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Original article

Synthesis and pharmacological evaluation of sulfamide-based analogues of anandamide

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ABSTRACT

Arachidonyl and linoleyl sulfamide derivatives have been synthesized and their potential cannabimimetic properties evaluated in in vitro functional and binding assays. Replacement of the ethanolamide moiety of anandamide by $-CH_2NHSO_2NH-R$ considerably reduces the CB1 receptor activity and only some of the compounds showed modest cannabinoid properties in binding assays. The new compounds were also tested as inhibitors of the FAAH enzyme but were inactive.

1. Introduction

The therapeutic properties of cannabinoids including plant derived compounds as well as synthetic derivatives are due to their interaction with the cannabinoid receptors CB1 and CB2 [1,2]. The identification of these G-protein coupled receptors [3] prompted the discovery of the endogenous ligands, of which the most important are anandamide (AEA) [4] (Fig. 1) and 2-arachidonoyl glycerol [5]. Recently new evidences are appearing to prove the existence of a third cannabinoid-like receptor called GPR55 [6].

The endocannabinoids together with the two proteins involved in their regulation mechanisms, the anandamide transporter (ANT) and the fatty acid amide hydrolase (FAAH), and the cannabinoid receptors responsible for many biochemical processes, integrate the endocannabinoid system and represent important therapeutic targets. Thus, many analogues of anandamide have been synthesized and their structure activity relationships are well established. Besides, improved metabolically stable derivatives and inhibitors of the transporter and of the FAAH have also been prepared and evaluated [7].

Oleylethanolamide (OEA) (Fig. 1) is another important acylethanolamide involved in metabolism pathways which acts through PPARα receptors [8]. We have reported the synthesis and biological evaluation of a series of novel sulfamide analogs of OEA that shows in vivo satiety inducing actions and PPARα activation, the most interesting compounds being *N*-oleyl-*N*′-propylsulfamide and *N*-propyl-*N*′-stearylsulfamide [9] (Fig. 2).

The fact that incorporation of the sulfamide moiety in OEA derivatives led to compounds with potent in vivo activity encouraged us to evaluate this replacement in the structurally related anandamide derivatives.

So, in the present study, we wish to report the synthesis and pharmacological evaluation of novel anandamide analogues with the particular feature that they incorporate an aminosulfonylamino group. Besides, the hydroxyethyl of the ethanolamide has been replaced by a propyl and, as a different approach to include highly lipophilic chains, adamantyl sulfamide derivatives have also been prepared. These compounds together with the OEA sulfamide analogs, previously described [9], have been tested for cannabinoid activity both in isolated tissue assays and in binding experiments.

On the other hand, alkane sulfonyl derivatives of anandamide are also of interest since fatty acid sulfonyl fluorides, such as palmitylsulfonylfluoride (AM374) (Fig. 2), are potent inhibitors of FAAH and thus they can enhance the action of endogenous

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Table 1
Structures and biological assays data of compounds **3**–**19** R^1–NH–SO_2–NH–R^2.

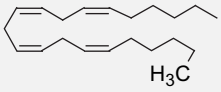
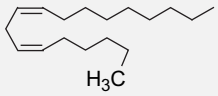
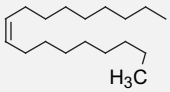
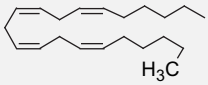
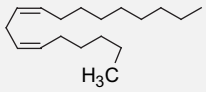
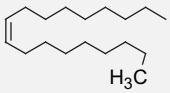
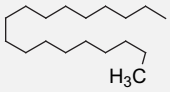
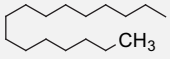
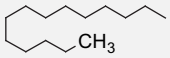
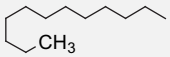
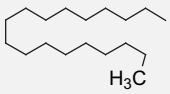
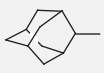
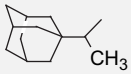
R^1	R^2	Compound	K_i (nM)[b]	FAAH activity (% of control)[c] [Compound] (M)	
				10^{-6}	10^{-7}
	H	3	4010 ± 825	ND[d]	
	H	4	6406 ± 267	ND[d]	
	Pr	8[a]	>10,000	96.5	101.1
	Pr	9	2339 ± 81	ND[d]	
	Pr	10	7190 ± 865	ND[d]	
	H	11[a]	1890 ± 360	101.7	100.2
	H	12[a]	>10,000	99.4	105.9
	H	13[a]	ND	102.9	113.5
	H	14[a]	ND	108.8	108.6
	H	15[a]	ND	99.3	107.5
	Pr	16[a]	9102 ± 140	109.8	108.8
	Pr	17[a]	6670 ± 1320	ND[d]	
	Pr	18[a]	>10,000	ND[d]	

Table 1 (*continued*)

R^1	R^2	Compound	K_i (nM)[b]	FAAH activity (% of control)[c] [Compound] (M)	
				10^{-6}	10^{-7}
	Pr	19[a]	8580 ± 669	92.6	90.4
–		AEA	132 ± 23	ND[d]	

[a] Described in Ref. [9].
[b] Affinity of compounds for the CB1 receptor was evaluated by displacing experiments using mouse cerebellum membrane and [^{3}H]SR141716A as CB1 receptor ligand.
[c] Membrane-bound FAAH activity was assayed using arachidonyl-[1-^{3}H]-ethanolamide as a substrate, and measuring metabolized [^{3}H]anandamide (as [^{3}H]ethanolamine) in the aqueous phase after chloroform extraction.
[d] ND: not determined.

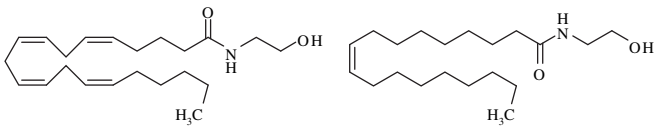


Fig. 1. Structures of anandamide and oleylethanolamide.

cannabinoids [10]. Due to the structural similarity of some of our compounds with these FAAH inhibitors, and to the fact that the sulphonamide group should, in principle, not be prone to the action of the amidohydrolase, the potential inhibition of FAAH by our compounds has also been evaluated.

2. Results and discussion

2.1. Chemistry

The synthesis of the long-chain sulfamoyl derivatives (Table 1) can be achieved starting from the amines of the corresponding fatty acids either by a transamination [11,12] with a sulfamide or by reaction with a sulfamoyl chloride [13].

First, *N*-arachidonylamine **1** was prepared from the parent acid following a described procedure [14] which involves mesylation, formation of the azide and subsequent reduction to the amine. *N*-Linoleylamine **2,** previously obtained by a different way [15], was prepared by following the procedure mentioned above (Scheme 1).

Then, reaction of **1** and **2** with sulfamide in water/ethanol afforded the corresponding *N*-monosubstituted derivatives **3** and **4,** as can be seen in Scheme 2. In a similar manner, *N*-2-adamantylamine and *N*-1(1-adamantylethyl)amine provided the adamantylsulfamides **5** and **6** (Scheme 2). (Compound **5** has been reported by a completely different synthetic procedure from the primary amine and *N*-(*tert*-butoxycarbonyl)-*N*-[4-(dimethylazaniumylidene)-1,4-dihydropyridin-1-ylsulfonyl]azanide [16]).

In a similar manner, it would be possible to obtain the desired *N*-propylsulfamide derivatives using propylsulfamide. However, when commercially available *N*-oleylamine reacted with *N*-propylsulfamide [11,12] the disubstitution product **7** was obtained, as the major compound, together with the mono oleyl propyl sulfamide **8** (Scheme 3) [9]. Therefore, another synthetic approach was envisaged which involved the use of *N*-propylsulfamoyl chloride prepared from propylamine and chlorosulfonic acid [17]. This was treated in situ with the fatty acid amines **1** and **2** in toluene with triethylamine as base and the corresponding derivative *N*-arachidonyl-*N*′-propylsulfamide (**9**) and derivative *N*-linoleyl-*N*′-propylsulfamide (**10**) were obtained (Scheme 2).

The structures of the novel compounds **3**–**6**, **9** and **10** were established according to their analytical and spectroscopic data.

2.2. Biological assays

Radioligand displacement assays were used to evaluate the affinity of the compounds to the CB1 receptor in rat cerebellar membranes using either ^{3}[H]WIN-55212-2 or ^{3}[H]SR141716A [18]. The cannabinoid properties of the compounds were also examined using the mouse vas deferens, a preparation supposed to contain cannabinoid receptors that can mediate an inhibitory effect of cannabinoid receptor agonists on electrically evoked contractions [19].

Binding affinity for the cannabinoid CB1 receptor of the new compounds here described, together with those of other fatty acid sulfamides previously synthesized in our group [9] are gathered in Table 1.

Examination of these data indicates that, in general, these sulfamoyl derivatives do not bind to the receptor or show only modest affinity. It is interesting to mention that contrary to what occurs with the sulfamide analogs of OEA, in which PPARα affinity and in vivo activity is kept, in the case of anandamide, the inclusion of sulfamide results in a loss of activity. In line with this, a structurally related compound, *N*-(2-hydroxyethyl)arachidonyl sulfonamide [20] turned out to be 2500-fold less potent than anandamide at the CB1 agonist site (Fig. 2).

We also performed binding assays using tritiated WIN 55,212-2. K_i values were usually higher using this agonist, as previously reported [21]. Thus, K_i values for compounds **16**, **17**, **18** and **19** were >10,000; > 10,000; >100,000 and 4229 ± 830 respectively. Since WIN 55,22-2 also labeled CB2 receptors and we did not observe any displacing curve fitting a two binding sites model, these results also

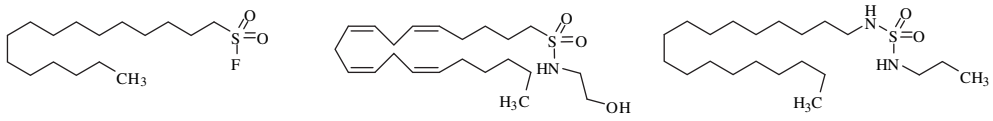
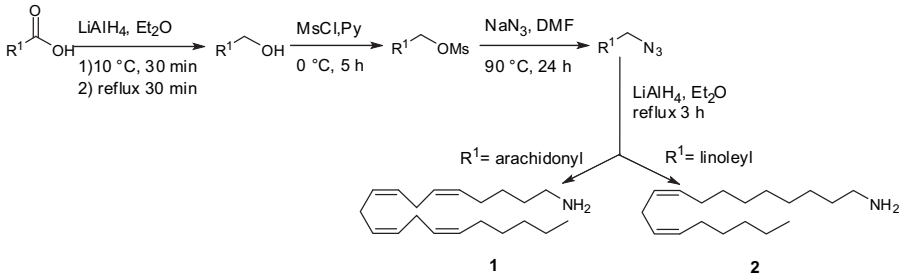
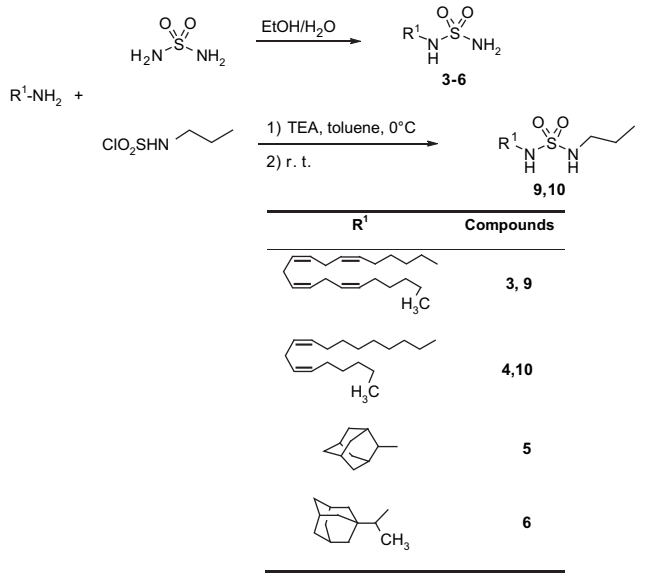


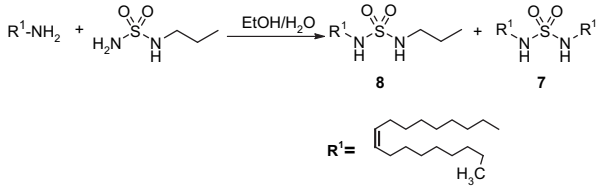
Fig. 2. Long chain derivatives as FAAH inhibitors and PPARα ligands.



Scheme 1. Synthesis of arachidonyl and linoleyl amines **1** and **2**.



Scheme 2. Synthesis of sulfamides **3**–**6**, **9** and **10**.



Scheme 3. Reaction of *N*-oleylamine with *N*-propylsulfamide to give **7** and **8**.

confirm the lack of binding affinities of these compounds to either CB1 or CB2 receptor.

The functional assays performed in compounds **3**–**6**, **8**–**12** and **16**–**19** confirmed also this lack of cannabinoid activity and so, none of the compounds were able to inhibit the electrically-evoked contractions on mouse isolated vas deferens (Fig. 3).

The negative results obtained in the binding and functional assays prompted us not to evaluate CB2 activity although an "inversion" for affinity in anandamide derivatives has been reported by Appendino et al. [22] who found CB2 selectivity for fatty acid derived compounds lacking polyunsaturation.

Due to the structural similarity with some FAAH inhibitors, the saturated long-chain derivatives (**12**–**16**), the oleyl derivatives **8** and **11** and the *N*-adamantyl-*N*′-propylsulfamide **19** were evaluated as FAAH inhibitors. None of the compounds showed significant activity (Table 1). Therefore, the lack of inhibition of fatty acid amidohydrolase by these compounds discards a potential indirect action on the endocannabinoid/oleylethanolamide system through interferences on their enzymatic degradation.

3. Conclusion

Novel sulfamoyl and propyl sulfamoyl derivatives with fatty acid and adamantyl chains have been synthesized and their cannabimimetic properties evaluated in functional and binding assays. Replacement of ethanolamide moiety of anandamide with $-CH_2NHSO_2NH-R$ considerably reduces the activity at the CB1 receptor and only some compounds showed modest cannabinoid properties in the binding assay. None of these compounds were inhibitors of FAAH.

4. Experimental

^1H and ^{13}C NMR spectra were recorded on Varian Unity 300 and 400 Varian Gemini and Bruker Avance 300 spectrometers. Chemical shifts are reported in ppm on the δ scale. The signal of the solvent was used as reference. Mass spectra (electrospray ionization) were determined on an MSD-Serie 1100 Hewlett Packard instrument. Melting points were determined on a Reichert Jung Thermovar melting point apparatus and are uncorrected. Elemental analyses were performed on a Heraeus CHN–O Rapid Analysis in our Analytical Services at Centro de Química Orgánica "Manuel Lora Tamayo" (CSIC). Flash column chromatography was carried out using Merck silica gel 60 (0.040–0.063). All starting materials were commercially available in Aldrich or Fluka and used without further purification. *N*-arachidonylamine and *N*-linoleylamine were obtained from their carboxylic acids purchased in Fluka, as described in the literature [14]. *N*-propylsulfamide **5** was prepared from the procedure reported by Paquin [11] and *N*-propylsulfamoyl chloride **7** was synthesized following the procedure of Kloek and Leschinsky [17].

4.1. General procedure for the preparation of monosubstituted sulfamides

The amine dissolved in EtOH was added dropwise to a solution of sulfamide in H_2O. The reaction mixture was refluxed and evaporated to dryness; the crude was purified by column chromatography on silica gel with CH_2Cl_2:MeOH/NH_3 9:1, giving the expected product.

4.1.1. *N*-(cis-5-cis-8-cis-11-cis-14-eicosatetraenyl)sulfamide (**3**)

From *N*-(*cis*-5-*cis*-8-*cis*-11-*cis*-14-eicosatetraenyl)amine **1** (0.100 g, 0.3 mmol), sulfamide (0.033 g, 0.3 mmol), H_2O (20 mL) and EtOH (10 mL); reaction time: 24 h; yield (0.020 g of yellow oil, 14%). Mp = oil. ^1H NMR (400 MHz, $CDCl_3$) δ: 5.37 (m, 8H, C*H*=C*H*); 4.60 (br

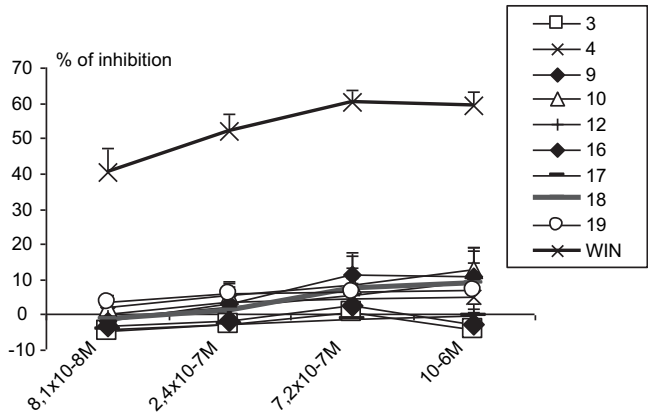


Fig. 3. Effect of the addition of increasing concentrations of WIN or of the new compounds in the contractile response induced by electrical stimulation of mouse vas deferens. Each point is the mean ± ESM of at least 6 data.

s, 2H, NH_2); 4.37 (br s, 1H, N*H*); 3.13 (m, 2H, CH_2NHSO$_2$); 2.81 (m, 6H, C=CH–CH_2–CH=C); 2.07 (m, 4H, CH=CH–CH_2CH$_2$); 1.60 (m, 2H, CH_2–CH$_2$NHSO$_2$); 1.43 (m, 2H, CH_2–CH$_2$CH$_2$NHSO$_2$); 1.30 (m, 6H, –CH_2–); 0.89 (t, 3H, J = 6.8 Hz, CH$_2$–CH_3). ^{13}C NMR (100 MHz, CDCl$_3$) δ: 130.7, 129.4, 128.6, 128.5, 128.2, 128.0, 127.8, 127.7 (CH=CH); 43.7 (CH$_2$NH); 31.8 (CH$_2$–CH$_2$CH$_3$); 29.8, 29.3 (CH$_2$–CH$_2$CH$_2$CH$_3$, CH_2–CH$_2$NHSO$_2$); 27.4 (CH=CH–CH$_2$CH$_2$); 26.8 (CH$_2$–CH$_2$CH$_2$NHSO$_2$); 25.6 (C=CH–CH_2–CH=C); 22.8 (CH$_2$–CH$_3$); 14.3 (CH$_2$–CH$_3$). MS (ES+) [M + H]$^+$ 369 (100%). Anal. calcd. for C20H36N2SO2: C 65.17, H 9.84, N 7.60, S 8.70; found: C 64.85, H 9.75, N 7.54, S 8.50.

4.1.2. *N-(*cis*-9-*cis*-12-octadecadienyl)sulfamide (**4**)*

From *N*-(*cis*-9-*cis*-12-octadecadienyl)amine **2** (0.044 g, 0.16 mmol), sulfamide (0.016 g, 0.16 mmol), H$_2$O (20 mL) and EtOH (10 mL); reaction time: 35 h; yield (0.007 g of white solid, 12%). Mp = 55–57 °C. ^{1}H NMR (400 MHz, CDCl$_3$) δ: 5.33 (m, 4H, C*H*=C*H*); 4.59 (br s, 2H, NH_2); 4.36 (br s, 1H, N*H*); 3.10 (m, 2H, CH_2NHSO$_2$); 2.76 (m, 2H, C=CH–CH_2–CH=C); 2.03 (m, 4H, CH=CH–CH_2CH$_2$); 1.56 (m, 2H, CH_2–CH$_2$NHSO$_2$); 1.29 (m, 16H, –CH_2–); 0.87 (t, 3H, J = 7.0 Hz, CH$_2$–CH_3). ^{13}C NMR (100 MHz, CDCl$_3$,) δ: 130.2, 130.0, 128.1, 127.9 (*C*H=*C*H); 43.6 (*C*H$_2$NH); 31.5 (*C*H$_2$–CH$_2$CH$_3$); 29.1 (*C*H$_2$–CH$_2$NHSO$_2$); 27.2 (CH=CH–*C*H$_2$CH$_2$); 29.6–26.6 (–*C*H$_2$–); 25.6 (C=CH–*C*H$_2$–CH=C); 22.5 (*C*H$_2$–CH$_3$); 14.0 (CH$_2$–*C*H$_3$). MS (ES+) [M + H]$^+$ 345 (100%). Anal. calcd. for C18H36N2SO2: C 62.79, H 10.46, N 8.14, S 9.30; found: C 62.90, H 10.80, N 8.03, S 9.51.

4.1.3. *N-(2-adamantyl)sulfamide (**5**)*

From *N*-(2-adamantyl)amine hydrochloride (0.190 g, 1.0 mmol), sulfamide (0.080 g, 0.8 mmol), H$_2$O (20 mL) and EtOH (10 mL), TEA 0.200 g (2.0 mmol), reaction time: 10 h; yield (0.059 g of white solid, 31%). Mp = 197–199 °C. ^{1}H NMR (400 MHz, CDCl$_3$) δ: 3.71 (m, 1H, *H*-2); 2.25 (m, 2H, *H*-1 and *H*-3); 2.21 (m, 2H, *H*-4ax, *H*-10ax); 1.97–2.12 (bm, 8H, *H*-5, *H*-6, *H*-7, *H*-8, *H*-9); 1.77 (bm, 2H, *H*-4ec and *H*-10eq). ^{13}C NMR (100 MHz, CDCl$_3$) δ: 59.3 (*C*-2); 38.7 (*C*-6); 38.5 (*C*-8 and *C*-9); 34.0 (*C*-3 and *C*-1); 32.2 (*C*-4 and *C*-10); 28.6 (*C*-5 and *C*-7). Anal. calcd. for C10H18N2SO2: C 52.15, H 7.88, N 12.16, S 13.92; found: C 51.97, H 7.71, N 11.98, S 13.40.

4.1.4. *N-(1-(1-adamantyl)ethyl)sulfamide (**6**)*

From *N*-(1-(1-adamantyl)ethyl)amine hydrochloride (0.172 g, 0.8 mmol), sulfamide (0.064 g, 0.6 mmol), H$_2$O (20 mL) and EtOH (10 mL); TEA 0.161 g (1.6 mmol), reaction time: 14 h; yield (0.085 g of white solid, 55%). Mp = 156–158 °C. ^{1}H NMR (400 MHz, CDCl$_3$) δ: 3.09 (q, 1H, *H*-1, J = 7.0 Hz); 2.16 (m, 3H, *H*-3); 1.93 (d, 3H, J = 12.1 Hz, *H*-4eq); 1.85 (d, 6H, J = 12.1 Hz, *H*-2ec and *H*-4ax); 1.71 (d, 3H, J = 12.1 Hz, *H*-2ax); 1.35 (d, 3H, J = 7.0 Hz, CH_3). ^{13}C NMR (100 MHz, CDCl$_3$) δ: 59.6 (C-1); 39.5 (C-4); 38.2 (C-2); 29.9 (C-3); 14.9 (CH$_3$). MS (ES+) [M-NHSO$_2$NH$_2$]$^+$ 163 (100%). Anal. calcd. for C12H22N2SO2: C 55.77, H 8.58, N 10.84, S 12.41; found: C 55.56, H 8.36, N 10.61, S 12.62.

4.2. *Preparation of N,N′-dioleylsulfamide (**7**) and N-oleyl-N′-propylsulfamide (**8**) from N-propylsulfamide*

0.68 mL of *N*-oleylamine (1.4 mmol) dissolved in EtOH was added dropwise to a solution of 0.200 g of *N*-propylsulfamide (1.4 mmol) in 2 mL of H$_2$O. The reaction mixture was refluxed for 48 h and evaporated to dryness; the crude was purified by column chromatography on silica gel with CH$_2$Cl$_2$, giving 0.260 g of white solid (**7**) and 0.120 g of desired white solid (**8**); Yield (**7**) 31%; Mp = 85–88 °C. ^{1}H NMR (400 MHz, CDCl$_3$) δ: 5.32 (m, 4H, C*H*=C*H*); 4.25 (br s, 2H, N*H*); 3.00 (m, 4H, CH_2NHSO$_2$); 1.99 (m, 8H, CH_2–CH=CH–CH_2); 1.53 (sextet, J = 7.3 Hz, 4H, CH_2CH$_3$); 1.26 (bm, 44H); 0.87 (t, 6H, J = 7.3 Hz, CH_3). ^{13}C NMR (100 MHz, CDCl$_3$) δ: 129.9, 129.7 (CH=CH); 43.2 (CH$_2$NHSO$_2$); 31.9 (CH$_2$–CH$_2$CH$_3$); 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 27.2, 26.7 (–CH$_2$); 22.6 (CH$_2$–CH$_3$); 14.0 (CH$_3$). MS (ES+) [M + H]$^+$ 598 (100%). Anal. calcd. for C36H72N2SO2: C 72.42, H 12.16, N 4.69, S 5.37; found: C 72.65, H 13.41, N 4.92, S 4.32. Yield (**8**) 22%; Mp = 82–83 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ: 5.30 (m, 2H, C*H*=C*H*); 4.25 (br s, 2H, N*H*); 2.97 (m, 4H, CH_2NHSO$_2$); 1.97 (m, 4H, CH_2–CH=CH–CH_2); 1.54 (sextet, J = 7.3 Hz, 2H, CH_2CH$_3$ (Pr)); 1.51 (m, 2H, CH_2CH$_2$NHSO$_2$); 1.21 (bm, 22H); 0.91 (t, 3H, J = 7.3 Hz, CH_3 (propyl)); 0.84 (t, 3H, J = 6.6 Hz, CH_3 (oleyl)). ^{13}C NMR (100 MHz, CDCl$_3$) δ: 129.9, 129.7 (CH=CH); 44.9 (NHSO$_2$NHCH$_2$ (Pr)); 43.2 (oleyl–CH$_2$NHSO$_2$); 31.8 (CH$_2$–CH$_2$CH$_3$); 29.8–28.9, 26.7 (–CH$_2$); 27.1 (CH$_2$–CH=CH–CH$_2$); 22.8 (CH$_2$–CH$_3$ (Pr)); 22.6 (CH$_2$–CH$_3$ (oleyl)); 14.1 (CH$_3$ (oleyl)); 11.2 (CH$_3$(Pr)). MS (ES+) [M + H]$^+$ 389 (100%); Anal. calcd. for C21H44N2SO2: C 64.86, H 11.32, N 7.20, S 8.23; found: C 65.06, H 11.60, N 7.34, S 8.33.

4.3. *General procedure for the preparation of disubstituted sulfamides*

Freshly distilled *N*-propylsulfamoyl chloride was added dropwise to a solution of the amine and TEA in dry toluene at 0 °C under nitrogen atmosphere. The reaction mixture was stirred at room temperature, the white solid TEA·HCl was filtered, the solvent was evaporated to dryness and the crude was purified by column chromatography on silica gel with CH$_2$Cl$_2$:MeOH/NH$_3$ 9:1, giving the expected product.

4.3.1. *N-(*cis*-5-*cis*-8-*cis*-11-*cis*-14-eicosatetraenyl)-N′-propylsulfamide (**9**)*

From *N*-(*cis*-5-*cis*-8-*cis*-11-*cis*-14-eicosatetraenyl)amine **1** (0.353 g, 1.2 mmol)), *N*-propylsulfamoyl chloride (0.192 g, 1.2 mmol), TEA (0.36 g, 3.6 mmol), toluene (30 mL); reaction time: 51 h; yield (0.090 g of yellow oil, 18%). ^{1}H NMR (400 MHz, CDCl$_3$) δ: 5.34 (m, 8H, CH=CH); 4.25 (br s, 1H, NH ((Pr)); 4.22 (br s, 1H, NH (arachidonyl)); 3.02 (m, 4H, CH$_2$NHSO$_2$); 2.81 (m, 6H, C=CH–CH$_2$–CH=C); 2.07 (m, 4H, CH=CH–CH_2CH$_2$); 1.54 (m, 2H, CH$_2$CH$_2$NHSO$_2$); 1.57 (sextet, 2H, J = 7.3 Hz, CH$_2$CH$_3$ (Pr)); 1.42 (m, 2H, CH$_2$–CH$_2$CH$_2$NHSO$_2$); 1.29 (bm, 6H, –CH$_2$–); 0.95 (t, 3H, J = 7.3 Hz, CH$_3$ (Pr)); 0.89 (t, 3H, J = 7.0 Hz, CH_3 (arachidonyl)). ^{13}C NMR (100 MHz, CDCl$_3$) δ: 130.5, 129.4, 128.6, 128.5, 128.3, 128.1, 127.8, 127.5 (CH=CH); 44.9 (CH$_2$NH (Pr)); 43.1 (CH$_2$NHSO$_2$ (arachidonyl)); 31.5 (CH$_2$–CH$_2$CH$_3$); 27.1 (CH=CH–CH$_2$CH$_2$); 29.3 (CH$_2$–CH$_2$CH$_2$CH$_3$); 26.8 (CH$_2$–CH$_2$CH$_2$NH SO$_2$); 25.6 (C=CH–CH$_2$–CH=C); 22.8 (CH$_2$–CH$_3$ (Pr)); 22.5 (CH$_2$–CH$_3$ (arachidonyl)); 14.3 (CH$_2$–CH$_3$ (arachidonyl)); 11.2 (CH$_2$–CH$_3$ (Pr)). MS (ES+) [M + H]$^+$

411 (100%). Anal. calcd. for C23H42N2SO2: C 67.31, H 10.24, N 6.82, S 7.80; found: C 67.24, H 10.35, N 6.88, S 8.02.

4.3.2. *N*-(cis-9-cis-12-octadecadienyl)-*N*′-propylsulfamide (**10**)

From *N*-(*cis*-9-*cis*-12-octadecadienyl)amine **2** (0.093 g, 0.35 mmol), *N*-propylsulfamoyl chloride (0.055 g, 0.35 mmol), TEA (0.050 g, 0.5 mmol), toluene (30 mL); reaction time: 24 h; yield (0.023 g of white solid, 17%). Mp = 70–75 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.34 (m, 4H, CH=CH); 4.27 (br s, 1H, NH, (Pr)); 4.25 (br s, 1H, NH (linoleyl)); 3.01 (m, 4H, CH_2NHSO_2); 2.77 (m, 2H, C=CH–CH_2–CH=C); 2.05 (m, 4H, CH=CH–CH_2CH_2); 1.60 (m, 4H, CH_2–CH_2NH); 1.29 (m, 16H, –CH_2–); 0.95 (t, 3H, J = 7.3 Hz, CH_3 (Pr)); 0.87 (t, 3H, J = 6.7 Hz, CH_3 (linoleyl)). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 130.2, 130.0, 128.0, 127.9 (CH=CH); 44.9 (SO_2NHCH_2 (Pr)); 43.2 (linoleyl–CH_2NH); 31.5 (CH_2–CH_2CH_3); 29.1 (CH_2–CH_2NHSO_2); 27.2 (CH=CH–CH_2CH_2); 29.6–26.6 (–CH_2–); 25.6 (C=CH–CH_2–CH=C); 22.8 (CH_2–CH_3 (Pr)); 22.5 (CH_2–CH_3 (linoleyl)); 14.0 (CH_3 (linoleyl)); 11.2 (CH_3 (Pr)). MS (ES+) $[M+H]^+$ 387 (100%). Anal. calcd. for C21H42N2SO2: C 65.28, H 10.88, N 7.25, S 8.29; found: C 65.30, H 11.12, N 7.22, S 8.40.

4.4. *In vitro assays*

4.4.1. *Agonistic activity*

1) Contractile responses in isolated tissues

The functional activity of the new compounds was tested on isolated tissues commonly used to study and characterize cannabinoid effects: mouse vas deferens Male CD-1mice weighing 25–30 g were used for this study. The mouse vas deferens was isolated as described by Hughes [23]. Tissues were suspended in a 10 mL organ bath containing 5 mL of Krebs solution (NaCl 118; KCl 4.75; $CaCl_2$ 2.54; KH_2PO_4 1.19; $MgSO_4$ 1.2; $NaHCO_3$ 25; glucose 11 mM). This solution was continuously gassed with 95% O_2 and CO_2. Tissues were kept under 0.5 g of resting tension at 32 °C and were electrically stimulated through two platinum ring electrodes. Mouse vas deferens were subjected to alternate periods of stimulation (5 min) and rest (10 min), during the stimulation period strains of 15 rectangular pulses of 70 V, 15 Hz and 2 ms duration for each min were applied. The isometric force was monitored by computer using a MacLab data recording and analysis systems.

Concentration–response curves for reference cannabinoid receptor agonists or the new compounds were constructed in a step-by-step manner. The interval between application of increasing concentrations was 15 min curves were constructed for the synthetic agonist WIN 55,212-2 (10^{-10} to 5×10^{-8} M), the natural agonist anandamide and the new compounds (10^{-8} to 10^{-6} mM) in mouse vas deferens.

The effect of the drugs was evaluated 15 min after the addition of each dose, as % of inhibition, taking the amplitude of the last contraction before the first addition of agonist as 100%. The agonists were added to the organ bath 15 min after the beginning of electrical stimulation. Each tissue was employed to construct only one concentration–response curve. In mice vas deferens the electrical stimulation was stopped, Krebs solution was replaced before the addition of each dose of the agonist and then, 10 min later, tissues were again stimulated during 5 min.

2) Radioligand binding experiments

A P2 membrane fraction from rat cerebellar homogenates was prepared. CB1 binding assays in rat cerebellar membranes were performed using either ^{3}[H]WIN-55212-2 or ^{3}[H]SR141716A (NEN-Dupont, Boston, MA, 40–60 Ci/mmol) as ligands. For competition analysis, drugs were dissolved in 100% DMSO to a final concentration of 10 nM. Further dilutions were made in assay buffer to reach concentrations spanning between 10^{-5} and 10^{-12} M (final concentration in tubes). Radioligand binding was initiated by the addition of 25 μg of protein to tubes in triplicates containing [^{3}H]-SR141716A (0.4 nM) or [^{3}H]-WIN-55212-2 (1 nM) and increasing concentrations of tested compounds in 0.5 mL of buffer HEPES 20 mM pH 7.4 and $MgCl_2$ 1 mM plus free fatty acids BSA 0.5%. Tubes were incubated at 30 °C during 90 min. The reaction was stopped by the addition of 2.5 mL of ice chilled buffer C, rapidly filtered in a Millipore vacuum manifold through Whatman GF/C glass-fiber filters previously soaked in assay buffer containing 5% BSA. The tubes were washed twice with 2.5 mL of ice-cold buffer C and the filters rinsed with 5 mL of the same buffer. Non-specific binding was assessed in the presence of 10 μM cold SR141716A. The filters were placed in vials containing liquid scintillation cocktail Ecolite (ICN Biomedicals), incubated at room temperature overnight and counted in a scintillation counter.

A single population of high-affinity binding sites was demonstrated using LIGAND analysis for both radioligands. K_i for the different drugs assayed were calculated from the equation of Cheng and Prusoff [24], using fixed K_d values for either ^{3}[H]WIN-55212-2 or ^{3}[H]SR141716A obtained from independent experimental assays. The different results (see Table 1) in terms of compound K_i for displacing the different CB1 tritiated ligands are based on: (1) the lower affinity of the cannabinoid agonist WIN-55212-2 with respect to ^{3}[H]SR141716A for the CB1 binding sites in rat cerebellar membranes, (2) the potential different recognition sites for displacing drugs.

4.5. *FAAH assay*

Brain tissues were homogenized in 50 mM Tris buffer, pH 8, containing 0.32 M sucrose. Homogenates were centrifuged first at 1000*g* (5 min), the pellet discarded and the supernatant centrifuged at 45,000*g* (30 min). The pellets obtained were solubilized at 0–4 °C in Tris buffer. Protein content in the membrane fraction was measured with the Bradford method [25]. All tissue samples and membrane fractions were stored at −70 °C until used. Both enzymatic assays were run under conditions that were linear with time and protein concentrations. We assayed membrane-bound FAAH activity using arachidonyl-[1-^{3}H]-ethanolamide as a substrate, and measuring metabolized [^{3}H]anandamide (as [^{3}H]ethanolamine) in the aqueous phase after chloroform extraction, as described [26].

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