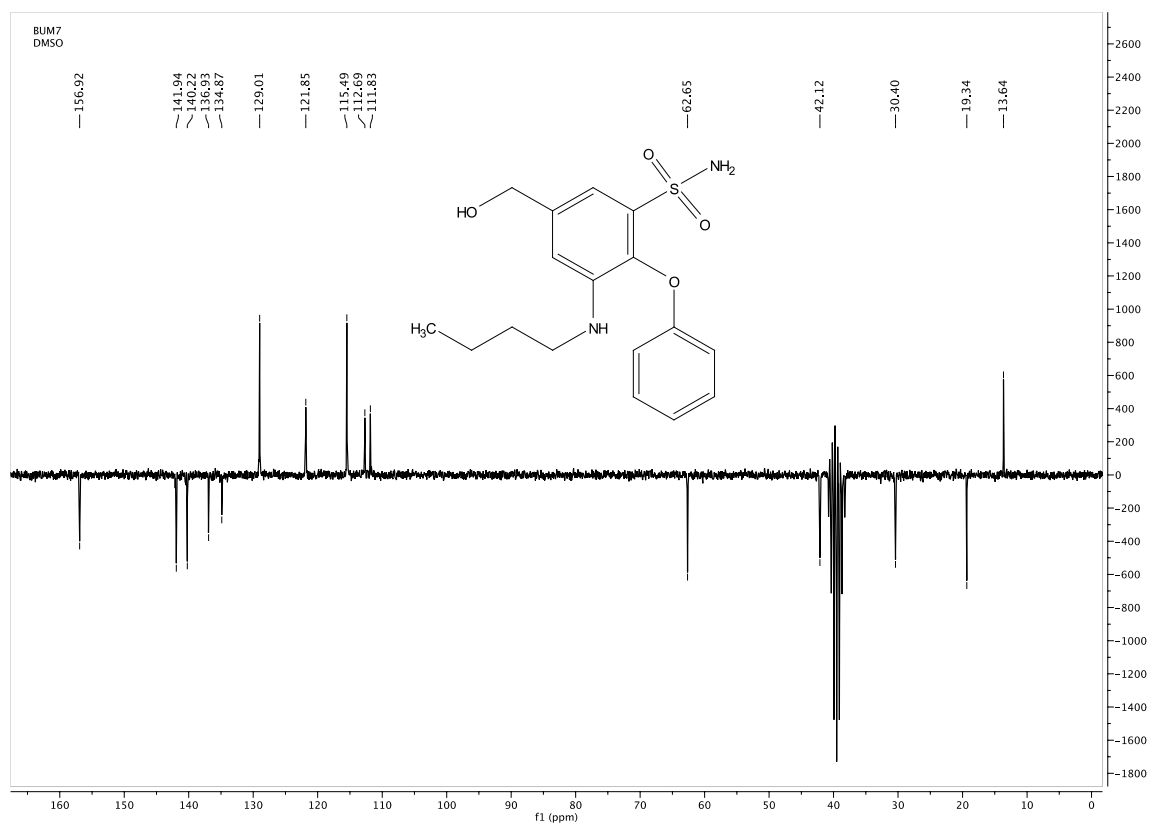
Compound 8 ¹³C-NMR (CDCl₃)



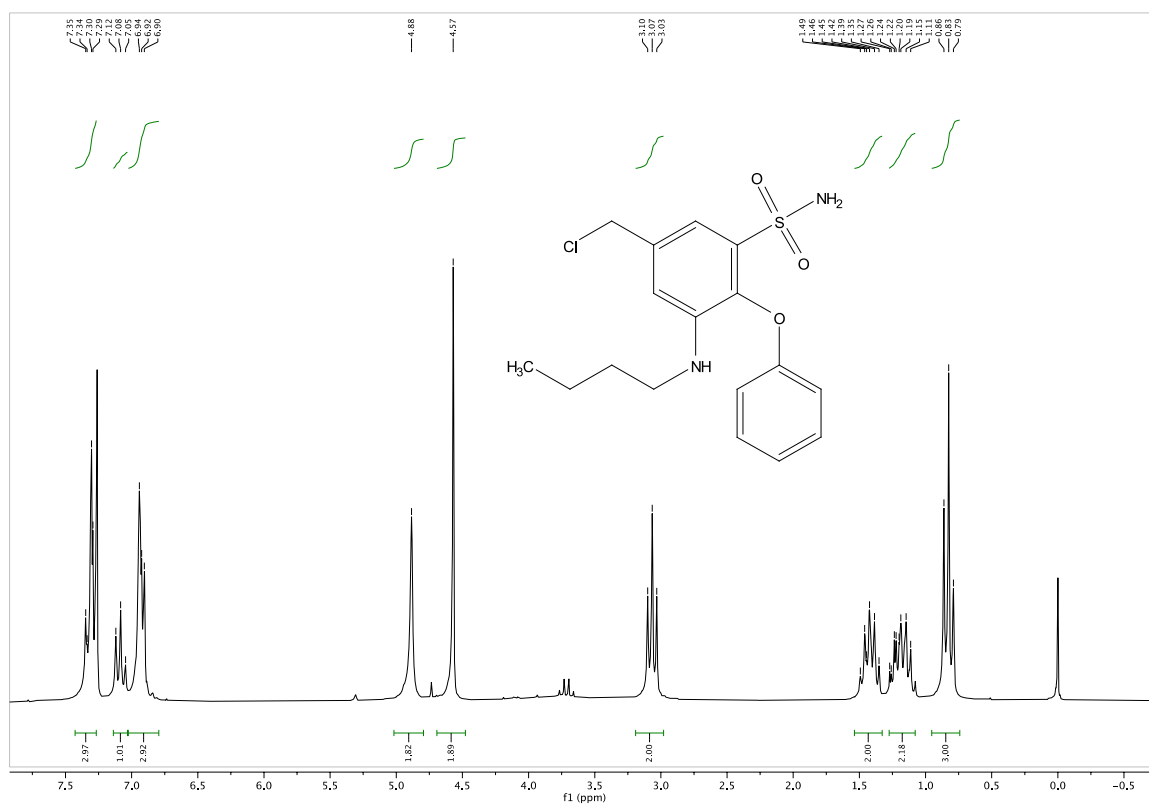
Compound 9 ¹H-NMR (DMSO-*d*₆)



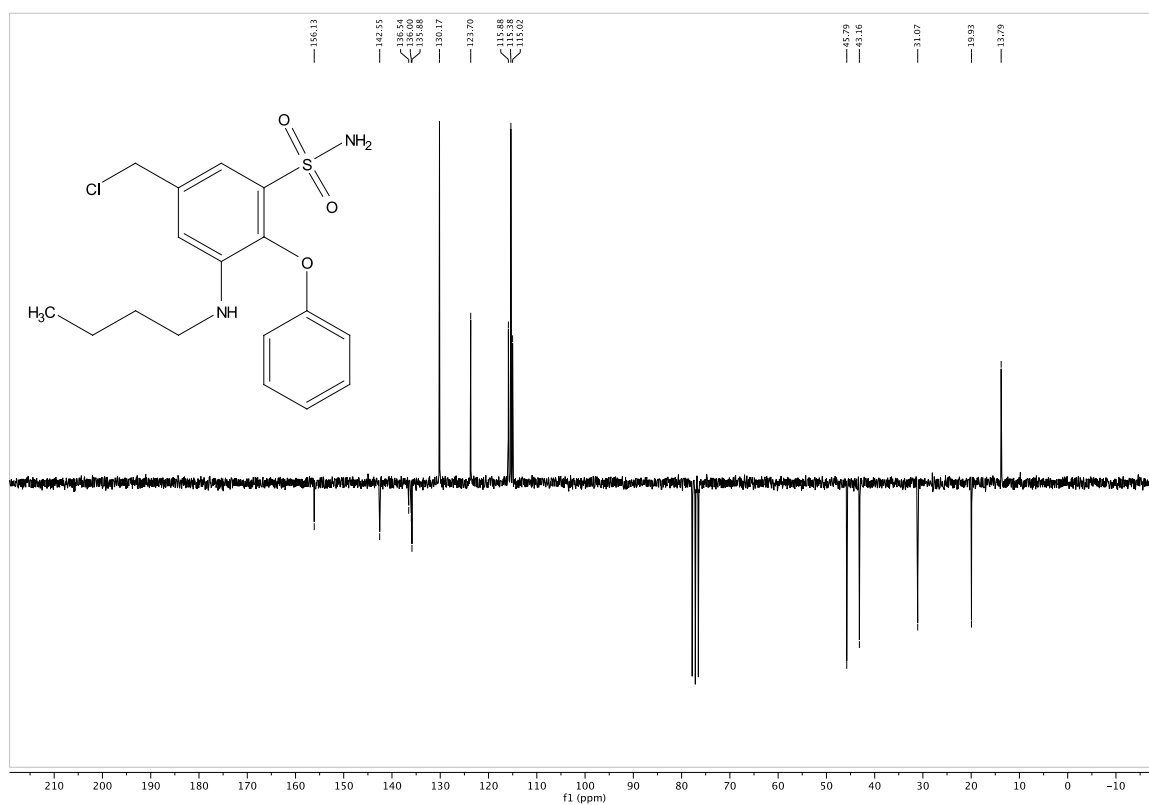
Compound 9 ¹³C-NMR (DMSO-*d*₆)



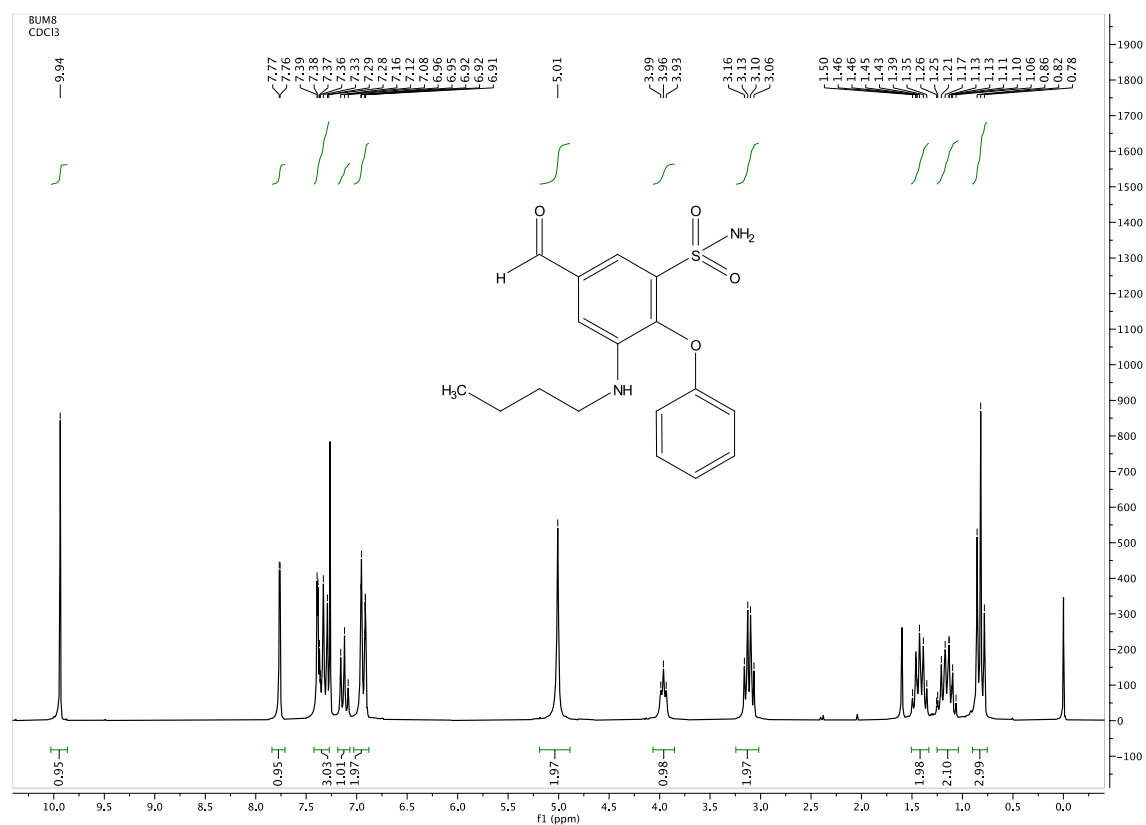
Compound 10 ^1H -NMR (CDCl_3)



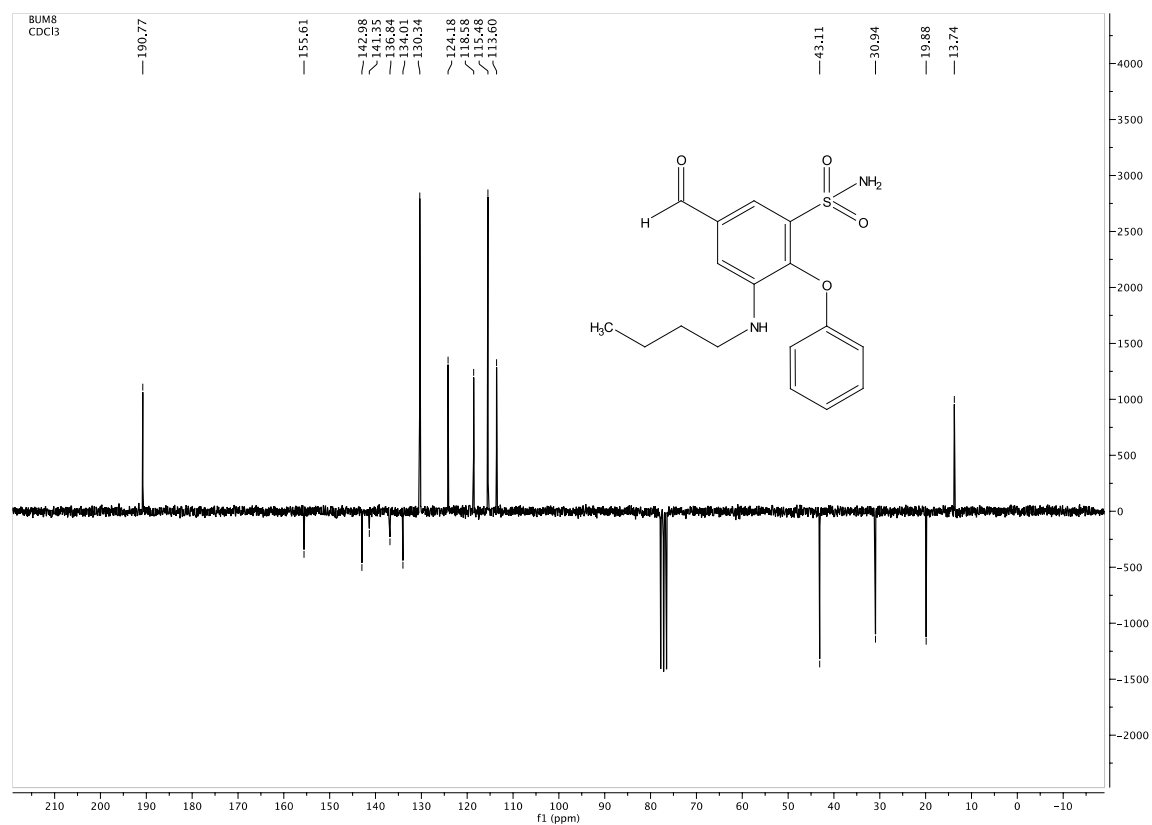
Compound 10 ^{13}C -NMR (CDCl_3)



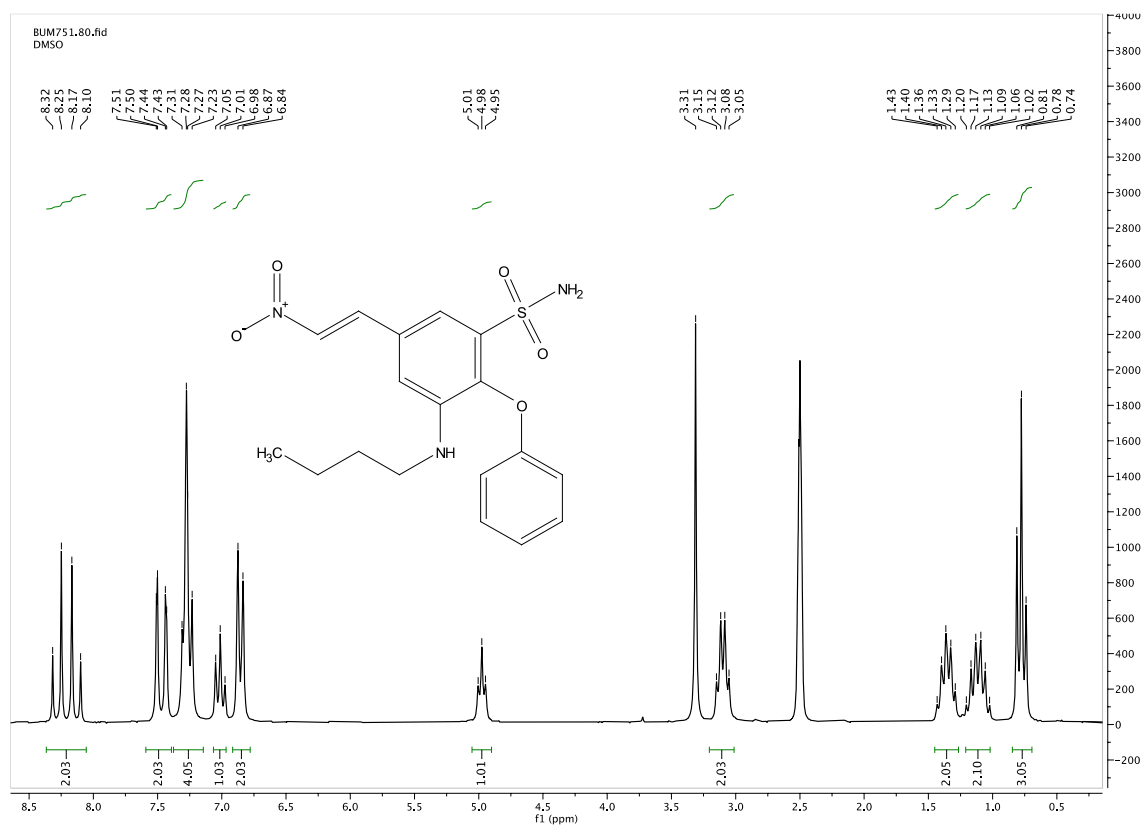
Compound 11 ¹H-NMR (CDCl₃)



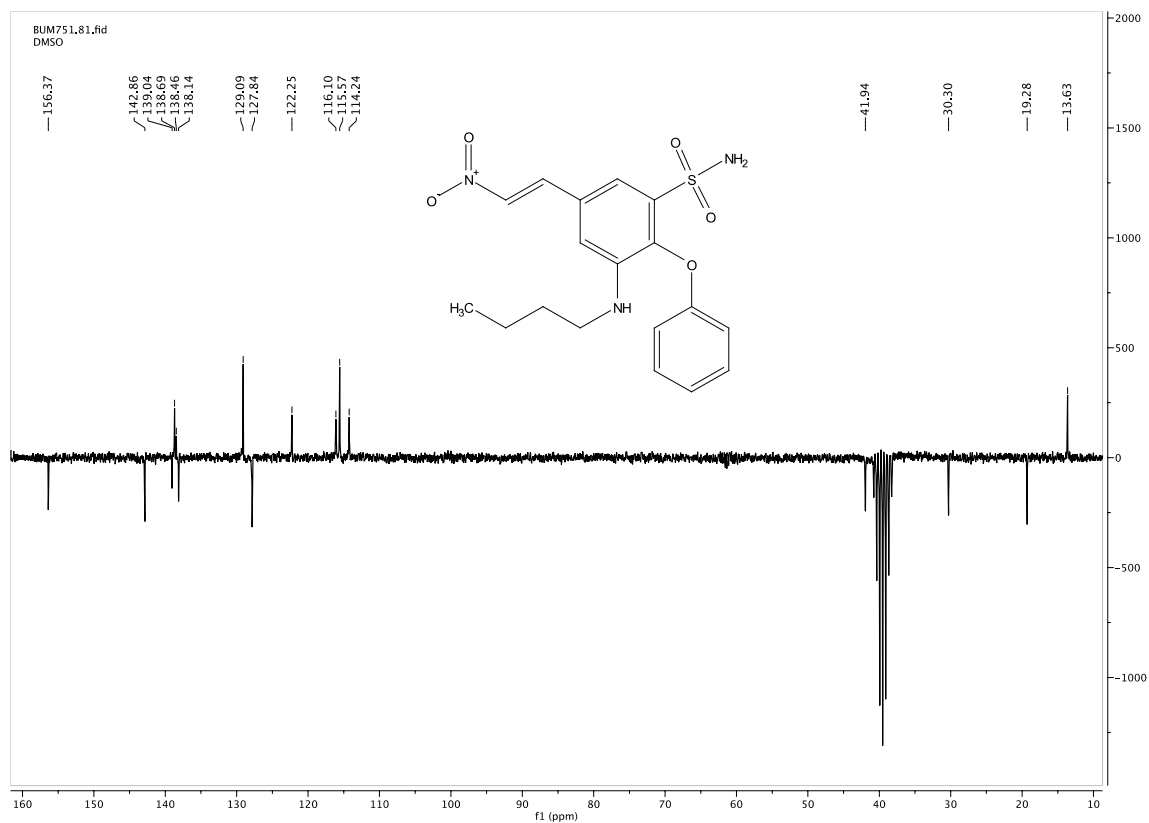
Compound 11 ¹³C-NMR (CDCl₃)



Compound 12 ¹H-NMR (DMSO-*d*₆)



Compound 12 ¹³C-NMR (DMSO-*d*₆)



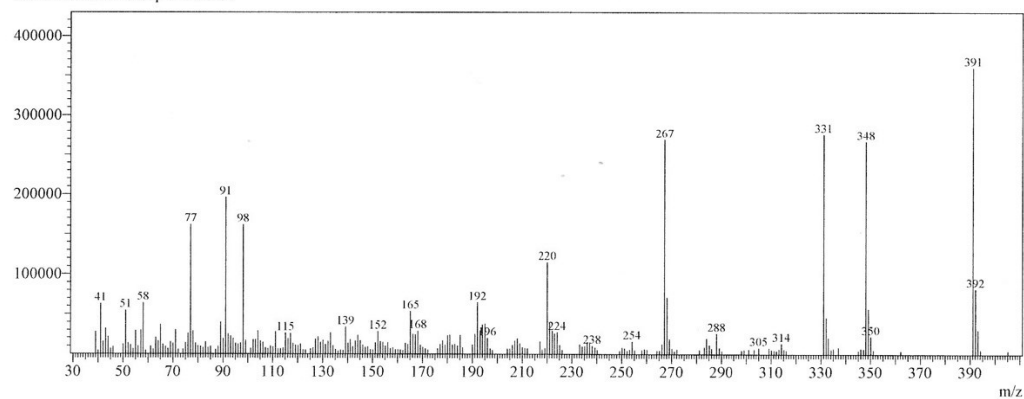
Compound 12 mass spectrum

Line#:1 R.Time:9.192(Scan#:1080)

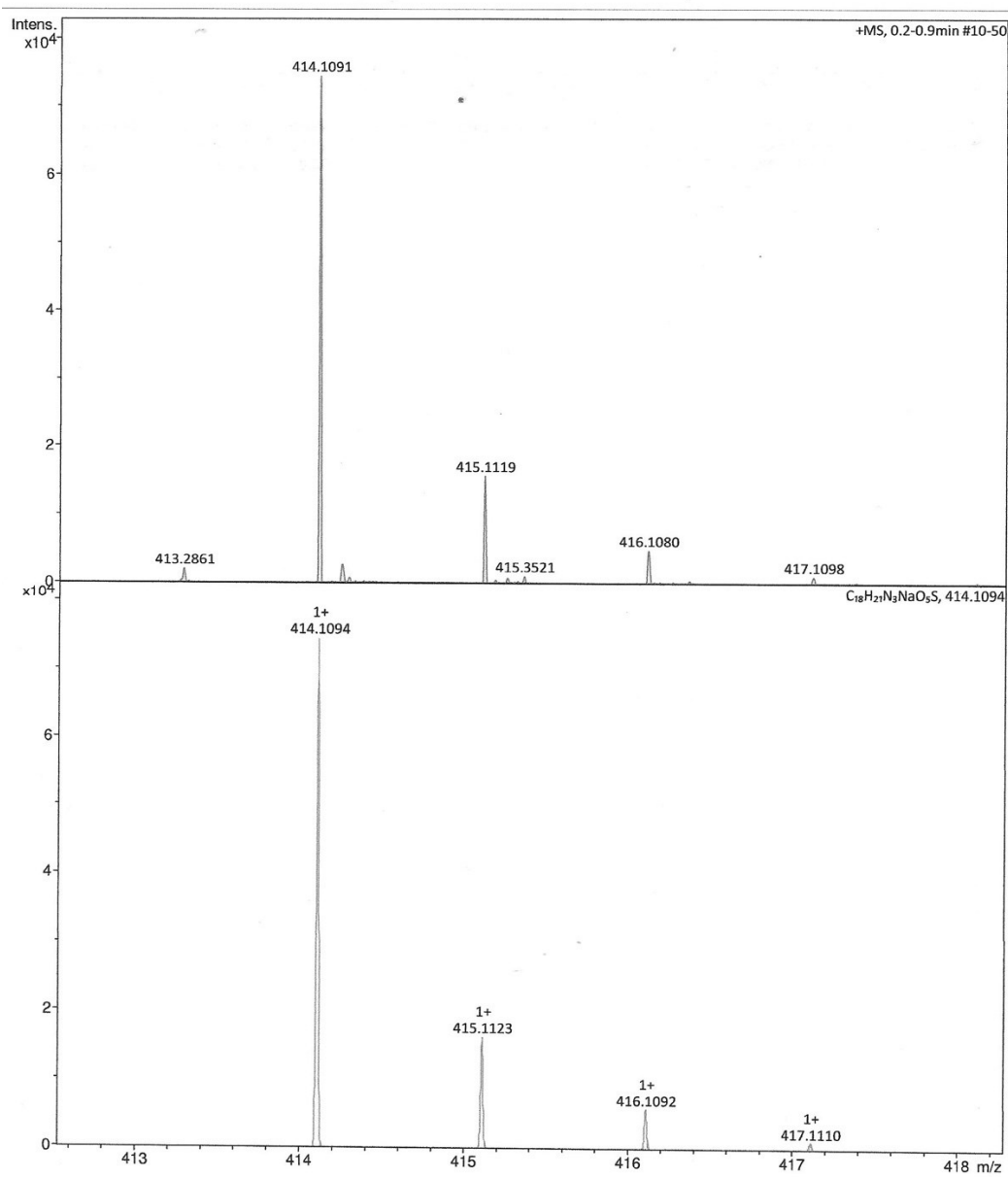
MassPeaks:232

RawMode:Single 9.192(1080) BasePeak:391.10(359703)

BG Mode:None Group 1 - Event 1



Compound 12 HRMS



7.1 Internal Codes for compounds 1-12

Compound 1	BUM 698
Compound 2	BUM 675
Compound 3	BUM 771
Compound 4	BUM 772
Compound 5	BUM 774
Compound 6	BUM 745
Compound 7	BUM 746
Compound 8	BUM 6
Compound 9	BUM 7
Compound 10	BUM 63
Compound 11	BUM 8
Compound 12	BUM 751

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