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Arachidonylsulfonyl Derivatives as Cannabinoid CB1 Receptor and Fatty Acid Amide Hydrolase Inhibitors

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Abstract—Arachidonylsulfonyl fluoride (**3**), reported here for the first time, is similar in potency to its known methyl arachidonylfluorophosphonate (**2**) analogue as an inhibitor of mouse brain fatty acid amide hydrolase activity (IC_{50} 0.1 nM) and cannabinoid CB1 agonist [^{3}H]CP 55,940 binding (IC_{50} 304–530 nM). Interestingly, **3** is much more selective than **2** as an inhibitor for fatty acid amide hydrolase relative to acetylcholinesterase, butyrylcholinesterase and neuropathy target esterase. *N*-(2-Hydroxyethyl)arachidonylsulfonamide (**4**) is at least 2500-fold less potent than *N*-(2-hydroxyethyl)arachidonamide (anandamide) (**1**) at the CB1 agonist site.

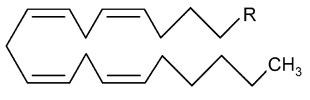
Arachidonic acid derivatives play key roles in the function and understanding of the brain cannabinoid system. *N*-(2-Hydroxyethyl)arachidonamide (anandamide) (**1**) is an important endogenous agonist for the cannabinoid CB1 receptor,[1] binding to the same site as Δ^9-tetrahydrocannabinol and [^{3}H]CP 55,940, the most widely used radioligand for the agonist target.[2] Anandamide is hydrolyzed by fatty acid amide hydrolase (FAAH) which is very sensitive to irreversible inhibition by methyl arachidonylfluorophosphonate (MAFP) (**2**).[3]

Agonist binding is also irreversibly inhibited by **2**,[3,4] involving several possible mechanisms: the arachidonyl moiety of **2** either occluding a site within the receptor which would otherwise be occupied by anandamide[3] or causing a conformational change; phosphonylation of an amino acid moiety in the vicinity of the agonist site. Alkanesulfonyl derivatives are also of interest since *n*-dodecanesulfonyl fluoride[5] and methyl *n*-dodecylfluorophosphonate[6] are similarly potent inhibitors of both FAAH and CB1. The exceptional activity of **2** suggests that arachidonylsulfonyl fluoride (**3**) might also be potent at FAAH or CB1 or both. This study incorporates the novel arachidonylsulfonyl substituent in both the fluoride and *N*-(2-hydroxyethyl)amide series and specifically reports the preparation and unusual potency and selectivity properties of **3** and **4** (Fig. 1).

The synthesis of **3** and **4** involved a three-step conversion of arachidonic acid, via the corresponding alcohol[7] and arachidonyl bromide,[8] to arachidonylsulfonyl chloride (**5**).[9] Compound **5** was converted to **3**[10] by treatment with $Bu_4N^+F^-$ and to **4**[11] with ethanolamine (Scheme 1).

The inhibitory potencies of arachidonyl derivatives **1**–**4** and two dodecyl analogues were compared for CB1 and FAAH of mouse brain (Table 1). The findings for **1** and the dodecyl analogues were generally similar to earlier studies with rat, thereby validating the assay methods for evaluation of the new arachidonylsulfonyl derivatives **3** and **4** (Table 1).

The most remarkable property of **3** is its outstanding potency as an inhibitor of mouse brain FAAH, with an IC_{50} of 0.1 nM as with the exceptionally-active **2**.[14]



1 R=C(O)NHCH$_2$CH$_2$OH
2 R=CH$_2$P(O)(OCH$_3$)F
3 R=CH$_2$SO$_2$F
4 R=CH$_2$SO$_2$NHCH$_2$CH$_2$OH

Figure 1. Structural relationship between anandamide (**1**), methyl arachidonylfluorophosphonate (**2**) and their sulfonyl analogues [arachidonylsulfonyl fluoride (**3**) and *N*-(2-hydroxyethyl)arachidonylsulfonamide (**4**)].

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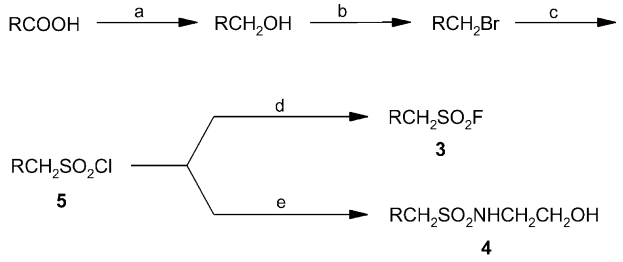
Lower potency is observed for the CB1 receptor with IC_{50} values for agonist binding of 530 nM for **2** and 304 nM for **3**.[15] Sulfonyl fluoride **3** is also much less potent and more selective than fluorophosphonate **2** as an inhibitor of other mouse brain esterases with IC_{50} values (nM) (15-min pre-incubation) as follows: **3** 780±120 and **2** 0.6±0.1[16] for neuropathy target esterase; **3** >100,000 and **2** 124±17[16] for acetylcholinesterase; **3** >100,000 and **2** 81 for butyrylcholinesterase.[17]

Inhibitory potencies were also compared for carboxamide **1** and sulfonamide **4** determined with [^{3}H]CP 55,940 in the absence and presence of phenylmethanesulfonyl fluoride (PMSF) to minimize hydrolysis by FAAH. Carboxamide **1** has an IC_{50} of 39 nM with PMSF (vs 40 nM for rat brain CB1 with PMSF)[3] or 12 μM without PMSF. Sulfonamide **4** (IC_{50} >100 μM) is at least 2500-fold less potent than **1** with PMSF. The difference could be due to either the sulfonyl replacement for the carbonyl group or the additional methylene of **4** compared with **1**.[18] Arachidonylsulfonamide **4** (IC_{50} ~10 μM) is presumably a competitive inhibitor of FAAH.

In conclusion, **3** has outstanding potency and selectivity for FAAH relative to other esterases and lower activity at the CB1 receptor. Sulfonamide **4** is also a moderately potent FAAH inhibitor with low potency for the agonist site. Thus, with FAAH the arachidonyl-type substituent very effectively positions the –C(O)NHCH$_2$–CH$_2$OH moiety of **1** for hydrolysis and the –CH$_2$SO$_2$F and –CH$_2$P(O)(OCH$_3$)F substituents of **3** and **2** for sulfonylation and phosphonylation, respectively. These relationships hold for CB1 although with lower inhibitory potency. However, replacement of the –C(O)– moiety of **1** with –CH$_2$SO$_2$– of **4** greatly reduces the activity at CB1. These findings are consistent with low potency of **4** for the agonist site but moderate potency of **2** and **3** for a coupled CB1 subsite.



Scheme 1. (a) $LiAlH_4$, Et_2O, 0 °C, N_2; (b) CBr_4, $(C_6H_5)_3P$, CH_2Cl_2, N_2, 0 °C; (c) (1) Mg, THF ($BrCH_2CH_2Br$), rt, 16 h; (2) SO_2Cl_2, THF/hexane, −60 °C, 30 min; (d) (1) $Bu_4N^+F^-$, THF, rt, 2 h; (2) 5% HCl; (e) $NH_2CH_2CH_2OH$, Et_3N, $CHCl_3$, 0 °C.

Table 1. Inhibitory potencies of arachidonyl derivatives **1–4** and two dodecyl analogues for [^{3}H]CP 55,940 agonist binding at the CB1 receptor and FAAH activity

	IC_{50}±SE (nM, n=3)			
	CB1		FAAH	
Compd	Mouse	Rat	Mouse	Rat
1	12,000±300			
1+PMSF	39±14	40[3]		
2	530±150	20[3]	0.10±0.02[12]	2.5[3]
3	304±23		0.11±0.05[a]	
4+PMSF	>100,000		~10,000	
n-$C_{12}H_{25}SO_2F$	6.9±3.6[13]	18[5]	2[13]	3[5]
n-$C_{12}H_{25}$P(O)(OR)F[b]	1.8±0.8[13]	2.5±0.3[6]	2[13]	3.0±0.2[6]

[a]The inhibition curve for **3**, but not **2**, is apparently biphasic, suggesting the involvement of two enzyme components of differential sensitivity to **3**.
[b]R = i-C_3H_7 and CH_3 for assays with mouse and rat brain membranes, respectively.

Acknowledgements

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9. ***(all-Z)*- Eicosa-5,8,11,14-tetraenesulfonyl chloride (arachidonylsulfonyl chloride) (5).** Mg turnings were cut into small pieces and purified by three washes with each of deionized water, MeOH, acetone and hexane in sequence, then stored under hexane. Freshly prepared Mg turnings (93. 6 mg, 3. 9 mmol) were covered with freshly dried THF (2 mL) under N_2 atmosphere. Dibromoethane (168 mg, 0. 9 mmol) was added and the slurry was stirred for 30 min (exothermic) until the Mg was activated. To that solution, arachidonyl bromide (1056 mg, 3 mmol) in THF (3 mL) was added and the slurry was stirred for 16 h at rt in a sealed flask. Addition of more THF (7 mL) dissolved all the salt formed during the preparation. The resulting Grignard reagent was filtered through oven dried glass wool and added dropwise to freshly distilled SO_2Cl_2 (402 mg, 3 mmol) in hexane (25 mL) over 30 min at −60 °C. A yellow precipitate was formed and the solution was stirred an additional 2 h at rt. The reaction was quenched with 5% HCl (30 mL) and the solution was extracted with $CHCl_3$ (3×20 mL). After removal of the solvent, the resulting colorless oil was chromatographed on a silica column eluted with $CHCl_3$/hexane (1:1). The major fraction (500 mg) had R_f 0. 95

on TLC/silica with the same solvent system and was identified by ^{1}H NMR [5.38 (8H, m), 2. 83 (6H, m), 2.06 (6H, m), 1.70 (2H, m), 1.33 (6H, m), 0. 90 (6H, br s)] and EI/MS (*m*/*z* 274, M^+) as eicosa-5,8,11,14-tetraene. The fraction at R_f 0.3–0.5 was collected and rechromatographed using similar conditions to give 120 mg of pure **5** as a colorless oil (11%). ^{1}H NMR: 5.38 (8H, m), 3.75 (2H, m), 2.83 (4H, m), 2.19 (2H, br q), 2.05 (4H, m), 1.60 (2H, m), 1.28 (8H, m), 0.89 (3H, br s). EI/MS: *m*/*z* 337, $M-Cl^+$ (20%), 273, $M-Cl-SO_2^+$ (20%), 79 (100%). EI-HRMS calculated for $C_{20}H_{33}SO_2$ ($M-Cl^+$): 337. 2201, found: 337. 2199.

10. ***(all-Z)*- Eicosa-5,8,11,14-tetraenesulfonyl fluoride (arachidonylsulfonyl fluoride) (3).** Using a syringe, $Bu_4N^+F^-$ (80 μL of 1 M solution in THF, 0.081 mmol) was introduced into a solution of **5** (20 mg, 0.054 mmol) in THF (1 mL). The solution became brown and was stirred for 2 h. It was then quenched into 5% HCl (20 mL) at 0 °C and extracted with $CHCl_3$ (2×25 mL), dried (Na_2SO_4), concentrated to 2 mL and passed with $CHCl_3$ through a short silica column to give 18 mg pure **3** (94%). ^{1}H NMR: 5.39 (8H, m), 3.37 (2H, m), 2.82 (6H, br quintet), 2.14 (2H, br q), 2.05 (2H, br q), 1.97 (2H, br quintet), 1.57 (4H, m), 1.31 (4H, m), 0.90 (3H, br s); ^{13}C NMR: 130. 78, 129. 62, 128. 93, 128. 72, 128. 51, 128. 12, 128. 00, 127. 76, 51. 10 (d, $J_{F\text{-}P}$ = 15.8 Hz), 38.50, 31.76, 30.59, 29.55, 27.99, 27.48, 26.58, 25.92, 23.23, 22.78, 14.24. EI/MS: *m*/*z* 356, M^+ (40%), 258, $M-F-SO_2-CH_2-H^+$ (15%), 79 (100%). EI-HRMS: calcd for $C_{20}H_{33}SO_2F$ (M^+):356. 2185 found: 356. 2195.

11. ***(all-Z)*- Eicosa-5,8,11,14-tetraenesulfonic acid *N*-(2-hydroxyethyl)amide [*N*-(2-hydroxyethyl)arachidonylsulfonamide] (4).** Arachidonylsulfonyl chloride (**5**, 50 mg, 0. 13 mmol) in $CHCl_3$ (2 mL, ethanol free) was added to a stirred and cooled (0 °C) solution of $NH_2CH_2CH_2OH$ (41 mg, 0.67 mmol) and Et_3N (14 mg, 0.13 mmol) in $CHCl_3$ (3 mL). A precipitate of $HOCH_2CH_2NH_2{\cdot}HCl$ was formed. Monitored by TLC (silica, EtOAc, R_f 0.85), the solution was stirred for 1 h at rt. The $CHCl_3$ was removed and the residue was dissolved in EtOAc (3 mL) and passed through a short (8 cm) silica column with EtOAc (50 mL). The solvent was distilled to leave 45 mg of pure **4** as a colorless oil (87%). The product gave one spot on TLC/silica developed with EtOAc/$CHCl_3$ 1:1 (R_f 0.5). ^{1}H NMR: 5.38 (8H, m), 5.11 (1H, t, J = 6.2 Hz), 3.75 (2H, br s), 3.25 (2H, q), 3.05 (2H, m), 2.81 (6H, m), 2.66 (1H, br s), 2.71 (4H, m), 1.83 (2H, m), 1.53 (2H, m), 1.30 (6H, m), 0.90 (3H, br s); ^{13}C NMR: 130.73, 129.14, 128.99, 128.84, 128.51, 128.30, 128.03, 127.73, 62.10, 52.94, 45.54, 31.70, 30.56, 29.52, 28.44, 27.42, 26.85, 25.86, 25.77, 23.47, 22.75, 14.27. EI/MS: *m*/*z* 397, M^+ (20%), 79 (100%). EI-HRMS: calcd for $C_{22}H_{39}NO_3S$ (M^+): 397.2651 found: 397.2658.

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14. FAAH inhibition was determined for mouse brain membranes (700 g supernatant, 10,000 g pellet; 8 μg protein) in Tris buffer (100 mM, pH 7.0, with 1 mM EDTA) incubated with test inhibitors (**2**–**4**) for 15 min, then with [^{14}C]oleamide (1 μM) for 15 min at 25 °C. An extraction procedure was used to analyze [^{14}C]oleic acid liberated by hydrolysis according to ref 12.

15. CB1 binding assays used the same mouse brain membranes (150–200 μg protein) as those for FAAH in Tris buffer (50 mM, pH 7.4, with 1 mM EDTA and 1 mM $MgCl_2$) and 3 mg/mL bovine serum albumin. Test inhibitors (**1**–**4**) or ligand for non-specific binding (10 μM **2**) were preincubated 15 min at rt prior to addition of the cannabinoid radioligand [^{3}H]CP 55,940 (10 nM). After incubation for 90 min at 30 °C, CB1-bound radiolabel was recovered by vacuum filtration for quantification. Potential hydrolysis of **1** and **4** by FAAH was examined and minimized by assaying with PMSF (50 μM, 15-min pre-incubation) or alone. Assay conditions of Quistad, G. B.; Nomura, D. K.; Sparks, S. E.; Segall, Y.; Casida, J. E. *Toxicol. Lett*. **2002**, *135*, 89.

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17. Assays according to refs 12 and 16.

18. Similar potency as an FAAH inhibitor is observed for **2** (Fig. 1) and its analogue lacking one methylene on the arachidonyl moiety [i.e., $RP(O)(OCH_3)F$] according to Seltzman, H. H.; Fleming, D. N.; Deutsch, D. G.; Glaser, S. T.; Stevenson, L.; Ross, R.; Pertwee, R. G. *Abstracts of Papers*, 2001 Symposium on the Cannabinoids, International Cannabinoid Research Society: Burlington, VT, 2001.