universität wien

DIPLOMARBEIT / DIPLOMA THESIS

Titel der Diplomarbeit / Title of the Diploma Thesis

„Design und Synthese von Bumetanid-Derivaten mit Benzylamin-Teilstruktur“

verfasst von/ submitted by

Medaris Mujkic

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Finally, I especially want to thank my parents, who made this study possible, not just by financial support, but also by helping me whenever possible, and also thanks to my friends for motivating me during all the time.

Abstract (English/German)

The objective of this diploma thesis was to synthesize bumetanide derivates. For most of the derivates the carboxylic acid function was substituted with different structures. The resulting products could possibly enter the blood-brain barrier more easily and then inhibit the Na^+-K^+-$2Cl^-$-Symporter in the CNS. This could lead to some promising effects, as an add-on to the current therapy of glioma and stroke. In total 13 compounds were synthesized, compounds **1 – 11** were made by different substitutions of the carboxylic group. Compound **12** and **13** just have the substructure of the sulfonamide group and the phenyl ring in common with bumetanide.

Das Ziel dieser Diplomarbeit war es Bumetanidderivate zu synthetisieren. Bei den meisten Derivaten wurde die Carbonsäure durch unterschiedliche Strukturen substituiert. Die daraus resultierenden Produkte könnten die Blut-Hirn-Schranke leichter durchqueren und dann den Na^+-K^+-$2Cl^-$-Symporter im ZNS inhibieren. Das könnte zu vielversprechenden Effekten führen, als Zusatz zur derzeitigen Therapie von Glioma und Schlaganfällen. Insgesamt wurden 13 Substanzen synthetisiert, wobei bei Substanz **1 – 11** die Carboxylgruppe von Bumetanid substituiert wurde. Substanz **12** und **13** haben nur die Teilstruktur der Sulfonamidgruppe und des Phenylringes mit Bumetanid gemeinsam.

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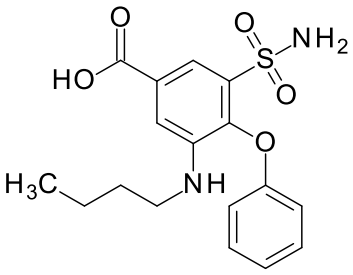
1 Introduction

1.1 Bumetanide

Scheme 1: Bumetanide



Bumetanide, a well-established loop diuretic belonging to the chemical group of aromatic carboxylic acids, is today mainly used in the therapy of severe heart diseases, including hypertonia, heart failure, but also for edema from multiple causes as it is able to drastically increase the diuresis in the kidney (Aktories, et al., 2009). The main effect of this is done by a reversible block of the Na^+-K^+-$2Cl^-$-cotransporter, which has two isoforms, NKCC1 and 2. NKCC 2 exists only in the kidney, which is the main isoform for the diuretic effect, NKCC 1 is expressed by many different cells in the body (Lykke, et al., 2015). Due to the high protein binding and the low pKa, only around 1 % of a given dose of bumetanide is able to reach the brain, which is probably a too low concentration to actively block NKCC 1 *in vivo* (Purskajov, et al., 2014). As all loop diuretics, also bumetanide has the typical side effects of hypomagnesemia, hypocalcemia, but especially the more dangerous hypokalemia and dehydration (Aktories, et al., 2009).

1.2 Glioma

Glioma consists of a heterogeneous group of tumors that represent around 30 % of tumors within the brain and the central nervous systems and 80 % of malignant cancers in the brain (Goodenberger, et al. 2012). The five-year survival rate for patients with glioma is the lowest among all cancers (Jemal, et al., 2010). The current treatment consists of surgery, radiation therapy and chemotherapy, but often fails due to the recurrence of the cancer (Xiaobing, et al., 2017).

- Tumor proliferation

A promising discovery is, as *in vitro* tests showed, that the cell migration of the highly invasive and most common glioma, the glioblastoma, is massively influenced by the NKCC1. In such glioblastomas the NKCC1 is highly up-regulated and knockdowns of the receptor illustrated a decreased cell migration. Bumetanide itself was tested on glioblastoma cell lines and there was significant evidence of lower invasion (Garzon-Muvdi, et al., 2012). The blockade by bumetanide mainly effects pathological neurons, as physiological low Cl^- concentrations are not altered (Ben-Ari, 2008). It is not able to eliminate the cancer *per se*, but it could help to reduce the recurrence of glioblastomas after surgery (Garzon-Muvdi, et al., 2012).

- Seizures

Another health problem is, that diffuse brain glioma often induce seizures as the chloride homeostasis is perturbed by the tumors cells. The chloride homeostasis is an important part for the activity of GABAergic receptors in the brain, which can cause epileptic symptoms and seizures due to the disturbed systems (Pallud, et al., 2014). There are mainly two types of GABA receptors: $GABA_A$, which is one of the most important inhibitory neurotransmitter receptors in the brain, and $GABA_B$, a G-protein-coupled receptor. The $GABA_A$ receptor is opened by γ-aminobutyric acid (GABA) and as this receptor-subtype has a central chloride ion-selective channel, it is understandable, why the system is so dependent on the chloride homeostasis, as an influx leads to a hyperpolarization and an efflux to a depolarization of the cell

membrane (Sigel & Steinmann, 2012). The chloride concentration within the brain cells is mainly controlled by the introversive Na^+-K^+-$2Cl^-$-cotransporter 1 and the outwardly going K^+-Cl^- co-transporter 2, so a blockade of the NKCC1 could stabilize chloride homeostasis (Hasbargen et al., 2010).

1.3 Stroke

Annually around 15 million people around the world suffer a stroke, mostly people at the age of 40 or more. Approximately five million of these die and five million are left permanently disabled. Strokes can be either induced by an obstruction of the blood flow to the brain, which is mainly caused by a blood clot, or by a burst of blood vessels (World Health Organization).

- Chloride homeostasis

Ischemia, due to a stroke, has a massive effect on the ionic homeostasis in neurons, especially Na^+, Cl^-, Ca^{2+} and K^+ ions are affected. The rise of the intracellular Cl^- concentration can lead directly to excitotoxic cell death within the brain, but also to GABA being less inhibitory, which means that the cells hyperpolarize, due to the influx of chloride, with similar effects as mentioned at 1.2 Glioma (Pond, 2006) (Rothman, 1985). *In vitro* tests with mouse hippocampal neurons have shown, that the application of bumetanide can reduce the increase of the chloride concentration, after an ischemic-like event was induced (Pond, 2006).

- Cell swelling

There have also been *in vivo* studies with male hypertensive rats, which have demonstrated, that the average total infarction volume, after a middle cerebral artery occlusion, was significantly reduced by bumetanide compared to placebo. After such an occlusion and ensuing ischemia, the Na^+-K^+-$2Cl^-$-cotransporters are up-regulated in cortical and striatal neurons (Yan, et al., 2001). High K+ ion concentrations, which

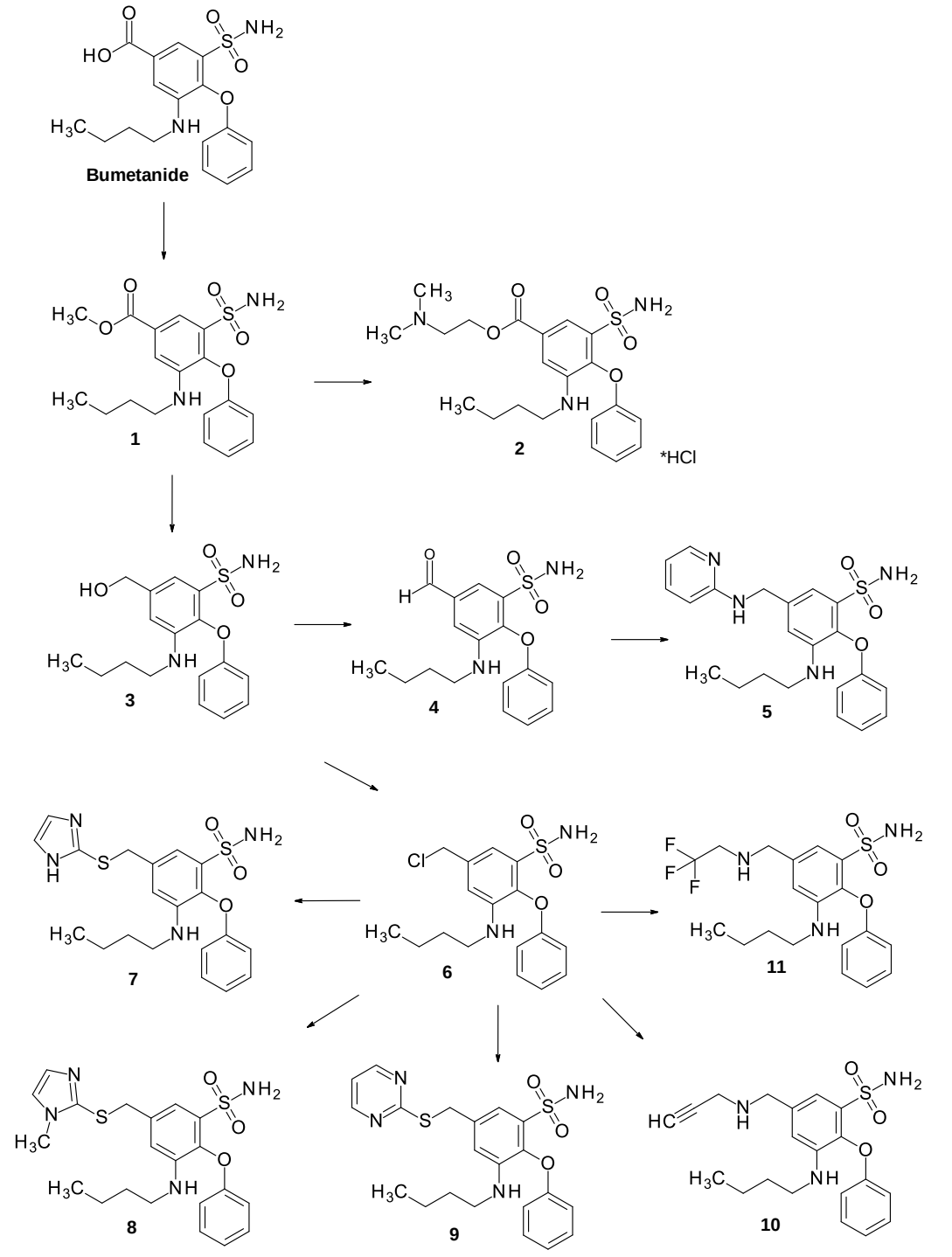
are known to rise during such events, play an important role in this up-regulation of the receptor (POND, 2006). On the other hand, *in vitro* studies represented, that the knock-out of NKCC1 in mice induced a lower K^+-influx, which is the main reason for cell swelling, into astrocytes at high K+ ion concentrations. (Gui, et al., 2001). In the *in vivo* study, the blocking of the hyperfunction of NKCC1, especially in astrocytes, lead to a decrease of the ischemic damage, due to a decrease of the cell swelling. It is important to mention, that this effect is not achieved by the systemic, especially the diuretic effect of the loop diuretic, but by the direct local interaction, as bumetanide was directly introduced into the brain *via* microdialysis. (Yan, et al., 2001)

2 Objective

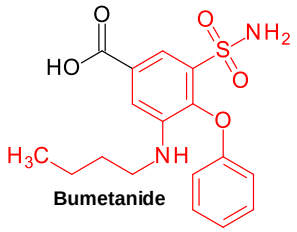
The objective of this diploma thesis was to find ways to synthetize various derivatives of bumetanide, most of which are substitutions of the carboxylic group, whereas two of them only have the partial structure of the phenyl and sulfonamide group in common with bumetanide. As the derivates probably still have the power to inhibit the NKCC1 receptor, we hope that they maybe also have a better affinity to the receptors in the brain and especially can enter through the blood-brain barrier more easily. If this was the case, it could be used as an additional treatment for people who suffer from stroke and glioma.

3 Synthesis of derivates of bumetanide

Scheme 2: Synthesis overview



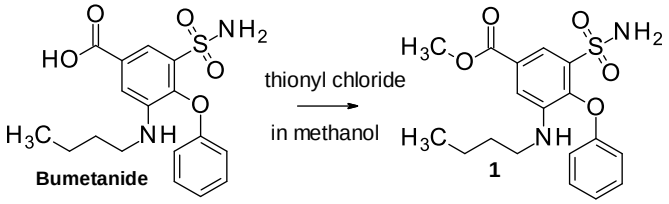
Scheme 3: Substructure of bumetanide for compounds **1 - 11**



The main objective of the diploma thesis was to synthesize derivates of bumetanide with a higher lipophilicity by replacing the very polar carboxylic group with different substituents for compounds **1 – 11**. While compound **2** is still a quite polar alcohol, all others are either a methyl ester (compound **1**), a chloride (compound **3**) or some sort of amine (compounds **4 – 11**). The substituents with a high lipophilicity are supposed to penetrate the blood brain barrier better than bumetanide itself, while hopefully still having the same effect on the receptor. The yields were quite high (up to 98 %) for compound **1**, **2** and **4**, while the other structures had mostly a much lower outcome, with yields down to only 9 %.

3.1 Compound **1**

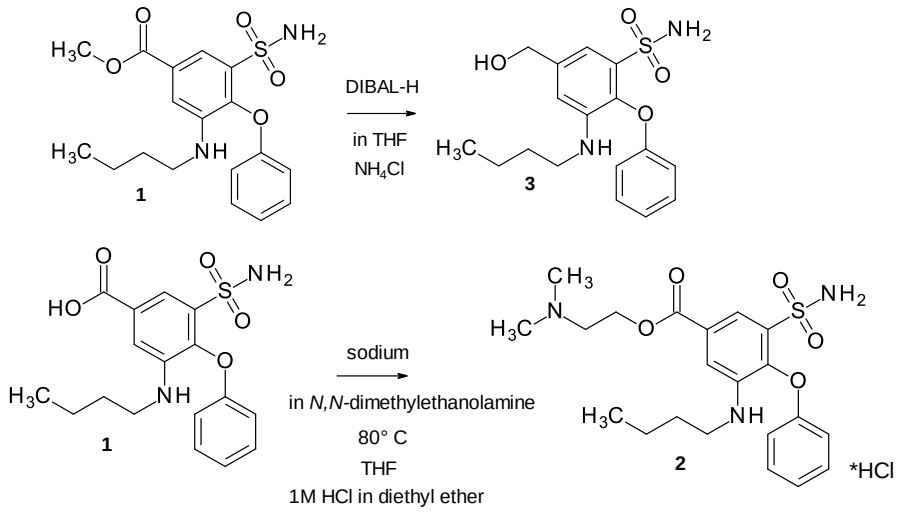
Scheme 4: Compound **1**



First of all, the objective was to synthetize a more lipophilic counterpart of bumetanide called compound **1**. Therefore, the carboxylic acid was methylated resulting in an ester. This reaction was done by adding thionyl chloride to a suspension of bumetanide in methanol and stirring it overnight. The reaction resulted in a high yield of 98% of white crystals.

3.2 Compounds **2** and **3**

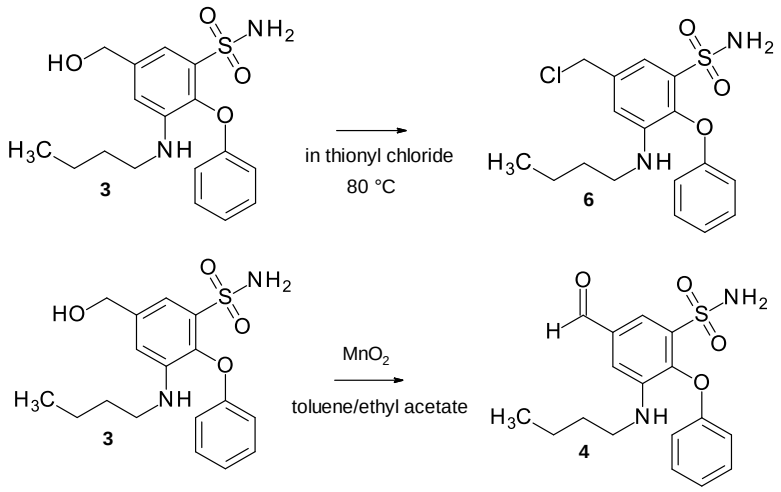
Scheme 5: Compounds **2** and **3**



The second step, for achieving the main precursor compound **6**, was to convert the methyl ester into a benzylic alcohol resulting in compound **3**. This was achieved by dissolving compound **1** in THF and gradually adding portions of diisobutylaluminium hydride in toluene. After the reaction was completed, which was tested by thin layer chromatography, the reactant was cooled to 0 °C and was quenched with ammonium chloride. The product had a yield of 93% of a slightly yellow solid. Compound **2**, is another derivate whose starting substance is compound **1**. Therefore compound **1** was dissolved in *N,N*-dimethylethanolamine and then elementary sodium, as a catalyst, was added and the mixture was heated to 80 °C. The solid, with a yield of 77 %, was then again dissolved in THF and precipitated with hydrogen chloride solution 1 M in diethyl ether.

3.3 Compounds **4** and **6**

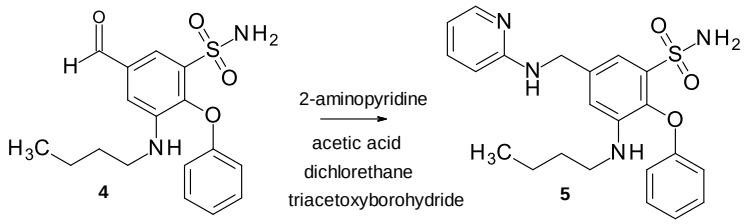
Scheme 6: Compounds **4** and **6**



Starting with compound **3**, compound **6**, our main precursor, was synthesized and later on also compound **4**. Compound **6**, where the hydroxide group was replaced by a chloride, was made by dissolving compound **3** in thionyl chloride and heating it up to 80 °C for three hours. After purifying it by column chromatography the yield was only 28% of brown crystals. Compound **4** is the only aldehyde made in the diploma thesis. It was made by dissolving compound **3** in toluene/ethyl acetate (1+1) and adding MnO_2. For this reaction it took five days to finish, whereas after three days again a portion of MnO_2 was added and the temperature was raised to 40 °C. The product yielded to 53 %.

3.4 Compound **5**

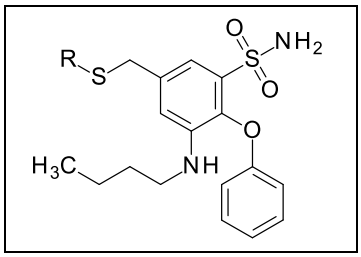
Scheme 7: Compound **5**

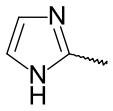
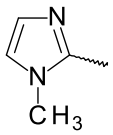
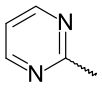


Compound **5** was made by reductive amination of compound **4**. 2-Aminopyridine and acetic acid were added to a solution of compound **5** in dichlorethane and were stirred for two hours. After that time sodium triacetoxyborohydride was added and the mixture was again stirred overnight. Then it was diluted, the organic phase evaporated and purified by column chromatography (ethyl acetate/petroleum ether 6+4) and recrystallization from 70 % EtOH, yielding to white crystals (yield 30 %).

3.5 Compounds **7 – 9**

Scheme 8: Compounds **7 - 9**

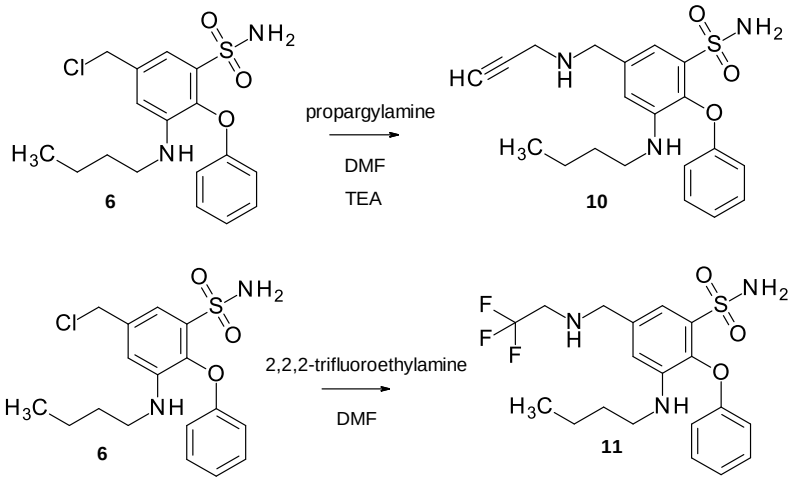


compound	R
7	
8	
9	

These three structures involve an aromatic ring with at least one nitrogen in it. Compound **7** and **8** have an imidazole ring, whereas compound **9** has a pyrimidine ring. To make the three desired products compound **6** was dissolved in DMF and triethylamine was added. For compound **7** 2-mercaptoimidazole, compound **8** 2-mercapto-1-methylimidazole and for compound **9** 2-mercaptopyrimidine were added, respectively, and stirred for at least one day. The yields were between 15 and 64 %:

3.6 Compounds **10** and **11**

Scheme 9: Compounds **10** and **11**

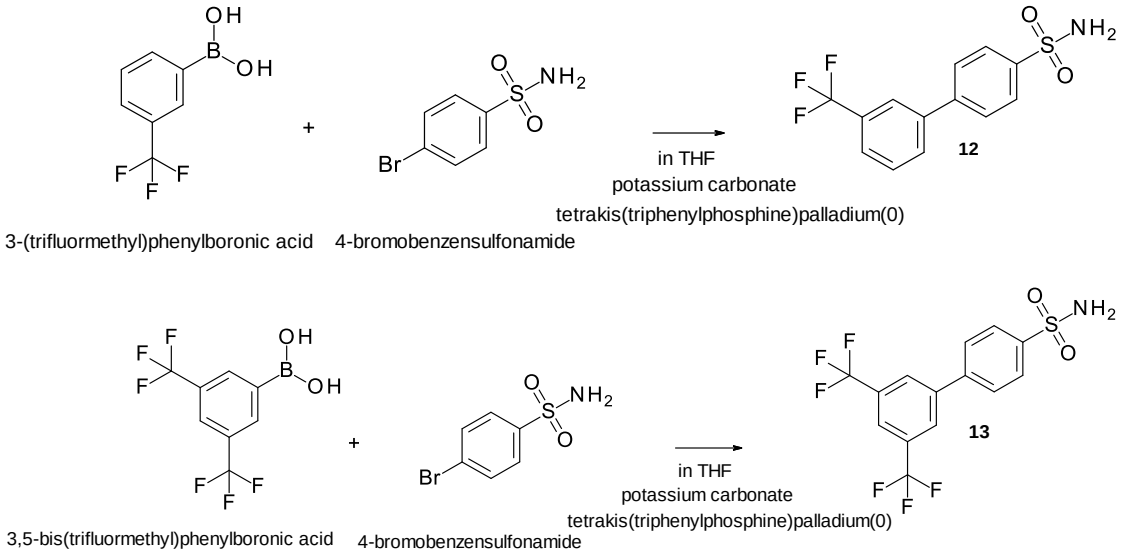


6 **10** **6** **11**

One of the main objectives of this diploma thesis was to synthesize derivates of bumetanide, which have a benzylamine substructure, like compound **10** and **11** have. They were made from compound **6**. To get compound **10** compound **6** was mixed with DMF and propargylamine, stirred for three days, but then again, a portion of propargylamine and also triethylamine, to make it more alkaline, were added and stirred for another day. The reaction was finished, but with a yield of only 9 %. Compound **11** was made by dissolving compound **6** in *N,N*-dimethylformamide, adding 2,2,2-trifluoroethylamine and stirring it for two days. After that again 2,2,2-trifluoroethylamine was added and got stirred for another day. After that the product was gained by column chromatography with a yield of only 13 %.

3.7 Compounds **12** and **13**

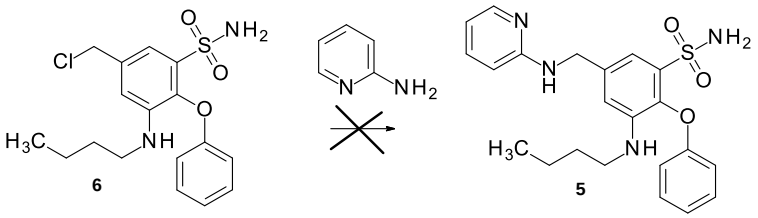
Scheme 10: Compounds **12** and **13**



These two substances differ quite a lot from the other products, as they only have some substructures of bumetanide. Both of them have at least three fluorine atoms at different positions. They were easily accessible by the Suzuki coupling. For compound **12** 3-(trifluoromethyl)phenylboronic acid and for compound **13** 3,5-bis(trifluoromethyl)phenylboronic acid were mixed with 4-bromobenzensulfonamide and solved in THF. After everything is solved, potassium carbonate, water and the catalyst tetrakis(triphenylphosphine)palladium(0) were added to the mixture and it was heated up to 70 °C and stirred overnight. The next day the solutions were extracted with ethyl acetate and water, evaporated and then purified *via* column chromatography with petroleum ether/ethyl acetate and were recrystallized by 50 to 80 % EtOH, with yields between 43 and 34 %.

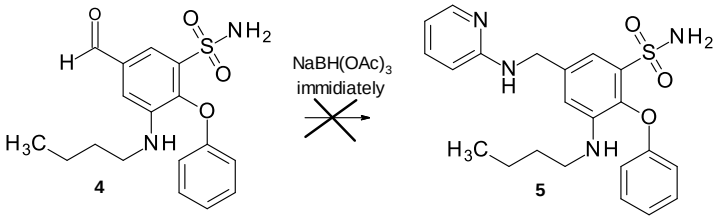
4 Discussion

Scheme 11: Compound **6** to compound **5**



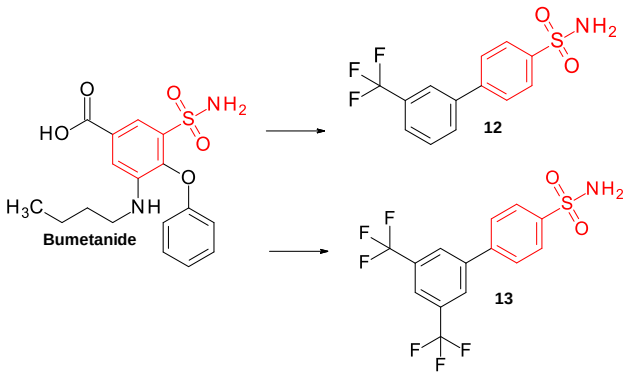
Compound **5**, one of the desired benzylamines, took many several experiments, until a way was found to synthesize it. One of our first attempts to synthesize compound **5** was by substitution of the chloride of compound **6** by 2-aminopyridine. Therefore compound **6** was dissolved in DMF, 2-aminopyridine was added and it was stirred for a few hours, but as nothing changed also some triethylamine was added, to become more basic, and it was stirred overnight. The next day it was heated up to 40 °C and then the mixture was diluted with ethyl acetate and water and evaporated under reduced pressure, purifying it with column chromatography (ethyl acetate/petroleum ether 6+4), but both products, tested with NMR, were not the desired compound **5**. The next attempt was to make the hydrochloride. Therefore, after the reaction was complete, the mixture was thrown onto ice for precipitation, filtered and after a failed attempt at recrystallization, evaporated again under reduced pressure, then dissolved in THF and precipitated with 1 M hydrogen chloride solution in diethyl ether, but also this was not a success.

Scheme 12: Compound **4** to compound **5**



After not being able to accomplish compound **5** *via* compound **6**, the reductive amination, as described earlier, was tried, which at the first attempt worked, but only at very low yields. After some attempts with changing amounts of the starting materials and temperatures, it was realized, that it is fundamental, that the sodium triacetoxyborohydride was added a few hours after all the other reactants. This is probably because of the fact, that the emerging imine needs time to develop. Finally, the synthesis was a success with yields of at least 30 %.

Scheme 13: Substructure of bumetanide for compounds **12** and **13**



Compound **12** and **13** differed quite a lot from the other structures, as they only had some substructures in common with bumetanide, the sulfonamide group and the phenyl ring. They were also the only two structures, whose starting material was not at any point bumetanide itself, but more simple structures connected to phenylboronic acid and sulfonamide *via* Suzuki coupling. Compound **12** has just one trifluormethyl-

group in meta position, while in compound **13** both meta positions are substituted with this lipophilic function.

Whether the increase in lipophilicity is enough to penetrate the brain more easily and still inhibit the NKCC1 can only be shown by *in vitro* and *in vivo* tests.

5 Experimental Section

5.1 General Methods

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

For monitoring the reactions *via* thin layer chromatography, silical gel F254 coated aluminum sheets from Merck were used.

For column chromatography, the stationary phase silicagel 60 70-230 mesh ASTM from Merck was used

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

Mass spectra were recorded on a Shimadzu (GC-17A; MS-QP5050A) spectrometer. The biggest signal was set to 100 %, while the other peaks were set in relative intensity to this.

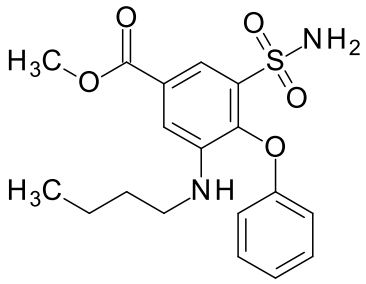
Elemental analyses were performed by Mag. Johannes Theiner at the University of Vienna and all reported values are within +/- 0.4 % of the calculated values.

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

5.2 Compound **1**: Methyl-3-(butylamino)-4-phenoxy-5-sulfamoyl-benzoate

(Germany Patentnr. DE 1966878, 1975)

Scheme 14: Compound **1**



Molecular formula:	$C_{18}H_{22}N_2O_5S$
Molecular weight:	378,44 g/mol

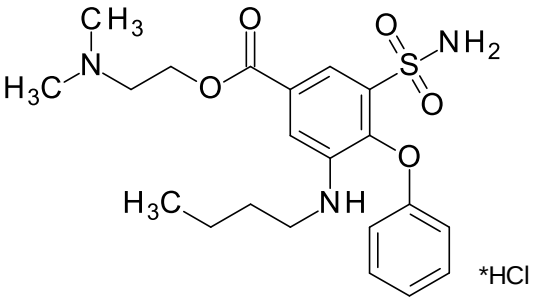
10 mmol (3.64 g) of bumetanide were mixed into 15 mL of MeOH. To this suspension 22 mmol (1.6 mL) of $SOCl_2$ were added, forming a clear, yellow solution, which was stirred at room temperature overnight. The next day the solution was extracted three times with ethyl acetate, washed with 5 % aqueous $NaHCO_3$-solution, water and brine. The washed organic phases were then dried over Na_2SO_4 and evaporated under reduced pressure to obtain 3.70 g of a white solid (98 % yield).

^{1}H NMR (200 MHz, $CDCl_3$) δ 7.96 (d, *J* = 2.0 Hz, 1H), 7.57 (d, *J* = 2.0 Hz, 2H), 7.42 – 7.26 (m, 2H), 7.10 (t, *J* = 7.4 Hz, 1H), 6.92 (d, *J* = 7.4 Hz, 2H), 4.93 (s, 2H), 3.94 (s, 3H), 3.10 (t, *J* = 6.9 Hz, 2H), 1.63 – 1.27 (m, 2H), 1.32 – 0.99 (m, 2H), 0.82 (t, *J* = 7.2 Hz, 3H).

5.3 Compound **2**: 2-(Dimethylamino)ethyl-3-(butylamino)-4-phenoxy-5-sulfamoyl-benzoate hydrochloride

(Toellner, et al., 2014)

Scheme 15: Compound **2**



Molecular formula:	$C_{21}H_{30}ClN_3O_5S$
Molecular weight:	471,99 g/mol

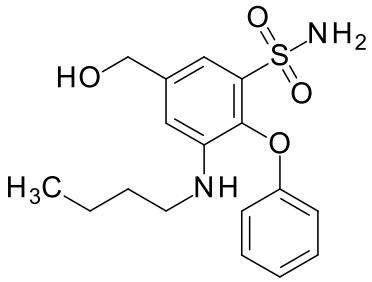
1 mmol of compound **1** was dissolved in 10 mL of *N,N*-dimethylethanolamine and a small amount of metallic sodium was added. After that the reaction was heated up to 80 °C for two hours and then the product was purified *via* column chromatography (ethyl acetate/triethylamine 6+4). The product was evaporated under reduced pressure and was then again dissolved in THF. Finally, the product was again precipitated as the hydrochloride with 1,5 mmol of 1 M HCl in diethyl ether, yielding to 363 mg of white crystals (77 % yield).

^{1}H NMR (200 MHz, MeOD) δ 7.92 (d, *J* = 2.0 Hz, 1H), 7.61 (d, *J* = 2.1 Hz, 1H), 7.36 – 7.21 (m, 2H), 7.16 – 7.01 (m, 1H), 6.96 – 6.85 (m, 2H), 4.79 – 4.65 (m, 2H), 3.80 – 3.51 (m, 2H), 3.13 (t, *J* = 6.7 Hz, 2H), 3.02 (s, 6H), 1.52 – 1.31 (m, 2H), 1.29 – 1.04 (m, 2H), 0.82 (t, *J* = 7.2 Hz, 3H).

5.4 Compound **3**: 3-(Butylamino)-5-(hydroxymethyl)-2-phenoxy-benzenesulfonamide

(Feit et al., 1978)

Scheme 16: Compound **3**



Molecular formula:	$C_{17}H_{22}N_2O_4S$
Molecular weight:	350,43 g/mol

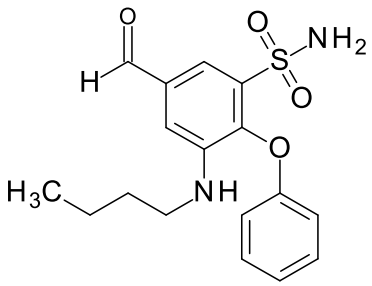
To 10 mmol of compound **1** 10 mL anhydrous THF were added to form a solution and was stirred under argon atmosphere at room temperature. 20 mL of a 1 M DIBAL-H solution in toluene were added once and then every hour, for four hours, another 10 mL of the same solution were added. After six hours the solution was quenched with 5 % aqueous NH_4Cl, while cooled down to 0 °C, forming a gel-like substance. The mixture was then again dissolved in 2 N HCl and extracted three times with ethyl acetate. The organic phases were washed three times with water and once with brine and were dried over Na_2SO_4. After that the substance was vacuum-dried to obtain 3.25 g of a slightly yellow solid (93 % yield).

^{1}H NMR (200 MHz, DMSO) δ 7.32 – 7.18 (m, 2H), 7.15 – 6.76 (m, 7H), 5.36 (t, *J* = 5.5 Hz, 1H), 4.66 (t, *J* = 5.7 Hz, 1H), 4.51 (d, 2H), 3.03 (q, *J* = 6.2 Hz, 2H), 1.47 – 1.26 (m, 2H), 1.23 – 0.99 (m, 2H), 0.77 (t, *J* = 7.2 Hz, 3H).

5.5 Compound **4**: 3-(Butylamino)-5-formyl-2-phenoxy-benzenesulfonamide

(Great Britain Patentnr. GB1523632, 1978)

Scheme 17: Compound **4**



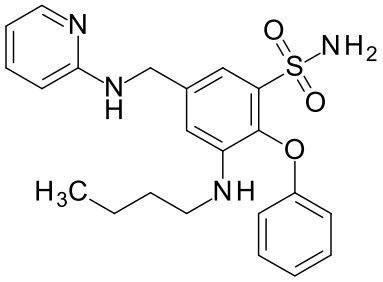
Molecular formula:	$C_{17}H_{20}N_2O_4S$
Molecular weight:	348,42 g/mol

10 mmol compound **3** were dissolved in 160 mL of toluene/ethyl acetate (1+1) and the solution was mixed with 45 mmol (3,90 g) of MnO2. The mixture was stirred at 40 °C for three days, after which the reaction was tested via thin layer chromatography, it was filtered and evaporated under reduced pressure. For the purification column chromatography (ethyl acetate/petroleum ether 1+1) was used, yielding to 1,84 g of yellow crystals (53 % yield).

^{1}H NMR (200 MHz, DMSO) δ 9.99 (s, 1H), 7.71 – 7.57 (m, 1H), 7.50 – 7.21 (m, 5H), 7.10 – 6.94 (m, 1H), 6.91 – 6.77 (m, 2H), 5.14 (t, *J* = 5.2 Hz, 1H), 3.25 – 2.93 (m, 2H), 1.47 – 1.26 (m, 2H), 1.25 – 0.98 (m, 2H), 0.76 (t, *J* = 7.1 Hz, 3H).

5.6 Compound **5**: 3-(Butylamino)-2-phenoxy-5-[(2-pyridylamino)methyl]benzenesulfonamide

Scheme 18: Compound **5**



Molecular formula:	$C_{22}H_{26}N_4O_3S$
Molecular weight:	426,53 g/mol

1 mmol (348 mg) of compound **4** was dissolved in 10 mL 1,2-dichlorethane and 1 mmol (29 µL) of acetic acid and 1,2 mmol 2-aminopyridine were added. After stirring the mixture for two hours, 1,5 mmol of triacetoxyborohydride ($NaBH(OAc)_3$) were added and the mixture was stirred overnight. The product was diluted with 40 mL of dichlormethane and 10 mL saturated $NaHCO_3$. After washing it with brine it was evaporated under reduced pressure. The purification was made by column chromatography (ethyl acetate/petroleum ether 6+4) and recrystallization out of 70 % EtOH, resulting in 130 mg of white crystals (30 % yield)

^{1}H NMR (200 MHz, DMSO) δ 8.06 – 7.88 (m, 1H), 7.51 – 6.30 (m, 12H), 4.76 – 4.61 (m, 1H), 4.55 – 4.41 (m, 2H), 3.13 – 2.87 (m, 2H), 1.40 – 1.20 (m, 2H), 1.19 – 0.97 (m, 2H), 0.74 (t, *J* = 7.0 Hz, 3H).

^{13}C NMR (50 MHz, DMSO) δ 159.0, 157.3, 147.9, 142.4, 138.9, 137.3, 137.3, 135.2, 129.5, 122.4, 115.9, 114.2, 112.8, 112.4, 108.6, 44.4, 42.6, 30.8, 19.8, 14.1.

Mass–Analysis

m/z	427	13%, M+
	132	51%
	78	73%
	77	100%
	41	54%

HRMS–Analysis

Meas.m/z	Formula	m/z	err [ppm]
427.1801	$C_{22}H_{27}N_4O_3S$	427.1798	-0.5

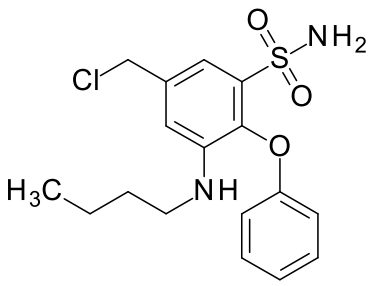
Elemental analysis ($C_{22}H_{26}N_4O_3S$) (*0,7 EtOH)

	C	H	N	S
Calculated (%)	61,26	6,63	12,21	7,52
Found (%)	61,09	6,52	12,3	7,74

5.7 Compound **6**: 3-(Butylamino)-5-(chloromethyl)-2-phenoxy-benzenesulfonamide

(Schreppel, 2015)

Scheme 19: Compound **6**



Molecular formula:	$C_{17}H_{21}ClN_2O_3S$
Molecular weight:	368,87 g/mol

10 mmol of compound **3** were dissolved in 5 mL of $SOCl_2$ and heated up to 80 °C for three hours. The thionyl chloride was evaporated under reduced pressure and the substance was purified by column chromatography (petroleum ether/ethyl acetate 7+3) and the extracted phases were evaporated under reduced pressure, resulting in 1.03 g of brown crystals (28 % yield)

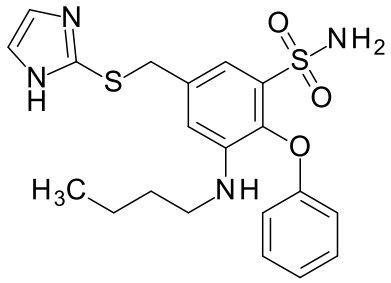
^{1}H NMR (200 MHz, $CDCl_3$) δ 7.37 – 7.22 (m, 3H), 7.14 – 7.01 (m, 1H), 7.00 – 6.80 (m, 3H), 4.88 (s, 2H), 4.57 (s, 2H), 3.06 (t, *J* = 6.9 Hz, 2H), 1.52 – 1.35 (m, 2H), 1.28 – 1.09 (m, 2H), 0.82 (t, *J* = 7.2 Hz, 3H).

^{13}C NMR (50 MHz, $CDCl_3$) δ 156.1, 142.5, 136.6, 136.0, 135.9, 130.2, 123.7, 115.9, 115.4, 115.0, 45.8, 43.2, 31.1, 19.9, 13.8.

5.8 Compound **7**: 3-(Butylamino)-5-(1H-imidazol-2-ylsulfanylmethyl)-2-phenoxy-benzenesulfonamide

(Schreppel, 2015)

Scheme 20: Compound **7**



Molecular formula:	$C_{20}H_{24}N_4O_3S_2$
Molecular weight:	432,56 g/mol

1 mmol of compound **6** was dissolved in 2 mL triethylamine and 3 mL DMF. Then 1 mmol of 2-mercaptoimidazole was added to the solution and the mixture was stirred for two days at room temperature. Tested by thin layer chromatography, the finished reaction was evaporated under reduced pressure and then purified by recrystallisation with 70 % MeOH yielding to 108 mg of a white solid (25 % yield).

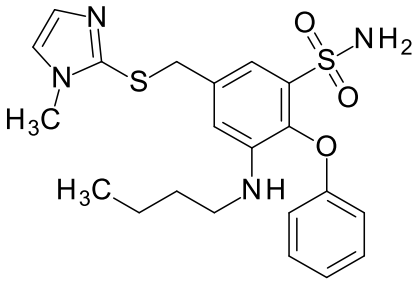
^{1}H NMR (200 MHz, MeOD) δ 7.35 – 7.17 (m, 2H), 7.15 – 6.93 (m, 4H), 6.94 – 6.79 (m, 2H), 6.66 – 6.59 (m, 1H), 4.15 (s, 2H), 2.94 (t, *J* = 6.7 Hz, 2H), 1.46 – 1.22 (m, 2H), 1.26 – 0.98 (m, 2H), 0.80 (t, *J* = 7.1 Hz, 3H).

^{13}C NMR (50 MHz, MeOD) δ 156.8, 142.3, 138.4, 136.5, 135.9, 129.1 (2C), 123.8, 122.3, 115.3, 115.1 (2C), 114.4, 42.3, 39.1, 30.6, 19.5, 12.6. One Cq could not be detected.

5.9 Compound **8**: 3-(Butylamino)-5-[(1-methylimidazol-2-yl)sulfanylmethyl]-2-phenoxy-benzenesulfonamide

(Schreppel, 2015)

Scheme 21: Compound **8**



Molecular formula:	$C_{21}H_{26}N_4O_3S_2$
Molecular weight:	446,59 g/mol

A solution was made by dissolving 1 mmol of compound **6** in 3 mL DMF and 2 mL of triethylamine. Then 1 mmol (114 mg) of 2-mercapto-1-methylimidazole was added and the mixture was stirred for two days at room temperature. After that, it was extracted with 20 mL ethyl acetate/water, the organic phases were evaporated under reduced pressure and purified *via* recrystallization with 70 % MeOH resulting in 67 mg of white crystals (15 % yield).

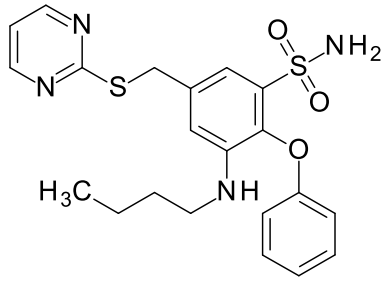
^{1}H NMR (200 MHz, $CDCl_3$) δ 7.37 – 7.19 (m, 2H), 7.16 – 6.97 (m, 3H), 6.96 – 6.74 (m, 3H), 6.70 – 6.50 (m, 1H), 5.27 (s, 2H), 4.11 (s, 2H), 3.76 (t, *J* = 5.4 Hz, 1H), 3.44 (s, 3H), 2.93 (q, *J* = 6.5 Hz, 2H), 1.48 – 1.20 (m, 2H), 1.28 – 1.00 (m, 2H), 0.81 (t, *J* = 7.2 Hz, 3H).

^{13}C NMR (50 MHz, $CDCl_3$) δ 156.1, 142.1, 140.1, 136.1, 135.9, 135.6, 129.9, 129.5, 123.3, 122.7, 116.0, 115.2, 115.0, 43.0, 39.4, 33.4, 31.0, 19.8, 13.7.

5.10 Compound **9**: 3-(Butylamino)-2-phenoxy-5-(pyrimidin-2-ylsulfanylmethyl)benzenesulfonamide

(Schreppel, 2015)

Scheme 22: Compound **9**



Molecular formula:	$C_{21}H_{24}N_4O_3S_2$
Molecular weight:	444,57 g/mol

Compound **6** was dissolved in 3 mL DMF and 2 mL triethylamine, where then 1 mmol (112 mg) of 2-mercaptopyrimidine were added. This mixture was stirred for one day and then evaporated under reduced pressure. The solid was purified *via* recrystallization with 70 % MeOH yielding in286 mg of white crystals (64 % yield).

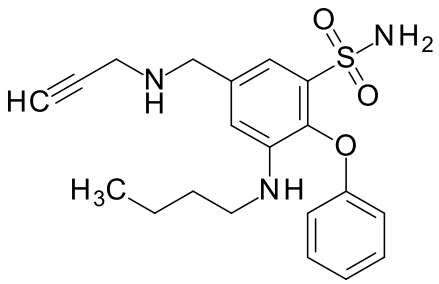
^{1}H NMR (200 MHz, $CDCl_3$) δ 8.53 (d, *J* = 4.9 Hz, 2H), 7.41 – 7.15 (m, 3H), 7.10 – 6.80 (m, 5H), 4.99 (s, 2H), 4.39 (s, 2H), 3.03 (t, *J* = 6.9 Hz, 2H), 1.51 – 1.26 (m, 2H), 1.31 – 1.00 (m, 2H), 0.80 (t, *J* = 7.1 Hz, 3H). NH could not be detected.

^{13}C NMR (50 MHz, $CDCl_3$) δ 171.6, 157.3, 156.2, 142.1, 136.2, 135.6, 129.9, 123.3, 116.9, 116.4, 115.5, 115.3, 114.8, 43.1, 34.9, 31.0, 19.8, 13.7.

5.11 Compound **10**: 3-(Butylamino)-2-phenoxy-5-[(prop-2-ynylamino)methyl]benzenesulfonamide

(Schreppel, 2015)

Scheme 23: Compound **10**



Molecular formula:	$C_{20}H_{25}N_3O_3S$
Molecular weight:	387,50 g/mol

1 mmol of compound **6** was mixed with 3 mL DMF and 1,2 mmol propargylamine and stirred at room temperature for two days. Then again 2,4 mmol propargylamine and 0,5 mL triethylamine were added and it was stirred for one more day. After that the mixture product was extracted with ethyl acetate/water and the organic phases were evaporated under reduced pressure. The crude product was purified *via* column chromatography (ethyl acetate/petroleum ether 1+1), resulting in 35 mg of white crystals (9 % yield).

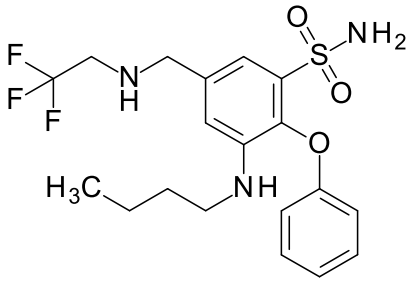
^{1}H NMR (200 MHz, CDCl3) δ 7.38 – 7.21 (m, 2H), 7.17 – 7.01 (m, 1H), 7.00 – 6.86 (m, 2H), 4.97 (s-br, 1H), 3.88 (s, 1H), 3.84 – 3.69 (m, 1H), 3.54 – 3.40 (m, 1H), 3.16 – 2.96 (m, 2H), 2.28 (s, 1H), 1.51 – 1.31 (m, 2H), 1.31 – 1.05 (m, 2H), 0.82 (t, *J* = 7.1 Hz, 3H).

^{13}C NMR (50 MHz, $CDCl_3$) δ 156.3, 142.2, 137.7, 135.6, 129.9, 123.3, 115.8, 115.2, 114.6, 81.7, 72.0, 51.9, 43.1, 37.4, 31.1, 19.8, 13.7.

5.12 Compound **11**: 3-(Butylamino)-2-phenoxy-5-[(2,2,2-trifluoroethylamino)methyl]benzenesulfonamide

(Schreppel, 2015)

Scheme 24: Compound **11**



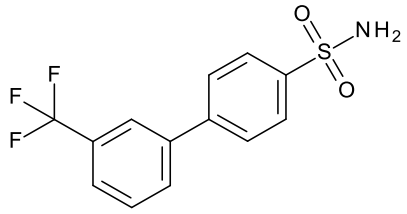
Molecular formula:	$C_{19}H_{24}F_3N_3O_3S$
Molecular weight:	431,47 g/mol

1 mmol (369 mg) of compound **6** was dissolved in 3 mL DMF, mixed with 2 mmol (157 µL) of 2,2,2-trifluorethylamine and stirred overnight. The next day again 2 mmol of 2,2,2-trifluorethylamine were added and stirred for two more days. The product was evaporated under reduced pressure and purified *via* column chromatography (ethyl acetate/petroleum ether 3,5+6,5) yielding to 56 mg of white crystals (13 % yield).

^{1}H NMR (200 MHz, MeOD) δ 7.36 – 7.15 (m, 3H), 7.09 – 6.98 (m, 2H), 6.97 – 6.80 (m, 2H), 3.87 (s, 2H), 3.19 (q, *J* = 9.8 Hz, 1H), 3.09 (t, *J* = 6.8 Hz, 2H), 1.60 – 1.29 (m, 2H), 1.30 – 1.03 (m, 2H), 0.81 (t, *J* = 7.2 Hz, 3H).

5.13 Compound **12**: 4-[3-(Trifluoromethyl)phenyl]benzenesulfonamide

Scheme 25: Compound **12**



Molecular formula:	$C_{13}H_{10}F_3NO_2S$
Molecular weight:	301,28 g/mol

1 mmol of 3-(trifluoromethyl)phenylboronic acid and 1 mmol 4-bromobenzensulfonamide were dissolved in 6 mL THF. Then 2 mmol (276 mg) of potassium carbonate, 4 mL water and 100 mg of tetrakis(triphenylphosphine)palladium(0) were added and the mixture was heated to 70 °C overnight. It was diluted the next day with 40 mL ethyl acetate and water and then washed with brine and dried over Na_2SO_4, before being evaporated under reduced pressure. Purification was made by column chromatography (ethyl acetate/petroleum ether 3,5+6,5) and recrystallization out of 50 % EtOH, yielding 148 mg of a white solid (43 % yield).

^{1}H NMR (200 MHz, MeOD) δ 8.04 (A part of AB system, J_{AB} = 8.4 Hz, 2H), 8.02 – 7,89 (m, 2H), 7.84 (B part of AB system, J_{AB} = 8.4 Hz, 2H), 7.77 – 7.63 (m, 2H).

^{13}C NMR (50 MHz, MeOD) δ 144.5, (d J = 6.9 Hz),144.4, 141.8, 132.7, 132.0 (d, J = 1.3 Hz), 131.0, 128.7, 128.3, 128.0, 125.93 (q, J = 3.9 Hz), 124.82 (q, J = 3.9 Hz), 122.9.

Mass–Analysis

m/z	301	100%, M+
	238	34%
	221	60%
	201	68%
	152	51%

HRMS–Analysis

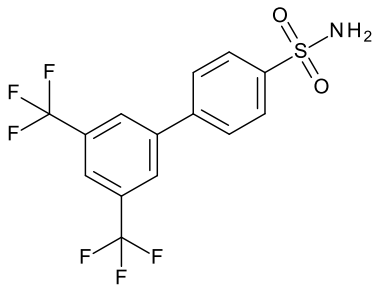
Meas.m/z	Formula	m/z	err [ppm]
324.0278	$C_{13}H_{10}F_3NNaO_2S$	324.0277	-0.5

Elemental analysis ($C_{13}H_{10}F_3NO_2S$)

	C	H	N	S
Calculated (%)	51,82	3,35	4,65	10,64
Found (%)	51,88	3,27	4,65	10,68

5.14 Compound **13**: 4-[3,5-Bis(trifluoromethyl)phenyl]benzenesulfonamide

Scheme 26: Compound **13**



Molecular formula:	$C_{14}H_9F_6NO_2S$
Molecular weight:	369,28 g/mol

1 mmol of 3,5-bis(trifluoromethyl)phenylboronic acid and 1 mmol 4-bromobenzenslufonamide were dissolved in 6 mL THF. Then 2 mmol (276 mg) of potassium carbonate, 4 mL water and 100 mg of tetrakis(triphenylphosphine)palladium(0) were added and the mixture was heated to 70 °C overnight. It was diluted the next day with 20 mL ethyl acetate and water and then washed with brine and dried over Na_2SO_4, before being evaporated under reduced pressure. Purification was made by column chromatography (ethyl acetate/petroleum ether 3+7) and recrystallization out of 80 % EtOH, yielding 126 mg of a white solid (34 % yield).

^{1}H NMR (200 MHz, MeOD) δ 8.26 (s, 2H), 8.14 – 8.00 (m, 3H), 7.98 – 7.88 (m, 2H).

^{13}C NMR (50 MHz, MeOD) δ 144.0, 142.0, 141.3, 132.10 (q, *J* = 33.3 Hz), 131,8, 127.7, 127.6 – 127.15 (m),126.8, 126.1, 121.36 (dt, *J* = 7.8, 4.0 Hz), 120.7.

Mass–Analysis

m/z	369	51%, M+
	269	74%
	81	49%
	69	100%
	41	51%

HRMS–Analysis

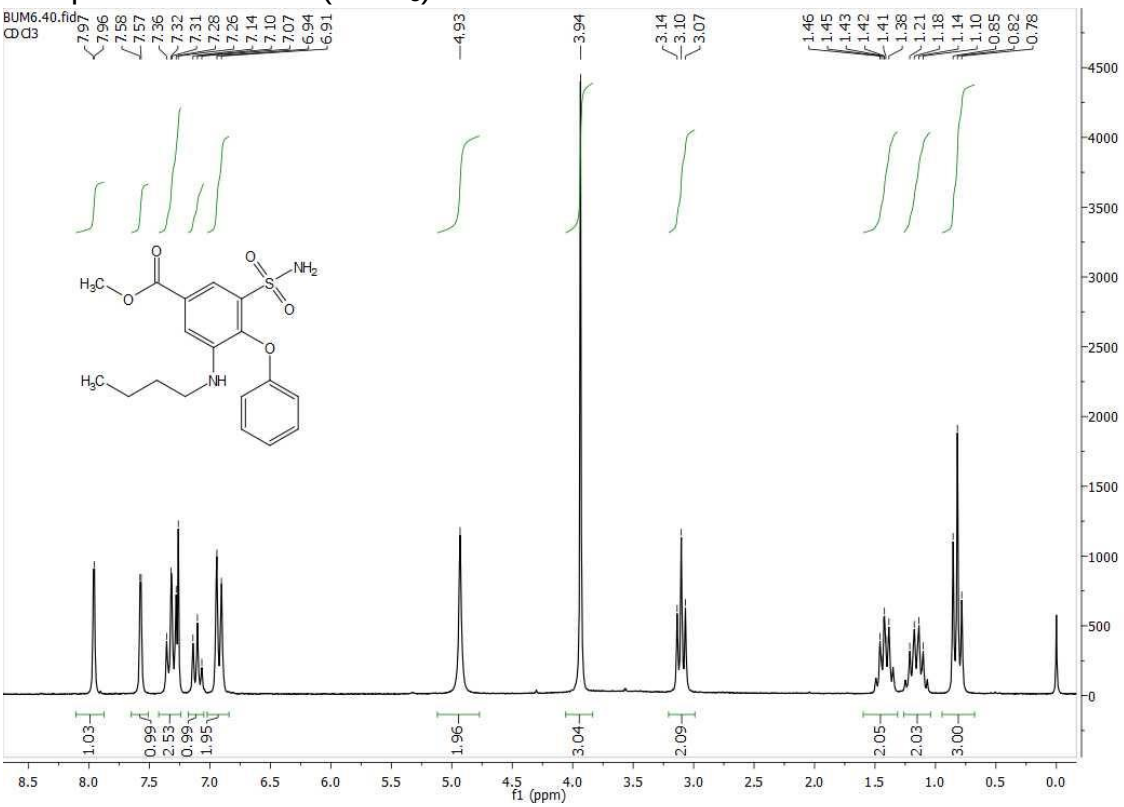
Meas.m/z	Formula	m/z	err [ppm]
392.0155	$C_{14}H_9F_6NNaO_2S$	392.0150	-1.2

Elemental analysis ($C_{14}H_9F_6NO_2S$)

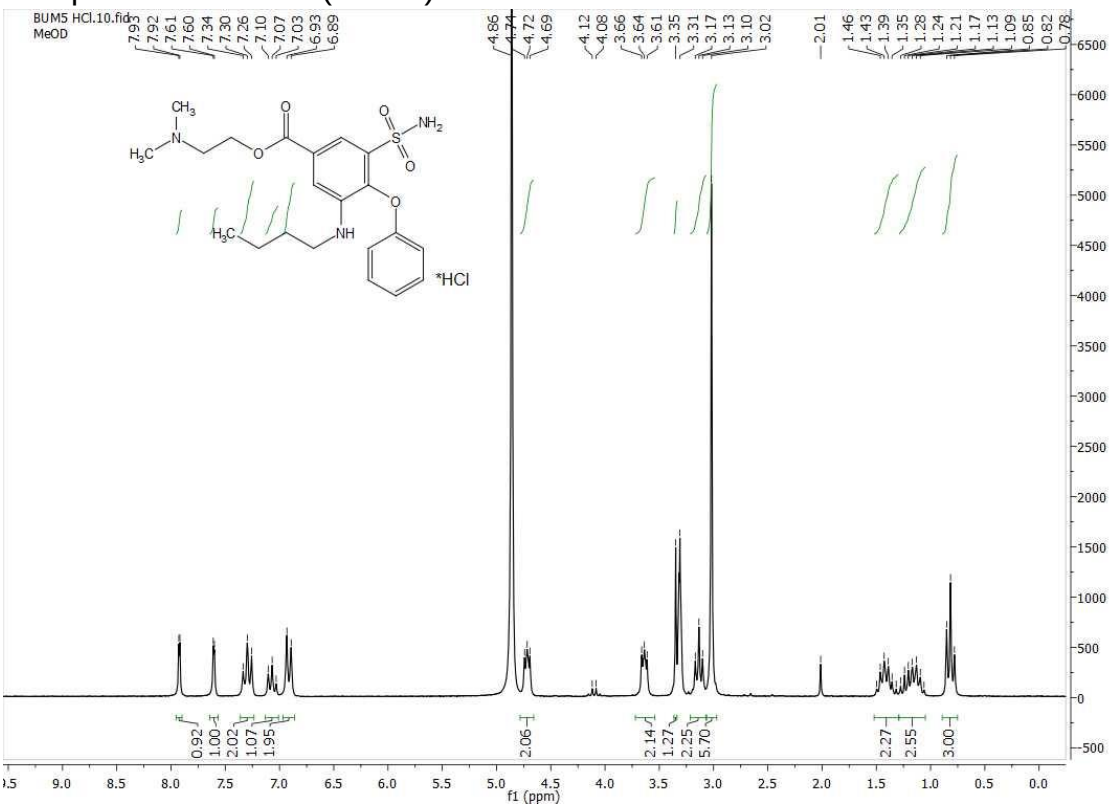
	C	H	N	S
Calculated (%)	45,53	2,46	3,79	8,68
Found (%)	45,82	2,50	3,73	8,61

6 Analytics

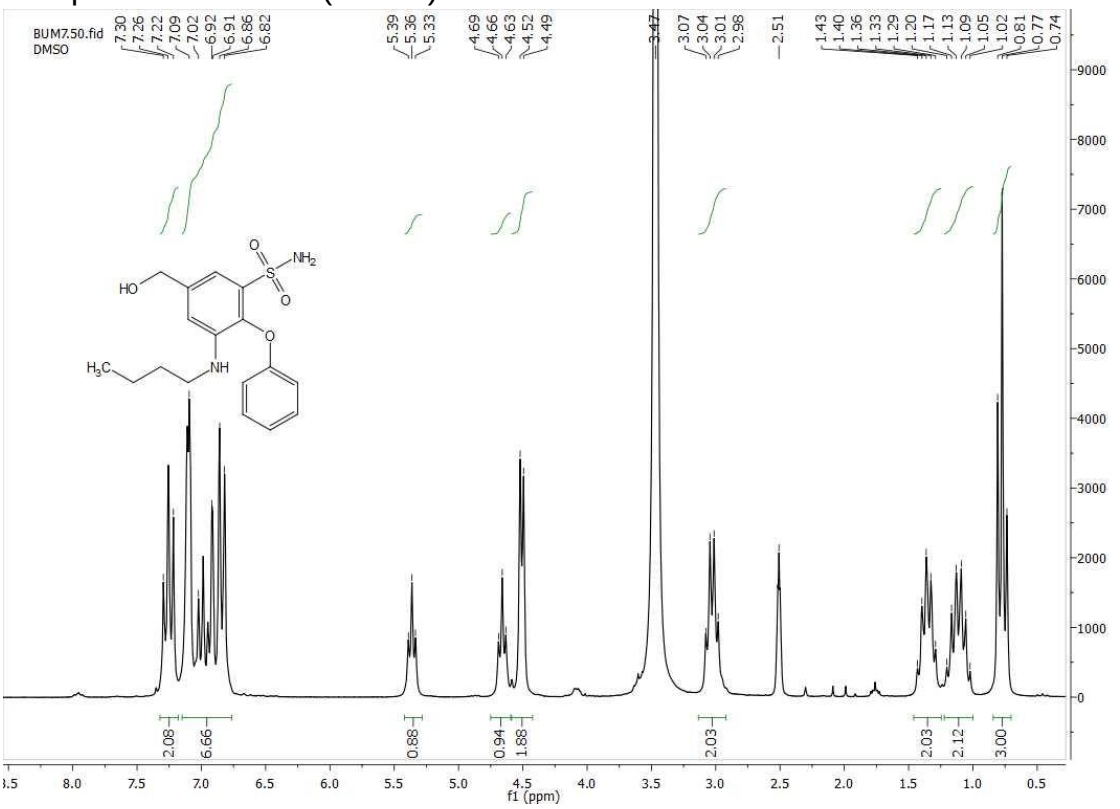
Compound **1** ^{1}H NMR ($CDCl_3$)



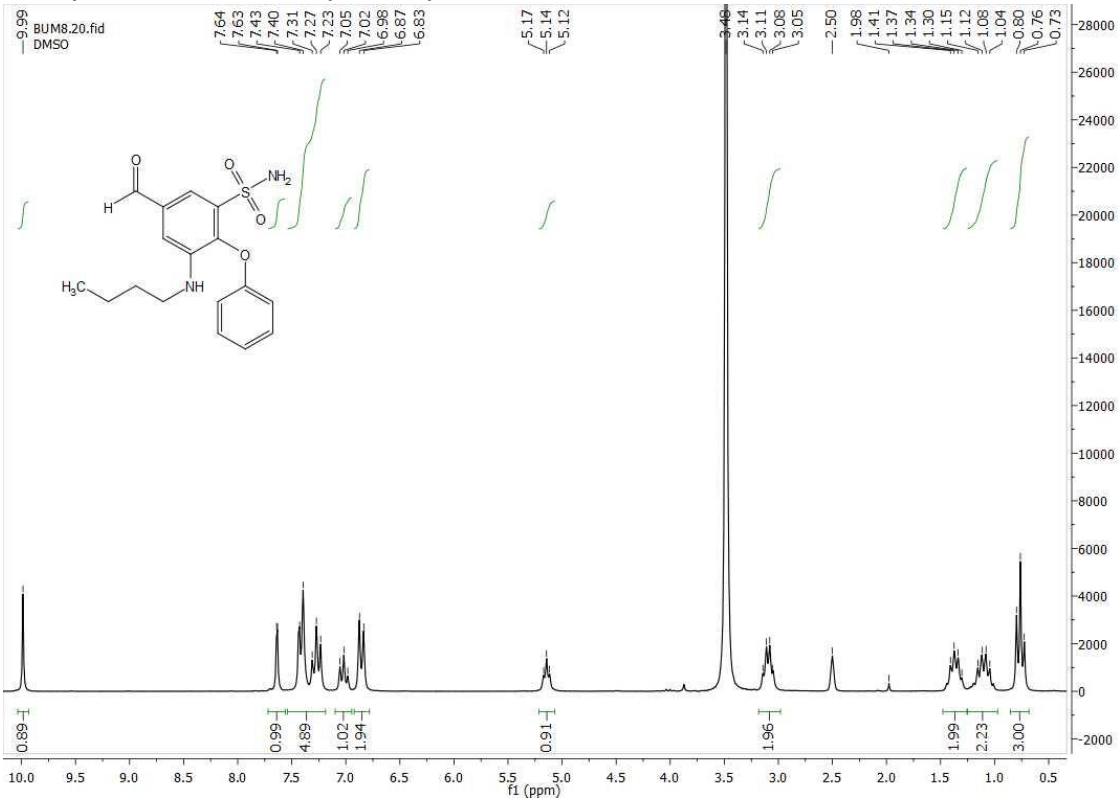
Compound **2** ^{1}H NMR (MeOD)



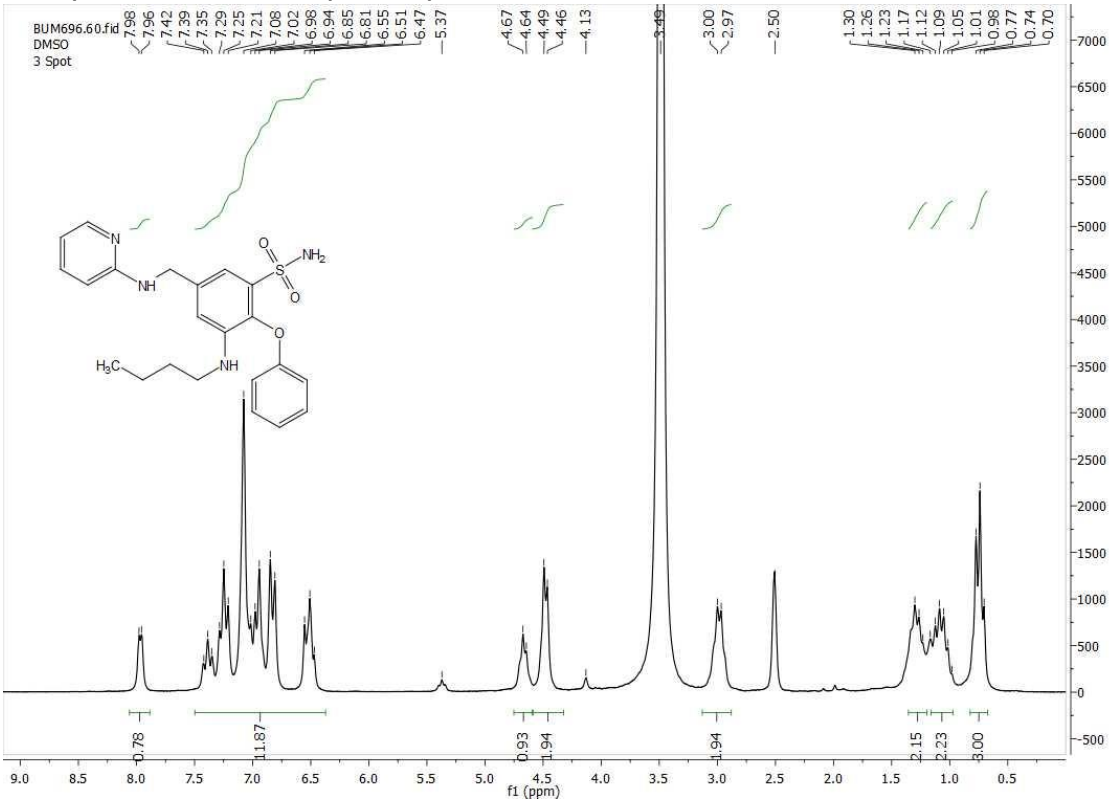
Compound **3** ^{1}H NMR (DMSO)



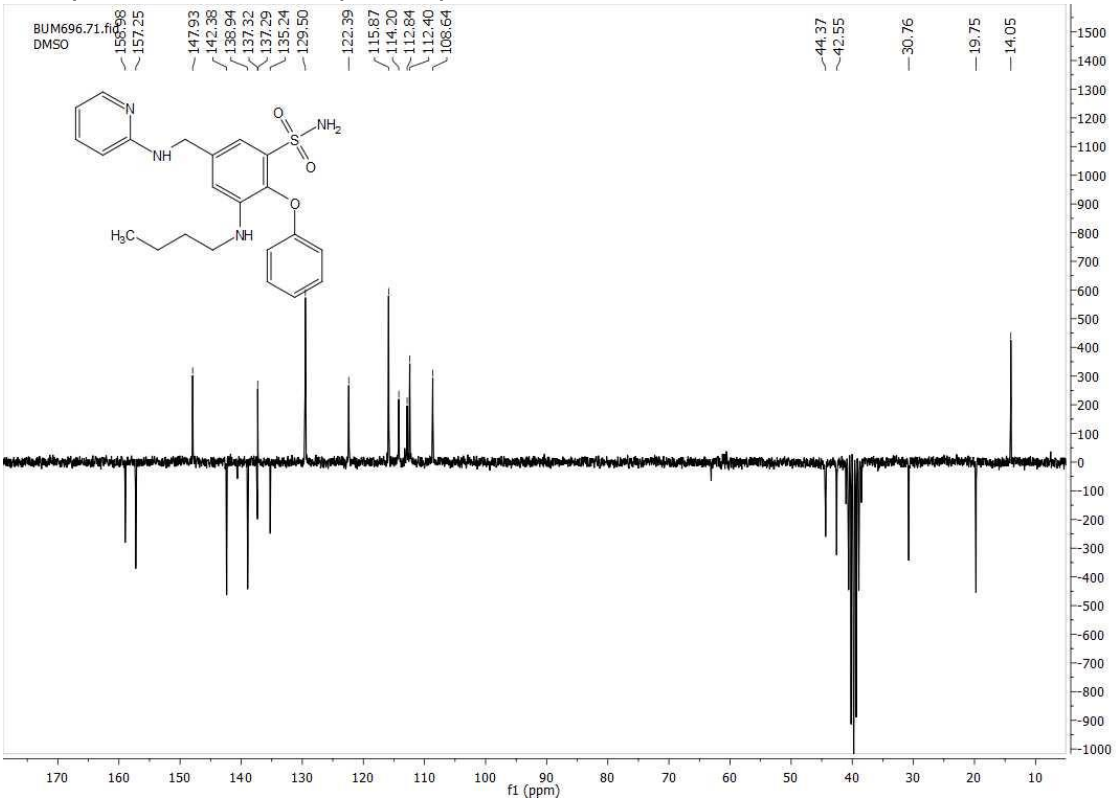
Compound **4** ^{1}H NMR (DMSO)



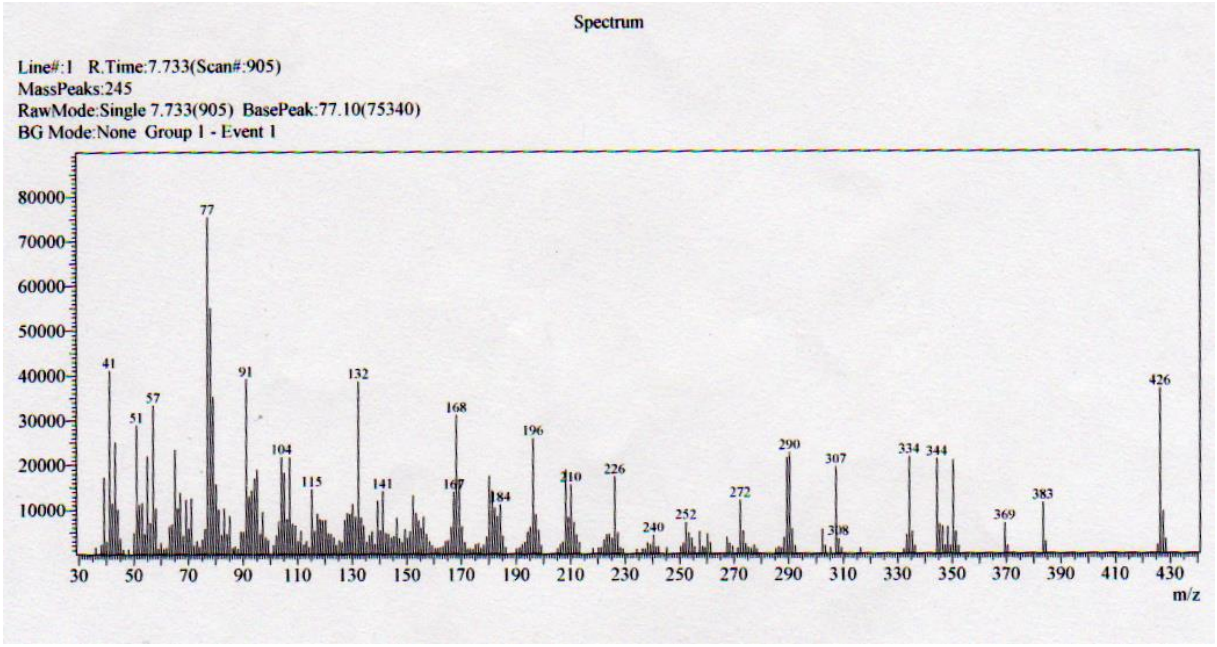
Compound **5** ^{1}H NMR (DMSO)



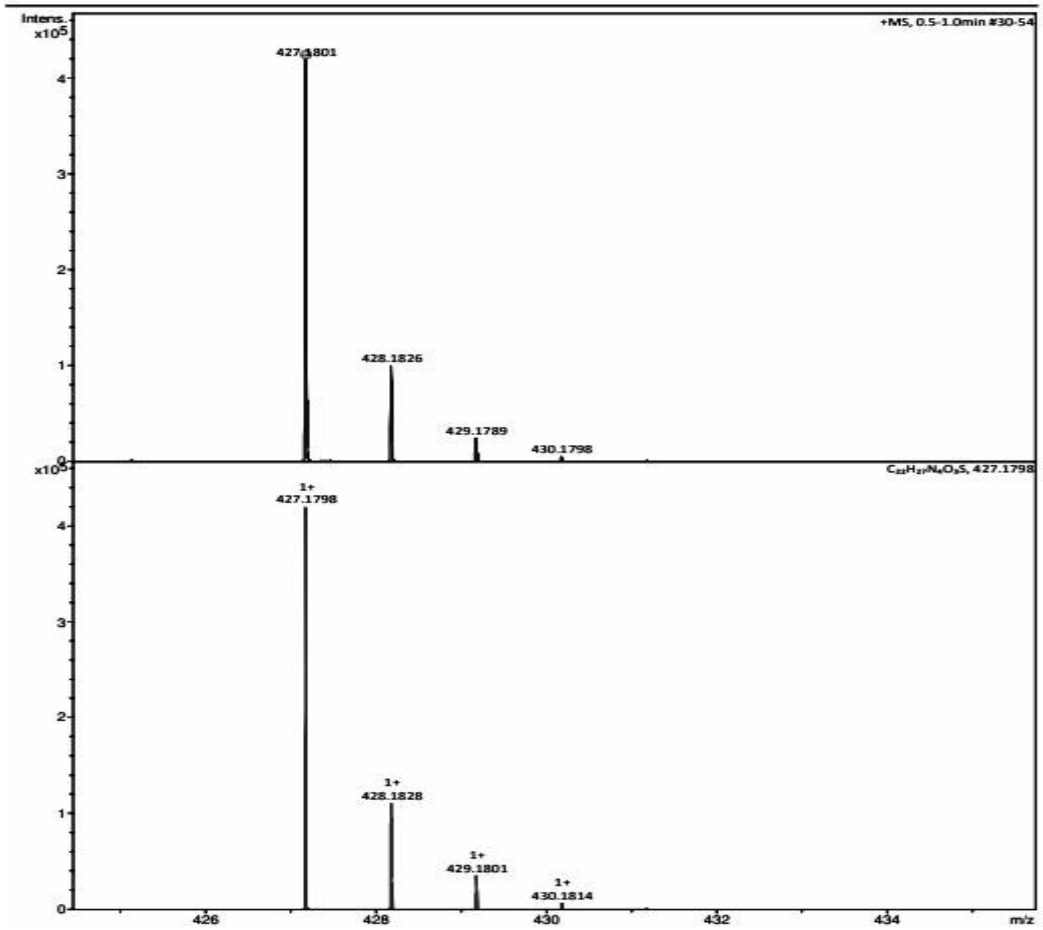
Compound **5** ^{13}C NMR (DMSO)



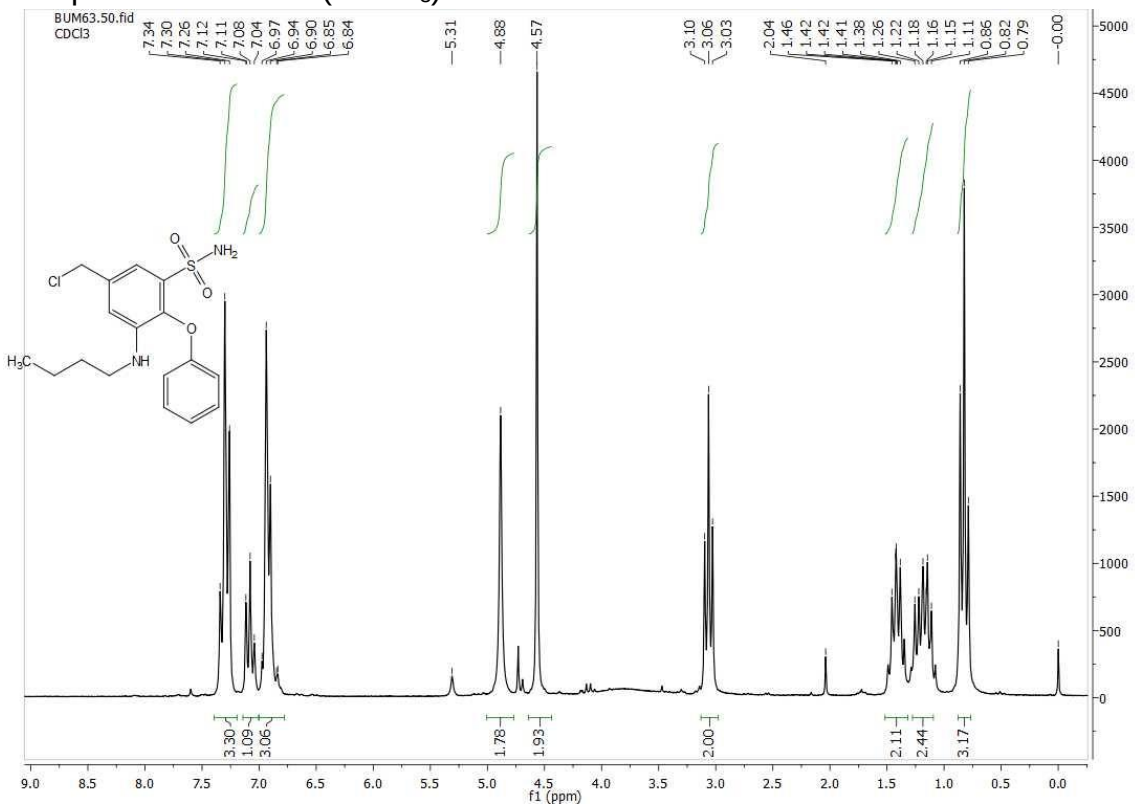
Compound **5** mass spectrum



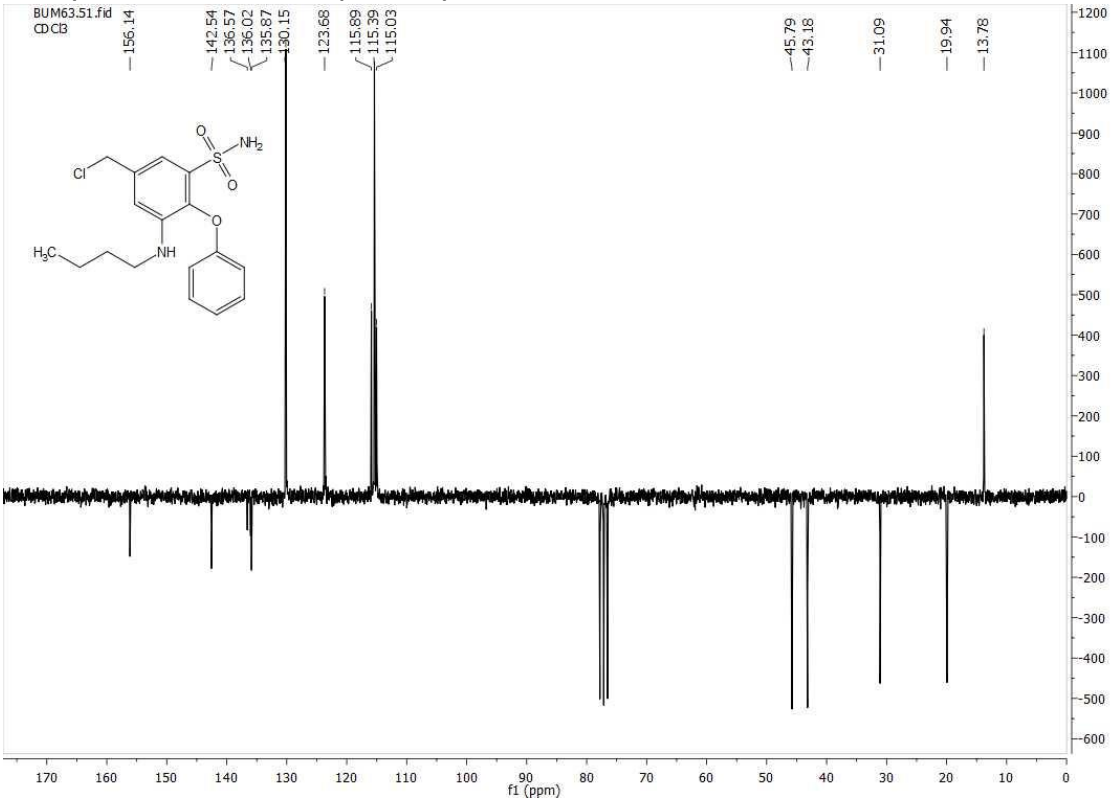
Compound **5** HRMS



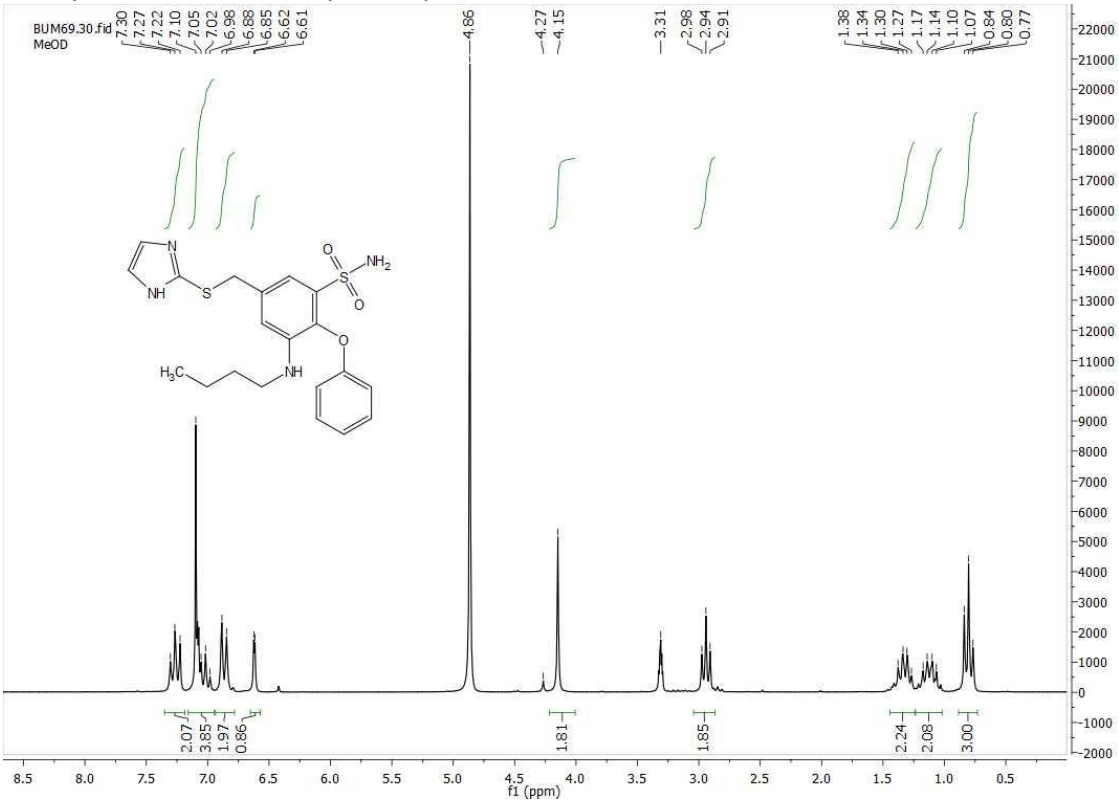
Compound **6** ^{1}H NMR ($CDCL_3$)



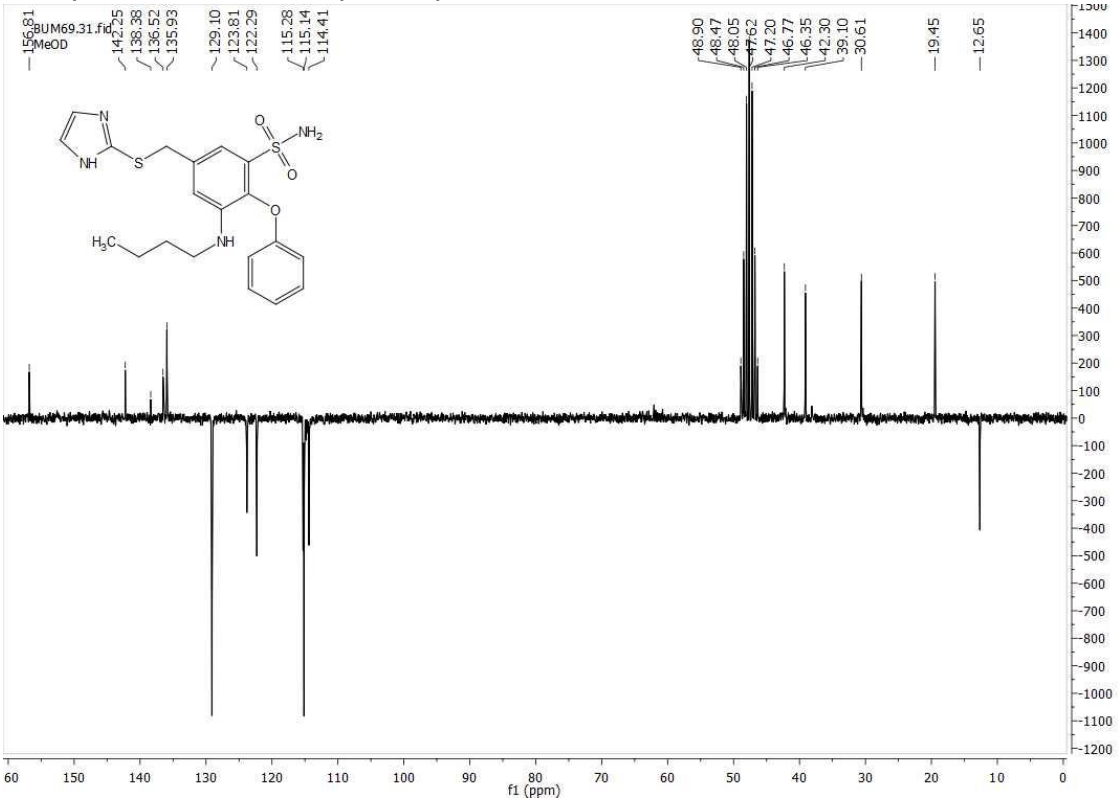
Compound **6** ^{13}C NMR ($CDCL_3$)



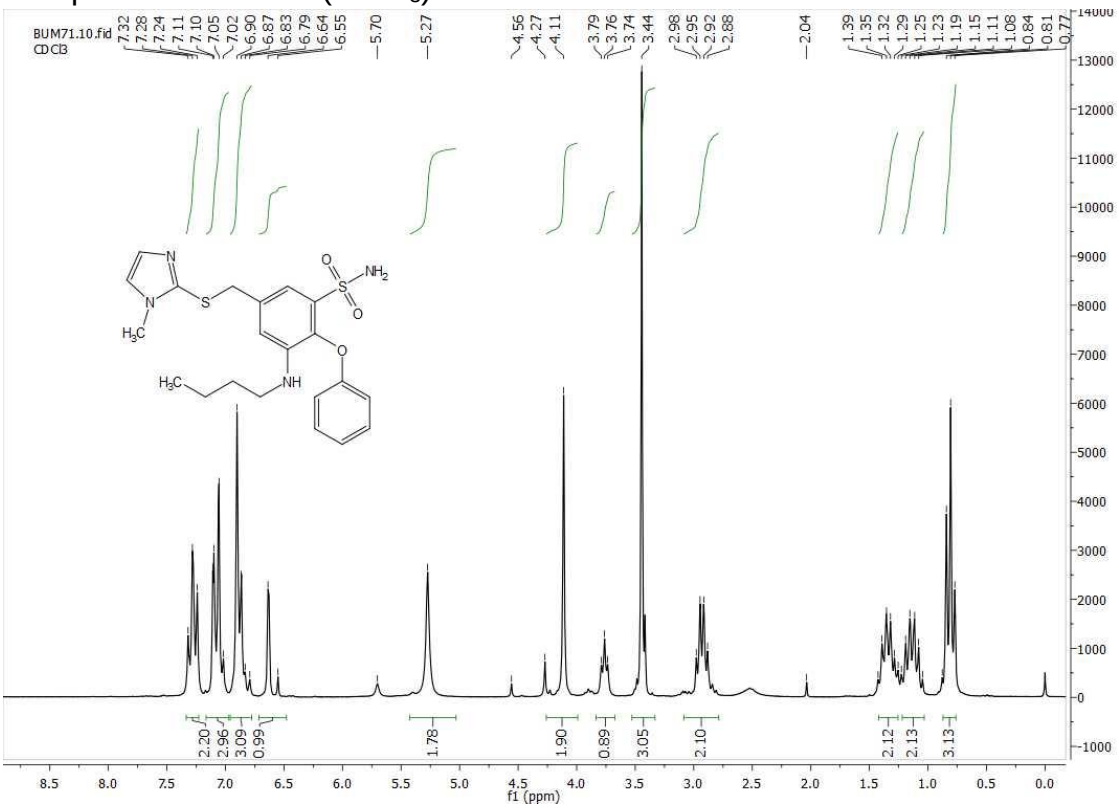
Compound **7** ^{1}H NMR (MeOD)



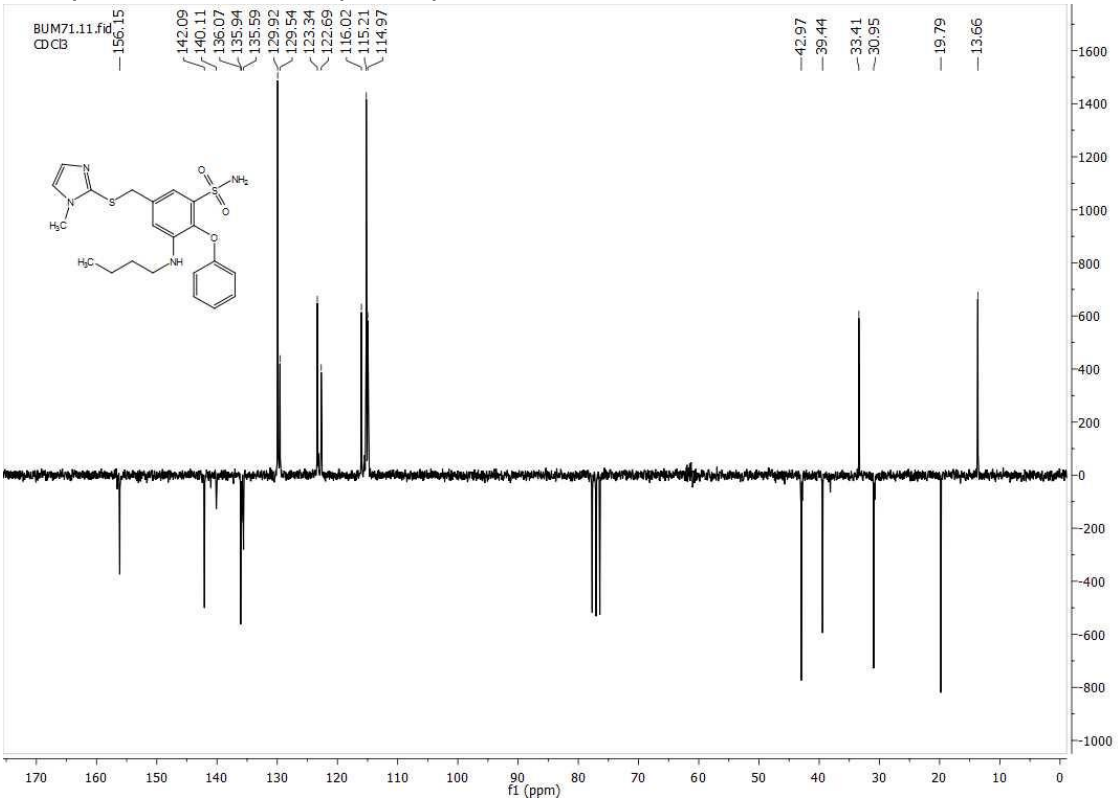
Compound **7** ^{13}C NMR (MeOD)



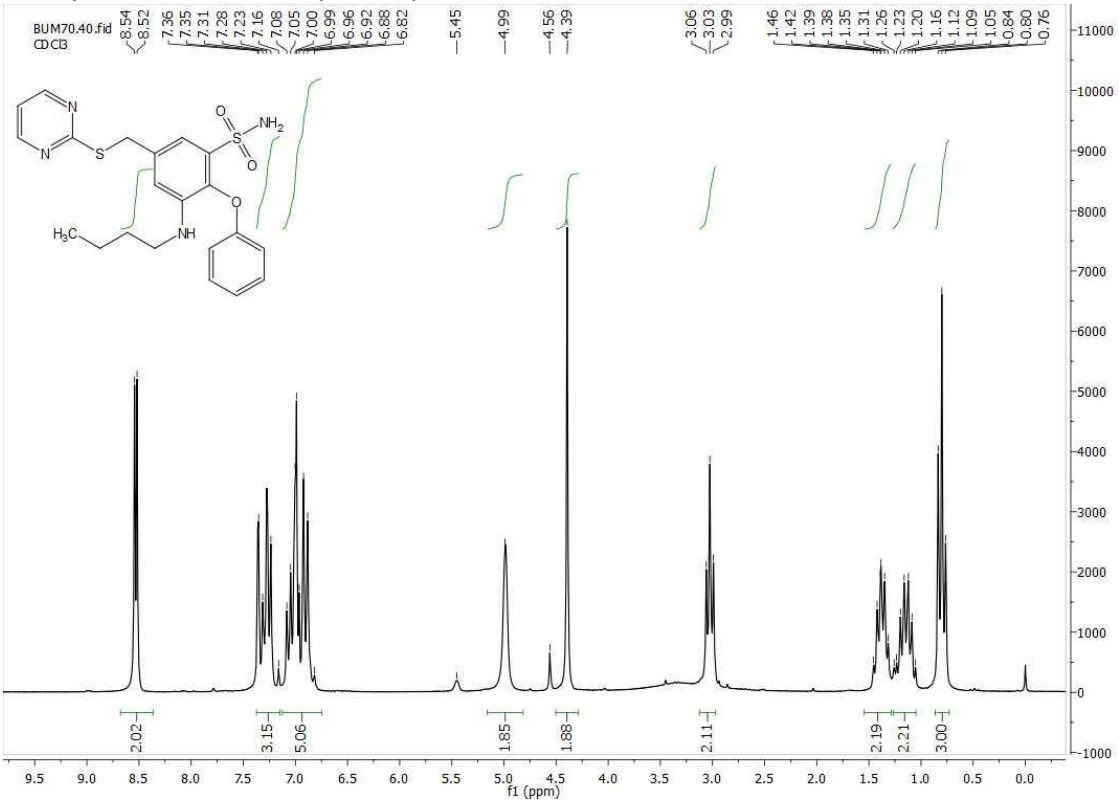
Compound **8** ^{1}H NMR ($CDCl_3$)



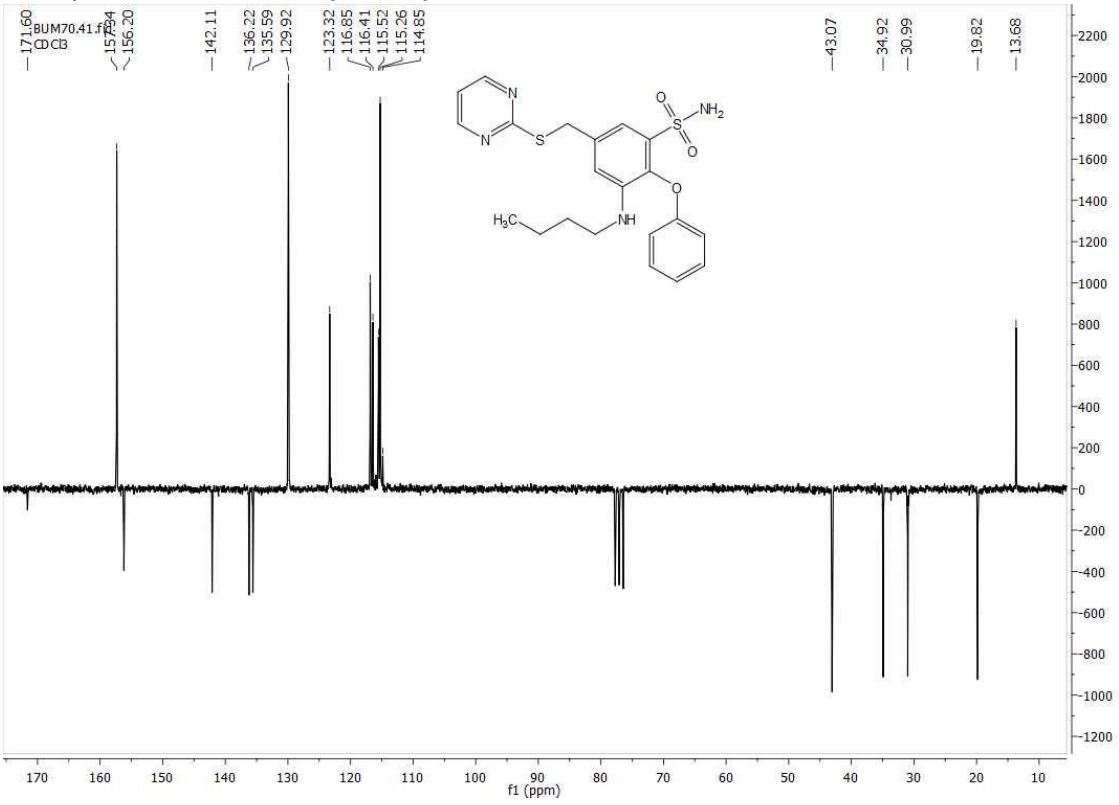
Compound **8** ^{13}C NMR ($CDCl_3$)



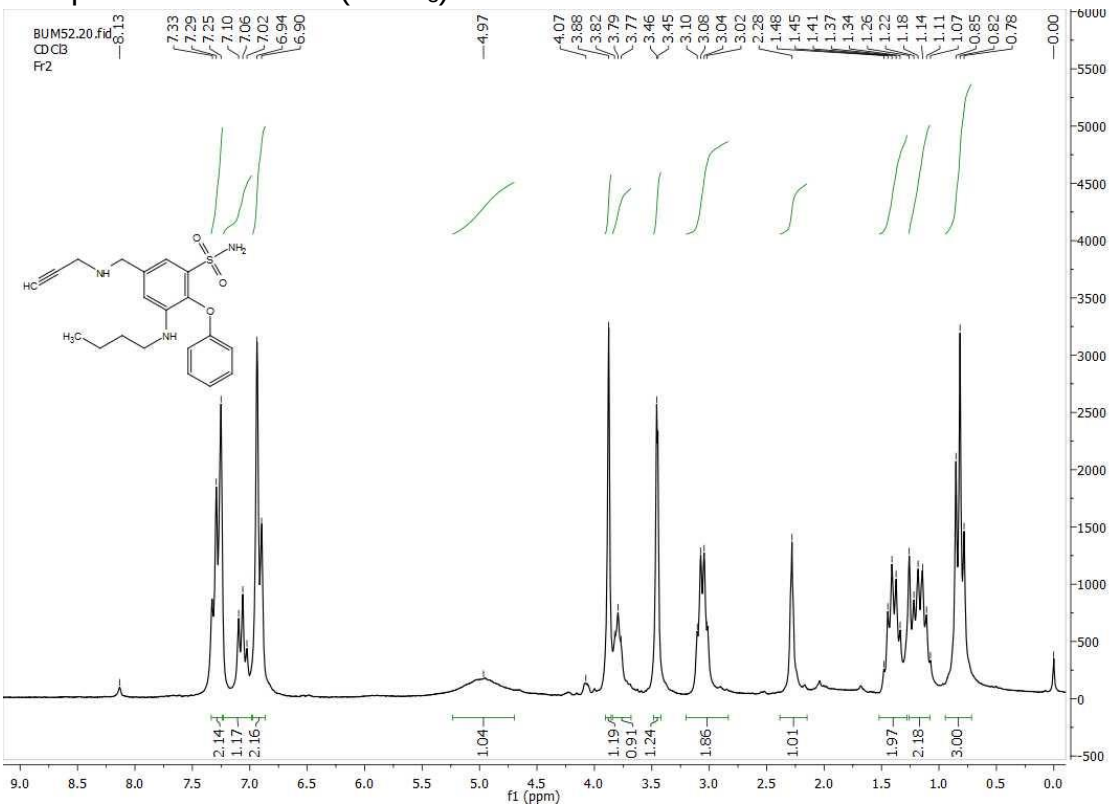
Compound **9** ^{1}H NMR($CDCl_3$)



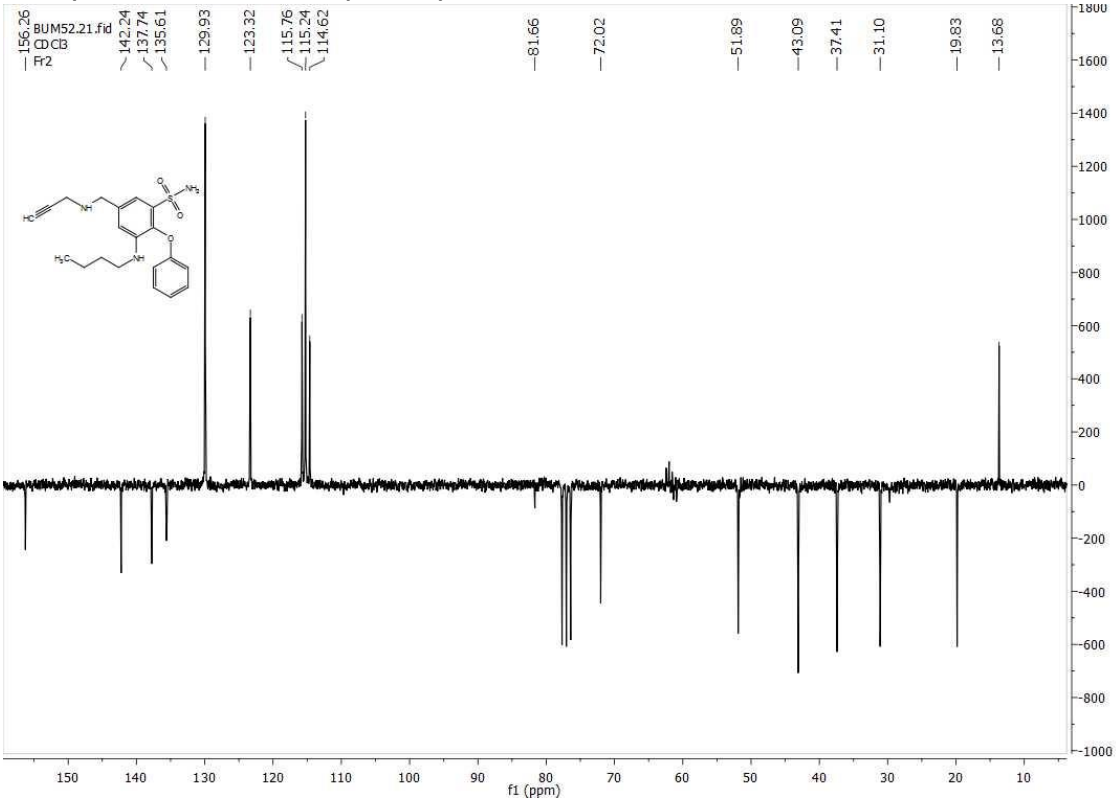
Compound **9** ^{13}C NMR($CDCl_3$)



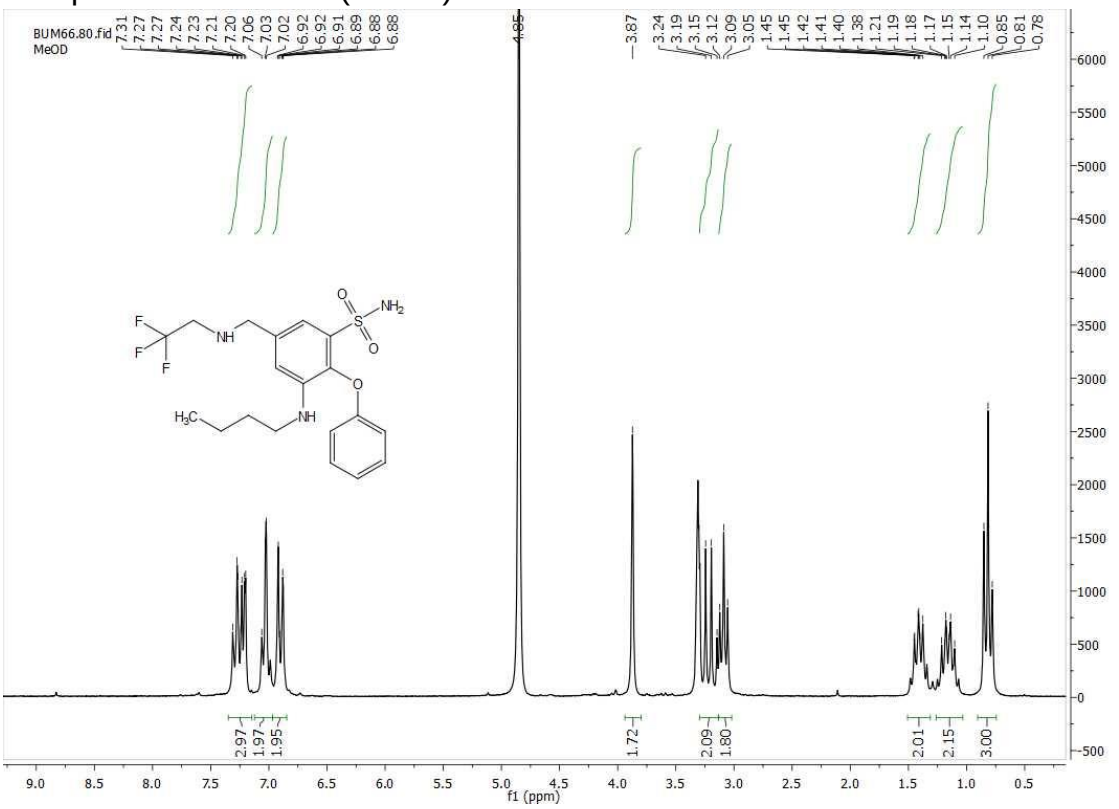
Compound **10** ^{1}H NMR ($CDCl_3$)



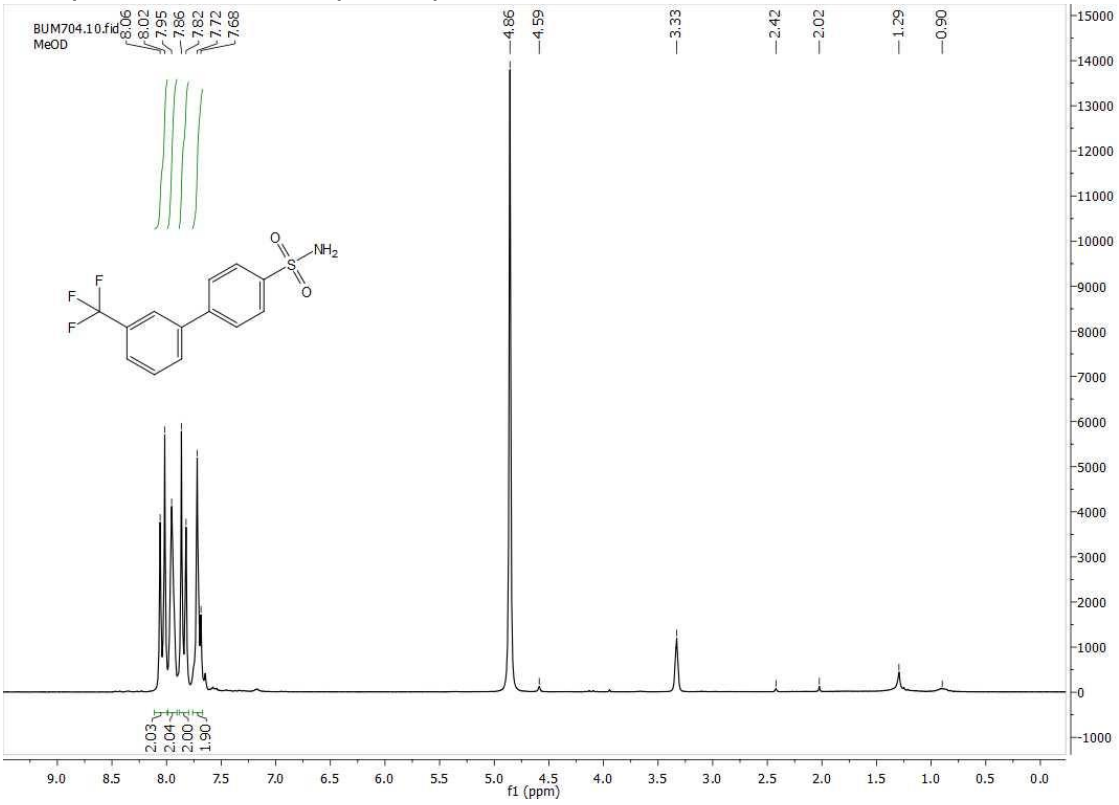
Compound **10** ^{1}C NMR ($CDCl_3$)



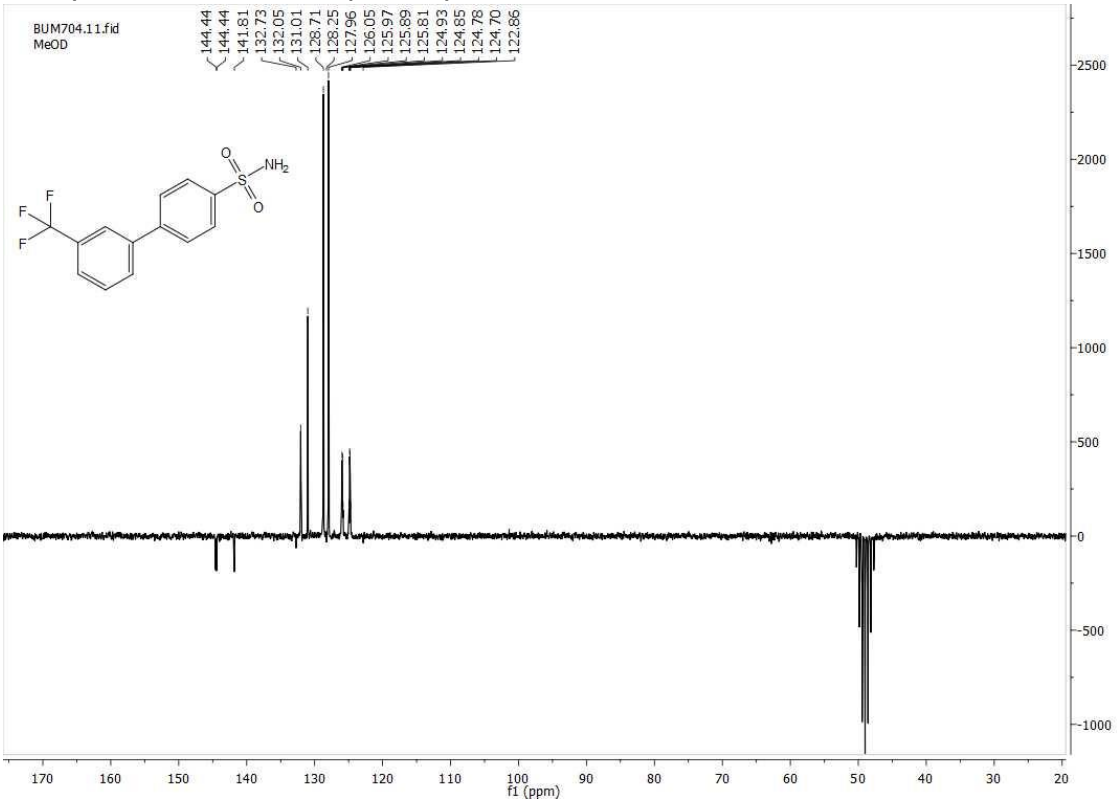
Compound **11** ^{1}H NMR (MeOD)



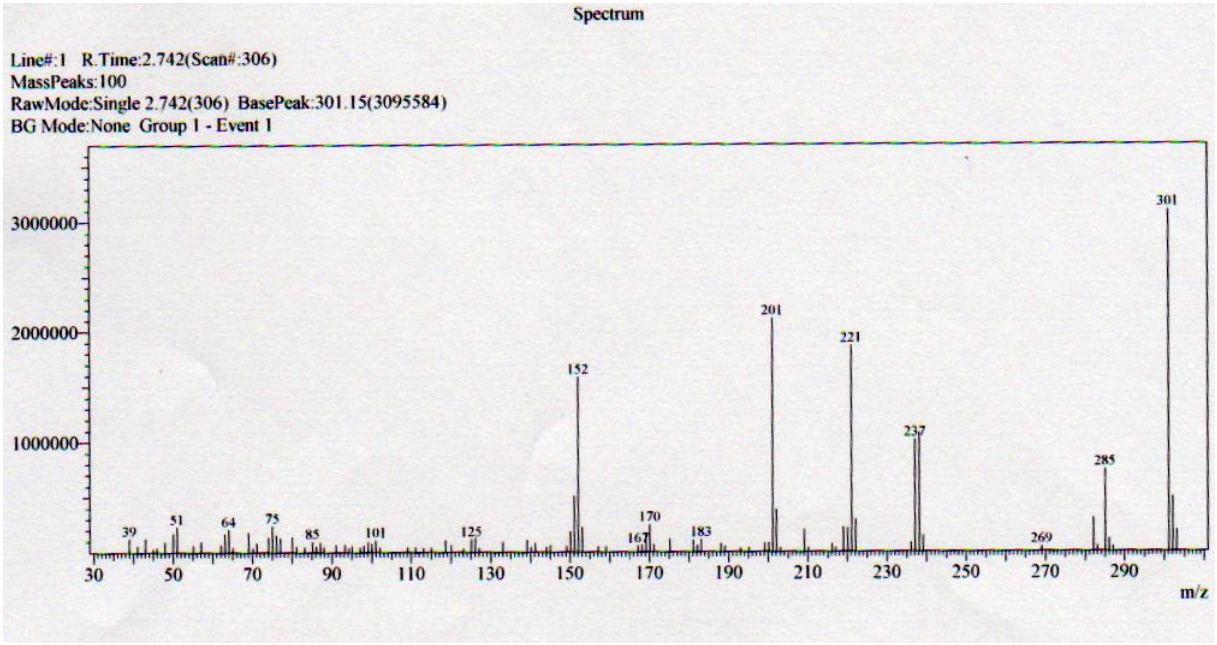
Compound **12** ^{1}H NMR(MeOD)



Compound **12** ^{13}C NMR(MeOD)

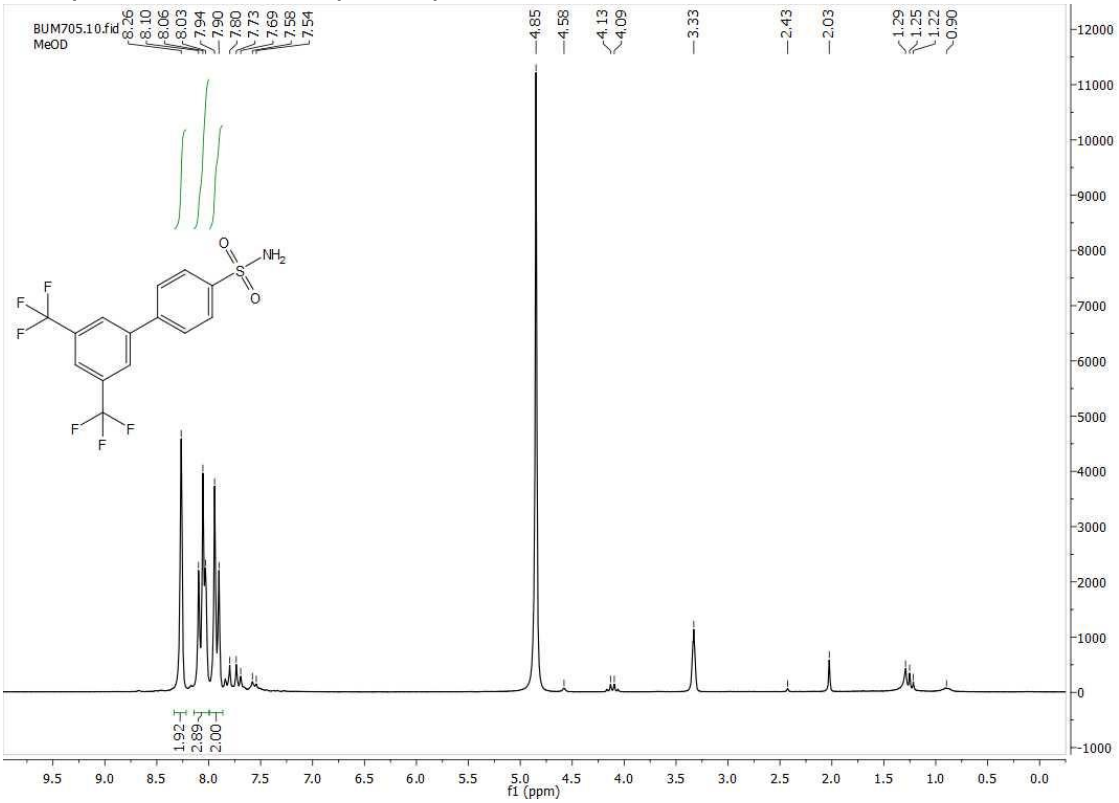


Compound **12** mass spectrum

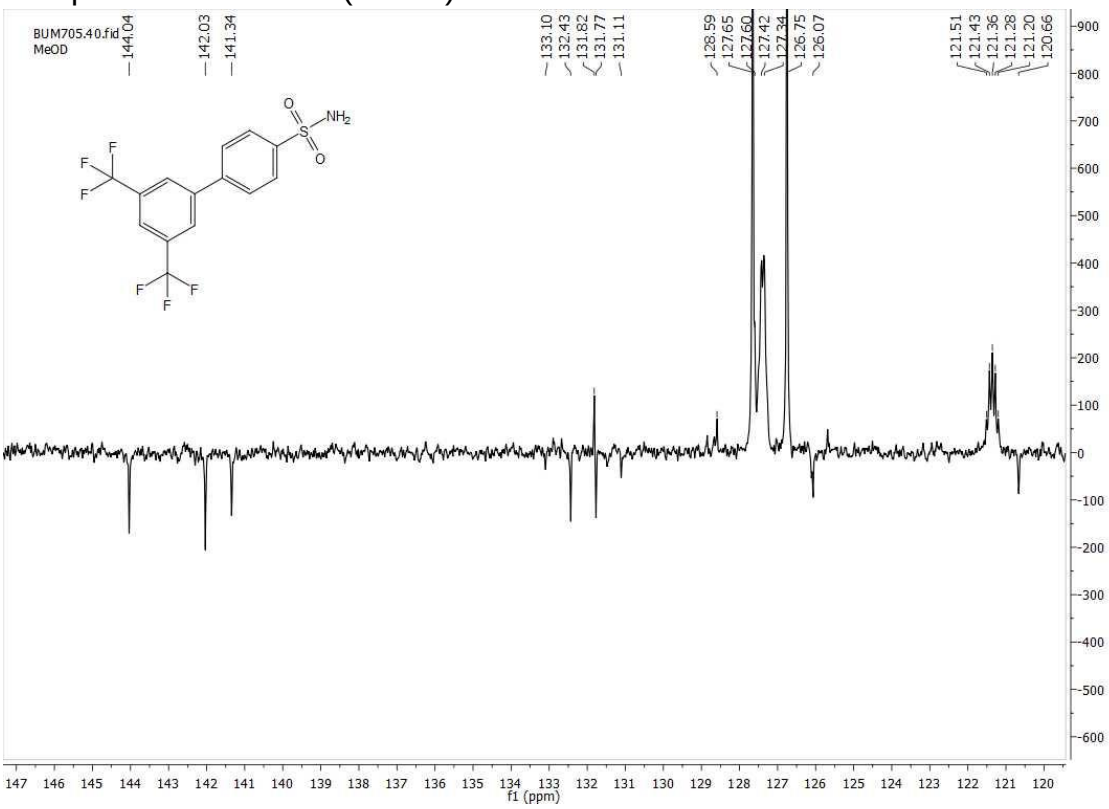


Compound **12** HRMS

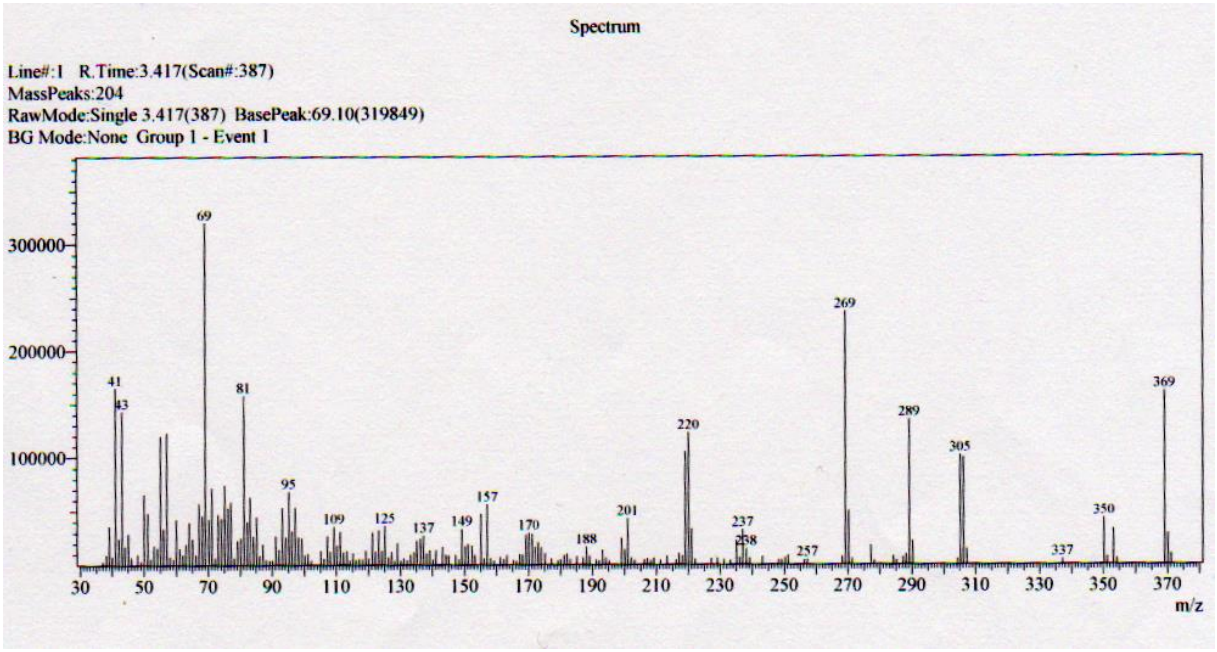
Compound **13** ^{1}H NMR(MeOD)



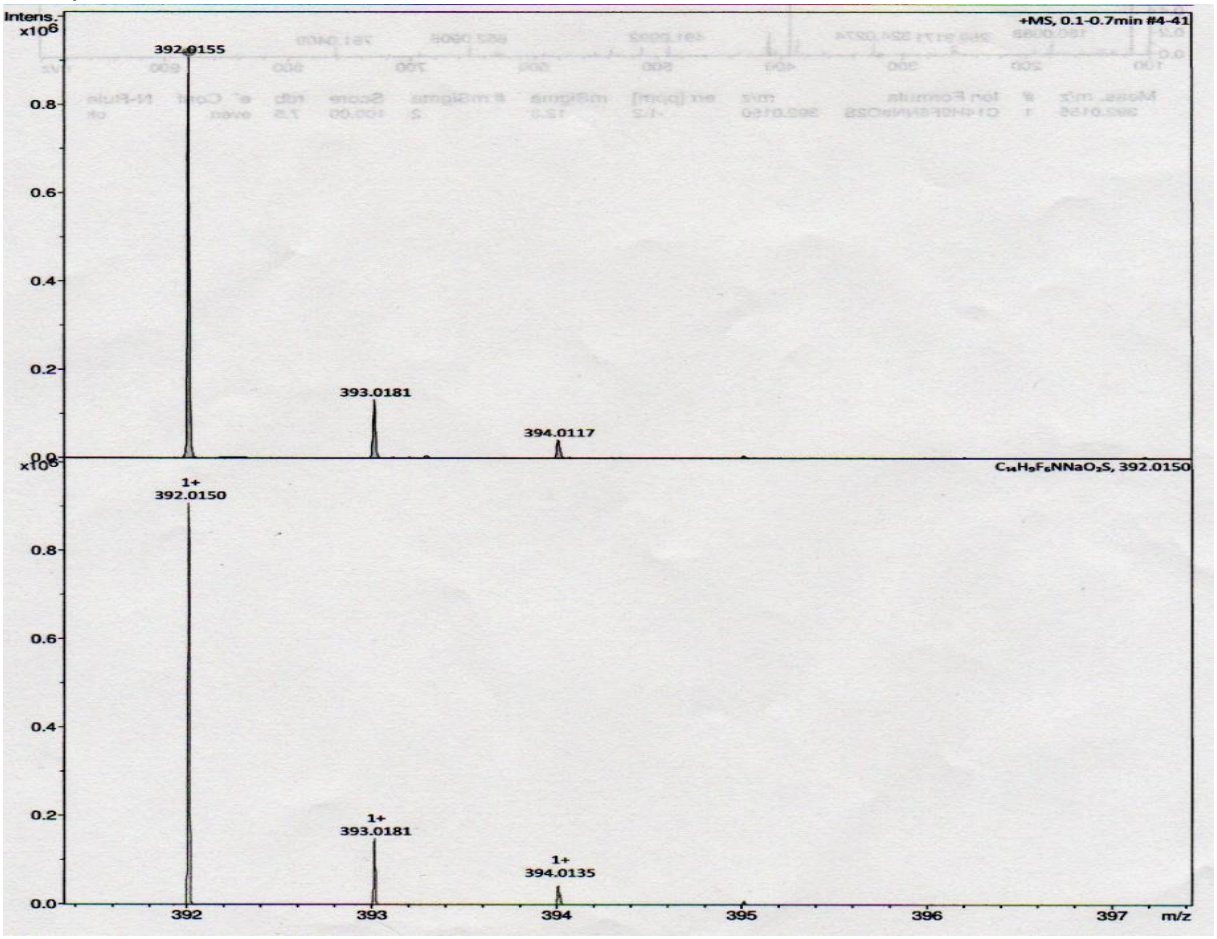
Compound **13** ^{13}C NMR(MeOD)



Compound **13** mass spectrum



Compound **13** HRMS



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