

New Resorcinol–Anandamide “Hybrids” as Potent Cannabinoid Receptor Ligands Endowed with Antinociceptive Activity in Vivo

Antonella Brizzi,^{*,§} Vittorio Brizzi,[§] Maria Grazia Cascio,^{‡,¶} Federico Corelli,^{*,§} Francesca Guida,[†] Alessia Ligresti,[‡] Sabatino Maione,[†] Adriano Martinelli,[⊥] Serena Pasquini,[§] Tiziano Tuccinardi,^{⊥,||} and Vincenzo Di Marzo[‡]

Dipartimento Farmaco Chimico Tecnologico, Università degli Studi di Siena, Via A. Moro 2, 53100 Siena, Italy, Endocannabinoid Research Group, Istituto di Chimica Biomolecolare, Consiglio Nazionale delle Ricerche, Via Campi Flegrei 34, 80078 Pozzuoli, Napoli, Italy, Dipartimento di Medicina Sperimentale, Sezione di Farmacologia “L. Donatelli”, Seconda Università di Napoli, Via Costantinopoli 16, 80138 Napoli, Italy, Dipartimento di Scienze Farmaceutiche, Università di Pisa, Via Bonanno 6, 56126 Pisa, Italy, and Sbarro Institute for Cancer Research and Molecular Medicine, Center for Biotechnology, College of Science and Technology, Temple University, Philadelphia, Pennsylvania 19122

Received December 22, 2008

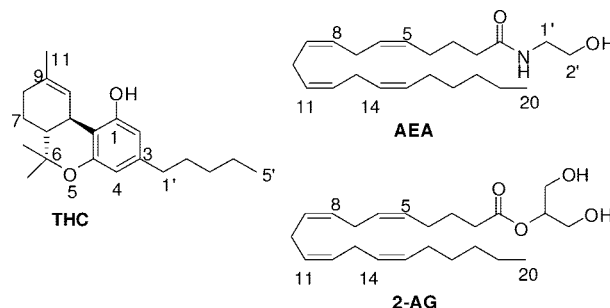
Bearing in mind the pharmacophoric requirements of both (–)-*trans*- Δ^9 -tetrahydrocannabinol (THC) and anandamide (AEA), we designed a novel pharmacophore consisting of both a rigid aromatic backbone and a flexible chain with the aim to develop a series of stable and potent ligands of cannabinoid receptors. In this paper we report the synthesis, docking studies, and structure–activity relationships of new resorcinol–anandamide “hybrids” differing in the side chain group. Compounds bearing a 2-methyloctan-2-yl group at position 5 showed a significantly higher affinity for cannabinoid (CB) receptors, in particular when an alkoxy chain of 7 or 10 carbon atoms was also present at position 1. Derivative **32** was a potent CB₁ and CB₂ ligand, with K_i values similar to that of WIN 55-212 and potent antinociceptive activity in vivo. Moreover, derivative **38**, although less potent, proved to be the most selective ligand for CB₂ receptor ($K_i(\text{CB}_1) = 1 \mu\text{M}$, $K_i(\text{CB}_2) = 35 \text{ nM}$).

Introduction

The possible therapeutic applications of *Cannabis sativa* L. have triggered intensive research on cannabinoid receptors and have aroused a considerable interest in the therapeutic potential of cannabimimetic ligands.¹ *Cannabis* mainly exerts its pharmacological effects via a group of typical diterpene C₂₁ compounds (now termed phytocannabinoids), and its most important component, THC^a (Chart 1), is rapidly absorbed and converted in the liver and lungs into a centrally active metabolite, 11-hydroxy- Δ^9 -THC.² Phytocannabinoids have been shown to produce a typical syndrome of effects on the human behavior via two G-protein-coupled receptors: CB₁,³ expressed at high levels in several brain areas but also present in peripheral tissues,⁴ including ileum, testis, urinary bladder, and vas deferens; CB₂, predominantly detected in the periphery, mainly in the immune system (spleen, tonsils, immune cells,⁵ and brain microglia under inflammatory conditions^{6,7}).

The discovery of cannabinoid receptors was followed by the discovery of a family of endogenous lipid modulators, the most studied members of which are AEA (Chart 1)⁸ and 2-arachi-

Chart 1. Chemical Structures of Main Cannabinoid Receptor Ligands



donylglycerol (2-AG, Chart 1),^{9–11} which are metabolically less stable and structurally very dissimilar from the plant-derived molecules.

Cannabinoid receptors, their endogenous ligands, and endocannabinoid proteins responsible for cellular uptake and inactivation, i.e., the putative anandamide membrane transporter (AMT)¹² and the fatty acid amide hydrolase (FAAH),¹³ or the monoacylglycerol lipase (MAGL)¹⁴ in the case of 2-AG, constitute the “endocannabinoid (EC) system” and represent potentially interesting targets¹⁵ for the development of new therapeutic agents to be employed in many pathologies, such as pain, loss of appetite in patients with AIDS, obesity, chemotherapy-induced nausea and vomiting, immune and inflammatory disorders, cardiovascular and gastrointestinal disorders, and neurodegenerative diseases.¹⁶ In fact, the EC system plays a role in a variety of physiological processes and in the past 2 decades it became clear that at least in mammals, the functions of this signaling system are not limited to the brain but are exerted in the whole organism. Moreover, during pathological states, the levels of these mediators in tissues change and their effects vary from those of protective endogenous compounds to those of dysregulated signals. These

* To whom correspondence should be addressed. For A.B.: phone, +39 577 234327; fax, +39 577 234333; e-mail, brizzi3@unisi.it. For F.C.: phone, +39 577 234308; fax, +39 577 234333; e-mail, corelli@unisi.it.

[§] Università degli Studi di Siena.

[‡] Istituto di Chimica Biomolecolare.

[¶] Present address: University of Aberdeen Medical School, Aberdeen, Scotland, U.K.

[†] Università di Napoli.

[⊥] Università di Pisa.

^{||} Temple University.

^a Abbreviations: THC, (–)-*trans*- Δ^9 -tetrahydrocannabinol; AEA, anandamide; CB, cannabinoid; SI, selectivity index; AG, arachidonylglycerol; AMT, anandamide membrane transporter; FAAH, fatty acid amide hydrolase; MAGL, monoacylglycerol lipase; EC, endocannabinoid; CBD, cannabidiol; DMF, *N,N*-dimethylformamide; DMSO, dimethyl sulfoxide; HOBt, 1-hydroxybenzotriazole; CMC, 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide methyl-*p*-toluenesulfonate.

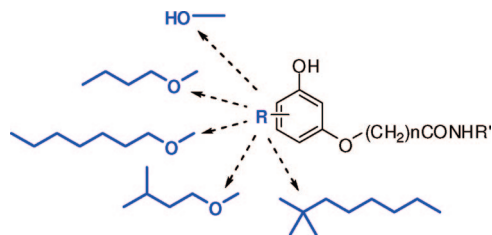
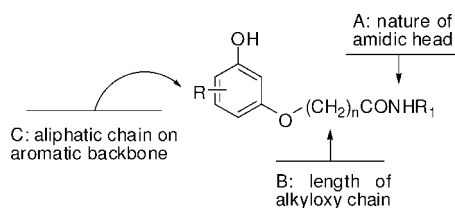


Figure 1. General structure of the synthesized compounds.

Chart 2. Proposed Pharmacophore Model for the Resorcinol–Anandamide “Hybrids”



observations have shown that both direct or indirect agonists and antagonists of cannabinoid receptors can produce beneficial effects, sometimes even in the same conditions, in agreement with the pleiotropic homeostatic function of this system.¹⁷

The use of marijuana preparations and phytocannabinoids for medicinal purposes has been limited because of their psychoactive properties, but in recent years a combination of THC and cannabidiol (CBD) from *Cannabis* extracts has been developed as an oromucosal spray for the treatment of neuropathic pain in multiple sclerosis and cancer. Several CB₁ receptor antagonists, i.e., SR141716¹⁸ (rimonabant¹⁹), have been developed to be used therapeutically for the treatment of obesity and associated metabolic disorders,²⁰ and they represent another example of endocannabinoid system-based drugs, although not without complications, since they seem to interfere with endocannabinoid-mediated adaptation to new stressful conditions. In fact, their chronic use in obese patients was associated with increased risk of developing signs of depression.²¹

On the basis of the pharmacophoric requirements²² of both AEA and THC for binding to cannabinoid CB₁ and CB₂ receptors, we have designed a novel pharmacophore (Chart 2) containing both a rigid backbone, as in THC, and a flexible chain, as in AEA, and developed a series of stable and potent ligands of cannabinoid receptors.^{23,24} Intriguingly, more recent studies²⁵ have shown that these compounds act as partial CB₁ agonists and CB₂ neutral antagonists. The results of cannabinoid receptor binding assays obtained with these derivatives, which can be considered as a new class of compounds with great affinity for cannabinoid receptors,²⁶ supplied us with useful information on their structure–activity relationships. The most critical requirements for the observation of high affinity appeared to be (a) the presence of a free phenolic hydroxy group, (b) the presence of an aliphatic chain of appropriate length on the aromatic ring, (c) the length of the alkyloxy chain carrying the amidic “head”, and (d) the nature of the amidic “head” (Chart 2).

On the ground of this pharmacophore model, in this paper we report the design and synthesis of twenty-one new derivatives, which maintain a free phenolic hydroxy group, an amidic “head” and a linear alkyloxy chain of 8, 11, or 12 carbon atoms, but contain modifications in the C portion, where an hydroxy group, a linear/branched alkyloxy chain, or a 1,1-dimethylheptyl aliphatic chain were introduced (Figure 1). These modifications were planned on the basis of the numerous reports showing that the lipophilic pentyl “tail” plays a pivotal role in determining

phytocannabinoid affinity and selectivity toward cannabinoid receptors, as well as their pharmacological potency,²⁷ suggesting that similar changes in the side chain of THC and the terminal alkyl position of AEA might lead to parallel changes in their biological activity.²⁸ Moreover, modifications of the side chain may give rise to high affinity compounds with either antagonist, partial agonist, or full agonist effects, while it is well-known that the branching of the side chain can increase functional potency.²⁹

Chemistry

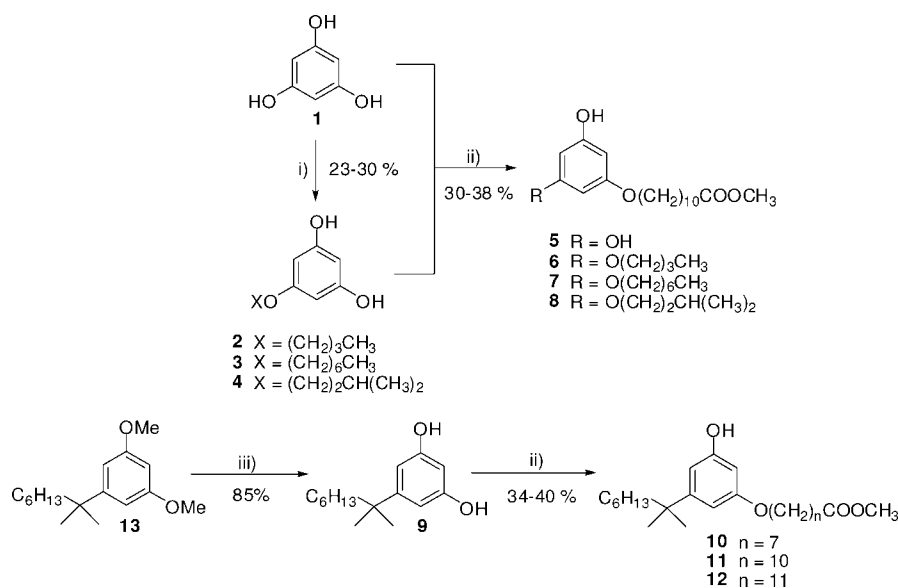
As shown in Scheme 1, the synthetic pathway started from phenols **1–4**, which were alkylated with 11-bromoundecanoic acid methyl ester in dry acetone in the presence of anhydrous potassium carbonate and potassium fluoride to furnish esters **5–8** in low yields (30–38%). Under the same conditions, 5-(2-methyloctan-2-yl)benzene-1,3-diol **9** reacted with the corresponding bromoalkyl methyl esters (8-bromooctanoic acid methyl ester, 11-bromoundecanoic acid methyl ester, and 12-bromododecanoic acid methyl ester) to give esters **10–12** in 34–40% yield.

5-Alkyloxybenzene-1,3-diols **2–4** were obtained from commercial phloroglucinol (or 1,3,5-trihydroxybenzene) **1**, which reacted in dry dimethyl sulfoxide (DMSO) with an alkyl bromide (1-bromobutane, 1-bromoheptane, and 1-bromo-3-methylbutane) in the presence of anhydrous potassium carbonate and potassium fluoride, while compound **9** was obtained with 85% yield by cleavage of the methoxy groups from 1,3-dimethoxy-5-(2-methyloctan-2-yl)benzene **13** using boron tribromide in dry dichloromethane.³⁰ As already described in a previous work,²³ O-alkylation of phenols afforded after column chromatography both monoalkylated and dialkylated products, although addition of potassium fluoride increased yields of the former over the latter, yields that were usually between 10% and 20%. Starting phenols were always recovered, too, by chromatographic purification of the crude reaction mixture. With the aim to improve the reaction yields, alkylation reactions were carried out using other solvents (*N,N*-dimethylformamide (DMF), DMSO, and acetone) and at different temperatures (reflux in acetone or at room temperature in DMF and DMSO) or by protracting the reaction time up to 120 h. However, regarding workup, reaction times, and yields, the best results were obtained using refluxing acetone for 48–72 h; because of its very low solubility, only phloroglucinol **1** was alkylated using DMSO as solvent.

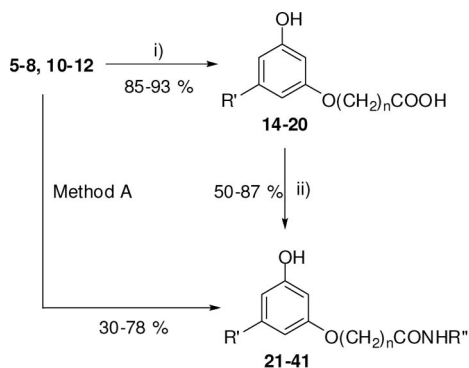
Final amides **21–41** were synthesized according to two methods (Scheme 2): (i) esters **5–8** and **10–12** were heated with redistilled ethanolamine as solvent to give the corresponding ethanolamides **21–27** in low (30%) to good (78%) yields (method A); (ii) acids **14–20**, obtained from hydrolysis of esters **5–8** and **10–12** with methanolic/aqueous sodium hydroxide solution in 85–93% yields, were reacted with the appropriate amines (cyclopropylamine, cyclopropanemethylamine, 3,4-dihydroxyphenethylamine hydrochloride, or 4-hydroxy-3-methoxybenzylamine hydrochloride) in the presence of 1-hydroxybenzotriazole (HOBt) and 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide methyl-*p*-toluenesulfonate (CMC) in dry dichloromethane or acetonitrile³¹ to provide the corresponding amides **28–41** in good yield (50–87%) (method B).

Biology

All the newly synthesized compounds that exhibited IC₅₀ ≤ 10 μM in the preliminary screening in radioligand binding assays for cannabinoid receptor were further evaluated for their affinity

Scheme 1. Synthesis of Intermediate Compounds 2–12^a

^a Reagents and conditions: (i) alkyl bromide, DMSO, K₂CO₃, KF, room temp, 48–72 h; (ii) appropriate bromoacid methyl ester, acetone, K₂CO₃, KF, room temp, 48–72 h; (iii) BBr₃, CH₂Cl₂, 0 °C to room temp.

Scheme 2. Synthesis of Final Amides 21–41^a

- 21 R' = OH, n = 10, R'' = CH₂CH₂OH
 22 R' = O(CH₂)₃CH₃, n = 10, R'' = CH₂CH₂OH
 23 R' = O(CH₂)₆CH₃, n = 10, R'' = CH₂CH₂OH
 24 R' = O(CH₂)₂CH(CH₃)₂, n = 10, R'' = CH₂CH₂OH
 25 R' = C(CH₃)₂C₆H₁₃, n = 7, R'' = CH₂CH₂OH
 26 R' = C(CH₃)₂C₆H₁₃, n = 10, R'' = CH₂CH₂OH
 27 R' = C(CH₃)₂C₆H₁₃, n = 11, R'' = CH₂CH₂OH
 28 R' = OH, n = 10, R'' = cC₃H₅
 29 R' = O(CH₂)₃CH₃, n = 10, R'' = cC₃H₅
 30 R' = O(CH₂)₆CH₃, n = 10, R'' = cC₃H₅
 31 R' = O(CH₂)₂CH(CH₃)₂, n = 10, R'' = cC₃H₅
 32 R' = C(CH₃)₂C₆H₁₃, n = 7, R'' = cC₃H₅
 33 R' = C(CH₃)₂C₆H₁₃, n = 10, R'' = cC₃H₅
 34 R' = C(CH₃)₂C₆H₁₃, n = 11, R'' = cC₃H₅
 35 R' = OH, n = 10, R'' = CH₂cC₃H₅
 36 R' = O(CH₂)₂CH(CH₃)₂, n = 10, R'' = CH₂cC₃H₅
 37 R' = C(CH₃)₂C₆H₁₃, n = 7, R'' = CH₂cC₃H₅
 38 R' = C(CH₃)₂C₆H₁₃, n = 10, R'' = CH₂cC₃H₅
 39 R' = C(CH₃)₂C₆H₁₃, n = 11, R'' = CH₂cC₃H₅
 40 R' = C(CH₃)₂C₆H₁₃, n = 10, R'' = 3,4-di-OH-phenethyl
 41 R' = C(CH₃)₂C₆H₁₃, n = 10, R'' = 3-OCH₃-4-OH-benzyl

^a Reagents and conditions. Method A: redistilled ethanolamine, 120–130 °C, 5 h. Method B: (i) MeOH/NaOH, reflux, 3 h; (ii) amine, HOBt, CMC, CH₃CN or CH₂Cl₂, room temp, overnight.

at recombinant human CB₁ and CB₂ receptors overexpressed in COS cells, and the results are summarized in Table 1. The preliminary screening was carried out using three concentrations (5, 10, and 25 μM) of each compound, membranes from HEK cells transfected with the human CB₁ or CB₂ receptor and [³H]-(-)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-

hydroxypropyl)cyclohexanol ([³H]CP-55,940) (*K*_d = 0.31 nM for CB₂ and 0.18 nM for CB₁ receptors) as the high affinity ligand as described by the manufacturer (Perkin-Elmer, Italia).³² Compounds that displaced [³H]CP-55,940 by more than 50% at 10 μM were further analyzed by carrying out a complete dose–response experiment. Displacement curves were generated by incubating drugs with [³H]CP-55,940 (0.084 μM for CB₂ and 0.14 nM for CB₁ binding assay). In all cases, *K*_i values were calculated by applying the Cheng–Prusoff equation to the IC₅₀ values (obtained by GraphPad) for the displacement of the bound radioligand by increasing concentrations of the test compounds. The antinociceptive activity of the compound with the highest affinity for cannabinoid receptors, i.e., **32**, was tested in mice treated with formalin, evaluating the effect of compound alone and in combination with SR141716A (rimonabant, a CB₁ antagonist) or AM630 (a CB₂ antagonist). A good agreement was observed between the cannabinoid receptor affinity of **32** and its effects in this *in vivo* assay.³³

Results and Discussion

CB₁ and CB₂ Receptors Affinity. With the exception of phloroglucinol derivatives (**21**, **28**, and **35**), that proved to be devoid of affinity for both the receptor subtypes, all the new compounds showed in the radioligand binding assays *K*_i values for either CB₁ or CB₂ receptor in the micromolar or submicromolar range. Analysis of the binding assay results for compounds **21–41** allowed us to assess with more accuracy the structure–activity relationships of this class of compounds, which can be summarized as follows. (a) In agreement with data from our previous study^{23,24} and the literature,³⁴ the cyclopropylamides (**29–34**) proved to be more potent than the corresponding ethanolamides (**22–27**), even though the insertion of a methylene group in the alkyl moiety of the amide head negatively affected affinity, the cyclopropylmethanilamides (**36–39**) being less potent than the corresponding cyclopropylamides (**29–34**). (b) In agreement again with our previous data, an aromatic group with a polar H-bond donor substituent in the amidic head caused a decrease in receptor affinity (**40**, **41**). (c) Replacement of the alkyl chain of olivetol compounds^{23,24} with a hydroxy group led to inactive compounds (**21**, **28**, **35**),

Table 1. Structure, CB₁ and CB₂ Receptor Affinity (K_i Values), and Selectivity of Derivatives **21–41** and Reference Compounds CB25, AEA, WIN 55,212-2, and HU-210^a

compd	<i>n</i>	R'	R''	CB ₁ K_i (μ M)	CB ₂ K_i (μ M)	CB ₁ /CB ₂ SI
21	10	OH	CH ₂ CH ₂ OH	na	na	
22	10	O(CH ₂) ₃ CH ₃	CH ₂ CH ₂ OH	2.41	1.62	1.5
23	10	O(CH ₂) ₆ CH ₃	CH ₂ CH ₂ OH	3.07	2.42	1.3
24	10	O(CH ₂) ₂ CH(CH ₃) ₂	CH ₂ CH ₂ OH	2.88	0.57	5.1
25	7	C(CH ₃) ₂ (CH ₂) ₅ CH ₃	CH ₂ CH ₂ OH	0.056	0.16	0.4
26	10	C(CH ₃) ₂ (CH ₂) ₅ CH ₃	CH ₂ CH ₂ OH	0.31	0.03	10.3
27	11	C(CH ₃) ₂ (CH ₂) ₅ CH ₃	CH ₂ CH ₂ OH	0.82	0.08	10.3
28	10	OH	<i>c</i> -C ₃ H ₅	na	na	
29	10	O(CH ₂) ₃ CH ₃	<i>c</i> -C ₃ H ₅	0.28	0.12	2.3
30	10	O(CH ₂) ₆ CH ₃	<i>c</i> -C ₃ H ₅	2.22	0.21	10.6
31	10	O(CH ₂) ₂ CH(CH ₃) ₂	<i>c</i> -C ₃ H ₅	0.22	0.13	1.7
32	7	C(CH ₃) ₂ (CH ₂) ₅ CH ₃	<i>c</i> -C ₃ H ₅	0.0056	0.0079	0.7
33	10	C(CH ₃) ₂ (CH ₂) ₅ CH ₃	<i>c</i> -C ₃ H ₅	0.021	0.0079	2.7
34	11	C(CH ₃) ₂ (CH ₂) ₅ CH ₃	<i>c</i> -C ₃ H ₅	0.17	0.02	8.5
35	10	OH	CH ₂ <i>c</i> -C ₃ H ₅	na	na	
36	10	O(CH ₂) ₂ CH(CH ₃) ₂	CH ₂ <i>c</i> -C ₃ H ₅	2.30	0.20	11.5
37	7	C(CH ₃) ₂ (CH ₂) ₅ CH ₃	CH ₂ <i>c</i> -C ₃ H ₅	0.10	0.034	2.9
38	10	C(CH ₃) ₂ (CH ₂) ₅ CH ₃	CH ₂ <i>c</i> -C ₃ H ₅	1.00	0.035	28.6
39	11	C(CH ₃) ₂ (CH ₂) ₅ CH ₃	CH ₂ <i>c</i> -C ₃ H ₅	5.40	0.60	9.0
40	10	C(CH ₃) ₂ (CH ₂) ₅ CH ₃	3,4-OH-phenethyl	3.4	5.0	0.7
41	10	C(CH ₃) ₂ (CH ₂) ₅ CH ₃	3-OCH ₃ -4-OH-benzyl	2.82	0.51	5.5
CB25	10	<i>n</i> -(CH ₂) ₄ CH ₃	<i>c</i> -C ₃ H ₅	0.0052	0.013	0.4
AEA				0.072		
WIN 55,212-2				0.021	0.0021	10.0
HU-210					0.15 × 10 ⁻³	

^a Data are mean values of $n = 3$ separate experiments and are expressed as K_i (μ M) for CB₁ and CB₂ binding assays. Reference compounds were tested under the same conditions in this study. Anandamide was tested in the presence of PMSF (100 nM). na = IC₅₀ > 10 in the preliminary screening carried out with rat brain and spleen membranes. Binding affinity constants of the most potent compounds ($K_i \leq 1 \mu$ M) are in bold as well as the most selective compounds for CB₁ and CB₂. Standard errors are not shown for the sake of simplicity and were never higher than 10% of the mean values.

further supporting the importance of the chain in determining cannabinoid receptor affinity. (d) Accordingly, introduction of a short alkoxy chain led to still active but less potent compounds (**22–24**, **29–31**, and **36**). (e) Very potent compounds were obtained by introduction of a 1,1-dimethylheptyl chain, which improved the CB₂ binding affinity compared to that of the olivetol analogues. Particularly, compounds **32** and **33** proved to be the most potent CB₁ (K_i of 5.6 and 21 nM, respectively) and CB₂ ligands ($K_i = 7.9$ nM for both compounds), with affinity constants comparable to those of WIN 55,212-2 (K_i (CB₁) = 21 nM and K_i (CB₂) = 2.1 nM) and our “lead compound” CB25 (K_i (CB₁) = 5.2 nM and K_i (CB₂) = 13 nM). (f) Some compounds, although less potent, showed selectivity for CB₂ receptors, in particular, compound **38** (K_i (CB₁) = 1000 nM and K_i (CB₂) = 35 nM), which still elicited a nanomolar affinity for CB₂ receptor and a selectivity index (SI), calculated as K_i (CB₁)/ K_i (CB₂), of 28.6. (g) Moreover, in the 1,1-dimethylheptyl series, receptor subtype selectivity was only exhibited when the methylene linker length was 10 or 11 carbon atoms.

Antinociceptive Activity in Mice. As further evidence that the new compounds likely behave in vivo as CB₁ receptor agonists, we found that the most potent compound described in the present study, i.e., **32** (denoted as CB86), exhibited potent antinociceptive effects against the second phase of the nociceptive response to formalin in mice (Figure 2). The compound was already maximally active at 1 mg/kg, ip, and its effect was fully antagonized by the CB₁ receptor antagonist rimonabant but not by the CB₂ receptor antagonist AM630. These observations are in agreement with our previous findings that potent compounds of similar structure act as CB₁ agonists or partial agonists and as neutral CB₂ antagonists in vivo.²⁵ Compound

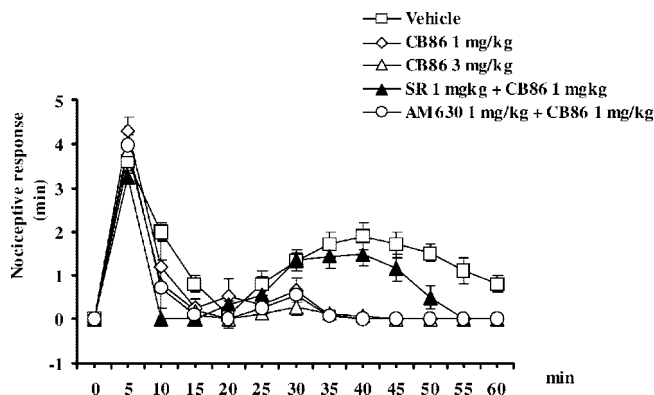


Figure 2. Antinociceptive effect of vehicle (10% DMSO in 0.9% NaCl) or of compound **32**, here denoted as CB86, in the formalin test in mice. Each point represents the mean \pm standard error of the mean (SEM) of 8–10 animals per group. Data were analyzed using the one-way ANOVA followed by the Bonferroni's test, and statistical significance was taken as $P < 0.05$. The effects of the CB₁ antagonist rimonabant (SR) and the CB₂ antagonist AM630 are also shown. Rimonabant reversed in a statistically significant manner the effect of the new compound on the second phase of the nociceptive response to formalin at all times between 30 and 45 min.

32, however, appeared to be more efficacious than previously reported compounds, possibly because of pharmacokinetic factors.

Molecular Modeling. A homology model of CB₁ and CB₂ receptors³⁵ and the hypothetical binding interactions of AEA into both receptors have been already described. In the CB₁ receptor model, AEA adopted a U-shaped molecular conformation placed among TM2-3-6-7 with the aliphatic chain directed toward the intracellular side of the receptor. In this disposition

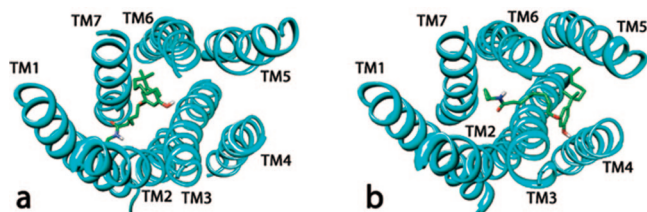


Figure 3. Compound **32** docked into the CB₁ (a) and CB₂ (b) receptor models (extracellular point of view).

the amide oxygen atom of AEA interacted with K3.28(192) and the hydroxy group formed a H bond with S7.39(383). In the CB₂ receptor, AEA was placed among TM3-4-5-6 with the aliphatic chain that interacted principally with W5.43 and W6.48. In this disposition the ligand did not interact with K3.28; it formed a H-bond with S3.31 through the amide oxygen atom, and the hydroxy group interacted with the oxygen backbone of L3.27.³⁵ The hypothetical binding disposition of AEA into both receptors was supported by the main mutagenesis data, which suggested an important role for K3.28 in the CB₁ and S3.31 in the CB₂ for the AEA interaction.³⁶

The direct interaction of AEA with K3.28 is supported by the work of Song and Bonner^{36b} who found that the endocannabinoid was unable to compete for [³H]WIN55,212-2 binding in a human CB₁ K3.28(192)A mutant and that its potency in inhibiting cAMP accumulation was reduced by more than 100-fold in this mutant. Moreover, the direct interaction of AEA with K3.28 is also supported by the CB₁-AEA binding hypothesis of McAllister and co-workers.³⁷

Figure 3 shows from an extracellular point of view the disposition of compound **32** into both CB₁ and CB₂ receptors. This compound is the most CB₁ and CB₂ active ligand among those reported.

In agreement with our previous studies,²⁴ in the CB₁ receptor model compound **32** was placed between TM1-2-3-6-7 with the carboxamide group directed towards TM1-2 and the phenolic group directed toward the extracellular side of TM3-6. As shown in Figure 4a, in the CB₁ receptor the phenolic substituent of compound **32** formed a H-bond with K3.28 and the 1,1-dimethylheptyl group showed lipophilic interactions with F3.25, M6.55, V6.59, and F7.35. The heptamethylene chain at position 1 was stabilized through the interaction with F2.57, V3.32, F3.36, and L7.43, the carboxamide group formed two H-bonds with T1.46 and S2.54, and the cyclopropyl substituent was inserted in a small lipophilic pocket mainly delimited by G1.43, V2.58, and L7.43. In the CB₂ receptor compound **32** showed a completely different binding mode; it was placed between TM3-4-5-6-7 with the phenolic group directed toward TM4-5 and the carboxamide group directed toward TM7 (see Figure 3b). As shown in Figure 4d, the phenolic substituent formed two H-bonds with S3.31 and the backbone of S4.57 and the 1,1-dimethylheptyl group established lipophilic interactions with W5.43 and F5.46. The alkyloxy chain at position 1 was stabilized through the interaction with L3.27, M6.55, and L6.59, the nitrogen atom of the carboxamide group formed a H-bond with S6.58 and the cyclopropyl substituent was stabilized by lipophilic interactions with I3.29 and L6.54.

The replacement of the heptamethylene chain with the decamethylene chain (compound **33**) caused only a slight decrease of CB₁ affinity, and the CB₂ affinity remained the same. As shown in Figure 4b and Figure 4e, all the main interactions already reported for compound **32** were maintained into both receptors. In particular, in the CB₁ receptor model compound **33** formed the three H-bonds with T1.46, S2.54, and K3.28 and

the 1,1-dimethylheptyl substituent was stabilized by the interaction with F3.25, M6.55, V6.59, and F7.35. In the CB₂ receptor the ligand formed the three H-bonds with S3.31, S4.57, and S6.58 and the 1,1-dimethylheptyl group interacted with W5.43 and F5.46. Thus, with both cannabinoid receptors, compound **33** showed the same interactions as compound **32**, in agreement with the experimental data, which highlighted an identical (CB₂) or very similar (CB₁) affinity of the two compounds for the two receptors.

Compound **38** was one of the most interesting compounds among those reported herein. It differed from compound **33** for the methylcyclopropylamide substitution and it possessed a good CB₂ affinity and a micromolar CB₁ affinity, resulting in a high CB₂ selectivity (CB₁/CB₂ = 28.6). In agreement with these data, in the CB₂ receptor this compound possessed all the main interactions showed also by compounds **32** and **33** (see Figure 4f), including the three H-bonds and the main lipophilic interactions. In the CB₁ receptor the methylcyclopropyl group was not able to interact with the small lipophilic pocket delimited by G1.43, V2.58, and L7.43. As shown in Figure 4c, the different arrangement of this substituent determined the loss of the two H-bonds of the carboxamide group with T1.46 and S2.54, thus explaining the low CB₁ affinity of this compound.

Taken together, all these results suggest that lipophilicity may play an important role in the CB affinity of analyzed compounds. To further investigate this aspect, lipophilic properties of the R' side chain and R'' amidic group, expressed as octanol/water partition coefficients (QlogP_{ow}), have been correlated with ligand potencies, expressed as pK_i(CB₁) or pK_i(CB₂) (see Table 6 and Figure 1 of Supporting Information). Results indicate some interesting correlations between CB₁ and CB₂ receptor affinities and the lipophilic nature of the R' aliphatic side chain, whereas affinity at either cannabinoid receptor does not seem to be affected by the more or less lipophilic character of the R'' amidic head.

Conclusions

In this study, 21 novel resorcinol-anandamide "hybrids" were designed and synthesized with the aim to better identify the structural modifications that influence either affinity or selectivity for cannabinoid receptors within this class of compounds, obtained by linking a rigid aromatic structure such as that of THC to a flexible chain carrying an amidic "head", mimicking that present in AEA. Many of the synthesized compounds exhibited high affinity for CB₁ and CB₂ receptors, and some of them, with the exception of those with the lowest K_i values for both receptors, exhibited also a reasonable CB₂ selectivity. Moreover, our results confirmed the pivotal role played by the aliphatic chain of the C region; its removal or shortening led to compounds that are inactive or less potent in the binding assays, whereas introduction of a 1,1-dimethylheptyl tail led to some compounds that are strong CB₂ ligands, still retaining high CB₁ affinity, confirming that changing the length and branching of the side pentyl chain can increase both potency and selectivity. Also, the nature and size of the amidic "head" significantly affected the ability of these hybrids to bind cannabinoid receptors. In fact, introduction of a polar H-bond donor substituent in both aliphatic and aromatic amines negatively affected the cannabinoid receptor affinities. These results are a significant starting point to define the structural requirements for the design of a second generation series of highly CB₁ or CB₂ selective ligands.

Interestingly, these resorcinol-anandamide "hybrids" acted in vivo as CB₁ agonists or partial CB₁ agonists, showing a potent

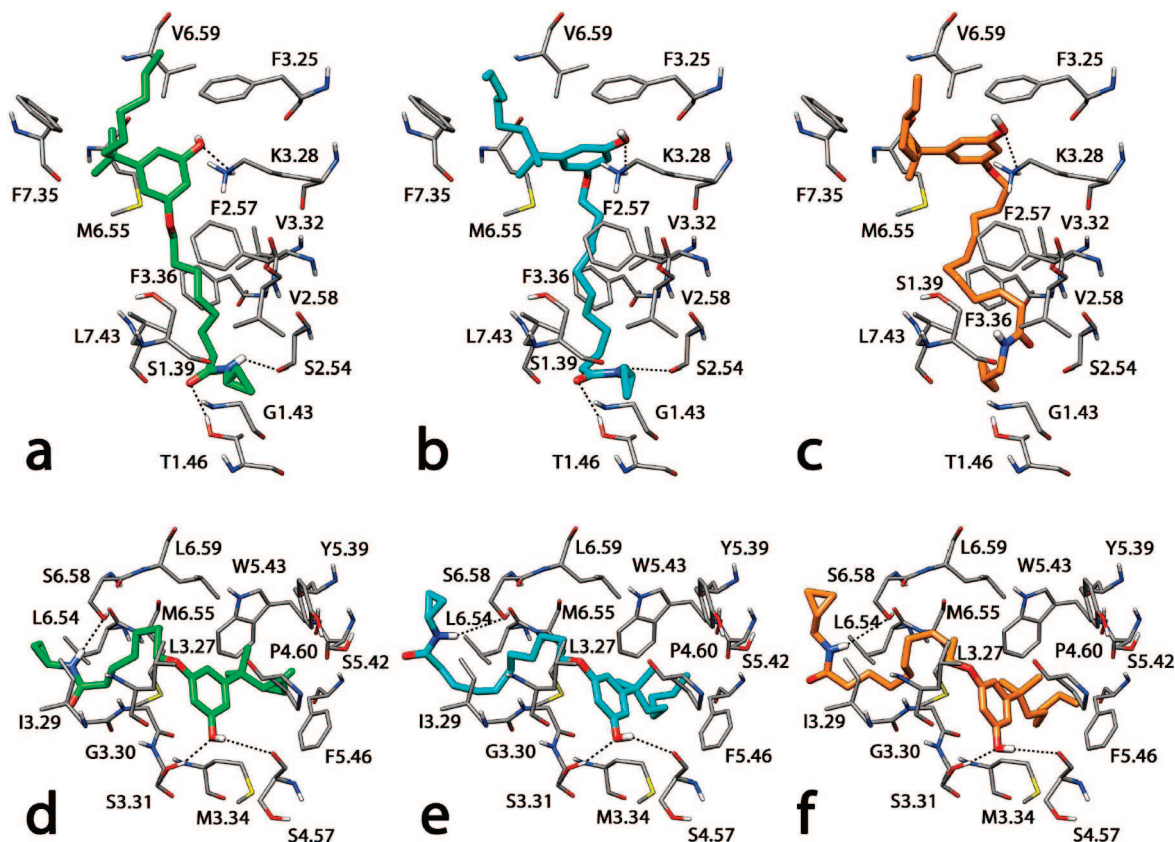


Figure 4. Compounds **32** (a, d), **33** (b, e), and **38** (c, f) docked into the CB₁ (a–c) and CB₂ (d–f) receptor models.

antinociceptive effect fully antagonized only by the CB₁ receptor antagonist/inverse agonist rimonabant but not by the CB₂ receptor antagonist AM630. This is in agreement with our previous report that compounds in this series behave in vitro mostly as partial CB₁ agonists and neutral CB₂ antagonists.²⁵

Furthermore, molecular modeling studies on the new compounds provided a reasonable hypothesis of their interaction mode with both CB₁ and CB₂ receptor binding sites. As already observed for AEA, the docking study suggested a completely different disposition of the compounds within the two receptors.³⁵ In the CB₁ receptor model, the carboxamide group of the most active compounds was directed toward TM1–2, the phenolic group was directed toward the extracellular side of TM3–6, and residues T1.46, S2.54, and K3.28 seemed to possess a key role because they formed H-bonds with the most active ligands. In the CB₂ receptor model, the analyzed compounds were placed between TM3–4–5–6–7 with the phenolic group directed toward TM4–5 and the carboxamide group directed toward TM7 and the nonconserved S3.31 and the backbone of S4.57 that formed two H-bonds with the ligands. Finally, an interesting correlation between the lipophilic nature of the aliphatic side chain and cannabinoid receptor potencies of the analyzed compounds was evidenced.

Experimental Section

Chemistry. All starting materials, reagents, and solvents were purchased from common commercial suppliers and were used as received unless otherwise indicated. Organic solutions were dried over anhydrous sodium sulfate and concentrated with a Büchi rotary evaporator R-110 equipped with a KNF N 820 FT 18 vacuum pump. Melting points were determined on a Kofler hot stage apparatus (K) or using a Mettler FPI apparatus (2 °C/min, M) and are uncorrected. Elemental analyses of all synthesized compounds were performed by our analytical laboratory using a Perkin-Elmer

elemental apparatus model 240 for C, H, N, and the data are within $\pm 0.4\%$ of the theoretical values. Proton nuclear magnetic resonance (¹H NMR) spectra were recorded in the indicated solvent at 25 °C on a Bruker AC200F employing TMS as internal standard, and chemical shifts are expressed as δ (ppm). Mass spectral data were determined by direct insertion at 70 eV with a VG70 spectrometer. All compounds were checked for purity by TLC on Merck 60 F₂₅₄ silica plates. For column chromatography, Merck 60 silica gel, 230–400 mesh, was used. Final products were purified by a Biotage flash chromatography system with 12.25 mm columns, packed with KP-Sil, 60A, 32–63 μ m.

General Procedure for the Synthesis of Ethers 2–4. A mixture of phloroglucinol **1** (0.63 g, 5.0 mmol), anhydrous potassium carbonate (0.35 g, 2.5 mmol), and potassium fluoride (0.29 g, 5.0 mmol) in dry DMSO (4 mL) was stirred for 30 min at room temperature under nitrogen atmosphere, and then a solution of the appropriate alkyl halide (5.0 mmol) in dry DMSO (2 mL) was added. After being stirred for a further 48–72 h, the reaction mixture was diluted with water (10 mL), neutralized by addition of diluted HCl, and extracted with chloroform. The organic layer was washed with brine, dried, and concentrated, and the residue was purified by silica gel column chromatography.

Example. 5-Butoxy-1,3-dihydroxybenzene (2). Eluent: CHCl₃/MeOH, 50/1. Yield: 30% (pasty yellow solid). ¹H NMR (CDCl₃): δ 6.84 (br s, 2H, disappears on treatment with D₂O), 5.98–5.94 (m, 3H), 3.67 (t, 2H, J = 6.4 Hz), 1.70–1.56 (m, 2H), 1.45–1.30 (m, 2H), 0.88 (t, 3H, J = 7.3 Hz). Anal. (C₁₀H₁₄O₃ (182.22)) C, H, N.

General Procedure for the Synthesis of Esters 5–8, 10–12. A mixture of the appropriate diphenol derivatives **2–4**, **9** (5.0 mmol), anhydrous potassium carbonate (0.35 g, 2.5 mmol), and potassium fluoride (0.29 g, 5.0 mmol) in dry acetone (20 mL) was refluxed under stirring and nitrogen atmosphere for 30 min. Then a solution of the appropriate bromoalkanoic acid methyl ester (5.0 mmol) in dry acetone (10 mL) was added, and the reaction mixture was kept at reflux temperature for a further 48–72 h. Afterward,

the reaction mixture was concentrated, diluted with saturated ammonium chloride solution (20 mL), and extracted with chloroform. The organic layers were collected, dried, and evaporated under reduced pressure to afford a residue, which was purified by silica gel column chromatography.

Example. 8-[3-Hydroxy-5-(2-methyloctan-2-yl)phenoxy]octanoic Acid Methyl Ester (10). Eluent: $\text{CHCl}_3/\text{MeOH}$, 100/0.5. Yield: 40% (pale-yellow oil). ^1H NMR (CDCl_3): δ 6.43 (s, 1H), 6.36 (d, 1H, $J = 1.5$ Hz), 6.21 (d, 1H, $J = 1.9$ Hz), 4.83 (s, 1H, disappears on treatment with D_2O), 3.89 (t, 2H, $J = 6.3$ Hz), 3.65 (s, 3H), 2.29 (t, 2H, $J = 7.3$ Hz), 1.77–1.45 (mm, 8H), 1.29–1.06 (mm, 24H), 0.82 (t, 3H, $J = 5.9$ Hz). MS m/z : 415 $[\text{M} + \text{Na}]^+$ (100). Anal. ($\text{C}_{24}\text{H}_{40}\text{O}_4$ (392.57)) C, H, N.

General Procedure for the Synthesis of Acids 14–20. A solution of the appropriate methyl esters **5–8**, **10–12** (2.0 mmol) in methanol (6 mL) and 3 N aqueous NaOH (2 mL, 6 mmol) was refluxed for 3 h. Then the reaction mixture was cooled in an ice bath and acidified to pH 3 with diluted HCl. The aqueous layer was extracted several times with ethyl acetate, and the collected organic solution was dried and evaporated to yield the crude acid, which was purified by silica gel column chromatography.

Example. 8-[5-(2-Methyloctan-2-yl)phenoxy]octanoic Acid (18). Eluent: $\text{CHCl}_3/\text{MeOH}$, 50/1. Yield: 80% (white solid), mp 111.1 °C (M). ^1H NMR (CDCl_3): δ 6.45 (s, 1H), 6.37 (d, 1H, $J = 1.6$ Hz), 6.21 (d, 1H, $J = 1.7$ Hz), 3.90 (t, 2H, $J = 6.6$ Hz), 2.36 (t, 2H, $J = 7.1$ Hz), 1.76–1.61 (mm, 4H), 1.56–1.40 (mm, 8H), 1.37–1.09 (mm, 14H), 0.82 (t, 3H, $J = 6.1$ Hz). MS m/z : 401 $[\text{M} + \text{Na}]^+$, 779 $[2\text{M} + \text{Na}]^+$ (100). Anal. ($\text{C}_{23}\text{H}_{38}\text{O}_4$ (378.55)) C, H, N.

General Procedure for the Synthesis of Ethanolamides 21–27. Method A. Esters **5–8**, **10–12** (2.0 mmol) were dissolved under nitrogen atmosphere and continuous stirring in redistilled ethanolamine (4 mL), and the solution was heated at 120–130 °C for 4 h. The reaction mixture was diluted with water, neutralized with diluted HCl, and extracted with chloroform. The collected extracts were washed with a solution of saturated ammonium chloride, dried, and evaporated, and the raw material so obtained was purified by silica gel column chromatography.

Example. 11-(3,5-Dihydroxyphenoxy)undecanoic Acid (2-Hydroxyethyl)amide (21). Eluent: $\text{CHCl}_3/\text{MeOH}$, 50/1. Yield 30% (pale-yellow solid), mp 125–130 °C (K). ^1H NMR (CDCl_3): δ 5.98–5.91 (m, 3H), 3.85 (t, 2H, $J = 6.2$ Hz), 3.54 (t, 2H, $J = 5.6$ Hz), 3.31–3.22 (m, 2H), 2.16 (t, 2H, $J = 7.8$ Hz), 1.69–1.54 (m, 4H), 1.48–1.29 (mm, 12H). MS m/z : 376 $[\text{M} + \text{Na}]^+$ (100). Anal. ($\text{C}_{19}\text{H}_{31}\text{NO}_5$ (353.45)) C, H, N.

General Procedure for the Synthesis of Amides 28–41. Method B. To a mixture of acids **14–20** (1.0 mmol), the appropriate amine (1.5 mmol), and 1-hydroxybenzotriazole (HOBt, 1.2 mmol) in dry dichloromethane (10 mL) kept at 0 °C in an ice bath, a solution in the same solvent (5 mL) of 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide methyl-*p*-toluenesulfonate (CMC, 1.5 mmol) was added dropwise under nitrogen atmosphere and continuous stirring. The solution was allowed to warm to room temperature, and stirring was continued for 24 h. The organic solution was washed with 5% aqueous NaHCO_3 , then with 1 N HCl, and dried. After evaporation of solvent, the crude product was purified by silica gel column chromatography.

Example. 8-[5-(2-Methyloctan-2-yl)phenoxy]octanoic Acid Cyclopropylamide (32). Eluent: $\text{CHCl}_3/\text{MeOH}$, 50/1. Yield 81% (pale-yellow oil). ^1H NMR (CDCl_3): δ 6.48–6.41 (m, 2H), 6.29–6.27 (m, 1H), 6.06 (br s, 1H), 5.61 (br s, 1H), 3.92 (t, 2H, $J = 6.7$ Hz), 2.75–2.66 (m, 1H), 2.14 (t, 2H, $J = 7.2$ Hz), 1.78–1.52 (mm, 6H), 1.48–1.09 (mm, 20H), 0.83 (t, 3H, $J = 6.2$ Hz), 0.79–0.73 (m, 2H), 0.57–0.43 (m, 2H). MS m/z : 440 $[\text{M} + \text{Na}]^+$, 857 $[2\text{M} + \text{Na}]^+$ (100). Anal. ($\text{C}_{26}\text{H}_{43}\text{NO}_3$ (417.62)) C, H, N.

Evaluation of Antinociceptive Activity in Mice. Formalin injection induces a biphasic stereotypical nocifensive behavior.³³ Nociceptive responses are divided into an early, short lasting first phase (0–7 min) caused by a primary afferent discharge produced by the stimulus, followed by a quiescent period and then a second, prolonged phase (15–60 min) of tonic pain. Mice received formalin (1.25% in saline, 30 μL) in the dorsal surface of one side of the

hind paw. Each mouse was randomly assigned to one of the experimental groups ($n = 8$ –10) and placed in a Plexiglas cage and allowed to move freely for 15–20 min. A mirror was placed at a 45° angle under the cage to allow full view of the hind paws. Lifting, favoring, licking, shaking, and flinching of the injected paw were recorded as nociceptive responses.³⁸ The total time of the nociceptive response was measured every 5 min and expressed as the total time of the nociceptive responses in minutes (mean \pm SEM). Recording of nociceptive behavior commenced immediately after formalin injection and was continued for 60 min. The version of the formalin test we applied is based on the fact that a correlational analysis showed that no single behavioral measure can be a strong predictor of formalin or drug concentrations on spontaneous behaviors.^{38,39} Consistently, we considered that a simple sum of time spent licking plus elevating the paw, or the weighted pain score,³³ is in fact superior to any single (lifting, favoring, licking, shaking, and flinching) measure (r ranging from 0.75 to 0.86³⁷). Treatments were as follows: groups of 8–10 animals per treatment were used, with each animal being used for one treatment only. Mice received intraperitoneal administration of vehicle (10% DMSO in saline) or two doses of compound **32** (1 or 3 mg/kg, ip) alone or compound **32** (1 mg/kg, ip) in combination with rimonabant (1 mg/kg, ip), a selective CB_1 receptor antagonist, or AM630 (1 mg/kg, ip), a selective CB_2 receptor antagonist. Compound **32** was administered 15 min before the peripheral injection of formalin. The CB_1 or CB_2 antagonists were administered 5 min before compound **32**.

Molecular Modeling. The ligands were submitted to a conformational search of 1000 steps. The algorithm used was the Monte Carlo method with MMFFs as the force field and a distance-dependent dielectric constant of 1.0. The ligands were then minimized using the conjugated gradient method until a convergence value of 0.05 kcal/(Å mol) was obtained, using the same force field and dielectric constant used for the conformational search.⁴⁰ Then the ligand was docked into CB_1 and CB_2 receptor using the AUTODOCK 3.0 program.⁴¹ AUTODOCK TOOLS⁴² was used to identify the torsion angles in the ligands, to add the solvent model, and to assign partial atomic charges (Gasteiger for the ligands and Kollman for the receptors). The regions of interest used by AUTODOCK were defined by considering for both CB_1 and CB_2 receptors T3.33 as the central residue of a grid of 60, 46, and 50 points in the x , y , and z directions. A grid spacing of 0.375 Å and a distance-dependent function of the dielectric constant were used for the energetic map calculations.

By use of the Lamarckian genetic algorithm, all docked compounds were subjected to 250 runs of the AUTODOCK search in which the default values of the other parameters were used. Cluster analysis was performed on the docked results, using an rms tolerance of 1.0 Å.

Acknowledgment. The authors from the University of Siena thank the Ministero dell'Università e della Ricerca (PRIN 2006, Prot. n 2006030948_002) for financial support. Molecular graphics images were produced using the UCSF Chimera package from the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco (supported by NIH Grant P41 RR-01081). The authors are grateful to Marco Allarà for technical assistance with the binding assays.

Supporting Information Available: Physical and spectral data for compounds **3–12**, **14–20**, and **22–41** and elemental analysis data for compounds **21–41**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

References

- (1) (a) Goutopoulos, A.; Makriyannis, A. From cannabis to cannabinergics: new therapeutic opportunities. *Pharmacol. Ther.* **2002**, *95*, 103–117. (b) Di Marzo, V.; Bifulco, M.; De Petrocellis, L. The endocannabinoid system and its therapeutic exploitation. *Nat. Rev. Drug Discovery* **2004**, *3*, 771–784. (c) Lambert, D. M.; Fowler, C. J. The endocannabinoid system: drug targets, lead compounds, and potential therapeutic

- applications. *J. Med. Chem.* **2005**, *48*, 5059–5087. (d) Di Marzo, V.; De Petrocellis, L. Plant, synthetic, and endogenous cannabinoids in medicine. *Ann. Rev. Med.* **2006**, *57*, 553–574. (e) Di Marzo, V. A brief history of cannabinoid and endocannabinoid pharmacology as inspired by the work of British scientists. *Trends Pharmacol. Sci.* **2006**, *27*, 134–140.
- (2) Abood, M. E.; Martin, B. R. Neurobiology of marijuana abuse. *Trends Pharmacol. Sci.* **1992**, *13*, 201–206.
 - (3) Devane, W. A.; Dysarz, F. A.; Johnson, M. R.; Melvin, L. S.; Howlett, C. A. Determination and characterization of a cannabinoid receptor in rat brain. *Mol. Pharmacol.* **1988**, *34*, 605–613.
 - (4) Herkenham, M. Localisation of Cannabinoid Receptors in Brain and Periphery. In *Cannabinoid Receptors*; Pertwee, R. G., Ed.; Academic Press: New York, 1995; pp 145–166.
 - (5) Munro, S.; Thomas, K. L.; Abu-Shaar, M. Molecular characterization of a peripheral receptor for cannabinoids. *Nature (London)* **1993**, *365*, 61–65.
 - (6) Walter, L.; Franklin, A.; Witting, A.; Wade, C.; Xie, Y.; Kunos, G.; Mackie, K.; Stella, N. Nonpsychotropic cannabinoid receptors regulate microglial cell migration. *J. Neurosci.* **2003**, *23*, 1398–1405.
 - (7) Benito, C.; Nunez, E.; Tolon, R. M.; Carrier, E. J.; Rabano, A.; Hillard, C. J.; Romero, J. Cannabinoid CB₂ receptors and fatty acid amide hydrolase are selectively overexpressed in neuritic plaque-associated glia in Alzheimer's disease brain. *J. Neurosci.* **2003**, *23*, 11136–11141.
 - (8) Devane, W. A.; Hanus, L.; Breuer, A.; Pertwee, R. G.; Stevenson, L. A.; Griffin, G.; Gibson, D.; Mandelbaum, A.; Etinger, A.; Mechoulam, R. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* **1992**, *258*, 1946–1949.
 - (9) Mechoulam, R.; Ben-Shabat, S.; Hanus, L.; Ligumsky, M.; Kaminski, N. E.; Schatz, A. R.; Gopher, A.; Almog, S.; Martin, B. R.; Compton, R. B.; Pertwee, R. G.; Griffin, G.; Bayewitch, M.; Barg, J.; Vogel, Z. Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem. Pharmacol.* **1995**, *50*, 83–90.
 - (10) Lee, M.; Yang, K. H.; Kaminski, N. E. Effects of putative cannabinoid receptor ligands, anandamide and 2-arachidonoyl-glycerol, on immune function in B6C3F1 mouse splenocytes. *J. Pharmacol. Exp.* **1995**, *275*, 529–536.
 - (11) Sugiura, T.; Kondo, S.; Sukagawa, A.; Nakane, S.; Shinoda, A.; Itoh, K.; Yamashita, A.; Waku, K. 2-Arachidonoyl-glycerol: a possible endogenous cannabinoid receptor ligand in brain. *Biochem. Biophys. Res. Commun.* **1995**, *215*, 89–97.
 - (12) Beltramo, M.; Stella, N.; Calignano, A.; Lin, S. Y.; Makriyannis, A.; Piomelli, D. Functional role of high-affinity anandamide transport, as revealed by selective inhibition. *Science* **1997**, *277*, 1094–1097.
 - (13) Cravatt, B. F.; Giang, D. K.; Mayfield, S. P.; Boger, D. L.; Lerner, R. A.; Gilula, N. B. Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. *Nature (London)* **1996**, *384*, 83–87.
 - (14) Di Marzo, V.; Petrosino, S. Endocannabinoids and the regulation of their levels in health and disease. *Curr. Opin. Lipidol.* **2007**, *18*, 129–140.
 - (15) Pacher, P.; Batkai, S.; Kunos, G. The endocannabinoid system as an emerging target of pharmacotherapy. *Pharmacol. Rev.* **2006**, *58*, 389–462.
 - (16) (a) Svíženská, I.; Dubový, P.; Šalcová, A. Cannabinoid receptors 1 and 2 (CB1 and CB2), their distribution, ligands and functional involvement in nervous system structures. A short review. *Pharmacol., Biochem. Behav.* **2008**, *90*, 501–511. (b) Micale, V.; Mazzola, C.; Drago, F. Endocannabinoids and neurodegenerative diseases. *Pharmacol. Res.* **2007**, *56*, 382–392.
 - (17) Di Marzo, V. Targeting the endocannabinoid system: to enhance or reduce? *Nat. Rev. Drug Discovery* **2008**, *7*, 438–455.
 - (18) Rinaldi-Carmona, M.; Barth, F.; Héaulme, M.; Shire, D.; Calandra, B.; Congy, C.; Martinez, S.; Maruani, J.; Nélat, G.; Caput, D.; Ferrara, P.; Soubrié, P.; Breliere, J. C.; Le Fur, G. SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. *FEBS Lett.* **1994**, *350*, 240–244.
 - (19) (a) Barth, F.; Casellas, P.; Congy, C.; Martinez, S.; Rinaldi, M.; Anne-Archard, G. Preparation of N-Piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methylpyrazole-3-carboxamide. Eur. Patent EP-656354, June 7, 1995; 1–12. (b) Barth, F.; Casellas, P.; Congy, C.; Martinez, S.; Rinaldi, M.; Anne-Archard, G. U.S. Patent 5,624,941, 1997.
 - (20) (a) Van Gaal, L.; Pi-Sunyer, X.; Despres, J. P.; McCarthy, C.; Scheen, A. Efficacy and safety of rimonabant for improvement of multiple cardiometabolic risk factors in overweight/obese patients: pooled 1-year data from the Rimonabant in Obesity (RIO) program. *Diabetes Care* **2008**, *31*, S229–S240. (b) Addy, C.; Li, S.; Agrawal, N.; Stone, J.; Majumdar, A.; Zhong, L.; Li, H.; Yuan, J.; Maes, A.; Rothemberg, P. Safety, tolerability, pharmacokinetics, and pharmacodynamic properties of taranabant, a novel selective cannabinoid-1 receptor inverse agonist, for the treatment of obesity: results from a double-blind, placebo-controlled, single oral dose study in healthy volunteers. *J. Clin. Pharmacol.* **2008**, *48*, 418–427.
 - (21) (a) Marketing authorization of European Medicines Agency (EMA) valid throughout the European Union: June 19, 2006. European Public Assessment Report (EPAR). Acomplia. Protocol EMEA/H/C/666. (b) Gelfand, E. V.; Cannon, C. P. Rimonabant: a selective blocker of the cannabinoid CB1 receptors for the management of obesity, smoking cessation and cardiometabolic risk factors. *Expert Opin. Invest. Drugs* **2006**, *15*, 307–315. (c) Sorbera, L. A.; Castaner, J.; Silvestre, J. S. Rimonabant hydrochloride: antiobesity drug, aid to smoking cessation, treatment of alcohol dependency, cannabinoid CB1 antagonist. *Drugs Future* **2005**, *30*, 128–137.
 - (22) (a) Thomas, B. F.; Adams, I. B.; Mascarella, S. W.; Martin, B. R.; Razdan, R. K. Structure–activity analysis of anandamide analogs: relationship to a cannabinoid pharmacophore. *J. Med. Chem.* **1996**, *39*, 471–479. (b) Tong, W.; Collantes, E. R.; Welsh, W. J. Derivation of a pharmacophore model for anandamide using constrained conformational searching and comparative molecular field analysis. *J. Med. Chem.* **1998**, *41*, 4207–4215. (c) Reggio, P. H.; Traore, H. Conformational requirements for endocannabinoid interaction with the cannabinoid receptors, the anandamide transporter and fatty acid amidohydrolase. *Chem. Phys. Lipids* **2000**, *108*, 15–35. (d) Bonechi, C.; Brizzi, A.; Brizzi, V.; Donati, A.; Francioli, M.; Rossi, C. Conformational analysis of the N-arachidonyl ethanolamide (anandamide) using nuclear magnetic resonance and theoretical calculations. *Nuclear Magn. Reson. Chem.* **2001**, *39*, 432–437.
 - (23) Brizzi, A.; Brizzi, V.; Cascio, M. G.; Bisogno, T.; Siriani, R.; Di Marzo, V. Design, synthesis and binding studies of new potent ligands of cannabinoid receptors. *J. Med. Chem.* **2005**, *48*, 7343–7350.
 - (24) Brizzi, A.; Cascio, M. G.; Brizzi, V.; Bisogno, T.; Dinatolo, M. T.; Martinelli, A.; Tuccinardi, T.; Di Marzo, V. Design, synthesis, binding, and molecular modeling studies of new potent ligands of cannabinoid receptors. *Bioorg. Med. Chem.* **2007**, *15*, 5406–5416.
 - (25) Cascio, M. G.; Bisogno, T.; Palazzo, E.; Thomas, A.; Van der Stelt, M.; Brizzi, A.; De Novellis, V.; Marabese, I.; Ross, R.; Van de Doelen, T.; Brizzi, V.; Pertwee, R.; Maione, S.; Di Marzo, V. In vitro and in vivo pharmacology of synthetic olivetol- or resorcinol-derived cannabinoid receptor ligands. *Br. J. Pharmacol.* **2006**, *149*, 431–440.
 - (26) Brizzi, A.; Brizzi, V.; Siriani, R.; Di Marzo, V.; Cascio, M. G.; Bisogno, T. New Potent and Selective Ligands of Cannabinoid Receptors. OC/ACT/PCT 90769, January 23, 2006.
 - (27) (a) Reggio, P. H. Pharmacophores for ligand recognition and activation/inactivation of the cannabinoid receptors. *Curr. Pharm. Des.* **2003**, *9*, 1607–1633. (b) Thakur, G. A.; Duclos, R. I., Jr.; Makriyannis, A. Natural cannabinoids: templates for drug discovery. *Life Sci.* **2005**, *78*, 454–466. (c) Makriyannis, A.; Rapaka, R. S. The molecular basis of cannabinoid activity. *Life Sci.* **1990**, *47*, 2173–2184. (d) Papahatjis, D. P.; Kourouli, T.; Abadji, V.; Goutopoulos, A.; Makriyannis, A. Pharmacophoric requirements for the cannabinoid side chains: multiple bond and C1'-substituted Δ^8 -tetrahydrocannabinols. *J. Med. Chem.* **1998**, *41*, 1195–1200.
 - (28) (a) Seltzman, H. H.; Fleming, D. N.; Thomas, B. F.; Gilliam, A. F.; McCallion, D. S.; Pertwee, R. G.; Compton, D. R.; Martin, B. R. Synthesis and pharmacological comparison of dimethylheptyl and pentyl analogs of anandamide. *J. Med. Chem.* **1997**, *40*, 3626–3634. (b) Ryan, J. W.; Banner, W. K.; Wiley, J. L.; Martin, B. R.; Razdan, R. K. Potent anandamide analogs: the effect of changing the length and branching of the end pentyl chain. *J. Med. Chem.* **1997**, *40*, 3617–3625.
 - (29) Razdan, R. K. Structure–activity relationships in the cannabinoids. *Pharm. Rev.* **1986**, *38*, 75–149.
 - (30) Dominianni, S. J.; Ryan, C. W.; DeArmitt, C. W. Synthesis of 5-(tert-alkyl)resorcinols. *J. Org. Chem.* **1977**, *42*, 344–346.
 - (31) Crescenza, A.; Botta, M.; Corelli, F.; Santini, A.; Tafi, A. Cyclic dipeptides. 3. Synthesis of methyl(R)-6[(tert-butoxycarbonyl)amino]-4,5,6,7-tetrahydro-2-methyl-5-oxo-1,4-thiazepine-3-carboxylate and its hexahydro analogues: elaboration of a novel dual ACE/NEP inhibitor. *J. Org. Chem.* **1999**, *64*, 3019–3025.
 - (32) Di Marzo, V.; Griffin, G.; De Petrocellis, L.; Brandi, I.; Bisogno, T.; Williams, W.; Grier, M. C.; Kulasegram, S.; Mahadevan, A.; Razdan, R. K.; Martin, B. R. A structure/activity relationship study on arvanil, an endocannabinoid and vanilloid hybrid. *J. Pharmacol. Exp. Ther.* **2002**, *300*, 984–991.
 - (33) Dubuisson, D.; Dennis, S. G. The formalin test: a quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats. *Pain* **1977**, *4*, 161–174.
 - (34) Di Marzo, V.; Bisogno, T.; De Petrocellis, L.; Melck, D.; Martin, B. R. Cannabimimetic fatty acid derivatives: the anandamide family and other endocannabinoids. *Curr. Med. Chem.* **1999**, *6*, 721–744.
 - (35) Tuccinardi, T.; Ferrarini, P. L.; Manera, C.; Ortore, G.; Saccomanni, G.; Martinelli, A. Cannabinoid CB2/CB1 selectivity. Receptor modeling and automated docking analysis. *J. Med. Chem.* **2006**, *49*, 984–994.
 - (36) (a) Manera, C.; Tuccinardi, T.; Martinelli, A. Indoles and related compounds as cannabinoid ligands. *Mini-Rev. Med. Chem.* **2008**, *8*, 370–

387. (b) Song, Z. H.; Bonner, T. I. A lysine residue of the cannabinoid receptor is critical for receptor recognition by several agonists but not WIN55212-2. *Mol. Pharmacol.* **1996**, *49*, 891–896. (c) Tao, Q.; McAllister, S. D.; Andreassi, J.; Nowell, K. W.; Cabral, G. A.; Hurst, D. P.; Bachtel, K.; Ekman, M. C.; Reggio, P. H.; Abood, M. E. Role of a conserved lysine residue in the peripheral cannabinoid receptor (CB2): evidence for a subtype specificity. *Mol. Pharmacol.* **1999**, *55*, 605–613.
- (37) McAllister, S. D.; Rizvi, G.; Anavi-Goffer, S.; Hurst, D. P.; Barnett-Norris, J.; Lynch, D. L.; Reggio, P. H.; Abood, M. E. An aromatic microdomain at the cannabinoid CB(1) receptor constitutes an agonist/inverse agonist binding region. *J. Med. Chem.* **2003**, *46*, 5139–5152.
- (38) Abbott, F. V.; Franklin, K. B.; Westbrook, R. F. The formalin test: scoring properties of the first and second phases of the pain response in rats. *Pain* **1995**, *60*, 91–102.
- (39) Saggi, G.; Abbott, F. V. The formalin test in the mouse: a parametric analysis of scoring properties. *Pain* **2000**, *89*, 53–63.
- (40) *Macromodel*, version 8.5; Schrodinger Inc.: New York, 1999.
- (41) Morris, G. M.; Goodsell, D. S.; Halliday, R. S.; Huey, R.; Hart, W. E.; Belew, R. K.; Olson, A. J. *J. Comput. Chem.* **1998**, *19*, 1639–1662.
- (42) Sanner, M. F. Python: a programming language for software integration and development. *J. Mol. Graphics Modell.* **1999**, *17*, 57–61.

JM8016255