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Novel, potent THC/anandamide (hybrid) analogs

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Abstract—The structure–activity relationship (SAR) of the end pentyl chain in anandamide (AEA) has been established to be very similar to that of Δ^9-tetrahydrocannabinol (Δ^9-THC). In order to broaden our understanding of the structural similarities between AEA and THC, hybrid structures **1**–**3** were designed. In these hybrids the aromatic ring of THC–DMH was linked to the AEA moiety through an ether linkage with the oxygen of the phenol of THC. Hybrid **1** (**O-2220**) was found to have very high binding affinity to CB1 receptors (K_i = 8.5 nM), and it is interesting to note that the orientation of the side chain with respect to the oxygen in the phenol is the same as in THCs. To further explore the SAR in this series the terminal carbon of the side chain was modified by adding different substituents. Several such analogs were synthesized and tested for their CB1 and CB2 binding affinities and in vivo activity (tetrad tests). The details of the synthesis and the biological activity of these compounds are described.

1. Introduction

During the last few decades there has been a surge of interest in cannabinoid pharmacology[1–5] driven by the identification of G-protein coupled cannabinoid receptors.[6,7] The CB1 receptor is located both inside and outside the CNS, and the CB2 receptor is located mainly in the periphery. The first potent CB1 receptor antagonist SR141716A[8] was reported by Sanofi, and they also reported the CB2 selective receptor antagonist SR144528.[9] Anandamide (AEA) was identified as the endogenous ligand for the CB1 receptor and another key endogenous ligand which has been identified is 2-arachidonyl-glycerol (2-Ara-Gl), which activates both CB1 and CB2 receptors. Both AEA and 2-Ara-Gl are present together with a family of related fatty acid glycerol esters and the potency of 2-Ara-Gl was found to be improved in the presence of the related 2-acyl-glycerols. This effect has been termed the 'entourage effect' which probably represents a pathway for regulation of endogenous cannabinoid activity.[10] Mechoulam's group isolated another endogenous ligand 'noladin ether' (2-arachidonylglyceryl ether) from porcine brain.[11] It binds to CB1 receptors and very weakly to CB2 receptors. The pharmacological activities of Δ^9-tetrahydrocannabinol (Δ^9-THC) and the endogenous ligand AEA have some key differences.[12–15] For example, the onset of action of AEA is much faster and the duration of action is much shorter when compared with Δ^9-THC. This is due to the enzymatic hydrolysis of the amide bond in AEA and the enzyme involved in the metabolic degradation of AEA, 'fatty acid amide hydrolase' (FAAH).

In order to improve the metabolic stability of AEA, several analogs have been synthesized which have greater binding affinities than AEA. Makriyannis and coworkers incorporated a methyl in the amide head group which dramatically improved the metabolic stability of AEA,[16–18] while our group improved the metabolic stability by introduction of substituents on the carbon in the 2-position of the arachidonic acid part of AEA.[19–21] Our ongoing program on the SAR of classical THC's has revealed that the alkyl side chain has a vital effect on pharmacological activity.[22–25] It has been well established by several groups, including ours, that the SAR of the end pentyl chain in anandamide (AEA) is often similar to that of the THC side chain. Branching of the side chain in THC's results in a dramatic increase in potency and efficacy, whereas this modification in the AEA series has produced only a moderate increase in potency.[26,27]

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It is also well known that AEA activates CB1 receptors and also acts as an agonist at vanilloid VR1 receptors, whereas Δ^9-THC activates both CB1 and CB2 receptors and acts as a partial agonist. Keeping these differences in mind we designed and synthesized hybrids of THC/AEA which we expect will provide ligands with novel pharmacological properties and will further our understanding of the role of the endocannabinoid system. We had previously incorporated the DMH (dimethylheptyl) side chain of THC into the AEA template, and to extend this approach we designed hybrid structures that incorporated the aromatic ring of THC–DMH and used the oxygen of the phenol/pyran ring of THC to include a part of the AEA moiety as an ether linkage. We designed and developed novel THC/AEA hybrid ligands (**1**–**8**) (Fig. 1) that incorporate conformational restraint in the AEA part, and the synthesis and SAR results are described in this paper.

2. Chemistry

Our general synthetic strategy is based on the introduction of the AEA moiety in the targets by coupling of appropriately substituted phenols with the key intermediate 11-methanesulfonyloxy-undeca-5,8-dienoic methyl ester **16** (Scheme 1).

11-Methanesulfonyloxy-undeca-5,8-dienoic methyl ester **16** has been efficiently synthesized in our labs starting from homopropargyl alcohol **9**, which was converted into its silyl ether **10** by treatment with *t*-BDPSiCl, imidazole. Treatment of **10** with formaldehyde in the presence of CuCl as catalyst gave the alcohol **11**.[28] Bromination gave the propargyl bromide **12**, which was then coupled with methyl hex-5-ynoate in the presence of CuI as catalyst to provide the diynoic acid methyl ester **13**.[29,30] Partial reduction of the diyne over 'nickel boride' catalyst provided the diene **14**.[31] Deprotection of the silyl ether, followed by treatment of the alcohol **15** with MsCl, Et_3N gave the desired mesylate **16** in 26% overall yield. 3-(2-Methyloctan-2-yl)phenol **25** which is required for the synthesis of hybrid **1** was synthesized starting from 3-methoxy benzoic acid (Scheme 2). The acid was converted into its corresponding Weinreb amide **19** which on treatment with hexyl magnesium bromide afforded the ketone **21**.[32] Introduction of the dimethyl group was accomplished by treatment of ketone **21** with $TiCl_4$, Me_2Zn to give **23**, which on demethylation with BBr_3 gave the phenol derivative **25**. A similar sequence was used for the synthesis of the 4-(2-methyloctan-2-yl)phenol **26** starting from 4-methoxybenzoic acid **18**. Condensation of the mesylate **16** with the phenols **25** and **26** in the presence of potassium hydroxide gave the desired ester precursors **27** and **28** for the target compounds **1** and **2**, respectively. Hydrolysis of the ester hybrid **27** with lithium hydroxide provided the acid which was then converted to the corresponding acid chloride by treatment with oxalyl chloride and coupled with (*R*)-2-aminopropanol to give the target compound **1** in 89%. The synthesis of target **2** was accomplished using ester precursor **28** and following the same strategy as in the synthesis of hybrid **1**. For the synthesis of hybrid target **3** (Scheme 3), the alcohol **15** was converted to the iodide **32** using triphenylphosphine and iodine. The iodide **32** was converted into the corresponding phosphonium iodide **33** by refluxing with triphenylposphine. Treatment of the phosphonium iodide **33** with NaHMDS converted it into the ylide which was then reacted with the aldehyde **31** in a Wittig reaction to afford the desired hybrid ester **34**. The ester **34** was treated with (*R*)-2-aminopropanol in the presence of a catalytic amount of sodium cyanide to provide the target compound **3**. The synthesis of

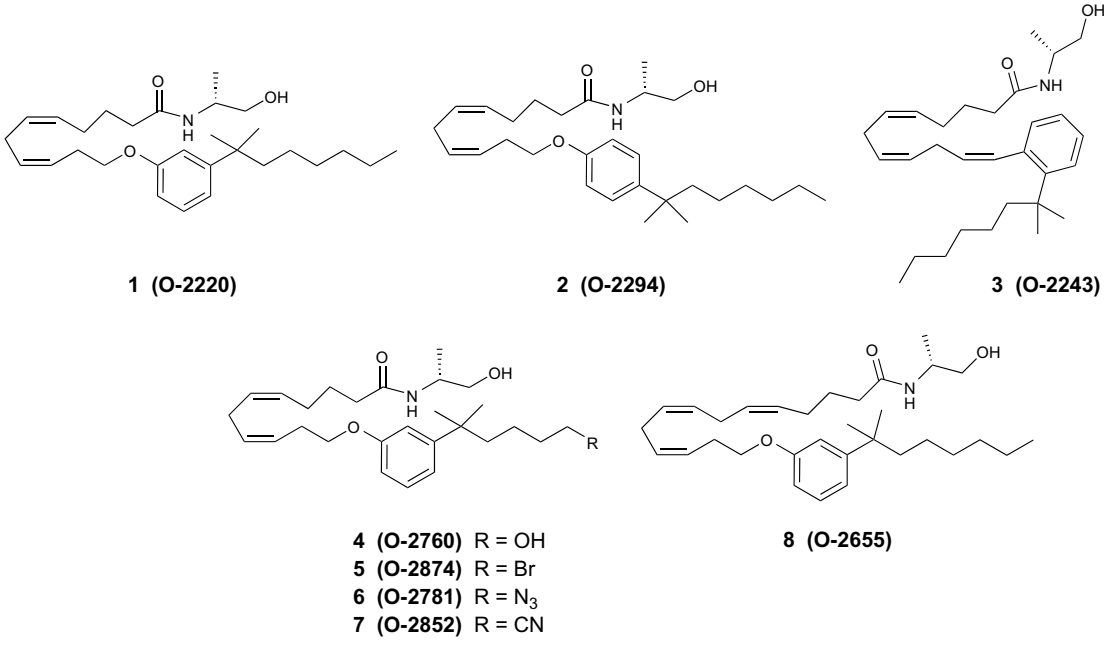
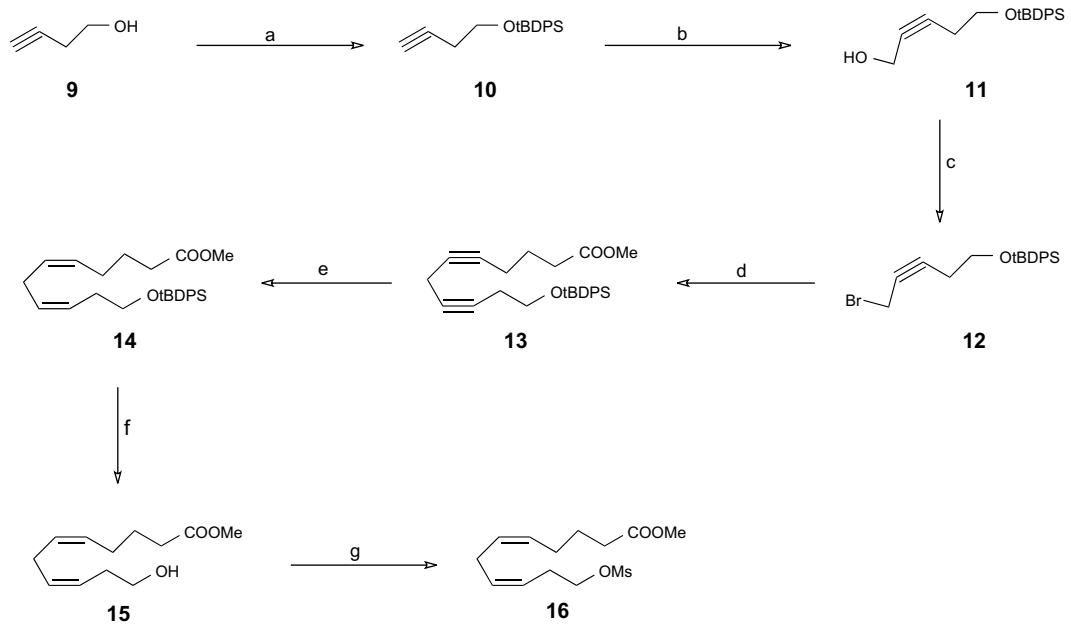
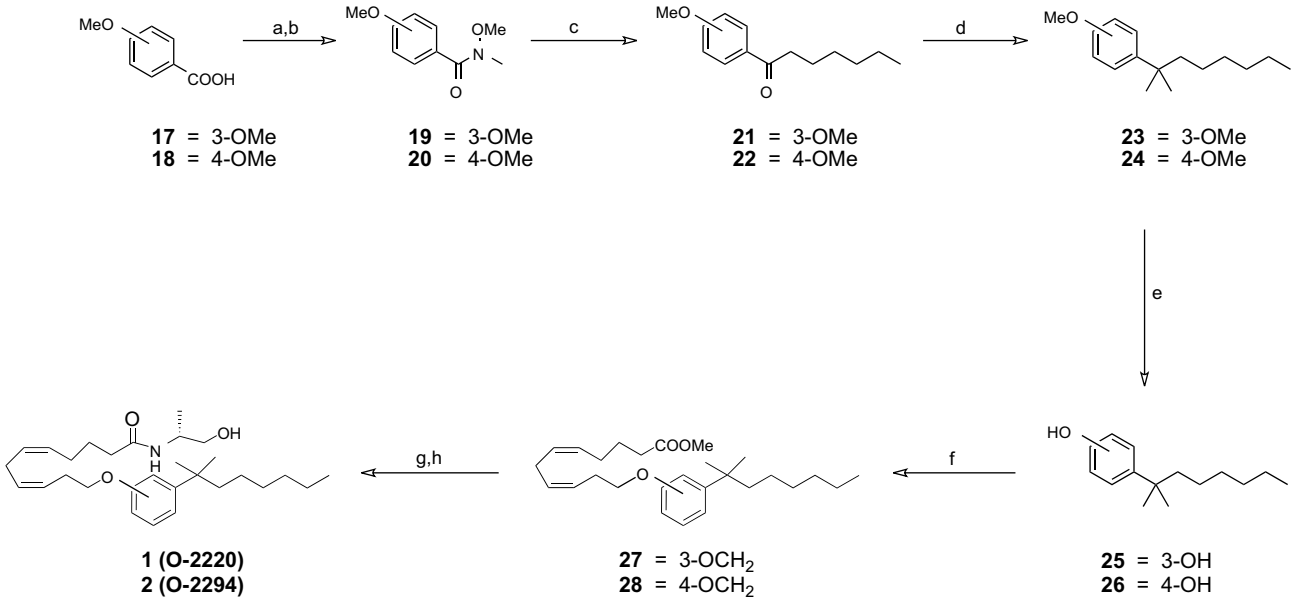


Figure 1. THC/anandamide hybrid ligands.



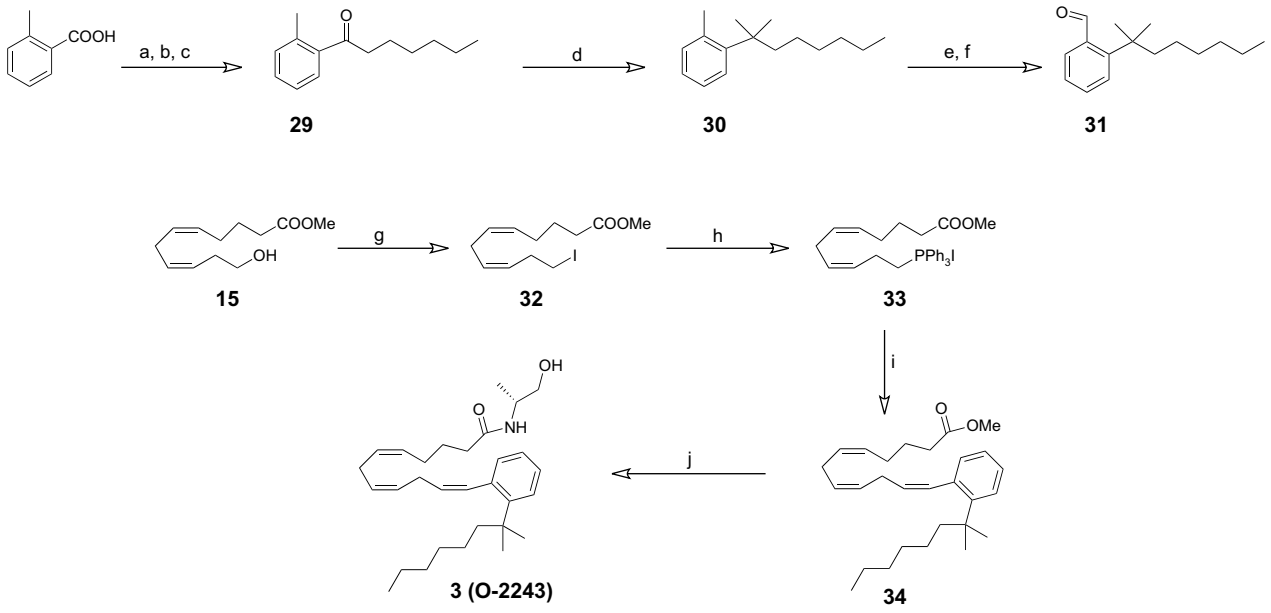
Scheme 1. Synthesis of the key intermediate **16**. Reagents and conditions: (a) *t*-BDPSiCl, imidazole, DMF, 81%; (b) 37% aq HCHO, CuCl, $CaCO_3$, H_2O, reflux, 79%; (c) CBr_4, Oct_3P, ether, 86%; (d) methyl hex-5-ynoate, CuI, NaI, K_2CO_3, DMF, 70%; (e) $Ni(OAc)_2$, $NaBH_4$, H_2, ethylenediamine, EtOH, 81%; (f) TBAF, AcOH, THF, 91%; (g) MsCl, Et_3N, CH_2Cl_2, 91%.



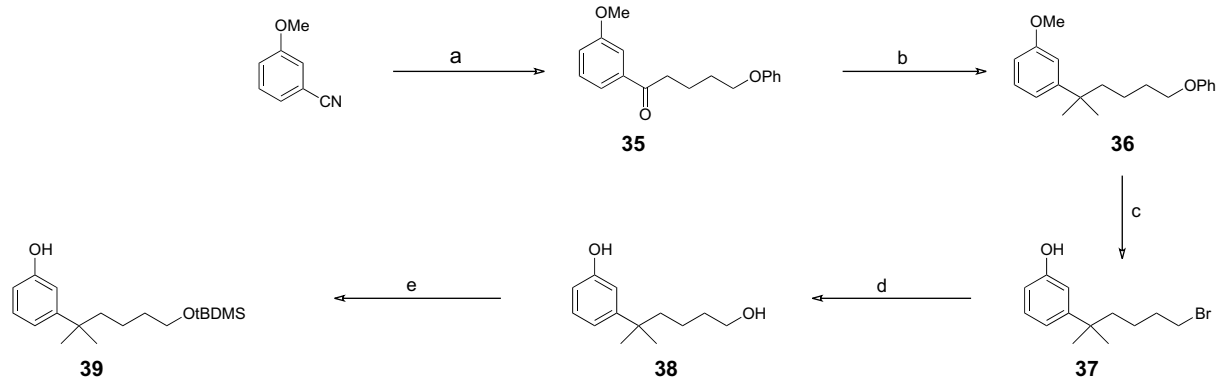
Scheme 2. Synthesis of hybrids **1** and **2**. Reagents and conditions: (a) $COCl_2$, DMF, CH_2Cl_2, 0 °C; (b) $CH_3ONHCH_3{\cdot}HCl$, Et_3N, CH (c) $CH_3(CH_2)_5MgBr$, THF, reflux; (d) $TiCl_4$, $(CH_3)_2Zn$, CH_2Cl_2, −78 to 0 °C; (e) BBr_3, CH_2Cl_2, 0 °C to rt; (f) **25** or **26**, KOH, DMF, 50 °C, −50%; (g) LiOH, MeOH, 50 °C, 100%; (h) oxalylchloride, DMF, benzene, (*R*)-2-aminopropanol, 64, 89%.

targets **4**–**7** was accomplished via the same strategy used in the synthesis of hybrid **1**. The appropriate phenol **39** (Scheme 4) for the synthesis of targets **4**–**7** was synthesized starting from the commercially available 3-methoxybenzonitrile. Treatment of 3-methoxy benzonitrile with (4-phenoxybutyl) magnesium bromide gave the desired ketone **35**. Introduction of the dimethyl group was accomplished by treatment of ketone **35** with $TiCl_4$, Me_2Zn to give **36**, which on demethylation with BBr_3 gave the phenol **37**. The bromide derivative **37** was converted to the alcohol derivative **38** using HMPA, H_2O which was then selectively protected with *tert*-butyldimethylsilyl chloride to give the desired phenol **39** in 14% overall yield.[33]

The phenol **39** on coupling with the mesylate **16** afforded the ester **40** (Scheme 5). Deprotection of the silyl protecting group in **40** followed by treatment of the alcohol **41** with MsCl, Et_3N provided the mesylate, which on further treatment with NaN_3 furnished compound **42**.



Scheme 3. Synthesis of hybrid **3**. Reagents and conditions: (a) $COCl_2$, DMF, CH_2Cl_2, 0 °C; (b) CH_3ONHCH_3·HCl, Et_3N, CH_2Cl_2, rt; (c) $CH_3(CH_2)_5MgBr$, THF, reflux, 55%, 3 steps; (d) $TiCl_4$, $(CH_3)_2Zn$, CH_2Cl_2, −78 to 0 °C, 90%; (e) NBS, AIBN, CCl_4, reflux, 85%; (f) $(Bu_4N)_2Cr_2O_7$, $CHCl_3$, reflux, 87%; (g) Ph_3P, Imidazole, I_2, CH_3CN–Et_2O, 0 °C, 90%; (h) Ph_3P, CH_3CN, reflux, 90%; (i) NHDMS, THF, HMPA, −78 °C, **31**, −78 °C to rt, 50%; (j) (*R*)-2-Amino propanol, NaCN·MeOH, sealed tube, 100%.



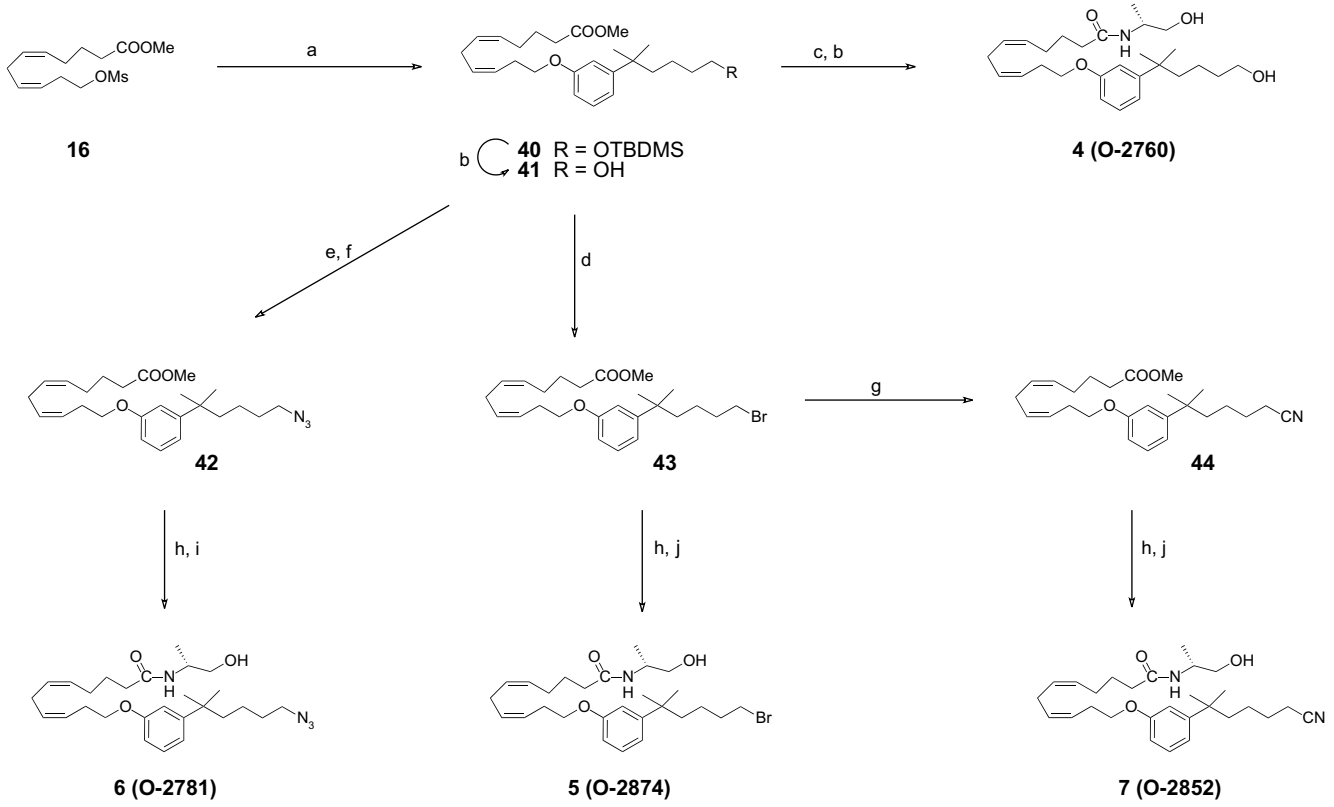
Scheme 4. Synthesis of phenolic intermediate **39**. Reagents and conditions: (a) $Br(CH_2)4OPh$, Mg, THF, 64%; (b) $TiCl_4$, $(CH_3)_2Zn$, CH_2Cl_2, −78 to −10 °C, 60%; (c) BBr_3, CH_2Cl_2, 0 °C to rt, 93%; (d) HMPA/H_2O (8:2), 100 °C, 70%; (e) *t*-BDMSCl, imidazole, DMF, 55%.

Bromo compound **43** was synthesized by bromination of **41** with CBr_4, Oct_3P. Treatment of the bromide **43** with NaCN gave the hybrid ester **44**. Target compound **4** was synthesized by treatment of **40** with (*R*)-2-aminopropanol in the presence of catalytic amount of NaCN followed by deprotection of the silyl protecting group with TBAF. The ester hybrids **43** and **44** were hydrolyzed to the corresponding acids and coupled with (*R*)-2-aminopropanol under the mixed anhydride condition using ClCOOEt to give the target compounds **5** and **7**, respectively. Hydrolysis of the ester hybrid **42** to the corresponding acid and coupling with (*R*)-2-aminopropanol under the acid chloride condition using oxalyl chloride afforded the target compound **6**. The synthesis of target compound **8** (Scheme 6) involved using synthon **45** which has been previously synthesized and reported by us.[29] Coupling of phenol **25** (Scheme 2) with the mesylate of alcohol **45** gave the desired ester **46** (Scheme 6). The ester hybrid **46** was hydrolyzed to the corresponding acid **47** and coupled with (*R*)-2-aminopropanol under the mixed anhydride condition using ClCOOEt to provide the target compound **8**.

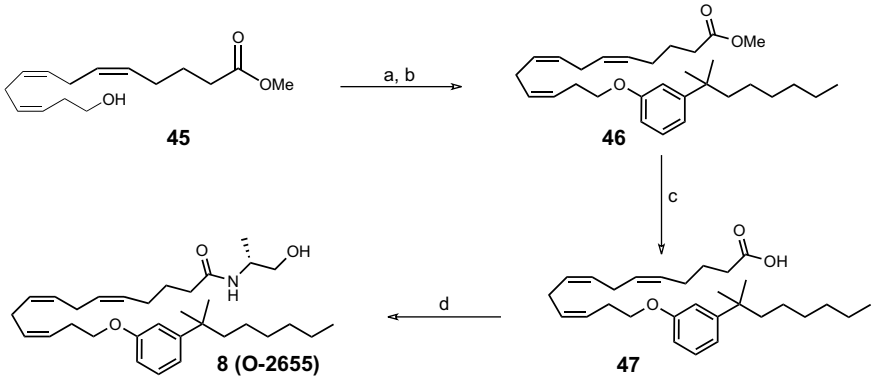
In summary we have developed a facile and convergent route for the synthesis of the hybrid analogs via the key intermediate **16**.

3. Results and discussion

The hybrid **1** (**O-2220**) (Table 1) was found to have good binding affinity to CB1 receptors (K_i = 8.5 nM). Comparison of the results obtained with the hybrids revealed that the orientation of the side chain in hybrid **1** was important, and it is interesting to note that in **1** the orientation of the side chain to the phenolic oxygen is the



Scheme 5. Synthesis of side chain modified hybrids. Reagents and conditions: (a) **39**, KOH, DMF, 50 °C, 62%; (b) TBAF, AcOH; (c) (*R*)-2-amino propanol, NaCN, MeOH, sealed tube, 64%; (d) CBr_4, Oct_3P, ether, 88%; (e) MsCl, Et_3N, CH_2Cl_2; (f) NaN_3, DMF, (g) NaCN, DMSO, 65%; (h) LiOH, MeOH; (i) $(COCl)_2$, DMF, CH_2Cl_2, 0 °C, (*R*)-2-mino propanol; (j) ClCOOEt, Et_3N, CH_3CN, 0 °C, (*R*)-2-amino propanol.



Scheme 6. Synthesis of hybrid analog **8**. Reagents and conditions: (a) MsCl, Et_3N, CH_2Cl_2, 73%; (b) **25**, KOH, DMF, 50 °C, 53%; (c) LiOH, MeOH, 50 °C, 60%; (d) ClCOOEt, Et_3N, CH_2Cl_2, 0 °C, (*R*)-2-aminopropanol, 75%.

same as in THC. When the orientation was changed to the C4-position as in **2** (**O-2294**) (CB1 $K_i = 195 \pm 39$), the binding affinity decreased 23-fold and activity in the tetrad tests was absent. The synthesis of the hybrid with an ether linkage at the C-2 position was attempted. However, our initial attempts were not successful due to the steric hinderance of the adjacent dimethylheptyl group. Hence, the hybrid **3** (**O-2243**) was synthesized in which we have a double bond in place of the ether linker. Hybrid **3** (**O-2243**) (CB1 $K_i = 86 \pm 8.7$) shows moderate binding affinity for CB1 receptors and moderate potency in two of the tetrad tests. In order to observe the effect of the change in length of the unsaturated backbone, we synthesized **8** (**O-2655**) (CB1 $K_i = 131 \pm 14$ nM). The introduction of the extra double bond was, however, found to be detrimental to the CB1 receptor binding affinity and in vivo activity.

To further explore the SAR of compound **1**, several analogs were synthesized (Fig. 1) with varying functional groups at the terminal end of the chain (Table 1). The SAR points out that the presence of a hydroxyl group on the terminal carbon of the chain (**O-2760**) decreased binding affinity to the CB1 receptors but slightly increased selectivity to CB2 receptors. In the tetrad tests, the potency in depressing spontaneous activity and

Table 1. Receptor affinity and pharmacological potency of the hybrid analogs

Compound	CB1 (K_i)	CB2 (K_i)	SA (ED_{50})	TF (ED_{50})	RT (ED_{50})	RI (ED_{50})
AEA	89 ± 10[35]	371 ± 102[35]	51.5[15]	17.8[15]	76.3[15]	55.0[15]
Δ^8-THC	44 ± 12[34]	44 ± 17[34]	2.9[35]	4.8[35]	4.5[35]	4.8[35]
Δ^8-THC–DMH	0.77 ± 0.11[35]	ND	0.27[35]	0.14[35]	0.15[35]	0.17[35]
O-2220	8.55 ± 1.83	21.48 ± 0.40	3.73 (2.63–5.30)	8.45 (6.26–11.41)	7.80 (4.46–13.62)	9.66 (7.78–11.98)
O-2294	194.85 ± 38.8	78.22 ± 9.26	44.9% at 30	30.7% at 30	−0.5 °C at 30	13.4% at 30
O-2243	86.33 ± 8.74	257.33 ± 3.49	25.7 (17.3–38.1)	50% at 30	−0.87 °C at 30	27.7 (18.9–40.4)
O-2760	78.97 ± 8.34	10.90 ± 0.94	1.1 (0.54–2.1)	0.6 (0.43–0.86)	2.8 (1.7–4.6)	0.7 (0.52–0.91)
O-2781	8.87 ± 2.44	7.46 ± 0.71	0.7 (0.5–1.0)	0.8 (0.61–0.97)	∼2.1	1.4 (1.1–1.7)
O-2874	2.74 ± 0.63	2.52 ± 0.35	0.2 (0.14–0.33)	0.2 (0.16–0.35)	∼1.95	0.3 (0.25–0.39)
O-2852	5.51 ± 0.50	3.13 ± 0.44	0.04 (0.02–0.07)	0.04 (0.02–0.07)	∼0.66	0.04 (0.02–0.07)
O-2655	131.33 ± 13.54	189 ± 23.39	55% at 30	61% at 30	−0.9 at 30	22% at 30

Binding affinity results are presented as K_i (nM); mice were injected iv with each analog and tested for suppression of spontaneous activity (SA), production of antinociception in the tail-flick procedure (TF), change in rectal temperature (RT) and production of ring immobility (RI). For compounds that produced dose-dependent effects, the results are presented as ED_{50}s (μmol/kg). Approximate ED_{50}s (indicated by ∼) are given when the highest dose tested produced half of the theoretical maximum response (e.g., temperature drop of −3 °C). When dose-dependence was not obtained and the maximum effect did not approach the theoretical maximum for the test, magnitude of maximal effect was indicated along with the dose (mg/kg) at which it occurred (e.g., −0.9 at 30 indicates −0.9 °C drop in body temperature at 30 mg/kg). Doses higher than 30 mg/kg were not tested. Superscript numbers in table indicate reference numbers for previously reported data.

rectal temperature was increased 3-fold whereas the potency in producing analgesia (TF) and relative immobility (RI) was increased 14-fold. The azido substituent (**O-2781**) on the other hand has no effect on the binding affinity but showed increased potency in the tetrad tests. However, the bromo (**O-2874**) and the cyano (**O-2852**) substituents produced an increase both in the binding affinity and potency. The latter results are consistent with our previous work that showed increases in affinity and potency through bromo and cyano substitutions to the terminal end of the side chain of Δ^8-THC.[34,35]

Our group has previously reported good AEA and Δ^9-THC overlays (Thomas's model), which could predict by QSAR the potencies of various AEA analogs with a reasonable degree of accuracy.[36] We had found that a looped conformation of these AEA analogs is energetically favorable and that a structural correlation between this low energy conformation and the classical cannabinoids can be obtained with the superposition of (1) the oxygen of the carboxamide with the pyran oxygen in Δ^9-THC, (2) the hydroxyl group of the ethanol with the phenolic hydroxyl group of Δ^9-THC, (3) the five terminal carbons and the pentyl side chain of Δ^9-THC, and (4) the polyolefin loop overlaying with the cannabinoid tricyclic ring. Our studies further showed that, when overlayed in this fashion, the potencies of AEA analogs are predictable by QSAR. The ability to incorporate the pharmacological potency of these AEA analogs into the cannabinoid pharmacophore model is also shown to support the relevance of this model. Preliminary molecular modeling studies of the hybrid **1** with Δ^9-THC, was carried out in collaboration with Dr. Brian Thomas. An initial overlay between the structures of **O-2220** and Δ^9-THC was obtained (see Fig. 2) by the superposition of (i) the dimethylheptyl side chain of the hybrid with the side chain of Δ^9-THC, (ii) oxygen of the ether of the hybrid with the pyran oxygen of Δ^9-THC, (iii) the terminal hydroxyl with the phenolic oxygen of Δ^9-THC, (iv) the chain with the olefinic bonds with the alicyclic ring of Δ^9-THC. However, when aligning the ring system as shown for **O-2220**, the side chains of **O-2294** and **O-2243** do not align as well as **O-2220** to the side chain orientation of Δ^9-THC (Fig. 3). The conformations obtained for **O-2220** were similar to those determined by Barnett-Norris et al.[37] using the method of conformational memories (CM) to study the conformations available to a series of ethanolamide fatty acid acyl chain congeners. These studies also suggested that anandamide and its congeners adopt tightly curved U/J-shaped conformations to interact with the TMH 2-3-7 region of CB1. We plan to develop a pharmacophore for our hybrid AEA analogs and subsequently we will use the model's predictive ability to design more compounds.

In summary, these analogs showed an increase in binding affinity and improvement in the pharmacological activity that is very similar to the results obtained with THC side chain modified analogs.

4. Conclusion

The high receptor affinity and pharmacological potency of **O-2220** provides further insight into the structural commonalities of THC and AEA that are required for biological activity. Incorporation of additional structural constraints in AEA will be necessary to establish its optimal conformation. However, the hybrids described herein illustrate the potential for developing new templates for cannabinoid receptor agonists and antagonists.

5. Experimental

5.1. General

All reagents were of commercial quality, reagent grade, and used without further purification. Anhydrous solvents were purchased from Aldrich and used without further purification. All reactions were carried out under N_2 atmosphere. Organic solutions were dried with

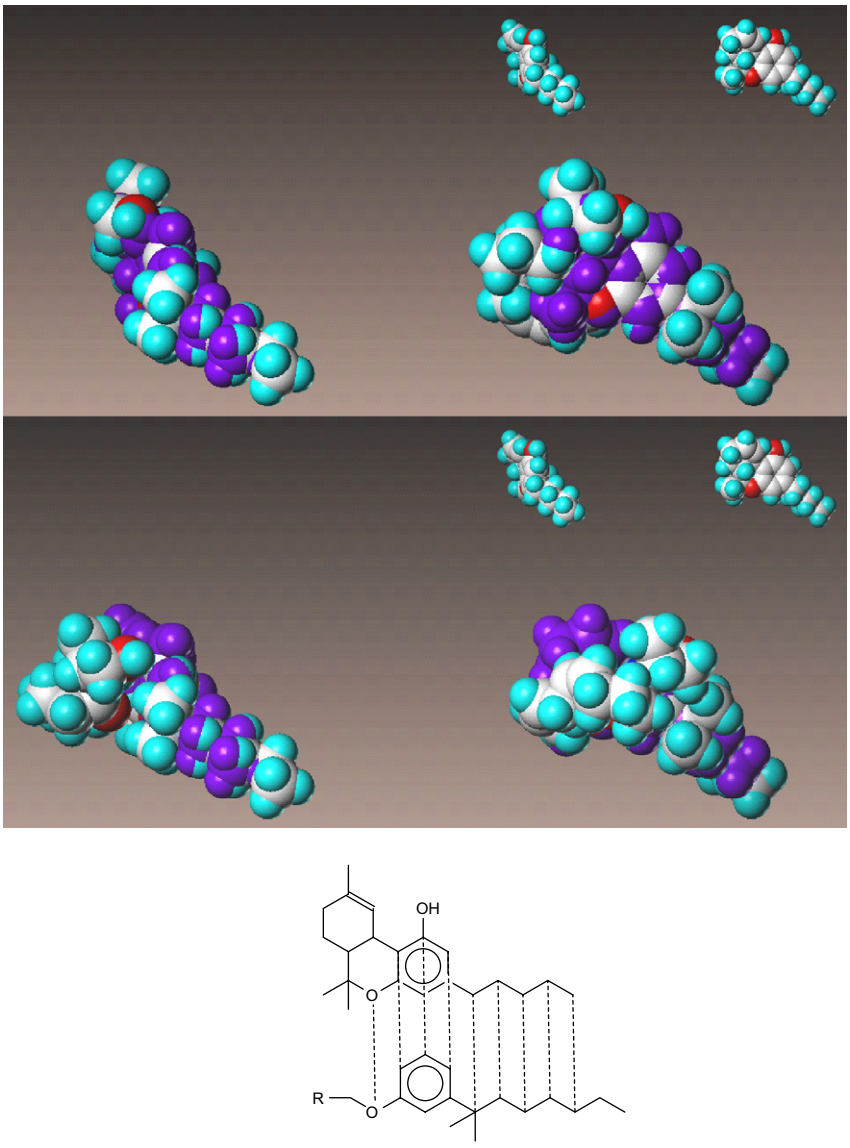


Figure 2. Superposition of two conformers of **O-2220** and Δ^9-THC. In the top panel, an orthogonal projection of space-filled models show a single conformation of **O-2220** overlayed with Δ^9-THC (in purple). The atoms in the **O-2220** conformation are colored by atom type (oxygen red, carbons white, hydrogens cyan, and nitrogens blue). Δ^9-THC is also shown in the upper right hand corner with its atoms colored by atom type to help orient the viewer to the overlay with Δ^9-THC in purple. In the lower panel, a similar presentation of an additional conformation of **O-2220** is presented. Finally, the basis for the initial superposition is shown in black and white.

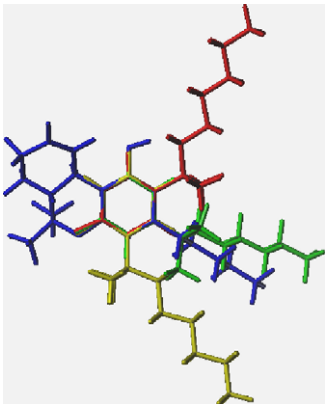


Figure 3. Superposition of the side chains of Δ^9-THC, **O-2220**, **O–2294** and **O–2243** based on the alignment described in Figure 2 for **O–2220**.

sodium sulfate. ^{1}H NMR spectra were recorded on a JEOL Eclipse 300 MHz spectrophotometer using $CDCl_3$ as the solvent with tetramethylsilane as an internal standard. MS data were obtained on an Agilent 1100 LC/MSD system. Thin-layer chromatography (TLC) was carried out on Baker Si 250F plates and was developed upon treatment with phosphomolybdic acid (PMA). Flash column chromatography was carried out on EM Science silica gel 60. Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, GA, and were found to be within ±0.4% of calculated values for the elements shown, unless otherwise noted.

5.2. *tert*-Butyl-but-3-ynyloxy-diphenylsilane (10)

To a stirred solution of 3-butyn-1-ol (5.0 g, 0.071 mol) in dry DMF (150 mL) at 0 °C, was added imidazole (9.7 g,

0.14 mol) and *tert*-butyldiphenylsilyl chloride (19.5 g, 0.072 mol) and the resulting solution was stirred overnight. On completion, the reaction was diluted with water followed by extraction with EtOAc (4 × 500 mL). The organic extracts were combined, washed with brine, dried and concentrated in vacuo. The residue was chromatographed on silica, eluting with 1% EtOAc–hexanes to afford 19.6 g of **10** as a oil in 90% yield. ^{1}H NMR δ 1.05 (s, 9H), 1.92 (t, 1H, J = 2.76 Hz), 2.41–2.47 (dt, 2H, J = 2.73, 7.14 Hz), 3.78 (t, 2H, J = 7.0 Hz), 7.34–7.41 (m, 6H), 7.66–7.71 (m, 4H).

5.3. 5-(*tert*-Butyl-diphenylsilanyloxy)pent-2-yn-1-ol (11)

Cuprous chloride (1.25 g, 0.0125 mol) was dissolved in 12% aqueous HCl (20 mL) to give a brown solution which was cooled to 0 °C. To this stirred solution, 40% KOH solution (20 mL) was added dropwise over the course of an hour precipitating an orange solid. This solid was filtered with suction and washed well with water. The moist solid was subsequently added to a suspension of **10** (17.8 g, 0.058 mol), $CaCO_3$ (0.062 g, 0.0006 mol), H_2O (1.25 mL) and 37% aq HCHO (6.2 mL) and the suspension heated at 110 °C. The reaction was monitored by TLC for 5 days, cooled to room temperature, diluted with EtOAc (800 mL), washed with water and brine. The organic extracts were dried and concentrated in vacuo to yield a brown residue which was chromatographed on silica, eluting with 20% EtOAc–hexanes to give 15.5 g of **11** in 79% yield as a colorless oil. ^{1}H NMR δ 1.05 (s, 9H), 2.48 (tt, 2H, J = 2.2, 7.14 Hz), 3.77 (t, 2H, J = 7.14 Hz), 4.19 (m, 2H), 7.35–7.45 (m, 6H), 7.65–7.7 (m, 4H).

5.4. 5-(Bromopent-3-ynyloxy)-*tert*-butyl-diphenylsilane (12)

To a stirred solution of **11** (14.6 g, 0.043 mol) in dry ether (150 mL) at 0 °C, was added CBr_4 (43.1 g, 0.13 mol) followed by dropwise addition of trioctyl phosphine (57.9 mL, 0.13 mol). The reaction was allowed to warm to room temperature and progress of the reaction was monitored by TLC. On completion the solvent was removed in vacuo and the residue subjected to column chromatography. On elution with 20% EtOAc–hexanes 15 g of **12** was obtained in 86% yield as yellow oil. ^{1}H NMR δ 1.05 (s, 9H), 2.48–2.50 (tt, 2H, J = 2.3, 6.87 Hz), 3.76 (t, 2H, J = 6.87 Hz), 3.78 (t, 2H, J = 2.1 Hz), 7.37–7.45 (m, 6H), 7.66–7.69 (m, 4H); MS (CI, m/z): 401, 403 [M^+].

5.5. 11-(*tert*-Butyldiphenylsilanyloxy)undeca-5,8-diynoic methyl ester (13)

A solution of **12** (8.68 g, 0.021 mol), K_2CO_3 (4.4 g, 0.032 mol), CuI (6.1 g, 0.032 mol), NaI (4.8 g, 0.032 mol) and methyl hex-5-ynoate (2.77 g, 0.022 mol) in dry DMF (60 mL) was stirred overnight at room temperature. On completion, the reaction mixture was diluted with EtOAc and filtered with suction to remove the inorganic solids. The filtrate was subsequently washed with water, brine and concentrated in vacuo. The residue thus obtained was filtered through a small pad of silica to afford 10 g of **13** as a pale yellow oil in 70% yield. ^{1}H NMR δ 1.05 (s, 9H), 1.79 (quint., 2H, J = 7.4 Hz), 2.16–2.25 (tt, 2H, J = 2.46, 6.87 Hz), 2.38–2.46 (m,4H), 3.08 (t, 2H, J = 2.1 Hz), 3.66 (s, 3H), 3.75 (t, 2H, J = 7.29 Hz), 7.37–7.42 (m, 6H), 7.66–7.69 (m, 4H).

5.6. 11-(*tert*-Butyldiphenylsilanyloxy)undeca-5,8-dienoic methyl ester (14)

To a stirred solution of $Ni(OAc)_2$ (10.9 g, 0.044 mol) in EtOH (500 mL) saturated with H_2 (g), was added $NaBH_4$ (1.66 g, 0.044 mol) in portions and the resulting solution saturated with H_2. To this was added ethylenediamine (3.71 mL, 0.055 mol) followed by a solution of **13** (10 g, 0.022 mol) in EtOH (50 mL), quickly maintaining the H_2 atmosphere after each addition. The reaction progress was monitored by TLC and on completion in 1 h was quenched with Et_2O (200 mL). The reaction solution was then filtered through Celite pad under suction and the filtrate concentrated in vacuo. The residue was filtered through a small pad of silica to give 8.22 g of **14** as a colorless oil in 81% yield. ^{1}H NMR δ 1.05 (s, 9H), 1.68 (quint., 2H, J = 7.4 Hz), 2.01–2.11 (m, 2H), 2.27–2.36 (m, 4H), 2.72 (t, 2H, J = 5.5 Hz), 3.64 (s, 3H), 3.67 (m, 2H), 5.31–5.42 (m, 4H), 7.32–7.44 (m, 6H), 7.65–7.71 (m, 4H).

5.7. 11-Hydroxyundeca-5,8-dienoic methyl ester (15)

To a solution of **14** (5.9 g, 0.013 mol), glacial AcOH (7.6 mL, 0.13 mol) in dry THF (70 mL), Bu_4NF (1 M in THF, 122.7 mL, 0.13 mol) was added dropwise and reaction was stirred at room temperature. The reaction was monitored by TLC and on completion the solvent was removed in vacuo. The oily residue obtained was extracted into ether, washed with water and brine, dried, concentrated and subjected to chromatographic purification on a silica column. 2.5 g of **15** was obtained on elution with 20% EtOAc–hexanes in 91% yield as a colorless oil. ^{1}H NMR δ 1.70 (quint., 2H, J = 7.4 Hz), 1.78 (br s, 1H), 2.07–2.15 (m, 2H), 2.28–2.38 (m, 4H), 2.81 (t, 2H, J = 5.76 Hz), 3.60–3.72 (m, 5H), 5.32–5.6 (m, 4H); MS (CI, m/z): 213 [$(M+H)^+$].

5.8. 11-methanesulfonyloxy-undeca-5,8-dienoic methyl ester (16)

To a stirred solution of **15** (2 g, 9.43 mmol) in dry CH_2Cl_2 (40 mL) at 0 °C was added Et_3N (4 mL, 28.7 mmol) followed by dropwise addition of methanesulfonyl chloride (1.1 mL, 14.2 mmol). The reaction was allowed to warm to room temperature with stirring and monitored by TLC. After three hours, the reaction solution was diluted with CH_2Cl_2 (150 mL), washed with water and brine, dried and concentrated in vacuo. The crude orange oily mesylate **16** (2.5 g, 91%) obtained was used as such in the next step without further purification. ^{1}H NMR δ 1.70 (quint., 2H, J = 7.4 Hz), 2.04–2.14 (m, 2H), 2.33 (t, 2H, J = 7.4 Hz), 2.51–2.57 (q, 2H, J = 6.8 Hz), 2.81 (br t, 2H, J = 7.14 Hz), 3.01 (s, 3H), 3.67 (s, 3H), 4.23 (t, 2H, J = 6.7 Hz), 5.32–5.6 (m, 4H).

5.9. *N*,3-Dimethoxy-*N*-methylbenzamide (19)

To a solution of 3-methoxybenzoic acid (10 g, 0.065 mol) in CH_2Cl_2 (70 mL) stirred at 0 °C was added DMF (0.8 mL) followed by oxalyl chloride (11.4 mL, 0.131 mol) dropwise. The reaction mixture was stirred and after 3–4 h was cannulated into addition funnel and added dropwise to a stirred solution of *N*,*O*-dimethylhydroxylamine hydrochloride (63.4 g, 0.65 mol) and Et_3N (95.6 mL, 0.65 mol) in CH_2Cl_2 (170 mL). The white heterogeneous mixture was left to stir vigorously at room temperature overnight. Water (200 mL) was added to the mixture followed by extraction with CH_2Cl_2. The combined organic extracts were washed with brine, dried and concentrated in vacuo. The residue was purified by column chromatography, eluting with 30% EtOAc–hexanes to give 38.1 g of the desired amide **19** in 81% yield. ^{1}H NMR δ 3.32 (s, 3H), 3.55 (s, 3H), 3.80 (s, 3H), 6.95–7.0 (m, 1H), 7.17–7.33 (m, 3H).

5.10. *N*,4-Dimethoxy-*N*-methylbenzamide (20)

This was prepared from *p*-anisic acid, oxalyl chloride, and *N*,*O*-dimethylhydroxylamine hydrochloride in quantitative yield by the same procedure as described for **19**. ^{1}H NMR δ 3.36 (s, 3H), 3.56 (s, 3H), 3.85 (s, 3H), 6.90 (d, 2H, J = 8.79 Hz), 7.73 (d, 2H, J = 8.79 Hz).

5.11. 1-(3-Methoxyphenyl)heptan-1-one (21)

To a solution of **19** (9.5 g, 0.048 mol) in THF (40 mL) was added dropwise a solution of hexylmagnesium bromide (2.0 M in ether, 26.4 mL, 0.0528 mol) and the reaction solution allowed to reflux for 6 h. After cooling to room temperature, the reaction was quenched with saturated NH_4Cl solution followed by extraction with EtOAc (4 × 100 mL). The organic extracts were combined, washed with brine, dried and concentrated in vacuo. The residue was purified by column chromatography, eluting with 25% EtOAc–hexanes to afford 6.5 g of ketone **21** as a colorless oil in 61% yield. ^{1}H NMR δ 0.90 (t, 3H, J = 6.87 Hz), 1.28–1.4 (m, 6H), 1.67–1.78 (quint., 2H, J = 7.14 Hz), 2.95 (t, 2H, J = 7.41 Hz), 3.85 (s, 3H), 7.08–7.12 (m, 1H), 7.36 (t, 1H, J = 7.8 Hz), 7.48–7.56 (m, 2H).

5.12. 1-(4-Methoxyphenyl)heptan-1-one (22)

This was prepared from **20** in 89% yield by similar procedure as described for **21**. ^{1}H NMR δ 0.90 (t, 3H, J = 6.9 Hz), 1.23–1.43 (m, 6H), 1.67–1.76 (quint., 2H, J = 7.14 Hz), 2.91 (t, 2H, J = 7.14 Hz), 3.87 (s, 3H), 6.93 (d, 2H, J = 9.06 Hz), 7.94 (d, 2H, J = 9.06 Hz).

5.13. 1-(1,1-Dimethylheptyl)-3-methoxybenzene (23)

A 1 M solution of $TiCl_4$ in CH_2Cl_2 (30.4 mL, 0.27 mol) was added to CH_2Cl_2 (125 mL) placed in a three-necked RB flask, fitted with an addition funnel and maintaining the temperature at −50 °C under a steady stream of N_2. This was followed by addition of $(CH_3)_2Zn$ (13.5 mL, 0.27 mol) via the addition funnel as quickly as possible while maintaining the temperature. The orange-brown solution obtained was stirred vigorously at −50 °C for one hour after which a solution of **21** (10 g, 0.045 mol) in dry CH_2Cl_2 (50 mL) was added dropwise. The reaction solution was allowed to stir for 2 h at −50 °C, then allowed to reach −10 °C before quenching by addition of ice-cold saturated NH_4Cl solution dropwise. The aqueous layer was then extracted with CH_2Cl_2 (5 × 200 mL), the organic extracts washed with brine, dried and concentrated in vacuo. The residue was purified by column chromatography, eluting with 1% EtOAc–hexanes to afford 10.2 g of **23** in 95% yield as a colorless oil. ^{1}H NMR δ 0.84 (t, 3H, J = 6.87 Hz), 1.06 (br s, 2H), 1.14–1.24 (m, 6H), 1.27 (s, 6H), 1.54–1.6 (m, 2H), 3.80 (s, 3H), 6.68–6.75 (m, 1H), 6.87–6.93 (m, 2H), 7.22 (t, 1H, J = 7.8 Hz).

5.14. 1-(1,1-Dimethylheptyl)-4-methoxybenzene (24)

This was prepared from **22**, $TiCl_4$ and $(CH_3)_2Zn$ in 25% yield by similar procedure as described for **23**. ^{1}H NMR δ 0.81 (t, 3H, J = 7.14 Hz), 1.05 (br s, 2H), 1.12–1.21 (m, 6H), 1.24 (s, 6H), 1.54 (m, 2H), 3.78 (s, 3H), 6.84 (d, 2H, J = 9.09 Hz), 7.23 (d, 2H, J = 8.79 Hz).

5.15. 3-(1,1-Dimethylheptyl)phenol 25

To a solution of BBr_3 (1.0 M in CH_2Cl_2, 33.3 mL, 0.033 mol) cooled to 0 °C was added a solution of **23** (6.9 g, 0.025 mol) in CH_2Cl_2 (100 mL) dropwise. The reaction was allowed to stir overnight at room temperature. The reaction solution was cooled to 0 °C and water (50 mL) was added dropwise. The aqueous layer was then extracted with EtOAc, the organic extracts combined, dried and concentrated in vacuo. The residue obtained was chromatographed on silica eluting with 3% EtOAc–hexanes to give 4 g of **25** as a viscous oil in 83% yield. ^{1}H NMR δ 0.84 (t, 3H, J = 6.6 Hz), 1.05 (br s, 2H), 1.14–1.22 (m, 6H), 1.25 (s, 6H), 1.52–1.57 (m, 2H), 6.60–6.65 (m, 1H), 6.81–6.90 (m, 2H), 7.15 (t, 1H, J = 7.8 Hz).

5.16. 4-(1,1-Dimethylheptyl)phenol (26)

This was prepared from **24** using BBr_3/CH_2Cl_2 in 95% yield by the same procedure as described for **25**. ^{1}H NMR δ 0.81 (t, 3H, J = 7.14 Hz), 1.05 (br s, 2H), 1.12–1.21 (m, 6H), 1.24 (s, 6H), 1.54 (m, 2H), 6.84 (d, 2H, J = 9.09 Hz), 7.23 (d, 2H, J = 8.79 Hz).

5.17. 11-[3-(1,1-Dimethylheptyl)phenoxy]undeca-5,8-dienoic acid methyl ester (27)

To a stirred solution of **25** (2.5 g, 11 mmol) and **16** (1.12 g, 3.8 mmol) in dry DMF (5 mL) was added KOH (0.65 g, 11 mmol). The reaction solution was stirred at 50 °C for 3 h and monitored by TLC. The reaction was worked up by diluting with water followed by extraction with EtOAc (5 × 100 mL). The organic extracts were washed with brine, dried and concentrated in vacuo. The brown residue obtained was chromatographed on silica eluting with 5% EtOAc–hexanes to give 0.85 g of **27** as a viscous oil in 53% yield. ^{1}H

NMR δ 0.84 (t, 3H, J = 6.87 Hz), 1.05 (br s, 2H), 1.14–1.22 (m, 6H), 1.26 (s, 6H), 1.52–1.59 (m, 2H), 1.73 (m, 2H), 2.10–2.22 (m, 2H), 2.3 (m, 2H), 2.58 (m, 2H), 2.83 (t, 2H, J = 5.76 Hz), 3.66 (s, 3H), 3.96 (t, 2H, J = 6.8 Hz), 5.30–5.58 (m, 4H), 6.65–6.7 (m, 1H), 6.86–6.92 (m, 2H), 7.2 (t, 1H, J = 7.8 Hz).

5.18. 11-[4-(1,1-Dimethylheptyl)phenoxy]undeca-5,8-dienoic acid methyl ester (28)

This was obtained from **26** and **16** by a procedure similar to **27** in 52% yield. ^{1}H NMR δ 0.83 (t, 3H, J = 6.67 Hz), 1.04 (br s, 2H), 1.14–1.23 (m, 6H), 1.25 (s, 6H), 1.50–1.57 (m, 2H), 1.70 (m, 2H), 2.08–2.22 (m, 2H), 2.32 (t, 2H, J = 7.41 Hz), 2.52 (q, 2H, J = 6.87, 5.22 Hz), 2.82 (t, 2H, J = 5.52 Hz), 3.65 (s, 3H), 3.95 (t, 2H, J = 6.87 Hz), 5.31–5.54 (m, 4H), 6.82 (d, 2H, J = 8.79 Hz), 7.21 (d, 2H, J = 8.79 Hz).

5.19. 11-[3-(1,1-Dimethylheptyl)phenoxy]undeca-5,8-dienoic acid [*R*(1-hydroxypropan-2-yl)]-amide 1 (O-2220)

LiOH (0.171 g, 0.0042 mol) was added to a solution of **27** (0.25 g, 0.0006 mol) in MeOH (18 mL) and H_2O (6 mL) and the reaction was left to stir overnight at 50 °C. On completion the reaction was cooled to room temperature and the solution acidified to pH 1.0 followed by extraction with ether. The organic extracts were dried and concentrated in vacuo to yield 0.24 g of the corresponding acid as a yellow oil to be used without further purification. ^{1}H NMR δ 0.82 (t, 3H, J = 6.87 Hz), 1.05 (br s, 2H), 1.14–1.22 (m, 6H), 1.26 (s, 6H), 1.57–1.75 (m, 4H), 2.08–2.18 (m, 2H), 2.24–2.32 (m, 2H), 2.50–2.58 (m, 2H), 2.87 (dt, 2H, J = 5.5 Hz), 3.97 (q, 2H, J = 6.6 Hz), 5.34–5.52 (m, 4H), 6.70–6.73 (m, 1H), 6.88–6.94 (m, 2H), 7.16 (t, 1H, J = 7.9 Hz), MS (CI, *m*/*z*): 401 [$(M+H)^+$].

Oxalyl chloride (0.155 g, 0.0012 mol) was added dropwise to a stirred solution of the above acid (0.24 g, 0.0006 mol) in benzene (2 mL) and DMF (1 drop) maintained at 0 °C. The reaction mixture was stirred for 2 h and the solvent was evaporated in vacuo and the flask was left to dry on high vacuum for 1 h. The residue obtained was dissolved in CH_2Cl_2 (2 mL), and added dropwise to an ice-cooled flask containing (*R*)-2-amino propanol (0.46 g, 0.006 mol) dissolved in CH_2Cl_2 and cooled to 0 °C. The reaction mixture was stirred overnight. The solvent was removed in vacuo and the residue chromatographed on silica eluting with $CHCl_3$ to afford 0.24 g of **1** as a yellow oil in 89% yield. ^{1}H NMR δ 0.83 (t, 3H, J = 6.3 Hz), 1.05 (br s, 2H), 1.13–1.18 (m, 9H), 1.26 (s, 6H), 1.53–1.59 (m, 2H), 1.68–1.78 (m, 2H), 2.09–2.22 (m, 4H), 2.56 (q, 2H, J = 5.22, 7.14 Hz), 2.73–2.85 (dt, 2H, J = 5.22 Hz), 3.48–3.69 (m, 2H), 3.97 (t, 2H, J = 6.8 Hz), 4.05–4.08(m, 1H), 5.37–5.56 (m, 4H), 6.67–6.71 (m, 1H), 6.85–6.92 (m, 2H), 7.20 (br t, 1H, J = 7.98 Hz), MS (CI, *m*/*z*): 458 [$(M+H)^+$]. Anal. Calcd for $C_{29}H_{47}O_3N{\cdot}1.2\ H_2O$: C, 72.67; H, 10.39; N, 2.92. Found: C, 72.68; H, 10.10; N, 2.69.

5.20. 11-[4-(1,1-Dimethylheptyl)phenoxy]undeca-5,8-dienoic acid [R(1-hydroxypropan-2-yl)]-amide 2 (O-2294)

The corresponding acid was obtained from **28** as a yellow oil in 93% yield by the same procedure as described for acid of **27**. ^{1}H NMR δ 0.84 (t, 3H, J = 6.60 Hz), 1.04 (br s, 2H), 1.12–1.21 (m, 6H), 1.25 (s, 6H), 1.51–1.57 (m, 2H), 1.66–1.79 (m, 2H), 2.10–2.17 (m, 2H), 2.34–2.39 (m, 2H), 2.55 (q, 2H, J = 6.60, 5.49 Hz), 2.83 (t, 2H, J = 5.49 Hz), 3.95 (t, 2H, J = 6.87 Hz), 5.38–5.51 (m, 4H), 6.83 (d, 2H, J = 8.79 Hz), 7.22 (d, 2H, J = 8.79 Hz). Target **2** was obtained in 64% yield from the above acid by similar procedure as described for **1**. ^{1}H NMR δ 0.83 (t, 3H, J = 6.8 Hz), 1.05 (br s, 2H), 1.14–1.24 (m, 9H), 1.26 (s, 6H), 1.53–1.59 (m, 2H), 1.68–1.78 (quint., 2H, J = 7.41 Hz), 2.09–2.22 (m, 4H), 2.56 (q, 2H, J = 6.57, 5.52 Hz), 2.83 (t, 2H, J = 5.5 Hz), 3.48–3.68 (m, 2H), 3.97 (t, 2H, J = 6.87 Hz), 4.05–4.08 (m, 1H), 5.37–5.52 (m, 4H), 5.58 (br s, 1H), 6.81–6.84 (d, 2H, J = 6.87 Hz), 7.22 (d, 2H, J = 6.87 Hz), MS (CI, *m*/*z*): 458 [$(M+H)^+$]. Anal. Calcd for $C_{29}H_{47}O_3N{\cdot}0.3\ H_2O$: C, 75.21; H, 10.36; N, 3.02. Found: C, 75.09; H, 10.51; N, 3.02.

5.21. 1-*o*-Tolylheptan-1-one (29)

This was prepared from *o*-toluic acid in 55% yield by the same procedure as described for **21**. ^{1}H NMR δ 0.88 (t, 3H, J = 6.3 Hz), 1.2–1.43 (m, 6H), 1.64–1.74 (m, 2H), 2.49 (s, 3H), 2.88 (t, 2H, J = 7.41 Hz), 7.23–7.26 (m, 2H), 7.3–7.41(m, 1H), 7.59–7.62 (m, 1H).

5.22. 1-(1,1-Dimethylheptyl)-2-methylbenzene (30)

This was prepared from **29** in 90% yield by the similar procedure as described for **23**. ^{1}H NMR δ 0.84 (t, 3H, J = 6.87 Hz), 1.03 (br s, 2H), 1.19–1.28 (m, 6H), 1.37 (s, 6H), 1.71–1.76 (m, 2H), 2.49 (s, 3H), 7.09–7.16 (m, 3H), 7.25–7.30 (m, 1H).

5.23. 2-(1,1-Dimethylheptyl)benzaldehyde (31)

NBS (1.55 g, 0.0086 mol) was added to a solution of **30** (1.57 g, 0.0072 mol) in CCl_4 (40 mL). Once the solid had dissolved, AIBN (0.05 g, 0.0018 mol) was added and the reaction mixture was refluxed overnight. The mixture was then filtered through a plug of silica and solvent removed in vacuo to yield 2.1 g of the benzyl bromide derivative as a clear oil in quantitative yield. ^{1}H NMR δ 0.83 (t, 3H, J = 6.87 Hz), 1.03 (br s, 2H), 1.18–1.3 (m, 6H), 1.4 (s, 6H), 1.71–1.76 (m, 2H), 4.81 (s, 2H), 7.18–7.3 (m, 3H), 7.41–7.46 (m, 1H). To a solution of the benzyl bromide derivative (2 g, 0.007 mol) in $CHCl_3$ (50 mL) was added $(Bu_4N)_2Cr_2O_7$ (9.9 g, 0.014 mol) and the mixture refluxed overnight. Silica (20 g) was added to the cooled reaction mixture and the solvent removed in vacuo. The red residue was chromatographed on silica eluting with 1–5% EtOAc–hexanes to yield 1.43 g of the aldehyde **31** in 87% yield. ^{1}H NMR δ 0.83 (t, 3H, J = 6.87 Hz), 1.03 (br s, 2H), 1.17–1.25 (s, 6H), 1.48 (s, 6H), 1.79–1.85 (m, 2H), 7.29–7.32 (t, 1H, J = 7.4 Hz), 7.39 (d, 1H, J = 1.1 Hz), 7.45–7.51 (m, 1H), 7.88–7.92 (dd, 1H, J = 1.65, 6.1 Hz).

5.24. 11-Iodoundeca-5,8-dienoic acid methyl ester (32)

To a stirred solution of **15** (2 g, 0.0094 mol), triphenylphosphine (5 g, 0.019 mol) and imidazole (1.3 g, 0.019 mol) in dry CH_3CN/Et_2O 1:2 (16.7:33.3 mL) at 0 °C was added I_2 (4.8 g, 0.019 mol) in portions. The reaction mixture was left to stir at room temperature for 1 h. The mixture was diluted with pentane/ether (4:1) and filtered through a silica plug. The colorless filtrate was dried and concentrated in vacuo and the residue was chromatographed on silica eluting with hexanes, then 20% EtOAc–hexanes to give 2.7 g of **32** in 90% yield. ^{1}H NMR δ 1.71 (quint., 2H, J = 7.4 Hz), 2.05–2.14 (m, 2H), 2.30–2.35 (t, 2H, J = 7.56 Hz), 2.62–2.79 (m, 4H), 3.15 (t, 2H, J = 7.14 Hz), 3.67 (s, 3H), 5.33–5.52 (m, 4H).

5.25. 11-Triphenylphosphoniumiodide-undeca-5,8-dienoic acid methyl ester (33)

A solution of **32** (2.7 g, 0.08 mol) and triphenylphosphine (2.4 g, 0.009 mol) in dry CH_3CN (50 mL) was refluxed overnight. The solvent was removed in vacuo and the viscous residue was purified by washing and decanting repeatedly with hexanes and benzene until most of the excess triphenylphosphine was removed. The viscous residue was dried in a vacuum oven for 24 h at 50 °C to give 4.2 g of **33** as a pale yellow gum in 90% yield. ^{1}H NMR δ 1.57–1.67 (m, 2H), 1.95 (q, 2H, J = 7.00 Hz), 2.25 (t, 2H, J = 7.41 Hz), 2.42–2.52 (m, 2H), 2.56 (t, 2H, J = 7.00 Hz), 3.63 (s, 3H), 3.81–3.90 (m, 2H), 5.18–5.66 (m, 4H), 7.25–7.36 (m, 6H), 7.78–7.94 (m, 9H).

5.26. 12-[2-(1,1-Dimethylheptyl)phenyl]dodeca-5,8,11-trienoic acid methyl ester (34)

To a stirring THF/HMPA solution (62 mL/8 mL) of **33** (3.78 g, 6.2 mmol) at −30 °C, was added NHMDS (6.5 mL, 6.2 mmol, 1 M in THF). The fluorescent red mixture was then allowed to warm to 0 °C. After 20 min the mixture was cooled to −78 °C and the aldehyde **31** (1.4 g, 6.0 mmol) dissolved in THF (30 mL) was added dropwise. The reaction mixture was warmed to room temperature and refluxed overnight. The mud brown mixture was diluted with hexanes (100 mL) and filtered through a plug of silica. The solvent was removed in vacuo and the residue was chromatographed on silica eluting with 5% EtOAc–hexanes to afford 1.3 g of **34** as a viscous oil in 50% yield. ^{1}H NMR δ 0.83 (t, 2H, J = 6.87 Hz), 0.97 (br s, 2H), 1.17–1.26 (m, 6H), 1.36 (s, 6H), 1.66–1.75 (m, 2H), 2.04–2.13 (m, 2H), 2.26–2.35 (m, 4H), 2.67–2.99 (m, 4H), 3.66 (s, 3H), 5.31–5.82 (m, 5H), 6.82 (d, 1H, J = 10.98 Hz), 7.02–7.38 (m, 4H), MS (CI, m/z): 411 [$(M+H)^+$].

5.27. 12-[2-(1,1-Dimethylheptyl)phenyl]dodeca-5,8,11-trienoic acid [*R*-(1-hydroxypropan-2-yl)]-amide 3 (O-2243)

A mixture of compound **34** (0.1 g, 0.26 mmol), NaCN (0.0013 g, 0.026 mmol) and (*R*)-2-aminopropan-1-ol (0.2 g, 2.6 mmol) in methanol (4 mL) was heated for 48 h in a sealed glass vial at 60 °C. The reaction progress was monitored by TLC and on completion was evaporated to dryness. The residue was purified by column chromatography, eluting with $CHCl_3$ to afford **3** in quantitative yield. ^{1}H NMR δ 0.83 (t, 2H, J = 6.87 Hz), 0.97 (br s, 2H), 1.12–1.21 (m, 9H), 1.34–1.38 (m, 6H), 1.66–1.76 (m, 4H), 2.05 (m, 2H), 2.16 (t, 2H, J = 7.44 Hz), 2.68–2.99 (m, 4H), 2.95–3.02 (m, 2H), 3.48–3.69 (m, 2H), 4.07 (m, 1H), 5.30–5.8 (m, 5H), 6.81–6.84 (d, 1H, J = 10.98 Hz), 7.03–7.34 (m, 4H), MS (CI, m/z): 454 [$(M+H)^+$]. Anal. Calcd for $C_{30}H_{47}O_2N{\cdot}1.1\ H_2O$: C, 76.09; H, 10.47; N, 2.96. Found: C, 76.03; H, 10.25; N, 2.96.

5.28. 1-(3-Methoxyphenyl)-5-phenoxypentan-1-one (35)

4-Bromophenoxybutane (10 g, 0.043 mol) and Mg powder (1.4 g, 325 mesh, 0.058 mol) in 500 mL THF was refluxed for 2 h. The reaction mixture was then cooled to room temperature and 3-methoxybenzonitrile (3.54 g, 0.029 mol) was added and again refluxed for 2 h. The imine formed was hydrolyzed with dropwise addition of 6 N HCl (150 mL) to the reaction solution cooled in ice-bath and reaction mixture was then refluxed overnight. The solvent was removed in vacuo and saturated NH_4Cl solution (600 mL) added. The aqueous layer was extracted with EtOAc (4 × 200 mL), organic extracts were combined, dried and concentrated in vacuo. The residue was purified by column chromatography, eluting with 10% EtOAc–hexanes to yield 8 g of **35** in 64% yield as a white crystalline solid. ^{1}H NMR δ 1.88–1.98 (m, 4H), 3.04 (t, 2H, J = 7.14 Hz), 3.84 (s, 6H), 4.0 (t, 2H, J = 5.77 Hz), 6.86–6.95 (m, 3H), 7.08–7.12 (dd, 1H, J = 2.9 Hz), 7.23–7.30 (m, 2H), 7.36 (t, 1H, J = 7.95 Hz), 7.48–7.55 (m, 2H), MS (CI, m/z): 285 [$(M+H)^+$].

5.29. 3-(5-Phenoxy-1,1-dimethylpentyl)-methoxyphenol (36)

This was prepared from ketone **35** in 60% yield in a procedure similar to **23**. ^{1}H NMR δ 1.18–1.25 (m, 2H), 1.29 (s, 6H), 1.62–1.68 (m, 4H), 3.80 (s, 3H), 3.84 (t, 2H, J = 6.6 Hz), 6.7 (m, 1H), 6.80–6.95 (m, 5H), 7.25 (t, 3H, J = 7.95 Hz), MS (CI, m/z): 299 [$(M+H)^+$].

5.30. 3-(5-Bromo-1,1-dimethylpentyl)phenol (37)

This was prepared from **36** using BBr_3/CH_2Cl_2 in 93% yield by the same procedure as described for **25**. ^{1}H NMR δ 1.20 (m, 2H), 1.28 (s, 6H), 1.56–1.62 (m, 2H), 1.75 (quint., 2H, J = 7.41 Hz), 3.32 (t, 2H, J = 6.87 Hz), 4.69 (br s, 1H), 6.62–6.66 (m, 1H), 6.80 (t, 1H, J = 2.2 Hz), 6.87–6.91 (m, 1H), 7.17 (t, 1H, J = 7.95 Hz).

5.31. 3-(5-Hydroxy-1,1-dimethylpentyl)phenol (38)

A solution of compound **37** (3.36 g, 0.012 mol) in 20% aqueous HMPA solution (70 mL) was stirred overnight at 110 °C. The reaction mixture was cooled to room temperature, diluted with water (150 mL) followed by extraction with EtOAc. The organic extracts were combined, dried and concentrated in vacuo. The residue was purified by column chromatography, eluting with 15–40% EtOAc–hexanes. The residue was subjected to column chromatography on silica to afford 1.8 g of **38** as a

yellow oil in 70% yield. ^{1}H NMR δ 1.12 (m, 2H), 1.26 (s, 6H), 1.48 (quint., 2H, J = 7.14 Hz), 1.56–1.62 (m, 2H), 3.58 (t, 2H, J = 6.45 Hz), 5.79 (br s, 1H), 6.62–6.65 (m, 1H), 6.80 (t, 1H, J = 2.1 Hz), 6.88 (m, 1H), 7.15 (t, 1H, J = 7.98 Hz), MS (CI, m/z): 207 [$(M-H)^+$].

5.32. 3-[5-(*tert*-Butyldimethylsilanyloxy)-1,1-dimethylpentyl]phenol (39)

To the phenol **38** (0.11 g, 0.53 mmol) dissolved in DMF (10 mL), was added imidazole (0.09 g, 1.4 mmol) and the reaction solution was cooled to 0 °C. To this was added *tert*-butyldimethylsilyl chloride (0.088 g, 0.53 mmol) and the reaction mixture was stirred for 7 h. The reaction was then diluted with water followed by extraction with EtOAc (4 × 50 mL). The organic extracts were combined, dried and concentrated. The residue was purified by column chromatography, eluting with 5–10% EtOAc–hexanes to give 0.1 g of the desired product **39** in 55% yield. ^{1}H NMR δ 0.009 (s, 6H), 0.85 (s, 9H), 1.10 (m, 2H), 1.25 (s, 6H), 1.42 (quint., 2H, J = 7.14 Hz), 1.52–1.58 (m, 2H), 3.52 (t, 2H, J = 6.6 Hz), 4.93 (br s, 1H), 6.59–6.63 (m, 1H), 6.78 (t, 1H, J = 2.2 Hz), 6.75 (m, 1H), 7.14 (t, 1H, J = 7.95 Hz), MS (CI, m/z): 323 [$(M+H)^+$].

5.33. 11-[3-(5-*tert*-Butyldimethylsiloxy-1,1-dimethylpentyl)phenoxy]undeca-5,8-dienoic acid methyl ester (40)

Compound **40** was prepared from **16** and **39** by the same procedure as described for **27** as a yellow oil in 62% yield. ^{1}H NMR δ 0.0 (s, 6H), 0.85 (s, 9H), 1.08 (m, 2H), 1.27 (s, 6H), 1.42 (quint. 2H, 7.14 Hz), 1.58 (m, 2H), 1.66–1.75 (m, 2H), 2.08–2.15 (m, 2H), 2.32 (t, 2H, J = 7.41 Hz), 2.53–2.59 (m, 2H), 2.83 (t, 2H, J = 5.5 Hz), 3.52 (t, 2H, J = 6.87 Hz), 3.66 (s, 3H), 3.96 (t, 2H, J = 6.87 Hz), 5.35–5.51 (m, 4H), 6.68–6.71 (m, 1H), 6.86–6.92 (m, 2H), 7.19 (t, 1H, J = 7.95 Hz), MS (CI, m/z): 517 [$(M+H)^+$].

5.34. 11-[3-(5-Hydroxy-1,1-dimethylpentyl)phenoxy]-undeca-5,8-dienoic acid [*R*-(1-hydroxypropan-2-yl)]amide 4 (O-2760)

A mixture of compound **40** (0.043 g, 0.083 mmol), NaCN (1 mg, 0.016 mmol) and (*R*)-2-aminopropan-1-ol (0.06 mL, 0.083 mmol) in methanol (3 mL) was heated for 48 h in a sealed glass vial at 60 °C. The reaction progress was monitored by TLC and on completion was evaporated to dryness. The residue was purified by column chromatography, eluting with 50% EtOAc–hexanes and 120 mg of the desired amide derivative was obtained as a colorless oil in 64% yield. ^{1}H NMR δ 0.0 (s, 6H), 0.86 (s, 9H), 1.1 (m, 2H), 1.16 (d, 3H, J = 6.87 Hz), 1.26 (s, 6H), 1.42 (m, 2H), 1.58 (m, 2H), 1.70–1.75 (m, 2H), 2.1–2.2 (m, 4H), 2.55–2.63 (m, 2H), 2.83 (m, 2H), 3.51 (t, 4H, J = 6.6 Hz), 3.96 (t, 2H, J = 6.87 Hz), 4.07 (m, 1H), 5.35–5.52 (m, 4H), 5.58 (br s, 1H), 6.69–6.71 (m, 1H), 6.86–6.92 (m, 2H), 7.19 (t, 1H, J = 7.98 Hz), MS (CI, m/z): 560 [$(M+H)^+$]. Tetrabutylammonium fluoride (2.15 mL, 1 M in THF, 0.21 mmol) was added dropwise to a solution of amide derivative (0.12 g, 0.21 mmol) in THF (10 mL), followed by AcOH (0.12 mL, 0.21 mmol) and reaction mixture stirred overnight. On completion, the reaction solution was diluted with water followed by extraction with ether. The organic extracts were combined, dried and concentrated in vacuo. The residue was purified through flash column chromatography on silica and 0.07 g of **4** was obtained as a yellow oil on elution with 2% MeOH–EtOAc in 73% yield. ^{1}H NMR δ 1.08–1.17 (m, 5H), 1.28 (s, 6H), 1.47 (m, 2H), 1.58–1.63 (m, 2H), 1.69–1.76 (m, 2H), 2.07–2.2 (m, 4H), 2.53–2.59 (m, 2H), 2.83 (t, 2H, J = 5.35 Hz), 3.48–3.67 (m, 4H), 3.97 (t, 2H, J = 6.87 Hz), 4.06 (s, 1H), 5.35–5.54 (m, 4H), 5.63 (s, 1H), 6.68–6.92 (m, 1H), 6.87–6.92 (m, 2H), 7.21 (t, 1H, J = 7.95 Hz), MS (CI, m/z): 446 [$(M+H)^+$]. Anal. Calcd for $C_{27}H_{43}O_4N{\cdot}0.2\ C_4H_8O_2$: C, 72.08; H, 9.70; N, 3.02. Found: C, 71.97; H, 9.96; N, 3.01.

5.35. 11-[3-(5-Hydroxy-1,1-dimethylpentyl)phenoxy]undeca-5,8-dienoic acid methyl ester (41)

This was obtained from **40** as a yellow oil by a procedure as described for **4** in 65% yield. ^{1}H NMR δ 1.12 (m, 2H), 1.28 (s, 6H), 1.47 (m, 2H), 1.58–1.63 (m, 2H), 1.68–1.75 (m, 2H), 2.04–2.16 (m, 2H), 2.32 (t, 2H, J = 7.41 Hz), 2.53–2.60 (m, 2H), 2.83 (br t, 2H, J = 5.22 Hz), 3.56 (t, 2H, J = 6.6 Hz), 3.65 (s, 3H), 3.96 (t, 2H, J = 6.87 Hz), 5.32–5.58 (m, 4H), 6.69–6.71 (m, 1H), 6.85–6.92 (m, 2H), 7.21 (t, 1H, J = 7.96 Hz), MS (CI, m/z): 403 [$(M+H)^+$].

5.36. 11-[3-(5-Azido-1,1-dimethylpentyl)phenoxy]undeca-5,8-dienoic acid methyl ester (42)

Methanesulfonyl chloride (0.15 mL, 1.9 mmol) was added dropwise to a solution of compound **41** (0.52 g, 1.3 mmol) and Et_3N (0.54 mL, 3.9 mmol) dissolved in CH_2Cl_2 (10 mL) and the reaction solution was cooled to 0 °C. The reaction was stirred for 3 h and allowed to reach room temperature. On completion, the reaction mixture was diluted with water (30 mL) followed by extraction with CH_2Cl_2. The organic extracts were combined, washed with brine, dried and concentrated to yield 0.62 g of the crude mesylate in quantitative yield. The mesylate was subsequently dried over vacuum and dissolved in anhydrous DMF (10 mL). NaN_3 (0.25 g, 3.8 mmol) was subsequently added and the reaction progress monitored by TLC. After 4 h, the reaction mixture was partitioned between water and ether. The organic layer was washed with brine, dried and concentrated to yield 0.48 g of **42** in 88% yield. ^{1}H NMR δ 1.13 (m, 2H), 1.28 (s, 6H), 1.5 (m, 2H), 1.58–1.62 (m, 2H), 1.68–1.73 (m, 2H), 2.05–2.15 (m, 2H), 2.32 (t, 2H, J = 7.41 Hz), 2.54–2.59 (m, 2H), 2.83 (br t, 2H, J = 5.76 Hz), 3.18 (t, 2H, J = 7.0 Hz), 3.64 (s, 3H), 3.97 (t, 2H, J = 6.87 Hz), 5.32–5.52 (m, 4H), 6.69–6.72 (m, 1H), 6.86–6.92 (m, 2H), 7.21 (t, 1H, J = 7.98 Hz), MS (CI, m/z): 428 [$(M+H)^+$].

5.37. 11-[3-(5-Bromo-1,1-dimethylpentyl)phenoxy]undeca-5,8-dienoic acid methyl ester (43)

The bromide **43** was obtained in 88% yield from **41** as a colored oil by a procedure similar to that described for **12**. ^{1}H

NMR δ 1.18–1.29 (m, 8H), 1.57–1.62 (m, 2H), 1.70–1.81 (m, 4H), 2.06–2.15 (m, 2H), 2.28–2.38 (m, 2H), 2.53–2.63 (m, 2H), 2.83 (br t, 2H, J = 5.76 Hz), 3.32 (t, 2H, J = 6.87 Hz), 3.67 (s, 3H), 3.92–3.99 (m, 2H), 5.35–5.6 (m, 4H), 6.68–6.73 (m, 1H), 6.86–6.92 (m, 2H), 7.21 (t, 1H, J = 7.98 Hz), MS (CI, m/z): 465, 467 [M^+].

5.38. 11-[3-(5-Cyano-1,1-dimethylpentyl)phenoxy]-undeca-5,8-dienoic acid methyl ester (44)

The bromide **43** (0.36 g, 0.8 mmol) was dissolved in 8 mL anhydrous DMSO followed by the addition of NaCN (0.14 g, 2.9 mmol) and the reaction progress was monitored by TLC. After 4 h at 50 °C, the reaction mixture was diluted with water followed by extraction with EtOAc. The organic extracts were combined, washed with brine, dried and concentrated in vacuo. The residue was purified by flash column and 0.2 g of **44** was obtained on elution with 15% EtOAc–hexanes in 65% yield as a colorless oil. ^{1}H NMR δ 1.16–1.32 (m, 8H), 1.51–1.63 (m, 4H), 1.68–1.76 (m, 2H), 2.06–2.15 (m, 2H), 2.23–2.35 (m, 4H), 2.54–2.64 (m, 2H), 2.83 (br t, 2H, J = 5.5 Hz), 3.66 (s, 3H), 3.92–3.99 (m, 2H), 5.35–5.54 (m, 4H), 6.70–6.73 (m, 1H), 6.85–6.91 (m, 2H), 7.21 (t, 1H, J = 7.98 Hz), MS (CI, m/z): 412 [$(M+H)^+$].

5.39. 11-[3-(5-Bromo-1,1-dimethylpentyl)phenoxy]-undeca-5,8-dienoic acid [*R*-(1-hydroxypropan-2-yl)]-amide 5 (O-2874)

The acid was obtained as an oily residue in 82% yield from **43** by a procedure similar to that described for acid of **1** except the reaction was performed at room temperature in this case. ^{1}H NMR δ 1.12–1.34 (m, 8H), 1.56–1.62 (m, 2H), 1.69–1.80 (m, 4H), 2.07–2.18 (m, 2H), 2.34–2.39 (m, 2H), 2.51–2.64 (m, 2H), 2.84 (br t, 2H, J = 5.5 Hz), 3.32 (t, 2H, J = 6.87 Hz), 3.92–3.99 (t, 2H, J = 6.85 Hz), 5.36–5.53 (m, 4H), 6.68–6.72 (m, 1H), 6.86–6.92 (m, 2H), 7.21 (t, 1H, J = 7.98 Hz), MS (CI, m/z): 451, 453 [M^+].

Ethylchloroformate (0.15 mL, 1.6 mmol) was added dropwise to a stirred solution of the above acid (0.29 g, 0.6 mmol) and triethylamine (0.26 mL, 1.8 mmol) in CH_2Cl_2 (5 mL) maintained at 0 °C. The reaction mixture was stirred for 3 h after which (*R*)-2-aminopropan-1-ol (0.14 mL, 1.8 mmol) dissolved in CH_2Cl_2 was added dropwise. The reaction mixture was stirred overnight. To the reaction mixture was added 10% HCl followed by extraction with EtOAc. The organic extracts were combined, washed with brine, dried and concentrated in vacuo. The residue was chromatographed on silica eluting with 50% EtOAc–hexanes to yield 0.156 g of **5** as a yellow oil in 48% yield. ^{1}H NMR δ 1.15–1.25 (m, 5H), 1.28 (s, 6H), 1.57–1.62 (m, 2H), 1.70–1.78 (m, 4H), 2.04–2.22 (m, 4H), 2.53–2.60 (m, 2H), 2.74–2.86 (m, 2H), 3.32 (t, 2H, J = 7.0 Hz), 3.48–3.56 (m, 1H), 3.63–3.67 (m, 1H), 3.95 (q, 2H, J = 6.7 Hz), 4.02–4.10 (m, 1H), 5.32–5.57 (m, 5H), 6.69–6.72 (m, 1H), 6.86–6.92 (m, 2H), 7.21 (t, 1H, J = 7.95 Hz), MS (CI, m/z): 508, 510 [M^+]. Anal. Calcd for $C_{27}H_{42}O_3NBr{\cdot}0.3\ H_2O$: C, 63.10; H, 8.35; N, 2.73. Found: C, 63.17; H, 8.54; N, 2.97.

5.40. 11-[3-(5-Azido-1,1-dimethylpentyl)phenoxy]undeca-5,8-dienoic acid [*R*-(1-hydroxypropan-2-yl)]-amide 6 (O-2781)

The acid was obtained as an oily residue in 95% yield from **42** by a procedure similar to that described for acid of **1**. ^{1}H NMR δ 1.08–1.15 (m, 2H), 1.28 (s, 6H), 1.45–1.52 (m, 2H), 1.57–1.63 (m, 2H), 1.69–1.74 (m, 2H), 2.09–2.17 (m, 2H), 2.36 (t, 2H, J = 7.42 Hz), 2.54–2.60 (m, 2H), 2.84 (br t, 2H, J = 5.5 Hz), 3.17 (t, 2H, J = 6.87 Hz), 3.97 (t, 2H, J = 6.84 Hz), 5.35–5.54 (m, 4H), 6.69–6.72 (m, 1H), 6.86–6.94 (m, 2H), 7.21 (t, 1H, J = 7.98 Hz), MS (CI, m/z): 414 [$(M+H)^+$].

Compound **6** was obtained as a yellow oil in 80% yield from the acid by the procedure as described for **5** ^{1}H NMR δ 1.08–1.18 (m, 5H), 1.28 (s, 6H), 1.45–1.52 (m, 2H), 1.57–1.63 (m, 2H), 1.68–1.76 (m, 2H), 2.05–2.22 (m, 4H), 2.54–2.60 (m, 2H), 2.76–2.86 (m, 2H), 3.18 (t, 2H, J = 7.0 Hz), 3.48–3.56 (m, 1H), 3.63–3.67 (m, 1H), 3.97 (t, 2H, J = 6.87 Hz), 4.03–4.09 (m, 1H), 5.34–5.56 (m, 5H), 6.69–6.73 (m, 1H), 6.86–6.92 (m, 2H), 7.21 (t, 1H, J = 7.95 Hz), MS (CI, m/z): 471 [$(M+H)^+$]. Anal. Calcd for $C_{27}H_{42}O_3N_4{\cdot}0.3\ H_2O$: C, 68.12; H, 9.02; N, 11.77. Found: C, 68.15; H, 9.18; N, 11.79.

5.41. 11-[3-(5-Cyano-1,1-dimethylpentyl)phenoxy]-undeca-5,8-dienoic acid [*R*-(1-hydroxypropan-2-yl)]-amide 7 (O-2852)

The acid was obtained in 96% yield as a colorless oil from **44** by similar procedure as described for acid of **1** except the reaction was performed at room temperature. ^{1}H NMR δ 1.15–1.33 (m, 8H), 1.57–1.62 (m, 2H), 1.69–1.80 (m, 4H), 2.06–2.18 (m, 2H), 2.32–2.39 (m, 2H), 2.53–2.60 (m, 2H), 2.84 (br t, 2H, J = 5.49 Hz), 3.32 (t, 2H, J = 7.00 Hz), 3.92–3.99 (q, 2H, J = 6.84 Hz), 5.34–5.52 (m, 4H), 6.68–6.72 (m, 1H), 6.86–6.91 (m, 2H), 7.21 (t, 1H, J = 7.96 Hz), MS (CI, m/z): 398 [$(M+H)^+$].

Compound **7** was obtained in 62% yield as a pale yellow oil by similar procedure as described for **5**. ^{1}H NMR δ 1.15–1.26 (m, 5H), 1.29 (s, 6H), 1.50–1.63 (m, 4H), 1.67–1.77 (m, 2H), 2.06–2.28 (m, 6H), 2.54–2.60 (m, 2H), 2.75–2.85 (m, 2H), 3.48–3.55 (m, 1H), 3.63–3.66 (m, 1H), 3.97 (t, 2H, J = 6.87 Hz), 4.02–4.08 (m, 1H), 5.34–5.60 (m, 5H), 6.69–6.73 (m, 1H), 6.85–6.91 (m, 2H), 7.22 (t, 1H, J = 7.95 Hz), MS (CI, m/z): 455 [$(M+H)^+$]. Anal. Calcd for $C_{28}H_{42}O_3N_2{\cdot}0.2\ H_2O$: C, 73.39; H, 9.33; N, 6.11. Found: C, 73.41; H, 9.50; N, 6.05.

5.42. (5*Z*,8*Z*,11*Z*)-*N*-((*R*)-1-hydroxypropan-2-yl)-14-(3-(2-methyloctan-2-yl)phenoxy)tetradeca-5,8,11-trienamide 8 (O-2655)

The alcohol **45**[29] was converted into the corresponding mesylate in 73% yield by similar procedure as described for **16**. ^{1}H NMR δ 1.30 (m, 2H), 1.68–1.73 (m, 2H), 2.08–2.16 (m, 3H), 2.30–2.33 (m, 2H), 2.50 (m, 1H), 2.79–2.90 (m, 2H), 3.01 (s, 3H), 3.68 (s, 3H), 4.21 (t, 2H, J = 6.6 Hz), 5.29–5.58 (m, 6H). Compound **46** was prepared from mesylate of **45** and **25** by using the same procedure as described for **27** in 53% yield. ^{1}H NMR δ

0.84 (t, 3H, $J = 6.87$ Hz), 1.05 (br s, 2H), 1.14–1.22 (m, 6H), 1.26 (s, 6H), 1.52–1.59 (m, 2H), 1.73 (m, 2H), 2.10–2.22 (m, 4H), 2.3 (m, 2H), 2.58 (m, 2H), 2.83 (m, 2H), 3.66 (s, 3H), 3.96 (m, 2H), 5.30–5.58 (m, 6H), 6.65–6.7 (m, 1H), 6.86–6.92 (m, 2H), 7.2 (t, 1H, $J = 7.9$ Hz); MS (CI, *m/z*): 455 [$(M+H)^+$]. The acid **47** was obtained in 60% yield by similar procedure as described for acid of **1**. ^{1}H NMR δ 0.82 (t, 3H, $J = 6.87$ Hz), 1.05 (br s, 2H), 1.14–1.22 (m, 6H), 1.26 (s, 6H), 1.57–1.75 (m, 4H), 2.08–2.18 (m, 4 H), 2.30–2.36 (m, 2H), 2.50–2.58 (m, 2H), 2.87 (dt, 2H, $J = 5.5$ Hz), 3.97 (m, 2H), 5.34–5.52 (m, 6H), 6.68–6.71 (m, 1H), 6.88–6.94 (m, 2H), 7.16 (t, 1H, $J = 7.9$ Hz), MS (CI, *m/z*): 441 [$(M+H)^+$]. Target **8** was obtained in 75% yield by similar procedure as described for **5**. ^{1}H NMR δ 0.83 (t, 3H, $J = 6.3$ Hz), 1.05 (br s, 2H), 1.13–1.18 (m, 9H), 1.21 (s, 6H), 1.26 (m, 1H), 1.53–1.59 (m, 2H), 1.68–1.78 (m, 2H), 2.09–2.22 (m, 4H), 2.56 (m, 2H), 2.73–2.85 (m, 3H), 3.54–3.71 (m, 2H), 3.97 (m, 2H), 4.05–4.08 (m, 1H), 5.37–5.56 (m, 6.5H), 6.67–6.71 (m, 1H), 6.85–6.92 (m, 2H), 7.20 (t, 1H, $J = 7.98$ Hz), MS (CI, *m/z*): 498 [$(M+H)^+$]. Anal. Calcd for $C_{32}H_{51}O_3N{\cdot}0.3\ H_2O$: C, 76.39; H, 10.34; N, 2.78. Found: C, 76.38; H, 10.59; N, 2.92.

5.43. Drug preparation and administration

For binding assays, the compounds were prepared as 1 mg/mL stock solutions in absolute ethanol and were stored at −20 °C. For behavioral assays, drugs were dissolved in a 1:1:18 mixture of ethanol, emulphor (GAF Corp., Linden, NJ), and saline (0.9% NaCl) and were administered intravenously (iv) in the mouse tail vein in volumes of 0.1 mL/10 g of body weight.

5.44. Membrane preparations

HEK-293 cells stably expressing the human CB1 receptor were cultured in DMEM with 10% FBS and Chinese Hamster Ovary (CHO) cells stably expressing the human CB2 receptor were cultured in DMEM with 10% FCS. Cells were harvested by replacement of the media with cold phosphate-buffered saline containing 1 mM EDTA followed by centrifugation at 1000*g* for 5 min at 4 °C. The pellet was resuspended in 50 mM Tris–HCl containing 320 mM sucrose, 2 mM EDTA and 5 mM $MgCl_2$ (pH 7.4) (centrifugation buffer), then centrifuged at 100*g* for 10 min at 4 °C and the resulting supernatant was saved. This process was repeated twice. The supernatant fractions were combined and centrifuged at 40,000*g* for 30 min at 4 °C. The resulting P2 pellet was resuspended in assay buffer (50 mM Tris–HCl (pH 7.4), 3 mM $MgCl_2$, 0.2 mM EGTA, and 100 mM NaCl) and protein was measured. Membranes were stored at −80 °C until use.

5.45. Receptor binding

Membrane homogenates (50 μg) were incubated with 0.5 nM [^{3}H]CP55,940 in the presence of varying concentrations (1 nM– 10 μM) of test compounds in 0.5 mL of buffer containing bovine serum albumin (5 mg/mL). Non-specific binding was measured in the presence of 1 mM CP55,940. The assay was incubated at 30 °C for 1 h and terminated by addition of ice-cold 50 mM Tris–HCl containing bovine serum albumin (1 mg/mL) (pH 7.4) followed by filtration under vacuum through Whatman GF/B glass fiber filters with three washes with cold Tris buffer. Bound radioactivity was determined by liquid scintillation spectrophotometry at 50% efficiency after extraction by shaking samples for 30–60 min with Budget-Solve scintillation fluid. Data are reported as meanS ± SEM of three experiments, each performed in triplicate. K_i values were calculated from displacement data using Equilibrium Binding Data Analysis (BIOSOFT, Milltown, NJ).

5.46. Behavioral evaluations

All mice were acclimated to the laboratory overnight. Each mouse was tested in two of the tetrad assays: locomotor activity and tail flick or rectal temperature and ring immobility. Prior to injection, baseline latency in the tail flick test was measured in mice in the first group. This procedure involved placing the mouse's tail on an ambient heat source (i.e., bright light) and recording latency (in seconds) for tail removal. Typical control latencies were 2–4 s. A 10-s maximal latency was used in order to avoid damage to the mouse's tail. After measurement of baseline tail flick latency, mice were injected iv with vehicle or drug. Four minUTES later they were re-tested in the tail flick procedure. Immediately thereafter, the mice were placed into individual photocell activity cages (11 × 6.5 in.) for assessment of spontaneous activity. For the next 10 min the total number of beam interruptions in the 16 photocell beams/cage was recorded using a Digiscan Animal Activity Monitor (Omnitech Electronics Inc., Columbus, OH). Spontaneous activity was expressed as percent of the control (vehicle) group's activity. Antinociception was expressed as the percent maximum possible effect (MPE) using a 10-s maximum test latency as described earlier. Each dose tested in the antinociception and hypomotility assays represents one group of animals (6 mice/group). Cannabinoid-induced hypothermia and immobility were determined in a separate group of animals. Prior to vehicle or drug administration, rectal temperature was determined by a thermistor probe (inserted 25 mm) and a telethermometer (Yellow Springs Instrument Co., Yellow Springs, OH). Four minutes after the i.v. injection of the drug, body temperature was measured again. The difference between pre- and postinjection rectal temperatures was calculated as change in body temperature. Immediately after measurement of body temperature, the mice were placed on a 5.5-cm ring attached at a height of 16 cm to a ring stand, and the amount of time the animals remained motionless during a 5-min period was recorded. The time that each animal remained motionless on the ring was divided by 300 s and multiplied by 100 to obtain a percent immobility rating.

5.47. Data analysis

Based on data obtained from numerous previous in vivo studies with cannabinoids, maximal cannabinoid effects in each in vivo pharmacological procedure were esti-

mated as follows: 90% inhibition of spontaneous activity, 100% MPE in the tail flick procedure, and −3 °C change in rectal temperature. Means and standard error (S.E.) were calculated for %MPE, number of photocell disruptions, % ring immobility and Δ°C. Analysis of variance (ANOVA) was used to determine significant differences between control and treatment groups followed by Dunnett's *t*-test post hoc analysis. Statistical analysis was performed using StatView, version 5.0 (SAS Institute, Cary, NC). Significance was defined as a $p < 0.05$. ED_{50}s were defined as the dose at which half maximal effect occurred. For drugs that produced one or more cannabinoid effect, ED_{50}s were calculated separately using least-squares linear regression on the linear part of the dose–effect curve for each measure in the mouse tetrad, plotted against log10 transformation of the dose. For the purposes of potency comparison, ED50s are expressed as μmol/kg.

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