universität wien

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„Beiträge zur Synthese von Thioanaloga des Bumetanids“

verfasst von / submitted by

Arne Cierpka

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Last but not least I want to thank my girlfriend and my family for the support throughout my studies.

2 Abstract (English / German)

During this thesis, bumetanide derivates were synthesized, which should show a higher biological activity than bumetanide itself, when given as a treatment for neonatal epilepsy. The sodium-potassium-chloride cotransporter (NKCC1) in immature brain cells should be inhibited by these derivatives, lowering high intracellular chloride concentrations ($[Cl^-]_i$), because of reduced influx. As a result, the opening of the $GABA_A$-ionic channel has no longer excitatory but hyperpolarizing effects in the neonatal brain. This would be expected to result in a lower risk for epileptic seizures and convulsions.

A total number of 8 compounds were synthesized. While compound **1-7** belong to the same row of synthesis, compound **8** was a wholly different attempt using also compound **1** as an educt.

Instead of the oxygen in Position 4 like in bumetanide, there is a sulfur as a different heteroatom in compounds **3-7** instead, which could potentially increase the efficacy. By generating an ester out of the carboxyl group in compound **1** the lipophilicity could also be increased.

Further *in vitro* and *in vivo* testing will be necessary to assess if the biological activity as well as the lipophilicity is sufficient for penetrating the blood-brain-barrier (BBB).

On compound **8** an aniline instead of a thiophenol was attached. Afterwards the attempt was to cyclisize the compound to achieve a higher rigidity. Despite numerous attempts this cyclization could not be performed.

Im Rahmen dieser Diplomarbeit wurden Derivate des Bumetanids synthetisiert, die eine erhöhte biologische Aktivität aufweisen sollten, wenn man sie zur Behandlung von neonataler Epilepsie einsetzt. Der Natrium-Kalium-Chlorid-Cotransporter (NKCC1) in unreifen Neuronen soll von diesen Derivaten inhibiert werden. Die Blockade des Ionenkanals hat zur Folge, dass die intrazelluläre Chlorid-Konzentration ($[Cl^-]_i$) sinkt. Dadurch wirkt die Öffnung des $GABA_A$-Ionenkanals hyperpolarisierend anstatt exzitatorisch im neonatalen Nervensystem, wodurch epileptische Anfälle und Krämpfe verringert werden können.

Insgesamt wurden 8 Derivate synthetisiert. Während die Verbindungen **1-7** zum selben Syntheseweg gehören, wurde bei Verbindung 8 ein ganzer anderer Ansatz versucht.
Im Gegensatz zu Bumetanid mit der Sauerstofffunktion in Position 4, wurde diese in Verbindung **3-7** durch einen Schwefel als Heteroatom ersetzt, was die Wirksamkeit erhöhen könnte. Durch die Esterbildung aus der Carboxylgruppe konnte bei Verbindung **1** auch die Lipophilie erhöht werden.
Ob die biologische Aktivität und auch die Lipophilie dieser Verbindungen ausreicht, um die Blut-Hirn-Schranke zu passieren, wird sich erst nach weiteren *in vitro* und *in vivo* Testungen zeigen.
Bei Verbindung **8** wurde anstelle des Thiophenols ein Anilin angebracht um in weiterer Folge das Molekül durch Cyclisierung zu rigidisieren. Diese Cyclisierung konnte jedoch trotz zahlreicher Versuche nicht umgesetzt werden.

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3 Introduction

3.1 Epilepsy

Epilepsy is among the most common neurological disorders. There are over 50 million people worldwide suffering from epilepsy (Sun et al., 2017).
Epilepsy is characterized by the predisposition to recurrent unprovoked seizures, which can lead to severe neurobiologic, cognitive, psychological and social consequences. An epileptic seizure as such emerges from abnormal excessive or synchronous neuronal activity in the brain (Kalbkhani & Shayesteh, 2016).
Basically, there are three different epilepsy types, which differ in etiology: Idiopathic, symptomatic and presumed symptomatic epilepsy. Idiopathic epilepsies originate from genetic abnormalities, which alter basic neuronal regulation. Symptomatic and presumed symptomatic epilepsies on the other hand have their origin in an epileptic lesion, which can be either a tumor or a defect in metabolism causing major damage in the brain (Löscher & Brandt, 2010).
Complex pathophysiological plastic changes in the brain, caused by an insult, such as stroke, traumatic brain injury, brain infection or exposure to a neurotoxin lead to the emergence of epilepsy, called epileptogenesis. Ignited by the initial injury phase, it is followed by the latent phase, which can last years without any symptoms. Eventually the chronic phase is the manifestation of recurrent seizures (Younus & Reddy, 2017).
In progression of the disease patients can develop drug resistance, suffer from exacerbation of seizures and onset of co-morbidities (Vezzani, Pascente & Ravizza, 2017).

3.2 Neonatal Seizures

The incidence of epileptic seizures in new-borns with 1.8 to 3.5 per 1000 live births, is higher than at any other age of life. In neonates, chances are even higher, especially in the first few days after birth. There are several possible causes for the epileptogenesis in neonates. The most prevalent conditions causing epileptic seizures are hypoxia-ischemia, metabolic disturbances, infections and intracranial structural abnormalities. The characterization of neonatal seizures differs from those of other ages, therefore

specialized treatments are necessary (Mizrahi, 2001). Due to severe injuries and long-term effects caused by seizures in neonates, immediate management and treatment is vital, once an epileptic condition is diagnosed (Wirrel, 2005).

3.3 GABA

In the adult brain γ- Aminobutyric acid (GABA) is the main inhibitory neurotransmitter (Kirmse et al., 2015).

When binding to the $GABA_A$ receptor, GABA induces a conformational change of this ligand-gated ion channel. Depending on the chloride (Cl^-) -equilibrium potential this results in a flux of Cl^- either into or out of the cell. Beside Cl^- there is also bicarbonate permeating through the $GABA_A$ channel, which slightly increases the depolarizing effect (Maa et al., 2011).

$[Cl^-]_i$ in neurons of adults are low. As a result, the membrane gets hyperpolarized, due to an influx of Cl^- into the cell when GABA induces the $GABA_A$-ionic channel. $[Cl^-]_i$ of immature neurons however are high, so that GABA triggers efflux of Cl^- and consequently membrane depolarization. While hyperpolarization implies inhibition, depolarization means excitation of the neuron (Kambli, et al., 2017).

In contrast with the adult brain, GABA therefore has an exciting effect on immature neurons, which can lead to seizures when overloaded (Maa et al., 2011).

3.4 Chloride homeostasis

In neuronal membranes there are two major Cl^--cotransporters (CCCs) expressed, belonging to the same family called SLC12A: The Na-K-2Cl cotransporter (NKCC1) and the K-Cl cotransporter (KCC2). NKCC1 imports two Cl^- along with one potassium (K^+) as well as one natrium (Na^+) following the Na^+ gradient. On the other hand, KCC2 exports Cl^- along with one K^+ out of the cell, using the K^+ gradient as a source of energy. The activity of both combined determine the $[Cl^-]_i$ (Maa et al., 2011).

In neonates the NKCC1 activity predominates due to higher expression which results in high $[Cl^-]_i$. In adults however $[Cl^-]_i$ is low because of a higher KCC2 expression. These findings suggest why in immature neurons GABA has an excitatory and not inhibitory

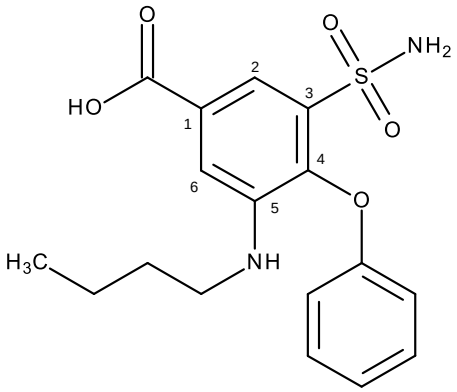
effect like in adults. (Maa et al., 2011). The altered balance between excitation and inhibition also explains the higher risk for seizures in neonates (Dzhala et al., 2008).

3.5 Current Therapy of Epilepsy and Neonatal Seizures

Drugs administered to treat neonatal seizures these days are barbiturates, benzodiazepines and other antiepileptic drugs (AEDs). Phenobarbital and phenytoin are still suggested as the first-line AEDs in neonates (Shetty, 2015).
However, there is scanty evidence for the efficacy of AEDs as a treatment for neonatal seizures. Furthermore, AEDs are associated with neurotoxicity and may alter normal brain development (Shetty, 2015).
Regarding the high $[Cl^-]_i$ in immature neurons due to the altered expression of SLC12A CCCs, these GABA-mimetic AEDs could even worsen seizures in neonates (Donovan et al., 2016). Therefore, there is a big medical need for better treatment options.

3.6 Bumetanide

Scheme 1: Bumetanide



Bumetanide is a loop diuretic, which is well-established as a treatment for heart failure, oedema with or without ascites and several nephrotic diseases like nephrotic syndrome (Ali & Duke, 1987).

Loop diuretics can inhibit NKCC1 as well as the other NKCC isoform NKCC2. As NKCC2 is mainly expressed in the thick ascending limb of Henle's loop in the kidney it is responsible for the diuretic effect of loop diuretics. NKCC1 on the other hand, which can be regarded as a new therapeutic target for neonatal seizures, is ubiquitously expressed including the brain (Lykke et al., 2016).

Bumetanide is the loop diuretic of interest for neonatal seizures because it is the only one that is selective for NKCCs vs. KCCs (Lykke et al., 2016).

However, the pharmacokinetics of bumetanide are unsatisfactory, as it shows a weak BBB penetration and a high degree of protein binding (Donovan et al., 2015).

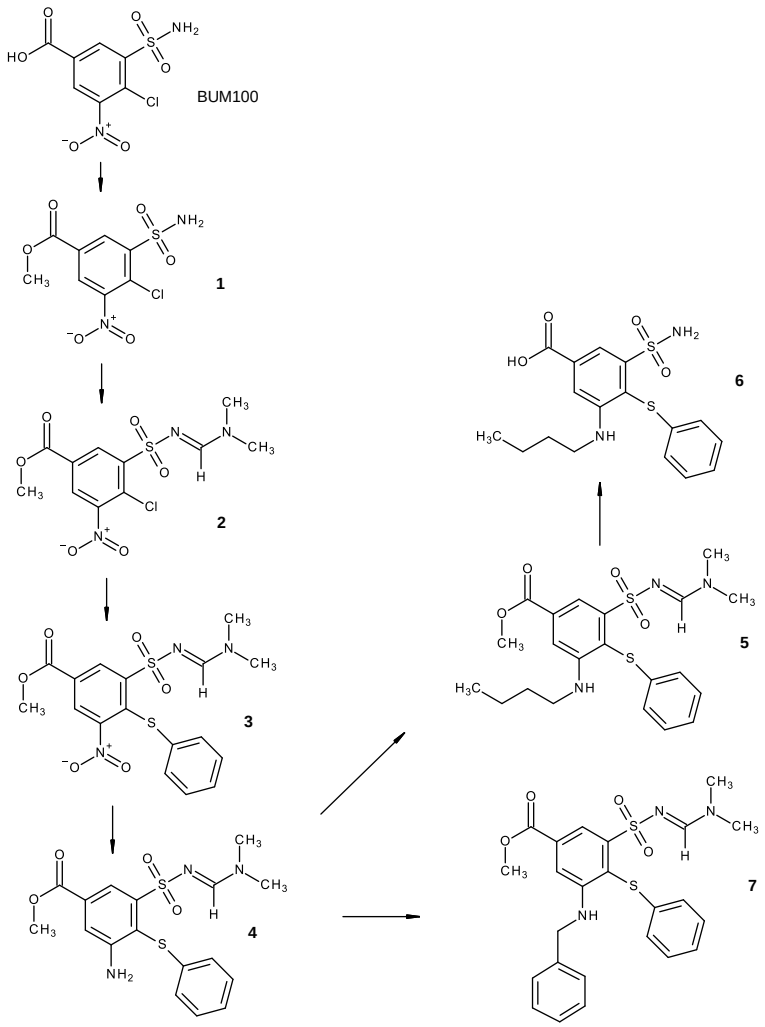
Furthermore, severe systemic side effects like increased diuresis, hypokalemic alkalosis and loss of hearing have to be expected when administered. Thus, a bumetanide derivate with better pharmacokinetics and less severe side effects could be a potential drug for treatment of neonatal seizures (Lykke et al., 2016).

4 Objective

In the neonatal brain bumetanide has a potential to lower the risk of epileptic seizures. Once the bumetanide derivatives pass the BBB, they are considered to inhibit the NKCC1 in immature neurons, where they could lower the $[Cl^-]_i$. As a result, the opening of the $GABA_A$-ionic channel is no longer excitatory but inhibiting in the neonatal brain. Compared to bumetanide with the oxygen in Position 4, the sulfur of the thiophenol in compound **3** pushes electrons into the aromatic ring, which could potentially increase the efficacy. On the other hand, the lipophilicity could be raised by generating an ester out of the carboxyl group in compound **1**. A higher lipophilicity should result in an easier penetration of the BBB.

5 Synthesis of Bumetanide Derivatives

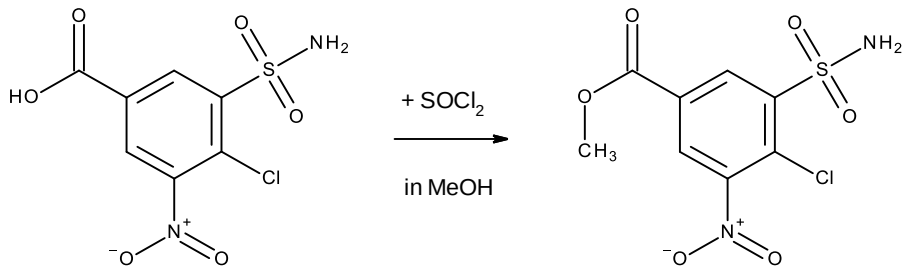
Scheme 1: Synthesis Overview for Compounds 1-7



5.1 Compounds **1-2**

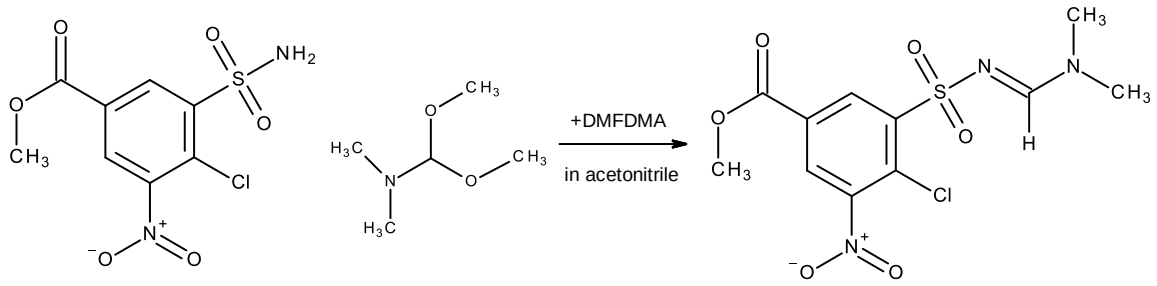
In the first two steps of the synthesis the functional groups, carboxylic acid and sulfonamide, were protected. In this way it could be ensured that further reactions took place in position 4. The esterification of the carboxylic group was the first step. After the educt (internal code: BUM100) was dissolved in MeOH thionyl chloride was added carefully. The sulfonamide group in the resulting compound **1** was protected with *N,N*-dimethylformamide dimethyl acetal. Both reactions were stirred overnight and went smoothly without any problems.

Scheme 3: Esterification of the carboxylic group (Wanaski, Collins & Kincaid, 2012)



BUM 100 Compound **1**

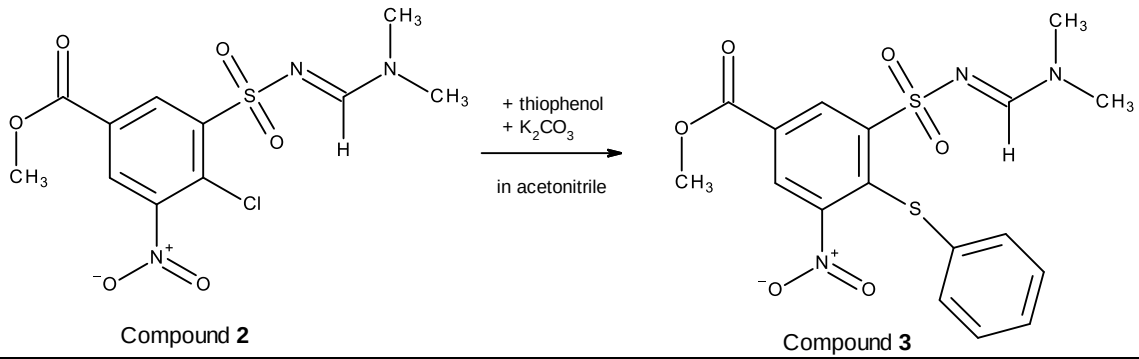
Scheme 4: Protecting the sulfonamide group (Wanaski, Collins & Kincaid, 2012)



Compound **1** DMF-DMA Compound **2**

5.2 Compound **3**

Scheme 5: Substituting the Cl^- group in Position 4 with thiophenol



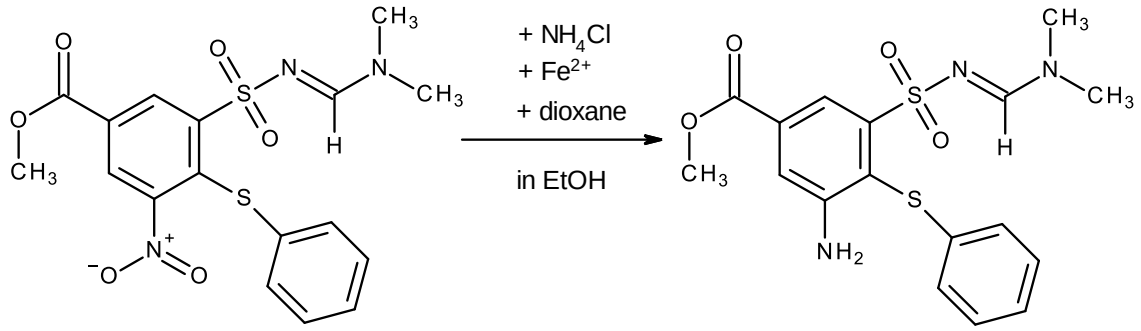
Compound **2** Compound **3**

After successfully protecting the functional groups, the objective was to substitute the Cl^- group in position 4 with thiophenol. As the sulfur of the thiophenol pushes electrons into the aromatic group this could potentially increase the biological activity.

Compound **2** was dissolved in acetonitrile and thiophenol was added in an alkali milieu by using K_2CO_3. The mixture was stirred for one day and delivered a decent yield of 85%.

5.3 Compound **4**

Scheme 6: Reducing the NO_2 group in position 5

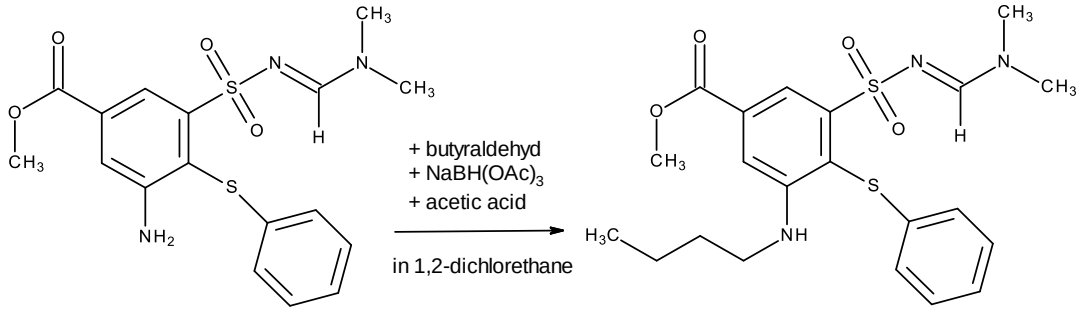


Compound **3** Compound **4**

In the next step of the synthesis the nitro (NO_2) -group was reduced to an amine (NH_2) group. This subsequently allowed attaching lipophilic structures in that position. Compound **3** was dissolved in EtOH and NH_4Cl as well as dioxane was added. Under reflux iron powder was added in three portions to the reactive mixture. After eight hours the reaction was complete with a yield of over 90%.

5.4 Compound **5**

Scheme 7: Attaching a butyl chain to the NH_2 group



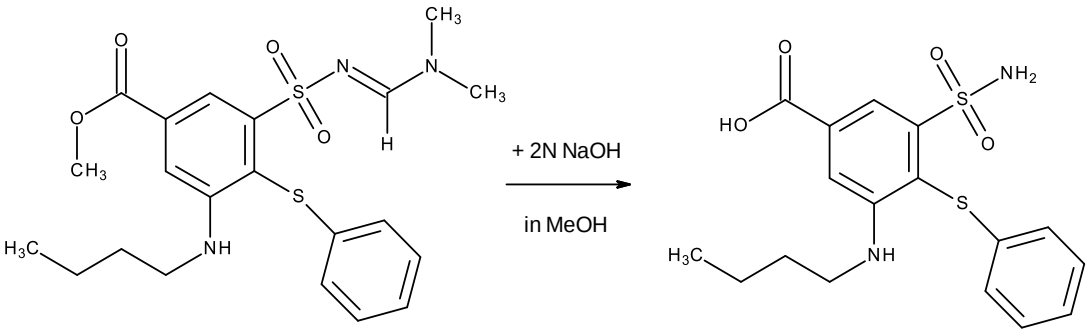
Compound **4** Compound **5**

After the NO_2 group was successfully reduced a butyl chain was attached to the NH_2 group.

Compound **4** was dissolved in a mixture of butyraldehyde and 1,2-dichlorethane and subsequently acetic acid was added. Afterwards sodium triacetoxyborohydride ($NaBH(OAc)_3$) was added to the reactive mixture in three portions with an interval of 30 minutes.

5.5 Compound **6**

Scheme 8: Removal of the protection groups

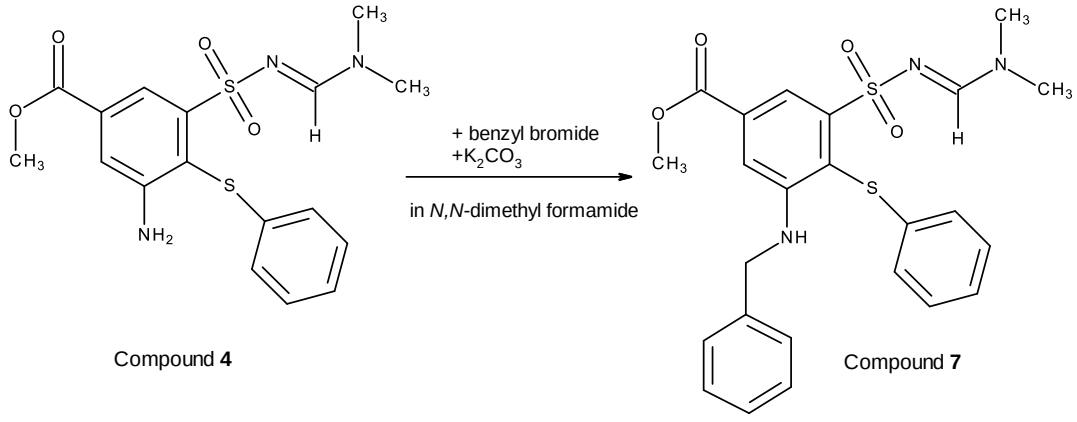


Compound **5** Compound **6**

As the functional groups are considered to be important for the binding process with NKCC1 the removal of the protection groups is crucial for a high biological activity. In order to remove those, compound **5** was dissolved in MeOH and 2N NaOH was added. After stirring overnight, it was acidified with 2N HCl and the resulting precipitate was filtered off.

5.6 Compound **7**

Scheme 9: Attaching a benzyl remnant to the NH_2 group



Compound **4** Compound **7**

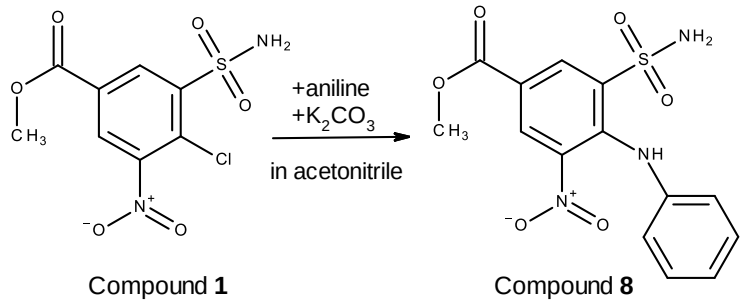
For compound **7** the objective was to attach a benzyl remnant to the NH_2 group instead of a butyl chain like in compound **5**.

After Compound **4** was dissolved in *N,N*-dimethyl formamide benzyl bromide was added in a basic milieu.

Although the reaction ran for two days the yield was below 50%.

5.7 Compound **8**

Scheme 10: Attaching an aniline in position 4



Compound **1** Compound **8**

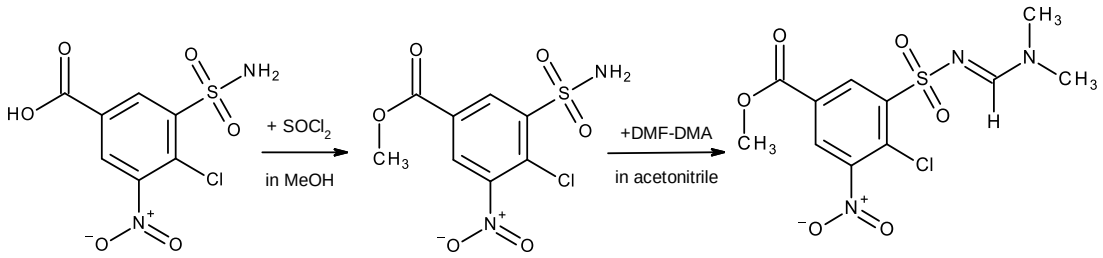
In a wholly different approach, the Cl^- of compound **1** in position 4 was substituted with an aniline instead of a thiophenol. Compound **1** was dissolved in acetonitrile with K_2CO_3 for a basic milieu. Aniline was added to the reactive mixture. It was stirred overnight at room temperature. The reaction ran without any problems and delivered a decent yield of 70%.

6 Discussion

The main objective was to synthesize thioanalogues of bumetanide, which should show a higher biological activity on NKCC1 than bumetanide itself. Other than that, better pharmacokinetics and less severe side effects would be important.

To realise a nucleophile substitution of thiophenol in position 4 the first step was to protect the functional groups of the precursor. A carboxylate ester and DMF-DMA were added without any issues.

Scheme 11: Applying the protection groups (Wanaski, Collins & Kincaid, 2012)

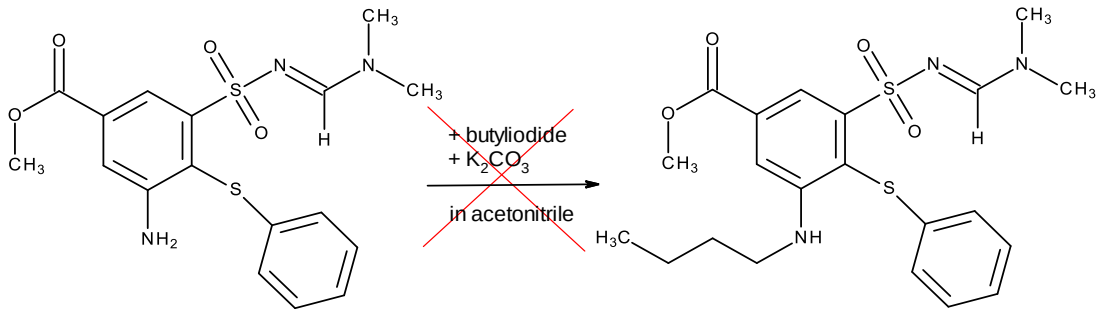


BUM 100 Compound **1** Compound **2**

With the protected functional groups, it was possible to add a thiophenol remnant in position 4. In the next step of the synthesis the NO_2 group was reduced to an NH_2 group which allowed attaching lipophilic structures in that position. To align the molecule to bumetanide a butyl chain was attached to the NH_2 group (compound **5**).

This step of the synthesis proved to be quite challenging.

Scheme 12: First failed attempt to add a butyl chain to the NH_2 group

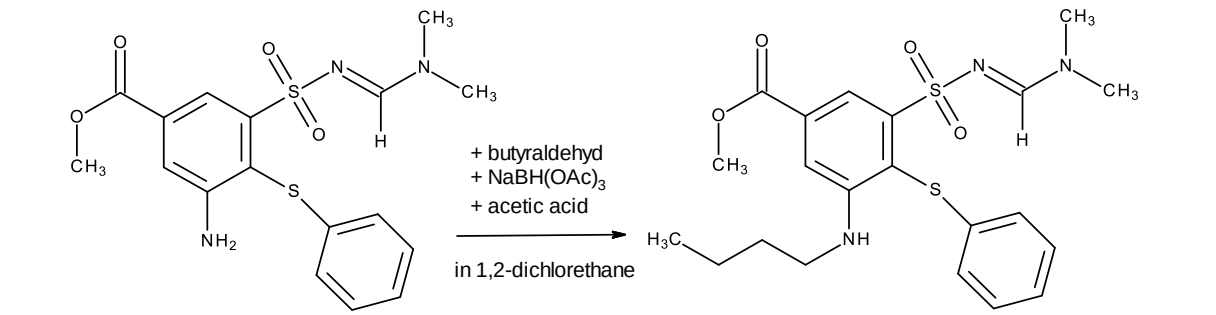


Compound **4** Compound **5**

Compound **4** was dissolved in acetonitrile and butyl iodide was added. The reaction ran overnight under reflux in an alkali milieu by using K_2CO_3. Even after several attempts with

increasing amounts of butyl iodide and K_2CO_3 and higher temperatures the reaction still failed.

Scheme 13: Second and successful attempt



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

In a new attempt we tried a reductive amination instead. Compound **4** was dissolved in a mixture of butyraldehyde and acetonitrile and subsequently acetic acid was added. Afterwards sodium triacetoxyborohydride ($NaBH(OAc)_3$) was added in three portions with an interval of 30 minutes. The mixture was stirred overnight. Unfortunately, the yield of 13% was quite low.

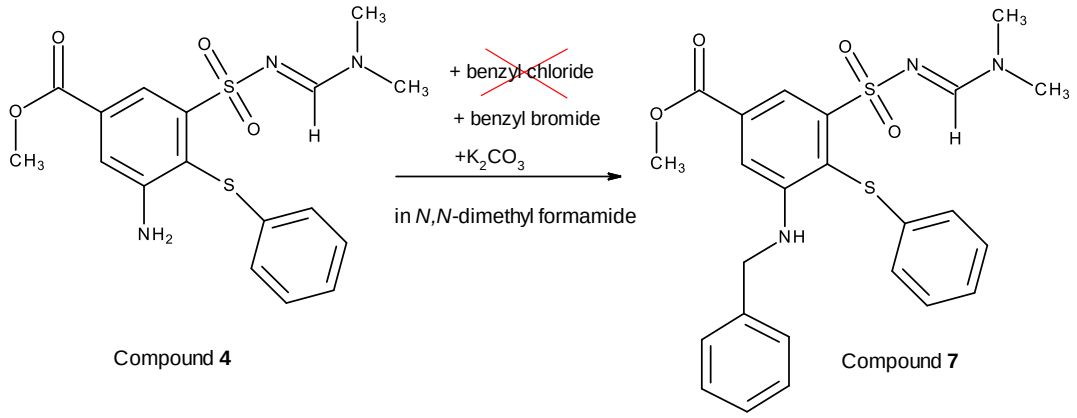
To examine the importance of this remnant a benzyl remnant was attached to the NH_2 group instead of a butyl chain like in compound **5**.

At first compound **4** was dissolved in *N,N*-dimethyl formamide.

Afterwards benzyl chloride was added in a basic milieu. As we did not see any progress in the reaction, we tried higher temperatures and adding more benzyl chloride with no success.

After the benzyl chloride was replaced with benzyl bromide the reaction could finally be realized.

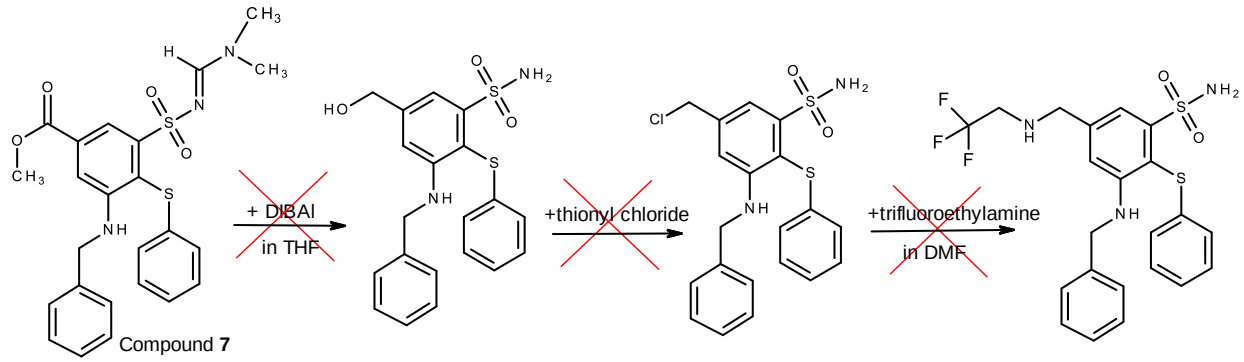
Scheme 14: Compound 7 failed with benzyl chloride, but was successful with benzyl bromide



Starting from compound **7** the thought was to add a trifluoroethylamine group for a higher lipophilicity. The more lipophilic the compound the better it passes the BBB, which is vital for reaching NKCC1 in the brain. Besides, similar compounds with that group delivered good results. In the first step compound **7** was dissolved in tetrahydrofuran (THF) and diisobutylaluminium hydride (DIBAl) was added in six portions with an interval of one hour under argon atmosphere. The product was not satisfactory, therefore further steps of this synthesis had to be neglected.

In the next step thionyl chloride would have been added to the product. In theory the resulting Cl^- group would have allowed attaching a trifluoroethylamine group by dissolving the product in dimethylformamide (DMF) and adding trifluoroethylamine.

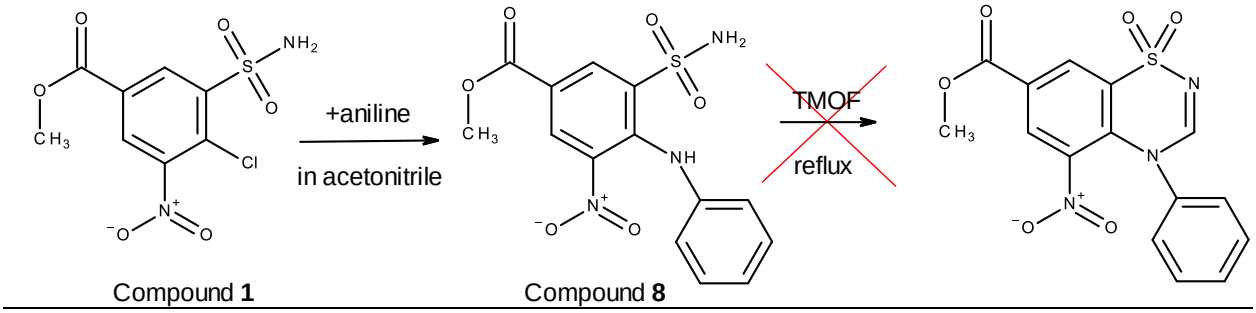
Scheme 15: Attempt to add a trifluoroethylamine remnant to compound 7



In the beginning of this thesis the objective actually was to synthesize bumetanide derivates with a higher rigidity. A higher rigidity could potentially result in a higher biological activity. To achieve that the thought was to cyclize the sulfonamide with the amine of the aniline.

After the aniline was attached in position 4 o-trimethyl-formic acid (TMOF) was added under reflux to compound **8**. This step failed despite numerous attempts. Therefore, the new objective was to synthesize thioanalogues of bumetanide instead.

Scheme 16: Failed cyclization



Compound **1** Compound **8**

7 Experimental Section

7.1 General methods

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

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.

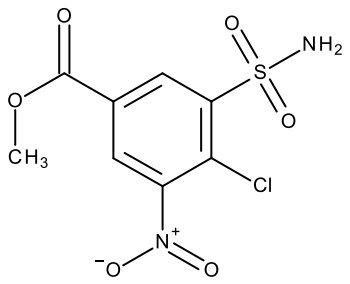
Melting points were measured on a Thermo Galen Kofler hot stage microscope. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker Advance (200 and 50 MHz respectively) and chemical shifts are reported in ppm relatively to the solvent residual line or tetramethylsilane as internal standard.

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

7.2 Compound **1**

Methyl 4-chloro-3-nitro-5-sulfamoyl-benzoate

Internal code: BUM150



Molecular formula	$C_8H_7ClN_2O_6S$
Molecular weight	294,67 g/mol

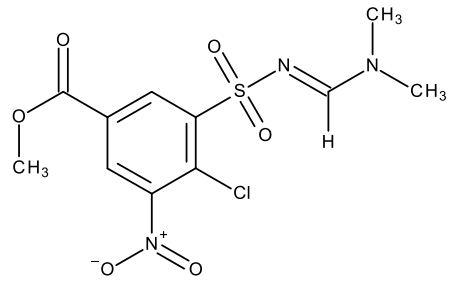
Wanaski, S.; Collins, S.; Kincaid, J. (2012).
Arylsulfonamide derivatives as sodium-potassium-chloride cotransporter inhibitors and their preparation, compositions, and methods of use.
PCT Int. Appl., 219 pp., Patent, 2012, CODEN: PIXXD2

To a solution of 30 mmol (8.42 g) methyl 4-chloro-3-nitro-5-sulfamoyl-benzoate (internal code: BUM100) in 45 mL MeOH 66 mmol (4.79 mL) $SOCl_2$ were added and the mixture was stirred overnight at room temperature. The mixture was then diluted with 5 % -sodium bicarbonate solution and extracted with ethyl acetate. The organic phase was dried over Na_2SO_4 and then evaporated under reduced pressure yielding 8.73 g of yellow crystals (98.8 % of compound **1**). ^{1}H NMR (200 MHz, DMSO-d_6) δ 8.70 (d, J = 8.6 Hz, 2H), 8.14 (s, 2H), 3.94 (s, 3H).

7.3 Compound **2**

Methyl 4-chloro-3-[(E)-dimethylaminomethyleneamino]sulfonyl-5-nitro-benzoate

Internal code: BUM151



Molecular formula	$C_{11}H_{12}ClN_3O_6S$
Molecular weight	349,75 g/mol
Melting point	130-133 °C

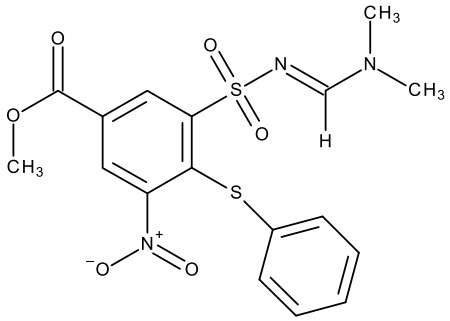
Wanaski, S; Collins, S., Kincaid, J. (2012).
Arylsulfonamide derivatives as sodium-potassium-chloride cotransporter inhibitors and their preparation, compositions, and methods of use.
PCT Int. Appl., 219 pp., Patent 2012, CODEN: PIXXD2

To a solution of 10 mmol (2.95 g) methyl 4-chloro-3-nitro-5-sulfamoyl-benzoate (**1**, internal code: BUM150) in 20 mL acetonitrile 10.49 mmol (1.40 mL) *N,N*-dimethylformamide dimethylacetal (DMFDMA) was added and stirred at room temperature overnight. After the reaction was complete, which was controlled *via* thin layer chromatography, acetonitrile was evaporated, the mixture was diluted with water and extracted with ethyl acetate. The organic phase was dried over Na_2SO_4 and then evaporated under reduced pressure. The resulting crude product was purified by recrystallization from EtOH yielding 1.57 g of yellow crystals (44.9 % of compound **2**). ^{1}H NMR (200 MHz, $CDCl_3$) δ 9.01 (s, 1H), 8.47 (s, 1H), 8.26 (s, 1H), 3.99 (s, 3H), 3.24 (s, 3H), 3.09 (s, 3H).

7.4 Compound **3**

Methyl 3-[(E)-dimethylaminomethyleneamino]sulfonyl-5-nitro-4-phenylsulfanyl-benzoate

Internal code: BUM290



Molecular formula	$C_{17}H_{17}N_3O_6S_2$
Molecular weight	423.46 g/mol
Melting point	170-173 °C

Bormann, D,; Merkel, W.; Muschaweck, R.; Mania, D. (1982).
Sulfamoylbenzoic acid derivatives.
Pat. Specif. (Aust.), 42 pp., Patent 1982, CODEN: ALXXAP

To a solution of 2 mmol (700 mg) methyl 4-chloro-3-[(E)-dimethylamino-methyleneamino]sulfonyl-5-nitro-benzoate (**2**, internal code: BUM151) in 10 mL acetonitrile 4 mmol K_2CO_3 (553 mg) and 2.4 mmol of thiophenol (245 µl) were added. The mixture was stirred at room temperature for one day and after the reaction was finished, which was controlled *via* thin layer chromatography, acetonitrile was evaporated, the mixture was diluted with water and extracted with ethyl acetate. The organic phase was dried over Na_2SO_4 and then evaporated under reduced pressure. The resulting crude product was purified by column chromatography (first 300 mL of petroleum ether, then 300 mL of ethyl acetate) yielding 723 mg of yellow crystals (85.4 % of compound **3**). ^{1}H NMR (200 MHz, $CDCl_3$) δ 9.10 (d, *J* = 1.9 Hz, 1H), 8.36 – 8.25 (m, 2H), 7.34 – 7.18 (m, 3H), 7.16 – 7.03 (m, 2H), 3.98 (s, 3H), 2.96 (s, 3H), 2.87 (s, 3H) ^{13}C NMR (50 MHz, $CDCl_3$)

δ 163.8, 161.3, 154.2, 147.0, 133.5, 132.6, 131.3, 129.9, 129.4, 128.4, 128.0, 77.2, 53.2, 41.6, 35.6.

Mass Analysis

m/z	423	M^+ 5%
	71	67%
	69	76%
	57	100%
	43	71%

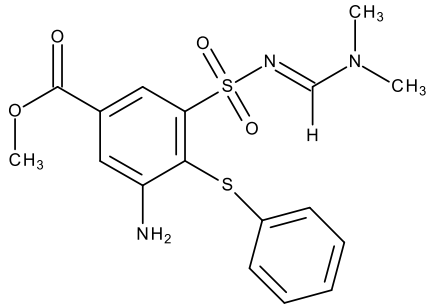
HRMS - Analysis

Mass.m/z	Molecular formula	m/z	err [ppm]
446.0454	$C_{17}H_{17}N_3NaO_6S_2$	446.0451	-0.8

7.5 Compound **4**

Methyl 3-amino-5-[(E)-dimethylaminomethyleneamino]sulfonyl-4-phenylsulfanyl-benzoate

Internal code: BUM291



Molecular formula	$C_{17}H_{19}N_3O_4S_2$
Molecular weight	393.48 g/mol
Melting point	147-150 °C

Englert, H.; Bormann, D. (1983).
Synthesis of piretanide analogs 1,3,4-triazoles.
Archiv der Pharmazie (Weinheim, Germany), Volume 316, Issue 5, Pages 460-3, Journal, 1983, CODEN: ARPMAS, ISSN: 0365-6233

To a solution of 1 mmol (423 mg) methyl 3-[(E)-dimethylaminomethyleneamino]sulfonyl-5-nitro-4-phenylsulfanyl-benzoate (**3**, internal code: BUM290) in 10 mL EtOH a solution of 10 mmol (535 mg) NH_4Cl in 30 mL H_2O was added. The mixture was stirred and heated under reflux. After adding 10 ml dioxane 4 mmol (223 mg) iron was added in three portions at intervals of three minutes. This mixture was heated for eight hours. After the reaction was complete, which was controlled *via* thin layer chromatography, EtOH was evaporated, the mixture was diluted with water and extracted with dichloromethane. The organic phase was dried over Na_2SO_4 and then evaporated under reduced pressure, yielding 390 mg of light yellow crystals (99.1 % of compound **4**). ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.39 (s, 1H), 8.22 (s, 1H), 7.65 (s, 1H), 7.26 – 7.06 (m, 3H), 6.99 – 6.84 (m, 2H), 4.25 (s br, 2H), 3.92 (s, 3H), 2.75 (s, 3H), 2.49 (s, 3H) ^{13}C NMR (50 MHz, $CDCl_3$) δ 165.9,

161.0, 150.1, 146.7, 134.7, 132.2, 129.3, 129.3, 125.7, 120.0, 119.9, 114.3, 52.6, 41.2, 34.9.

Mass Analysis

m/z	393	M^+ 13%
	73	100%
	71	19%
	44	63%
	43	31%

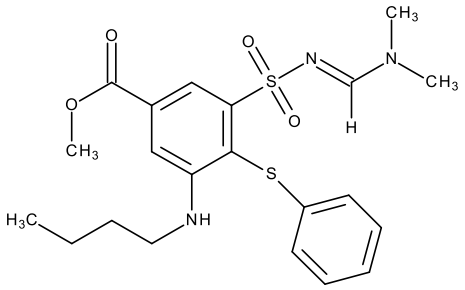
HRMS - Analysis

Mass.m/z	Molecular formula	m/z	err [ppm]
416.0713	$C_{17}H_{19}N_3NaO_4S_2$	416.0709	-0.9

7.6 Compound **5**

Methyl 3-(butylamino)-5-[(E)-dimethylaminomethyleneamino]sulfonyl-4-phenylsulfanyl-benzoate

Internal code: BUM292



Molecular formula	$C_{21}H_{27}N_3O_4S_2$
Molecular weight	449.59 g/mol
Melting point	157-160 °C

To a solution of 1.5 mmol (135 µl) butyraldehyde in 10 mL 1,2 dichloroethane 1 mmol (393 mg) methyl 3-amino-5-[(E)-dimethylaminomethyleneamino]sulfonyl-4-phenyl-sulfanyl-benzoate (**4**, internal code**:** BUM291) was added. To this solution 1 mmol acetic acid (58 µl) and 1.5 mmol (318 mg) sodium triacetoxyborohydride ($NaBH(OAc)_3$) were added and the mixture was stirred at room temperature overnight. After the reaction was complete, which was controlled *via* thin layer chromatography, the mixture was diluted with water and extracted with dichloromethane. The organic phase was dried over Na_2SO_4 and then evaporated under reduced pressure. The resulting crude product was purified by column chromatography (ethyl acetate/petroleum ether 1+1) yielding 60 mg of a beige powder (13.3 % of compound **5**). ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.31 (s, 1H), 8.22 (s, 1H), 7.49 (s, 1H), 7.33 – 7.04 (m, 3H), 7.01 – 6.83 (m, 2H), 3.93 (s, 3H), 3.23 -3.01 (m, 2H), 2.74 (s, 3H), 2.49 (s, 3H), 1.51 – 1.28 (m, 2H), 1.21 – 0.98 (m, 2H), 0.87 - 0.69 (m, 3H) ^{13}C NMR (50 MHz, $CDCl_3$) δ 166.4, 161.0, 150.2, 146.7, 134.8, 132.3, 129.2, 129.2, 125.9, 125.8, 117.9, 114.8, 52.6, 43.4, 41.1, 34.9, 31.0, 19.9, 13.7.

Mass Analysis

m/z	449	M^+ 25%
	334	26%
	313	34%
	270	62%
	73	100%

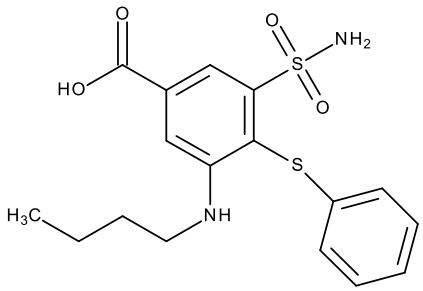
HRMS - Analysis

Mass.m/z	Molecular formula	m/z	err [ppm]
472.1336	$C_{21}H_{27}N_3NaO_4S_2$	472.1335	-0.9

7.7 Compound **6**

3-(Butylamino)-4-phenylsulfanyl-5-sulfamoyl-benzoic acid

Internal code: BUM293



Molecular formula	$C_{17}H_{20}N_2O_4S_2$
Molecular weight	380.48 g/mol
Melting point	169-170 °C

Feit, P. W. (1976).
Sulfamoylbenzoic acid derivatives.
U.S., 19 pp., Division of U.S. 3,806,534. Patent 1976, CODEN: USXXAM

To a solution of 0.5 mmol (225 mg) methyl 3-(butylamino)-5-[(E)-dimethylamino-methyleneamino]sulfonyl-4-phenylsulfanyl-benzoate (5, internal code: BUM292) in 3 mL MeOH 2 mL 2N NaOH was added and stirred at room temperature overnight. When the reaction was complete, which was controlled by thin layer chromatography, the mixture was acidified with 2 mL 2N HCl. The precipitate was filtered off yielding 80 mg of a beige solid product (42.1 % of compound 6). ^{1}H NMR (200 MHz, DMSO-d_6) δ 7.86 (s, 1H), 7.45 (s, 2H), 7.35 (s, 1H), 7.31 – 7.06 (m, 4H), 5.53 (t, J = 5.6 Hz, 1H), 3.20 – 2.98 (m, 2H), 1.41 – 1.18 (m, 2H), 1.12 – 0.88 (m, 2H), 0.82 – 0.58 (m, 3H) ^{13}C NMR (50 MHz, DMSO) δ 166.7, 149.9, 148.8, 134.4, 133.1, 129.0, 127.4, 126.6, 114.9, 114.1, 113.5, 42.3, 30.1, 19.1, 13.6.

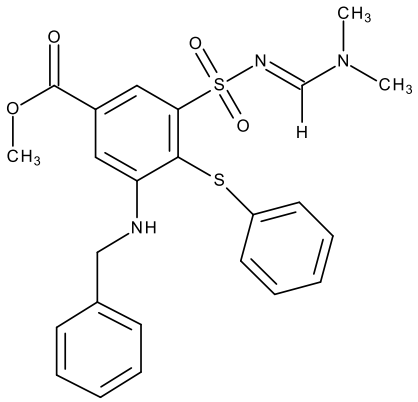
HRMS - Analysis

Mass.m/z	Molecular formula	m/z	err [ppm]
403.0753	$C_{17}H_{20}N_2NaO_4S_2$	403.0757	0.9

7.8 Compound **7**

Methyl 3-(benzylamino)-5-[(E)-dimethylaminomethyleneamino]sulfonyl-4-phenylsulfanyl-benzoate

Internal code: BUM294



Molecular formula	$C_{24}H_{25}N_3O_4S_2$
Molecular weight	483,60 g/mol
Melting point	178-181 °C

To a solution of 1 mmol (393 mg) (**6**, internal code: BUM291) in 3.3 mL *N,N*-dimethyl formamide 2 mmol (493 µl) benzyl bromide and 0.5 mmol (69 mg) K_2CO_3 were added. The mixture was stirred under reflux for two days. After the reaction was complete, which was controlled *via* thin layer chromatography, the mixture was diluted with water and extracted with ethyl acetate. The organic phase was dried over Na_2SO_4 and then evaporated under reduced pressure. The resulting crude product was purified by column chromatography (ethyl acetate/petroleum ether 6+4) yielding 220 mg of a beige solid product (45.5 % of compound **7**). ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.25 (d, *J* = 1.8 Hz, 1H), 8.16 (s, 1H), 7.40 (d, *J* = 1.8 Hz, 1H), 7.23 – 7.03 (m, 6H), 6.92 – 6.76 (m, 4H), 5.46 (t, *J* = 5.6 Hz, 1H), 4.25 (d, *J* = 5.6 Hz, 2H), 3.82 (s, 3H), 2.66 (s, 3H), 2.44 (s, 3H) ^{13}C NMR (50 MHz, $CDCl_3$) δ 166.1, 160.9, 149.8, 146.7, 137.7, 134.7, 132.2, 129.2, 129.1, 128.7, 127.5, 127.0, 126.0, 126.0, 118.3, 114.8, 114.3, 52.5, 47.7, 41.1, 34.9.

Mass Analysis

m/z	483	M^+ 11%
	270	35%
	91	95%
	73	100%
	44	42%

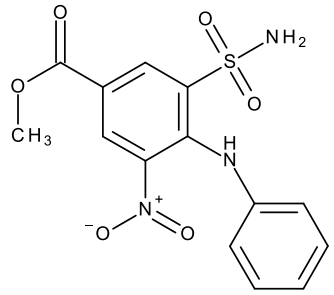
HRMS - Analysis

Mass.m/z	Molecular formula	m/z	err [ppm]
506.1183	$C_{24}H_{25}N_3NaO_4S_2$	506.1179	-0.8

7.9 Compound **8**

Methyl 4-anilino-3-nitro-5-sulfamoyl-benzoate

Internal code: BUM280



Molecular formula	$C_{14}H_{13}N_3O_6S$
Molecular weight	351.33 g/mol
Melting point	197-200 °C

To a solution of 10 mmol (2.947 g) methyl 3-(benzylamino)-5-[(E)-dimethylamino-methyleneamino]sulfonyl-4-phenylsulfanyl-benzoate (**7**, internal code: BUM150) in 50 mL acetonitrile 24 mmol aniline (2.191 mL) and 20 mmol K_2CO_3 (2.764 g) were added. The mixture was stirred for two hours at room temperature. After the reaction was complete, which was controlled by thin layer chromatography, the mixture was diluted with water and extracted with ethyl acetate. The organic phase was dried over Na_2SO_4 and then evaporated under reduced pressure. The resulting crude product was purified by recrystallization from EtOH (70%) yielding 3.40 g of orange crystals (96.8 % of compound **8**). ^{1}H NMR (200 MHz, DMSO-*d*$_6$) δ 8.60 (d, *J* = 2.1 Hz, 1H), 8.46 (s, 1H), 8.41 (d, *J* = 2.1 Hz, 1H), 8.11 (s, 2H), 7.37 – 7.22 (m, 2H), 7.18 – 7.04 (m, 1H), 7.03 – 6.89 (m, 2H), 3.90 (s, 3H) ^{13}C NMR (50 MHz, DMSO) δ 163.8, 140.2, 139.3, 137.7, 133.9, 133.2, 131.1, 129.3, 124.5, 119.4, 119.3, 52.6.

Mass Analysis

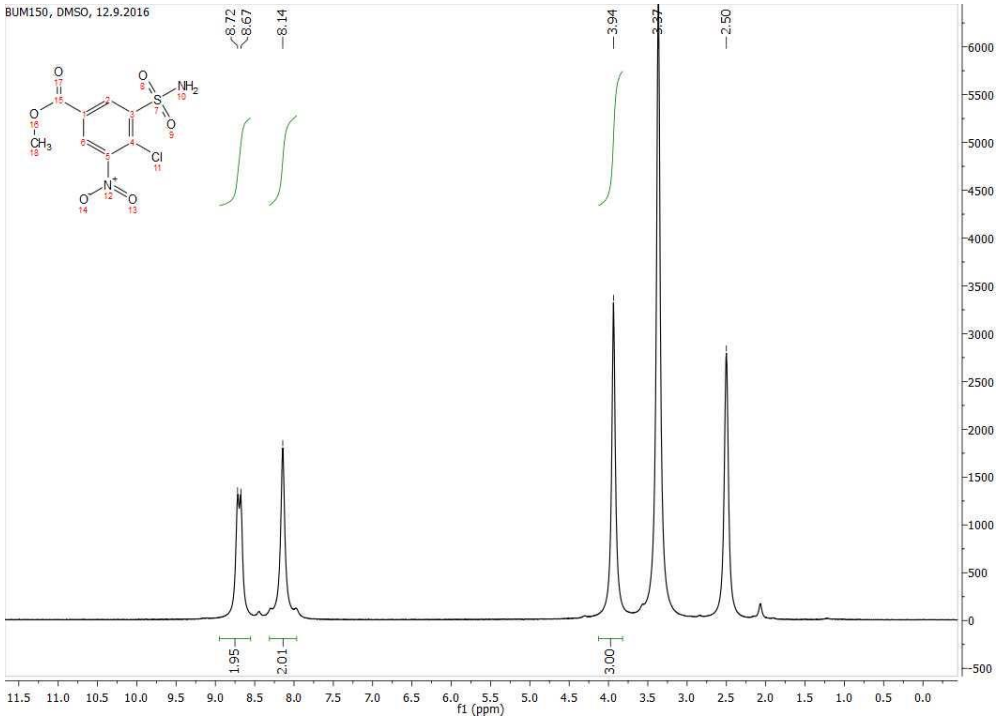
m/z	351	M^+ 91%
	239	76%
	165	100%
	139	86%
	77	97%

HRMS - Analysis

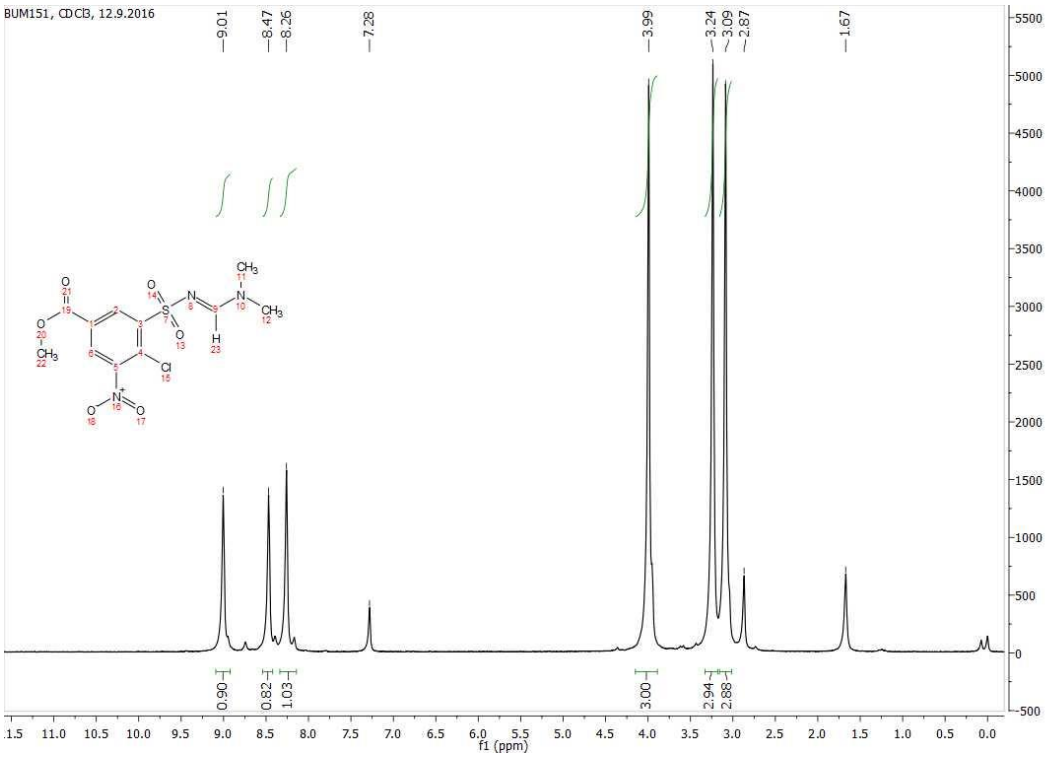
Mass.m/z	Molecular formula	m/z	err [ppm]
374.0419	$C_{14}H_{13}N_3NaO_6S$	374.0417	-0.3

8 Analytics

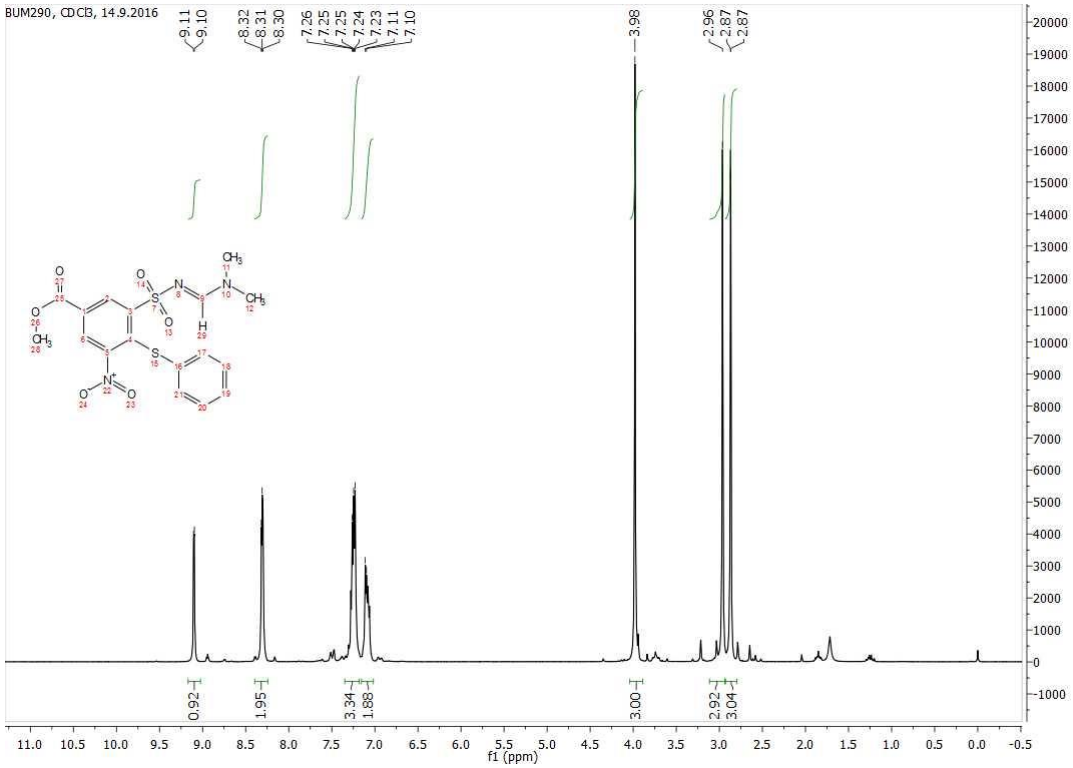
Compound **1** ^{1}H-NMR (DMSO-d_6)



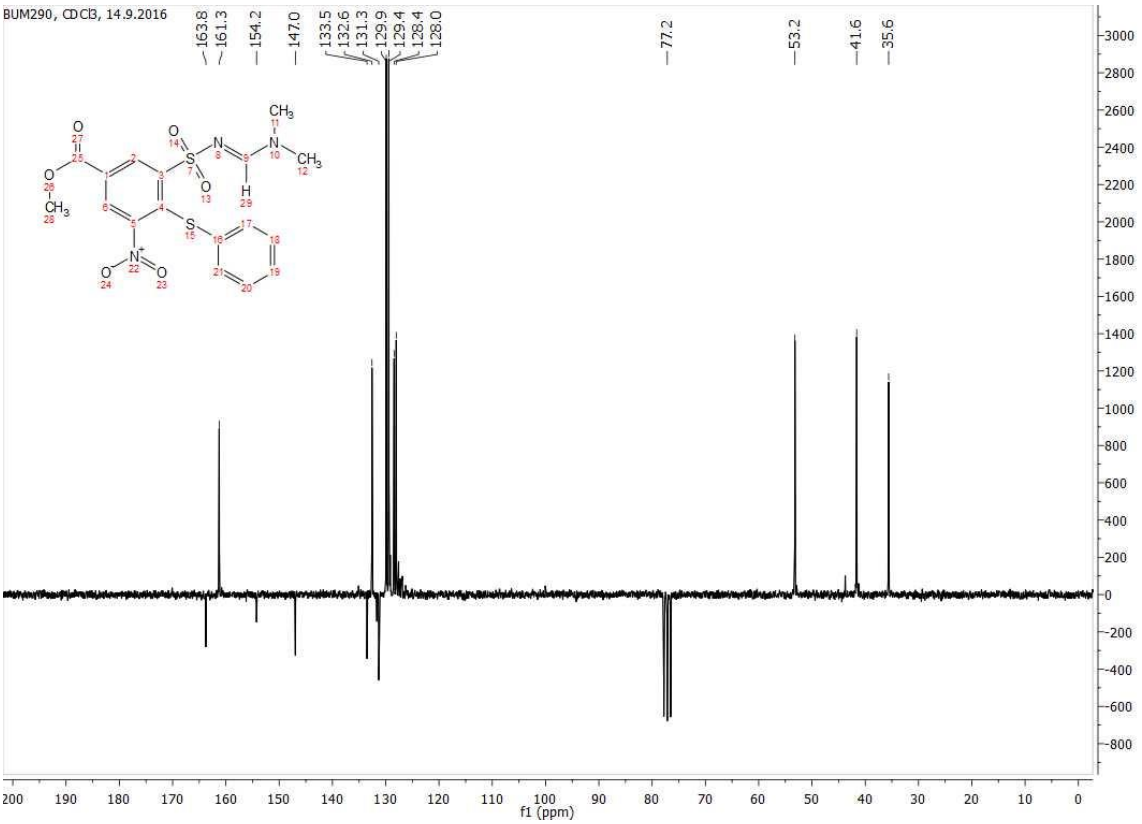
Compound **2** ^{1}H-NMR ($CDCl_3$)



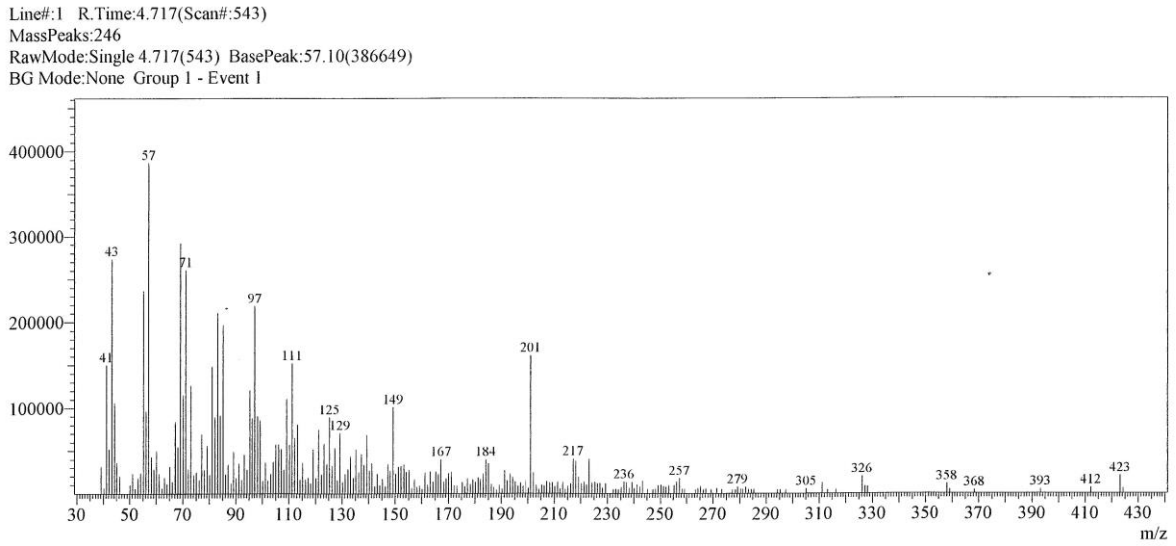
Compound **3** ^{1}H-NMR ($CDCl_3$)



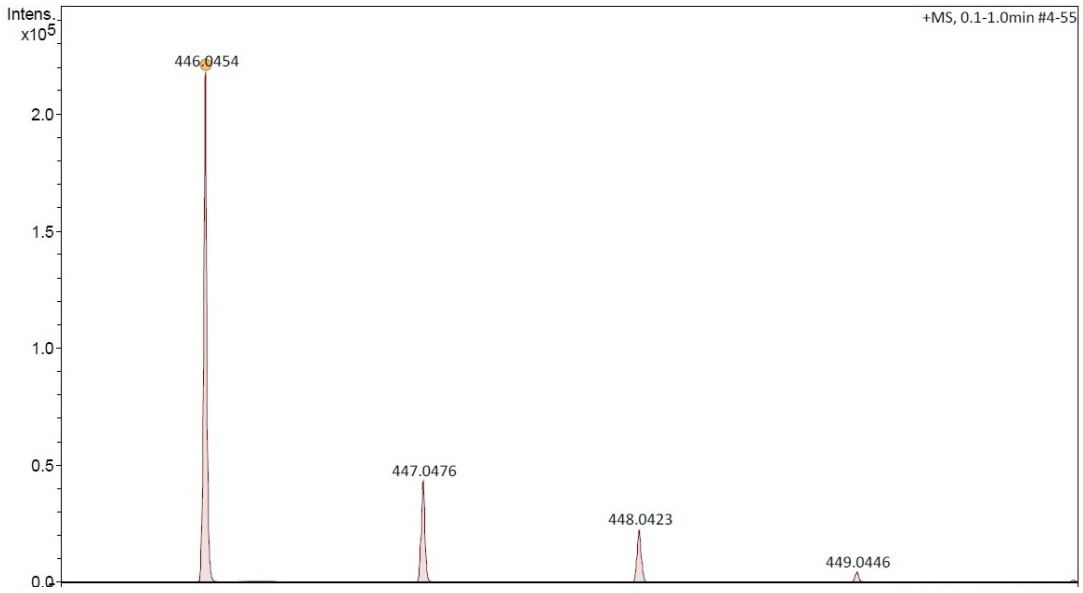
Compound **3** ^{13}C-NMR ($CDCl_3$)



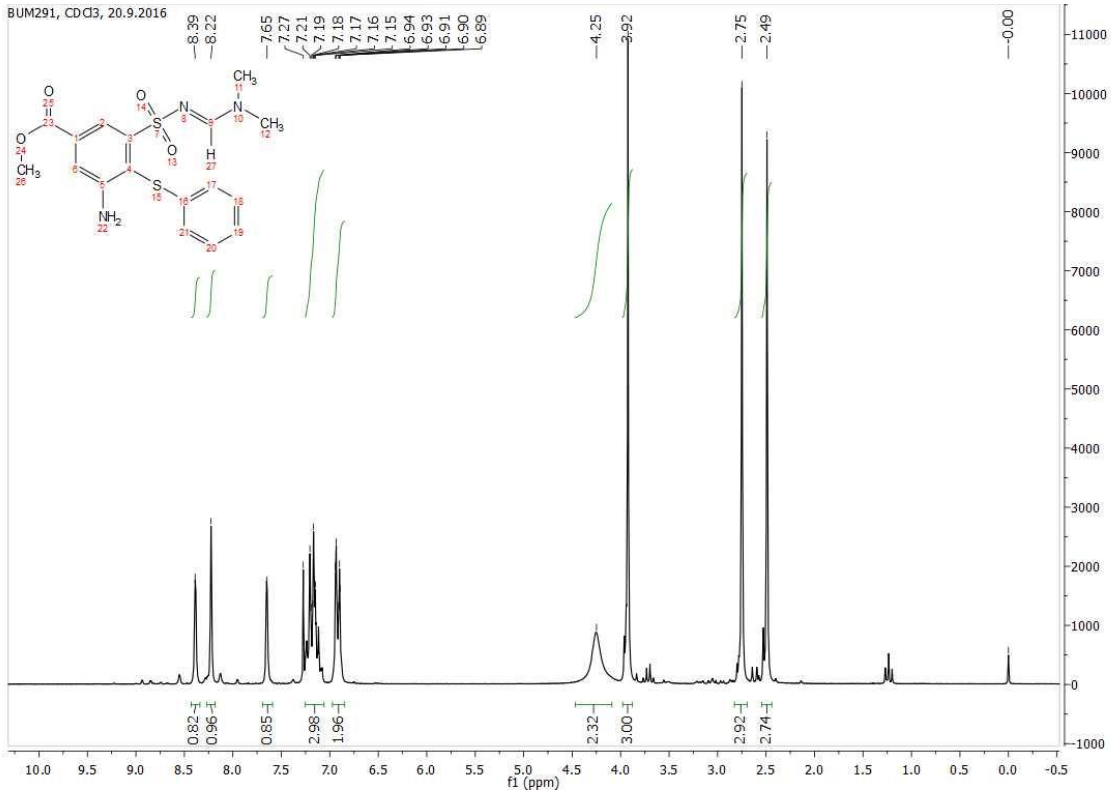
Compound **3** mass spectrum



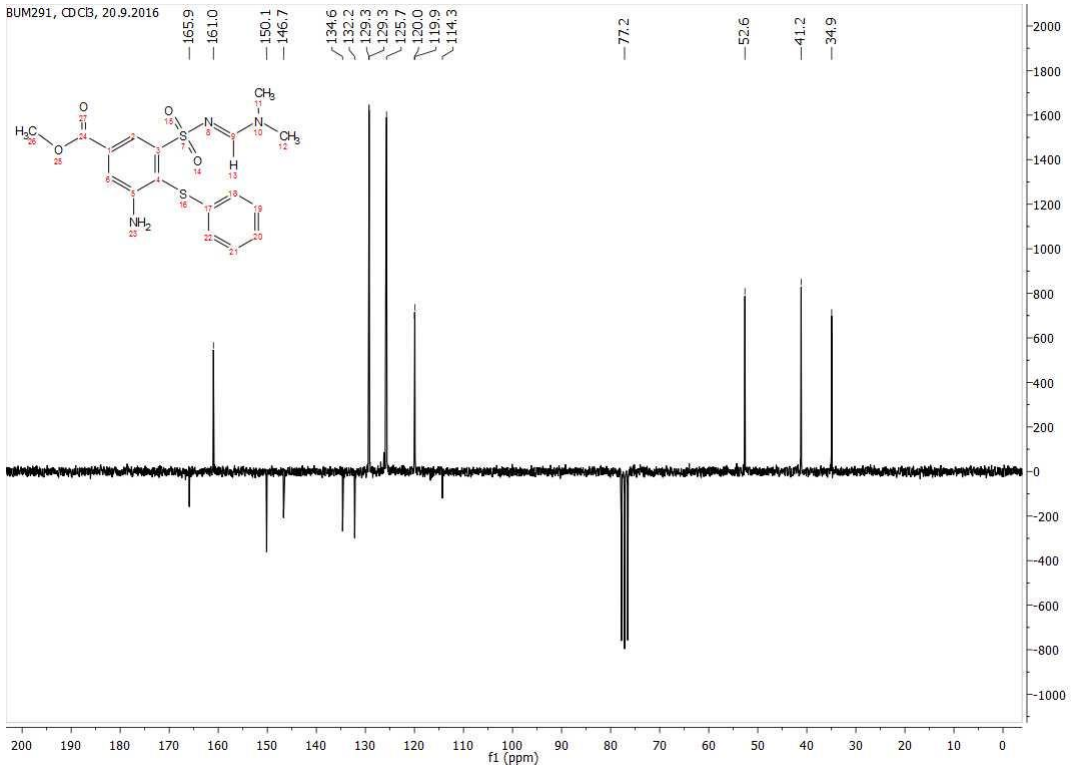
Compound **3** HRMS



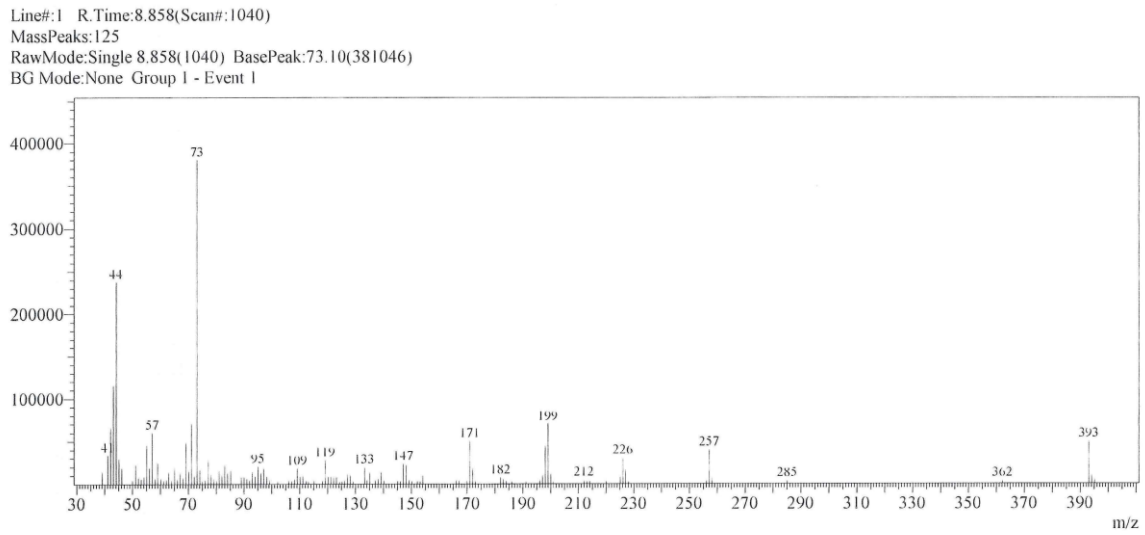
Compound **4** ^{1}H-NMR ($CDCl_3$)



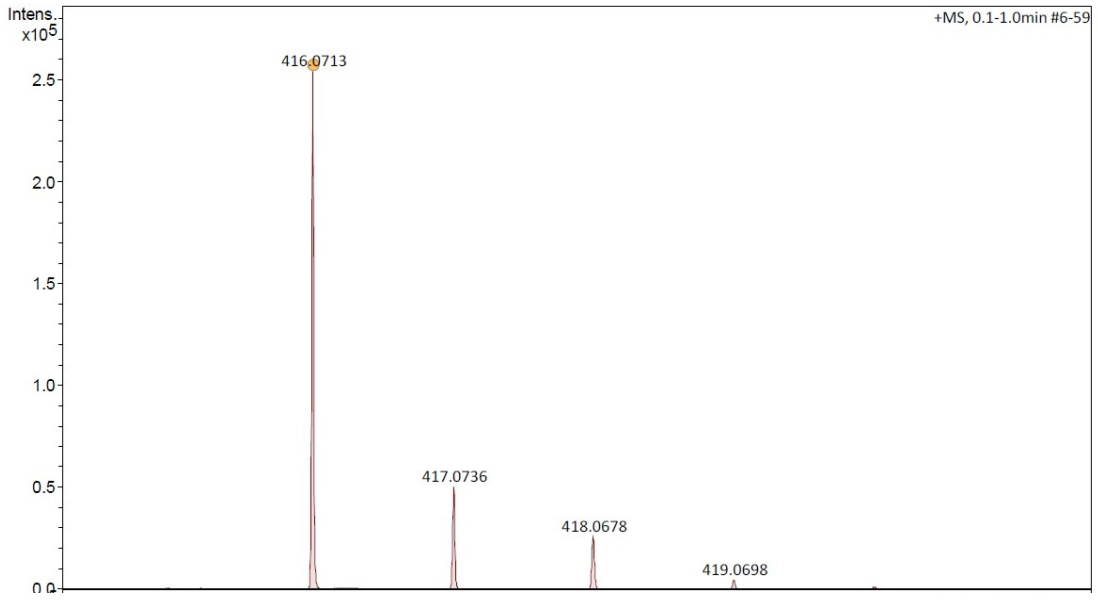
Compound **4** ^{13}C-NMR ($CDCl_3$)



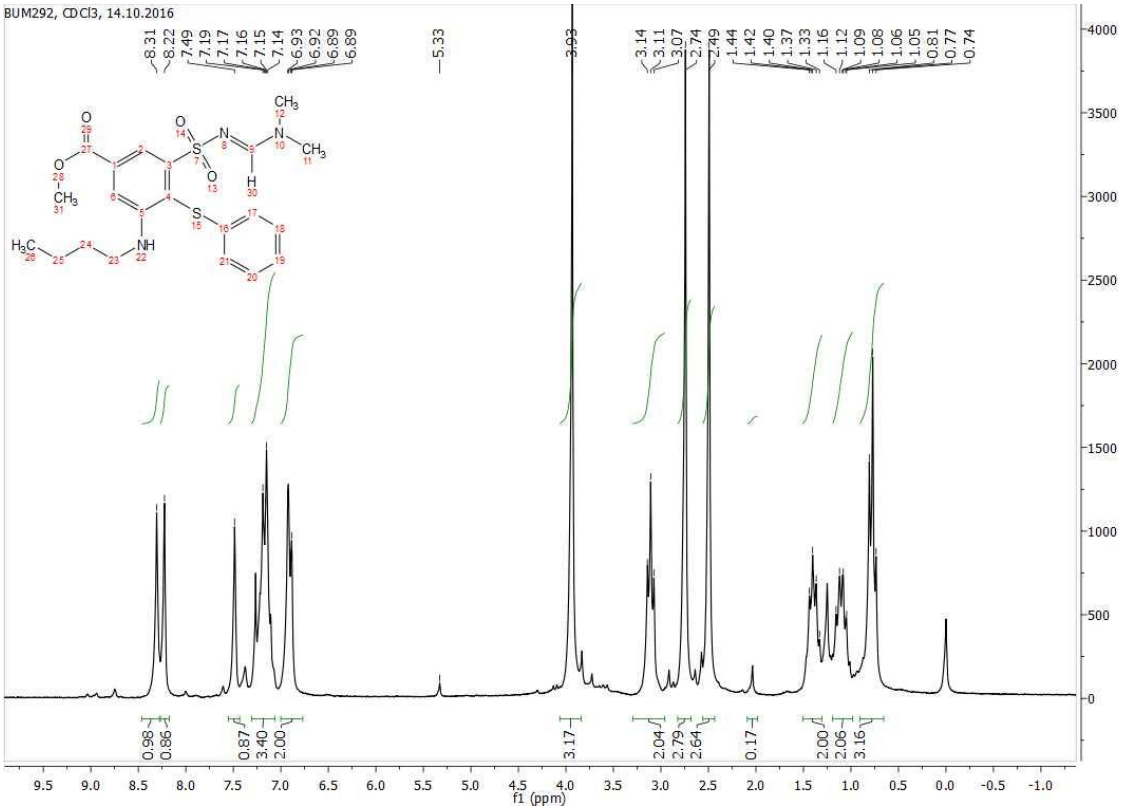
Compound **4** mass spectrum



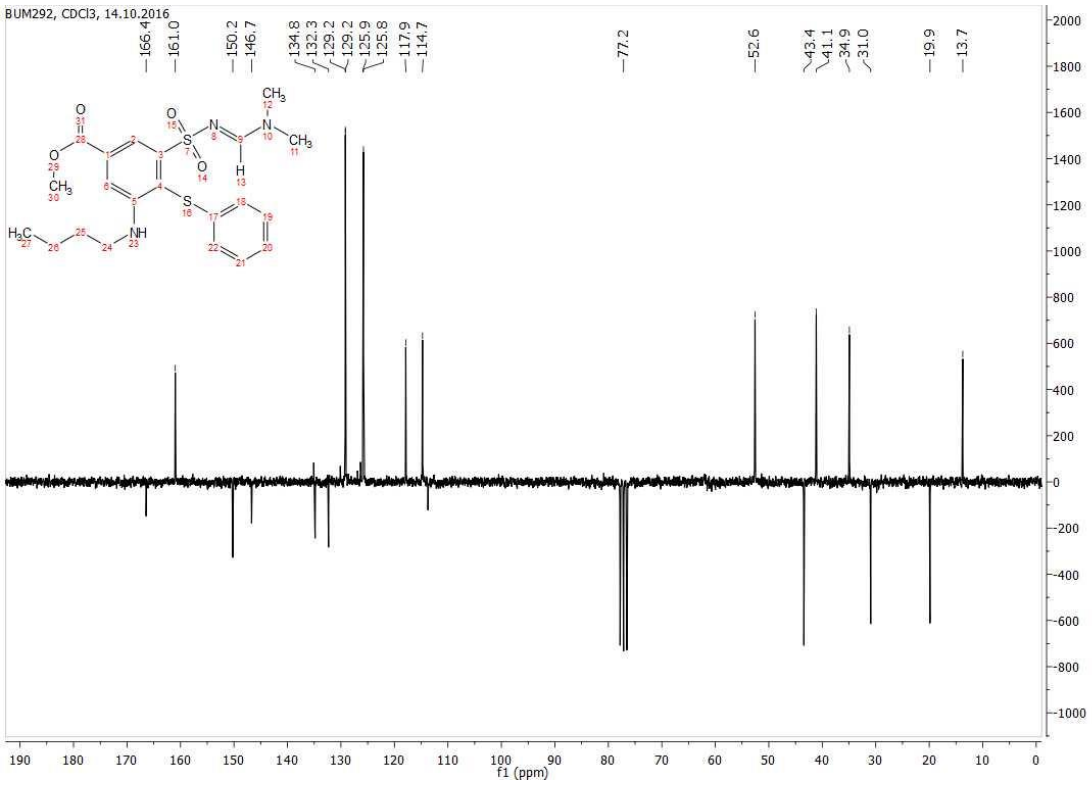
Compound **4** HRMS



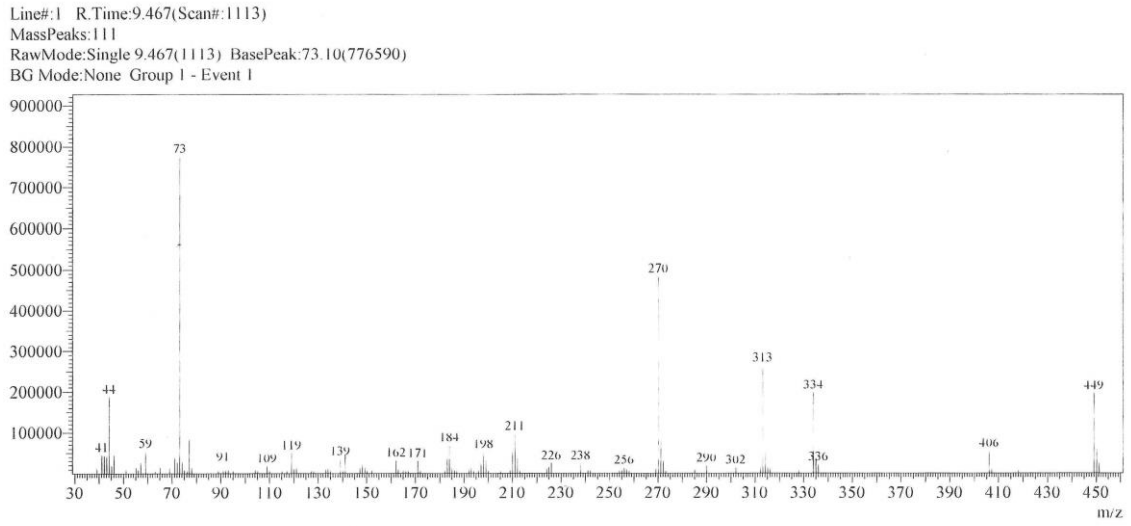
Compound **5** ^{1}H-NMR ($CDCl_3$)



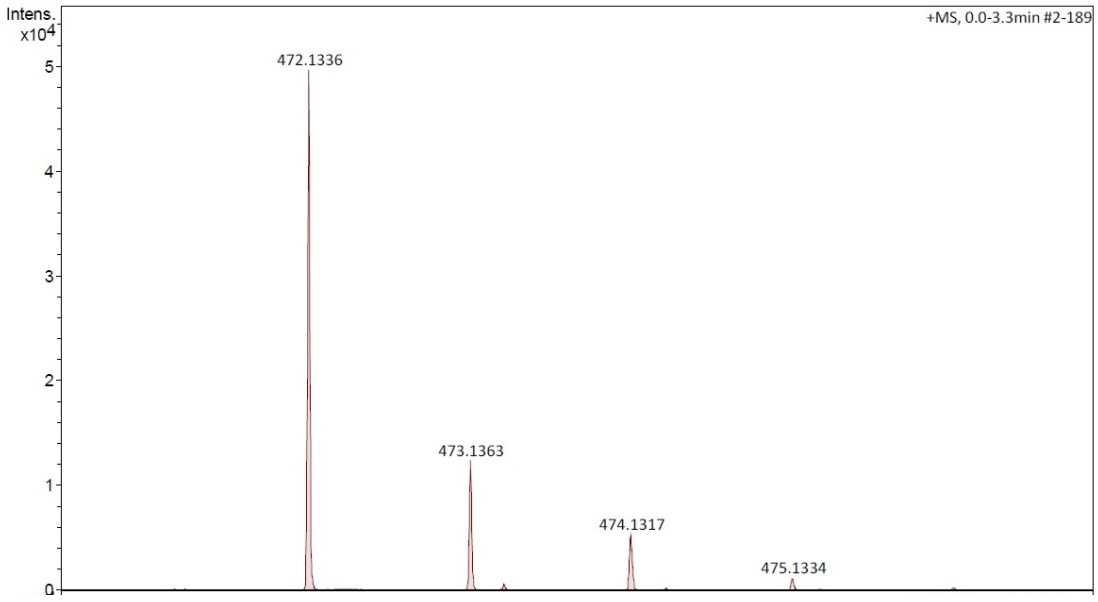
Compound **5** ^{13}C-NMR ($CDCl_3$)



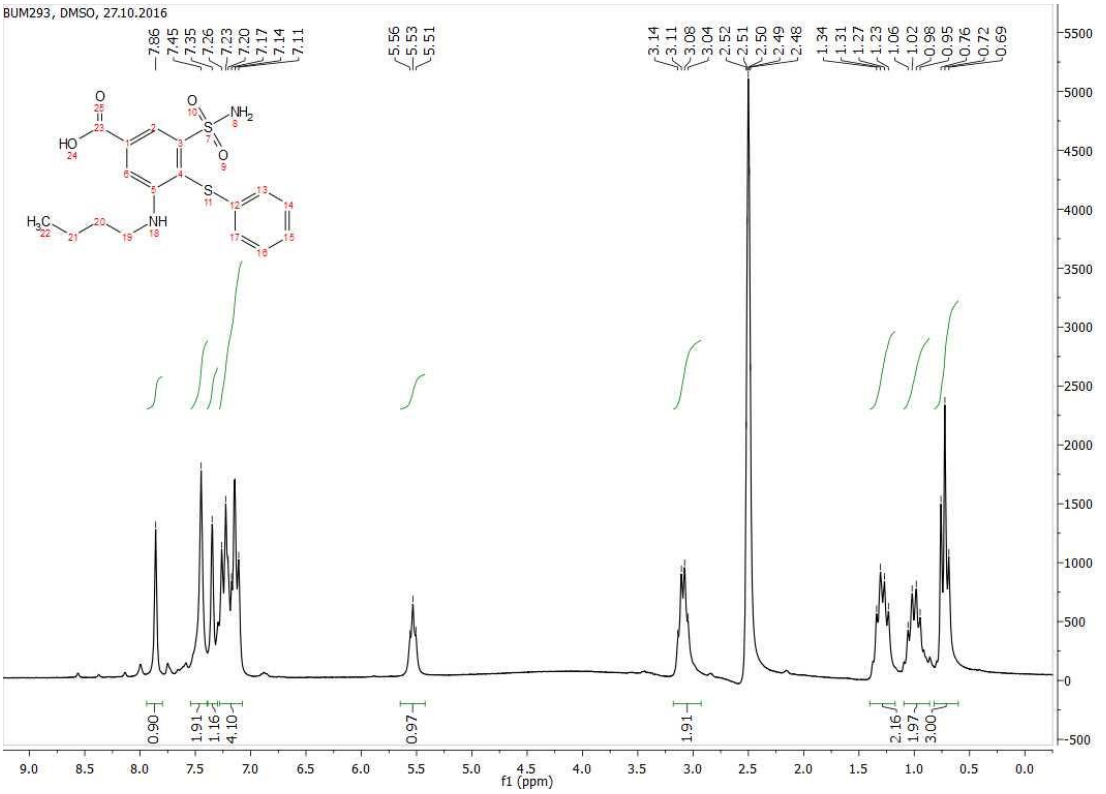
Compound **5** mass spectrum



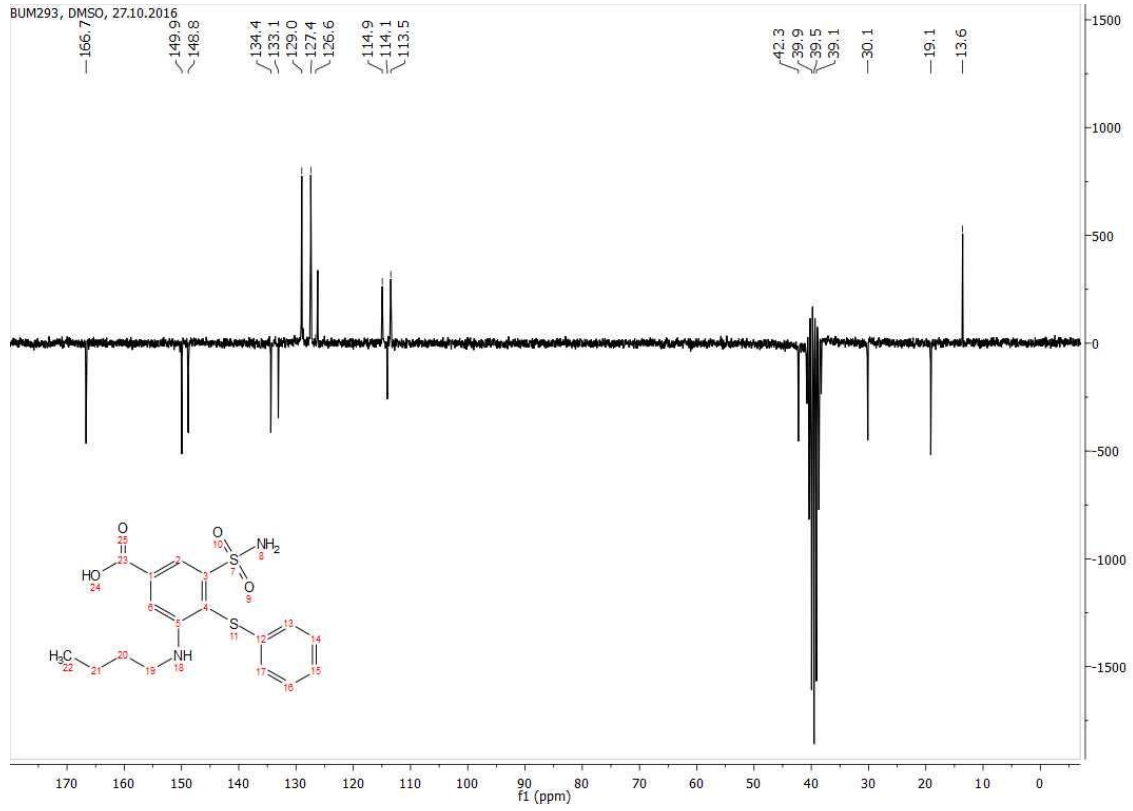
Compound **5** HRMS



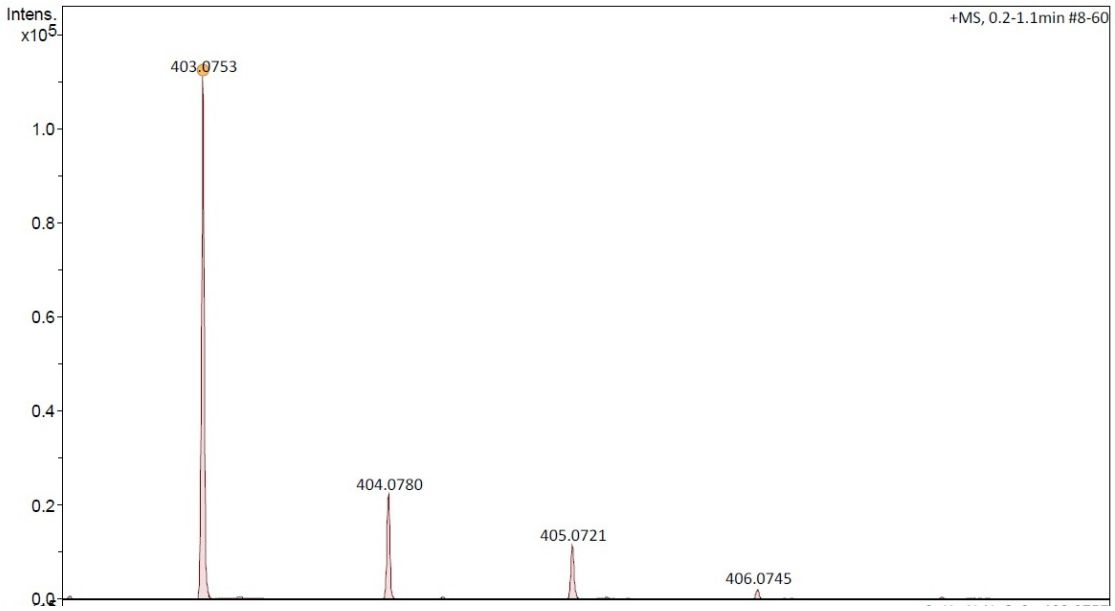
Compound **6** ^{1}H-NMR (DMSO-d_6)



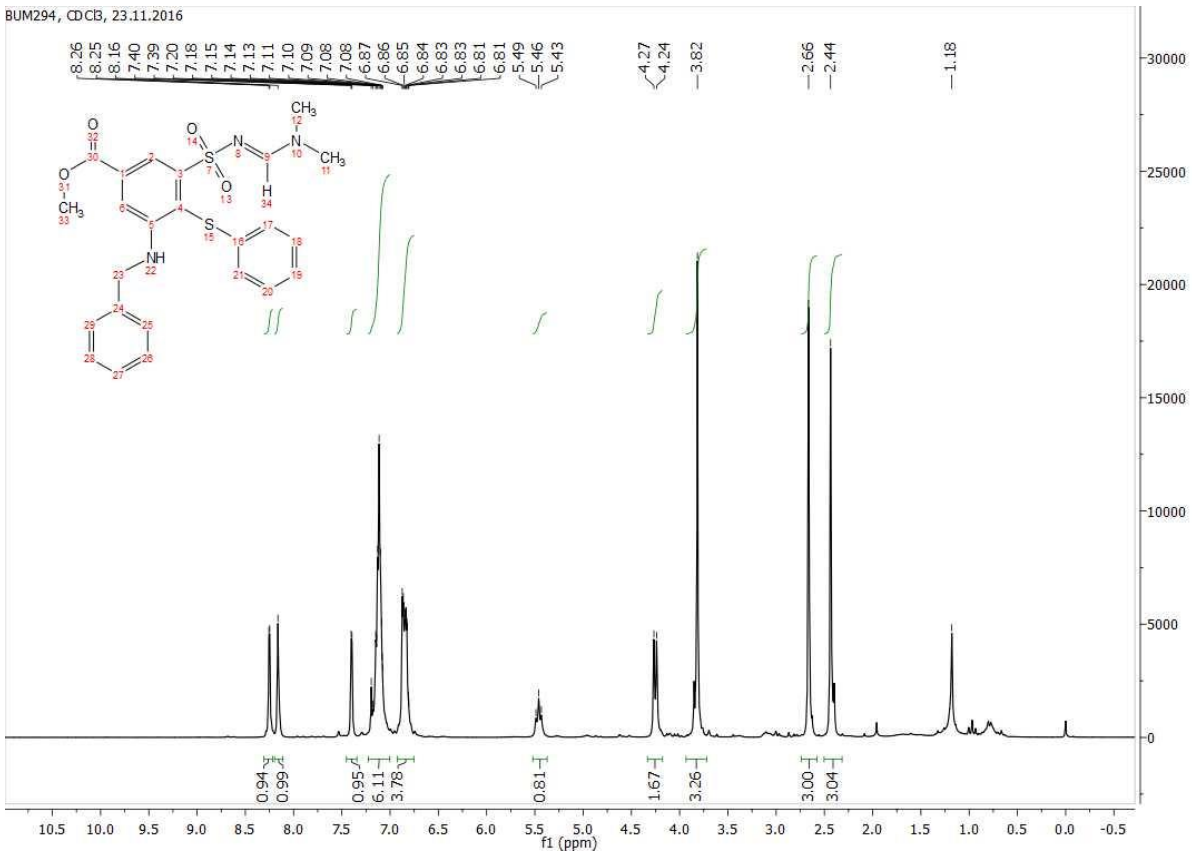
Compound **6** ^{13}C-NMR (DMSO-d_6)



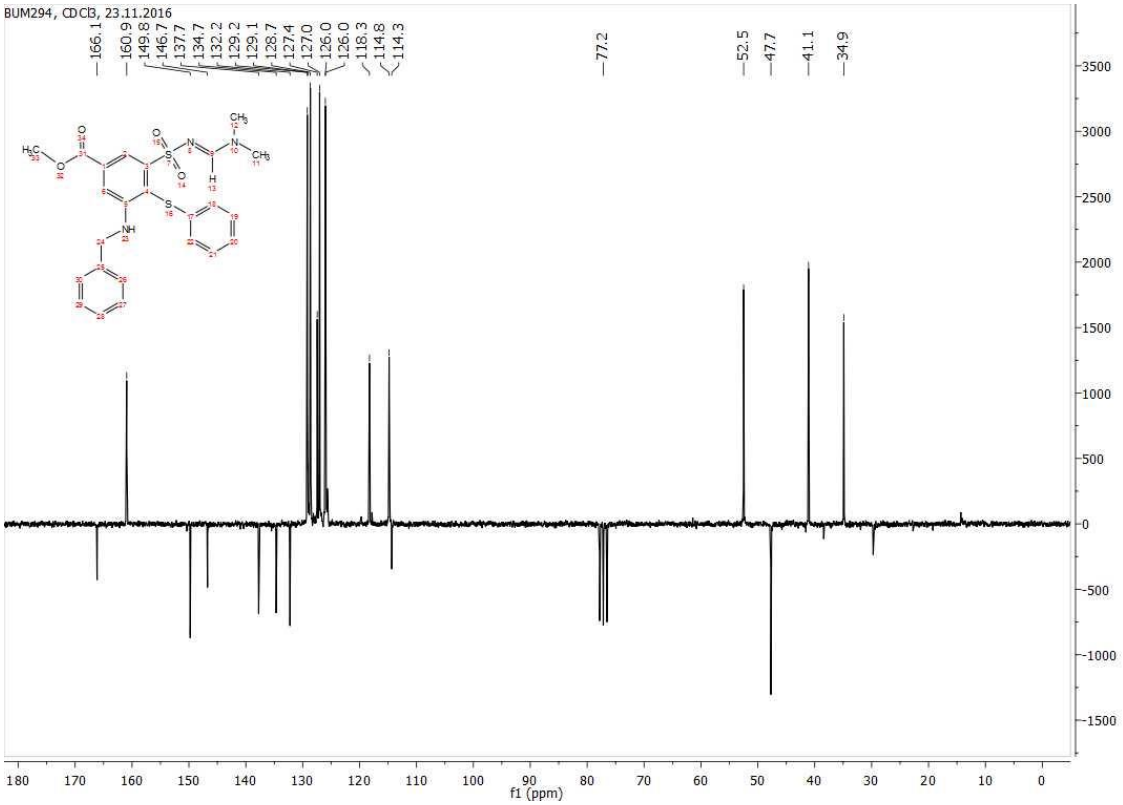
Compound **6** HRMS



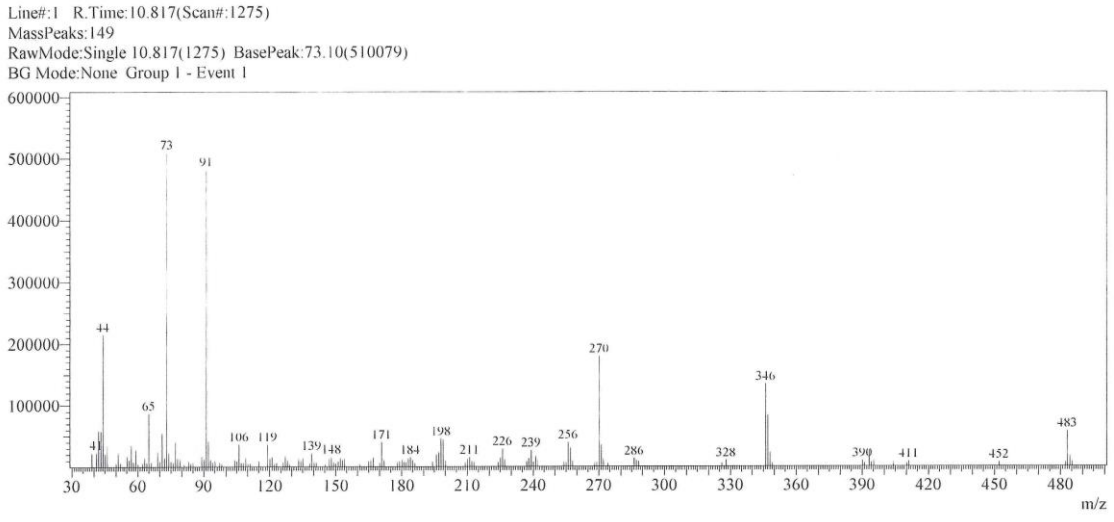
Compound **7** ^{1}H-NMR ($CDCl_3$)



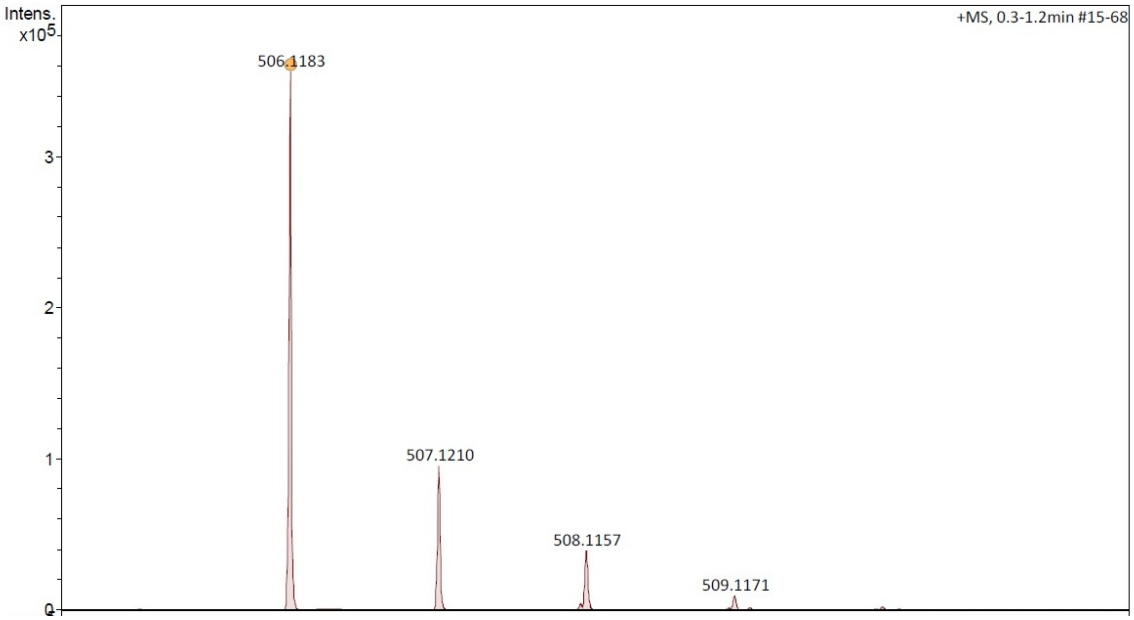
Compound **7** ^{13}C-NMR ($CDCl_3$)



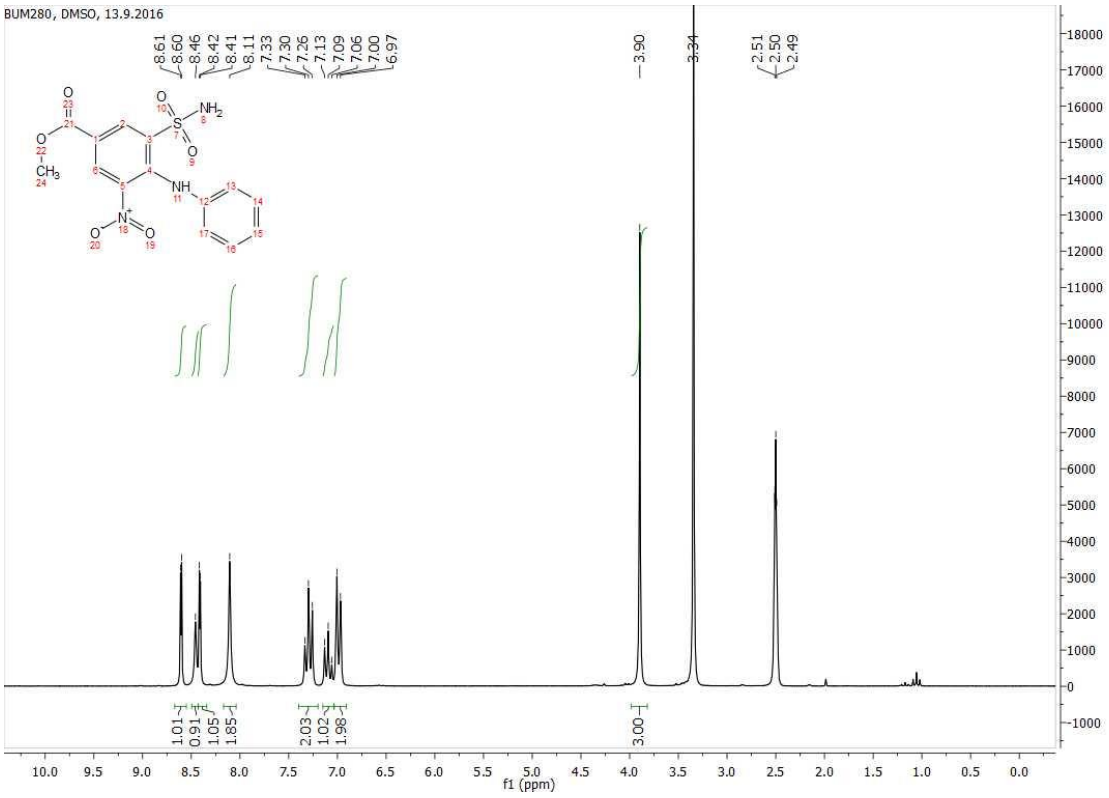
Compound **7** mass spectrum



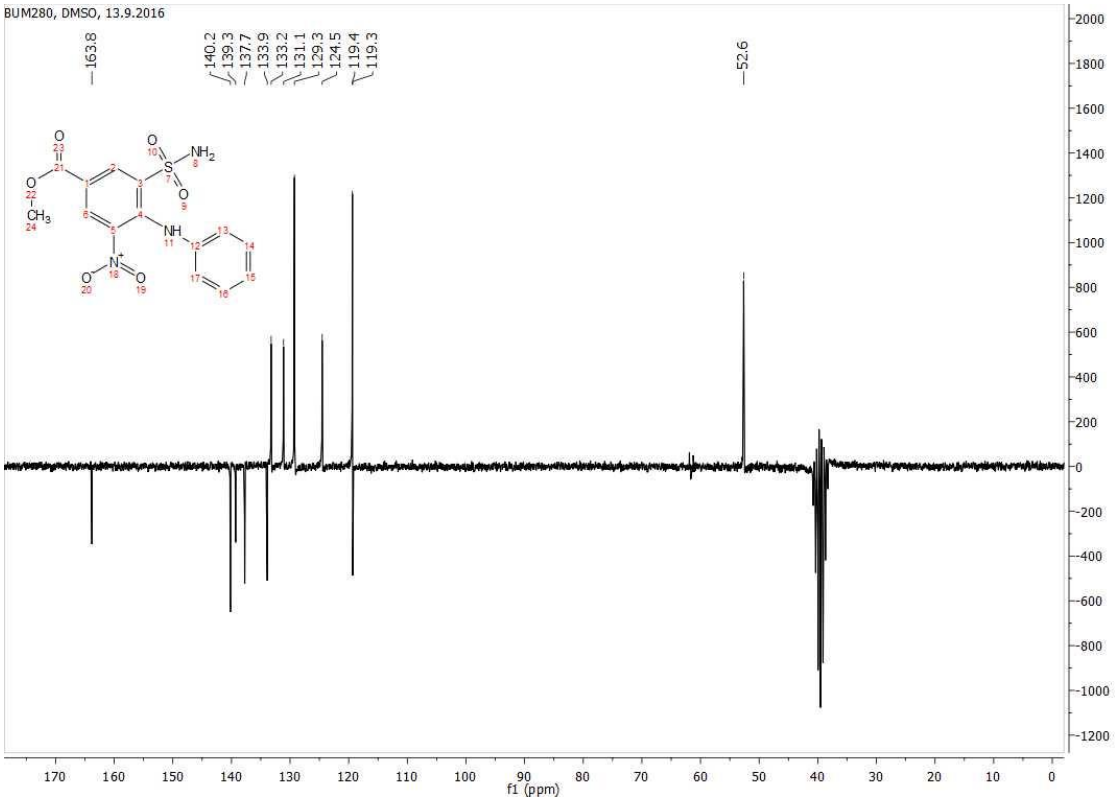
Compound **7** HRMS



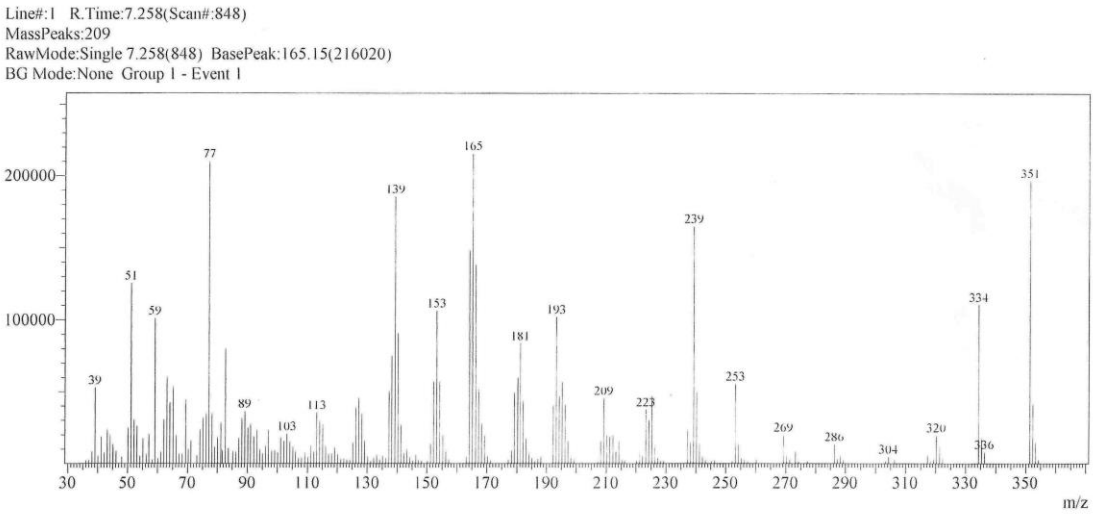
Compound **8** ^{1}H-NMR (DMSO-d_6)



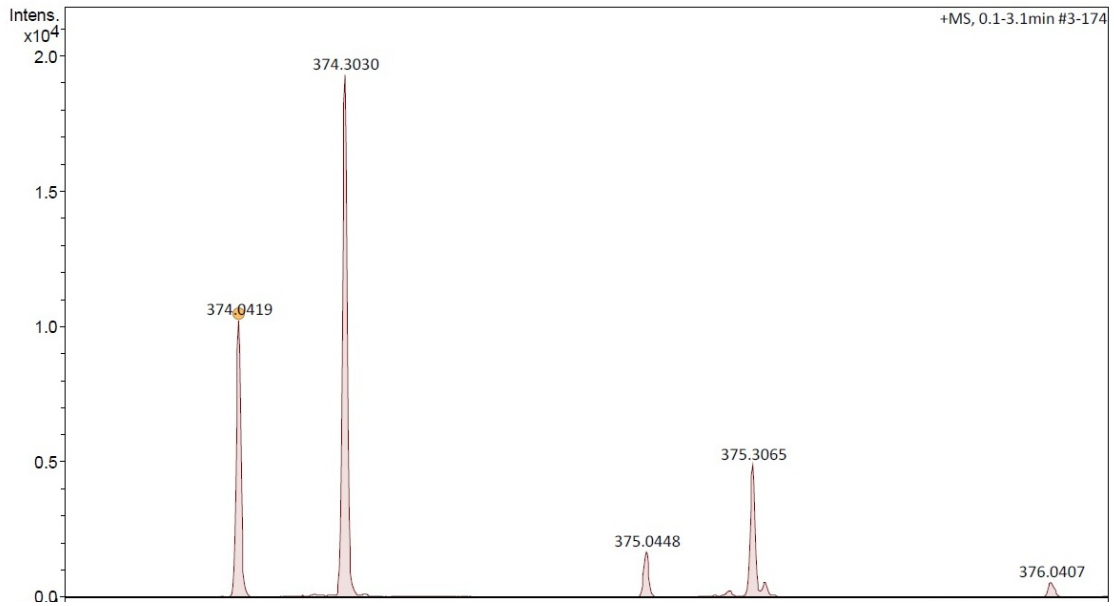
Compound **8** ^{13}C-NMR (DMSO-d_6)



Compound **8** mass spectrum



Compound **8** HRMS



8.1 Internal codes of compounds **1** - **8**

Compound **1** = BUM150

Compound **2** = BUM151

Compound **3** = BUM290

Compound **4** = BUM291

Compound **5** = BUM292

Compound **6** = BUM293

Compound **7** = BUM294

Compound **8** = BUM280

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