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Design, synthesis, binding, and molecular modeling studies of new potent ligands of cannabinoid receptors

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Abstract—In our ongoing program aimed at the design, synthesis, and biological evaluation of novel cannabinoid receptor ligands derived from olivetol and hexyl-resorcinol, we have designed a structural model for new derivatives on the basis of a previous study. Here we report the synthesis, binding, and molecular modeling studies of new potent compounds with high affinity toward CB_1 and CB_2 receptors. Compounds with amidic 'heads' with alkyloxy chains varying in length from 8 to 12 carbon atoms showed nanomolar affinity for both receptors, depending on the type of aromatic backbone. Two of the new compounds, although not very potent, exhibit selectivity for CB_1 receptors (CB_1/CB_2 = 0.07 and 0.08, respectively). Molecular modeling studies fitted this new class of cannabinoid ligands into a CB_1 receptor model, and the qualitative analysis of the results was in general agreement with the CB_1 affinity constants observed experimentally for these derivatives.

1. Introduction

In the last few years the therapeutic potential of *Cannabis sativa* L. for the treatment of many pathologies has been more and more appreciated.[1] Its most important component, (−)-*trans*-Δ^9-tetrahydrocannabinol (THC, Chart 1), mainly exerts its pharmacological properties by binding to two G-protein-coupled receptors, CB_1,[2] expressed at high levels in several brain areas but also present in peripheral tissues,[3] and CB_2, predominantly found in the periphery,[4] mainly in the immune system (spleen, tonsils, immune cells), but recently also in the brain.[5,6]

The discovery and characterization of CB_1 and CB_2 receptors and of their major endogenous ligands, that is, the endocannabinoids anandamide (AEA)[7] and 2-arachidonoylglycerol (2-AG) (Chart 1),[8–10] as well as the pharmacological characterization of the mechanisms responsible for endocannabinoid cellular uptake and inactivation, that is, the putative anandamide membrane transporter (AMT)[11] and the fatty acid amide hydrolase (FAAH)[12] or the monoacylglycerol lipase, in the case of 2-AG, offer areas for the development of new therapeutic interventions.[13] Despite their different chemical structures, THC, a very rigid molecule, and AEA, a very flexible[14] and easily oxidized compound, have several common pharmacological properties. Bearing in mind the pharmacophore requirements[15] of both AEA and THC, necessary for the binding to cannabinoid CB_1 and CB_2 receptors, in a previous paper[16] we have described a series of metabolically stable and potent ligands of cannabinoid receptors, which consist of both a rigid aromatic portion, as in THC, and a flexible chain, as in AEA. Furthermore, our recent studies[17] indicate that our compounds act as partial CB_1 agonists and CB_2 neutral antagonists. The binding assay results of those derivatives, which can be considered as a new

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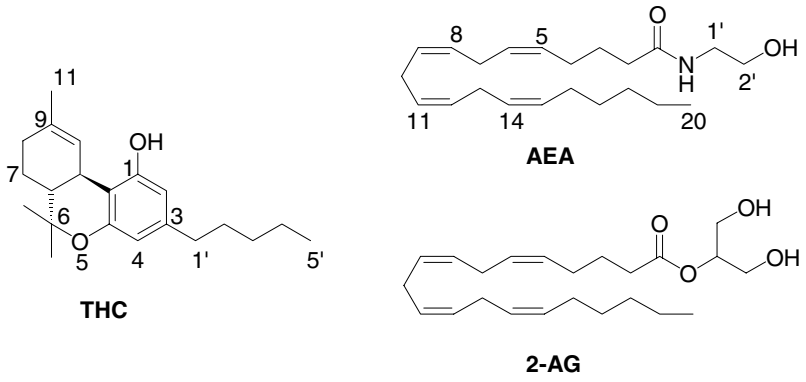


Chart 1.

class of compounds with a great affinity for cannabinoid receptors,[18] supplied us with useful information on their structure–activity relationships. The most important critical points for the observation of high affinity appeared to be: (a) the presence of a free phenolic hydroxyl group; (b) the presence of an aliphatic chain on the aromatic ring and its length; (c) the length of the alkyloxy chain carrying the amidic 'head'; (d) the nature of the amidic 'head' (Fig. 1). Thanks to the information gained in our previous study it has now been possible to design a basic structure for the development of new compounds of this class with high affinity for cannabinoid receptors.

With the aim to investigate these features in more detail, in the present study we have designed and synthesized 21 new derivatives which maintained a free phenolic hydroxyl group and a linear aliphatic chain of five or six carbon atoms in the C portion, but with some modifications in the A and B portions (Fig. 1). In our previous study,[16] we found that the length of the alkyloxy chain plays a very important role in the affinity of this class of compounds for cannabinoid receptors: in fact, the 15-carbon atom derivatives were inactive and the majority of the five-carbon atom derivatives were also inactive, with some exceptions. Therefore, the length of the alkyloxy chain was kept at 8, 11, and 12 carbon atoms. Moreover, for the amidic 'head', besides ethanolamine and cyclopropylamine, three new amines, that is, methylcyclopropylamine, 3-hydroxytyramine (3,4-dihydroxyphenethylamine), and 3-methoxy-4-hydroxybenzylamine, were introduced (Fig. 2).

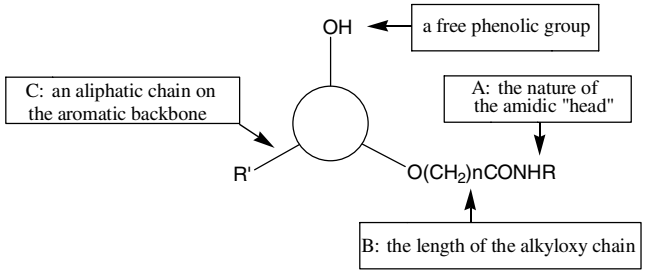


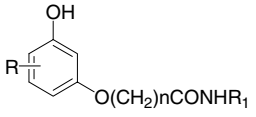
Figure 1. Proposed pharmacophoric model of this new class of cannabinoid receptor ligands.

Finally a molecular modeling and docking study was carried out in order to explain the structure–activity relationships experimentally observed. For this purpose a previously developed model of the CB_1 receptor in its activated form[19] was used. A series of compounds were docked into this receptor model and the observed ligand–receptor interactions were discussed also taking into account the site-directed mutagenesis data available for this receptor subtype.

2. Chemistry

As shown in Scheme 1, the synthesis of the new compounds starts from olivetol or 4-hexylresorcinol, which are reacted with a bromoalkylmethylester (8-bromo-hexanoic acid methylester or 11-bromo-undecanoic acid methylester or 12-bromo-dodecanoic acid methylester) in dry acetone in presence of anhydrous K_2CO_3 and KF, yielding esters **1**–**9**. The final amides are obtained by either of two simple methods. Method A: esters were refluxed with methanolic/aqueous sodium hydroxide solution to give the corresponding acids, which were used as such and reacted with the amines in presence of 1-hydroxybenzotriazole (HOBt) and 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide methyl-*p*-toluenesulfonate (CMC) in dichloromethane or acetonitrile.[20] Method B: esters were warmed up with ethanolamine as solvent.

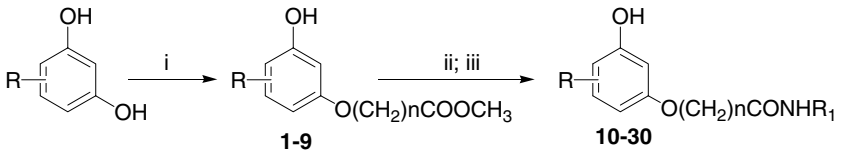
The O-alkylation of phenols afforded all possible alkylated products at the two free hydroxyl groups; moreover, O-alkylation of 4-hexylresorcinol afforded two



n = 7, 11;
R = nC_5H_{11}, nC_6H_{13};
R_1 = CH_2CH_2OH, cyclopropyl.

n = 10;
R = nC_5H_{11}, nC_6H_{13};
R_1 = methylcyclopropyl, 3,4-dihydroxyphenethyl, 3-methoxy-4-hydroxy-benzyl.

Figure 2. Structures of the synthesized compounds **10**–**30**.



Scheme 1. Reagents and conditions: (i) Br-$(CH_2)_n$$COOCH_3$, acetone, K_2CO_3, KF, reflux, 48–72 h; (ii) method A: MeOH/NaOH, reflux, 3 h; amine, HOBt, CMC, rt, overnight; (iii) method B: $H_2NCH_2CH_2OH$, 120–130 °C, 5 h.

different monoalkylated regioisomers. The reaction occurred mainly on the hydroxyl in C-1, the less hindered position (Scheme 2), irrespectively of the bromomethylester used, and independently from the length of its chain. As described in our previous paper,[16] the structure of two regioisomers has been definitively assigned by NOESY experiments.

3. Pharmacological evaluation

3.1. Binding assays

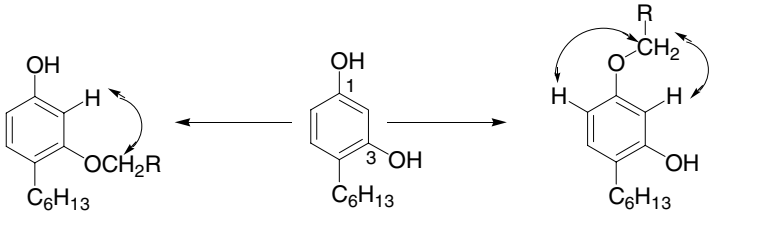
For CB_1 and CB_2 receptor binding assays, the new compounds were first subjected to a preliminary screening carried out using three concentrations of the compounds (5, 10, and 25 μM), membranes from HEK cells transfected with either the human CB_1 or CB_2 receptor, and [^{3}H]-(−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)-phenyl]-*trans*-4-(3-hydroxy-propyl)-cyclohexanol ([^{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).[21] 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 curve. Displacement curves were generated by incubating drugs with [^{3}H]CP-55,940 (0.084 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[22] to the IC_{50} values (obtained by GraphPad) for the displacement of the bound radioligand by increasing concentrations of the test compounds.

3.2. Fatty acid amide hydrolase assays

The effect of compounds on the enzymatic hydrolysis of [^{14}C]anandamide (6 μM) was studied by using membranes prepared from rat brain incubated with increasing concentrations of compounds in 50 mM Tris–HCl, pH 9, for 30 min at 37 °C.[20] [^{14}C]ethanolamine produced from [^{14}C]anandamide hydrolysis was measured by scintillation counting of the aqueous phase after extraction of the incubation mixture with 2 volumes of $CHCl_3$/CH_3OH (2:1 by volume). In most cases, only the most potent compounds in the binding assays were subjected to this assay.

4. Results and discussion

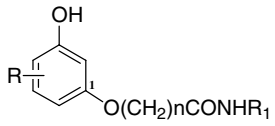
All the newly synthesized compounds that exhibited $IC_{50} \leqslant 10$ μM in the preliminary screening for cannabinoid receptor binding activity were evaluated in radioligand binding assays for affinity at recombinant human CB_1, CB_2 over-expressed in COS cells, or for their inhibitory action on FAAH. The results are summarized in Table 1. Analysis of the binding assay results of the compounds described here allows us to assess with more accuracy the structure–activity relationships in this class of compounds, summarized as follows: (a) in accordance with data from our previous study[16] and from the literature[23] cyclopropylamides (**11**, **18**, **23**, **25**, **30**) were more potent than the respective ethanolamides (**10**, **17**, **22**, **24**, **29**) in the binding assays (with the exception of compound **15** that was more potent than **16**); (b) the amidic 'head' must be of little steric hindrance. Amine size increase always causes a decrease (**13**, **14**, **20**, **21**) or the loss (**27** and **28**) of receptor affinity; (c) the length of the alkyloxy chain carrying the amidic 'head' can vary from 8 to 12 carbon atoms, leading to compounds with nanomolar affinity, depending on the type of aromatic backbone (**11**, n = 7; **15**, n = 11; **23**, n = 11; **25**, n = 7), even though the best ligand was still **CB 25** (n = 10); (d) two compounds (**17** and **24**), although not very potent in the binding assays, show selectivity for CB_1 receptors (CB_1/CB_2 = 0.07 and 0.08, respectively). Worth of note is that these compounds are the two regioisomers of 4-hexlresorcinol with n = 7, and both are ethanolamides; (e) 4-hexylresorcinol derivatives with alkylic chain *ortho* to the hydroxyl group (**24**–**30**) show generally less receptor affinity, suggesting that the



alkylation OH in C-3 alkylation OH in C-1

Scheme 2.

Table 1. Radioligand CB_1 and CB_2 binding assays and selectivity over FAAH of the synthetized compounds[a]



Compound	R	*N*	R_1	CB_1	CB_2	CB_1/CB_2	FAAH
10	5-*n*-Pentyl	7	CH_2CH_2OH	**0.13**	**0.39**	1.35	>50
11	5-*n*-Pentyl	7	*c*-C_3H_5	**0.016**	**0.039**	0.33	>50
12	5-*n*-Pentyl	10	CH_2-*c*-C_3H_5	**0.51**	**0.08**	6.4	>50
13	5-*n*-Pentyl	10	3,4-OH-Phenylethyl	**0.56**	na		nt
14	5-*n*-Pentyl	10	3-OH-4-OCH_3-benzyl	2.1	1.65	1.3	=10
15	5-*n*-Pentyl	11	CH_2CH_2OH	**0.06**	**0.039**	1.5	>50
16	5-*n*-Pentyl	11	*c*-C_3H_5	**0.75**	**0.31**	2.4	=50
17	2-*n*-Hexyl	7	CH_2CH_2OH	**0.26**	3.94	**0.07**	=25
18	2-*n*-Hexyl	7	*c*-C_3H_5	**0.31**	1.8	0.17	=50
19	2-*n*-Hexyl	10	CH_2-*c*-C_3H_5	**0.75**	**0.47**	1.6	>50
20	2-*n*-Hexyl	10	3,4-OH-Phenylethyl	2.1	7.9		nt
21	2-*n*-Hexyl	10	3-OH-4-OCH_3-Benzyl	2.82	2.1		=50
22	2-*n*-Hexyl	11	CH_2CH_2OH	**0.17**	1.42	0.12	=50
23	2-*n*-Hexyl	11	*c*-C_3H_5	**0.013**	**0.039**	0.33	>50
24	4-*n*-Hexyl	7	CH_2CH_2OH	**0.19**	2.4	**0.08**	=50
25	4-*n*-Hexyl	7	*c*-C_3H_5	**0.056**	**0.28**	0.2	>50
26	4-*n*-Hexyl	10	CH_2-*c*-C_3H_5	na	2.9		>50
27	4-*n*-Hexyl	10	3,4-OH-Phenylethyl	na	na		nt
28	4-*n*-Hexyl	10	3-OH-4-OCH_3-benzyl	na	na	1.3	>50
29	4-*n*-Hexyl	11	CH_2CH_2OH	na	na		=10
30	4-*n*-Hexyl	11	*c*-C_3H_5	na	3.94		>50
CB 25	5-*n*-Pentyl	10	*c*-C_3H_5	**0.0052**	**0.013**	0.4	>50
Anandamide				0.072			
WIN 55,212-2				0.021	0.0021		
HU-210					$0.15\ 10^{-3}$		

[a] Data are means of $n = 3$ separate experiments and are expressed as K_i (μM), for CB_1 and CB_2 binding assays, and in IC_{50} (μM) for FAAH assays. Reference compounds were tested under the same conditions in this study. Anandamide was tested in the presence of PMSF (100 nM). na, $IC_{50} > 10$ μM in the preliminary screening carried out with rat brain and spleen membranes; nt, not tested. Binding affinity constants of the most potent compounds ($K_i \leqslant 1$ μM) are highlighted 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 means.

phenolic group must be not hindered; (g) regarding the interaction of the most potent cannabinoid receptor ligands with FAAH, the data obtained here confirm the results of the previous paper[16] that all such compounds are essentially unable to inhibit the enzyme.

4.1. Molecular modeling

As previously reported, the docking of AEA into our CB_1 receptor model suggested that it was placed among TM2-3-6-7 with the aliphatic chain directed toward the intracellular side of the receptor. The amide oxygen atom of the ligand interacted with K3.28, in agreement with site-directed mutagenesis studies,[24] and the hydroxy group formed a H bond with S7.39 (see Fig. 3a).

The docking of compound **CB 25** ($K_i = 5.2$ nM, Fig. 4), which is the most CB_1 active compound among those tested here and previously,[16] revealed a similar occupation of the binding site. However, as shown in Figure 3b and differently from AEA, the carboxamide system was directed toward TM1 and TM2 and formed two H bonds with T1.46 and S2.54, while K3.28 formed a H bond with the phenolic substituent.

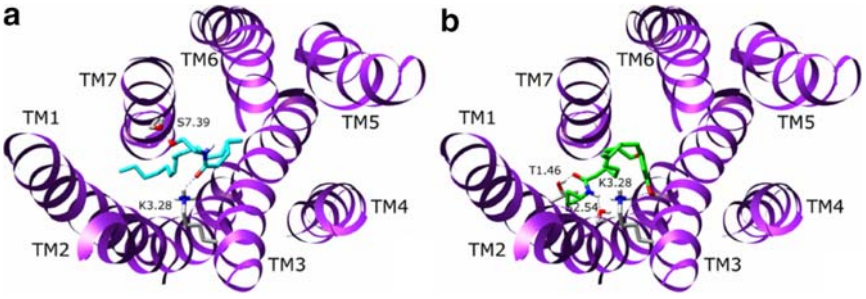


Figure 3. AEA (a) and compound **CB 25** (b) docked into the CB_1 receptor (extracellular point of view).

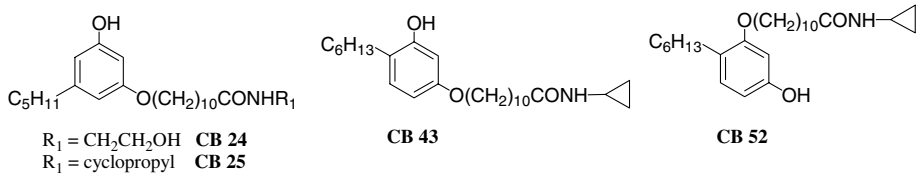


Figure 4. Previously published compounds[14] docked into CB_1 receptor.

With regard to lipophilic interactions, the *n*-pentyl substituent was stabilized by F3.25, V6.59, and F7.35, the decyl-alkyloxy chain was stabilized through the interaction with F2.57, V3.32, F3.36, and L7.43, whereas the cyclopropyl substituent was inserted in a lipophilic pocket principally delimited by V2.58, L7.43 and the backbone of TM1 (see Fig. 5a).

The reduction of the alkyloxy chain from ten to seven C atoms (compound **11**) determined only a threefold reduction of CB_1 affinity and in fact the docking studies revealed that this compound showed all the H bonds observed for compound **CB 25**, even though the H bond with T1.46 was weaker since the distance between the amide oxygen atom of the ligand and the polar hydrogen of the threonine was 2.8 Å while for compound **CB 25** it was 1.8 Å (see Fig. 5b).

The increase of the length of the alkyloxy chain, adding only one C to the 10 C atoms of compound **CB 25**, determined a 144-fold decrease of affinity (**16**, K_i = 750 nM). As shown in Figure 5c the docking of compound **16** into our CB_1 receptor model revealed a different rearrangement of the lipophilic alkyloxy chain with the maintenance of the H bond with T1.46 and S2.54. However with this disposition the ligand was not able to interact with K3.28, whose mutagenesis data have suggested to be a fundamental residue for ligand recognition, thus explaining its low CB_1 affinity.

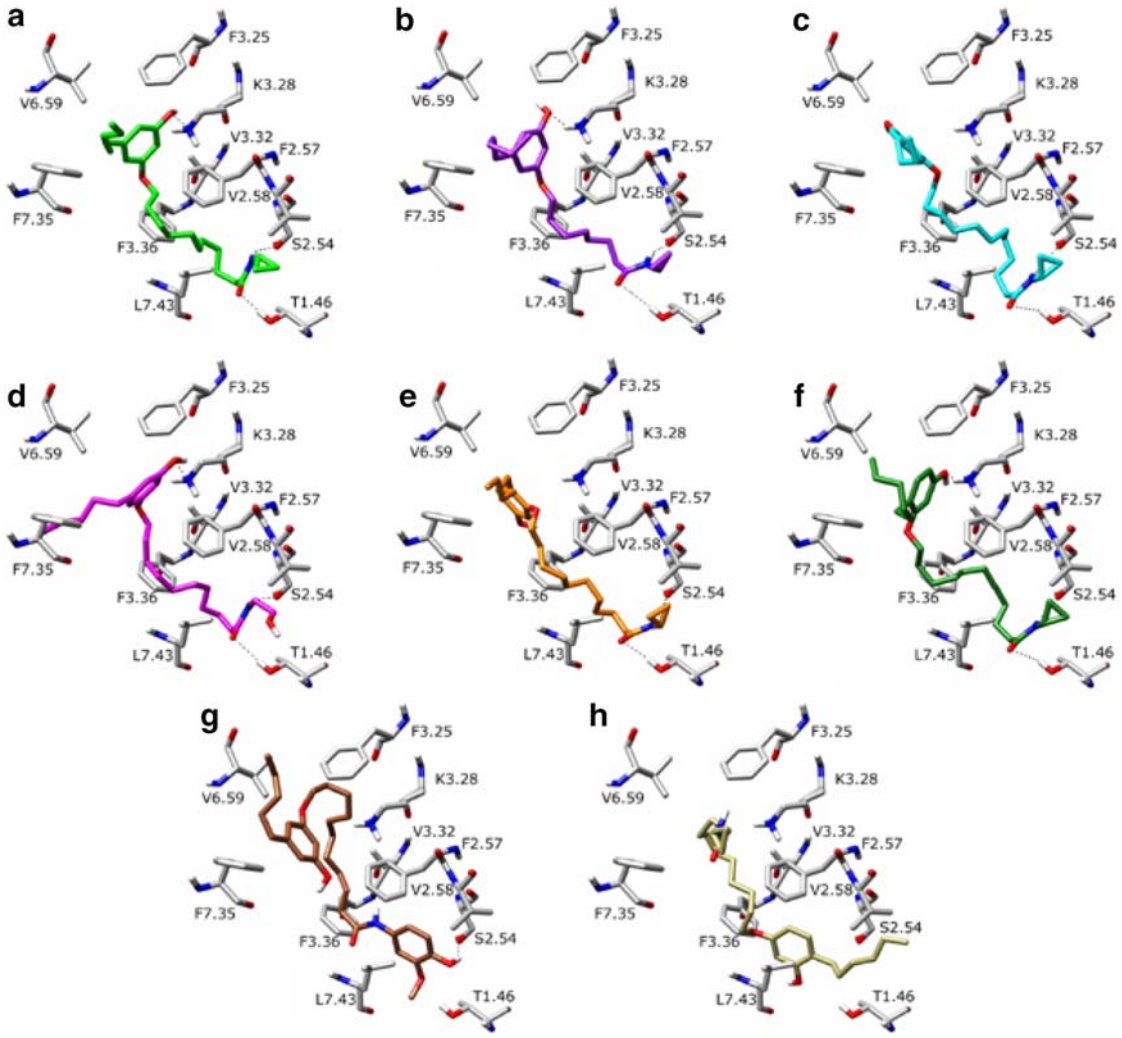


Figure 5. Compounds **CB 25** (a), **11** (b), **16** (c), **CB 24** (d), **CB 52** (e), **23** (f), **14** (g), **CB 43** (h) docked into the CB_1 receptor model.

Table 2. Chemical and physical data of the synthetized esters

Compound	Purification[a]	Yield %	^{1}H NMR ($CDCl_3$) δ (ppm)
1	Yellow oil ($CHCl_3$)	40	6.30–6.20 (m, 3H), 3.88 (t, 2H, *J* = 6.5 Hz), 3.65 (s, 3H), 2.48 (t, 2H, *J* = 7.3 Hz), 2.30 (t, 2H, *J* = 7.2 Hz), 1.76–1.57 (m, 6H), 1.52–1.25 (mm, 10H), 0.87 (t, 3H, *J* = 6.4 Hz)
2[16]	Yellow oil ($CHCl_3$/MeOH) 100:1	45	^{1}H NMR ($CDCl_3$) δ (ppm): 6.29–6.21 (m, 3H), 5.06 (s, 1H), 3.89 (t, 2H, *J* = 6.4 Hz), 3.65 (s, 3H), 2.48 (t, 2H, *J* = 7.6 Hz), 2.29 (t, 2H, *J* = 7.4 Hz), 1.80–1.69 (m, 2H), 1.66–1.49 (m, 4H), 1.41–1.28 (mm, 16H), 0.87 (t, 3H, *J* = 6.6 Hz)
3	Dark yellow oil ($CHCl_3$)	38	6.30 (s, 1H), 6.22–6.20 (m, 2H), 4.97 (s, 1H), 3.89 (t, 2H, *J* = 6.4 Hz), 3.65 (s, 3H), 2.48 (t, 2H, *J* = 7.6 Hz), 2.29 (t, 2H, *J* = 7.6 Hz), 1.80–1.70 (m, 2H), 1.66–1.49 (m, 4H), 1.40–1.28 (mm, 18H), 0.87 (t, 3H, *J* = 6.6 Hz)
4	Yellow oil ($CHCl_3$)	20	6.91 (d, 1H, *J* = 7.9 Hz), 6.36–6.30 (m, 2H), 3.86 (t, 2H, *J* = 6.2 Hz), 3.66 (s, 3H), 2.49 (t, 2H, *J* = 8.1 Hz), 2.31 (t, 2H, *J* = 7.3 Hz), 1.75–1.59 (m, 4H), 1.46–1.29 (mm, 14H), 0.86 (t, 3H, *J* = 6.2 Hz)
5[16]	Yellow oil ($CHCl_3$/MeOH) 50:1	30	6.92 (d, 1H, *J* = 8.2 Hz), 6.36–6.27 (m, 2H), 5.12 (s, 1H), 3.88 (t, 2H, *J* = 6.3 Hz), 3.65 (s, 3H), 2.50 (t, 2H, *J* = 7.5 Hz), 2.29 (t, 2H, *J* = 7.4 Hz), 1.79–1.47 (mm, 10H), 1.44-1.29 (mm, 14H), 0.86 (t, 3H, *J* = 6.2 Hz)
6	Pale yellow oil ($CHCl_3$)	25	6.92 (d, 1H, *J* = 8.0 Hz), 6.36 (s, 1H), 6.30 (dd, 1H, *J* = 2.6 Hz, *J* = 8.0 Hz), 5.00 (s, 1H), 3.89 (t, 2H, *J* = 6.1 Hz), 3.66 (s, 3H), 2.50 (t, 2H, *J* = 7.0 Hz), 2.30 (t, 2H, *J* = 7.2 Hz), 1.83–1.73 (m, 2H), 1.70–1.52 (mm, 6H), 1.48–1.28 (mm, 18H), 0.87 (t, 3H, *J* = 6.2 Hz)
7	Pale yellow oil ($CHCl_3$)	30	6.96 (d, 1H, *J* = 8.2 Hz), 6.42–6.34 (m, 2H), 4.95 (s br, 1H), 3.88 (t, 2H, *J* = 6.4 Hz), 3.65 (s, 3H), 2.50 (t, 2H, *J* = 7.5 Hz), 2.30 (t, 2H, *J* = 7.8 Hz), 1.76–1.55 (m, 4H), 1.46–1.29 (mm, 14H), 0.86 (t, 3H, *J* = 6.4 Hz)
8[16]	White solid ($CHCl_3$/MeOH) 50:1 mp 60.8 °C (M)	35	6.96 (d, 1H, *J* = 8.3 Hz), 6.43–6.35 (m, 2H), 5.22 (s, 1H), 3.90 (t, 2H, *J* = 6.4 Hz), 3.66 (s, 3H), 2.50 (t, 2H, *J* = 7.9 Hz), 2.30 (t, 2H, *J* = 7.5 Hz), 1.76–1.52 (mm, 6H), 1.48–1.28 (mm, 18H), 0.87 (t, 3H, *J* = 6.5 Hz)
9	Cream solid ($CHCl_3$) mp73.2 °C (M)	35	6.96 (d, 1H, *J* = 8.1 Hz), 6.42–6.34 (m, 2H), 5.05 (s br, 1H), 3.88 (t, 2H, *J* = 6.7 Hz), 3.66 (s, 3H), 2.50 (t, 2H, *J* = 7.3 Hz), 2.30 (t, 2H, *J* = 7.2 Hz), 1.79–1.69 (m, 2H), 1.66–1.52 (m, 4H), 1.48–1.26 (mm, 20H), 0.87 (t, 3H, *J* = 6.5 Hz)

[a] Column chromatography eluent.

The substitution of the cyclopropyl substituent with ethanol determined a 152-fold decrease of affinity, as compound **CB 24** (Fig. 4) showed a 800 nM CB_1 affinity.[16] The docking of this compound highlighted a very similar disposition to the one observed for compound **CB 25**. However, as shown in Figure 5d, the ethanol/cyclopropyl substitution determined the loss of the lipophilic interaction with V2.58 and L7.43. Furthermore, the hydroxy group of the ethanol substituent did not seem to form any electrostatic interaction, thus explaining the strong decrease of affinity.

The displacement of the alkyl chain from the 5′ to the 2′ position determined a general decrease of the CB_1 affinity, as typified by compound **CB 52**[16] (Fig. 4), which presented a *n*-hexyl chain in 2′ position and displayed a 40-fold decrease of CB_1 affinity (K_i = 210 nM) with respect to **CB 25**. The docking of compound **CB 52** revealed that, as for **CB 25**, the carboxamide group formed two H bonds with T1.46 and S2.54, whereas the *n*-hexyl chain interacted with F3.25, V6.59, and F7.35. However, with this disposition of the substituents, the phenolic oxygen did not interact with K3.28 (see Fig. 5e).

Very interestingly, differently from the 3′,5′ disubstituted derivatives where the increase of the length of the alkyloxy chain from ten to eleven carbons determined a decrease of affinity, for the 2′,5′ disubstituted compounds this change determined an increase of affinity.

Docking studies of compound **23**, which possesses an alkyloxy chain of eleven carbons, highlighted not only all the main interactions of compound **CB 52**, but also the H bond between K3.28 and the phenolic oxygen of the ligand (see Fig. 5f), as for **CB 25**. These results were in agreement with the CB_1 binding affinity of this compound, which showed a K_i of 13 nM.

With regard to the substitution of the cyclopropyl group with the 4-hydroxy-3-methoxy-benzyl, in one compound (**14**) this determined a great decrease of CB_1 affinity (K_i = 2100 nM). The reason could be the high dimension of the aromatic substituent, compared with the cyclopropyl group. In fact, as shown in Figure 5g, the interactions of the aromatic group inside the lipophilic pocket principally constituted of V2.58 and L7.43 determined a different rearrangement of the decyl alkyloxy chain with the consequent loss of the H bond between the phenolic substituent and K3.28.

Finally the displacement of the alkyl chain from the 5′ (compound **CB 25**, K_i = 0.0052) to the 4′ position (compound **CB 43**,[16] Fig. 4) determined a complete loss of affinity (K_i > 10 μM). The docking analysis revealed that the *n*-hexyl chain was unable to interact with F3.25, V6.59, and F7.35, and the disposition of the ligand was completely overturned: the phenolic system was directed toward the TM2 and the carboxamide group was directed toward the extracellular side of the receptor (see Fig. 5h).

5. Conclusions

In this study, we have designed and synthetized 21 new olivetol and hexylresorcinol-derived compounds with the aim to identify the structural modifications that define affinity for cannabinoid receptors in this class of compounds, which are obtained by linking a stable and rigid structure, such as THC, to a flexible chain carrying an amidic 'head', as in AEA. The range of carbon atom length in the alkyloxy chain and the structural features of the amidic 'head' necessary to observe high affinity have been characterized. In fact, although compound **CB 25** still shows the lowest K_i values, many of the newly synthesized compounds exhibited strong affinity for CB_1 and CB_2 receptors and two of them exhibited also a reasonable selectivity for CB_1 versus CB_2 receptors. Moreover, molecular modeling studies fitted this new class of cannabinoid ligands into a CB_1 receptor model, and the qualitative analysis of the results was in general agreement with the CB_1 affinity constants observed experimentally for these derivatives.

6. Experimental

6.1. General information

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 in a Perkin-Elmer elemental apparatus Mod. 240 for C, H, N and the data are within ±0.4% of the theoretical values. ^{1}H NMR spectra were recorded 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 T.L.C. 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 columns 12.25 mm, packed with KP-Sil, 60A, 32–63 μM. Reagents were purchased from Sigma–Aldrich Srl (Italy) and used as received unless otherwise stated.

6.2. General method for esters

A mixture of phenolic compound (5.0 mmol), anhydrous potassium carbonate (2.5 mmol), and potassium fluoride (5.0 mmol) in dry acetone was refluxed under nitrogen atmosphere and continuous stirring for half an hour, and then a solution of the corresponding bromoalkylmethylester (5.0 mmol) in dry acetone was added, refluxing for another 48–72 h and checking the reaction by TLC. Afterwards the reaction mixture was concentrated, diluted with water, and extracted with chloroform. The extracts were collected, dried ($MgSO_4$), evaporated under reduced pressure, and the residue purified by column chromatography on silica gel. Chemical and physical data of all synthetized esters are reported in Table 2.

6.3. General methods for the final products

6.3.1. Method A. Each ester (1.0 mmol) was refluxed in a methanolic/aqueous sodium hydroxide solution (0.1 M, 3.0 equiv) for 3 h. Then the reaction mixture was allowed to get to room temperature, made acidic (pH 3–4) by adding diluted HCl, and finally extracted with ethyl acetate; the organic layer was dried and the solvent evaporated to yield the crude acid, which was thoroughly dried under vacuum before being subjected to the subsequent reaction without further manipulation. To a mixture of the crude acid (1.0 mmol), the appropriate amine (1.5 mmol), and 1-hydroxybenzotriazole (HOBt, 1.2 mmol) in dry dichloromethane or in dry acetonitrile, kept in an ice bath, a solution, in the same solvent, of 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide methyl-*p*-toluensulfonate (CMC, 1.5 mmol) was added dropwise under nitrogen atmosphere and continuous stirring. The reaction mixture was left to room temperature and stirring was continued for 24 h. The solvent was removed under reduced pressure and the residue was diluted with chloroform; the organic layer was washed with 5% aqueous $NaHCO_3$, then with 1N HCl and dried ($MgSO_4$). After drying and evaporation of the solvent, a crude residue was obtained and purified by column chromatography on silica gel.

6.3.2. Method B. Each ester (0.150 g) was dissolved, under nitrogen atmosphere and stirring, in redistilled ethanolamine (4 mL) and warmed at 120–130 °C for 4–6 h. The resulting mixture was diluted with water and extracted with chloroform; the extracts were washed with a solution of ammonium chloride, dried ($MgSO_4$), evaporated, and the raw material purified by column chromatography on silica gel.

6.3.3. 8-(3-Hydroxy-5-pentyl-phenoxy)-octanoic acid (2-hydroxy-ethyl)-amide (10). Pale pink oil (ethyl acetate) (65% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 6.32–6.25 (m, 3H), 3.87 (t, 2H, J = 6.4 Hz), 3.69 (t, 2H, J = 4.9 Hz), 3.43–3.35 (m, 2H), 2.47 (t, 2H, J = 7.3 Hz), 2.18 (t, 2H, J = 7.3 Hz), 1.73–1.56 (mm, 6H), 1.48–1.28 (mm, 10H), 0.86 (t, 3H, J = 6.1 Hz). MS m/z: 388 $[M+Na]^+$ (100), 753 $[2M+Na]^+$. Anal. Calcd for $C_{21}H_{35}NO_4$: C, 69.01; H, 9.65; N, 3.83. Found: C, 68.80; H, 9.98; N, 3.75.

6.3.4. 8-(3-Hydroxy-5-pentyl-phenoxy)-octanoic acid cyclopropylamide (11). Pale yellow oil ($CHCl_3$/MeOH = 48:2) (60% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 6.79 (s, 1H), 6.27–6.25 (m, 3H), 5.74 (s br, 1H), 3.87 (t, 2H, J = 6.6 Hz), 2.72–2.64 (m, 1H), 2.46 (t, 2H, J = 7.4 Hz), 2.12 (t, 2H, J = 7.2 Hz), 1.73–1.55 (mm, 6H), 1.34–1.23 (mm, 10H), 0.85 (t, 3H, J = 6.6 Hz), 0.80–0.70 (m, 2H), 0.49–0.44 (m, 2H). MS m/z: 362 $[M+1]^+$ (100). Anal. Calcd for $C_{22}H_{35}NO_3$: C, 73.09; H, 9.76; N, 3.87. Found: C, 72.91; H, 9.58; N, 3.90.

6.3.5. 11-(3-Hydroxy-5-pentyl-phenoxy)-undecanoic acid cyclopropylmethyl-amide (12). Pasty pale yellow solid ($CHCl_3$/MeOH = 50:1) (96% yield): mp 54–55 °C (K). ^{1}H NMR ($CDCl_3$) δ (ppm): 6.26 (s, 3H), 5.72 (s br, 1H), 3.88 (t, 2H, J = 6.3 Hz), 3.13–3.07 (m, 2H), 2.46 (t, 2H, J = 7.7 Hz), 2.18 (t, 2H, J = 7.5 Hz), 1.74–1.52 (mm, 8H), 1.48–1.26 (mm, 14H), 0.96–0.92 (m, 1H), 0.86 (t, 3H, J = 6.3 Hz), 0.53–0.44 (m, 2H), 0.21–0.16 (m, 2H). MS m/z: 418 $[M+1]^+$ (100). Anal. Calcd for $C_{26}H_{43}NO_3$: C, 74.77; H, 10.38; N, 3.35. Found: C, 74.52; H, 10.57; N, 3.18.

6.3.6. 11-(3-Hydroxy-5-pentyl-phenoxy)-undecanoic acid [2-(3,4-dihydroxy-phenyl)-ethyl]-amide (13). Yellow oil ($CHCl_3$/MeOH = 50:1) (70% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 6.77 (d, 1H, J = 7.9 Hz), 6.71 (d, 1H, J = 1.3 Hz), 6.53 (d, 1H, J = 8.0 Hz), 6.28–6.22 (m, 3H), 5.70 (t br, 1H), 3.90 (t, 2H, J = 6.4 Hz), 3.60–3.41 (m, 2H), 2.66 (t, 2H, J = 6.8 Hz), 2.48 (t, 2H, J = 7.6 Hz), 2.12 (t, 2H, J = 7.5 Hz), 1.71–1.56 (mm, 8H), 1.32–1.03 (mm, 14H), 0.87 (t, 3H, J = 6.5 Hz). MS m/z: 522 $[M+Na]^+$ (100). Anal. Calcd for $C_{30}H_{45}NO_5$: C, 72.11; H, 9.08; N, 2.80. Found: C, 71.88; H, 9.12; N, 2.75.

6.3.7. 11-(3-Hydroxy-5-pentyl-phenoxy)-undecanoic acid (3-methoxy-4-hydroxy-phenyl)-amide (14). Pale yellow oil ($CHCl_3$/MeOH = 50:1) (88% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 6.86–6.76 (m, 3H), 6.26–6.24 (m, 3H), 6.17 (s, 1H), 5.72 (t br, 1H), 5.63 (s, 1H), 4.34 (d, 2H, J = 5.7 Hz), 3.88 (t, 2H, J = 6.6 Hz), 3.84 (s, 3H), 2.47 (t, 2H, J = 7.5 Hz), 2.19 (t, 2H, J = 7.3 Hz), 1.75–1.52 (mm, 8H), 1.49–1.26 (mm, 14H), 0.86 (t, 3H, J = 6.2 Hz). MS m/z: 522 $[M+Na]^+$ (100). Anal. Calcd for $C_{30}H_{45}NO_5$: C, 72.11; H, 9.08; N, 2.80. Found: C, 71.92; H, 9.03; N, 2.69.

6.3.8. 12-(3-Hydroxy-5-pentyl-phenoxy)-dodecanoic acid (2-hydroxy-ethyl)-amide (15). Transparent oil (ethyl acetate) (66% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 6.27–6.24 (m, 3H), 5.98 (s br, 1H), 3.89 (t, 2H, J = 6.4 Hz), 3.71 (t, 2H, J = 4.9 Hz), 3.45–3.37 (m, 2H), 2.48 (t, 2H, J = 7.5 Hz), 2.19 (t, 2H, J = 7.4 Hz), 1.69–1.79 (m, 2H), 1.66–1.53 (m, 4H), 1.49–1.26 (mm, 18H), 0.86 (t, 3H, J = 6.6 Hz). MS m/z: 444 $[M+Na]^+$ (100). Anal. Calcd for $C_{25}H_{43}NO_4$: C, 71.22; H, 10.28; N, 3.32. Found: C, 71.08; H, 10.41; N, 3.23.

6.3.9. 12-(3-Hydroxy-5-pentyl-phenoxy)-dodecanoic acid cyclopropylamide (16). White solid ($CHCl_3$/MeOH = 50:1) (91% yield): mp 75.5 °C (M). ^{1}H NMR ($CDCl_3$) δ (ppm): 6.27–6.26 (m, 3H), 5.78 (s br, 1H), 3.88 (t, 2H, J = 6.5 Hz), 2.73–2.62 (m, 1H), 2.47 (t, 2H, J = 7.6 Hz), 2.12 (t, 2H, J = 7.5 Hz), 1.75–1.49 (mm, 6H), 1.32–1.25 (mm, 18H), 0.86 (t, 3H, J = 6.6 Hz), 0.79–0.70 (m, 2H), 0.50–0.42 (m, 2H). MS m/z: 418 $[M+1]^+$ (100). Anal. Calcd for $C_{26}H_{43}NO_3$: C, 74.77; H, 10.38; N, 3.35. Found: C, 74.56; H, 10.22; N, 3.24.

6.3.10. 8-(2-Hexyl-5-hydroxy-phenoxy)-octanoic acid (2-hydroxy-ethyl)-amide (17). Pale yellow oil (ethyl acetate) (45% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 6.90 (d, 1H, J = 8.1 Hz), 6.37 (d, 1H, J = 2.1 Hz), 6.24 (dd, 1H, J = 2.2 Hz, J = 8.0 Hz), 6.13 (s br, 1H), 3.87 (t, 2H, J = 6.5 Hz), 3.69 (t, 2H, J = 4.9 Hz), 3.43–3.35 (m, 2H), 2.54–2.43 (m, 2H), 2.18 (t, 2H, J = 7.3 Hz), 1.76–1.58 (m, 4H), 1.1.46–1.28 (mm, 14H), 0.85 (t, 3H,

J = 6.3 Hz). MS m/z: 402 $[M+Na]^+$ (100), 781 $[2M+Na]^+$. Anal. Calcd for $C_{22}H_{37}NO_4$: C, 69.62; H, 9.83; N, 3.69. Found: C, 69.81; H, 9.90; N, 3.57.

6.3.11. 8-(2-Hexyl-5-hydroxy-phenoxy)-octanoic acid cyclopropylamide (18). White solid ($CHCl_3$/MeOH = 48:2) (75% yield): mp 53–55 °C (K). ^1H NMR ($CDCl_3$) δ (ppm): 6.90 (d, 1H, J = 8.0 Hz), 6.40 (s, 1H), 6.33 (dd, 1H, J = 2.4 Hz, J = 8.1 Hz), 5.64 (s br, 1H), 3.89 (t, 2H, J = 6.6 Hz), 2.71–2.66 (m, 1H), 2.48 (t, 2H, J = 7.0 Hz), 2.13 (t, 2H, J = 7.2 Hz), 1.77–1.53 (mm, 6H), 1.50–1.37 (mm, 6H), 1.36–1.25 (mm, 6H), 0.85 (t, 3H, J = 6.4 Hz), 0.80–0.70 (m, 2H), 0.49–0.41 (m, 2H). MS m/z: 376 $[M+1]^+$ (100). Anal. Calcd for $C_{23}H_{37}NO_3$: C, 73.56; H, 9.93; N, 3.73. Found: C, 73.72; H, 9.81; N, 3.62.

6.3.12. 11-(2-Hexyl-5-hydroxy-phenoxy)-undecanoic acid cyclopropylmethyl-amide (19). Pasty pale yellow solid ($CHCl_3$/MeOH = 48:2) (96% yield): mp 49–50 °C (K). ^1H NMR ($CDCl_3$) δ (ppm): 6.90 (d, 1H, J = 7.9 Hz), 6.70 (s br, 1H), 6.40–6.31 (m, 2H), 5.68 (s br, 1H), 3.86 (t, 2H, J = 6.3 Hz), 3.13–3.07 (m, 2H), 2.48 (t, 2H, J = 7.5 Hz), 2.17 (t, 2H, J = 7.5 Hz), 1.77–1.43 (mm, 8H), 1.39–1.02 (m, 16H), 0.98–0.92 (m, 1H), 0.85 (t, 3H, J = 6.0 Hz), 0.53–0.40 (m, 2H), 0.21–0.13 (m, 2H). MS m/z: 432 $[M+1]^+$ (100). Anal. Calcd for $C_{27}H_{45}NO_3$: C, 75.13; H, 10.51; N, 3.24. Found: C, 74.95; H, 10.73; N, 3.40.

6.3.13. 11-(2-Hexyl-5-hydroxy-phenoxy)-undecanoic acid [2-(3,4-dihydroxy-phenyl)-ethyl]-amide (20). Pale yellow oil ($CHCl_3$/MeOH = 47:3) (70% yield). ^1H NMR ($CDCl_3$) δ (ppm): 6.91 (d, 1H, J = 7.9 Hz), 6.78 (d, 1H, J = 8.0 Hz), 6.71 (d, 1H, J = 2.1 Hz), 6.55 (dd, 1H, J = 2.1, J = 8.1 Hz), 6.37–6.31 (m, 2H), 5.65 (t br, 1H), 3.85 (t, 2H, J = 6.3 Hz), 3.46–3.42 (m, 2H), 2.65 (t, 2H, J = 6.9 Hz), 2.49 (t, 2H, J = 7.4 Hz), 2.12 (t, 2H, J = 7.5 Hz), 1.74–1.70 (m, 2H), 1.58–1.47 (m, 4H,), 1.42–1.24 (mm, 18H), 0.85 (t, 3H, J = 6.3 Hz). MS m/z: 514 $[M+1]^+$ (100). Anal. Calcd for $C_{31}H_{47}NO_5$: C, 72.48; H, 9.22; N, 2.73. Found: C, 72.25; H, 9.48; N, 2.89.

6.3.14. 11-(2-Hexyl-5-hydroxy-phenoxy)-undecanoic acid (3-methoxy-4-hydroxy-phenyl)-amide (21). Pale pink oil ($CHCl_3$/MeOH = 50:2) (50% yield). ^1H NMR ($CDCl_3$) δ (ppm): 6.92–6.71 (m, 4H), 6.38 (d, 1H, J = 2.3 Hz), 6.32 (dd, 1H, J = 2.3 Hz, J = 8.0 Hz), 5.75 (t br, 1H), 5.67 (s, 1H), 4.34 (d, 2H, J = 5.5 Hz), 3.87 (t, 2H, J = 6.3 Hz), 3.84 (s, 3H), 2.49 (t, 2H, J = 7.1 Hz), 2.19 (t, 2H, J = 7.3 Hz), 1.81–1.63 (mm, 6H), 1.59–1.43 (m, 4 H), 1.39–1.27 (mm, 14H), 0.86 (t, 3H, J = 6.5 Hz). MS m/z: 514 $[M+1]^+$ (100). Anal. Calcd for $C_{31}H_{47}NO_5$: C, 72.48; H, 9.22; N, 2.73. Found: C, 72.33; H, 9.40; N, 2.84.

6.3.15. 12-(2-Hexyl-5-hydroxy-phenoxy)-dodecanoic acid (2-hydroxy-ethyl)-amide (22). Pasty white solid ($CHCl_3$/MeOH = 50:1) (50% yield): mp 57–58 °C (K). ^1H NMR ($CDCl_3$) δ (ppm): 6.91 (d, 1H, J = 8.00 Hz), 6.37 (s, 1H), 6.32 (dd, 1H, J = 2.0 Hz, J = 8.0 Hz), 6.08 (s br, 1H), 3.88 (t, 2H, J = 6.3 Hz), 3.72 (t, 2H, J = 4.9 Hz), 3.45–3.37 (m, 2H), 2.50 (t, 2 H, J = 7.4 Hz), 2.19 (t, 2H, J = 7.5 Hz), 1.79–1.69 (m, 2H), 1.65–1.44 (m, 4H), 1.41–1.27 (mm, 20H), 0.87 (t, 3H, J = 6.1 Hz). MS m/z: 458 $[M+Na]^+$ (100). Anal. Calcd for $C_{26}H_{45}NO_4$: C, 71.68; H, 10.41; N, 3.22. Found: C, 71.52; H, 10.56; N, 3.37.

6.3.16. 12-(2-Hexyl-5-hydroxy-phenoxy)-dodecanoic acid cyclopropylamide (23). White bright solid ($CHCl_3$/MeOH = 50:1) (90% yield): mp 53–55 °C (K). ^1H NMR ($CDCl_3$) δ (ppm): 6.91 (d, 1H, J = 8.0 Hz), 6.39–6.30 (m, 2H), 5.57 (s br, 1H), 3.88 (t, 2H, J = 6.3 Hz), 2.70–2.66 (m, 1H, CH), 2.49 (t, 2H, J = 7.1 Hz), 2.11 (t, 2H, J = 7.2 Hz), 1.79–1.51 (mm, 6H), 1.47–1.26 (mm, 20H), 0.86 (t, 3H, J = 6.2 Hz), 0.77–0.71 (m, 2H), 0.50–0.42 (m, 2H). MS m/z: 432 $[M+1]^+$ (100). Anal. Calcd for $C_{27}H_{45}NO_3$: C, 75.13; H, 10.51; N, 3.24. Found: C, 74.95; H, 10.66; N, 3.41.

6.3.17. 8-(4-Hexyl-3-hydroxy-phenoxy)-octanoic acid (2-hydroxy-ethyl)-amide (24). White solid ($CHCl_3$/MeOH = 47:3) (88% yield): mp 63–65 °C (K). ^1H NMR ($CDCl_3$) δ (ppm): 7.80 (s br, 1H), 6.93 (d, 1H, J = 8.1 Hz), 6.66 (s br, 1H), 6.38–6.31 (m, 2H), 3.82 (t, 2H, J = 5.8 Hz), 3.66–3.64 (m, 2H), 3.43–3.35 (m, 2H), 2.50 (t, 2H, J = 6.7 Hz), 2.15 (t, 2H, J = 6.3 Hz), 1.66–1.54 (m, 6H), 1.45–1.27 (mm, 12H), 0.85 (t, 3H, J = 6.3 Hz). MS m/z: 402 $[M+Na]^+$ (100). Anal. Calcd for $C_{22}H_{37}NO_4$: C, 69.62; H, 9.83; N, 3.69. Found: C, 69.78; H, 9.94; N, 3.55.

6.3.18. 8-(4-Hexyl-3-hydroxy-phenoxy)-octanoic acid cyclopropylamide (25). White bright solid ($CHCl_3$/MeOH = 48:2; recrystallized n-hexane/ethyl acetate) (81% yield): mp 123.4 °C (M). ^1H NMR ($CDCl_3$) δ (ppm): 9.50 (s, 1H), 8.15 (s br, 1H), 6.84 (d, 1H, J = 8.1 Hz), 6.29 (d, 1H, J = 2.5 Hz), 6.23 (dd, 1H, J = 2.4 Hz, J = 8.4 Hz), 3.80 (t, 2H, J = 6.5 Hz), 2.59–2.50 (m, 1H), 2.38 (t, 2H, J = 7.0 Hz), 1.96 (t, 2H, J = 7.1 Hz), 1.62–1.41 (m, 6H), 1.37–1.22 (mm, 12H), 0.82 (t, 3H, J = 6.4 Hz), 0.59–0.49 (m, 2H), 0.35–0.30 (m, 2H). MS m/z: 376 $[M+1]^+$ (100). Anal. Calcd for $C_{23}H_{37}NO_3$: C, 73.56; H, 9.93; N, 3.73. Found: C, 73.41; H, 9.98; N, 3.80.

6.3.19. 11-(4-Hexyl-3-hydroxy-phenoxy)-undecanoic acid cyclopropylmethyl-amide (26). White solid ($CHCl_3$/MeOH = 50:1) (83.0% yield): mp 87.3 °C (M). ^1H NMR ($CDCl_3$) δ (ppm): 8.90 (s, 1H), 7.63 (s br, 1H), 6.80 (d, 1H, J = 8.4 Hz), 6.26 (d, 1H, J = 2.3 Hz), 6.16 (dd, 1H, J = 2.1 Hz, J = 8.3 Hz), 3.76 (t, 2H, J = 6.2 Hz), 2.90 (t, 2H, J = 6.1 Hz), 2.36 (t, 2H, J = 7.3 Hz), 2.00 (t, 2H, J = 7.3 Hz), 1.62–1.42 (mm, 6H), 1.40–1.20 (mm, 18H), 0.83–0.77 (m, 4H), 0.38–0.31 (m, 2H), 0.11–0.04 (m, 2H). MS m/z: 432 $[M+1]^+$ (100). Anal. Calcd for $C_{27}H_{45}NO_3$: C, 75.13; H, 10.51; N, 3.24. Found: C, 74.98; H, 10.69; N, 3.38.

6.3.20. 11-(4-Hexyl-3-hydroxy-phenoxy)-undecanoic acid [2-(3,4-dihydroxy-phenyl)-ethyl]-amide (27). Pale yellow bright solid ($CHCl_3$/MeOH = 50:1) (70.0% yield): mp 97–100 °C (K). ^1H NMR ($CDCl_3$) δ (ppm): 6.96 (d, 1H, J = 8.9 Hz), 6.83–6.66 (m, 3H), 6.54 (d, 1H,

J = 8.2 Hz), 6.40–6.36 (m, 1H), 5.63 (t br, 1H), 3.88 (t, 2H, J = 6.3 Hz), 3.52–3.42 (m, 2H), 2.67 (t, 2H, J = 6.9 Hz), 2.51 (t, 2H, J = 7.6 Hz), 2.13 (t, 2H, J = 7.5 Hz), 1.74–1.68 (m, 2H), 1.60–1.52 (m, 4H), 1.40–1.24 (mm, 18H), 0.86 (t, 3H, J = 6.6 Hz). MS *m/z*: 514 $[M+1]^+$ (100). Anal. Calcd for $C_{31}H_{47}NO_5$: C, 72.48; H, 9.22; N, 2.73. Found: C, 72.30; H, 9.45; N, 2.86.

6.3.21. 11-(4-Hexyl-3-hydroxy-phenoxy)-undecanoic acid (3-methoxy-4-hydroxy-phenyl)-amide (28). White solid ($CHCl_3$/MeOH = 50:1) (84.0% yield): mp 103–104 °C (K). ^1H NMR ($CDCl_3$) δ (ppm): 7.00–6.71 (m, 3H), 6.42–6.34 (m, 2H), 6.19 (s, 1H), 5.76 (t br, 1H), 5.67 (s, 1H), 4.34 (d, 2H, J = 5.7 Hz), 3.87 (t, 2H, J = 6.5 Hz), 3.84 (s, 3H), 2.51 (t, 2H, J = 7.1 Hz), 2.19 (t, 2H, J = 7.1 Hz), 1.74–1.51 (mm, 8H), 1.48–1.07 (mm, 16H), 0.86 (t, 3H, J = 6.4 Hz). MS *m/z*: 514 $[M+1]^+$ (100). Anal. Calcd for $C_{31}H_{47}NO_5$: C, 72.48; H, 9.22; N, 2.73. Found: C, 72.28; H, 9.46; N, 2.89.

6.3.22. 12-(4-Hexyl-3-hydroxy-phenoxy)-dodecanoic acid (2-hydroxy-ethyl)-amide (29). White solid (ethyl acetate) (40.0% yield): mp 80.5 °C (M). ^1H NMR ($CDCl_3$) δ (ppm): 6.95 (d, 1H, J = 8.9 Hz), 6.42–6.36 (m, 2H), 5.96 (s br, 1H), 3.88 (t, 2H, J = 6.3 Hz), 3.72 (t, 2H, J = 4.4 Hz), 3.45–3.38 (m, 2H), 2.50 (t, 2H, J = 7.2 Hz), 2.20 (t, 2H, J = 7.3 Hz), 1.76–1.51 (mm, 6H), 1.48–1.25 (mm, 20H), 0.86 (t, 3H, J = 6.5 Hz). MS *m/z*: 436 $[M+1]^+$, 458 $[M+Na]^+$ (100). Anal. Calcd for $C_{26}H_{45}NO_4$: C, 71.68; H, 10.41; N, 3.22. Found: C, 71.49; H, 10.58; N, 3.40.

6.3.23. 12-(4-Hexyl-3-hydroxy-phenoxy)-dodecanoic acid cyclopropylamide (30). White bright solid ($CHCl_3$/MeOH = 50:1) (90.0% yield): mp 115.9 °C (M). ^1H NMR ($CDCl_3$) δ (ppm): 6.96 (d, 1H, J = 7.9 Hz), 6.41–6.36 (m, 2H), 5.57 (s br, 1H), 3.90 (t, 2H, J = 6.4 Hz), 2.73–2.69 (m, 1H), 2.52 (t, 2H, J = 7.2 Hz), 2.12 (t, 2H, J = 7.4 Hz), 1.77–1.61 (mm, 6H), 1.57–1.28 (mm, 20H), 0.87 (t, 3H, J = 6.5 Hz), 0.81–0.72 (m, 2H), 0.51–0.43 (m, 2H). MS *m/z*: 432 $[M+1]^+$ (100). Anal. Calcd for $C_{27}H_{45}NO_3$: C, 75.13; H, 10.51; N, 3.24. Found: C, 74.98; H, 10.63; N, 3.38.

6.4. Molecular modeling

The ligands were submitted to a conformational search of 1000 steps with an energy window for saving structure of 10 kJ/mol. The algorithm used was the Montecarlo method with MMFFs as the forcefield 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/Åmol, using the same forcefield and dielectric constant used for the conformational search.[25] Then the ligand was docked into both receptors using the AUTODOCK 3.0 program.[26] The regions of interest used by AUTODOCK were defined by considering T3.33 as the central residue of a grid of 56, 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.

Using the Lamarckian Genetic Algorithm, the compound was 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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