New Resorcinol−Anandamide "Hybrids" as Potent Cannabinoid Receptor Ligands Endowed with Antinociceptive Activity in Vivo

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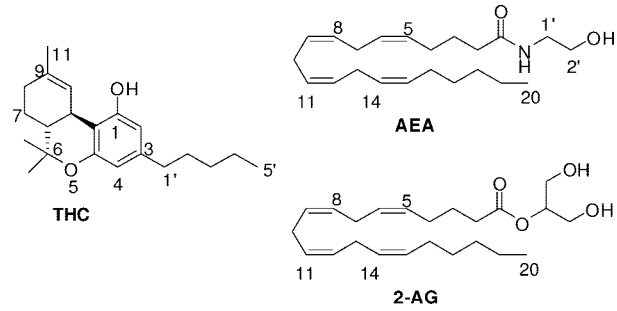
Bearing in mind the pharmacophoric requirements of both (−)-*trans*-Δ^9-tetrahydrocannabinol (THC) and anandamide (AEA), we designed a novel pharmacophore consisting of both a rigid aromatic backbone and a flexible chain with the aim to develop a series of stable and potent ligands of cannabinoid receptors. 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 alkyloxy chain of 7 or 10 carbon atoms was also present at position 1. Derivative **32** was a potent CB_1 and CB_2 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 CB_2 receptor ($K_i(CB_1) = 1\ \mu M$, $K_i(CB_2) = 35$ 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 C_{21} 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: CB_1,[3] expressed at high levels in several brain areas but also present in peripheral tissues,[4] including ileum, testis, urinary bladder, and vas deferens; CB_2, 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-arachidonylglycerol (2-AG, Chart 1),[9−11] which are metabolically less stable and structurally very dissimilar from the plant-derived molecules.

Chart 1. Chemical Structures of Main Cannabinoid Receptor Ligands



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 targets[15] 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

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[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.

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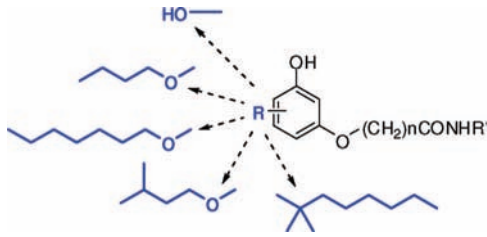
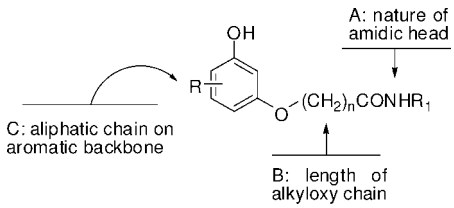


Figure 1. General structure of the synthesized compounds.

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 CB_1 receptor antagonists, i.e., SR141716[18] (rimonabant[19]), 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 requirements[22] of both AEA and THC for binding to cannabinoid CB_1 and CB_2 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 studies[25] have shown that these compounds act as partial CB_1 agonists and CB_2 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.

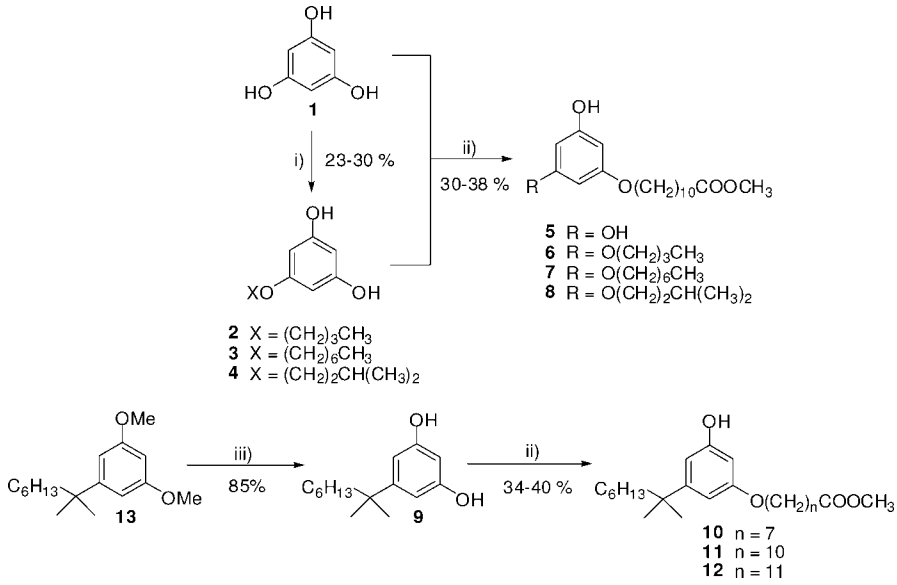
5-Alkyloxybenzene-1,3-diols **2**−**4** were obtained from commercial phloroglucinol (or 1,3,5-trihydroxybenzene) **1**, which reacted in dry dimethyl sulfoxide (DMSO) with an alkyl bromide (1-bromobutane, 1-bromoheptane, and 1-bromo-3-methylbutane) in the presence of anhydrous potassium carbonate and 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 acetonitrile[31] to provide the corresponding amides **28**−**41** in good yield (50−87%) (method B).

Biology

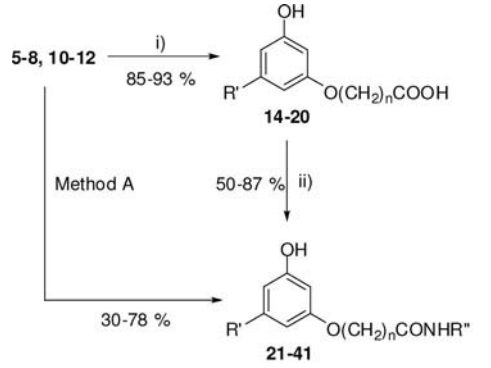
All the newly synthesized compounds that exhibited $IC_{50} \leq 10\ \mu M$ in the preliminary screening in radioligand binding assays for cannabinoid receptor were further evaluated for their affinity

Scheme 1. Synthesis of Intermediate Compounds **2**−**12**[a]



[a] Reagents and conditions: (i) alkyl bromide, DMSO, K_2CO_3, KF, room temp, 48−72 h; (ii) appropriate bromoacid methyl ester, acetone, K_2CO_3, KF, room temp, 48−72 h; (iii) BBr_3, CH_2Cl_2, 0 °C to room temp.

Scheme 2. Synthesis of Final Amides **21**−**41**[a]



21 R' = OH, n = 10, R" = CH_2CH_2OH
22 R' = $O(CH_2)_3CH_3$, n = 10, R" = CH_2CH_2OH
23 R' = $O(CH_2)_6CH_3$, n = 10, R" = CH_2CH_2OH
24 R' = $O(CH_2)_2CH(CH_3)_2$, n = 10, R" = CH_2CH_2OH
25 R' = $C(CH_3)_2C_6H_{13}$, n = 7, R" = CH_2CH_2OH
26 R' = $C(CH_3)_2C_6H_{13}$, n = 10, R" = CH_2CH_2OH
27 R' = $C(CH_3)_2C_6H_{13}$, n = 11, R" = CH_2CH_2OH
28 R' = OH, n = 10, R" = cC_3H_5
29 R' = $O(CH_2)_3CH_3$, n = 10, R" = cC_3H_5
30 R' = $O(CH_2)_6CH_3$, n = 10, R" = cC_3H_5
31 R' = $O(CH_2)_2CH(CH_3)_2$, n = 10, R" = cC_3H_5
32 R' = $C(CH_3)_2C_6H_{13}$, n = 7, R" = cC_3H_5
33 R' = $C(CH_3)_2C_6H_{13}$, n = 10, R" = cC_3H_5
34 R' = $C(CH_3)_2C_6H_{13}$, n = 11, R" = cC_3H_5
35 R' = OH, n = 10, R" = $CH_2cC_3H_5$
36 R' = $O(CH_2)_2CH(CH_3)_2$, n = 10, R" = $CH_2cC_3H_5$
37 R' = $C(CH_3)_2C_6H_{13}$, n = 7, R" = $CH_2cC_3H_5$
38 R' = $C(CH_3)_2C_6H_{13}$, n = 10, R" = $CH_2cC_3H_5$
39 R' = $C(CH_3)_2C_6H_{13}$, n = 11, R" = $CH_2cC_3H_5$
40 R' = $C(CH_3)_2C_6H_{13}$, n = 10, R" = 3,4-di-OH-phenethyl
41 R' = $C(CH_3)_2C_6H_{13}$, n = 10, R" = 3-OCH_3-4-OH-benzyl

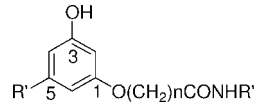
[a] Reagents and conditions. Method A: redistilled ethanolamine, 120−130 °C, 5 h. Method B: (i) MeOH/NaOH, reflux, 3 h; (ii) amine, HOBt, CMC, CH_3CN or CH_2Cl_2, room temp, overnight.

at recombinant human CB_1 and CB_2 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 CB_1 or CB_2 receptor and [^{3}H]-(−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol ([^{3}H]CP-55,940) (K_d = 0.31 nM for CB_2 and 0.18 nM for CB_1 receptors) as the high affinity ligand as described by the manufacturer (Perkin-Elmer, Italia).[32] Compounds that displaced [^{3}H]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 [^{3}H]CP-55,940 (0.084 μM for CB_2 and 0.14 nM for CB_1 binding assay). In all cases, K_i values were calculated by applying the Cheng−Prusoff equation to the IC_{50} 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 CB_1 antagonist) or AM630 (a CB_2 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

CB_1 and CB_2 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 CB_1 or CB_2 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 cyclopropylmethylamides (**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, CB_1 and CB_2 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	*n*	R′	R″	CB_1 K_i (μM)	CB_2 K_i (μM)	CB_1/CB_2 SI
21	10	OH	CH_2CH_2OH	na	na	
22	10	$O(CH_2)_3CH_3$	CH_2CH_2OH	2.41	1.62	1.5
23	10	$O(CH_2)_6CH_3$	CH_2CH_2OH	3.07	2.42	1.3
24	10	$O(CH_2)_2CH(CH_3)_2$	CH_2CH_2OH	2.88	**0.57**	5.1
25	7	$C(CH_3)_2(CH_2)_5CH_3$	CH_2CH_2OH	**0.056**	**0.16**	0.4
26	10	$C(CH_3)_2(CH_2)_5CH_3$	CH_2CH_2OH	**0.31**	**0.03**	**10.3**
27	11	$C(CH_3)_2(CH_2)_5CH_3$	CH_2CH_2OH	**0.82**	**0.08**	**10.3**
28	10	OH	*c*-C_3H_5	na	na	
29	10	$O(CH_2)_3CH_3$	*c*-C_3H_5	**0.28**	**0.12**	2.3
30	10	$O(CH_2)_6CH_3$	*c*-C_3H_5	2.22	**0.21**	**10.6**
31	10	$O(CH_2)_2CH(CH_3)_2$	*c*-C_3H_5	**0.22**	**0.13**	1.7
32	7	$C(CH_3)_2(CH_2)_5CH_3$	*c*.C_3H_5	**0.0056**	**0.0079**	0.7
33	10	$C(CH_3)_2(CH_2)_5CH_3$	*c*-C_3H_5	**0.021**	**0.0079**	2.7
34	11	$C(CH_3)_2(CH_2)_5CH_3$	*c*-C_3H_5	**0.17**	**0.02**	8.5
35	10	OH	CH_2*c*-C_3H_5	na	na	
36	10	$O(CH_2)_2CH(CH_3)_2$	CH_2*c*-C_3H_5	2.30	**0.20**	**11.5**
37	7	$C(CH_3)_2(CH_2)_5CH_3$	CH_2*c*-C_3H_5	**0.10**	**0.034**	2.9
38	10	$C(CH_3)_2(CH_2)_5CH_3$	CH_2*c*-C_3H_5	1.00	**0.035**	**28.6**
39	11	$C(CH_3)_2(CH_2)_5CH_3$	CH_2*c*-C_3H_5	5.40	**0.60**	9.0
40	10	$C(CH_3)_2(CH_2)_5CH_3$	3,4-OH-phenethyl	3.4	5.0	0.7
41	10	$C(CH_3)_2(CH_2)_5CH_3$	3-OCH_3-4-OH-benzyl	2.82	**0.51**	5.5
CB25	10	*n*.$(CH_2)_4CH_3$	*c*-C_3H_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 (μM) for CB_1 and CB_2 binding assays. Reference compounds were tested under the same conditions in this study. Anandamide was tested in the presence of PMSF (100 nM). na = $IC_{50} > 10$ in the preliminary screening carried out with rat brain and spleen membranes. Binding affinity costants of the most potent compounds ($K_i \leq 1$ μM) are in bold as well as the most selective compounds for CB_1 and CB_2. 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 alkyloxy 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 CB_2 binding affinity compared to that of the olivetol analogues. Particularly, compounds **32** and **33** proved to be the most potent CB_1 (K_i of 5.6 and 21 nM, respectively) and CB_2 ligands (K_i = 7.9 nM for both compounds), with affinity constants comparable to those of WIN 55,212-2 ($K_i(CB_1)$ = 21 nM and $K_i(CB_2)$ = 2.1 nM) and our "lead compound" CB25 ($K_i(CB_1)$ = 5.2 nM and $K_i(CB_2)$ = 13 nM). (f) Some compounds, although less potent, showed selectivity for CB_2 receptors, in particular, compound **38** ($K_i(CB_1)$ = 1000 nM and $K_i(CB_2)$ = 35 nM), which still elicited a nanomolar affinity for CB_2 receptor and a selectivity index (SI), calculated as $K_i(CB_1)/K_i(CB_2)$, 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 CB_1 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 nocifensive 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 CB_1 receptor antagonist rimonabant but not by the CB_2 receptor antagonist AM630. These observations are in agreement with our previous findings that potent compounds of similar structure act as CB_1 agonists or partial agonists and as neutral CB_2 antagonists in vivo.[25] Compound

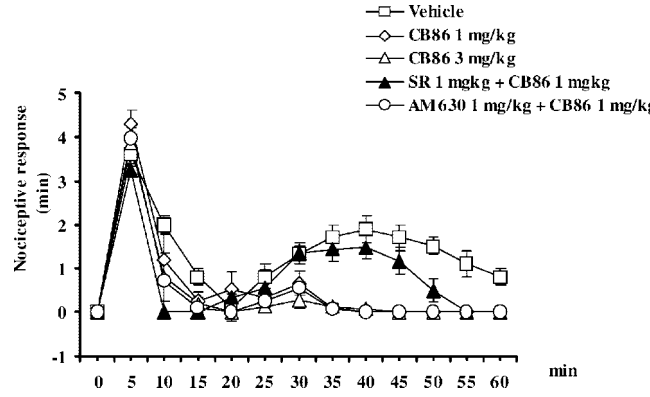


Figure 2. Antinociceptive effect of vehicle (10% DMSO in 0.9% NaCl) or of compound **32**, here denoted as CB86, in the formalin test in mice. Each point represents the mean ± standard error of the mean (SEM) of 8−10 animals per group. Data were analyzed using the one-way ANOVA followed by the Bonferroni's test, and statistical significance was taken as $P < 0.05$. The effects of the CB_1 antagonist rimonabant (SR) and the CB_2 antagonist AM630 are also shown. Rimonabant reversed in a statistically significant manner the effect of the new compound on the second phase of the nocifensive 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 CB_1 and CB_2 receptors[35] and the hypothetical binding interactions of AEA into both receptors have been already described. In the CB_1 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

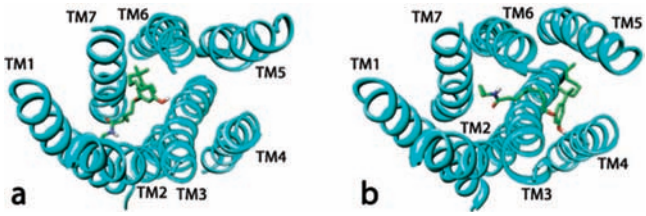


Figure 3. Compound **32** docked into the CB_1 (a) and CB_2 (b) receptor models (extracellular point of view).

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 CB_2 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 CB_1 and S3.31 in the CB_2 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 [^{3}H]WIN55,212-2 binding in a human CB_1 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 CB_1-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 CB_1 and CB_2 receptors. This compound is the most CB_1 and CB_2 active ligand among those reported.

In agreement with our previous studies,[24] in the CB_1 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 CB_1 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 CB_2 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 CB_1 affinity, and the CB_2 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 CB_1 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 CB_2 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 (CB_2) or very similar (CB_1) 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 CB_2 affinity and a micromolar CB_1 affinity, resulting in a high CB_2 selectivity ($CB_1/CB_2 = 28.6$). In agreement with these data, in the CB_2 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 CB_1 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 CB_1 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 ($QPlogP_{o/w}$), have been correlated with ligand potencies, expressed as $pK_i(CB_1)$ or $pK_i(CB_2)$ (see Table 6 and Figure 1 of Supporting Information). Results indicate some interesting correlations between CB_1 and CB_2 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 CB_1 and CB_2 receptors, and some of them, with the exception of those with the lowest K_i values for both receptors, exhibited also a reasonable CB_2 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 CB_2 ligands, still retaining high CB_1 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 CB_1 or CB_2 selective ligands.

Interestingly, these resorcinol−anandamide "hybrids" acted in vivo as CB_1 agonists or partial CB_1 agonists, showing a potent

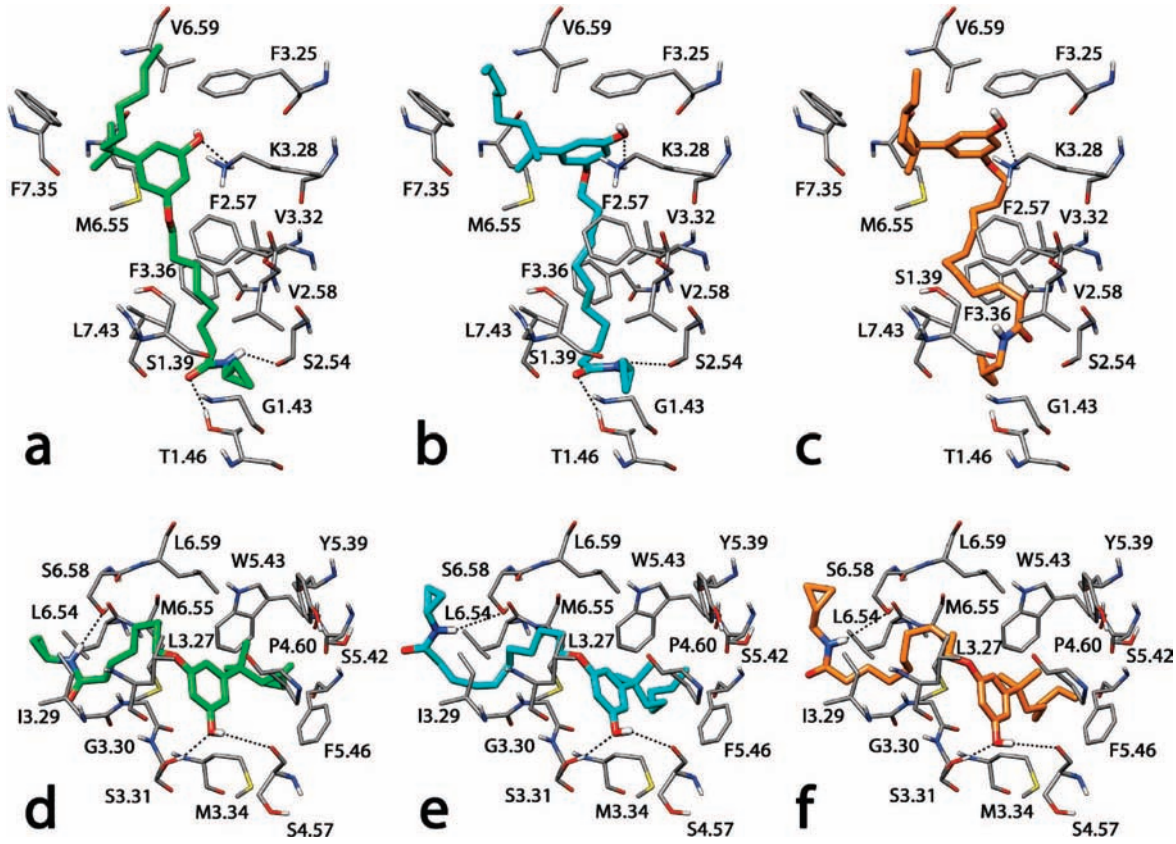


Figure 4. Compounds **32** (a, d), **33** (b, e), and **38** (c, f) docked into the CB_1 (a−c) and CB_2 (d−f) receptor models.

antinociceptive effect fully antagonized only by the CB_1 receptor antagonist/inverse agonist rimonabant but not by the CB_2 receptor antagonist AM630. This is in agreement with our previous report that compounds in this series behave in vitro mostly as partial CB_1 agonists and neutral CB_2 antagonists.[25]

Furthermore, molecular modeling studies on the new compounds provided a reasonable hypothesis of their interaction mode with both CB_1 and CB_2 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 CB_1 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 CB_2 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 ±0.4% of the theoretical values. Proton nuclear magnetic resonance (^{1}H 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 F_{254} 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 μM.

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: $CHCl_3$/MeOH, 50/1. Yield: 30% (pasty yellow solid). ^{1}H NMR ($CDCl_3$): δ 6.84 (br s, 2H, disappears on treatment with D_2O), 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. ($C_{10}H_{14}O_3$ (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: $CHCl_3$/MeOH, 100/0.5. Yield: 40% (pale-yellow oil). ^{1}H 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 $[M + Na]^+$ (100). Anal. ($C_{24}H_{40}O_4$ (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: $CHCl_3$/MeOH, 50/1. Yield: 80% (white solid), mp 111.1 °C (M). ^{1}H 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 $[M + Na]^+$, 779 $[2M + Na]^+$ (100). Anal. ($C_{23}H_{38}O_4$ (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: $CHCl_3$/MeOH, 50/1. Yield 30% (pale-yellow solid), mp 125−130 °C (K). ^{1}H 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 $[M + Na]^+$ (100). Anal. ($C_{19}H_{31}NO_5$ (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*-toluensulfonate (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: $CHCl_3$/MeOH, 50/1. Yield 81% (pale-yellow oil). ^{1}H 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 $[M + Na]^+$, 857 $[2M + Na]^+$ (100). Anal. ($C_{26}H_{43}NO_3$ (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 ± 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 0.86[37]). 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 TOOLS[42] 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.

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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.

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