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# Toxicological and Structural Features of Organophosphorus and Organosulfur Cannabinoid CB1 Receptor Ligands

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**Potent cannabinoid CB1 receptor ligands include anandamide [*N*-(2-hydroxyethyl)arachidonamide], $\Delta^9$-tetrahydrocannabinol, and $^3$H-CP 55,940 at the agonist site and selected organophosphorus esters (including some pesticides) and organosulfur compounds at a proposed closely coupled "nucleophilic" site. This study considers the toxicological and structural features of alkylfluorophosphonates, benzodioxaphosphorin oxides, alkanesulfonyl fluorides, and analogs acting at the nucleophilic site. Binding at the agonist site, using $^3$H-CP 55,940 in assays with mouse brain membranes, is inhibited by *O*-isopropyl dodecylfluorophosphonate (compound 2), dodecanesulfonyl fluoride (compound 14) and dodecylbenzodioxaphosphorin oxide with $IC_{50}$ values of 2–11 nM. Compounds 2 and 14 are also effective *in vivo*, with 84% inhibition of mouse brain CB1 binding 4 h after intraperitoneal dosage at 30 mg/kg. Compound 14-inhibited CB1 in mouse brain requires about 3–4 days for recovery of 50% activity, suggesting covalent derivatization. Delayed toxicity (mortality in 0.3–5 days) from compounds 2, 14, and octanesulfonyl fluoride (18) is more closely associated with *in vivo* inhibition of brain neuropathy target esterase–lysophospholipase (NTE-LysoPLA) than with that of CB1 or acetylcholinesterase. NTE-LysoPLA inhibited by sulfonyl fluorides 14 and 18 cannot "age," a proposed requirement for NTE phosphorylated by organophosphorus-delayed neurotoxicants. Several octane- and dodecanesulfonamides with *N*-(2-hydroxyethyl) and other substituents based on anandamide give depressed mobility and recumbent posture in mice, but the effects do not correlate with potency for CB1 inhibition *in vitro*. Specific toxicological responses are not clearly associated with organophosphorus- or organosulfur-induced inhibition of the proposed CB1 nucleophilic site in mouse brain. On the other hand, the most potent CB1 inhibitors examined here are also NTE-LysoPLA inhibitors and cause delayed toxicity in mice.**

*Key Words:* **cannabinoid receptor; CB1; dodecanesulfonyl fluoride; *O*-isopropyl dodecylfluorophosphonate; neuropathy target esterase; lysophospholipase; fatty acid amide hydrolase.**

The toxicity of many organophosphorus esters (including some pesticides) and a few organosulfonyl fluorides is attributed to inhibition of acetylcholinesterase (AChE) for acute effects and neuropathy target esterase (NTE) for delayed neuropathy (Aldridge and Reiner, 1972; Johnson and Glynn, 2001; Wu and Casida, 1996) (Fig. 1). The NTE target for delayed toxicity (Wu and Casida, 1996) and hyperactivity (Winrow *et al.*, 2003) in mice is a lysophospholipase (LysoPLA) referred to as NTE-LysoPLA (Quistad *et al.*, 2003). The organophosphorus and organosulfonyl compounds referred to above also inhibit two components of the cannabinoid system; i.e., they phosphorylate and probably sulfonylate fatty acid amide hydrolase (FAAH), which hydrolyzes the endogenous agonist *N*-(2-hydroxyethyl)arachidonamide (anandamide) (Bracey *et al.*, 2002; Devane *et al.*, 1992; Quistad *et al.*, 2001, 2002b), and they block agonist binding at the CB1 receptor either directly or via a coupled "nucleophilic" site or subsite (Deutsch *et al.*, 1997a,b; Martin *et al.*, 2000; Quistad *et al.*, 2002a). The structure-function relationships between the proposed nucleophilic site of the CB1 receptor and its agonist site are largely unknown relative to pharmacological actions and toxic effects (if any) of its organophosphorus and organosulfur inhibitors.

The agonist site of the cannabinoid CB1 receptor (Devane *et al.*, 1992; Felder *et al.*, 1993) binds $\Delta^9$-tetrahydrocannabinol (THC) and many of its analogs (Khanolkar *et al.*, 2000), including several radioligands, the most commonly utilized being $^3$H-CP 55,940 (Hillard and Campbell, 1997; Ross *et al.*, 1998). Methyl arachidonylfluorophosphonate (MAFP, compound 3) is a potent inhibitor of CP 55,940 binding with apparent covalent derivatization at a nucleophilic site (Deutsch *et al.*, 1997b). Other long-chain methyl alkylfluorophosphonates and alkanesulfonyl fluorides (especially the dodecyl analogs) are also potent inhibitors of CP 55,940 binding (Deutsch *et al.*, 1997a; Martin *et al.*, 2000), possibly at the same nucleophilic site. Although methyl octylfluorophosphonate is ineffective in inhibition of CP 55,940 binding, it is a very potent CB1 agonist based on pharmacologic responses compared to other alkylfluorophosphonates (Martin *et al.*, 2000). Thus, octyl and dodecyl are preferred chain lengths in these two series for interaction with CB1.

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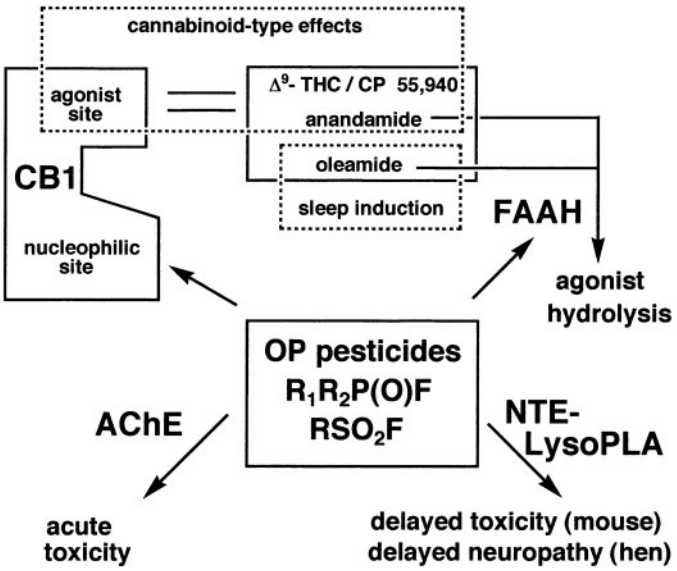


**FIG. 1.** Interactions of agonists and toxicants with the CB1 receptor. The relationship between the CB1 agonist and nucleophilic sites is unknown. The organophosphorus or organosulfonyl compounds block agonist binding at CB1 either directly or via a coupled nucleophilic site or subsite. Cannabinoids acting at the agonist site are $\Delta^9$-THC, anandamide, and oleamide. $^3$H-CP 55,940 is the agonist radioligand used in this study for assay of the agonist site and coupled nucleophilic site. The toxicants are selected organophosphorus (OP) pesticides, alkanesulfonyl fluorides, and alkylfluorophosphonates. Alternative targets for the organophosphorus and organosulfur compounds are FAAH to inhibit agonist hydrolysis, AChE leading to acute toxicity, and NTE-LysoPLA resulting in delayed toxicity (mortality in 0.3–5 days) in mice and delayed neuropathy (a central peripheral distal axonopathy) in hens. NTE-LysoPLA (assayed with lysolecithin as the substrate) is synonymous with NTE (assayed with phenyl valerate) in mouse brain and probably also in hen and human brain (Lush *et al*., 1998; Quistad *et al*. 2003; Winrow *et al*., 2003).

This study considers the toxicological and structural aspects of organophosphorus and organosulfur esters as cannabinoid CB1 receptor ligands acting at the proposed nucleophilic site. Octyl and dodecyl derivatives are emphasized not only as fluorophosphonates and sulfonyl fluorides but also as benzodioxaphosphorin oxides (BDPOs) (which are known to inhibit NTE and NTE-LysoPLA [Quistad *et al*., 2003; Wu and Casida, 1992] and FAAH [Quistad *et al.*, 2001]). Comparisons are made with mice for inhibition *in vitro* and/or *in vivo* between CB1, NTE-LysoPLA, and AChE. The *N*-(2-hydroxyethyl) substituent, important in anandamide binding at CB1 and agonist action (Hillard and Campbell, 1997; Khanolkar and Makriyannis, 1999), is also examined here in the sulfonamide series along with several analogs for possible inhibition of $^3$H-CP 55,940 binding or pharmacological activity.

## MATERIALS AND METHODS

***Caution.*** Some of the organophosphorus and organosulfur compounds used herein are known or suspected to be acute and/or delayed neurotoxicants and should therefore be used under careful containment conditions. They can be destroyed by treatment with sodium hydroxide in aqueous ethanol.

***Chemicals.*** The test chemicals used for this study are listed in Tables 1–3. Sources for the chemicals were: $^3$H-CP 55,940 (120 Ci/mmol) from New England Nuclear (Boston, MA); MAFP (compound **3**) from Cayman Chemical (Ann Arbor, MI); diisopropyl fluorophosphate (DFP, compound **6**) from Sigma Chemical (St. Louis, MO). Compounds described before and available from previous or analogous syntheses in this laboratory were: alkylfluorophosphonates **1** and **2** (Segall *et al*., 2003a); **4** (Wu and Casida, 1995); **5**, **7,** and **18** (Quistad *et al*., 2002b); BDPOs **8–13** (Quistad *et al*., 2001; Wu and Casida, 1992); and alkane- and alkenesulfonyl fluorides **14–16** (Segall *et al*., 2003a,b).

Dodecanesulfonyl azide (**17**) was synthesized by dropwise addition of a freshly prepared solution of dodecanesulfonyl chloride (804 mg, 3 mmol) in absolute ethanol (15 ml) to a stirred solution of sodium azide (390 mg, 6 mmol in water [1.5 ml]). The mixture was stirred for 1 h at 25°C while a precipitate of sodium chloride was formed. The alcohol was distilled, and ethyl acetate (30 ml) was added followed by water (20 ml). The organic layer was separated, dried (sodium sulfate), and the solvent evaporated to leave a colorless oil (790 mg, 96%) that solidified on standing for 15 min at 25°C. Recrystallization from hexane, followed by overnight cooling at 4°C, gave pure **17,** m.p. 31°C. Thirteen 1-dodecanesulfonamides and ten 1-octanesulfonamides (not designated by number) were prepared by adding dodecane- or octanesulfonyl chloride (4 mmol in 20 ml dry ethanol-free chloroform) to a cooled (0°C) and stirred solution of the appropriate amine (4 equivalents or 2–3 fold excess in 20 ml chloroform) and dry triethylamine (2–4 equivalents). After stirring for 1 h at room temperature, the organic layer was treated with 0.5 *N* hydrochloric acid (30 ml), dried (sodium sulfate), and the chloroform was evaporated. The resulting solid (usually 80–100% yield), was recrystallized from hexane. All compounds were identified by $^1$H and $^{13}$C nuclear magnetic resonance spectroscopy.

***Animal studies.*** Male Swiss-Webster mice (22–28 g) from Harlan Laboratories (Indianapolis, IN) were used for studies in accordance with the Guiding Principles in the Use of Animals in Toxicology as adopted by the

**TABLE 1**
**Inhibitory Potency for CB1 Receptor *in Vitro***

| Compound (number and structure) | $IC_{50}$ (nM)[a] |
|---|---|
| Alkylfluorophosphonates | |
| **1** $C_{12}H_{23}P(O)(OC_3H_7\text{-}i)F$ (11-ene) | 1.0 ± 0.1[b] |
| **2** $C_{12}H_{25}P(O)(OC_3H_7\text{-}i)F$ | 1.8 ± 0.8[b] |
| **3** $C_{20}H_{33}P(O)(OCH_3)F$ | (MAFP) 530 ± 150 |
| **4** $C_8H_{17}P(O)(OC_2H_5)F$ | 11000 ± 5000 |
| **5** $C_{12}H_{25}P(O)F_2$ | 36000 ± 1600 |
| **6** $(i\text{-}C_3H_7O)_2P(O)F$ (DFP) | > 10000 (41,38)[c] |
| **7** $C_8H_{17}P(O)F_2$ | > 100000 |
| Benzodioxaphosphorin oxides (BDPOs) | |
| **8** $C_{18}H_{35}$BDPO (*cis*-9) (oleyl) | 9.7 ± 0.5 |
| **9** $C_{12}H_{25}$BDPO (dodecyl) | 11 ± 5 |
| **10** $C_{18}H_{37}$BDPO (stearyl) | 80 ± 48 |
| **11** $C_8H_{17}$BDPO (octyl) | 120 ± 43 |
| **12** $C_8H_{17}$OBDPO (*O*-octyl) | 2900 ± 1700 |
| **13** $C_6H_5$BDPO (phenyl) | 12000 ± 1700 |
| Alkanesulfonyl fluorides and azide | |
| **14** $C_{12}H_{25}SO_2F$ | 6.9 ± 3.6[b] |
| **15** $C_{12}H_{23}SO_2F$ (11-ene) | 29 ± 15[b] |
| **16** $C_{20}H_{33}SO_2F$ (arachidonyl) | 304 ± 23[d] |
| **17** $C_{12}H_{25}SO_2N_3$ | 420 ± 180 |
| **18** $C_8H_{17}SO_2F$ | 1300 ± 300 |

[a] Mean ± SE ($n$ = 3).
[b] Segall *et al.* (2003a).
[c] Individual values for % inhibition at 10000 nM.
[d] Segall *et al.* (2003b).

**TABLE 2**
**Inhibitory Potency for CB1 Receptor, NTE-LysoPLA, and AChE *in Vivo* Relative to Delayed Toxicity and Pharmacologic Response**

| Compound (number and structure) | Inhibition and delayed toxicity: mg/kg (*n*)[b] | Inhibition (%) 4 h[a]: CB1 | NTE-LysoPLA | AChE | Mortality (0.3–5d) dead/total | Pharmacologic response[c] (400 mg/kg, ip) |
|---|---|---|---|---|---|---|
| **2** $C^{12}H_{25}P(O)(OC_3H_7\text{-}i)F$ | | | | | | 3 (3) |
| | 10 (3) | 18 ± 13 | 98 ± 2 | 0 ± 0 | 4/4 | |
| | 30 (5) | 84 ± 13 | 99 ± 1 | 37 ± 5 | 4/4 | |
| **14** $C_{12}H_{25}SO_2F$ | | | | | | 3 (3) |
| | 1 (4) | 15 ± 5 | | | | |
| | 3 (4) | 29 ± 17 | | | | |
| | 10 (4) | 50 ± 6 | 34 ± 17 | 0 ± 0 | 0/4 | |
| | 30 (6) | 84 ± 8 | 84 ± 3 | 9 ± 5 | 0/8 | |
| | 100 (3) | 87 ± 4 | 98 ± 3 | 28 ± 7 | 7/7 | |
| **18** $C_8H_{17}SO_2F$ | | | | | | 2 (1–3) |
| | 10 | | 46, 39[d] | 5 ± 6 | 0/10 | |
| | 30 | | 55 ± 4[d] | 0 ± 0[d] | 3/15 | |
| | 100 (2) | 27, 22[c] | 87 ± 4[d] | 4 ± 5[d] | 8/10 | |

[a]Mean ± SE ($n = 3$) or individual values.
[b]Number of mice individually analyzed.
[c]Rated at 15 min as mean and range (in parentheses) with grading scale 0–4 for no effect to mobility. See Methods for conditions and ratings.
[d]Quistad *et al.* (2002b).

**TABLE 3**
**Inhibitory Potency for CB1 Receptor *in Vitro* and Pharmacologic Responses for Dodecane- and Octanesulfonamides Modeled on Anandamide**

| R | CB1 receptor $IC_{50}$ ($\mu$M)[a,b]: $C_{12}H_{25}SO_2R$ | $C_8H_{17}SO_2R$ | Pharmacologic response[b,c] (400 mg/kg, ip): $C_{12}H_{25}SO_2R$ | $C_8H_{17}SO_2R$ |
|---|---|---|---|---|
| $NHCH_2CH_2OH$ | >10 (8 ± 5) | >10 (11,8) | 2 (2) | 3.7 (2–4) |
| $NHC\overline{HCH_2CH_2}$ | 10 ± 4 | >10 (0,0) | 1 (1) | 1 (1) |
| $NHCH_2CH_2Cl$ | 14 ± 6 | >10 (21,8) | 0.7(0–1) | 2 (2) |
| $NHCH(CH_3)_2$ | 16 ± 3 | >10 (15,8) | 0.7(0–1) | 1.8 (1–3) |
| $NHCH_2CH_2Br$ | 20 ± 5 | | 1 (1) | |
| $NHCH_2CH_3$ | 22 ± 5 | | 2 (2) | |
| NHPh-4-$N(CH_3)_2$ | 26 ± 12 | | 1 (1) | |
| $NHCH_2CH_2F$ | 32 ± 12 | >10 (14,2) | 1.3 (1–2) | 4 (4) |
| $NHCH_2CH_2N(C_2H_5)_2$ | 49 ± 5 | | 2 (2) | |
| $NHC\overline{H{=}NCH_2CH_2S}$ | >10 (43,34) | >10 (6 ± 7) | 1 (1) | 4 (4) |
| $NH_2$ | >10 (20,17) | | 1 (1) | |
| $NHCH_2CH(CH_3)_2$ | >100 | | insol. | |
| $N(CH_2CH_2OH)_2$ | >10 (0,0) | | 4 (4) | |
| imidazole | | >10 (24,24) | | 2.5 (2–4) |
| morpholine | | >10(0 ± 0) | | 2 (2) |
| $NHC\overline{H{=}NCH{=}CHS}$ | | >10 (0,0) | | 1 (1) |
| $N{=}C\overline{SCH_2CH_2S}$ | | >10 (0,0) | | 1 (1) |

[a]Mean ± SE ($n = 3$). In parentheses % inhibition at highest dose is given as mean ± SE ($n = 3$) or as individual values. Anandamide analog $K_i$ values ($\mu$M in the presence of 50–150 mM PMSF) as follows: $NH_2$ 9.6 (Felder *et al.*, 1993), $NHCH_2CH_2OH$ 0.089 and $NHCH_2CH_2F$ 0.0086 (Adams *et al.*, 1995), $NHC\overline{HCH_2CH_2}$ 0.0022, and $NHCH_2CH_2Cl$ 0.0014 (Hillard *et al.*, 1999).
[b]$IC_{50}$ (nM) and pharmacologic response for standards: $\Delta^9$-THC 48 ± 20, 3 (3); anandamide > 10,000 (no PMSF), 0 (0).
[c]Rated at 15 min as mean and range (in parentheses) with grading scale of 0–4 for no effect to immobility. See Methods for conditions and ratings.

Society of Toxicology in 1989. Test compounds were administered ip as solutions in dimethyl sulfoxide (10–100 $\mu$l) in comparison with dimethyl sulfoxide alone as a control. For possible cannabinoid or delayed toxic effects, mice were maintained and observed for up to 5 days.

***Cannabinoid and other pharmacologic effects.*** Mice were treated ip with compounds **2**, **14**, **18**, and several dodecane- and octanesulfonamides as above for observation of depression of activity and recumbent posture at 15 min (Quistad *et al*., 2001). They were scored individually on a scale of 0–4 for activity depression (no deaths during 0–15 min): 0, no effect; 1, minimal depression; 2, moderate depression but sternal posture; 3, recumbent posture; 4, total immobility. These signs are consistent with the enhanced effects produced by anandamide administered to mice lacking FAAH, which adopt a flattened, rigid posture and remain completely motionless (Cravatt *et al*., 2001). Results are given as the mean followed in parentheses by the range (a single number indicates the same rating for all mice), $n$ = 3–6. Potential analgesic activity was determined with the mouse hot plate assay 15 min after ip administration of the test compound at 5 or 10 mg/kg following the procedure used earlier (Tomizawa *et al*., 2001).

***Inhibition of CB1 receptor* in vitro *and* in vivo.** Mouse brain membranes were used for the CB1 receptor binding assay with $^3$H-CP 55,940 as the radioligand determining nonspecific binding with MAFP for the nucleophilic site or WIN 55,212–2 mesylate for the agonist site (Quistad *et al.*, 2002a). The assays involved brain membranes (150–200 $\mu$g protein, pooled from 10 mice) in incubation buffer (50 mM Tris [pH 7.4] containing 1 mM EDTA, 1 mM $MgCl_2$, and 3 mg/ml bovine serum albumin) (475 ul) to which was added in sequence the test inhibitor in $Me_2SO$ (5 $\mu$l) or MAFP or WIN 55,212–2 (10 or 1 $\mu$M final concentration, respectively) in $Me_2SO$ (10 $\mu$l). After incubation at room temperature for 15 min, the radioligand (10 nM final concentration) was added in $Me_2SO$ (10 $\mu$l). Incubations were carried out for 90 min at 30°C before termination by the addition of ice-cold wash buffer (0.9% NaCl with 1 mg/ml bovine serum albumin) (1 ml) and vacuum filtration using Whatman GF/B glass-fiber filters that were presoaked in wash buffer for 2 h at 4°C. The filters were rinsed three times with 4 ml of ice-cold wash buffer prior to scintillation counting. In some cases, potential hydrolysis of candidate inhibitors by FAAH was examined and minimized by assaying with phenylmethanesulfonyl fluoride (PMSF) (50 $\mu$M, 15 min preincubation) or alone (Compton and Martin, 1997). The concentration of compound inhibiting 50% of the $^3$H-CP 55,940 binding ($IC_{50}$) was derived usually from threefold dose differentials giving 15–85% inhibition. Mean $IC_{50}$ and SE values reported represent at least three experiments.

***Assays of mouse brain FAAH, NTE, NTE-LysoPLA, and AChE activities.*** The methods have been described (Quistad *et al*., 2001, 2002b, 2003; Wu and Casida, 1996). Hydrolysis assays used specific substrates. FAAH activity was assayed with $^{14}$C-oleamide. NTE activity is defined as paraoxon-resistant and mipafox-sensitive using phenyl valerate. This enzyme is referred to here as NTE-LysoPLA to recognize that NTE utilizes lysolecithin as a substrate (i.e., has LysoPLA activity) (Quistad *et al*., 2003). AChE activity is determined using acetylthiocholine with Ellman's reagent.

## RESULTS

### *Inhibitory Potency for CB1 Receptor* in Vitro

Two types of organophosphorus compounds were examined, i.e., alkylfluorophosphonates (**1–7**) and BDPOs (**8–13**). Among the alkylfluorophosphonates, the $C_{12}$–$C_{20}$ monofluorides are the most active, with exceptional potency for $C_{12}H_{25}P(O)(OC_3H_7$-*i*$)F$ (**2**) and its unsaturated analog (**1**) ($IC_{50}$ = 1.0–1.8 nM) and moderate effectiveness for MAFP (**3**) ($IC_{50}$ = 520 nM). Smaller alkyl ($C_8$ and $C_3$) monofluorides (**4** and **6**) and $C_8$ and $C_{12}$ difluorides (**5** and **7**) are much less potent ($IC_{50}$ = > 10000 nM). The long-chain oleyl- and dodecyl-BDPOs (**8** and **9**) give $IC_{50}$ values of 10–11 nM, but there is major activity loss with the stearyl- and octylphosphonates (**10** and **11**) and particularly with the *O*-octylphosphate (**12**) and phenylphosphonate (**13**). The alkane- and alkenesulfonyl fluorides (**14–16, 18**) are optimal with the dodecane- and dodecene substituents (**14** and **15** with $IC_{50}$ 7 and 29 nM, respectively). Much lower potency is found with the arachidonyl (**16**) and octane (**18**) analogs and with octanesulfonyl azide (**17**) ($IC_{50}$ = 304–1300 nM).

### *Inhibitory Potency for CB1 Receptor, NTE-LysoPLA and AChE* in Vivo *Relative to Pharmacologic Response and Delayed Toxicity*

Two very potent (**2** and **14**) and one moderately effective (**18**) CB1 inhibitors *in vitro* were examined for pharmacologic response at 15 min and CB1 inhibition *in vivo* 4 h after treatment (Table 2). The dodecyl compounds **2** and **14** both cause recumbent posture in mice at 400 mg/kg ip and inhibit CB1 strongly at 30 mg/kg, whereas octanesulfonyl fluoride (**18**) is much less active (requiring 100 mg/kg). Additional compounds giving less than 50% inhibition of CB1 receptor at 4 h with indicated dose (mg/kg) are: alkylfluorophosphonates **4** and **5** at 30, BDPOs **8** at 30, **9** and **11** at 10, and **13** at 100. After dosage of **14** at 30 mg/kg, mouse brain CB1 gave the following percentage inhibition as a function of time ($n$ = 3–4): 87 ± 4 at 2 day and 17 ± 12 at 5 day. Thus, with **14** the half-time for CB1 activity recovery is estimated to be 3–4 days. Alkylfluorophosphonates **4** and **5** in this study and BDPOs **8** and **13** at 30 or 100 mg/kg are less active *in vivo* CB1 inhibitors than dodecyl compounds **2** and **14**.

The *in vivo* studies with compounds **2**, **14,** and **18** not only examined the dose dependency for CB1 inhibition but also considered NTE-LysoPLA and AChE inhibition and mortality. NTE-LysoPLA was of similar sensitivity to CB1 with sulfonyl fluoride **14** and more sensitive than CB1 with fluorophosphonate **2** and sulfonyl fluoride **18**. Brain AChE is much less sensitive than CB1 or NTE-LysoPLA to the test compounds. Delayed toxicity, indicated by mortality at 0.3–5 days, more closely follows NTE-LysoPLA inhibition than CB1 or AChE inhibition.

### *Inhibitory Potency for CB1 Receptor* in Vitro *and Pharmacologic Responses for Dodecane- and Octanesulfonamides Modeled on Anandamide*

A series of 23 $C_{12}$ and $C_8$ sulfonamide analogs of anandamide was synthesized as candidate agonists acting at CB1. The compounds were also tested for pharmacologic response in mice. Dodecanesulfonamides with cyclopropyl, 2-chloroethyl, isopropyl, and 2-fluoroethyl substituents on nitrogen (but not the 2-hydroxyethyl analog) showed moderate inhibition of CB1 binding ($IC_{50}$ = 10–32 $\mu$M), whereas the corresponding octanesulfonamides were inactive. Pharmacologic effects in

mice at 15 min (hypomotility, but not death) were observed after a high ip dose (400 mg/kg) with most analogs, but $C_8$ sulfonamides are generally more active than $C_{12}$. The exception was a dodecanesulfonamide with bis(2-hydroxyethyl) substitutions at nitrogen. Stronger effects were observed for $C_8H_{17}SO_2$-imidazole and $C_8H_{17}SO_2N$- bound to 2-hydroxyethyl, 2-fluoroethyl, and 2-thiazolyl: recumbent posture or total immobility at 400 mg/kg and (although not tabulated) moderate reduction of mobility at 40 mg/kg. Overall, depressed mobility did not correlate to CB1 inhibition. Use of PMSF at 50 $\mu$M to inhibit possible FAAH hydrolysis of the sulfonamides (and thereby possibly enhance activity) had no effect on CB1 inhibitory potency ($C_{12}H_{25}SO_2$–F, –$NHCH_2CH_2Cl$, and –$NHCH_2CH_2F$).

Six compounds were tested for possible analgesic activity in the mouse hot plate assay. No activity was observed at 5 mg/kg for $C_8H_{17}SO_2NHCH_2CH_2OH$ and at 10 mg/kg for $C_{12}H_{25}SO_2$-$NHCH_2CH_2OH$, the $C_8$ and $C_{12}$ sulfonamides with the 2-fluoroethyl substituent, the $C_{12}$ analog with bis(2-hydroxyethyl), and $C_{12}H_{25}SO_2N_3$ (**17**).

## DISCUSSION

### *Organophosphorus and Organosulfur Compounds Active* in Vitro *at CB1*

Cannabinoid agonist binding to the CB1 receptor is sensitive to *in vitro* inhibition by selected organophosphorus and organosulfur compounds with higher potency conferred by $C_{12}$–$C_{20}$ than by $C_8$ alkyl substituents. The most information is available on alkylfluorophosphonates and BDPOs. The highest potency ($IC_{50}$ = 2–17 nM) is found for $C_{12:0}$, $C_{18:0}$, $C_{20:1}$, and $C_{20:2}$ alkylfluorophosphonates, particularly methyl and isopropyl 1-dodecylfluorophosphonates (Martin *et al.*, 2000; Segall *et al.*, 2003a; this study). MAFP (**3**) ($C_{20:4}$), the first reported OP inhibitor of agonist binding (Deutsch *et al.*, 1997b), is somewhat less active (this report). The alkyl chain specificity is also evident in the BDPO series with a potency decrease in the order $C_{18:1} > C_{12:0} > C_{18:0} > C_{8:0}$ (this study). Organophosphorus pesticides examined earlier are generally less active, with two interesting exceptions: the $IC_{50}$ of the insecticide metabolite chlorpyrifos oxon is 14 nM, but replacing its diethyl substituents with dipentyl greatly lowers the potency ($IC_{50} >$ 100 nM); the insecticide dichlorvos is of low potency, but its dipentyl analog is much more active, i.e., $IC_{50}$ values of 4200 and 92 nM, respectively (Quistad *et al.*, 2002a). Clearly the optimal chain length for OP potency is dependent on the other molecular substituents.

Alkanesulfonyl fluorides can achieve essentially the same potency as their organophosphorus counterparts and, again, with specific chain length requirements. $C_{12}$ is optimal in a $C_{12}$–$C_{20}$ series (Deutsch *et al.*, 1997a). The terminal unsaturation in **15** appears to reduce the potency versus the $C_{12}$ compound **14,** and arachidonylsulfonyl fluoride (**16**) with four *cis* double bonds is even less potent (Segall *et al.,* 2003b).

### *Selectivity for CB1 Receptor Relative to FAAH* in Vitro

Since anandamide is both a ligand for CB1 and a substrate for FAAH, mutual inhibitors might be expected (Table 4). Long-chain ($C_{18}$ and $C_{20}$) alkylphosphonates have the highest selectivity for CB1 versus FAAH, while retaining high potency for CB1 (Martin *et al.*, 2000). In this investigation four series of $C_{12}$ and $C_8$ analogs (fluorophosphonates, BDPOs, phosphonic difluorides, and sulfonyl fluorides) were compared for selectivity between CB1 and FAAH. In each series, $C_8$ versus $C_{12}$ chain length has less effect on inhibition of FAAH activity than on CP 55,940 binding to CB1, where $C_{12}$ is always preferred. Although $C_{12}$ monofluorophosphonates were equally potent on CB1 and FAAH, all other $C_8$ and $C_{12}$ analogs were FAAH selective. Long-chain alkanesulfonyl fluorides are much more potent for FAAH inhibition compared to CB1, but compound **14** is somewhat more selective, favoring FAAH inhibition *in vitro* using rat brain by only 6-fold (Deutsch *et al.*, 1997a). Compound **18** is the most selective inhibitor of FAAH (Quistad *et al.*, 2002b). PMSF and palmitylsulfonyl fluoride are known to potentiate anandamide action at CB1 by inhibiting FAAH (Compton and Martin, 1997; Gifford *et al.,* 1999).

### *Organophosphorus and Organosulfur Compounds Active* in Vivo *at CB1 Receptor*

The plant defoliant tribufos inhibits CB1 by 50% *in vivo* in mice at 50 mg/kg (ip), whereas 5 other OP pesticides are less inhibitory at symptomatic but sublethal doses (Quistad *et al.*, 2002a). The more potent dodecyl compounds **2** and **14** are active *in vivo* with $ED_{50}$ doses of 10–20 mg/kg, and octanesulfonyl fluoride (**18**) is less effective both *in vitro* and *in vivo* (this study). Delayed toxicity (death in 0.3–5 days) from compounds **2**, **14,** and **18** correlates to inhibition of NTE-LysoPLA, but contrary to the accepted mechanism for organophosphorus inhibitors such as **2** and **6,** NTE-LysoPLA inhibited by alkanesulfonyl fluorides **14** and **18** cannot "age," which is proposed (Glynn, 2000; Johnson and Glynn, 2001) to be a requirement for "toxic gain of function" and progression to delayed neuropathy. As a class, longer-chain alkanesulfonyl fluorides (exemplified by $C_8$ and $C_{12}$) are delayed toxicants in mice, as noted previously for PMSF in both mice (Wu and Casida, 1996) and hens (Lotti *et al*., 1993), but the level of NTE-LysoPLA inhibition must be high for onset of neuropathy (Lotti *et al*., 1993). Many other alkylfluorophosphonates and BDPOs in the present investigation were not active *in vivo*, perhaps due to transport, metabolism, or stability problems for these lipophilic compounds.

In conclusion, the most potent organophosphorus and organosulfur CB1 inhibitors examined here are also NTE-LysoPLA inhibitors and cause delayed toxicity in mice.

**TABLE 4**
**Selectivity for CB1 Receptor Relative to FAAH *in Vitro* and *in Vivo***

| Compound | $IC_{50}$ (nM) | | | $ED_{50}$ (mg/kg ip) | |
|---|---|---|---|---|---|
| | CB1 | FAAH | CB1/FAAH | CB1 | FAAH |
| CB1 selective | | | | | |
| **19** $C_{20}H_{37}$ P(O)$(OCH_3)$F | 3[a] | 55[a] | 0.05 | | |
| **20** $C_{20}H_{39}$ P(O)$(OCH_3)$F | 17[a] | 103[a] | 0.2 | | |
| **21** $C_{18}H_{37}$ P(O)$(OCH_3)$F | 10[a] | 48[a] | 0.2 | | |
| Nonselective | | | | | |
| **2** $C_{12}H_{25}$P(O)$(OC_3H_7$-*i*$)$F | 2[b] | 2[b] | 1 | 20 | |
| **22** $C_{12}H_{25}$P(O)$(OCH_3)$F | 3[a] | 3[a] | 1 | | |
| FAAH selective | | | | | |
| **3** $C_{20}H_{33}$P(O)$(OCH_3)$F | 530[c] | 0.10[d] | 5300 | | >10[d] |
| **4** $C_8H_{17}$P(O)$(OC_2H_5)$F | 11000 | 0.6[d] | 18000 | >30 | 0.5[d] |
| **5** $C_{12}H_{25}$P(O)$F_2$ | 36000 | 20 | 1800 | >30 | |
| **7** $C_8H_{17}$P(O)$F_2$ | >100,000 | 9[e] | >11,000 | | |
| **9** $C_{12}H_{25}$BDPO | 11 | 0.5[d] | 22 | >10 | 2[d] |
| **11** $C_8H_{17}$BDPO | 120 | 8[d] | 15 | >10 | 2[e] |
| **14** $C_{12}H_{25}SO_2F$ | 7[b](18)[f] | 0.5[b](3)[f] | 14(6) | 10 | <10 |
| **16** $C_{20}H_{33}SO_2F$ | 304[c] | 0.11[c] | 2800 | | |
| **18** $C_8H_{17}SO_2F$ | 1300 | 2[e] | 650 | >100 | 0.2[e] |
| **23** $C_8H_{17}$P(O)$(OCH_3)$F | >10,000[a] | 15[a] | >670 | | |

*Note.* Data from this study (Table 2 and Results) except where indicated otherwise.
[a]Martin *et al.* (2000) ($K_i$ for CB1at 30°C and $IC_{50}$ for FAAH at 37°C, rat); **19**, *cis*-11, *cis*-14; **20**, *cis*-11.
[b]Segall *et al.* (2003a).
[c]Segall *et al.* (2003b).
[d]Quistad *et al.* (2001).
[e]Quistad *et al.* (2002b).
[f]Deutsch *et al.* (1997a) (rat).

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