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Resorcinol-*sn*-Glycerol Derivatives: Novel 2-Arachidonoylglycerol Mimetics Endowed with High Affinity and Selectivity for Cannabinoid Type 1 Receptor

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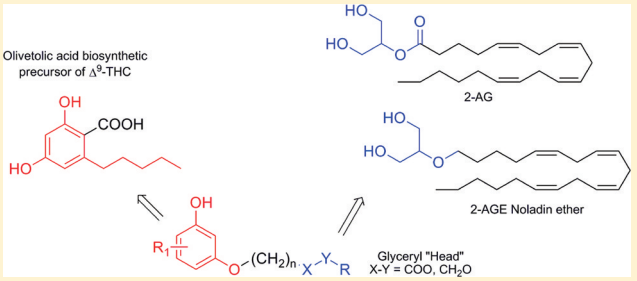
Supporting Information

ABSTRACT: Since the discovery of the endocannabinoid system, evidence has been progressively accumulating to suggest that 2-arachidonoylglycerol (2-AG) rather than anandamide (AEA) is the endogenous ligand for both cannabinoid (CB) receptors. Moreover, other studies have shown that another lipid molecule, 2-arachidonyl-glycerol ether (2-AGE, noladin ether), which acts as a full agonist at cannabinoid receptors, might occur in tissues. Having previously designed a resorcinol−AEA hybrid model, in this paper we have explored the cannabinoid receptor binding properties, the CB_1 functional activity, and the stability to plasma esterases of a novel series of compounds characterized by the conversion of the amide head into the glycerol-ester or glycerol-ether head, typical of 2-AG or the "putative" endocannabinoid 2-AGE, respectively. Glyceryl esters **39** and **41** displayed greater potency for CB_1 (K_i in the nanomolar range) than for CB_2 receptors plus the potential to be exploited as useful hits for the development of novel 2-AG mimetics.



INTRODUCTION

Only in the last 20 years have the endogenous counterparts of (−)-*trans*-Δ^9-tetrahydrocannabinol (THC), the main psychoactive principle in marijuana, been identified, opening the way for the identification of a rather complex innate signaling system of local lipid mediators, better known as the endocannabinoid system (ES).[1] Interest in investigating and understanding more about the (patho)physiological functions of this plastic system, characterized by at least two specific G-protein-coupled receptors, named CB_1[2] and CB_2,[3] their endogenous ligands,[4] and the enzymatic pathways for their biosynthesis[5] and degradation,[5a,6] is increasing due to the multiplicity of endocannabinoid actions and targets and the complexity of endocannabinoid regulation and signaling. Indeed, information about this lipid signaling system is still "evolving" as new bioactive endocannabinoid-related molecules, non-CB_1 non-CB_2 receptors for endocannabinoids and enzymes for endocannabinoid pathways are being recognized every year.[7]

Two arachidonoyl-derivatives are still referred to as the "major" endocannabinoids, i.e. anandamide (AEA, *N*-arachidonoyl-ethanolamine)[4a] and 2-AG (2-arachidonoyl-glycerol),[4b,c] whose pharmacological activity and metabolic pathways have been carefully investigated; moreover, the brain seems to produce at least three other chemically similar "endocannabinoids": 2-arachidonyl-glycerol ether (2-AGE, noladin ether),[8] *N*-arachidonoyl-dopamine (NADA),[9] and virodhamine[10] (Chart 1). More recently, a nonapeptide known as "hemopressin" has been proposed to be the first endogenous antagonist/inverse agonist of CB_1 receptors, following its detection in the brain.[11]

2-AG is a unique monoglyceride of arachidonic acid esterified at the *sn*-2 position, the levels of which in the brain are much higher than those of AEA.[4c,12] It is able to bind as a full agonist to both CB_1 and CB_2 cannabinoid receptors.[13] While 2-AG exhibits a lower CB_1 affinity than anandamide, evidence has progressively accumulated to suggest that it may be the main endogenous agonist for both cannabinoid receptors, eliciting biological activities as a signaling molecule, for example in the

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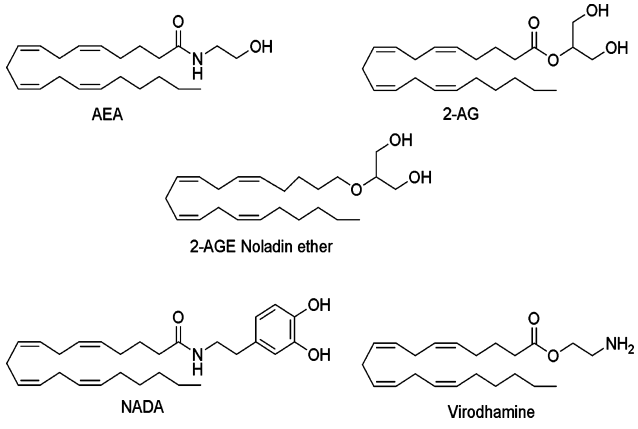
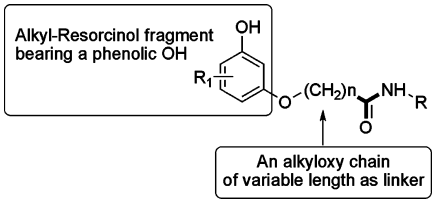
Chart 1. Chemical Structures of AEA, 2-AG, and the other Proposed Endocannabinoids



nervous system, i.e. in neuroprotection and neuromodulation, in the immune system, in cell proliferation, in inflammatory responses, and in the cardiovascular system.[14] Because 2-AG is a metabolically labile molecule, rapidly hydrolyzed by the monoglyceride lipase (MAGL),[15] it has been somewhat challenging to assess its biological activity precisely, especially in vivo; hence, 2-AGE, the ether analogue of 2-AG, was synthesized[4b,16] as a stable chemical tool and tested for its binding activity at cannabinoid receptors.[8a] It has also been postulated to be a novel endocannabinoid.[8a] However, its proposed role as an endogenously occurring compound is still under debate[8b,17] and therefore it is better referred to as a "putative" endocannabinoid, 2-AGE has been shown to act as a full agonist at both cannabinoid receptor subtypes.[8a,18,19] Furthermore, 2-AGE exerts typical cannabimimetic activity in vivo (i.e., hypothermia, antinociception, decreased locomotor activity) and has been shown to reduce the intraocular pressure through the ocular CB_1 receptor.[20]

There has been a growing interest in the development of new compounds that selectively regulate and/or modify the action and the levels of endocannabinoids, and many attempts have been made to obtain stable structural analogues of 2-AG[21] and novel homologues and isomers of 2-AGE[22] to investigate the structure–affinity relationships underlying the biological activity of alkyl glyceryl ethers. During our research in the cannabinoid field, we have designed and developed an attractive medicinal chemistry template[23] which turned out to produce potent cannabimimetic ligands (Chart 2). To validate our

Chart 2. Proposed Pharmacophore Model for the Resorcinol-AEA "Hybrids"



resorcinol–AEA hybrid model, a novel series of 23 compounds chemically characterized by the conversion of the amide head into the glycerol-ester or glycerol-ether head (Figure 1), typical of 2-AG and 2-AGE, respectively, were synthesized and their cannabimimetic properties evaluated. Important chemical

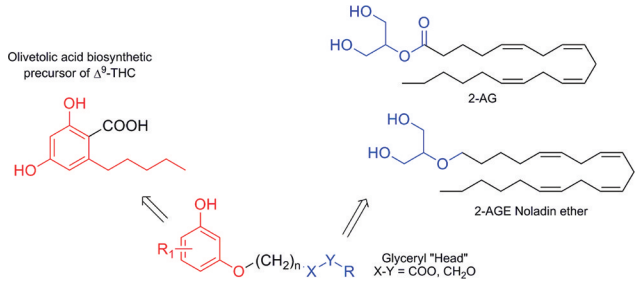


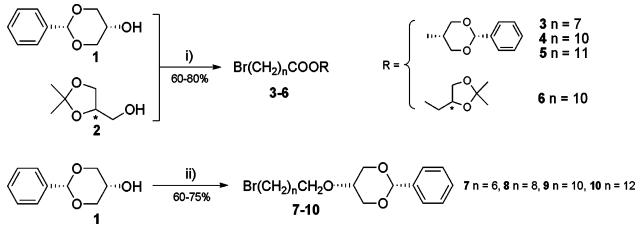
Figure 1. General chemical structure of new synthesized compounds.

features of these molecules are: (i) the rigid aromatic backbone, (ii) the linear or branched aliphatic tail, and (iii) the flexible alkyloxy chain bearing the ester/ether glyceryl head. These modifications were carried out to replace the metabolically unstable polyunsaturated fatty acid,[24] typical of endocannabinoids, with a stable aromatic scaffold, characteristic of the phytocannabinoid THC, containing the suitable pharmacophore determinants for cannabinoid receptor recognition. Moreover, because the available literature on the structure–activity relationships of arachidonoylglycerol analogues suggested that 1(3)-arachidonoylglycerol may exhibit CB_1 and CB_2 receptor binding affinities similar to or only slightly lower than that of 2-AG,[25] *sn*-1(3)-glycerol ester regioisomers were prepared and tested in cannabinoid receptor binding assays. In view of the generally high susceptibility of the ester functionality to plasma esterases, the in vitro stability of one of the new compounds in the presence of human plasma was also assessed. Finally, because the cytochrome enzyme superfamily is involved in the primary oxidation of several endogenous and/or exogenous compounds,[26] with lipophilicity being one of the major factors in the molecular recognition of different substrates,[27] the metabolic stability of the same compound was investigated, using an in vitro human recombinant cytochrome *P*450 3A4 assay (hCYP3A4) coupled with MS detection.[28]

CHEMISTRY

As depicted in Scheme 1, the bromoalkyl-glycerol esters **3**–**6** were obtained in 60–80% yield by reacting commercially

Scheme 1. Synthesis of Protected Compounds 3–6 and 7–10[a]



[a]Reagents: (i) $Br(CH_2)_nCOOH$, DCC, DMAP, dry THF, rt, overnight; (ii) $Br(CH_2)_nBr$, NaH, dry THF, 0 °C to reflux, overnight

available *cis*-1,3-benzylideneglycerol **1** or (±)-solketal **2** with the appropriate bromoacids in the presence of *N*,*N*′-dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino)-pyridine (DMAP). On the other hand, etherification of various dibromoalkanes with *cis*-1,3-benzylideneglycerol **1** in dry THF in the presence of sodium hydride afforded the bromoalkyl-glycerol ethers **7**–**10** in 60–75% yield.

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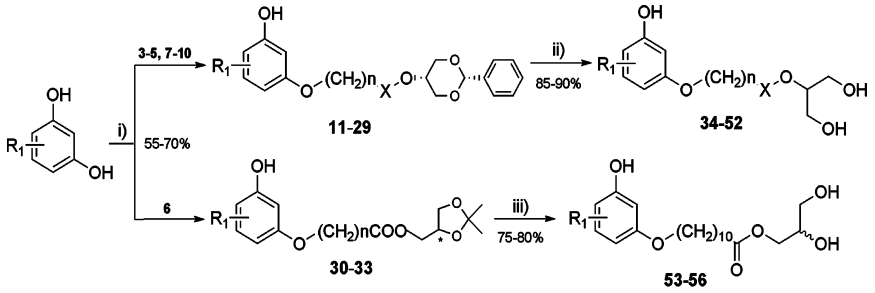
Scheme 2. Synthesis of Tested Compounds 34−52 and 53−56[a]



11, **34** R_1 = 5-C_5H_{11}, n = 7, X = CO
12, **35** R_1 = 5-C_5H_{11}, n = 10, X = CO
13, **36** R_1 = 4-C_6H_{13}, n = 7, X = CO
14, **37** R_1 = 4-C_6H_{13}, n = 10, X = CO
15, **38** R_1 = 2-C_6H_{13}, n = 7, X = CO
16, **39** R_1 = 2-C_6H_{13}, n = 10, X = CO
17, **40** R_1 = 5-$C(CH_3)_2C_6H_{13}$, n = 7, X = CO
18, **41** R_1 = 5-$C(CH_3)_2C_6H_{13}$, n = 10, X = CO
19, **42** R_1 = 5-$C(CH_3)_2C_6H_{13}$, n = 11, X = CO
20, **43** R_1 = 5-C_5H_{11}, n = 5, X = CH_2
21, **44** R_1 = 5-C_5H_{11}, n = 7, X = CH_2
22, **45** R_1 = 5-C_5H_{11}, n = 9, X = CH_2
23, **46** R_1 = 5-C_5H_{11}, n = 11, X = CH_2
24, **47** R_1 = 4-C_6H_{13}, n = 5, X = CH_2
25, **48** R_1 = 4-C_6H_{13}, n = 9, X = CH_2
26, **49** R_1 = 5-$C(CH_3)_2C_6H_{13}$, n = 5, X = CH_2
27, **50** R_1 = 5-$C(CH_3)_2C_6H_{13}$, n = 7, X = CH_2
28, **51** R_1 = 5-$C(CH_3)_2C_6H_{13}$, n = 9, X = CH_2
29, **52** R_1 = 5-$C(CH_3)_2C_6H_{13}$, n = 11, X = CH_2
30, **53** R_1 = 5-C_5H_{11}
31, **54** R_1 = 4-C_6H_{13}
32, **55** R_1 = 2-C_6H_{13}
33, **56** R_1 = 5-$C(CH_3)_2C_6H_{13}$

[a]Reagents: (i) acetone, K_2CO_3, KF, reflux, 48 h; (ii) EtOH/H_2O = 1/1, 37% HCl, 80−90 °C, 3 h; (iii) 60% acetic acid, EtOH, 40−50 °C, 3 h.

The synthetic route for the final products **34−56** is shown in Scheme 2. Appropriate alkyl-resorcinols, i.e. 5-pentylresorcinol (olivetol), 4-hexylresorcinol, and 5-(1,1-dimethylheptyl)-resorcinol, were alkylated with intermediates **3−10** in dry acetone in the presence of potassium carbonate and potassium fluoride to furnish the protected compounds **11−33** in moderate (55%) to good (70%) yields. Although *O*-alkylation of various resorcinols usually affords both monoalkylated and dialkylated products,[23] in this case, no dialkylated compound was isolated.

Finally, cleavage of the benzylidene or acetonide protecting groups by treatment with concentrated HCl[29] and 60% acetic acid,[22a] respectively, gave the final compounds **34−56** in 75−90% yield.

RESULTS AND DISCUSSION

In Vitro CB_1 and CB_2 Receptor Binding Assay. All the newly synthesized compounds were evaluated for their affinity at recombinant human CB_1 and CB_2 receptors stably overexpressed in HEK-293 cells, and the results are summarized in Table 1.

In the series of resorcinol-*sn*-2-glycerol esters **34−42**, almost all compounds, with the only exception of **34**, **36**, and **37**, behaved as potent cannabinoid receptor ligands, exhibiting affinity values in the submicromolar or nanomolar range of concentration. In agreement with our previous structure−affinity relationship observations,[23a,c] the most potent compounds were those characterized by the presence of a 5-(2-methyloctan-2-yl) tail (**40−42**) and/or an alkyloxy flexible chain of 10 carbon atoms (**35**, **39**, **41**): compound **41** proved to be the most potent CB_1 ligand (K_i 9.0 nM) endowed also with a good CB_1 selectivity (SI = 88, calculated as K_i CB_2/K_i CB_1 ratio). Very interesting was the opposite behavior of the two regioisomers derived from 4-hexylresorcinol; in fact, while the 4-hexyl-*sn*-2-glycerol esters (**36** and **37**) were weak CB_1 cannabinoid ligands, the 2-hexyl-*sn*-2-glycerol ester regioisomers (**38** and **39**) showed submicromolar or nanomolar CB_1 affinity values (K_i CB_1 0.8 μM and 56 nM, respectively) and a moderate or good selectivity toward CB_2. These binding data suggest that the length of the alkyloxy spacer markedly influences CB_1 receptor affinity, while it does not alter the interaction with CB_2 receptor. In fact, lengthening the chain from 10 (**39** and **41**) to 11 (**42**) or shortening it to seven carbon atoms (**38** and **40**) dramatically decreased CB_1 K_i values from 10 (**38**) to a thousand times (**40** and **42**) with no change in CB_2 affinity.

Regarding the group of resorcinol-*sn*-2-glycerol ethers **43−52**, replacement of the carbonyl functionality with a methylene group proved to be detrimental for cannabinoid receptor affinity properties. Only compounds **43**, **49**, and **50** behaved as quite potent cannabinoid receptor ligands, showing submicromolar affinity values for both receptor subtypes. In good agreement with previous published results for the 2-AGE series,[21b] the shorter alkyl chain, characterizing all these derivatives, seemed to yield the best results, whereas, once again, the 3-hydroxy-5-(1-methyloctan-2-yl)phenoxy backbone (**49** and **50**) possessed the optimal chemical properties for interacting with both cannabinoid receptors.

Finally, the regioisomeric resorcinol-*sn*-1(3)-glycerol esters **53−56** possessed a weak ability to interact with cannabinoid receptors. In fact, the shift of the acyl "head" from position 2 to position 1(3) of glycerol diminished significantly the cannabinoid receptor affinities and specifically caused a complete loss of interaction with the CB_1 receptor subtype, independently of the aromatic scaffold and the alkyloxy chain length. However, all these derivatives, though not very potent, yielded ligands selective toward CB_2, exhibiting K_i values in the low micromolar range. In agreement with our previous results,[23c] the best receptor interaction occurs when a 5-(2-methyloctan-2-yl) tail (compound **56**, K_i CB_2 = 0.31 μM) is present.

Functional Activity of 39 and 41 at CB_1 Receptors: MF1 Mouse Brain and hCB_1−CHO Cell Membranes. The CB_1 receptor activity of the compounds with the highest affinity for this receptor subtype, i.e. **39** and **41**, was evaluated by estimating their capacity to stimulate or inhibit [^{35}S]GTPγS

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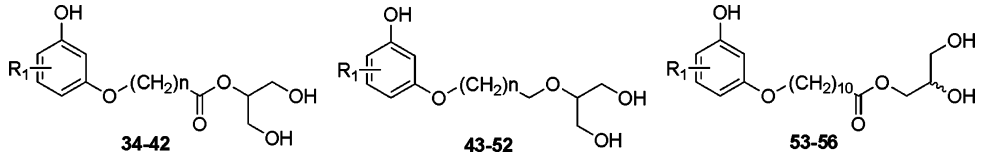
Table 1. Structure, CB_1 and CB_2 Receptor Affinity (K_i Values), and Selectivity of Derivatives 34−56 and Reference Compounds AEA, WIN 55,212-2, 2-AG, and 2-AGE[a]



compd	n	R′	CB_1 K_i (μM)	CB_2 K_i (μM)	CB_2/CB_1 SI
34	7	5-$(CH_2)_4CH_3$	5.60 ± 1.43	7.87 ± 2.01	
35	10	5-$(CH_2)_4CH_3$	5.60 ± 2.03	0.87 ± 0.06	
36	7	4-$(CH_2)_5CH_3$	>10	7.87 ± 1.54	
37	10	4-$(CH_2)_5CH_3$	>10	7.87 ± 3.21	
38	7	2-$(CH_2)_5CH_3$	**0.80** ± 0.32	1.80 ± 0.22	
39	10	2-$(CH_2)_5CH_3$	**0.06** ± 0.01	1.80 ± 0.21	**32**
40	7	5-$C(CH_3)_2(CH_2)_5CH_3$	1.12 ± 0.05	**0.79** ± 0.08	
41	10	5-$C(CH_3)_2(CH_2)_5CH_3$	**0.01** ± 0.002	**0.79** ± 0.07	**88**
42	11	5-$C(CH_3)_2(CH_2)_5CH_3$	2.71 ± 0.78	**0.80** ± 0.08	
43	5	5-$(CH_2)_4CH_3$	**0.43** ± 0.03	**0.91** ± 0.02	
44	7	5-$(CH_2)_4CH_3$	1.86 ± 0.28	1.16 ± 0.09	
45	9	5-$(CH_2)_4CH_3$	>10	>10	
46	11	5-$(CH_2)_4CH_3$	3.78 ± 1.68	>10	
47	5	4-$(CH_2)_5CH_3$	>10	1.13 ± 0.96	
48	9	4-$(CH_2)_5CH_3$	>10	2.39 ± 1.02	
49	5	$C(CH_3)_2(CH_2)_5CH_3$	**0.17** ± 0.08	**0.19** ± 0.05	
50	7	$C(CH_3)_2(CH_2)_5CH_3$	**0.27** ± 0.06	**0.11** ± 0.01	
51	9	$C(CH_3)_2(CH_2)_5CH_3$	2.94 ± 1.01	**0.38** ± 0.03	
52	11	$C(CH_3)_2(CH_2)_5CH_3$	1.04 ± 0.04	1.67 ± 0.10	
53		5-$(CH_2)_4CH_3$	>10	7.91 ± 1.13	
54		4-$(CH_2)_5CH_3$	>10	10.44 ± 0.55	
55		2-$(CH_2)_5CH_3$	>10	4.82 ± 0.90	
56		5-$C(CH_3)_2(CH_2)_5CH_3$	>10	**0.31** ± 0.06	
AEA			0.07		
WIN 55,212−2			0.02	0.002	0.1
2-AG[b]			0.47	1.4	3
2-AGE[c]			0.02	>3	

[a]Data represent mean value ± SEM for three separate experiments performed in duplicate 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 constants of the most potent compounds ($K_i \leq 1$ μM) are highlighted in bold as well as the most selective compounds for CB_1 and CB_2. [b]Reference 24. [c]Reference 8a.

binding to G proteins using MF1 mouse brain membranes. Dose−response curves for both compounds and the potent nonselective CB_1/CB_2 receptor agonist CP-55940 are shown in Figure 2.

Concentrations ranging from 1 nM up to 10 μM were tested and both EC_{50} and E_{max} values were considered (95% confidence limits in brackets). Compounds **41** and **39** stimulated [^{35}S]GTPγS binding to mouse whole brain membranes with high potency (EC_{50} = 6.3 nM (0.6 and 66.9) and 78.4 nM (9.7 and 635), respectively) (Figure 2). However, they exhibited very low efficacy, E_{max} = 14.7% (10.5 and 19.0) and 11.4% (5.5 and 17.3) as compared to the potent full cannabinoid receptor agonist CP-55,940 [EC_{50} = 42 nM (22.4 and 78.8) and E_{max} = 104% (93.3 and 115.2)]. Of the two tested compounds, **41** displayed higher potency than compound **39**.

Because both compounds showed a very low efficacy when tested per se for their effect on [^{35}S]GTPγS binding to mouse membranes, their ability to antagonize CP-55940-induced stimulation of [^{35}S]GTPγS binding to mouse brain membranes was also investigated. Experimental results showed that at 100 nM (compound **41**) and 1 μM (compound **39**), these compounds did not produce any significant rightward shift in the log concentration−response curve of CP-55940 (Figure 3). Next, we have evaluated the ability of both compounds to stimulate or inhibit [^{35}S]GTPγS binding to G proteins using CHO cells stably transfected with the human CB_1 receptor and the nonselective CB_1/CB_2 receptor agonist CP-55940 as active control (data not shown). Experimental results showed that both compounds did not produce any detectable effect in this assay (Figure 4), thus suggesting that they are more efficacious at antagonizing the mouse versus the human receptor, although behaving overall as very weak CB_1 antagonists. This rather surprising result led us to evaluate the chemical stability of compound **41** under the experimental conditions by incubating it in buffer at 30 °C for 90 min and checking by mass analysis after 60 and 90 min the presence of hydrolysis products in the sample. The experimental results indicated that **41** was stable under the conditions routinely used for this biological assay, thus arguing against the possibility that chemical ester hydrolysis was the cause of the low efficacy of the compound. However, the hypothesis of a rapid acyl migration from the

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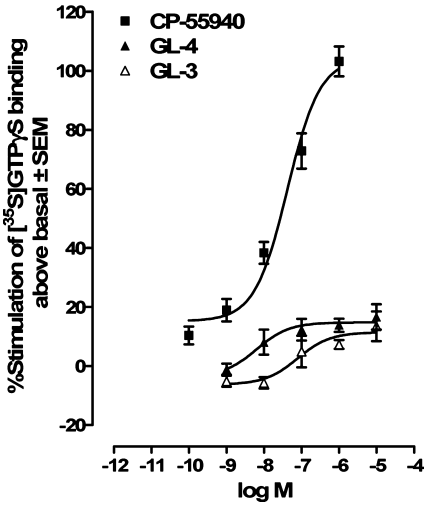

Figure 2. Effect of CP-55940 (n = 6), **41** (GL-4, n = 8), and **39** (GL-3, n = 6) on the level of [^{35}S]GTPγS binding to mouse whole brain membranes.

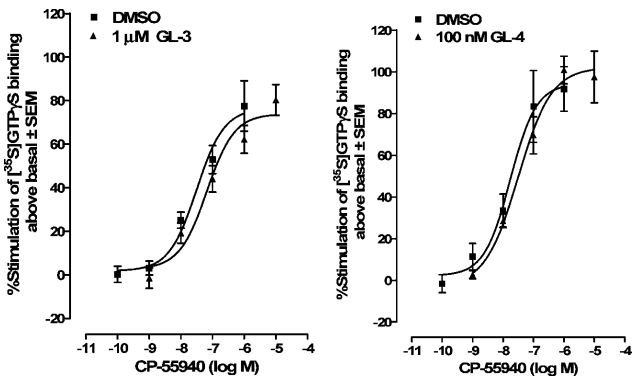

Figure 3. Effect of 1 μM of **39** (GL-3, n = 12) and of 100 nM of **41** (GL-4, n = 4) on the mean log concentration−response curve of CP-55940 (n = 12, n = 4, respectively) for stimulation of [^{35}S]GTPγS binding to mouse whole brain membranes.

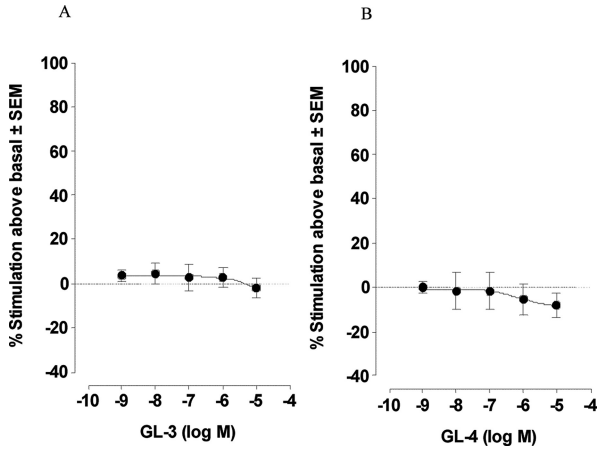

Figure 4. Mean log concentration−reponse curves of **39** (GL-3, (A)) and **41** (GL-4, (B)) (n = 6,7). Each symbol represents the mean percentage change in binding of [^{35}S]GTPγS to hCB$_1$−CHO cell membranes ± SEM.

2-position to the 1- or 3-position could still be possible. In fact, this process is characteristic of 2-monoglycerides[30] and the conversion of 2-AG to 1(3)-AG is well-known and highly dependent on the experimental conditions.[12,31] Acyl migration for these compounds would be relevant in the functional assays because the two regioisomers, i.e. **41** versus **56** and **39** versus **55**, showed a significant difference in the relative potencies (see Table 1).

Stability to Plasma Esterases. The enzymatic stability study was performed in human plasma and in water as control, according to the method previously described[32] with slight modifications. Known aliquots of compound **41** from a stock solution were added to plasma or water (final concentrations of **41** being 1×10^{-4} and 2×10^{-4} M) and incubated at 37 °C for specified time intervals (0, 15, 30, 60, and 120 min). At the end of the incubation period, the reaction was stopped by addition of acetonitrile and each sample, performed in triplicate, was subjected to mass analysis.

Independently of the test compound concentration, reverse-phase LC-MS analysis showed no significant hydrolytic activity of human plasma toward compound **41** compared to the water control (Figure 5).

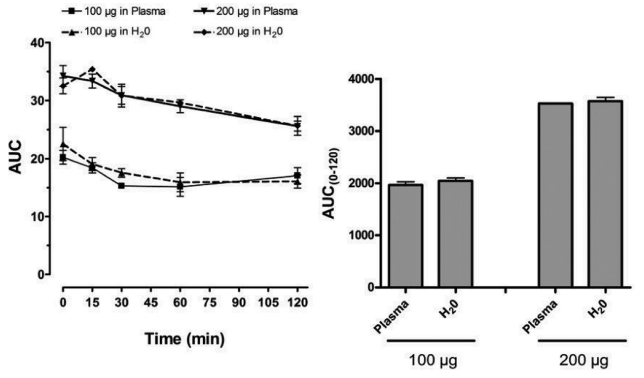

Figure 5. Mean (± SEM) changes in **41** concentration determined in plasma or water solutions incubated at 37 °C for different times (0−120 min) (A). The compound was tested at two different concentrations (■ 100 μM in plasma; ▲ 100 μM in water; ▼ 200 μM in plasma; ◆ 200 μM in water). Changes of the UV peak area under the experimental curve ($AUC_{(0-120\ min)}$) of **41** relative to each concentration was also shown (B). The comparison between $AUC_{(0-120\ min)}$ of water vs plasma samples at 100 or 200 μM **41** concentration was performed by using Student's t test.

Metabolic Stability. A known concentration of compound **41** was incubated with the hCYP3A4 in the presence or absence of NADPH as cofactor, the final enzyme concentration corresponding to 0.1−0.2 mg/mL of microsomal protein. After 1 h incubation, the remaining percentage of compound **41** was 24% compared to the typical value of 10% for test compounds. Therefore, a low substrate conversion was measured under these experimental conditions, indicating a notably metabolic stability for compound **41**.

■ CONCLUSIONS

Analysis of the binding assay results for this novel series of 23 compounds was multifaceted, and it was not possible to find a linear and rational trend in the structure−affinity relationships. In fact, even if all the synthesized compounds shared the hypothesized key pharmacophoric features for interaction with cannabinoid receptors, most of them behaving as cannabinoid receptor ligands, replacement of the amide head with a glycerol-ester or a glycerol-ether head affected the affinity in a complex manner, thus leading to compounds expressing very different

affinity values and cannabinoid receptor subtype selectivity. The carbonyl group seemed to play a crucial role in the cannabinoid receptor interaction because derivatives having an ester linkage as a structural feature still behaved as cannabinoid ligands, while those characterized by an ether bridge showed weak cannabinoid ligand properties. Interestingly, unlike endocannabinoids, in which the metabolically stable glyceryl ether moiety still endowed the compounds with high cannabinoid receptor affinity, our hybrids appeared to be substantially more sensitive to structural changes in the amide moiety, yielding ether derivatives with low affinities. Moreover, our results underline the significant influence of the alkyloxy-chain length and the lipophilic tail on the cannabinoid receptor interaction. The in vitro functional data presented here suggest that these compounds exhibit only little efficacy in the [^{35}S]GTPγS binding to mouse brain membranes and did not show any detectable effect in the same assay to hCB$_1$−CHO cell membranes. Last, because it is obvious that any investigation of the functional activity of these resorcinol-*sn*-glycerol derivatives must take into consideration their stability profile, our findings suggest that these novel compounds are not very sensitive to chemical and enzymatic hydrolysis and possess acceptable metabolic stability. These latter data, while indicating that our novel compounds may represent useful leads for the future development of novel 2-AG mimetics, also leave open the question of the reasons underlying their very weak functional activity, at least in our in vitro assays. It is possible that, like 2-AG,[31] compound **41** undergoes rapid isomerization to the 1(3)-glycerol ester during the GTPγS binding assay and that this leads to loss of functional activity.[33]

EXPERIMENTAL SECTION

Chemicals, Materials, and Methods. All starting materials, reagents, and solvents were purchased from common commercial suppliers and were used as received unless otherwise indicated. 5-(1,1-Dimethylheptyl)resorcinol was obtained with 85% yield by cleavage of the methoxy groups from commercial 1,3-dimethoxy-5-(2-methyloctan-2-yl)benzene using boron tribromide in dry dichloromethane, as already described in our previous work.[23c] 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. ^{1}H NMR spectra and ^{13}C NMR spectra were recorded in the indicated solvent at 25 °C on a Bruker AC200F or on a Bruker Advance DPX400 employing TMS as internal standard and chemical shifts are expressed as δ (ppm). The chromatography−mass spectrometry (LC-MS) system consisted of an Agilent 1100 series liquid chromatograph system including a 1100 MSD model VL benchtop mass spectrometer with API-ES interface, a binary high-pressure gradient pump (0.4 mL/min low flow rate, employing a binary solvent system of 95/5 methanol/water), and a vacuum solvent degassing unit. The nebulizer gas, the drying gas, the capillary voltage, and the vaporizer temperature were set at 40 psi, 9 L/min, 3000 V, and 350 °C, respectively. Nitrogen (purity 99.995%) was used as nebulizer gas and drying gas. UV detection was monitored at 254 nm. Mass spectra were acquired in positive mode scanning over the mass range *m*/*z* of 105−1500. The structures of final compounds were unambiguously assessed by ^{1}H NMR, ^{13}C NMR, and MS. 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 columns 12.25 mm, packed with KP-Sil, 60A, 32−63 μM. Compound purity was assessed by elemental analysis on a Perkin-Elmer elemental apparatus model 240 for C, H, N, and the data are within ±0.4% of the theoretical values. All the tested compounds possessed a purity >95.0%.

General Procedure for the Synthesis of Bromoesters 3−6. To a mixture of the appropriate bromoacid (1.2 equiv), (±)-solketal (or *cis*-1,3-benzylideneglycerol, 1.0 equiv), and DMAP (0.3 equiv) in dry THF (15 mL), a solution of DCC (1.2 equiv) in the same dry solvent (5 mL) was added, under stirring and nitrogen atmosphere; then, the reaction mixture was kept at room temperature overnight. Afterward, the crude material, obtained from evaporation of solvent, was taken up in cold chloroform (15 mL) and the resulting cloudy solution was filtered, washed with water (5 × 8 mL), and dried over sodium sulfate. Chloroform was evaporated under reduced pressure to afford a residue, which was purified by silica gel column chromatography.

Example: *cis*-2-Phenyl-1,3-dioxan-5-yl 8-bromooctanoate (3). Eluent: $CHCl_3$. Yield: 70% (transparent oil). ^{1}H NMR ($CDCl_3$): δ 7.48−7.44 (m, 2H), 7.35−7.30 (m, 3H), 5.46 (s, 1H), 4.60 (s, 1H), 4.18 (d, 2H, *J* = 12.6 Hz), 4.03 (d, 2H, *J* = 12.8 Hz), 3.30 (t, 2H, *J* = 6.9 Hz), 2.36 (t, 2H, *J* = 7.4 Hz), 1.89−1.72 (m, 2H), 1.69−1.55 (m, 2H), 1.43−1.20 (mm, 6H). Anal. ($C_{18}H_{25}BrO_4$ (385.29)) C, H, N.

Example: (±)-(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl 11-Bromoundecanoate (6). Eluent: $CHCl_3$. Yield: 75% (white pasty solid); mp <30 °C (K). ^{1}H NMR ($CDCl_3$): δ 4.24−4.21 (m, 1H), 4.05 (t, 2H, *J* = 4.0 Hz), 3.99 (dd, 1H, *J* = 6.1 Hz, *J* = 7.7 Hz), 3.66 (dd, 1H, *J* = 6.3 Hz, *J* = 7.9 Hz), 3.32 (t, 2H, *J* = 6.9 Hz), 2.26 (t, 2H, *J* = 7.4 Hz), 1.81−1.71 (m, 2H), 1.55−1.51 (m, 2H), 1.35 (s, 3H), 1.29 (s, 3H), 1.27−1.10 (mm, 12H). Anal. ($C_{17}H_{31}BrO_4$ (379.33)) C, H, N.

General Procedure for the Synthesis of bromoethers 7−10. A mixture of *cis*-1,3-benzylidenglycerol (1.0 equiv) and NaH (1.2 equiv) in dry THF (10 mL) was stirred at 0 °C on an ice bath for an half hour, under nitrogen atmosphere; then, a solution of the appropriate dibromoalkane (1.0 equiv) in dry THF (10 mL) was added dropwise and the reaction mixture refluxed overnight. Afterward, the organic solvent was evaporated under reduced pressure, the residue diluted with a saturated aqueous solution of ammonium chloride, and the aqueous layer extracted with chloroform (4 × 15 mL). The collected extracts were washed with water, dried, evaporated, and the raw material so obtained was purified by silica gel column chromatography.

Example: *cis*-5-(6-Bromohexyloxy)-2-phenyl-1,3-dioxane (7). Eluent: $CHCl_3$. Yield: 72% (transparent oil). ^{1}H NMR ($CDCl_3$): δ 7.52−7.47 (m, 2H), 7.39−7.31 (m, 3H), 5.54 (s, 1H), 4.32 (d, 2H, *J* = 11.4 Hz), 4.03 (d, 2H, *J* = 11.5 Hz), 3.54 (t, 2H, *J* = 6.5 Hz), 3.39 (t, 2H, *J* = 6.8 Hz), 3.25−3.23 (m, 1H), 1.92−1.78 (m, 2H), 1.69−1.59 (m, 2H), 1.53−1.42 (m, 4H). MS *m*/*z*: 344 [M + H]$^+$ (25), 366 [M + Na]$^+$ (100). Anal. ($C_{16}H_{23}BrO_3$ (343.26)) C, H, N.

General Procedure for the Synthesis of Esters 11−19, 30−33, and Ethers 20−29. A mixture of the appropriate resorcinol derivative (olivetol, 4-hexylresorcinol, or 5-(1,1-dimethylheptyl)-resorcinol, 5.0 mmol), anhydrous potassium carbonate (0.35 g, 2.5 mmol), and potassium fluoride (0.29 g, 5.0 mmol) in dry acetone (30 mL) was heated to reflux with stirring under nitrogen atmosphere for 30 min; then, a solution of the appropriate bromo derivative (**3**−**6** and **7**−**10**, 5.0 mmol) in dry acetone (10 mL) was added, and the reaction mixture was maintained at reflux temperature for further 48 h. Afterward, the reaction mixture was concentrated, diluted with saturated ammonium chloride solution (20 mL), and extracted with chloroform (3 × 15 mL). The organic layers were collected, dried, and evaporated under reduced pressure to afford a residue, which was purified by silica gel column chromatography.

Example: *cis*-2-Phenyl-1,3-dioxan-5-yl 11-[3-Hydroxy-5-(2-methyloctan-2-yl)phenoxy]undecanoate (18). Eluent: $CHCl_3$/MeOH = 50/1. Yield: 65% (yellow oil). ^{1}H NMR ($CDCl_3$): δ 7.50−7.48 (m, 2H), 7.40−7.33 (m, 3H), 6.43 (s, 1H), 6.36 (s, 1H), 6.19 (s, 1H), 5.54 (s, 1H), 4.70 (s, 1H), 4.27 (d, 2H, *J* = 12.6 Hz), 4.14 (d, 2H, *J* = 13.0 Hz), 3.87 (t, 2H, *J* = 6.5 Hz), 2.41 (t, 2H, *J* = 7.4 Hz), 1.73−1.31 (mm, 6H), 1.51−1.21 (mm, 26H), 0.84 (t, 3H, *J* = 6.3 Hz). MS *m*/*z*: 605 [M + Na]$^+$ (100). Anal. ($C_{36}H_{54}O_6$ (582.81)) C, H, N.

Example: *cis*-3-(2-Methyloctan-2-yl)-5-[10-(2-phenyl-1,3-dioxan-5-yloxy)decyloxy]phenol (28). Eluent: $CHCl_3$/MeOH = 50/0.5. Yield: 58% (pale-yellow oil). ^1H NMR ($CDCl_3$): δ 7.56–7.52 (m, 2H), 7.46–7.33 (m, 3H), 6.41 (s, 1H), 6.36 (s, 1H), 6.20 (s, 1H), 5.53 (s, 1H), 4.32 (d, 2H, J = 12.6 Hz), 4.02 (d, 2H, J = 12.8 Hz), 3.87 (t, 2H, J = 6.5 Hz), 3.38 (t, 2H, J = 6.9 Hz), 3.24 (s, 1H), 1.85–1.73 (m, 2H), 1.69–1.50 (m, 4H), 1.46–1.20 (mm, 26H), 0.81 (t, 3H, J = 6.8 Hz). MS m/z: 577 $[M + Na]^+$ (100). Anal. ($C_{35}H_{54}O_5$ (554.80)) C, H, N.

Example: (±)-(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl 11-[3-hydroxy-5-(2-methyloctan-2-yl)phenoxy]undecanoate (33). Eluent: $CHCl_3$/MeOH = 50/2. Yield: 63% (yellow oil). ^1H NMR ($CDCl_3$): δ 6.37 (s, 1H), 6.33 (s, 1H), 6.20–6.16 (m, 1H), 4.29–4.18 (m, 1H), 4.07–3.95 (m, 3H), 3.87–3.78 (m, 2H), 3.66 (dd, 1H, J = 6.2 Hz, J = 7.9 Hz), 2.26 (t, 2H, J = 7.4 Hz), 1.67–1.55 (m, 2H), 1.51–1.41 (m, 2H), 1.35 (s, 3H), 1.28 (s, 3H), 1.22–1.14 (mm, 28H), 0.76 (t, 3H, J = 6.1 Hz). MS m/z: 557 $[M + Na]^+$ (100). Anal. ($C_{32}H_{54}O_6$ (534.77)) C, H, N.

General Procedure for the Synthesis of Compounds 34–52. To a solution of each benzylidene derivative (0.100 g) in ethanol/water (9 mL, 2/1) was added 37% HCl (0.5 mL) and the reaction mixture stirred at 80–90 °C. After 3 h, the solvent was evaporated under reduced pressure and the residue was diluted with ethyl acetate. The organic phase was washed with brine, dried over sodium sulfate, filtered, and finally concentrated to dryness, affording a crude material purified by silica gel column chromatography.

1,3-Dihydroxypropan-2-yl 8-(3-Hydroxy-5-pentylphenoxy)-octanoate (34). Eluent: $CHCl_3$/MeOH = 48/2. Yield 87% (yellow oil). ^1H NMR ($CDCl_3$): δ 6.27–6.20 (m, 3H), 4.14 (d, 1H, J = 5.2 Hz), 3.97–3.78 (m, 4H), 3.72–3.53 (m, 2H), 2.46 (t, 2H, J = 7.7 Hz), 2.31 (t, 2H, J = 7.4 Hz), 1.73–1.55 (mm, 6H), 1.34–1.24 (mm, 10H), 0.86 (t, 3H, J = 6.4 Hz). ^{13}C NMR ($CDCl_3$): δ 174.30, 160.03, 156.66, 145.70, 107.90, 107.18, 99.32, 70.31, 67.81, 65.17, 63.38, 36.03, 34.06, 31.51, 30.83 (×2), 28.99, 28.69, 25.70, 24.70, 22.53, 14.01. MS m/z: 397 $[M + H]^+$ (20), 419 $[M + Na]^+$ (100). Anal. ($C_{22}H_{36}O_6$ (396.52)) C, H, N.

1,3-Dihydroxypropan-2-yl 11-(3-hydroxy-5-pentylphenoxy)undecanoate (35). Eluent: $CHCl_3$/MeOH = 50/2. Yield 85% (yellow oil). ^1H NMR ($CDCl_3$): δ 6.29 (s, 1H), 6.23–6.21 (m, 2H), 4.18–4.14 (m, 1H), 3.95–3.81 (m, 4H), 3.69 (dd, 1H, J = 4.0 Hz, J = 11.6 Hz), 3.58 (dd, 1H, J = 5.8 Hz, J = 11.7 Hz), 2.45 (t, 2H, J = 7.6 Hz), 2.33 (t, 2H, J = 7.5 Hz), 1.76–1.69 (m, 2H), 1.66–1.52 (m, 4H), 1.19–1.28 (mm, 16H), 0.86 (t, 3H, J = 6.6 Hz). ^{13}C NMR ($CDCl_3$): δ 174.39, 160.09, 156.71, 145.59, 107.80, 107.12, 99.30, 70.29, 67.75, 65.21, 63.47, 36.01, 34.20, 31.44, 30.82 (×2), 29.32, 29.21, 29.12 (×2), 29.02, 25.78, 24.83, 22.51, 14.03. MS m/z: 439 $[M + H]^+$ (100). Anal. ($C_{25}H_{42}O_6$ (438.60)) C, H, N.

1,3-Dihydroxypropan-2-yl 8-(4-hexyl-3-hydroxyphenoxy)-octanoate (36). Eluent: $CHCl_3$/MeOH = 47/3. Yield 89% (yellow oil). ^1H NMR ($CDCl_3$): δ 6.95 (d, 1H, J = 8.0 Hz), 6.40–6.35 (m, 2H), 4.15 (d, 1H, J = 4.7 Hz), 4.07–3.79 (m, 4H), 3.69 (dd, 1H, J = 3.9 Hz, J = 11.7 Hz), 3.58 (dd, 1H, J = 5.9 Hz, J = 11.6 Hz), 2.50 (t, 2H, J = 7.5 Hz), 2.33 (t, 2H, J = 7.4 Hz), 1.74–1.51 (mm, 6H), 1.47–1.28 (mm, 12H), 0.86 (t, 3H, J = 6.5 Hz). ^{13}C NMR ($CDCl_3$): δ 174.32, 158.33, 154.28, 130.40, 123.09, 106.44, 102.31, 70.33, 68.10, 65.22, 63.28, 34.21, 31.82, 30.02, 29.31, 29.08, 28.98, 28.81, 25.87, 24.92, 24.70, 22.58, 14.08. MS m/z: 433 $[M + Na]^+$ (100). Anal. ($C_{23}H_{38}O_6$ (410.54)) C, H, N.

1,3-Dihydroxypropan-2-yl 11-(4-Hexyl-3-hydroxyphenoxy)-undecanoate (37). Eluent: $CHCl_3$/MeOH = 45/5. Yield 90% (white solid); mp = 68–70 °C (K). ^1H NMR ($CDCl_3$): δ 6.46 (d, 1H, J = 8.2 Hz), 6.42–6.35 (m, 2H), 4.16 (dd, 1H, J = 5.4 Hz, J = 2.4 Hz), 3.95–3.80 (m, 4H), 3.73–3.69 (dd, 1H, J = 3.8 Hz, J = 11.2 Hz), 3.58 (dd, 1H, J = 5.8 Hz, J = 11.4 Hz), 2.50 (t, 2H, J = 7.5 Hz), 2.33 (t, 2H, J = 7.4 Hz), 1.76–1.52 (mm, 6H), 1.44–1.28 (mm, 18H), 0.87 (t, 3H, J = 6.5 Hz).

^{13}C NMR ($CDCl_3$): δ 174.47, 158.35, 154.24, 130.36, 120.71, 106.35, 102.24, 70.28, 68.00, 65.18, 63.32, 34.14, 31.74, 30.00, 29.26 (×2), 29.18, 29.12 (×2), 29.03 (×2), 25.90, 24.83, 24.68, 22.62, 14.07. MS m/z: 475 $[M + Na]^+$ (100). Anal. ($C_{26}H_{44}O_6$ (452.62)) C, H, N.

1,3-Dihydroxypropan-2-yl 8-(2-Hexyl-5-hydroxyphenoxy)-octanoate (38). Eluent: $CHCl_3$/MeOH = 47/3. Yield 85% (yellow oil). ^1H NMR ($CDCl_3$): δ 6.91 (d, 1H, J = 7.9 Hz), 6.35–6.27 (m, 2H), 4.16 (dd, 1H, J = 5.1 Hz, J = 2.2 Hz), 3.91–3.80 (m, 4H), 3.68 (dd, 1H, J = 3.7 Hz, J = 11.3 Hz), 3.58 (dd, 1H, J = 5.8 Hz, J = 11.7 Hz), 2.49 (t, 2H, J = 7.5 Hz), 2.34 (t, 2H, J = 7.3 Hz), 1.76–1.64 (mm, 6H), 1.49–1.28 (mm, 12H), 0.86 (t, 3H, J = 5.0 Hz). ^{13}C NMR ($CDCl_3$): δ 174.58, 157.80, 154.72, 130.08, 123.42, 106.29, 99.77, 70.40, 67.87, 65.13, 63.09, 34.25, 31.81, 30.22, 29.59, 29.32, 29.11, 29.00, 28.73, 26.01, 24.78, 22.60, 14.10. MS m/z: 433 $[M + Na]^+$ (100). Anal. ($C_{23}H_{38}O_6$ (410.54)) C, H, N.

1,3-Dihydroxypropan-2-yl 11-(2-Hexyl-5-hydroxyphenoxy)-undecanoate (39). Eluent: $CHCl_3$/MeOH = 45/5. Yield 87% (pasty cream solid). mp <30 °C (K). ^1H NMR ($CDCl_3$): δ 6.91 (d, 1H, J = 7.9 Hz), 6.35 (d, 1H, J = 2.3 Hz), 6.29 (dd, 1H, J = 2.6 Hz, J = 7.9 Hz), 4.16 (dd, 1H, J = 5.0 Hz, J = 2.1 Hz), 3.90–3.80 (m, 4H), 3.69 (dd, 1H, J = 3.9 Hz, J = 11.4 Hz), 3.58 (dd, 1H, J = 5.9 Hz, J = 11.7 Hz), 2.49 (t, 2H, J = 7.5 Hz), 2.32 (t, 2H, J = 7.4 Hz), 1.79–1.47 (mm, 6H), 1.44–1.28 (mm, 18H), 0.86 (t, 3H, J = 6.4 Hz). ^{13}C NMR ($CDCl_3$): δ 174.55, 157.81, 154.64, 130.12, 123.48, 106.23, 99.75, 70.41, 67.84, 65.19, 63.27, 34.14, 31.80, 30.14, 29.52, 29.39, 29.20, 29.18 (×2), 29.09, 28.97 (×2), 26.00, 24.83, 22.71, 14.08. MS m/z: 475 $[M + Na]^+$ (100). Anal. ($C_{26}H_{44}O_6$ (452.62)) C, H, N.

1,3-Dihydroxypropan-2-yl 8-[3-Hydroxy-5-(2-methyloctan-2-yl)phenoxy]octanoate (40). Eluent: $CHCl_3$/MeOH = 47/3. Yield 88% (transparent oil). ^1H NMR ($CDCl_3$): δ 6.41–6.38 (m, 2H), 6.20–6.19 (m, 1H), 4.14 (d, 1H, J = 5.3 Hz), 4.07–3.81 (m, 4H), 3.69 (dd, 1H, J = 3.7 Hz, J = 11.6 Hz), 3.58 (dd, 1H, J = 6.1 Hz, J = 11.5 Hz), 2.32 (t, 2H, J = 7.4 Hz), 1.75–1.57 (m, 4H), 1.53–1.34 (mm, 6H), 1.32–1.19 (mm, 16H), 0.81 (t, 3H, J = 6.5 Hz). ^{13}C NMR ($CDCl_3$): δ 174.15, 160.02, 156.37, 152.98, 105.65, 105.53, 98.60, 70.34, 67.81, 65.20, 63.36, 44.50, 37.83, 34.06, 31.78, 30.02, 29.04 (×2), 28.90, 28.84 (×2), 25.73, 24.72, 24.63, 22.66, 14.06. MS m/z: 453 $[M + H]^+$ (10), 475 $[M + Na]^+$ (100). Anal. ($C_{26}H_{44}O_6$ (452.62)) C, H, N.

1,3-Dihydroxypropan-2-yl 11-[3-Hydroxy-5-(2-methyloctan-2-yl)phenoxy]undecanoate (41). Eluent: $CHCl_3$/MeOH = 50/2. Yield 86% (yellow oil). ^1H NMR ($CDCl_3$): δ 6.41 (s, 1H), 6.37 (s, 1H), 6.21 (d, 1H, J = 1.7 Hz), 4.14 (d, 1H, J = 5.8 Hz), 3.94–3.80 (m, 4H), 3.68 (dd, 1H, J = 3.8 Hz, J = 11.6 Hz), 3.57 (dd, 1H, J = 6.0 Hz, J = 11.3 Hz), 2.31 (t, 2H, J = 7.4 Hz), 1.76–1.73 (m, 2H), 1.69–1.45 (m, 4H), 1.40–1.26 (mm, 12H), 1.20–1.04 (mm, 14H), 0.81 (t, 3H, J = 6.5 Hz). ^{13}C NMR ($CDCl_3$): δ 174.33, 160.00, 156.37, 152.91, 105.64, 105.48, 98.62, 70.29, 67.90, 65.18, 63.34, 44.50, 37.86, 34.15, 31.78, 30.02 (×2), 29.30, 29.17 (×2), 29.02 (×2), 28.89 (×2), 25.92, 24.85, 24.6, 22.66, 14.06. MS m/z: 517 $[M + Na]^+$ (100). Anal. ($C_{29}H_{50}O_6$ (494.70)) C, H, N.

1,3-Dihydroxypropan-2-yl 12-[3-Hydroxy-5-(2-methyloctan-2-yl)phenoxy]dodecanoate (42). Eluent: $CHCl_3$/MeOH = 50/1. Yield 85% (yellow oil). ^1H NMR ($CDCl_3$): δ 6.43 (s, 1H), 6.35 (s, 1H), 6.20 (s, 1H), 4.19–4.14 (m, 1H), 3.92–3.84 (m, 4H), 3.67–3.55 (m, 2H), 2.33 (t, 2H, J = 7.3 Hz), 2.03–1.82 (m, 2H), 1.77–1.52 (mm, 10H), 1.47–1.07 (mm, 22H), 0.82 (t, 3H, J = 6.3 Hz). ^{13}C NMR ($CDCl_3$): δ 174.25, 160.08, 156.29, 152.90, 105.46, 105.50, 98.56, 70.36, 67.88, 65.20, 63.28, 44.50, 37.90, 34.08, 31.81, 30.09 (×2), 29.50, 29.42, 29.20 (×2), 29.02 (×2), 28.89 (×2), 25.90, 24.85, 24.64, 22.70, 14.08. MS m/z: 509 $[M + H]^+$ (10), 531 $[M + Na]^+$ (100). Anal. ($C_{30}H_{52}O_6$ (508.73)) C, H, N.

2-[6-(3-Hydroxy-5-pentylphenoxy)hexyloxy]propane-1,3-diol (43). Eluent: $CHCl_3$/MeOH = 47/3. Yield 86% (yellow oil). ^1H NMR ($CDCl_3$): δ 6.27 (d, 1H, J = 1.3 Hz), 6.24 (s, 1H), 6.27 (d, 1H, J = 1.8 Hz), 3.89 (t, 2H, J = 6.3 Hz), 3.78 (dd, 2H, J = 4.4 Hz, J = 11.7 Hz), 3.68 (dd, 2H, J = 4.7 Hz, J = 11.7 Hz), 3.56 (t, 2H, J = 6.4 Hz), 3.49–3.42 (m, 1H), 2.47 (t, 2H, J = 7.7 Hz), 1.77–1.67 (m, 2H), 1.64–1.52 (m, 4H), 1.48–1.41 (m, 4H), 1.31–1.24 (m, 4H), 0.86 (t, 3H, J = 6.4 Hz). ^{13}C NMR ($CDCl_3$): δ 160.02, 156.60, 145.68, 107.82, 107.21, 99.35, 78.83, 70.37, 68.64, 63.40, 62.28, 36.12, 31.50, 30.72, 30.50, 30.05, 29.62, 26.04, 22.37, 14.06. MS m/z: 355 $[M + H]^+$ (20), 377 $[M + Na]^+$ (100). Anal. ($C_{20}H_{34}O_5$ (354.48)) C, H, N.

2-[8-(3-Hydroxy-5-pentylphenoxy)octyloxy]propane-1,3-diol (44). Eluent: $CHCl_3$/MeOH = 48/2. Yield 88% (yellow oil). ^1H

NMR ($CDCl_3$): δ 6.28 (s, 1H), 6.23 (s, 1H), 6.21 (d, 1H, *J* = 1.9 Hz), 3.89 (t, 2H, *J* = 6.4 Hz), 3.77 (dd, 2H, *J* = 4.4 Hz, *J* = 11.6 Hz), 3.67 (dd, 2H, *J* = 4.8 Hz, *J* = 11.7 Hz), 3.55 (t, 2H, *J* = 6.5 Hz), 3.50−3.42 (m, 1H), 2.48 (t, 2H, *J* = 7.7 Hz), 1.76−1.69 (m, 2H), 1.66−1.49 (m, 4H), 1.41−1.23 (mm, 12H), 0.86 (t, 3H, *J* = 6.4 Hz). ^{13}C NMR ($CDCl_3$): δ 160.18, 156.70, 145.72, 107.89, 107.20, 99.37, 78.75, 70.31, 68.58, 63.50, 62.26, 36.02, 31.53, 30.80, 30.54, 29.80, 29.63 (×2), 29.22, 25.89, 22.50, 14.08. MS *m*/*z*: 383 $[M + H]^+$ (20), 405 $[M + Na]^+$ (100). Anal. ($C_{22}H_{38}O_5$ (382.53)) C, H, N.

2-[10-(3-Hydroxy-5-pentylphenoxy]decyloxy)propane-1,3-diol (45). Eluent: $CHCl_3$/MeOH = 48/2. Yield 90% (yellow oil). ^{1}H NMR ($CDCl_3$): δ 6.28 (s, 1H), 6.23 (s, 1H), 6.20 (d, 1H, *J* = 1.7 Hz), 3.88 (t, 2H, *J* = 6.5 Hz), 3.77 (dd, 2H, *J* = 4.6 Hz, *J* = 11.6 Hz), 3.67 (dd, 2H, *J* = 4.8 Hz, *J* = 11.7 Hz), 3.54 (t, 2H, *J* = 6.5 Hz), 3.49−3.42 (m, 1H), 2.47 (t, 2H, *J* = 7.7 Hz), 1.76−1.66 (m, 2H), 1.56−1.48 (m, 4H), 1.45−1.28 (mm, 16H), 0.86 (t, 3H, *J* = 6.5 Hz). ^{13}C NMR ($CDCl_3$): δ 160.12, 156.80, 145.61, 107.78, 107.18, 99.35, 78.77, 70.45, 68.50, 63.45, 62.28, 36.07, 31.54, 30.72, 30.48, 29.91, 29.80 (×2), 29.61 (×2), 29.28, 26.00, 22.58, 14.06. MS *m*/*z*: 411 $[M + H]^+$ (15), 433 $[M + Na]^+$ (100). Anal. ($C_{24}H_{42}O_5$ (410.59)) C, H, N.

2-[12-(3-Hydroxy-5-pentylphenoxy)dodecyloxy]propane-1,3-diol (46). Eluent: $CHCl_3$/MeOH = 50/2. Yield 87% (pale-yellow oil). ^{1}H NMR ($CDCl_3$): δ 6.28 (s, 1H), 6.22 (s, 1H), 6.19 (d, 1H, *J* = 1.9 Hz), 3.88 (t, 2H, *J* = 6.4 Hz), 3.77 (dd, 2H, *J* = 4.7 Hz, *J* = 11.7 Hz), 3.67 (dd, 2H, *J* = 4.8 Hz, *J* = 11.6 Hz), 3.55 (t, 2H, *J* = 6.6 Hz), 3.49−3.42 (m, 1H), 2.47 (t, 2H, *J* = 7.7 Hz), 1.79−1.66 (m, 2H), 1.60−1.49 (m, 4H), 1.48−1.26 (mm, 20H), 0.86 (t, 3H, *J* = 6.5 Hz). ^{13}C NMR ($CDCl_3$): δ 160.08, 156.75, 145.56, 107.78, 107.16, 99.40, 78.81, 70.69, 68.42, 63.45, 62.20, 36.08, 31.52, 30.81, 30.02 (×2), 29.84, 29.75, 29.60 (×2), 29.53 (×2), 29.38, 29.12, 22.49, 14.10. MS *m*/*z*: 461 $[M + Na]^+$ (100). Anal. ($C_{26}H_{46}O_5$ (438.64)) C, H, N.

2-[6-(4-Hexyl-3-hydroxyphenoxy)hexyloxy]propane-1,3-diol (47). Eluent: $CHCl_3$/MeOH = 47/3. Yield 88% (pale-yellow oil). ^{1}H NMR ($CDCl_3$): δ 6.96 (d, 1H, *J* = 8.0 Hz), 6.41−6.35 (m, 2H), 3.90 (t, 2H, *J* = 6.3 Hz), 3.78 (dd, 2H, *J* = 4.3 Hz, *J* = 11.5 Hz), 3.67 (dd, 2H, *J* = 4.7 Hz, *J* = 11.5 Hz), 3.57 (t, 2H, *J* = 6.3 Hz), 3.49−3.42 (m, 1H), 2.49 (t, 2H, *J* = 7.6 Hz), 1.77−1.44 (mm, 10H), 1.42−1.23 (mm, 6H), 0.86 (t, 3H, *J* = 6.3 Hz). ^{13}C NMR ($CDCl_3$): δ 158.20, 154.19, 130.45, 122.90, 106.43, 102.34, 78.75, 70.26, 68.18, 63.35, 62.28, 31.74, 30.12, 29.90, 29.58, 29.35, 29.03, 25.90 (×2), 22.61, 14.10. MS *m*/*z*: 391 $[M + Na]^+$ (100). Anal. ($C_{21}H_{36}O_5$ (368.51)) C, H, N.

2-[10-(4-Hexyl-3-hydroxyphenoxy)decyloxy]propane-1,3-diol (48). Eluent: $CHCl_3$/MeOH = 48/2. Yield 86% (yellow oil). ^{1}H NMR ($CDCl_3$): δ 6.91 (d, 1H, *J* = 8.0 Hz), 6.34 (dd, 1H, *J* = 8.0 Hz, *J* = 4.0 Hz), 6.29 (d, 1H, *J* = 3.7 Hz), 3.83 (t, 2H, *J* = 7.8 Hz), 3.71 (dd, 2H, *J* = 4.2 Hz, *J* = 11.8 Hz), 3.62 (dd, 2H, *J* = 4.3 Hz, *J* = 11.7 Hz), 3.51 (t, 2H, *J* = 7.7 Hz), 3.43−3.40 (m, 1H), 2.45 (t, 2H, *J* = 6.5 Hz), 1.76−1.64 (m, 4H), 1.57−1.46 (m, 4H), 1.38−1.20 (mm, 16H), 0.81 (t, 3H, *J* = 6.3 Hz). ^{13}C NMR ($CDCl_3$): δ 158.32, 154.28, 130.55, 123.03, 106.37, 102.20, 78.74, 70.30, 68.08, 63.33, 62.41, 31.80, 30.08, 29.87 (×2), 29.60 (×2), 29.41 (×2), 29.35, 29.22, 29.01, 26.00, 22.62, 14.08. MS *m*/*z*: 425 $[M + H]^+$ (15), 447 $[M + Na]^+$ (100). Anal. ($C_{25}H_{44}O_5$ (424.61)) C, H, N.

2-{6-[3-Hydroxy-5-(2-methyloctan-2-yl)phenoxy]hexyloxy}-propane-1,3-diol (49). Eluent: $CHCl_3$/MeOH = 47/3. Yield 87% (yellow oil). ^{1}H NMR ($CDCl_3$): δ 6.42 (s, 1H), 6.37 (s, 1H), 6.21 (d, 1H, *J* = 1.7 Hz), 3.90 (t, 2H, *J* = 6.3 Hz), 3.78 (dd, 2H, *J* = 4.5 Hz, *J* = 11.6 Hz), 3.68 (dd, 2H, *J* = 4.6 Hz, *J* = 11.6 Hz), 3.58 (t, 2H, *J* = 6.3 Hz), 3.50−3.43 (m, 1H), 1.79−1.69 (m, 2H), 1.66−1.59 (m, 4H), 1.55−1.37 (mm, 6H), 1.21 (s, 6H), 1.18−1.03 (mm, 6H), 0.82 (t, 3H, *J* = 6.3 Hz). ^{13}C NMR ($CDCl_3$): δ 160.05, 156.56, 152.92, 105.70, 105.55, 98.71, 78.80, 70.52, 68.60, 63.48, 62.31, 44.60, 37.82, 31.80, 30.15, 30.04, 29.93, 29.62, 29.00 (×2), 25.89, 25.83, 22.70, 14.10. MS *m*/*z*: 425 $[M + H]^+$ (15), 447 $[M + Na]^+$ (100). Anal. ($C_{24}H_{42}O_5$ (410.59)) C, H, N.

2-{8-[3-Hydroxy-5-(2-methyloctan-2-yl)phenoxy]octyloxy}-propane-1,3-diol (50). Eluent: $CHCl_3$/MeOH = 48/2. Yield 88% (yellow oil). ^{1}H NMR ($CDCl_3$): δ 6.42 (t, 1H, *J* = 1.7 Hz), 6.37 (t, 1H, *J* = 1.7 Hz), 6.20 (t, 1H, *J* = 1.8 Hz), 5.54 (br s, 1H, disappears on treatment with D_2O), 3.89 (t, 2H, *J* = 6.4 Hz), 3.77 (dd, 2H, *J* = 4.6 Hz, *J* = 11.6 Hz), 3.67 (dd, 2H, *J* = 4.8 Hz, *J* = 11.6 Hz), 3.56 (t, 2H, *J* = 6.7 Hz), 3.50−3.43 (m, 1H), 2.20 (br s, 2H, disappears on treatment with D_2O), 1.77−1.67 (m, 4H), 1.62−1.50 (mm, 6H), 1.48−1.34 (mm, 6H), 1.21 (s, 6H), 1.17−1.03 (mm, 6H), 0.82 (t, 3H, *J* = 6.4 Hz). ^{13}C NMR ($CDCl_3$): δ 160.06, 156.47, 152.90, 105.61, 105.49, 98.71, 78.85, 70.52, 68.58, 63.60, 62.21, 44.55, 37.80, 31.66, 30.08, 29.90, 29.83, 29.61 (×2), 29.45, 29.24, 29.02 (×2), 26.00, 22.67, 14.08. MS *m*/*z*: 425 $[M + H]^+$ (15), 447 $[M + Na]^+$ (100). Anal. ($C_{26}H_{46}O_5$ (438.64)) C, H, N.

2-{10-[3-Hydroxy-5-(2-methyloctan-2-yl)phenoxy]-decyloxy}propane-1,3-diol (51). Eluent: $CHCl_3$/MeOH = 48/2. Yield 86% (yellow oil). ^{1}H NMR ($CDCl_3$): δ 6.42 (d, 1H, *J* = 1.5 Hz), 6.36 (d, 1H, *J* = 1.5 Hz), 6.20 (t, 1H, *J* = 2.0 Hz), 3.89 (t, 2H, *J* = 6.5 Hz), 3.77 (dd, 2H, *J* = 4.4 Hz, *J* = 11.8 Hz), 3.67 (dd, 2H, *J* = 4.9 Hz, *J* = 11.8 Hz), 3.55 (t, 2H, *J* = 6.4 Hz), 3.50−3.43 (m, 1H), 1.81−1.67 (m, 4H), 1.61−1.46 (mm, 6H), 1.41−1.23 (mm, 8H), 1.21 (s, 6H), 1.17−1.02 (mm, 8H), 0.82 (t, 3H, *J* = 6.5 Hz). ^{13}C NMR ($CDCl_3$): δ 160.10, 156.45, 152.83, 105.56, 105.35, 98.67, 78.70, 70.44, 68.43, 63.61, 62.30, 44.62, 37.88, 31.80, 30.21, 30.04, 29.92 (×2), 29.60 (×2), 29.55, 29.43, 29.34, 29.00 (×2), 26.09, 22.65, 14.10. MS *m*/*z*: 467 $[M + H]^+$ (20), 489 $[M + Na]^+$ (100). Anal. ($C_{28}H_{50}O_5$ (466.69)) C, H, N.

2-{12-[3-Hydroxy-5-(2-methyloctan-2-yl)phenoxy]-dodecyloxy}propane-1,3-diol (52). Eluent: $CHCl_3$/MeOH = 49/1. Yield 87% (pale-yellow oil). ^{1}H NMR ($CDCl_3$): δ 6.43 (s, 1H), 6.36 (s, 1H), 6.20 (t, 1H, *J* = 1.7 Hz), 3.89 (t, 2H, *J* = 6.4 Hz), 3.77 (dd, 2H, *J* = 4.7 Hz, *J* = 11.7 Hz), 3.67 (dd, 2H, *J* = 4.7 Hz, *J* = 11.5 Hz), 3.55 (t, 2H, *J* = 6.6 Hz), 3.48−3.43 (m, 1H), 1.78−1.67 (m, 4H), 1.61−1.46 (mm, 6H), 1.44−1.26 (mm, 12H), 1.21 (s, 6H), 1.17−1.02 (mm, 8H), 0.82 (t, 3H, *J* = 6.4 Hz). ^{13}C NMR ($CDCl_3$): δ 160.15, 156.55, 152.80, 105.61, 105.34, 98.78, 78.90, 70.63, 68.45, 63.50, 62.16, 44.50, 37.85, 31.76, 30.30, 30.08, 30.03, 29.82 (×2), 29.60 (×2), 29.54 (×2), 29.45, 29.17, 29.01 (×2), 26.04, 22.70, 14.06. MS *m*/*z*: 495 $[M + H]^+$ (10), 517 $[M + Na]^+$ (100). Anal. ($C_{30}H_{54}O_5$ (494.75)) C, H, N.

General Procedure for the Synthesis of Final Compounds 53−56. To a solution of each acetonide (0.100 g) in ethanol (10 mL) was added 60% aqueous acetic acid (5 mL) and the reaction mixture mildly heated (40−50 °C). After 3 h, the solvent was concentrated and the residue partitioned between ethyl acetate and water, the organic layer dried over sodium sulfate, filtered, and finally concentrated to dryness, affording a crude material purified by silica gel column chromatography.

2,3-Dihydroxypropyl 11-(3-Hydroxy-5-pentylphenoxy)-undecanoate (53). Eluent: $CHCl_3$/MeOH = 47/3. Yield 80% (yellow oil). ^{1}H NMR ($CDCl_3$): δ 6.27−6.20 (m, 3H), 4.14 (d, 1H, *J* = 5.1 Hz), 3.96−3.78 (m, 4H), 3.72−3.53 (m, 2H), 2.46 (t, 2H, *J* = 7.2 Hz), 2.31 (t, 2H, *J* = 7.4 Hz), 1.75−1.68 (m, 2H), 1.63−1.52 (m, 4H), 1.48−1.26 (mm, 16H), 0.86 (t, 3H, *J* = 6.5 Hz). ^{13}C NMR ($CDCl_3$): δ 174.64, 160.22, 156.82, 145.59, 107.92, 106.97, 99.37, 70.27, 67.92, 65.11, 63.36, 36.05, 34.13, 31.53, 30.84 (×2), 29.30, 29.17, 29.06 (×2), 29.02, 25.92, 24.83, 22.54, 14.02. MS *m*/*z*: 461 $[M + Na]^+$ (100). Anal. ($C_{25}H_{42}O_6$ (438.60)) C, H, N.

2,3-Dihydroxypropyl 11-(4-Hexyl-3-hydroxyphenoxy)-undecanoate (54). Eluent: $CHCl_3$/MeOH = 47/3. Yield 78% (white solid); mp 30−32 °C (K). ^{1}H NMR ($CDCl_3$): δ 6.95 (dd, 1H, *J* = 2.0 Hz, *J* = 7.9 Hz), 6.38−6.34 (m, 2H), 4.14 (d, 1H, *J* = 5.5 Hz), 3.92−3.77 (m, 4H), 3.71−3.52 (m, 2H), 2.49 (t, 2H, *J* = 7.5 Hz), 2.32 (t, 2H, *J* = 7.4 Hz), 1.75−1.64 (m, 4H), 1.60−1.51 (mm, 6H), 1.47−1.27 (mm, 14H), 0.86 (t, 3H, *J* = 6.4 Hz). ^{13}C NMR ($CDCl_3$): δ 174.35, 158.26, 154.32, 130.39, 123.05, 106.36, 102.26, 70.08, 68.01, 65.20, 63.35, 34.15, 31.76, 30.02, 29.28 (×2), 29.18, 29.14 (×2), 29.06 (×2), 25.92, 25.13, 24.85, 22.63, 14.10. MS *m*/*z*: 453 $[M + H]^+$ (50), 475 $[M + Na]^+$ (100). Anal. ($C_{26}H_{44}O_6$ (452.62)) C, H, N.

2,3-Dihydroxypropyl 11-(2-Hexyl-5-hydroxyphenoxy)-undecanoate (55). Eluent: $CHCl_3$/MeOH = 47/3. Yield 75% (pasty cream solid); mp <30 °C (K). ^{1}H NMR ($CDCl_3$): δ 6.90 (d, 1H, *J* = 7.9 Hz), 6.34 (s, 1H), 6.29 (dd, 1H, *J* = 1.9 Hz, *J* = 8.00 Hz), 4.14 (d, 1H, *J* = 5.0 Hz), 3.96−3.78 (m, 4H), 3.72−3.53 (m, 2H), 2.48 (t, 2H, *J* = 7.5 Hz), 2.31 (t, 2H, *J* = 7.4 Hz), 1.81−1.67 (m, 4H), 1.63−

1.46 (mm, 6H), 1.43−1.27 (mm, 14H), 0.86 (t, 3H, J = 6.3 Hz). ^{13}C NMR ($CDCl_3$): δ 174.60, 157.85, 154.84, 130.03, 123.53, 106.38, 99.69, 70.32, 67.87, 65.12, 63.38, 34.10, 31.85, 30.22 (×2), 29.65, 29.41, 29.20 (×2), 29.12, 28.98 (×2), 26.03, 24.85, 22.61, 14.08. MS m/z: 475 $[M + Na]^+$ (100). Anal. ($C_{26}H_{44}O_6$ (452.62)) C, H, N.

2,3-Dihydroxypropyl 11-[3-Hydroxy-5-(2-methyloctan-2-yl)-phenoxy]undecanoate (56). Eluent: $CHCl_3$/MeOH = 48/2. Yield 75% (transparent oil). ^{1}H NMR ($CDCl_3$): δ 6.43 (s, 1H, disappears on treatment with D_2O), 6.37 (s, 1H), 6.25 (s, 1H), 6.21 (s, 1H), 5.27 (br s, 2H, disappears on treatment with D_2O), 4.16−4.14 (m, 1H), 4.07−3.82 (m, 4H), 3.80−3.52 (m, 2H), 2.32 (t, 2H, J = 7.4 Hz), 1.92−1.83 (m, 2H), 1.76−1.48 (mm, 8H), 1.40−1.06 (mm, 22H), 0.82 (t, 3H, J = 6.3 Hz). ^{13}C NMR ($CDCl_3$): δ 174.60, 159.98 156.51, 152.87, 105.66, 105.31, 98.70, 70.29, 67.92, 65.13, 62.06, 44.50, 37.81, 34.13, 31.78, 30.02 (×2), 29.32, 29.22 (×2), 29.08 (×2), 29.02 (×2), 25.95, 24.83, 24.64, 22.66, 14.06. MS m/z: 517 $[M + Na]^+$ (100). Anal. ($C_{29}H_{50}O_6$ (494.70)) C, H, N.

CHO Cells. Chinese hamster ovary cells (CHO) stably transfected with cDNA encoding human cannabinoid CB_1 receptors were maintained at 37 °C and 5% CO_2 Dulbecco's Modified Eagle's Medium nutrient mixture F-12 HAM supplemented with 1 mM L-glutamine, 10% fetal bovine serum, and 0.6% penicillin−streptomycin together with G418 (600 μ mL^{-1}). These cells were passaged twice a week using nonenzymatic cell dissociation solution.

Binding Assay. The preliminary screening was carried out using three concentrations (5, 10, and 25 μM) of each compound, membranes from HEK-293 cells stably transfected with either the human CB_1 or CB_2 receptors and [^{3}H]-(−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)-phenyl]-*trans*-4-(3-hydroxypropyl)-cyclohexanol ([^{3}H]CP-55,940) (K_d = 0.18 nM for CB_1 and 0.31 nM for CB_2 receptors) as the high affinity ligand as described by the manufacturer (Perkin-Elmer, Italy).[34] 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 (using 1, 10, 50, 100, 500, 1000 nM concentrations). Displacement curves were generated by incubating drugs with [^{3}H]CP-55,940 (0.14 nM for CB_1 and 0.084 μM for CB_2 binding assay). In all cases, K_i values were calculated by applying the Cheng−Prusoff equation[35] to the IC_{50} values (obtained by GraphPad) for the displacement of the bound radioligand by increasing concentrations of the test compounds. The concentrations used to carry out the dose−responses allow us to calculate IC_{50}, and hence K_i, with at least a 10 nM resolution. Data are reported as mean value ± SEM of three separate experiments performed in duplicate.

[^{35}S]GTPγS Assay. The method for measuring agonist-stimulated [^{35}S]GTPγS binding to cannabinoid CB_1 receptors was adapted from previous described methods.[36] The assays were carried out with GTPγS binding buffer (50 mM Tris-HCl, 50 mM Tris-Base, 5 mM $MgCl_2$, 1 mM EDTA, 100 mM NaCl, 1 mM dithiothreitol, 0.1% BSA) in the presence of [^{35}S]GTPγS and GDP, in a final volume of 500 μL. Binding was initiated by the addition of [^{35}S]GTPγS to the wells. Nonspecific binding was measured in the presence of 30 μM GTPγS. The drugs were incubated in the assay for 60 min at 30 °C. The reaction was terminated by addition of ice-cold Tris-binding buffer and a rapid vacuum filtration. The filters were oven-dried for 60 min and then placed in 5 mL of scintillation fluid. Radioactivity was quantified by liquid scintillation spectrometry. In all assays have been used 0.1 nM [^{35}S]GTPγS, 30 μM GDP, and 5 μg of protein per well for mouse brain membranes and 50 μg per well for CHO−hCB_1 cell membranes. Additionally, mouse brain membranes were preincubated for 30 min at 30 °C with 0.5 U mL^{-1} adenosine deaminase (200 U mL^{-1}) to remove endogenous adenosine. Net agonist stimulated [^{35}S]GTPγS binding values were calculated by subtracting basal binding values (obtained in absence of agonist) from agonist-stimulated values (obtained in the presence of agonist) as detailed elsewhere.[37] Values for EC_{50}, maximal effect (E_{max}) and SEM or 95% confidence limits of these values have been calculated by nonlinear regression analysis using the equation for a sigmoid concentration−reponse curve (GraphPad Prism). Agonists and antagonists were stored at −20 °C as 10 mM stock solutions dissolved in DMSO. Chemical stability in the binding buffer was assessed by diluting 200 μL of a standard solution of compound **41** (2.1 mM in DMSO) in a 1 mL volumetric flask with the biological buffer and incubating for 90 min at 30 °C. At 60 and 90 min time intervals, 5 μL of the incubated solution were injected in a liquid chromatography−mass spectrometry system consisted of a Varian apparatus (Varian Inc.) including a vacuum solvent degassing unit, two pumps (212-LC), a triple quadrupole MSD (model 320-LC) mass spectrometer with ES interface and Varian MS Workstation System Control Vers. 6.9 software. The instrument operated in positive mode and ESI parameters were: detector 1640 V, drying gas pressure 25.0 psi, desolvation temperature 300.0 °C, nebulizing gas 45.0 psi, needle 5000 V, and shield 600 V. Nitrogen was used as nebulizer gas and drying gas. The chromatographic separation was performed using a Lichrospher 100 C18 column (125 mm × 4.0 mm) with 5 μm particle size. Chromatographic analysis was carried out using gradient elution with eluent A being acetonitrile and eluent B consisting of an aqueous solution of formic acid (0.1%); the analysis started with 5% of eluent A, which was linearly increased up to 95% in 20 min, being the flow rate 0.4 mL/min.

Human Plasma/Water Stability Assay. A stock solution of compound **41** (2.2×10^{-2} M) was prepared in DMSO and stored at −20 °C. Standard solutions for plasma esterase assay were prepared at a final concentration of 1×10^{-3} M or 2×10^{-3} M by serial dilution of the stock solution with water immediately prior to use. After being drawn from the volunteers, human blood samples were placed in a tube containing a sodium citrate solution as anticoagulant (3.8% p/v, 1:9) and centrifuged immediately to separate the plasma. An aliquot of 225 μL of human plasma was placed in an Eppendorff tube containing 25 μL of 1×10^{-3} or 2×10^{-3} M solution of compound **41**, obtaining a final concentration of 1×10^{-4} and 2×10^{-4} M. After vortex-mixed for 10 s, samples were incubated at 37 °C for specified time intervals (0, 15, 30, 60, and 120 min). Control samples were prepared in water at the same final concentration of the tested compound, and all experiments were done in triplicates. At the end of incubation time, Eppendorff tubes were placed in ice and 0.4 mL of acetonitrile was added to stop the reaction, vortexed for 2 min, and then centrifuged at 17800g for 5 min. The supernatant was aspirated off and stored at −20 °C until analysis. The chromatographic separation was performed on a DB-C_8 Zorbax Eclipse analytical column (4.6 mm × 150 mm; 5 μm), using a binary mobile phase of 92/8 methanol/water at a flow rate of 0.6 mL/min. Analysis was carried out following both UV (254 nm) and MS signals (positive ion mode, Agilent 1100 LC/MSD VL system G1946C). The sample were injected (20 μL) after filtration. In these chromatographic conditions, retention times were 4.77 min for compound **41** and 3.52 min for its corresponding acid. The retention time values were assessed by injecting 20 μL of standard solutions of both compound **41** and its corresponding acid, prepared by dissolving each compound in acetonitrile in a volumetric flask.

Data are reported as mean ± SEM of samples run in triplicate. The area under the experimental curve (AUC, UV signal) was calculated by a combined linear logarithmic trapezoidal method using Graphpad-Prism V program (GraphPad Software Inc., San Diego, CA, USA). The comparison between $AUC_{(0-120\ min)}$ of water vs plasma samples was performed by using Student's t test. A P value <0.05 was considered significant.

Metabolic Stability Assay. Compound **41** in 10 mM DMSO solution was added to an incubation mixture in a 96-well microplate containing 20 pmol/mL of hCYP3A4 (0.1−0.2 mg/mL protein). The mixture was split in two aliquots: one receiving a NADPH regenerating system, the other an equal amount of phosphate buffer. The final substrate concentration was 1 μM along with 0.25% of organic solvent. Incubation proceeded for 1 h at 37 °C and was stopped by addition of acetonitrile to precipitate proteins. Metabolic stability is given as the percent remaining following incubation with cofactor (NADPH) with reference to the incubation mixture without NADPH: % remaining = Area NADPH × 100/Area ctrl, where Area NADPH is the MS peak area of the sample solution with NADPH and Area ctrl is the MS peak area of the sample solution without NADPH. The % CV obtained was typically within 10%. Mass analysis was performed using a Waters UPLC Acquity system with a qTOF LCT Premiere interface.

■ ASSOCIATED CONTENT

Supporting Information

Physical and spectral data for compounds **4**–**5**, **8**–**10**, **11**–**17**, **19**, **20**–**27**, **29**, **30**–**32** and elemental analyses for all final compounds **34**–**56**. LC-MS chromatogram of **41** incubated in the biological buffer. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

AG, arachidonoylglycerol; AEA, anandamide; CB, cannabinoid; AGE, arachidonyl glyceryl ether; THC, (−)-*trans*-Δ^9-tetrahydrocannabinol; ES, endocannabinoid system; NADA, *N*-arachidonoyl-dopamine; MAGL, monoglyceride lipase; THF, tetrahydrofuran; DCC, *N*,*N*′-dicyclohexylcarbodiimide; DMAP, 4-(dimethylamino)pyridine; hCYP3A4, human recombinant cytochrome *P*450 3A4

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