Amino Acids
DOI 10.1007/s00726-015-2099-6

ORIGINAL ARTICLE

Mass spectrometry study of *N*-alkylbenzenesulfonamides with potential antagonist activity to potassium channels

Carina C. Martins[1] · Carlos A. Zanutto Bassetto Jr.[1] · Jandyson M. Santos[2] · Marcos N. Eberlin[2] · Alvicler Magalhães[3] · Wamberto Varanda[4] · Eduardo R. Perez Gonzalez[1]

Received: 7 September 2015 / Accepted: 9 September 2015

Abstract Herein, we report the synthesis and mass spectrometry studies of several *N*-alkylbenzenesulfonamides structurally related to sulfanilic acid. The compounds were synthesized using a modified Schotten–Baumann reaction coupled with Meisenheimer arylation. Sequential mass spectrometry by negative mode electrospray ionization (ESI(−)-MS/MS) showed the formation of sulfoxylate anion (*m*/*z* 65) observed in the mass spectrum of *p*-chloro-*N*-alkylbenzenesulfonamides. Investigation of the unexpected loss of two water molecules, as observed by electron ionization mass spectrometry (EI-MS) analysis of *p*-(*N*-alkyl)lactam sulfonamides, led to the proposal of corresponding fragmentation pathways. These compounds showed loss of neutral iminosulfane dioxide molecule (M-79) with formation of ions observed at *m*/*z* 344 and 377. These ions were formed by rearrangement on ESI(+)-MS/MS analysis. Some of the molecules showed antagonistic activity against Kv3.1 voltage-gated potassium channels.

Keywords *N*-alkylbenzenesulfonamides · Mass spectrometry · ESI-MS/MS · Kv3.1

Handling Editor: J. Leban.

Electronic supplementary material The online version of this article (doi:10.1007/s00726-015-2099-6) contains supplementary material, which is available to authorized users.

✉ Eduardo R. Perez Gonzalez
eperez@fct.unesp.br

[1] Laboratório de Química Orgânica Fina, Departamento de Física, Química e Biologia, Faculdade de Ciências e Tecnologia, Universidade Estadual Paulista (UNESP), C.P. 467, Presidente Prudente, SP 19060-900, Brazil

[2] ThoMSon Mass Spectrometry Laboratory, Institute of Chemistry, University of Campinas, UNICAMP, Campinas, SP 13083-970, Brazil

[3] Departamento de Química Inorgânica, Instituto de Química, Universidade de Campinas, UNICAMP, Campinas 13083-970, SP, Brazil

[4] Department of Physiology, School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP 14049-900, Brazil

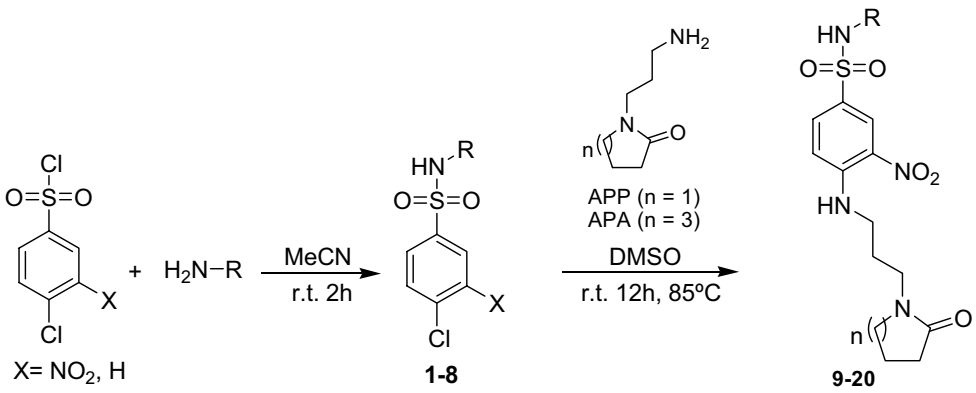
Introduction

The benzene sulfonamides are amides of sulfanilic acid, characterized by the presence of Ar–SO_2–NR′R″ groups. The most widely used synthetic method for the preparation of sulfonamides is by using Schotten–Baumann reaction conditions, which involves the condensation of acid chloride with an amine in the presence of a base (Deng and Mani 2006; Smith and March 2001). These compounds are known to be antibacterial agents (Joshi and Khosla 2003) and are also reported to inhibit the activity of carbonic anhydrase and cyclooxygenases (COX-1 and COX-2) (Supuran et al. 1998; Dannhardt and Kiefer 2001; Li et al. 1995). They also have anticancer (Scozzafava et al. 2002), anti-HIV (Bromidge et al. 2002), antifungal and antituberculosis (Goodman 1996) activity.

The structure of sulfonamides is very similar to *para*-aminobenzoic acid (PABA), and they inhibit the bacterial growth and multiplication by competition with PABA for the active site of the dihydropteroate synthase enzyme, which is an essential precursor for bacterial synthesis of folic acid, an important precursor in the synthesis of nucleic acids (Supuran et al. 1998). PABA derivatives and analogs are known to be local anesthetics (LA) (Gonçalves et al. 2011) and block nerve conduction, reversibly binding

Published online: 22 September 2015

Scheme 1 Synthesis of *p*-chloro-*N*-alkylbenzenesulfonamides (**1**–**8**) and *p*-(*N*-alkyl) lactam sulfonamides (**9**–**20**)



R = furfuryl, butyl, benzyl, ciclohexyl, 4-clorobenzyl and 2,4-diclorobenzyl.

to voltage-dependent sodium channels present in excitable cell membranes (Gouauax and Mackinnon 2005).

Ion channels are a class of transmembrane proteins responsible for promoting the selective passage of ions, such as sodium (Na^+), potassium (K^+), chloride (Cl^-), and calcium (Ca^{2+}) (Gonçalves et al. 2011; Lü and Frank 2008). The selective movement of ions across cell membranes is related to many physiological processes, such as the control of the neurons and muscle excitability, the generation and propagation of action potentials, release of neurotransmitters, and setting of cardiac rhythms and muscle tones. They also play an important role in hormone secretion, cell volume regulation, gene expression, immune response, and cell division. Ion channel dysfunctions are involved in several pathological conditions such as hypertension, pain, insomnia, diabetes, and constipation (Lü and Frank 2008). In this context, it has been shown that malfunctioning of voltage-dependent potassium channels (Kv) is implicated in cardiac arrhythmia, epilepsy, multiple sclerosis, diabetes, and arthritis and thus can be taken as important therapeutic targets (Wulf et al. 2009) for the control of such pathological conditions. In the face of these findings, the development of novel compounds whose therapeutic action is directed to these types of voltage-dependent ionic channels seems crucial. Benzene sulfonamides were reported as potent and selective blockers of Kv1.5 (Gross et al. 2007; Lloyd et al. 2007; Olsson et al. 2014) and its use has been suggested in the treatment of atrial fibrillation (Wulf et al. 2009), the most common type of cardiac arrhythmia. Recently, Olsson et al. (2014) reported a series of lactam sulfonamides as potent inhibitors of Kv1.5 (Ye et al. 2009).

Herein, we show the synthesis and chemical characterization by different spectroscopic and spectrometric techniques of several benzene sulfonamides with potential activity against potassium ion channels. A number of synthesized sulfonamides have the *N*-(aminoalkyl) lactam moiety in their structures. Lactam groups were *N*-(3-aminopropyl)-2-azepanone (APA) and *N*-(3-aminopropyl)-2-pyrrolidinone (APP), which have been described as inhibitors of human tryptase, an important mediator in asthma (Zhao et al. 2004), and HIV-1 protease, respectively (Ghosh et al. 2009).

All sulfonamides were studied by mass spectrometry technique using ion fragmentation in the gas phase providing useful information regarding the reaction mechanism, in addition to classical analyses using electron ionization as ion source (EI-MS) (Irikura and Todua 2014). There are some studies using electrospray ionization mass spectrometry (ESI-MS) of the cationic sulfonamide derivatives (Sun et al. 2007; Hu et al. 2010), and only recently investigations into negative ion conditions have been reported (Hibbs et al. 2013; Hu et al. 2008).

The techniques used in these studies are: mass spectrometry with electron ionization (EI-MS), high-resolution mass spectrometry (HRMS) in the positive (ESI(+)-MS) and negative (ESI(−)-MS) modes and tandem experiments (ESI-MS/MS).

Results and discussion

Synthesis of *N*-alkylbenzenesulfonamides

Synthesis of the *p*-chloro-*N*-alkylbenzenesulfonamides (**1**–**8**) was performed by reaction between a benzenesulfonyl chloride with different amines, according to the literature (Lü and Frank 2008). Because of the pharmacological importance of the lactam group (Zhao et al. 2004; Ghosh et al. 2009) and due to a recent report of Olsson et al. (2014) about lactam sulfonamides as potent inhibitors of Kv1.5 (Olsson et al. 2014) channels, the second step of this work was to use *p*-chloro-*N*-alkylbenzenesulfonamides as intermediate for the synthesis of *p*-(*N*-alkyl)lactam sulfonamides (**9**–**20**) (Scheme 1). The formation of *p*-(*N*-alkyl) lactam sulfonamides was performed through a nucleophilic

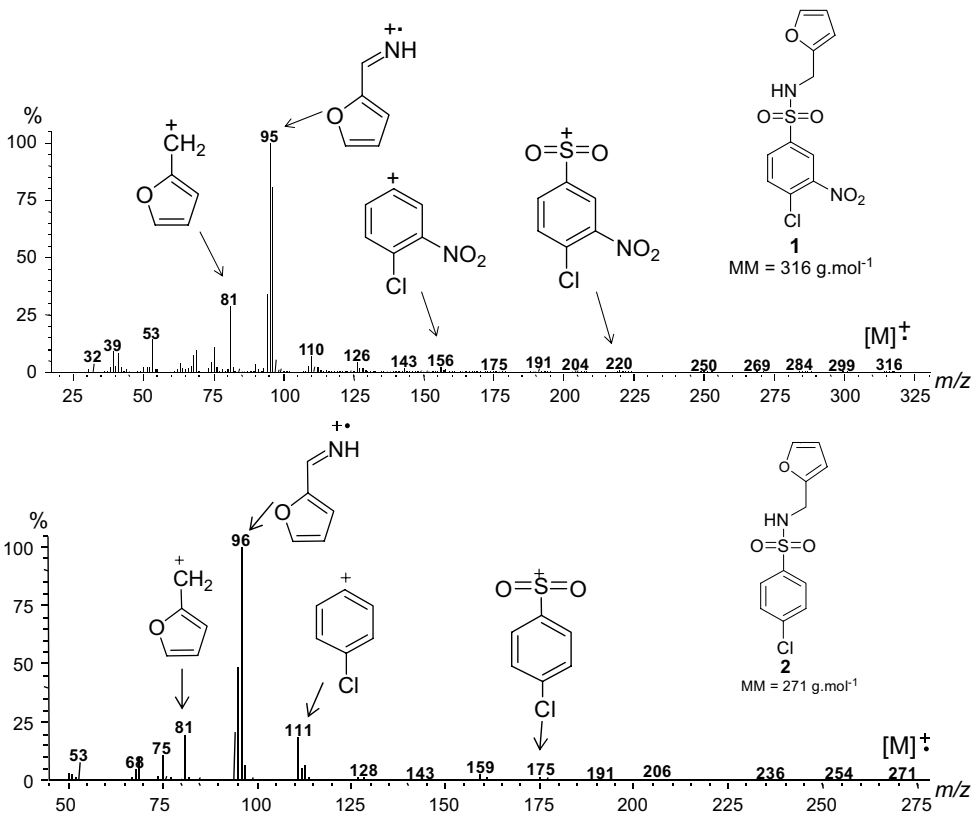


Fig. 1 EI-MS spectrum of compounds **1** and **2**

aromatic substitution reaction (S_NAr) by modifying the methodology of S_NAr reactions previously described by Gonçalves et al. (2011). Compounds **2** and **4** were also reacted with amino lactams, but due to the absence of nitro group no reaction occurred.

Mass spectrometry study of the *p*-chloro-*N*-alkylbenzenesulfonamides (1–8)

An MS study was performed for *p*-chloro-*N*-alkylsulfonamides (**1**–**8**). As an example of the results obtained, two cases can be discussed, compounds 4-chloro-3-nitro-*N*-furfurylbenzenesulfonamide (**1**) and 4-chloro-*N*-furfurylbenzenesulfonamide (**2**). These compounds were chosen for comparison of their MS spectra to know the influence of the nitro group in the fragmentation mechanisms.

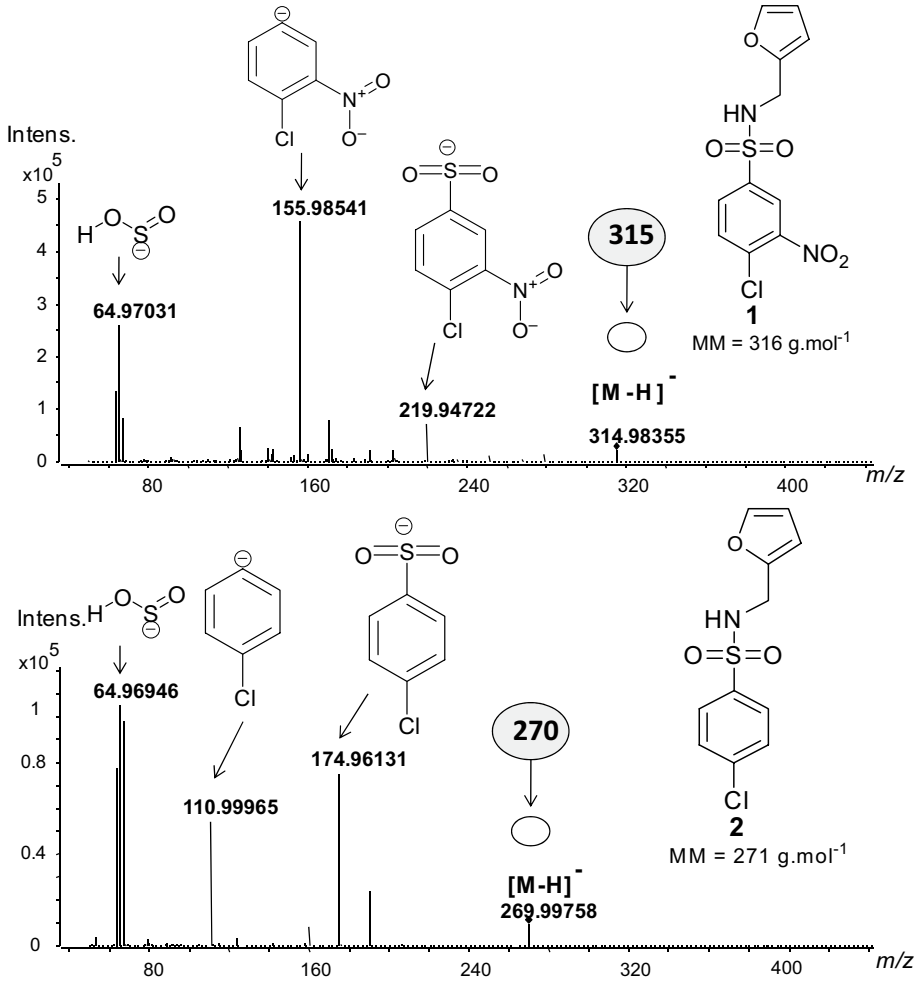
MS study of compounds **1** and **2** was performed by EI-MS, ESI(−)-MS and ESI(−)-MS/MS. Figure 1 shows the EI-MS spectrum of compound **1**. Molecular ion was observed at *m*/*z* 316. The base ion at *m*/*z* 95 corresponds to furfurylimine. Furfuryl ion of *m*/*z* 81 and other fragment ions were also observed in the spectrum.

The EI-MS spectrum of compound **2** showed the same fragmentation pattern as described for compound **1** (spectrum is shown in supplementary material) and, therefore, the group at position 3 in the benzene ring (H or NO_2) seems not to change the fragmentation mechanism of *p*-chloro-*N*-alkylbenzenesulfonamides (**1**–**8**).

The ESI-MS in the negative ion mode of the compounds **1** and **2** allowed the observation of compounds as intact deprotonated molecules with their corresponding isotopic patterns of the ions with *m*/*z* 315 and *m*/*z* 317 for **1** and *m*/*z* 270 and *m*/*z* 272 for **2** (corresponding to chlorine isotope pattern). The deprotonated molecules were selected and fragmented by sequential MS experiments (ESI(−)-MS/MS). Spectrum of **1** showed the 4-chloro-3-nitrobenzyl anion of *m*/*z* 156 as the 100 %. Fragment anions of *m*/*z* 220 (4-chloro-3-nitrobenzenesulfonyl anion) and *m*/*z* 65 corresponding to sulfoxylate anion were also observed (Fig. 2). On the other hand, spectrum of **2** showed sulfoxylate anion (*m*/*z* 65) as the base anion. Fragment anions of *m*/*z* 111 and 175 were attributed to 4-chlorobenzyl and 4-chlorobenzenesulfonyl anions, respectively (Fig. 3).

The fragmentation path shown by anionic sulfonamides involved the formation of sulfoxylate ion of *m*/*z* 65 as described in Fig. 2, differently from the loss of neutral molecule of sulfur dioxide (SO_2) (Irikura and Todua 2014; Sun et al. 2007; Hibbs et al. 2013; Lloyd et al. 2007).

In view of the differences between the fragments observed (formation of sulfoxylate anion) and that reported by Hibbs et al. (2013), it is possible to propose a fragmentation mechanism to explain the formation of the anion HSO_2^- with *m*/*z* 65. The proposed mechanism (Scheme 2) showed the loss



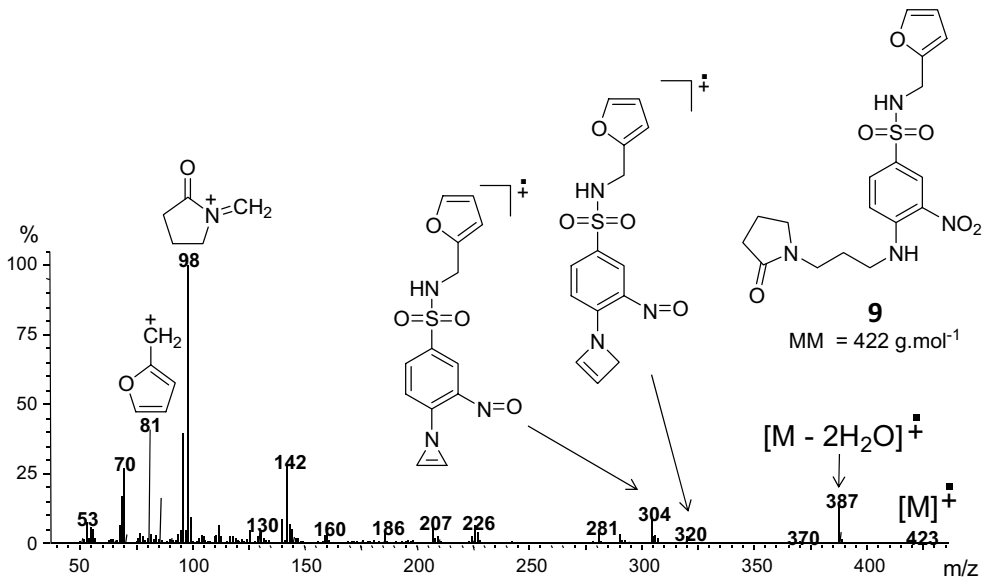
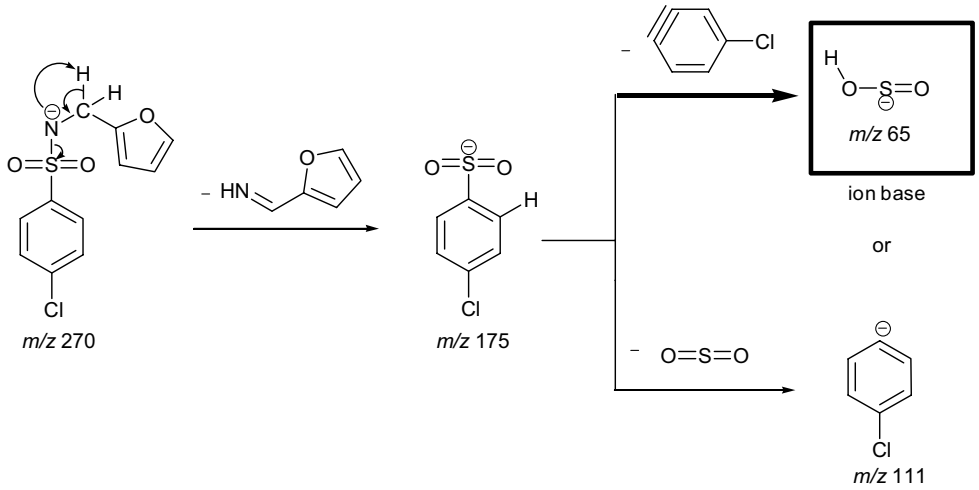


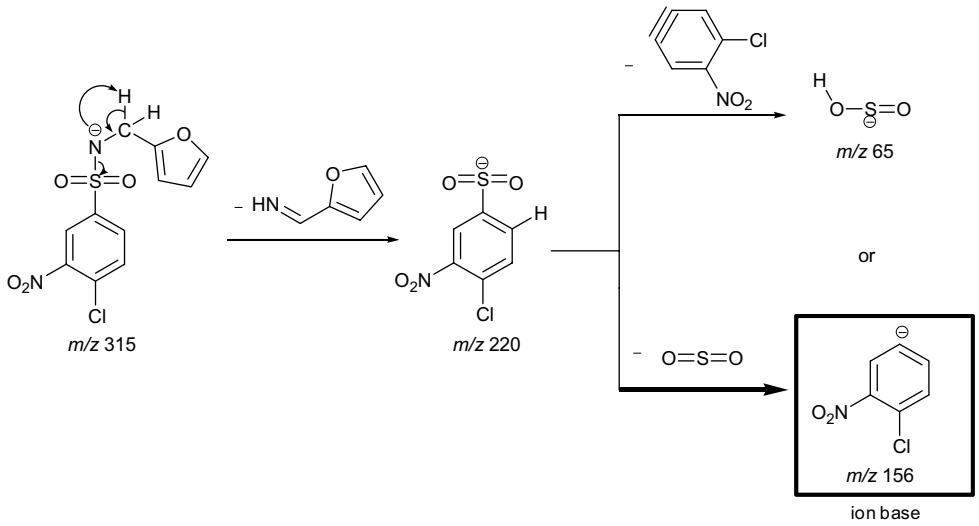
Fig. 2 ESI(−)-MS/MS spectra of compounds **1** and **2**

Fig. 3 EI-MS spectrum of compound **9**

Scheme 2 The proposed mechanism for the formation of sulfoxylate anion (SO_2H^- with *m/z* 65) of compound **2**



Scheme 3 The proposed mechanism for the formation of the sulfoxylate anion (SO_2H^- with *m/z* 65) for compound **1**



of neutral furfurylimine molecule generating the fragment anion of *m/z* 175, which was attributed to 4-chlorobenzenesulfonyl anion. This anion can undergo an intramolecular rearrangement process resulting in a neutral loss of chlorobenzyne and formation of the sulfoxylate anion with *m/z* 65. The same fragment lost a neutral molecule of SO_2 and gave rise to the 4-chlorobenzyl anion of *m/z* 111 following the mechanism reported by Hibbs et al. (2013).

In the case of compound **2** (Scheme 2), where there is not a strong π-electron withdrawing group (as the NO_2 group) at position 3 of the benzene ring, the formation of anion fragment *m/z* 65 (base ion in the spectrum of **2**) and the neutral loss of chlorobenzyne molecule are the most favored processes.

In the spectrum of compound **1,** the presence of nitro group stabilized 2-chloronitrophenyl anion of *m/z* 156 (main anion) and the neutral loss of SO_2 as reported by Hibbs et al. (2013) (Scheme 3).

Our proposed mechanism (Schemes 2, 3) explains the behavior of a series of *p*-chloro-*N*-alkylsulfonamides under ionization at negative mode conditions. The formation of sulfoxylate anion was observed for all *p*-chloro-*N*-alkylsulfonamides (**1**–**8**). Synthesis and complete characterization can be found in the Supplementary Material.

Mass spectrometry study of the *p*-(*N*-alkyl) lactam sulfonamides (9–20)

Compounds **9** and **10** were chosen to exemplify the MS study of compounds **9**–**20**. EI-MS spectra of **9** and **10** (Figs. 3 and 4, respectively) show their respective molecular ions of *m/z* 423 and *m/z* 451. The formation of the base peak of *m/z* 98 to compound **9** and *m/z* 126 for compound **10** involves the breaking of bonds in the alkyl chain of the *N*-substituent (Fiedorow et al. 2005). The ion fragments formed by the transfer of two hydrogen atoms via

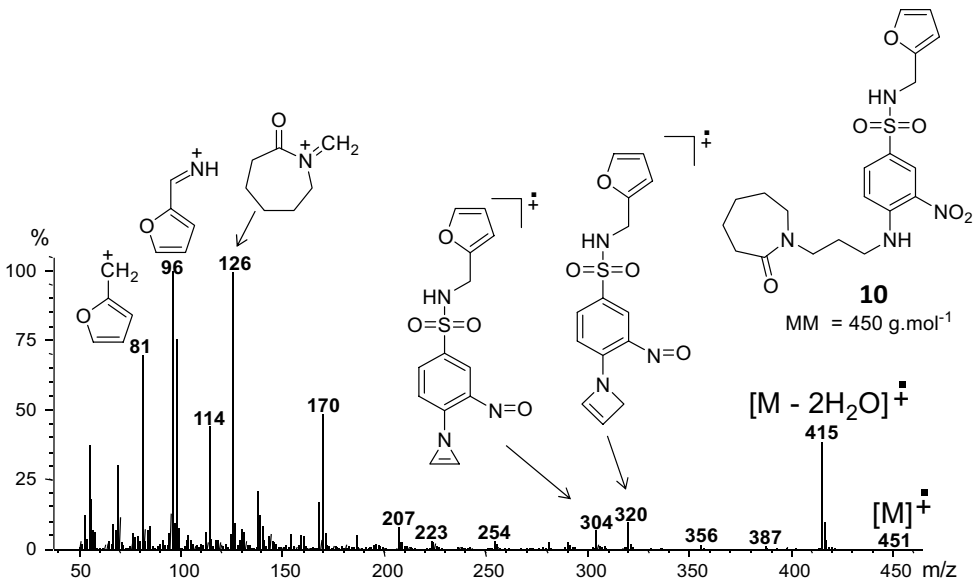
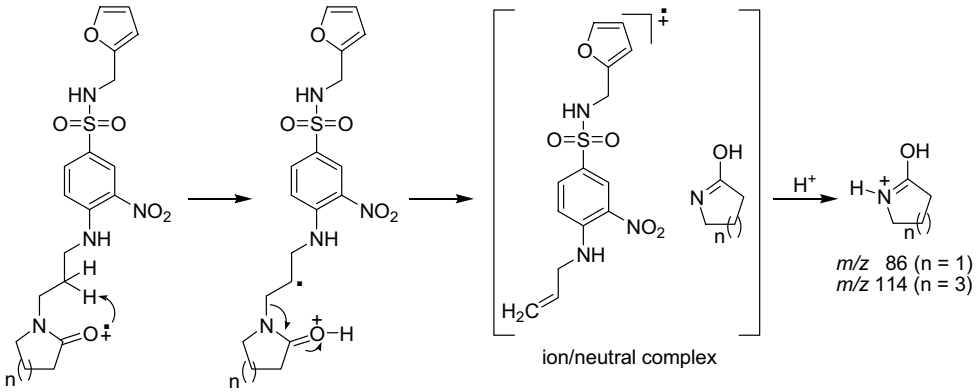


Fig. 4 EI-MS spectrum of compound **10**



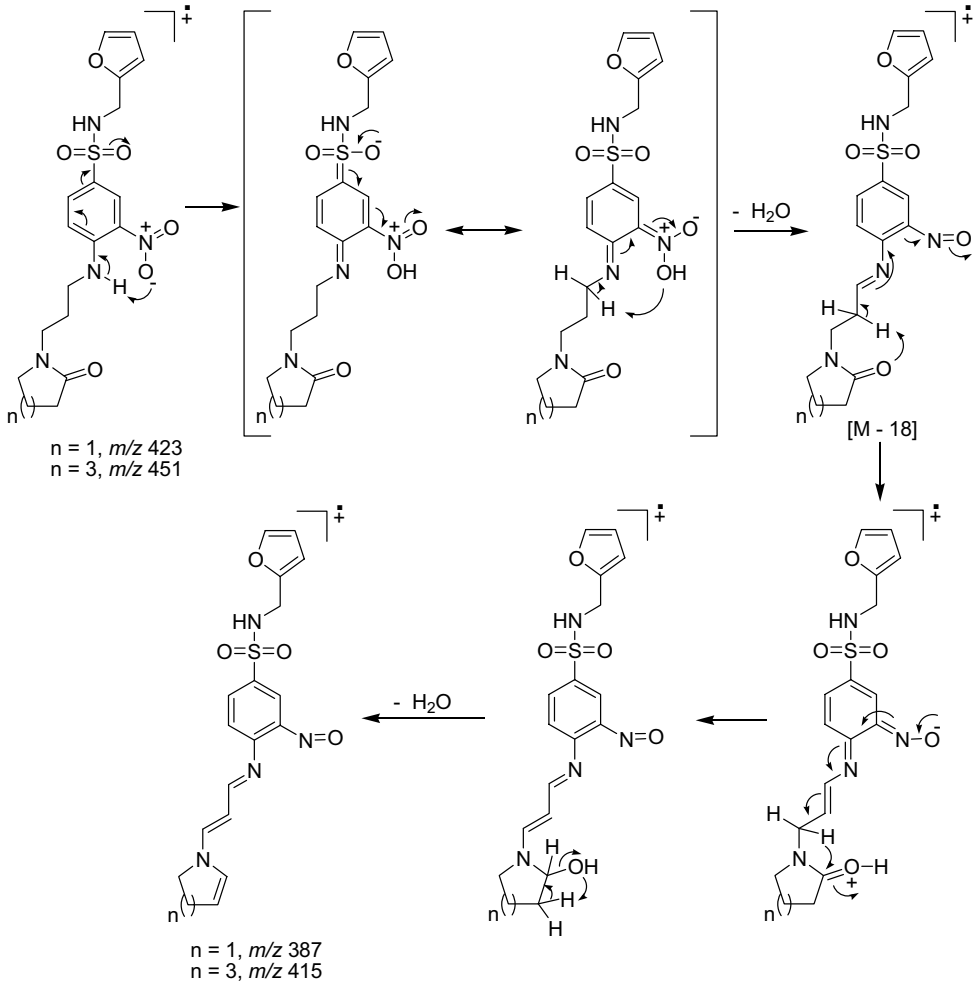
Scheme 4 Mechanism of formation of fragment ion of *m/z* 86 and *m/z* 114 observed in the EI-MS spectrum of compounds **9** and **10**, respectively (Fiedorow et al. 2005)

McLafferty rearrangement (*m/z* 86 for **9** and *m/z* 114 for **10**) were also observed. This type of double rearrangement proceeds via formation of ion/neutral complex, followed by abstraction of the hydrogen atom from the amino group (Zhao et al. 2004; Fiedorow et al. 2005; Yamaoka et al. 2004) as shown in Scheme 4.

Interestingly, it was observed that the fragment ions M−36 attributed to the loss of two water molecules (*m/z* 387 for **9** and *m/z* 415 for **10**) by EI-MS analysis. The loss of a water molecule is the result of so-called "ortho effect" characteristic of the fragmentation of *N*-alkyl-ortho-nitroanilines (Danikiewicz 1998). This fragmentation occurs from the rearrangement of a hydrogen atom from the amino group to oxygen atom of the nitro group, resulting in an sp^2 bond between nitrogen and carbon. After delocalization of negative charge, the transfer of the other hydrogen atom (from α-alkyl carbon) occurs to oxygen of the nitro group, resulting in a loss of the first water molecule (Danikiewicz 1998) generating the fragment M−18 (Scheme 5). The second loss of water molecule occurs by the rearrangement of a hydrogen atom of the β-lactam carbon to the lactam oxygen atom via a McLafferty rearrangement. After delocalization of negative charge, the hydrogen is transferred from α-lactam carbon to carbonyl lactam, followed by an elimination of the second water molecule generating the fragment ions M−32 of *m/z* 387 for **9** and *m/z* 415 for **10**.

The fragment ions at *m/z* 304 and 320 present in both compounds (independent of the lactam ring) correspond to consecutive loss of water molecule (Fiedorow et al. 2005) followed by a McLafferty rearrangement with formation of three or four heterocyclic fragment members. The loss of water molecule occurs by ortho effect characteristic of the fragmentation of *N*-alkyl-ortho-nitroanilines (Danikiewicz 1998) generating the M−18 fragment as shown in Scheme 5, followed by neutral loss of methyl lactam (*m/z* 304) or lactam (*m/z* 320). Scheme 6 summarizes the possibilities of fragmentation of the M−18 species.

Scheme 5 The proposed mechanism for consecutive loss of two water molecules for compounds **9** and **10**



The ESI-MS analysis in the positive ion mode of compounds **9** and **10** allowed their detection as intact protonated molecules with their corresponding isotopic patterns with *m/z* 423 for **9** and *m/z* 451 for **10**. The sodium cations $[M+Na]^+$ were also observed with *m/z* 445 and *m/z* 473, respectively.

The protonated molecules were selected and fragmented by sequential MS experiments (ESI(+)-MS/MS) (Fig. 5). The base peak observed for both **9** and **10** was the furfuryl ion of *m/z* 81. Both compounds showed the fragment ion corresponding to the respective *N*-allyl-lactam ring of *m/z* 126 for **9** (*N*-allyl-lactam five-members ring) and *m/z* 154 for **10** (*N*-allyl lactam seven-member ring), formed by neutral loss of the anilines R–Ph–NH_2 (Gonçalves et al. 2011).

Differently from the results observed in the EI-MS study, the ESI-MS results did not show the fragment ions which correspond to the two loss of water (M−36). Instead, the compounds showed an interesting pattern with the fragment ions M−46 and M−79. The pattern M−46 was attributed to a neutral loss of nitrogen dioxide molecule (NO_2) (Gonçalves et al. 2011; Morgan et al. 2014) (Scheme 7) generating the fragment ions of *m/z* 377 for compound **9** and *m/z* 405 for compound **10**.

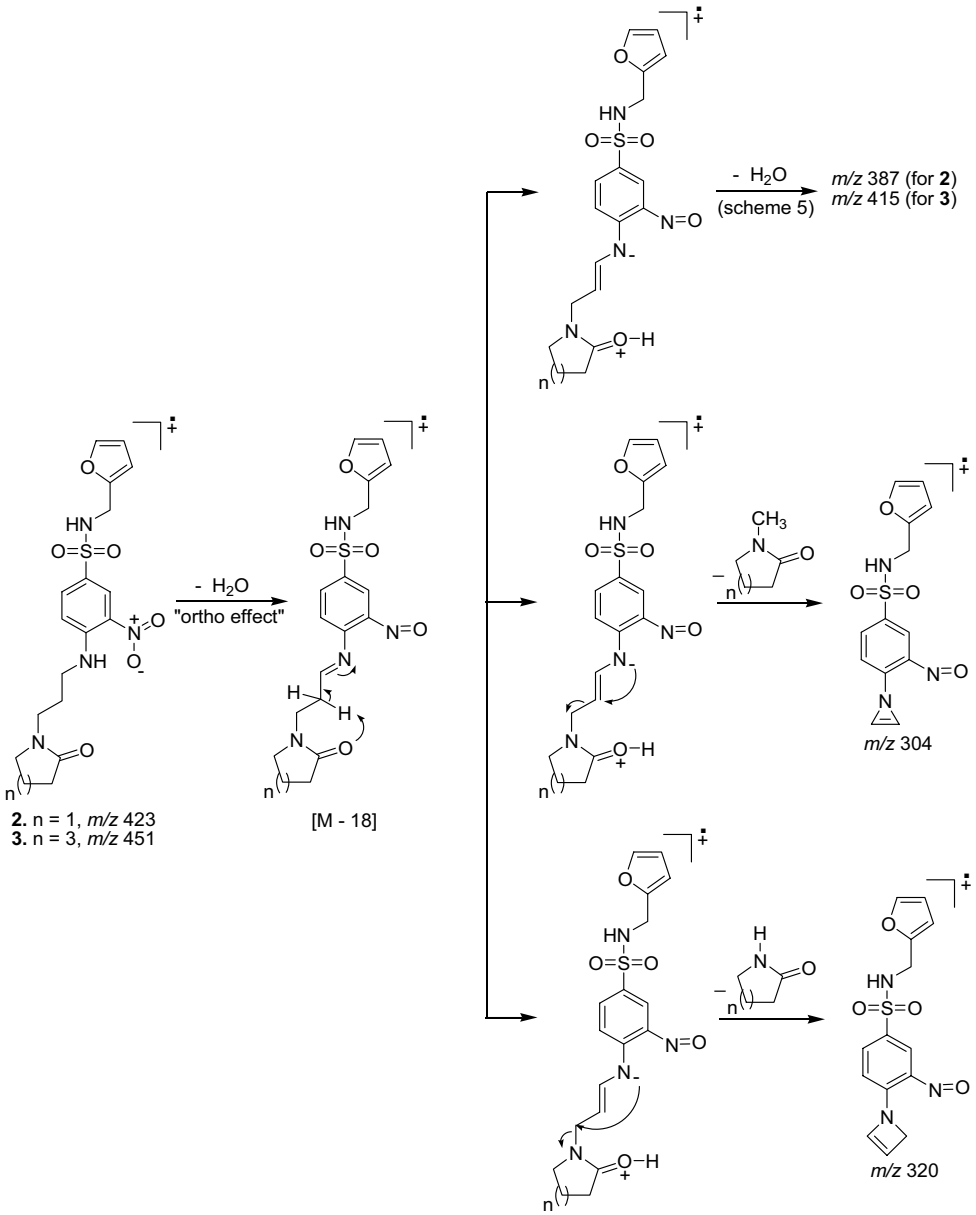
The second pattern observed for the compounds **9**–**20** was the loss of neutral iminosulfane dioxide molecule (M−79) which generated the fragment ions *m/z* 344 for **9** and *m/z* 372 for **10**. This fragmentation pathway occurs through intramolecular rearrangement displayed in Scheme 8.

The fragmentation pattern showed for compounds **9** and **10** were observed in the ESI(+)-MS/MS mass spectra of all synthesized *p*-(*N*-alkyl)lactam sulfonamides (**9**–**20**).

*Biological activity of compounds **1**, **3** and **9***

A series of *N*-alkylbenzenesulfonamides were tested for their effects on potassium ion channels by using electrophysiological recordings of ionic currents with the patch clamp technique (Hamill et al. 1981). As shown in Fig. 6, compounds **1**, **3** and **9** are blockers of Kv3.1 channels. In

Scheme 6 Possible pathways for fragmentation of M−18 species



the presence of the drugs, the current rises and then decays to a new steady state, suggesting an interaction with the channel in the open state.

Dose–response relationships were constructed for each of the compounds referred in Fig. 6, the Hill equation was fitted to the data points and their IC_{50} and Hill coefficients are shown in Table 1.

The binding of the molecule to the channel is reversible upon washout of the drug from the bath solution. Preliminary studies of the effects of these compounds on Kv1.3 and Kv1.4 shows a much lower affinity than that reported above for the Kv3.1 channels. Detailed characterization of the mechanism of action of the compounds on the ion channels are being carried out.

Conclusions

We showed that the nitro group does not affect the fragmentation pattern of compounds (**1**–**8**) by ESI(−)-MS/MS; however, it influences the mechanism of formation of sulfoxylate with *m/z* 65. EI-MS spectra for the *p*-substituted aminolactams (**9**–**20**) showed the loss of two water molecules, which is associated with the ortho effect present in the *N*-alkyl-ortho-nitroanilines and a McLafferty rearrangement. ESI(+)-MS/MS spectra of such compounds showed the loss of M−79, which corresponds to iminosulfane dioxide (SO_2NH) and also the loss of nitro group (M−46). The synthesized molecules are under current investigation for their inhibitory activity of potassium

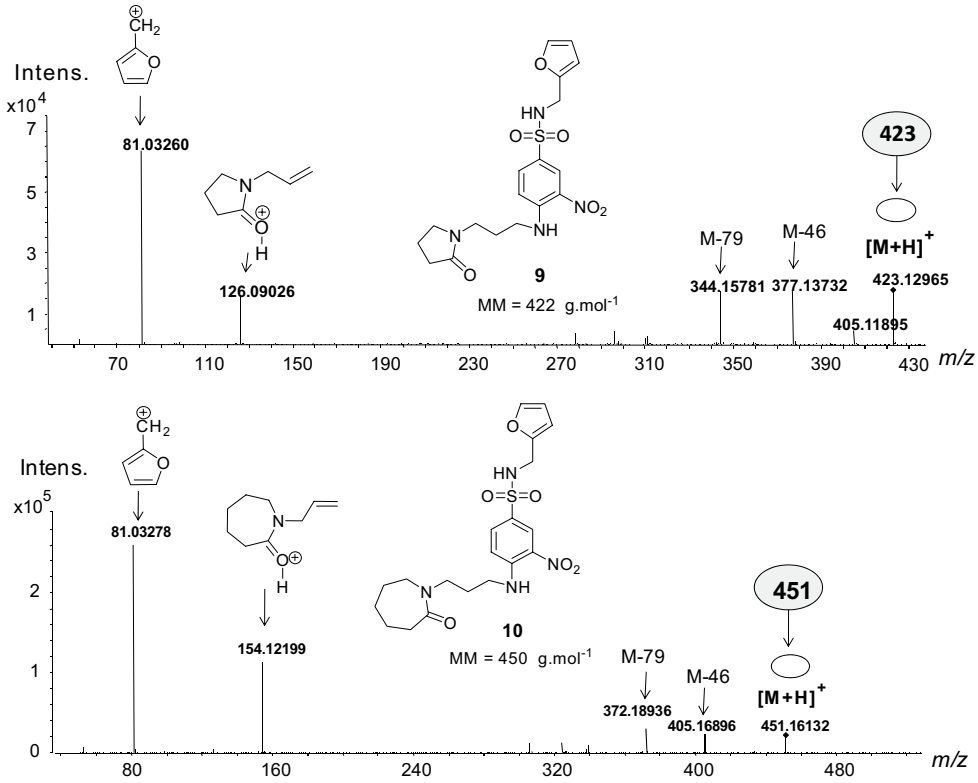
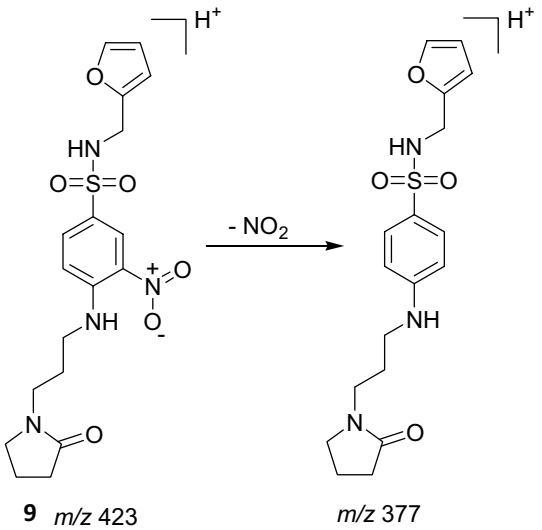


Fig. 5 ESI(+)-MS/MS of compounds **9** and **10**



Scheme 7 Loss of neutral NO_2 molecule from compound **9**

channels and some molecules have already shown positive results.

Experimental section

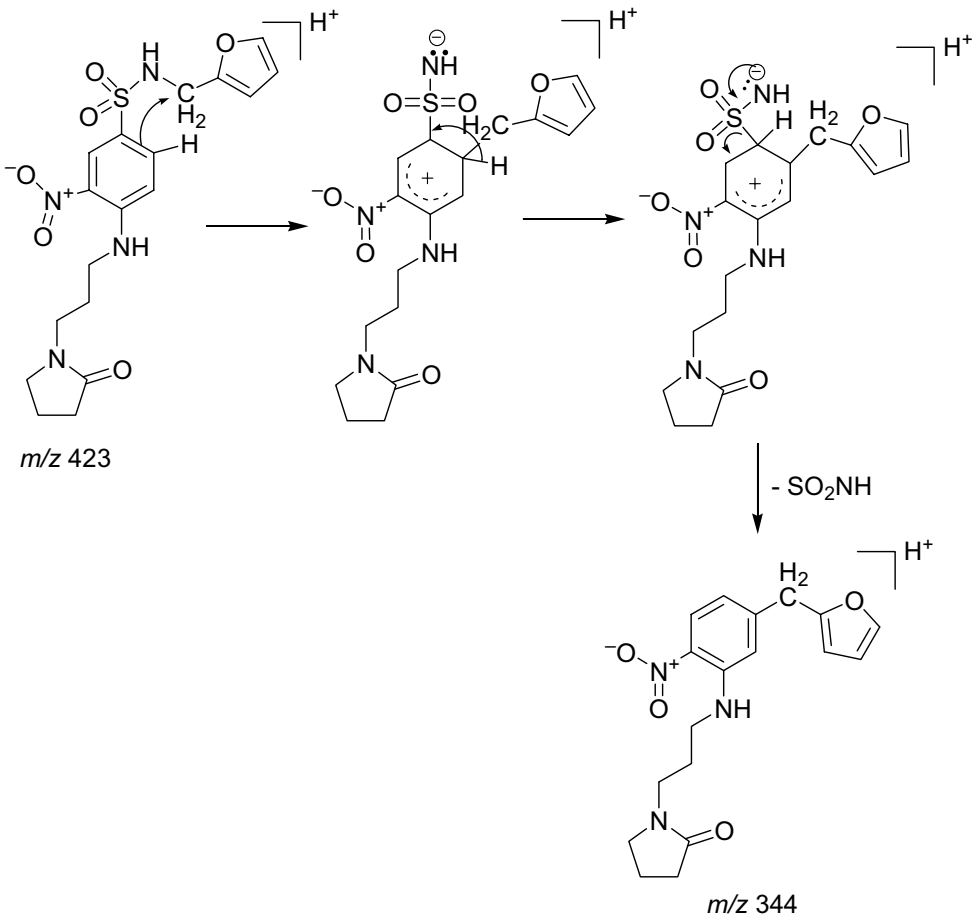
Materials and methods

The reagents used in this work (4-chlorobenzenesulfonic chloride, *N*-(3-aminopropyl)-2-pyrrolidone (APP), benzylamine, butylamine, furfurylamine, cyclohexylamine, 4-chlorobenzylamine, 2,4-dichlorobenzylamine, hydrochloric acid and solvents: 99 % ethanol, acetonitrile and dimethylsulfoxide) were all commercially obtained and used without further purification.

*General procedure for the synthesis of p-chloro-N-alkylb enzenesulfonamides (**1–8**)*

4-Chloro-3-nitrobenzenesulfonyl chloride (1 equiv.) and different amines (2 equiv.) were added to a 50 mL round-bottomed flask and dissolved in acetonitrile (25 mL). The contents were then stirred for 1–2 h at room temperature. Afterward, the solvent was removed under vacuum and the product was washed with a dilute solution (5 %) of $NaHCO_3$ and filtered.

Scheme 8 The proposed mechanism for the loss of iminosulfane dioxide molecule (SO_2NH) for compound **9**



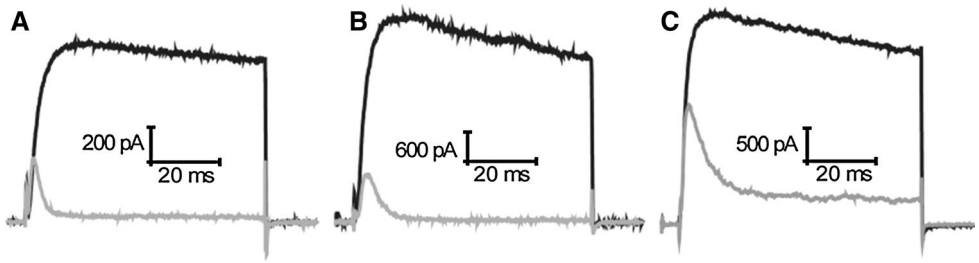


Fig. 6 Compounds 1 (**a**), 3 (**b**) and 9 (**c**) block ionic currents through Kv3.1 channels. The currents were elicited by a voltage pulse from −88 to +32 mV (70 ms). *Black lines* represent the currents in control conditions and *gray lines* are the current responses in the presence of the particular compound at a concentration of 80 μM

Table 1 Characteristics of sulfonamides **1**, **3** and **9** blockade of Kv3.1 channel

Identification	IC_{50} (μM)	Hill coefficient
Compound 1 ($N = 5$)	16.9 ± 0.6	1.5 ± 0.06
Compound 3 ($N = 9$)	12.5 ± 0.5	1.72 ± 0.05
Compound 9 ($N = 5$)	34.3 ± 0.9	1.61 ± 0.06

N is the number of cells tested in each experiment

*General procedure for the synthesis of p-(N-alkyl)lactam sulfonamides (**9–20**)*

p-Chloro-*N*-alkylbenzenesulfonamides (**1–8**) (1 equiv.) and aminolactam *N*-(3-aminopropyl)-2-pyrrolidinone (APP) (1.25 equiv.) or *N*-(3-aminopropyl)-2-azepanone (APA) (1.25 equiv.) were added to a 50 mL round-bottomed flask and dissolved in dimethylsulfoxide (10 mL) for a nucleophilic aromatic substitution reaction (S_NAr). The contents

were then stirred in a reflux system for 1–2 h at 85 °C. Afterward, the solvent was removed with frozen water and the product was treated with diluted HCl (5 %) for removal of amino-lactam excess. The crude product was filtered off and recrystallized from 1:1 ethyl ether–petroleum ether (bp 60–80 °C). All obtained compounds were structurally confirmed by ^{13}C and ^{1}H NMR analyses (see Supplementary material).

Methodology of the study by mass spectrometry

EI-MS spectra of all compounds were obtained by direct introduction of the samples in the mass spectrometer GC–MS Shimadzu QP-2010 Plus. The parameters used for these analyzes were: interface temperature, 240 °C; ionization chamber temperature, 300 °C; solvent cutting time, 0.25 min; start time, 0.30 min; end time, 25.0 min. DI temperature program: initial temperature of 50 °C, with heating at 20 °C min^{-1} to 350 °C and hold time of 10 min.

ESI-MS spectra were obtained using a mass spectrometer Agilent ifunnel Q-TOF 6550 LC–MS with source Dual Agilent Jet Stream ESI (Dual AJS-ESI). The solution of the samples was directly injected into the ESI source using an autosampler FIA at flow rate of 1 µL min^{-1} at 25 °C. The solutions were eluted in a gradient of 20 % water (0.1 % formic acid) and 80 % methanol (0.1 % formic acid) for ESI(+), and gradient 20 % water (0.1 % ammonium acetate) and 80 % methanol (0.1 % ammonium acetate) for ESI(−). Mass spectra of sulfonamide by ESI-MS and ESI-MS/MS were acquired using the following operating conditions: capillary voltage ±3 kV, drying gas flow 10 L min^{-1}, drying gas at 250 °C, nebulizer gas at 50 psi. The system was operated for acquisitions in the mass range of 50–1500 *m/z*. For MS/MS experiments, collision energy varied between 5 and 25 V using N_2. Acquisition and data processing were performed using the Agilent MassHunter Workstation software (B.06.01 version).

Methodology of the study of biological activity

For the in vitro studies, we used the L-929 cell line stably expressing Kv 1.3 and 3.1 channels and CHO cell line, stably expressing Kv1.4. Cells were voltage clamped at a holding potential of −88 mV and pulsed to +32 mV (70 ms) to activate the potassium currents, essentially as described in Rodrigues et al. (2003). The bath solution had the following composition (in mM): 155 NaCl, 1.0 $MgCl_2$, 2.0 $CaCl_2$, 5.0 HEPES and 4.5 KCl, pH 7.4 (NaOH) and osmolality of 300–310 mOsm/kg H_2O. The pipette solution was composed of (in mM): 155 KF, 5.0 EGTA, 10 HEPES, 2.0 $MgCl_2$ and 1.0 $CaCl_2$, pH 7.2 (KOH) and osmolality of 290–300 mOsm/kg H_2O. The series resistance was electronically compensated up to 80 % when currents exceeded 2.0 nA. The signal was low-pass filtered at 5.0 kHz and acquired at 20 kHz through an AxoPacth 200B amplifier (Axon Instruments®, Foster City, CA, USA) connected to an A/D and D/A converter Digidata 1440A (Axon Instruments®). The data were analyzed with Origin 8.0 software (OriginLab®, Northampton, MA, USA). Chemicals used for electrophysiological studies were purchased from Sigma (St Louis, MO, USA).

*4-Chloro-3-nitro-N-furfurylbenzenesulfonamide (base consulted = CSID:2362584) (**1**)*

Beige solid (0.434 g, 68.7 %), mp = 131.8–132.4 °C; MM = 316.17 g mol^{-1}. ^{1}H NMR (600 MHz, DMSO) δ 8.62 (s, 1H), 8.31 (d, $J = 2.1$ Hz, 1H), 7.96 (d, $J = 2.0$ Hz, 1H), 7.95 (s, 1H), 7.40 (dd, $J = 1.8$, 0.8 Hz, 1H), 6.24 (dd, $J = 3.2$, 1.9 Hz, 1H), 6.17 (d, $J = 3.2$ Hz, 1H), 4.13 (s, 2H). ^{13}C NMR (151 MHz, DMSO) δ 149.87, 147.13, 142.59, 141.09, 132.82, 131.25, 129.01, 123.86, 110.27, 108.51, 39.16. EI-MS *m/z* found: 316. ESI(−)-MS *m/z* found: 314.98674, *m/z* calculated for $[C_{11}H_9ClN_2O_5S-H]^-$: 314.98479.

*4-Chloro-N-furfurylbenzenesulfonamide (base consulted = CSID:91535) (**2**)*

Brown solid (0.320 g, 59 %), mp = 118.7–119.9 °C; MM = 271.72 g mol^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76–7.74 (m, 1H), 7.74–7.72 (m, 1H), 7.45–7.44 (m, 1H), 7.44–7.42 (m, 1H), 7.22 (dd, $J = 1.8$, 0.7 Hz, 1H), 6.22 (dd, $J = 3.2$, 1.9 Hz, 1H), 6.09 (dd, $J = 3.2$, 0.6 Hz, 1H), 4.84 (t, $J = 5.5$ Hz, 1H), 4.21 (d, $J = 6.0$ Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 149.31, 149.30, 142.73, 139.20, 138.69, 138.67, 129.37, 128.64, 110.53, 108.60, 108.59, 40.20. EI-MS *m/z* found: 271. ESI(−)-MS *m/z* found: 270.00074, *m/z* calculated for $[C_{11}H_{10}ClNO_3S-H]^-$: 269.99972.

*4-Chloro-3-nitro-N-butylbenzenesulfonamide (CAS No. 96-61-7) (**3**)*

White yellow solid. (0.450 g, 77 %), mp = 68–69 °C; MM = 292.73 g mol^{-1}. ^{1}H NMR (400 MHz, DMSO) δ 8.42 (d, $J = 1.8$ Hz, 1H), 8.05 (dd, $J = 8.5$, 2.0 Hz, 1H), 8.02 (d, $J = 8.3$ Hz, 1H), 7.95 (t, $J = 5.8$ Hz, 1H), 2.80 (dd, $J = 12.8$, 6.9 Hz, 2H), 1.40–1.32 (m, 2H), 1.24 (sext, $J = 14.0$, 7.0 Hz, 2H), 0.81 (t, $J = 7.3$ Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 147.45, 140.88, 133.09, 131.26, 129.12, 123.86, 42.21, 31.05, 19.12, 13.40. EI-MS *m/z* found: 392. ESI(−)-MS *m/z* found: 291.02251, *m/z* calculated for $[C_{10}H_{13}ClN_2O_4S-H]^-$: 291.02118.

*4-Chloro-N-butylbenzenesulfonamide (CAS No. 6419-73-4) (**4**)*

White solid. (0.164 g, 33.1 %); mp = 46–46.5 °C; MM = 247.74 g mol^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84–7.76 (m, 2H), 7.53–7.44 (m, 2H), 4.81 (t, *J* = 5.9 Hz, 1H), 2.93 (dd, *J* = 13.3, 6.9 Hz, 2H), 1.47–1.40 (m, 2H), 1.32–1.23 (m, 2H), 0.84 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.16, 138.65, 129.49, 128.64, 43.07, 31.67, 19.78, 13.62. EI-MS *m/z* found: 247. ESI(−)-MS *m/z* found: 246.03720, *m/z* calculated for $[C_{10}H_{14}ClNO_2S-H]^-$: 246.0361.

*4-Chloro-3-nitro-N-benzylbenzenesulfonamide (CAS No. 82835-65-2) (**5**)*

Yellow solid (0.465 g, 71.2 %), mp = 93–95 °C; MM = 326.75 g mol^{-1}. ^{1}H NMR (600 MHz, DMSO) δ 8.60 (t, *J* = 6.3 Hz, 1H), 8.30 (d, *J* = 2.1 Hz, 1H), 7.98 (dd, *J* = 8.4, 2.1 Hz, 1H), 7.92 (d, *J* = 8.4 Hz, 1H), 7.25–7.15 (m, 5H), 4.10 (d, *J* = 6.2 Hz, 2H). ^{13}C NMR (151 MHz, DMSO) δ 147.13, 141.17, 136.85, 132.94, 131.31, 129.09, 128.19, 127.80, 127.23, 123.98, 46.22. EI-MS *m/z* found: 325. ESI(−)-MS *m/z* found: 325.00784, *m/z* calculated for $[C_{13}H_{11}ClN_2O_4S-H]^-$; 325.00553.

*4-Chloro-3-nitro-N-cyclohexylbenzenesulfonamide (base consulted = CSID:5876919) (**6**)*

Yellow solid (0.473 g, 74.4 %), mp = 39–40 °C; MM = 318.04 g mol^{-1}. ^{1}H NMR (400 MHz, DMSO) δ 8.45 (d, *J* = 2.1 Hz, 1H), 8.09–8.06 (m, 1H), 8.02 (t, *J* = 7.4 Hz, 2H), 3.08–3.00 (m, 1H), 1.59 (d, *J* = 9.5 Hz, 4H), 1.19–1.02 (m, 5H). ^{13}C NMR (101 MHz, DMSO) δ 147.36, 142.57, 133.10, 131.01, 128.97, 123.66, 52.25, 33.18, 24.74, 24.23. EI-MS *m/z* found: 318. ESI(−)-MS *m/z* found: 317.03874, *m/z* calculated for $[C_{12}H_{15}ClN_2O_4S-H]^-$: 317.03683.

*4-Chloro-3-nitro-N-4-chlorobenzylbenzenesulfonamide (**7**)*

Yellow solid (0.500 g, 69.2 %), mp = 107–109 °C; MM = 361.20 g mol^{-1}. ^{1}H NMR (600 MHz, DMSO) δ 8.71–8.65 (m, 1H), 8.29 (s, 1H), 8.00–7.95 (m, 1H), 7.92 (d, *J* = 8.4 Hz, 1H), 7.30–7.24 (m, 2H), 7.21 (d, *J* = 8.5 Hz, 2H), 4.09 (d, *J* = 6.0 Hz, 2H). ^{13}C NMR (151 MHz, DMSO) δ 147.57, 141.53, 136.43, 133.41, 132.39, 132.03, 131.77, 131.19, 130.08, 129.62, 128.59, 124.43, 123.01, 45.88. EI-MS *m/z* found: 360. ESI(−)-MS *m/z* found: 358.96628, *m/z* calculated for $[C_{13}H_{11}Cl_2N_2O_4S-H]^-$: 358.96656.

*4-Chloro-3-nitro-N-2,4-dichlorobenzylbenzenesulfonamide (**8**)*

Beige solid (0.483 g, 61 %), mp = 135–136.5 °C; MM = 395.65 g mol^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (d, *J* = 2.2 Hz, 1H), 7.86–7.83 (m, 1H), 7.62 (d, *J* = 8.4 Hz, 1H), 7.28 (d, *J* = 2.0 Hz, 1H), 7.24 (s, 1H), 7.19 (dd, *J* = 8.2, 2.0 Hz, 1H), 5.21 (t, *J* = 6.2 Hz, 1H), 4.33 (d, *J* = 6.4 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 140.65, 135.44, 134.33, 132.90, 131.75, 131.71, 131.61, 130.88, 129.70, 127.65, 124.45, 45.14. EI-MS *m/z* found: 395. ESI(−)-MS *m/z* found: 394.92664, *m/z* calculated for $[C_{13}H_9Cl_3N_2O_4S-H]^-$: 392.92758.

*4-[N-(3′-Aminopropyl)-2-pyrrolidone]-3-nitro-N-furfurylbenzenesulfonamide (**9**)*

Yellow solid (0.463 g, 54.8 %), mp = 115–117.8 °C; MM = 422.45 g mol^{-1}. ^{1}H NMR (600 MHz, DMSO) δ 8.64 (t, *J* = 6.0 Hz, 1H), 8.31 (d, *J* = 2.3 Hz, 1H), 8.19 (t, *J* = 6.4 Hz, 1H), 7.71 (dd, *J* = 9.2, 2.3 Hz, 1H), 7.29 (d, *J* = 8.5 Hz, 2H), 7.22 (d, *J* = 8.4 Hz, 2H), 7.17 (d, *J* = 16.1 Hz, 1H), 4.01 (d, *J* = 6.3 Hz, 2H), 3.44–3.39 (m, 2H), 3.38–3.36 (m, 2H), 3.28 (t, *J* = 6.7 Hz, 2H), 2.24 (t, *J* = 8.1 Hz, 2H), 1.98–1.90 (m, 2H), 1.79 (qu, *J* = 6.7 Hz, 2H). ^{13}C NMR (151 MHz, DMSO) δ 174.71, 150.74, 147.04, 142.90, 133.65, 130.16, 126.51, 115.77, 110.72, 108.55, 46.83, 40.53, 39.77, 39.67, 30.88, 26.33, 18.00. EI-MS *m/z* found: 423. ESI(+)-MS *m/z* found: 23.13222, *m/z* calculated for $[C_{18}H_{22}N_4O_6S+H]^+$: 423.13273.

*4-[N-(3′-Aminopropyl)-2-azepanone]-3-nitro-N-furfurylbenzenesulfonamide (**10**)*

Yellow solid (0.381 g, 42.3 %), mp = 145–148 °C; MM = 450.50 g mol^{-1}. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.69 (t, *J* = 6.0 Hz, 1H), 8.35 (d, *J* = 2.3 Hz, 1H), 8.13 (t, *J* = 6.1 Hz, 1H), 7.73 (dd, *J* = 9.1, 2.3 Hz, 1H), 7.43 (dd, *J* = 1.8, 0.8 Hz, 1H), 7.17 (d, *J* = 9.3 Hz, 1H), 6.26 (dd, *J* = 3.2, 1.8 Hz, 1H), 6.16 (dd, *J* = 3.2, 0.7 Hz, 1H), 4.01 (d, *J* = 6.1 Hz, 2H), 3.40–3.35 (m, 6H), 2.46–2.41 (m, 2H), 1.74 (qu, *J* = 6.8 Hz, 2H), 1.66 (qu, *J* = 5.8 Hz, 2H), 1.55 (dq, *J* = 11.9, 7.0, 6.4 Hz, 4H). ^{13}C NMR (151 MHz, DMSO) δ 174.96, 145.05, 135.13, 133.97, 131.58, 130.75, 129.29, 125.03, 123.13, 122.52, 114.03, 48.54, 44.75, 40.38, 39.86, 36.46, 29.22, 28.32, 26.84, 23.02. EI-MS *m/z* found: 451. ESI(+)-MS *m/z* found: 451.16137, *m/z* calculated for $[C_{20}H_{26}N_4O_6S+H]^+$: 451.16458.

*4-[N-(3′-Aminopropyl)-2-pyrrolidone]-3-nitro-N-butylbenzenesulfonamide (**11**)*

Yellow solid (0.414 g, 52 %), mp = 111–113.7 °C; MM = 398.47 g mol^{-1}. ^{1}H NMR (600 MHz, DMSO) δ 8.63 (t, *J* = 5.9 Hz, 1H), 8.42 (d, *J* = 2.2 Hz, 1H), 7.78 (dd, *J* = 9.1, 1.9 Hz, 1H), 7.52 (t, *J* = 5.8 Hz, 1H), 7.24 (d, *J* = 9.2 Hz, 1H), 3.40 (q, *J* = 6.6 Hz, 2H), 3.35 (d, *J* = 6.9 Hz, 2H), 3.27 (t, *J* = 6.6 Hz, 2H), 2.71 (dd, *J* = 13.1, 6.8 Hz, 2H), 2.22 (t, *J* = 8.1 Hz, 2H), 1.95–1.89 (m, 2H), 1.79 (qu, *J* = 6.7 Hz, 2H), 1.37–1.32 (m, 2H), 1.26–1.20 (m, 2H), 0.79 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (151 MHz, DMSO) δ 174.22, 146.63, 133.21, 129.79, 126.01, 125.90, 115.54, 46.34, 42.14, 40.10, 39.30, 30.99, 30.40, 25.86, 19.22, 17.53, 13.43. ESI(+)-MS *m/z* found: 399.16556, *m/z* calculated for $[C_{17}H_{26}N_4O_5S+H]^+$: 399.16967.

*4-[N-(3′-Aminopropyl)-2-azepanone]-3-nitro-N-butylbenzenesulfonamide (**12**)*

Yellow solid (0.272 g, 31.9 %), mp = 124.4–126 °C; MM = 426.53 g mol^{-1}. ^{1}H NMR (400 MHz, DMSO) δ 8.69 (t, *J* = 6.0 Hz, 1H), 8.42 (d, *J* = 2.2 Hz, 1H), 7.78 (dd, *J* = 9.1, 2.1 Hz, 1H), 7.51 (t, *J* = 5.8 Hz, 1H), 7.24 (d, *J* = 9.3 Hz, 1H), 3.38 (dt, *J* = 9.4, 6.4 Hz, 6H), 2.71 (dd, *J* = 13.0, 6.8 Hz, 2H), 2.45–2.40 (m, 2H), 1.75 (qu, *J* = 6.6 Hz, 2H), 1.70–1.62 (m, 2H), 1.55 (d, *J* = 4.2 Hz, 4H), 1.39–1.31 (m, 2H), 1.23 (dd, *J* = 15.0, 7.2 Hz, 2H), 0.80 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 175.03, 146.63, 133.20, 129.79, 125.92, 115.50, 48.53, 44.67, 42.13, 36.48, 30.98, 29.26, 28.34, 26.74, 23.03, 19.26, 13.45. EI-MS *m/z* found: 427. ESI(+)-MS *m/z* found: 427.19950, *m/z* calculated for $[C_{19}H_{30}N_4O_5S+H]^+$: 427.20097.

*4-[N-(3′-Aminopropyl)-2-pyrrolidone]-3-nitro-N-benzylbenzenesulfonamide (**13**)*

Yellow solid (0.441 g, 51 %), mp = 151–153.9 °C; MM = 432.49 g mol^{-1}. ^{1}H NMR (600 MHz, DMSO) δ 8.62 (t, *J* = 6.0 Hz, 1H), 8.32 (d, *J* = 2.4 Hz, 1H), 8.13 (t, *J* = 6.4 Hz, 1H), 7.73 (dd, *J* = 9.1, 2.2 Hz, 1H), 7.25–7.15 (m, 6H), 3.99 (d, *J* = 6.5 Hz, 2H), 3.40–3.34 (m, 4H), 3.27 (dt, *J* = 6.7, 3.3 Hz, 2H), 2.23 (t, *J* = 8.0 Hz, 2H), 1.96–1.90 (m, 2H), 1.78 (qu, *J* = 6.8 Hz, 2H). ^{13}C NMR (151 MHz, DMSO) δ 174.27, 146.57, 137.28, 133.15, 129.69, 128.11, 127.67, 126.96, 126.12, 115.37, 46.37, 40.05, 39.30, 30.42, 25.87, 17.55. EI-MS *m/z* found: 433. ESI(+)-MS *m/z* found: 433.15016, *m/z* calculated for $[C_{20}H_{24}N_4O_5S+H]^+$: 433.15402.

*4-[N-(3′-Aminopropyl)-2-azepanone]-3-nitro-N-benzylbenzenesulfonamide (**14**)*

Yellow solid (0.389 g, 42.2 %), mp = 157.4–160 °C; MM = 460.54 g mol^{-1}. ^{1}H NMR (400 MHz, DMSO) δ 8.68 (t, *J* = 6.0 Hz, 1H), 8.33 (d, *J* = 2.2 Hz, 1H), 8.12 (t, *J* = 6.4 Hz, 1H), 7.73 (dd, *J* = 9.2, 2.1 Hz, 1H), 7.25–7.15 (m, 6H), 3.99 (d, *J* = 6.3 Hz, 2H), 3.38 (dd, *J* = 12.1, 6.3 Hz, 6H), 2.49–2.40 (m, 2H), 1.74 (qu, *J* = 6.6 Hz, 2H), 1.66 (d, *J* = 5.0 Hz, 2H), 1.62–1.49 (m, 4H). ^{13}C NMR (101 MHz, DMSO) δ 175.07, 146.57, 137.29, 133.14, 129.68, 128.11, 127.67, 126.96, 126.14, 115.34, 48.55, 46.15, 44.65, 36.49, 29.27, 28.36, 26.75, 23.04. EI-MS *m/z* found: 461. ESI(+)-MS *m/z* found: 461.18232, *m/z* calculated for $[C_{22}H_{28}N_4O_5S+H]^+$: 461.18532).

*4-[N-(3′-Aminopropyl)-2-pyrrolidone]-3-nitro-N-cyclohexylbenzenesulfonamide (**15**)*

Yellow solid (0.356 g, 42 %), mp = 148–151.8 °C; MM = 424.51 g mol^{-1}. ^{1}H NMR (600 MHz, DMSO) δ 8.62 (t, *J* = 6.0 Hz, 1H), 8.45 (d, *J* = 2.3 Hz, 1H), 7.82 (dd, *J* = 9.2, 2.2 Hz, 1H), 7.62 (d, *J* = 7.2 Hz, 1H), 7.25 (d, *J* = 9.3 Hz, 1H), 3.41 (dd, *J* = 13.2, 6.6 Hz, 2H), 3.37–3.34 (m, 2H), 3.28 (t, *J* = 6.7 Hz, 2H), 2.92 (d, *J* = 6.7 Hz, 1H), 2.22 (t, *J* = 8.1 Hz, 2H), 1.97–1.88 (m, 2H), 1.80 (qu, *J* = 6.8 Hz, 2H), 1.60 (dd, *J* = 10.2, 5.2 Hz, 4H), 1.14 (dd, *J* = 13.7, 8.9 Hz, 4H). ^{13}C NMR (151 MHz, DMSO) δ 174.26, 146.58, 136.38, 133.11, 131.67, 129.66, 129.52, 128.04, 126.16, 126.12, 115.37, 46.37, 45.41, 40.05, 39.30, 30.42, 25.93, 17.54. EI-MS *m/z* found: 425. ESI(+)-MS *m/z* found: 425.18190, *m/z* calculated for $[C_{19}H_{28}N_4O_5S+H]^+$: 425.18532.

*4-[N-(3′-Aminopropyl)-2-azepanone]-3-nitro-N-cyclohexylbenzenesulfonamide (**16**)*

Yellow solid (0.430 g, 47.5 %), mp = 156.3–159.1 °C; MM = 452.56 g mol^{-1}. ^{1}H NMR (600 MHz, DMSO) δ 8.62 (t, *J* = 6.0 Hz, 1H), 8.45 (dd, *J* = 5.6, 3.7 Hz, 1H), 7.85–7.77 (m, 1H), 7.62 (d, *J* = 7.2 Hz, 1H), 7.25 (d, *J* = 9.3 Hz, 1H), 3.39 (d, *J* = 6.6 Hz, 5H), 3.36–3.33 (m, 1H), 3.27 (d, *J* = 6.7 Hz, 1H), 2.91 (s, 1H), 2.23 (s, 1H), 1.94 (s, 1H), 1.83–1.76 (m, 1H), 1.59 (dd, *J* = 10.1, 5.4 Hz, 1H), 1.13 (d, *J* = 10.9 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ 175.06, 147.40, 142.67, 133.16, 131.12, 129.03, 123.73, 54.15, 52.35, 48.56, 45.39, 44.67, 36.49, 33.24, 29.26, 28.36, 26.73, 24.80, 24.31, 23.04. EI-MS *m/z* found: 453. ESI(+)-MS *m/z* found: 453.21379, *m/z* calculated for $[C_{21}H_{32}N_4O_5S+H]^+$: 453.21662.

*4-[N-(3′-Aminopropyl)-2-pyrrolidone]-3-nitro-N-4-chlorobenzylbenzenesulfonamide (**17**)*

Yellow solid (0.361 g, 38.7 %), mp = 167.7–170.5 °C; MM = 466.93. ^{1}H NMR (600 MHz, DMSO) δ 8.64 (t, *J* = 6.0 Hz, 1H), 8.31 (d, *J* = 2.3 Hz, 1H), 8.19 (t, *J* = 6.4 Hz, 1H), 7.71 (dd, *J* = 9.2, 2.3 Hz, 1H), 7.29 (d, *J* = 8.5 Hz, 2H), 7.22 (d, *J* = 8.4 Hz, 2H), 7.16 (s, 1H), 4.01 (d, *J* = 6.3 Hz, 2H), 3.43–3.39 (m, 2H), 3.38–3.36 (m, 2H), 3.28 (t, *J* = 6.7 Hz, 2H), 2.24 (t, *J* = 8.1 Hz, 2H), 1.99–1.90 (m, 2H), 1.79 (qu, *J* = 6.7 Hz, 2H). ^{13}C NMR (151 MHz, DMSO) δ 174.26, 146.58, 136.38, 133.11, 131.67, 129.66, 129.52, 128.04, 126.16, 126.12, 115.37, 46.37, 45.41, 40.05, 39.30, 30.42, 25.93, 17.54. EI-MS *m/z* found: 467. ESI(+)-MS *m/z* found: 467.11199, *m/z* calculated for $[C_{20}H_{23}N_4ClO_5S+H]^+$: 467.11504.

*4-[N-(3′-aminopropyl)-2-azepanone]-3-nitro-N-4-chlorobenzylbenzenesulfonamide (**18**)*

Yellow solid (0.368 g, 37.2 %), mp = 165.6–171.7 °C; MM = 494.99 g mol^{-1}. ^{1}H NMR (400 MHz, DMSO) δ 8.71 (t, *J* = 6.0 Hz, 1H), 8.33 (d, *J* = 2.2 Hz, 1H), 8.19 (t, *J* = 6.4 Hz, 1H), 7.72 (dd, *J* = 9.2, 2.2 Hz, 1H), 7.30 (d, *J* = 8.5 Hz, 2H), 7.24 (d, *J* = 8.4 Hz, 2H), 7.17 (d, *J* = 9.3 Hz, 1H), 4.02 (d, *J* = 6.4 Hz, 2H), 3.40 (qua, *J* = 6.1 Hz, 6H), 2.49–2.42 (m, 2H), 1.76 (qu, *J* = 6.6 Hz, 2H), 1.68 (d, *J* = 5.0 Hz, 2H), 1.58 (dd, *J* = 9.1, 4.4 Hz, 4H). ^{13}C NMR (101 MHz, DMSO) δ 175.04, 146.57, 136.39, 133.09, 131.64, 129.64, 129.60, 129.51, 128.14, 128.03, 126.13, 126.06, 115.33, 48.55, 45.38, 44.67, 36.48, 29.26, 28.35, 26.73, 23.03. EI-MS *m/z* found: 487. ESI(+)-MS *m/z* found: 495.14268, *m/z* calculated for $[C_{22}H_{27}ClN_4O_5S+H]^+$: 495.14634.

*4-[N-(3′-Aminopropyl)-2-pyrrolidone]-3-nitro-N-2,4-dichlorobenzylbenzenesulfonamide (**19**)*

Yellow solid (0.270 g, 27 %); mp = 142.2–145 °C; MM = 501.38 g mol^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.55 (d, *J* = 2.2 Hz, 1H), 8.51 (t, *J* = 4.8 Hz, 1H), 7.68 (dd, *J* = 9.1, 2.2 Hz, 1H), 7.27 (s, 1H), 7.24 (s, 1H), 7.15 (dd, *J* = 8.2, 2.1 Hz, 1H), 6.81 (d, *J* = 9.1 Hz, 1H), 5.10 (t, *J* = 6.5 Hz, 1H), 4.25 (d, *J* = 6.4 Hz, 2H), 3.46–3.35 (m, 6H), 2.41 (t, *J* = 8.1 Hz, 2H), 2.05 (dd, *J* = 15.1, 7.6 Hz, 2H), 1.93 (dd, *J* = 13.3, 6.7 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.09, 147.08, 134.55, 134.20, 133.57, 132.65, 131.54, 130.78, 129.40, 127.55, 127.42, 126.43, 114.31, 47.63, 44.78, 40.88, 40.40, 30.97, 26.74, 18.10. EI-MS *m/z* found: 501. ESI(+)-MS *m/z* found: 501.07316, *m/z* calculated for $[C_{20}H_{22}Cl_2N_4O_5S+H]^+$: 501.07607.

*4-[N-(3′-Aminopropyl)-2-azepanone]-3-nitro-N-2,4-dichlorobenzylbenzenesulfonamide (**20**)*

Yellow solid (0.497 g, 46.9 %), mp = 154–160 °C; MM = 529.44 g mol^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.64 (t, *J* = 5.2 Hz, 1H), 8.56 (d, *J* = 2.3 Hz, 1H), 7.69 (dd, *J* = 9.1, 2.3 Hz, 1H), 7.29 (d, *J* = 8.3 Hz, 1H), 7.16 (dd, *J* = 8.2, 2.1 Hz, 1H), 6.83 (d, *J* = 9.2 Hz, 1H), 5.27 (t, *J* = 6.5 Hz, 1H), 4.26 (d, *J* = 6.3 Hz, 2H), 3.53 (t, *J* = 6.4 Hz, 2H), 3.41–3.36 (m, 4H), 2.58–2.53 (m, 2H), 1.90 (dd, *J* = 13.1, 6.6 Hz, 2H), 1.77–1.64 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.00, 147.14, 133.50, 132.68, 131.63, 131.58, 130.95, 129.43, 127.58, 127.42, 126.17, 124.48, 114.27, 50.04, 45.84, 44.81, 40.64, 37.16, 30.05, 28.76, 27.44, 23.49. EI-MS *m/z* found: 529. ESI(+)-MS *m/z* found: 529.10526, *m/z* calculated for $[C_{22}H_{26}Cl_2N_4O_5S+H]^+$: 529.10737.

Acknowledgments The authors would like to thank the Brazilian Research Foundations FAPESP (research fund 2013/24487-6), CNPq and CAPES for their financial assistance. We are also grateful to Post-graduation Program POSMAT at São Paulo State University (UNESP) for the post-graduation fellowship.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing financial interests.

References

Bromidge SM, Charke SE, King FD, Lovell PJ, Newman H, Riley G, Routledge C, Serafinowska HT, Smith DR, Thomas DR (2002) Bicyclic piperazinylbenzenesulphonamides are potent and selective 5-HT6 receptor antagonists. Bioorg Med Chem Lett 12(10):1357–1360. doi:10.1016/S0960-894X(02)00172-5

Danikiewicz W (1998) Electron ionization-induced fragmentation of *N*-alkyl-*o*-nitroanilines: observation of new types of *ortho* effects. Eur J Mass Spectrom 4:167

Dannhardt G, Kiefer W (2001) Cyclooxygenase inhibitors—current status and future prospects. Eur J Med Chem 36(2):109–126. doi:10.1016/S0223-5234(01)01197-7

Deng X, Mani NS (2006) A facile, environmentally benign sulfonamide synthesis in water. Green Chem 8:835–838. doi:10.1039/b606127c

Fiedorow P, Franski R, Gierczyk B, Schroeder G (2005) Fragmentation and skeletal rearrangements of products of the reaction between fluorobenzenes and bicyclic *N*-bases studied by electron ionization mass spectrometry. Int J Mass Spectrom 240:7–15. doi:10.1016/j.ijms.2004.10.001

Ghosh AK, Leshchenko-Yashchuk S, Anderson DD, Baldridge A, Noetzel M, Miller HB, Tie Y, Wang Y, Koh Y, Weber IT, Mitsuya H (2009) Design of HIV-1 protease inhibitors with pyrrolidinones and oxazolidinones as novel P1′-ligands to enhance backbone-binding interactions with protease: synthesis, biological evaluation, and protein-ligand X-ray studies. J Med Chem 52(13):3902–3914. doi:10.1021/jm900303m

Gonçalves RS, Abdelnur PV, Santos VG, Simas RC, Eberlin MN, Magalhães A, Gonzalez ERP (2011) Synthesis of potentially

bioactive PABA-related *N*-(aminoalkyl)lactamic amino acids and esters via selective SNAr reactions. Amino Acids 40(1):197–204. doi:10.1007/s00726-010-0634-z

Goodman A (1996) As bases farmacológicas da terapêutica. McGraw Hill, Cidade do México, p 1436

Gouauax E, Mackinnon R (2005) Principles of selective ion transport in channels and pumps. Science, Washington 310(5753):1461–1465. doi:10.1126/science.1113666

Gross MF, Beaudoin S, McNaughton-Smith G, Amato GS, Castle NA, Huang C, Zou A, Yu W (2007) Aryl sulphonamido indane inhibitors of the Kv1.5 ion channel. Med Chem Lett 17(10):2849–2853. doi:10.1016/j.bmcl.2007.02.052

Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ (1981) Improved patch-clamp techniques for high resolution current recording from cells and cell-free membrane patch. Pfluegers Arch 391(2):85–100. doi:10.1007/BF00656997

Hibbs JA, Jariwala FB, Weisbecker CS, Attygalle ABJ (2013) Gas-phase fragmentations of anions derived from *N*-phenyl benzenesulfonamides. Am Soc Mass Spectrom 24:1280–1287. doi:10.1007/s13361-013-0671-4

Hu N, Liu P, Jiang K, Zhou Y, Pan Y (2008) Mechanism study of SO_2 elimination from sulfonamides by negative electrospray ionization mass spectrometry. Rapid Commun Mass Spectrom 22:2715–2722. doi:10.1002/rcm.3672

Hu N, Tu Y, Jiang K, Pan Y (2010) Intramolecular charge transfer in the gas phase: fragmentation of protonated sulfonamides in mass spectrometry. J Org Chem 74:4244–4250. doi:10.1007/s13361-014-0935-7

Irikura KK, Todua NG (2014) Facile Smiles-type rearrangement in radical cations of *N*-acylarylsulfonamides and analogs. Rapid Commun Mass Spectrom 28:829–834. doi:10.1002/rcm.6850

Joshi S, Khosla N (2003) QSAR study on antibacterial activity of sulphonamides and derived Mannich bases. Bioorg Med Chem Lett 13:3747–3751. doi:10.1016/j.bmcl.2003.08.017

Li JJ, Anderson QD, Burton EG, Cogburn JN, Collins JT, Garland DJ, Gregory SA, Huang HC, Isakson PC, Kobaldt CM, Longush EN, Norton MB, Perkns WE, Reinhard EJ, Serbert K, Veenhuizen AW, Zang Y, Rertz DB (1995) 1,2-Diarylcyclopentenes as selective cyclooxygenase-2 inhibitors and orally active antiinflammatory agents. J Med Chem 38(22):4570–4578

Lloyd J, Atwal KS, Finlay HJ, Nyman M, Huynh T, Bhandaru R, Kover A, Schmidt J, Vaccaro W, Conder ML, Jenkins-West T, Levesque P (2007) Benzopyran sulphonamides as Kv1.5 potassium channel blockers. Bioorg Med Chem Lett 17(12):3271–3275. doi:10.1016/j.bmcl.2007.04.020

Lü Q, Frank W (2008) Impact of novel screening technologies on ion channel drug discovery. Comb Chem High Throughput Screen 11(3):185–194. doi:10.2174/138620708783877735

Morgan KM, Ashline DJ, Morgan JP, Greenberg A (2014) Electrospray ionization (ESI) fragmentations and Dimethyldioxirane reactivities of three diverse lactams having full, half, and zero resonance energies. J Org Chem 79(2):517–528

Olsson RI, Jacobson I, Iliefski T, Boström J, Davidsson Ö, Fjellström O, Björe A, Olsson C, Sundell J, Gran U, Gyll J, Malmberg J, Hidestål O, Emtenäs H, Svensson T, Yuan Z, Strandlund G, Åstrand A, Lindhardt E, Linhardt G, Forsström E, Högberg Å, Persson F, Andersson B, Rönnborg A, Löfberg B (2014) Lactam sulfonamides as potent inhibitors of the Kv1.5 potassium ion channel. Bioorg Med Chem Lett 24(5):1269–1273. doi:10.1016/j.bmcl.2014.01.067

Rodrigues ARA, Arantes EC, Monje F, Stuhmer W, Varanda WA (2003) Tityustoxin-K(alpha) blockade of the voltage-gated potassium channel Kv1.3. Br J Pharmacol 139(6):1180–1186. doi:10.1038/sj.bjp.0705343

Scozzafava A, Mastrolorenzo A, Supuran CT (2002) Arylsulfonyl-*N*,*N*-diethyldithiocarbamates: a novel class of antitumor agents. Bioorg Med Chem Lett 10:1887–1891. doi:10.1016/S0960-894X(00)00375-9

Smith MB, March J (2001) March's advanced organic chemistry. Wiley, New York

Sun M, Dai W, Liu DQ (2007) Fragmentation of aromatic sulfonamides in electrospray ionization mass spectrometry: elimination of SO_2 via rearrangement. J Mass Spectrom 43:383–393. doi:10.1002/jms.1335

Supuran CT, Scozzafava A, Jurca BC, Ilies MA (1998) Carbonic anhydrase inhibitors—part 49: synthesis of substituted ureido and thioureido derivatives of aromatic/heterocyclic sulfonamides with increased affinities for isozyme I. Eur J Med Chem 33:83–93. doi:10.1016/S0223-5234(98)80033-0

Wulf H, Castle NA, Pardo LA (2009) Voltage-gated potassium channels as therapeutic targets. Nat Rev Drug Discov Lond 8(12):982–999. doi:10.1038/nrd2983

Yamaoka H, Kusagi I, Isa K, Maekawa Y, Nibbering NMM (2004) On the double benzylic hydrogen migration in the molecular ion of *N*-(5-phenylvaleryl)-1-azacyclopentane-2-thione. Int J Mass Spectrom 234:171

Ye D, Wang J, Yu K, Zhou Y, Jiang H, Chen K, Liu H (2009) Current strategies for the discovery of K^+ channel modulators. Curr Top Med Chem 9:348–361. doi:10.2174/156802609788317865

Zhao G, Bolton SA, Kwon C, Hartl KS, Seiler SM, Slusarchyk WA, Sutton JC, Bisacchi GS (2004) Synthesis of potent and selective 2-azepanone inhibitors of human tryptase. Bioorg Med Chem Lett 14:309–312. doi:10.1016/j.bmcl.2003.11.016