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Novel sulfamides and sulfamates derived from amino esters: Synthetic studies and anticonvulsant activity

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ABSTRACT

We report herein the design and optimization of a novel series of sulfamides and sulfamates derived from amino esters with anticonvulsant properties. The structures were designed based on the pharmacophoric pattern previously proposed, with the aim of improving the anticonvulsant action. The compounds were obtained by a new synthetic procedure with microwave assisted heating and the use of adsorbents in the isolation process. All the derivatives showed protection against the maximal electroshock seizure test (MES test) in mice at the lowest dose tested (30 mg/kg) but they did not show significant protection against the chemical induced convulsion by pentylenetetrazole. These results verify the ability of the computational model for designing new anticonvulsants structures with anti-MES activity. Additionally, we evaluated the capacity of the synthesized structures to bind to the benzodiazepine binding site (BDZ-bs) of the γ-aminobutiric acid receptor ($GABA_A$ receptor). Some of them showed medium to low affinity for the BDZ-bs.

1. Introduction

Epilepsy is not one condition, but a complex set of cerebral disorders that have in common the occurrence and recurrence of seizures (Fisher et al., 2014). About 65 million people worldwide currently live with epilepsy, and only 70% of them control the seizures with the available medication (Moshé et al., 2015), at expenses of the significant adverse side effects that increase their toxic actions when a lifelong medication is required (Bialer et al., 2013; Löscheran and Schmidt, 2011). The remaining one third of the patients are still resistant to the current anticonvulsant drugs, condition known as refractory epilepsy (French, 2007). Under this scenario, there is a genuine need for new antiepileptic compounds with more efficacy and safety.

Research from our group and others has focused on sulfamide derivatives as new targets of anticonvulsant drugs. (Gavernet et al., 2009, 2007a, 2007b; McComsey et al., 2013). Particularly, our previous findings allowed us to define a new pharmacophoric pattern for amino acid derived sulfamides (Fig. 1) (Gavernet et al., 2009), with anticonvulsant action evidenced in the Maximal electroshock seizure test in mice (MES test, Porter et al., 1984).

The most promising compounds were two β-Alanine sulfamide derivatives: the methyl [N-(N′-2-propylpentyl)-sulfamoyl]-β-alaninate and the methyl [N-(N′butyl)-sulfamoyl]-β-alaninate (compounds 1 and 2, Fig. 2). They exhibit some structural similarities with the anticonvulsant drug Valrocemide (Isoherranen et al., 2001), a valproic acid derivative with a glycinamide moiety linked to the valpromide function (compound 3, Fig. 2).

We report herein the synthesis and anticonvulsant activity of a new set of β-alanine derived sulfamides (compounds 4–9, Fig. 3). Additionally, we replaced β-alanine moiety of Methyl [N-(p-fluorobenzyl)-sulfamoyl] β-alaninate (compound 9, Fig. 3) by L-valine and L-phenyl alanine skeleton (compounds 10 and 11, Fig. 3). As will be explained in Section 4, compound 9 showed promising anticonvulsant action, so we synthesized structures 10 and 11 in order to explore the influence of other amino acid chains to the activity.

To obtain the new structures we design here a new synthetic protocol by using microwave assisted synthesis. As will be described in the next section, the synthetic routes of sulfamides involved the previous preparation of ester sulfamates. Sulfamates also comply with the pharmacopohore requirements for the polar

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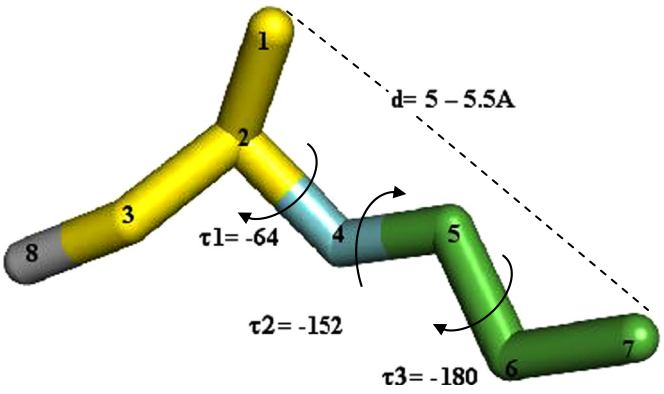

Fig. 1. Pharmacophoric pattern proposed by Gavernet et al. (2009). The anti-MES requirements can be summarized as: (1) a polar moiety (atoms 1–3, in yellow), (2) a hydrophobic chain (atoms 5–7 in green) placed in a conformation defined by *t*1, *t*2, *t*3 and *d*, and connected to the polar moiety through a link atom (atom 4, in cyan), (3) any group attached to atom 3 should be non polar or H.

group and they present a similar electronic distribution than sulfamides (Gavernet et al., 2007a). In fact, the anticonvulsant drug Topiramate presents this function into its chemical structure. For that reason we included the sulfamates showed in Fig. 4 in the protocols for biological assays.

It is worth mentioning that sulfamides and sulfamates are versatile functions that have been employed in medicinal chemistry to construct active compounds with different applications. These functionalities interact with molecular targets of several diseases, such as aspartic proteases (HIV-1 protease, γ-secretase), serine proteases, metalloproteinases, steroid sulfatases and 5-HTID Receptors among others (Nussbaumer and Billich, 2005; Reitz et al., 2009; Winum et al., 2006). They have also successfully tested as carbonic anhydrase inhibitors due to their bioisosteric correspondence with sulfonamide, the classical functional group that inhibit the active isoforms through its direct interaction with the active site (Gavernet et al., 2013; McKenna and Supuran, 2014).

2. Materials and methods

2.1. Chemistry

In previous investigations we synthesized amino acid derived sulfamides by modifying the synthesis of aril/alkyl sulfamides via catechol sulfate (DuBois and Stephenson, 1980). Catechol sulfate (prepared with catechol and sulfuryl chloride) reacts with the salt of the amino ester under controlled conditions, to yield a sulfamate ester derivative (Scheme 1). Then, the resulting sulfamate reacts with the alkyl/aryl amine to yield the amino acid derived sulfamide (Scheme 1, Gavernet et al., 2009). Unlike previous methodologies, we employed here microwave assisted synthesis in both steps of the reaction. With this method, we incorporated reactions under solvent free conditions, which is more benign to the environment than the use of the traditional reaction media.

The synthetic route outlined in Scheme 1 was employed for the synthesis of β-Alanine methyl ester sulfamides. However, our attempts to obtain the corresponding sulfamates from L-valine methyl ester and L-phenylalanine methyl ester were not successful, even when traditional heating and different catechol/amino ester ratio were used.

Particularly, the synthesis of the sulfamate of L-valine methyl ester (compound 13) with the traditional heating gave the N,N′-disubstituted sulfamide, the N,N′-Sulfonyl bis-L-valine dimethyl ester (Gavernet et al., 2009), as the main product of the reaction; and small quantities of compound 13 (which was purified and tested). This result is consistent with the analysis of the products obtained from the synthesis of other alkyl and aryl sulfamates (DuBois and Stephenson, 1980). Finally, we prepared the sulfamides 10 and 11 by inverting the synthetic route: first we obtained the sulfamate of p-fluorobenzyl amine and then we added the corresponding amino ester (Scheme 2).

The synthetic methods outlined in Schemes 1 and 2 involve the production of equimolar quantities of sulfamide and catechol. In our experience, the isolation and purification of the sulfamide from the relative large amounts of catechol causes difficulties during the work up process. Traditionally, we washed the crude product and then we performed at least one column chromatography process, which require large amounts of silica gel and dichloromethane as the elution solvent (Gavernet et al., 2009). In this investigation we replaced the standard work up process by an heterogeneous filtration process. We directly added to the reaction mixture an insoluble inorganic compound with the capacity to absorb selectively catechol and then filtered the solid.

Details about the synthesis of the new sulfamides and sulfamates, their physicochemical characteristics and their spectroscopic characterization are summarized next.

2.1.1. General information

Melting points were determined using capillary tubes with an electrothermal melting point apparatus and were uncorrected. Thin-layer chromatography (TLC) was performed with aluminum backed sheets with silica gel 60 F254 (Merck, ref 1.05554), and the spots were visualized with 254 nm UV light and 5% aqueous solution of ammonium molybdate (VI) tetrahydrate. Column chromatography was performed on silica gel 60 (70–230 mesh, Merck, ref 1.07734.2500). ^{1}H and ^{13}C NMR spectra were recorded for compounds 3, 4 and 6 on a Varian Gemini 200 spectrometer at 200 and 50 MHz; whereas we employed a BRUKER AVANCE II 500 spectrometer at 500 and 126 MHz for the rest of the structures. The chemical shifts were reported in ppm (δ scale) relative to internal TMS, and coupling constants were reported in Hertz (Hz). FTIR spectra were performed using a Bruker EXINOX 55 equipment. Spectra were recorded at room temperature in the 4000–400 cm^{-1} range, and the samples were prepared in form of wafers with KBr. Absorption values were expressed as wavenumbers (cm^{-1}) and only significant absorption bands are given. Analytical grade solvents were employed for crystallization, while pure solvents were used for the reactions, extractions and column chromatography. Commercial amines were distilled prior to their

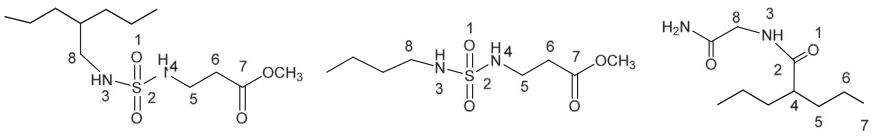

Fig. 2. Compounds with anticonvulsant properties analyzed in previous investigations by Gavernet et al. (2009). Atoms are numbered for the centers that define the pharmacophoric pattern shown in Fig. 1.

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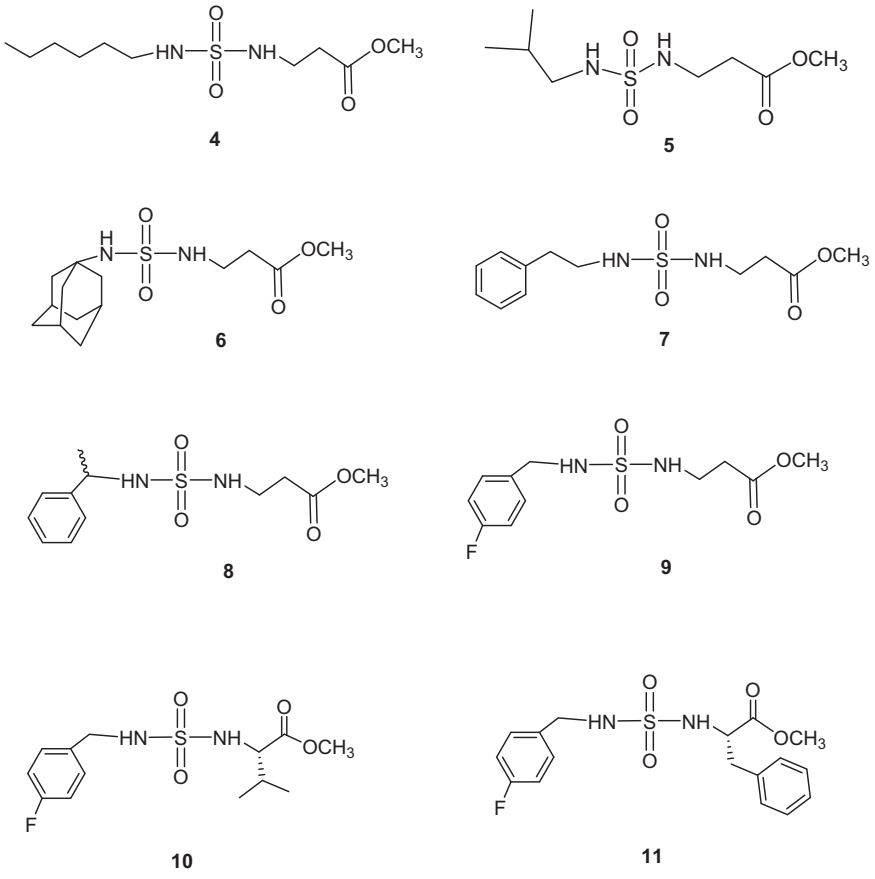


Fig. 3. Amino acid derived sulfamides synthesized and tested in this investigation.

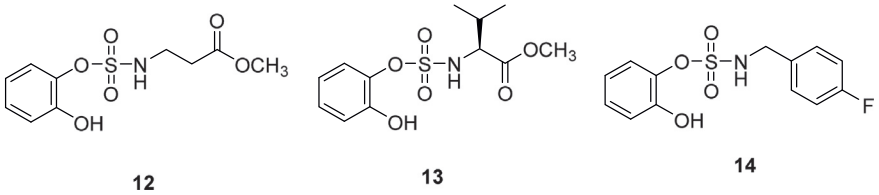
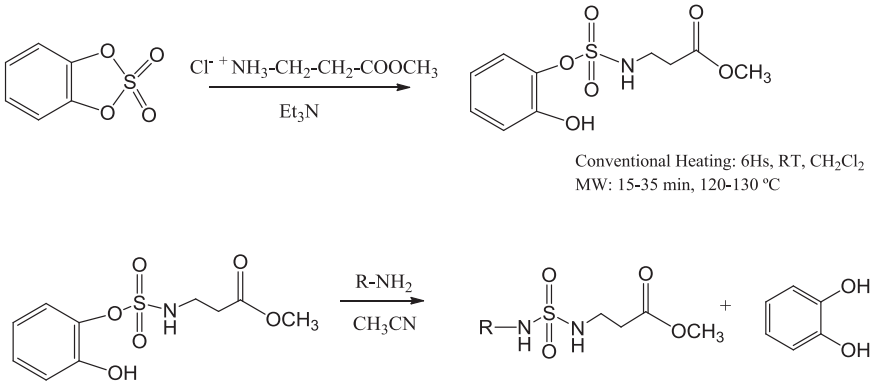


Fig. 4. Sulfamates synthesized and tested in this investigation.

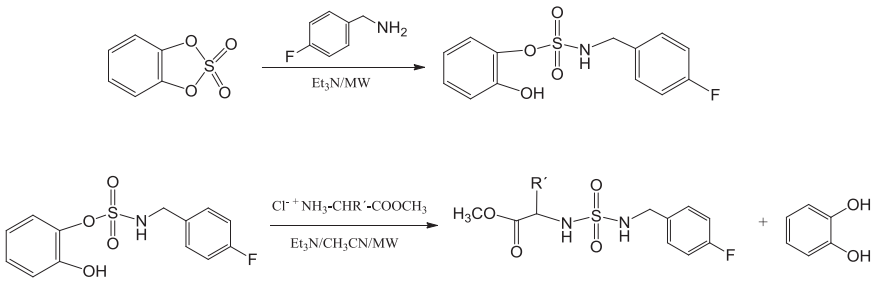


Scheme 1. Traditional and alternative conditions for the synthetic routes of β-Alanine methyl ester sulfamides. *R*=hexyl (compound 4), isobutyl (compound 5), adamantyl (compound 6), phenethyl (compound 7), α-metilbenzyl (compound 8), p-fluorobenzyl (compound 9).

use. All reagents employed have analytical grade (Sigma-Aldrich, Fluka, J.Backer). Microwave reactions were carried out in an Anton-Paar-monowave-300 reactor (monowave, maximum power 850 W, temperature control via IR-sensor, vial volume: 10–30 ml). Mass spectra were obtained on an Agilent Technologies LC/MSD Trap SL. Specific rotations of chiral structures 10, 11 and 13 were measured on PerKin Elmer 343 at λ: 589 nm and 25 °C.

2.1.2. Synthesis of 2-Hydroxyphenyl-N-β-alanine-methyl ester sulfamate (12)

Triethylamine (6.10 ml, 44.6 mmol) was added dropwise to a solution of H-β-Ala-OMe.HCl (1.99 g, 22.30 mmol) and catechol



Scheme 2. Synthesis of compound 10 (R´: isopropyl) and 11 (R´: benzyl).

sulfate (4.20 g, 25.12 mmol, prepared with catechol and sulfuryl chloride according to DuBois and Stephenson, 1980). The resulting solution was placed into a microwave vial containing a stirrer bar. The vial was sealed after purging with N_2 for some min. The reaction was then heated to 90 °C for 15 min using the microwave reactor. After that, dichloromethane (35 ml) was added and the solution was washed with 15 ml of 5 N hydrochloric acid (2 ×) and brine (1 ×). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was then purified by silica column chromatography (15:1 CH_2Cl_2/MeOH). Crystallization with CH_2Cl_2 afforded the product as a white solid (3.60 g, 59.0% yield). Melting point 102–104 °C, Rf: 0.59 (15:1 CH_2Cl_2/MeOH) (Gavernet et al., 2009).

2.1.3. Synthesis of 2-hydroxyphenyl-N-L-valine-methyl ester sulfamate (13)

The compound was synthesized according to the method previously reported for 2-Hydroxyphenyl-N-β-alanine-methyl Ester Sulfamate (12) (Gavernet et al., 2009). A solution of catechol sulfate (2.80 g, 16.28 mmol) in 10.0 ml of CH_2Cl_2 was added dropwise to a solution of L-Val-OMe.HCl (2.72 g, 16.28 mmol) and triethylamine (4.89 ml, 35.2 mmol) in 10.0 ml of CH_2Cl_2 with vigorous stirring at 0 °C under N_2.The reaction medium was then warmed to room temperature and stirred for 18 h, concentrated under reduced pressure. The crude product was purified by column chromatography (CH_2Cl_2/MeOH 20:1). Crystallization with CH_2Cl_2/petroleum ether afforded the product as a white solid (0.28 g, 28.0% yield). Melting point: 69–71 °C. Rf: 0.57 (20:1 CH_2Cl_2/MeOH). $[\alpha]_D^{25}$: 29.0° (*c* 0.5, $CHCl_3$).^{1}H NMR ($CDCl_3$) δ 6.90–7.29 (m, 4H, H–Ar), 6.82 (s, 1H, OH), 5.55 (s, 1H, NH), 4.14 (s, 1H, CH–N), 3.85 (s, 3H, OCH_3), 2.19–2.25 (m, 1H, CH), 0.96–1.06 (dd, *J*=6.8 Hz, 6.9 Hz, 6H, CH_3). ^{13}C NMR ($CDCl_3$) δ 172.36 (C=O ester), 148.16 (C–OH), 137.72, 128.30, 122.99, 120.82, 118.24 ($C^{1,3,4,5,6}$ Ar), 62.74 (C–N), 53.07 (OCH_3), 31.48 (CH), 18.79 (CH_3), 17.37 (CH_3). IR (KBr) 3272 (O–H), 2983–2881(C–H,OCH_3), 1714 (C=O), 1371, 1168 (SO_2). Elemental analysis calculated for $C_{10}H_{22}N_2O_4S$: C 47.5, H 5.6, N 4.6, S 10.6 % Found: C 47.7, H 5.8, N 4.7, S 10.6%.

2.1.4. General procedure for the synthesis of sulfamides 4 to 9

The corresponding amine was added to one solution of the sulfamate 12 in acetonitrile (5.00 ml). The mixture was placed into a microwave vial containing a stirrer bar, which was sealed after purging with N_2 for some min. The resulting solution was heated to 110–130 °C for 15–35 min (depending on the amine). The reaction medium was then diluted with acetonitrile (20 ml) and γ-Al_2O_3 was added to remove catechol. The acetonitrile solution was filtered and the solvent was removed under reduced pressure. The products were purified by column chromatography and/or crystallization.

2.1.4.1. Methyl [N-(N´hexyl)-sulfamoyl]β-alaninate (4). The compound was obtained following the general procedure, from sulfamate 12 (0.800 g, 2.90 mmol), acetonitrile, and hexylamine (0.46 ml, 3.19 mmol). The reaction mixture was heated to 120 °C for 15 min in a microwave reactor. Then the reaction medium was diluted with 20 ml of acetonitrile and 4.00 g of γ-Al_2O_3 (x3) was employed to remove catechol. The product was purified by column chromatography (CH_2Cl_2/MeOH 20:1) followed by crystallization with CH_2Cl_2/petroleum ether, affording the compound as a white solid (0.418 g, 54.0%). Melting point: 47–47.5 °C. Rf: 0.72 (20:1 CH_2Cl_2/MeOH). ^{1}H-NMR ($CDCl_3$) δ 5.01 (t, *J*=6.5 Hz, 1H, NH-β-Ala), 4.50 (*t*, *J*=6.1 Hz, 1H, NH-hexyl), 3.72 (s ,3H, O–CH_3), 3.31 (AM_2X_2 , *J*=6.5, 6.2 Hz, 2H, N-CH_2-β-Ala), 3.04–2.97 (m, 2H, N–CH_2–hexyl), 2.64 (*t*, *J*=6.1 Hz, 2H, CH_2-CO-β-Ala), 1.58–1.48 (m, 2H, CH_3–CH_2–hexyl), 1.29 (m, 6H, CH_2–CH_2–CH_2), 0.92–0.86 (m, 3H, CH_3-hexyl). ^{13}C NMR ($CDCl_3$) δ 172.46 (C=O ester), 51.96 (O–CH_3), 43.45 (N–C–hexyl), 38.91 (N-C-β-Ala), 34.10 (C–CO), 31.45 (α-C-hexyl), 29.86 (β-C-hexyl), 26.59 (γ– C-hexyl), 22.74 (δ–C-hexyl), 14.22 (CH_3-hexyl). IR (KBr): 3465–3296 (N–H), 2956–2852 (C–H, OCH_3), 1741 (C=O), 1332, 1199 (SO_2). Elemental analysis calculated for $C_{10}H_{22}N_2O_4S$: C 45.1, H 8.3, N 10.5, S 12.0%. Found: C 45.3, H 8.4, N 10.4, S 11.9%.

2.1.4.2. Methyl [N-(N´isobutyl)-sulfamoyl]β-alaninate (5). The compound was obtained following the general procedure, from sulfamate 12 (1.02 g, 3.64 mmol), acetonitrile and isobutylamine (0.44 ml, 4.36 mmol). The reaction mixture was heated to 120 °C for 20 min in a microwave reactor. Then the crude reaction medium was diluted with 20 ml of acetonitrile and 4.24 g of γ-Al_2O_3 (x3) was employed to remove catechol. The product was purified by crystallization with ether, to afford compound 5 as a white solid (0.416 g, 48.0%). Melting point: 42–43 °C. Rf: 0.66 (20:1 CH_2Cl_2 /MeOH). ^{1}H NMR ($CDCl_3$) δ 5.02 (*t*, *J*=6.3 Hz, 1H, NH-β-Ala), 4.58 (*t*, *J*=6.2 Hz, 1H, NH-isobutyl), 3.72 (s, 3H, O–CH_3), 3.30 (dd, *J*=6.3, 6.3 Hz, 2H, N–CH_2–β-Ala), 2.85 (*t*, *J*=6.5 Hz, 2H, CH_2-CO-β-Ala), 2.64 (*t*, *J*=6.1 Hz, 2H, CH_2-isobutyl), 1.91–1.70 (m, 1H, CH-isobutyl), 0.95 (*d*, *J*=6.3 Hz, 6H, CH_3).^{13}C NMR ($CDCl_3$) δ 172.83 (CO), 52.19 (O–CH_3), 50.78 (N-C-isobutyl), 38.91(N-C-β-Ala), 34.12 (C–CO), 28.49 (CH-isobutyl), 20.23 (CH_3). IR (KBr) 3280 (N–H), 2958–2873 (C–H, OCH_3),1749 (C=O), 1311, 1132 (SO_2). MS (ES^+): m/z calculated for $C_8H_{18}N_2O_4S$: 238.1 $[M+H]^+$, 499.2 $[2M+Na]^+$. Found: 239.1, 498.8. Elemental analysis calculated for $C_8H_{18}N_2O_4S$: C 40.3, H 7.6, N 11.7, S 13.4%. Found: C 40.5, H 7.6, N 11.5, S 13.0%.

2.1.4.3. Methyl [N-(N´1-adamantyl)-sulfamoyl] β-alaninate (6). The compound was obtained following the general procedure. Triethylamine (1.10 ml, 8.0 mmol) was added dropwise to a solution of the sulfamate 12 (1.14 g, 4.00 mmol), amantadine hydrochloride (0.76 g, 4.00 mmol) and 5.00 ml acetonitrile. The reaction medium was then heated to 120 °C for 20 min in a microwave reactor. After that, the crude product was diluted with acetonitrile (20 ml) and 9.25 g of γ-Al_2O_3 (x3) were used to remove catechol. Then the acetonitrile solution was filtered, and the solvent was removed under reduced pressure. The product was purified by column chromatography (CH_2Cl_2/MeOH 15:1) and crystallization with CH_2Cl_2/hexane, affording compound 6 as a white solid (0.534 g,

42.0%). Melting point: 86–87 °C. Rf: 0.54 (40:1 CH_2Cl_2/ MeOH). ^{1}H NMR ($CDCl_3$) δ 4.82 (s, 1H, NH-β-Ala), 3.73 (s, 3H, O–CH_3), 3.33 (*t*, *J*=6.0 Hz, 2H, CH_2–NH), 2.68 (*t*, *J*=6.0 Hz, 2H, N–CH_2–β-Ala), 2.12 (s, 3H , 3CH-adamantyl), 1.96 (*d*, *J*=2.3 Hz, 6H, 3 α–CH_2-adamantyl), 1.68 (*t*, 6H ,3 β–CH_2-adamantyl).^{13}C NMR ($CDCl_3$), δ 172.32 (C=O ester), 55.74 (O–CH3), 51.91 (N-C-adamantyl), 42.42(C-α-adamantyl), 38.95 (N-C-β-Ala), 35.89 (CH–adamantyl), 33.76 (C–CO), 29.58 (C-β-Adamantyl). IR (KBr) 3330–3284 (N–H), 2921-2861 (C–H, OCH_3), 1737 (C=O), 1321, 1143 (SO_2). Elemental analysis calculated for $C_{14}H_{24}N_2O_4S$: C 53.1, H 7.6, N 8.8, S 10.0 %. Found: C 52.9, H 7.7, N 8.8, S 9.8%.

2.1.4.4. Methyl [N-(N´phenethyl)-sulfamoyl]β-alaninate (7). The compound was obtained following the general procedure from sulfamate 12 (0.80 g, 2.90 mmol), acetonitrile and phenethylamine (0.36 ml, 2.92 mmol). The reaction mixture was heated to 130 °C for 15 min in a microwave reactor. Then the crude reaction medium was diluted with 20 ml of acetonitrile, and 4.00 g of γ-Al_2O_3 (x3) was employed to remove catechol. The product was purified by crystallization with CH_2Cl_2/petroleum ether, to afford compound 7 as a white solid (0.39 g, 47.0%). Melting point: 72–73 °C . Rf: 0.57 (20:1 CH_2Cl_2 /MeOH). ^{1}H-NMR ($CDCl_3$) δ 7.36–7.18 (m, 5H, Ar–H), 4.86 (s, broad, 1H, NH-βAla), 4.26 (s, broad, 1H, NH-phenethyl), 3.70 (s, 3H, O-CH_3), 3.31 (*t*, *J*=6.5 Hz, 2H, N-CH_2-phenethyl), 3.17 (s, broad, 2H, CH_2-benzyl), 2.87 (*t*, *J*=6.5 Hz, 2H, N-CH_2-β-Ala), 2.55 (*t*, *J*=6.1 Hz, 2H ,CH_2-CO-β-Ala). ^{13}C NMR (50 MHz, $CDCl_3$) δ 172.75 (C=O ester) 138.45 (C^1 Ar), 129.29, 129.01, 127.23 ($C^{2,3,4}$ Ar), 51.90 (O–CH_3), 44.81(CH_2–NH), 39.35 (N-C-β-Ala), 35.64 (CH_2-benzyl), 34.39 (C–CO). IR (KBr) 3477-3269 (N–H), 2954–2896 (C–H, OCH3), 1757 (C=O), 1319, 1145 (SO2). Elemental Analysis calculated for C12H18N2O4S: C 50.4, H 6.3, N 9.8, S 11.2 %. Found: C 50.7, H 6.5, N 9.6, S 11.1%.

2.1.4.5. Methyl [N-(N´α-methylbenzyl)-sulfamoyl]β-alaninate (8). The compound was obtained following the general procedure from sulfamate 12 (0.961g, 3.49 mmol), acetonitrile, and L(+)α-methylbenzylamine (0.49 ml, 3.84 mmol). The reaction mixture was heated to 120 °C for 25 min in a microwave reactor. Then the crude reaction medium was diluted with 20 ml of acetonitrile, and 4.28g of γ-Al2O3 (x3) was employed to remove catechol. The crude product was purified by chromatography (Hexane/ethyl acetate 1:1) affording compound 8 as colorless oil (0.401 g, 40.0%). Rf: 0.62 (CH_2Cl_2/MeOH 20:1). ^{1}H NMR ($CDCl_3$) δ 7.40–7.29 (m, 5H, C–Ar), 4.53–4.57 (m, 1H, CH-benzyl), 3.68 (s, 3H, O–CH_3) 3.18–2.96 (m, 2H, N–CH_2-β-Ala), 2.49–2.34 (m, 2H, CH_2-CO β-Ala), 1.56 (*d*, *J* = 6.3 Hz, 3H, CH_3).^{13}C NMR ($CDCl_3$) δ 172.07 (C=O ester) 142.25 (C^1 Ar), 128.30, 127.63, 126.43 ($C^{2,3,4}$ Ar), 53.41 (N–C–Ar), 51.57 (O–CH_3), 38.81 (N-C-β-Ala), 33.80 (C–CO), 23.55 (CH_3). IR (KBr) 3311 (N–H), 2977 (C–H, OCH_3), 2370 (C–H Ar) 1735 (C=O), 1440 (C–C Ar), 1330, 1161(SO_2) . MS (ES^+): m/z calculated for $C_{12}H_{18}N_2O_4S$: 286.3 [M+H^+], 595.7 [2M+Na]$^+$. Found 286.9, 594.8. Elemental analysis calculated for $C_{12}H_{18}N_2O_4S$: C 50.3, H 6.3, N 9.8, S 11.2%. Found: C 50.2, H 6.3, N 9.7, S 10.9%.

2.1.4.6. Methyl [N-(p-fluorobenzyl)-sulfamoyl]β-alaninate (9). The compound was obtained following the general procedure, from sulfamate 12 (0.904 g, 3.42 mmol), acetonitrile and p-fluorobenzylamine (0.76 ml, 3.76 mmol). The reaction was heated to 120 °C for 35 min in a microwave reactor. Then the crude reaction medium was diluted with 20 ml of acetonitrile, and 5.28 g of γ-Al_2O_3 (x4) was employed to remove catechol. The crude product was purified by column chromatography (Hexane/ethyl acetate 6:4) followed by crystallization with CH_2Cl_2/hexane; to give the product as a white solid (0.449 g, 45.0%). Melting point 87–88 °C. Rf: 0.58 (20:1 CH_2Cl_2/MeOH). ^{1}H NMR ($CDCl_3$) δ 7.33–7.36 (m, 2H, $C^{4,5}$–Ar), 7.04–7.08 (m, 2H, $C^{2,6}$–Ar), 4.20 (s, 2H, CH_2-benzyl), 3.71 (s, 3H, O–CH_3), 3.29 (*t*, *J*=6.0 Hz, 2H, N-CH_2-β-Ala), 2.60 (*t*, *J*=6.0 Hz, 2H ,CH_2–CO β-Ala).^{13}C NMR ($CDCl_3$) δ 172.57 (C=O ester), 163.47–161.70 (C–F), 132.46, 129.78, 115.62 ($C^{3,2,1}$–Ar), 51.77 (O–CH_3), 46.59 (C-benzyl), 38.69 (N-C-β-Ala), 33.67 (C–CO). IR (KBr) 3488–3288 (N–H), 2960–2860 (C–H, OCH_3), 1743 (C=O), 1510 (C–F), 1310, 1141 (SO_2). Elemental analysis calculated for $C_{11}H_{15}FN_2O_4S$: C 45.5, H 5.2, N 9.6, S 11.0 %. Found: C 45.5, H 5.3, N 9.5, S 11.1 %.

2.1.5. Synthesis of 2-hydroxyphenyl-N-4-fluorobenzyl sulfamate (14)

Triethylamine (2.30 ml, 16.8 mmol) was added dropwise to a cold solution of 4-fluorobenzylamine (1.70 ml, 16.83 mmol) and catechol sulfate (2.91g, 16.3 mmol, prepared with catechol and sulfuryl chloride). The reaction mixture was placed into a microwave vial containing a stirrer bar. The vial was sealed after purging with N_2 for some min. The reaction was then heated to 55 °C for 3 min in a microwave reactor. The crude product was purified by column chromatography (40:1 CH_2Cl_2/MeOH). Crystallization with CH_2Cl_2 afforded the product as a white solid (1.91 g, 38.3% yield). Melting point 129–130 °C. Rf: 0.53 (40:1 CH_2Cl_2/MeOH). ^{1}H NMR (DMSO) δ 7.39–6.77 (m, 8H, Ar), 6.97 (s, 1H ,OH), 4.28 (s, 1H, NH), 3.31 (S, 2H, CH_2). ^{13}C NMR (DMSO) δ 163.55, 160.33 (C–F), 150.31 (COH), 138.18 (C–O), 134.32, 130.27, 127.70 ($C^{3,2,1}$–Ar–F) 123.95, 119.52, 117.87,115.40 ($C^{3,4,5,6}$–Ar–OH), 39.97 (CH_2). IR (KBr) 3423 (O–H), 3282 (N–H), 2958–2860 (C–H, OCH_3), 1363, 1155 (SO_2), 1220 (C–F). Elemental analysis calculated for $C_{13}H_{12}FNO_4S$: C 52.5, H 4.1, N 4.7, S 10.8%. Found: C 52.3, H 4.2, N 4.8, S 10.9%.

2.1.6. Synthesis of methyl [N-(N´-4-fluorobenzyl)]-sulfamoyl]L-valinate (10)

Triethylamine (0.65 ml, 5.4 mmol) was added dropwise to a solution of H-Val-OMe.HCl (0.390 g, 2.37 mmol) and 2-Hydroxyphenyl-N-4-fluorobenzyl sulfamate 14 (0.705 mg, 2.37 mmol) in acetonitrile (5 ml). The reaction mixture was stirred and heated to 120 °C for 20 min in a microwave reactor. After that, dichloromethane (35 ml) was added and the solution was washed with 15 ml of 2.5 N hydrochloric acid (1 ×) and brine (1 ×). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. Then the crude product was diluted with 20 ml of acetonitrile, and 3.75 g of γ-Al_2O_3 (x2) was employed to remove catechol. Finally the product was purified by crystallization with CH_2Cl_2/hexane, to afford compound 10 as a white solid (0.338g, 44.7 %). Melting point: 70–71 °C. Rf: 0.56 (40:1 CH_2Cl_2/MeOH). $[\alpha]_D^{25}$: −9.0° (*c* 0.5, MeOH). ^{1}H NMR ($CDCl_3$) δ 7.32–7.29 (m, 4H, H–Ar), 5.10 (*d*, *J*=6.8 Hz, 1H, NH-Val), 4.57 (s, 1H, NH), 4.23 – 4.16 (s, 2H, CH_2 benzyl), 3.87–3.85 (m, 1H, CH), 3.75 (s, 3H, OCH_3), 2.17–2.10 (m, 1H, CH-isopropyl), 1.04-0.93 (dd, *J*=6.8 Hz, 6.9 Hz, 6H). IR (KBr) 3313 (N–H), 2973–2881 (C–H, OCH_3), 1743 (C=O), 1321, 1143 (SO_2), 1234 (C–F). Elemental analysis calculated for $C_{13}H_{19}FN_2O_4S$: C 49.0, H 6.0, N 8.8, S 10.1. Found: C 49.3, H 6.1, N 8.5, S 10.1%.

2.1.7. Synthesis of Methyl [N-(N´-4-fluorobenzyl)]-sulfamoyl] L-phenylalaninate (11)

Triethylamine (0.93 ml, 6.76 mmol) was added dropwise to a solution of H-Phe-OMe.HCl (0.730 g, 3.38 mmol) and 2-Hydroxyphenyl-N-4-fluorobenzyl sulfamate (1.0g, 3.38 mmol), in acetonitrile (5 ml). The reaction mixture was placed into a microwave vial containing a stirrer barand it was heated to 120 °C for 25 min. After that, dichloromethane (35 ml) was added, and the solution was washed with 5 ml of 2.5 N hydrochloric acid (1 ×) and brine (1 ×). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. Then the crude reaction medium was diluted with 30 ml of acetonitrile and 5.58 g of γ-Al_2O_3 (x2) was employed to remove catechol. The product was purified by crystallization with CH_2Cl_2/ether, to afford compound

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11 as a white solid (0.495 g, 41.3%). Melting point: 64–65 °C. Rf: 0.71 (40:1 CH_2Cl_2 /MeOH). $[\alpha]_D^{25}$: −16.8° (*c* 0.5, MeOH). ^{1}H NMR (DMSO) 7.33–6.96 (m, 9H, H–Ar), 4.95 (*d*, *J*=6.4 Hz, 1H, NH), 4.42 (broad, 1H, NH), 4.30–4.23 (m, 1H, CH), 4.01–3.84 (dd, 2H, CH_2–Ar–F), 3.60 (s, 3H, O–CH_3), 3.15–2.94 (m, 2H, CH_2-benzyl).^{13}C NMR (DMSO) δ 173.17 (C=O ester), 162.73, 160.60 (C–F), 134.75, 129.91, 115.23 ($C^{3,2,1}$–Ar–F), 137.43, 129.09, 127.24, 115.40 ($C^{5,6,7,8}$–Ar), 57.63 (CH), 52.24 (OCH_3), 44.81 (CH_2–Ar–F), 38.41 (CH_2-benzyl). IR (KBr) 3280 (N–H), 2962–2873 (C–H, OCH_3), 1726 (C=O), 1326, 1145 (SO_2), 1216 (C–F). Elemental analysis calculated for $C_{18}H_{21}FN_2O_4S$: C 55.7, H 5.2, N 7.6, S 8.7%. Found: C 55.4, H 5.2, N 7.5, S 8.5%.

2.2. Pharmacology

Biological evaluation was performed following the standard procedures proposed by The NIH anticonvulsant drug development (ADD) program, via the anticonvulsant screening project (Krall et al., 1978; see *Experimental section*). We employed the most popular seizure models, the MES test and the PTZ test in mice (Porter et al., 1984). The MES test (maximal electroshock seizure test) is associated with the electrical induction of the seizure, whereas PTZ test (pentilenetetrazol test) involves a chemical induction to generate the convulsion. Toxicity was also evaluated by using the standardized RotoRod test, which is also included in the primary phase of ADD program (Dunham and Miya, 1957; Porter et al., 1984). Additionally, a radioligand binding assay was used to evaluate the putative action of the compounds on the benzodiazepine binding site of the $GABA_A$ receptor complex. Details of the experiments are given in the next section.

2.2.1. Animals for MES, PTZ and Rototod test

Adult male Swiss mice (18–25 g) were employed in the pharmacological assays. They were obtained from the Faculty of Veterinary, National University of La Plata. The animals were maintained on a 12 h light/dark cycle and allowed free access to food and water, except during the time they were removed from their cages for testing. They were daily manipulated during one week before the experiment, to minimize stress caused by handling at the time of trial. Housing, handling, and experimental procedures complied with the recommendations provided by the National Institutes of Health Guide for Care and Use of Laboratory Animals.

2.2.2. MES test

To evaluate protection in the MES test, Maximal Electroshock Seizures were elicited in mice by delivering a 60 Hz/50 mA electrical-stimulus for 0.2 s via ear clip electrodes, at 0.5 and 4 h after the drug injection, using an UGO Basile equipment. A drop of saline solution was applied on each ear before placing the electrodes to ensure adequate electrical contact. In these conditions, maximal seizures are produced in virtually all normal mice. The maximal seizure typically consists of a short period of initial tonic flexion and a prolonged period of tonic extension (especially of the hind limbs) followed by terminal clonus. The typical seizure lasts approximately 22 s. Failure to extend the hind limbs to an angle with the trunk greater than 90° is defined as protection.

2.2.3. PTZ test

The induced seizure entailed the subcutaneous (sc) administration of 85 mg/kg of PTZ in 0.9% saline in the posterior midline of the mice. The animals were observed for 30 min. Protection was defined as the failure to observe a single episode of clonic spasms of at least 5 s duration.

2.2.4. Rotorod test

It was used to determine possible neurotoxic effects of the test drugs. This test is designed to detect minimal neurological deficit. The mouse is placed on a 1 inch diameter knurled plastic rod rotating at 6 rpm. Normal mice remain indefinitely on a rod rotating at this speed. Neurological deficit (e.g., ataxia, sedation, hyperexcitability) is indicated by the inability of the animal to maintain equilibrium on the rod for at least 1 min in each of three trials (Dunham and Miya,1957).

2.2.5. $GABA_A$ binding assay

The binding of [^{3}H]-flunitrazepam (81.8 Ci/mmol; obtained from PerkinElmer Life and Analytical Sciences, Boston, MA, USA) to the benzodiazepine binding site was performed in washed crude synaptosomal membranes from rat cerebral cortex. Membranes were prepared according to Wasowski et al. (2012). The compounds were added to 0.2–0.3 mg membrane protein suspended in 1 ml of 25 mM Tris–HCl buffer in the presence of [^{3}H]-flunitrazepam 0.3 nM. In the screening assays each compound was tested at 300 μM in triplicate. In the competition assays, the incubations were done with 3–900 μM of each compound. Diazepam was used as positive control in concentrations between 1 and 100 nM. Non-specific binding was measured in the presence of flunitrazepam 10 μM and represented 5–15% of the total binding. The incubations were carried out at 4 °C for 1 h. After incubation, the assays were terminated by filtration under vacuum through Whatman GF/B glass-fiber filters followed by washing three times with 3 ml each of incubation medium. Individual filters were incubated overnight with scintillation cocktail (OptiPhase 'HiSafe' 3) before measuring radioactivity in a Wallac Rackbeta 1214 liquid scintillation counter.

3. Results

3.1. Chemistry

We first prepared the intermediate 2-hydroxyphenyl-*N*-β-alanine-methyl ester sulfamate (Scheme 1). The best conditions were achieved after several experiments that differ from each other in the reaction temperatures, reaction times and presence/absence of solvent. The best yield, 59%, was obtained by stirring the reactants (without solvent) under microwave radiation at 90 °C for 15 min (traditional synthesis needs 6 h of reaction according to Gavernet et al., 2009). The yield obtained from MW heating is lower than the one achieved from traditional heating, but the reaction is faster and cleaner.

Regarding the sulfamide products synthesized from 2-hydroxyphenyl-*N*-β-alanine-methyl ester sulfamate, they also were prepared via microwave assisted heating. However, compounds 4, 5 and 7 were also obtained following the standard procedures, to compare the performance of the methodologies. Table 1 summarizes the conditions employed for the preparation of the compounds and the results obtained. Microwave assisted preparation over performs the traditional procedure in both yields and

Table 1
Comparison of the result obtained from conventional and microwave assisted synthesis of sulfamide derivatives. γ-Al_2O_3 was used for the work up processes in all cases. The yields were calculated from the pure products.

Compound R	Heating	Time reaction	Temperature(°C)	Yield(%)
Hexyl	Conventional	10 h	80-100	40
	MW	15 min	120	54
Isobutyl	Conventional	22 h	45	0
	MW	20 min	120	48
Phenetyl	Conventional	22 h	90	41
	MW	15 min	130	46

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Table 2
Results obtained from the adsorption experiments. In all cases we employed a solution of 0.0166 mM of catechol in dichloromethane as initial sample.

Adsorbent	Mass of adsorvent (mg)	% Adsortion
α-Al_2O_3	100	2
ZrO_2	100	57
ZrO_2	200	68
ZrO_2	100–100	69
2 adsorptions		
ZrO_2	100–100–100	86
3 adsorptions		
γ-Al_2O_3	100	93
γ-Al_2O_3	100–100	100
2 adsorptions		
$Cu(OAc)_2$–ZrO_2	100	28
$Cu(OAc)_2$–α–Al_2O_3	100	61

reaction times.

We employed heterogeneous filtration to eliminate the catechol produced in the last step of the reactions. In order to find the optimal conditions, we initially tested the capacity of several solids to retain catechol. We evaluated zirconia (ZrO_2), α–alumina (α-Al_2O_3) and γ-alumina (γ-Al_2O_3) because these solids are widely studied as adsorbents suitable for effluent treatment in industrial processes (Lin and Juang, 2009). Additionally, we impregnate the selected oxides with cupric acetate (to obtain oxide supported cupric acetate), since the cupric salt itself was used to absorb catechol in old synthetic procedures (DuBois and Stephenson, 1980). Characteristics of the solids are given as Supporting information.

Table 2 shows the results obtained from the absorption of equimolar samples of catechol. The quantification was performed by gas chromatography with a Shimadzu GC-2014 chromatograph equipped with a FID detector. We employed a semi-polar Supelco SPB-5 column. It was heated from room temperature to 210 °C with a temperature ramp program of 10° Cm^{-1} and linear velocity of 30 cm s^{-1}. The capacity of the absorption was expressed as the percent of catechol adsorbed in each experiment. We performed more than one adsorption experiments over the same sample of catechol for the most promising solids (ZrO_2 and γ-Al_2O_3).

According to the results shown in Table 2, the best adsorbent for catechol is γ-Al_2O_3, which quantitatively retains the catechol after two adsorption processes. This solid present the highest capability of adsorption for the tested adsorbents, and it can be handled without modification with cupric acetate. It presents a high specific area and it is a low priced and accessible commercial reactant.

3.2. Pharmacology

The results of the biological evaluation of the synthesized compounds are shown in Table 3. We also incorporated into the table the biological profile of compounds 1, 2 and 12 for comparison. All compounds of the set showed positive response in the MES test. In fact, all sulfamides presented protection at the lowest dose proposed by the ADD program (30 mg/kg), with at least 1/3 animal protected at 0.5 and/or 4 h after administration. Moreover, compounds 5, 6 and 10 showed anticonvulsant activity for all tested animals at 30 mg/kg. No sedative effects were observed for most of the molecules with exception of compound 4 (1/3 mice at

Table 3
Pharmacological profile (Phase I) of the synthesized compounds.

Compound	Doses (mg/kg)	[a]MES activity time (h)		[b]PTZ activity time (h)		[c]TOX time (h)		[d]Class	Binding inhibition [Ki (μM)][e]
		0.5	4	0.5	4	0.5	4		
1	30[f]	1/3	3/3	1/2	0/2	0/5	0/5	1	+[g]
	100[f]	0/3	0/3	2/2	0/2	0/5	0/5		
2	30[f]	1/3	1/3	0/3	0/3	0/6	0/6	1	nt
	100[f]	1/3	1/3	0/3	0/3	0/3	0/6		
4	30	1/3	2/3	0/2	0/2	1/3	0/3	4	++
	100	0/3	0/3	0/2	0/2	0/3	0/3		[140 ± 31]
5	30	0/3	3/3	0/2	0/2	0/3	0/3	1	–
	100	1/3	3/3	0/2	0/2	0/3	0/3		
6	30	0/3	3/3	0/2	0/2	0/3	0/3	1	–
	100	2/3	2/3	0/2	0/2	0/3	0/3		
7	30	2/3	1/3	0/2	0/2	0/3	0/3	1	++
	100	3/3	1/3	0/2	1/2	0/3	0/3		[85 ± 21]
8	30	1/3	1/3	0/2	0/2	0/3	0/3	1	–
	100	1/3	1/3	0/2	0/2	0/3	0/3		
9	30	0/3	1/3	0/2	0/2	0/3	0/3	1	++
	100	2/3	2/3	0/2	0/2	0/3	0/3		[123 ± 15]
10	30	1/3	3/3	0/2	0/2	0/3	0/3	1	++
	100	3/3	1/3	nt	nt	0/3	0/3		[61 ± 21]
11	30	1/2	2/4	0/2	0/2	0/2	0/2	1	+++
	100	0/2	3/4	0/2	0/2	1/2	2/4		[14 ± 1]
12	30[f]	0/3	0/3	1/3	0/3	0/3	0/3	1	+
	100[f]	1/3	1/3	0/3	0/3	0/3	0/3		
13	30	1/4	0/4	0/2	0/2	0/2	0/2	1	–
	100	1/4	2/4	0/2	0/2	0/2	0/2		
14	30	1/2	0/2	0/2	0/2	0/4	0/2	1	–
	100	2/4	3/4	0/2	0/2	2/4	1/4		

[a] Maximal Electroshock Seizure test.
[b] Pentilenetetrazol test
[c] Toxicity evaluated in Rotorod test.
[d] The compounds can be classified into four classes according to their activity in MES test.
[e] nt: Not tested. [e]Capacity of the compounds, at 300 μM, to inhibit the binding of [^{3}H]-flunitrazepam to the benzodiazepine binding site of the $GABA_A$ receptor indicated as: inhibition > 80% (+++); inhibition; 40–80% (++); inhibition 20–40% (+) and inhibition < 20% (–). Ki ± standard error of the mean values are means of 2–3 independent determinations. Diazepam, used as a control, gave a Ki value of 0.0070 ± 0.0005 μM (n=5).
[f] Anticonvulsant data already reported by Gavernet et al (2009)
[g] Value previously reported by Wasowski et al (2012).

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30 mg/Kg), 11 and 13 (at higher doses). The absence of toxic effects of most structures at this early stage of the evaluation process supports the use of amino acids as suitable functionalities for the design of anticonvulsant compounds.

According to the biological results, the molecules can be grouped into one of the four classes proposed to classify the activity at the initial evaluation (Malawska et al., 2004): Class 1 involves those compounds with anticonvulsant activity at 100 mg/kg or less; whereas class 2 represents structures with anticonvulsant activity at doses higher than 100 mg/kg. Class 3 covers inactive molecules at any doses up to 300 mg/kg and class 4 implicates inactive compounds at 300 mg/kg and toxic at 30 mg/kg or less. Table 3 shows the classification for the new structures. Most of them belong to class 1, except for compound 3 which presents anticonvulsant activity but we decided to classify it as class 4 due to its sedative effects at the lower doses.

4. Discussion

The anticonvulsant activity found in MES-test justifies further evaluations for the most promising compounds (i.e. phase 2 of the ADD program: median effective doses calculations). However, with the biological results obtained in phase 1 we can arrive to some preliminary conclusions.

Compound 4 shows a similar profile than its homologous 2 at the lower dose tested (Gavernet et al., 2009), showing low influence of the length of the alkyl chain to the activity (at least with 2 more carbon atoms). However, the evidence of sedative effects found for structure 4 suggests that it is not the most promising candidate and more experiments might be performed at lower doses to see if the anticonvulsant action prevails without these adverse effects.

On the other hand, the branched isomer of 2, compound 5, presents a better profile than structures 1, 2 and 4, with all animals protected at 4 h. With these results in mind, an adamantane sulfamide derivative was also included in the set (compound 6), which has a very bulky aliphatic substituent near the polar end. The results at this preliminary phase showed good activity, with 100% protection at the lowest dose tested.

Interesting results were also obtained for the aromatic sulfamide derivatives, compared with the ones previously reported that showed weak (or none) protection in phase 1 (Gavernet et al., 2009). Signs of anticonvulsant activity in MES test were detected before only for the methyl [N-(N'benzyl)-sulfamoyl]-β-alaninate, which was active at 100 mg/Kg in 1/3 mice tested (Gavernet et al., 2009). Here we evaluated a homolog of this compound (compound 7), its branched isomer (compound 8) and a p-fluorine-substituted benzyl sulfamide derivative (compound 9). All new structures showed protection at the lowest doses (30 mg/Kg).

Based on the results obtained from Phase 1, compounds 5 and 9 were selected for further analysis. Phase 2 of the ADD program includes the calculations of median effective doses (ED50). ED50 measures the dose of drug that is effective in 50% of the tested animals. This value is calculated at the time of peak effect (TPE), which has to be previously identified (Porter et al., 1984). The TPE was identified for compound 5 (1 h) but no apparent dose–response relationship was found, so the ED50 value cannot be calculated. On the contrary, ED50 was determined for 9 with a final value of 152 μmol/Kg (TPE = 4 h). This pharmacological results are interesting, since compound 9 at least duplicates the protection found for other aromatic sulfamate derivative of β-Alanine (2-hydroxyphenyl-N-β-alanine-methyl ester sulfamate, ED50 = 374 μmol/Kg according to Gavernet et al., 2009). However, it is less active than aliphatic sulfamides of the family (compound 1, ED50 = 79 μmol/Kg and compound 2, ED50 = 71 μmol/Kg; Gavernet et al., 2009).

In relation to Valrocemide (which ED50 in MES test is 755 μmol/Kg, according to Isoherranen et al., 2001) compound 9 increases 5 times the antiepileptic action at this preclinical stage. For that reason, we synthesized other amino acid derived sulfamides that retains the p-fluorobenzyl substituent. We replaced the β-alanine moiety of 9 by L-valine and L-phenyl alanine skeleton (compounds 10 and 11) in order to explore the influence of other amino acid chains to the activity. Compound 11 showed similar anticonvulsant profile in MES test than 9 with higher activity at 4 h after administration, but it presented neurotoxic effects. Structure 10 showed an interesting anticonvulsant action in MES test with 3/3 mice protected at 30 mg/Kg (at 4 h) and 3/3 mice protected at 100 mg/Kg (at 0.5 h). However, 75% of the animals tested with compound 10 against PTZ assay died after the experiment. For that reason we did not complete the biological profile at 100 mg/Kg.

Sulfamates also showed anticonvulsant activity against MES test but, according to the preliminary results obtained in this investigation, they give the impression of having less potency than sulfamides. However, more biological studies (and more structures) will be necessary to confirm this hypothesis. Toxicity was observed for the sulfamate with p-fluorobenzyl substituent.

Regarding the PTZ assays, the new compounds did not show significant protection against this chemically induced convulsion. Similar results were found in previous investigations for other sulfamides and sulfamates (Gavernet et al., 2009, 2007a, 2007b). As the PTZ test could provide equivocal results in contrast to clinical studies (Löscher et al., 1991), we included an in vitro test to evaluate the capacity of the structures to bind the BDZ-bs of the $GABA_A$ receptor in washed crude synaptosomal membranes of rat cerebral cortex (Table 3). The best results were achieved from compounds 10 and 11; which suggest that β-alanine is not the best amino acid to promote the binding to BDZ-bs. Again, more biological studies and new structures will be necessary to verify this hypothesis.

Additionally, we tested the anxiolytic effect of compounds 9 and 11, since previous results from our laboratories demonstrated the combined anticonvulsant and anxiolytic like activities of some other sulfamide derivatives (Wasowski et al., 2012). However, compounds 9 and 11 showed no effect in the plus maze in mice (1 mg/kg i.p.), revealing no anxiolytic effect (data not shown).

5. Conclusions

The structures tested as anticonvulsants in this investigation were selected based on one pharmacophoric pattern previously defined, with the aim of improving the knowledge about the influence of the second substitution of the sulfamide moiety for the anticonvulsant action. The results of this investigation support the strategy of designing active compounds that include the β-alanine sulfamide as main structure. All the derivatives tested showed protection against MES test, confirming the ability of the pharmacophore model for designing new anticonvulsants compounds.

The set of new structures were obtained after optimizing synthetic methodologies in both chemical reactions and work up stages, and these results could be useful for future preparations of new derivatives of the family.

Based on the biological results, we suggest that the second substitution of the sulfamide function by branched alkyl groups would increase the anti-MES activity. However more biological test will be performed in future investigations, to confirm our hypothesis.

Regarding the binding to BDZ-bs, some of the compounds

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showed medium to low affinity for this receptor active site. We conjecture that this is not the main mechanism of the anticonvulsant action of the studied compounds.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.ejphar.2016.02.001.

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