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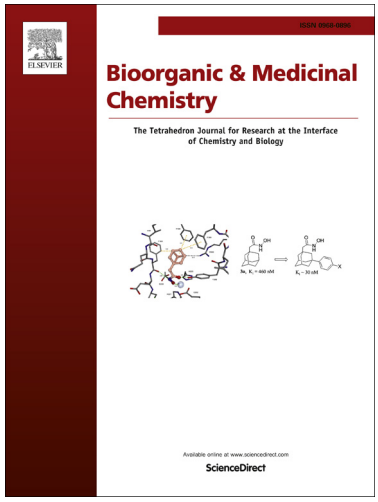

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Structure-affinity relationships and pharmacological characterization of new alkyl-resorcinol cannabinoid receptor ligands: identification of a dual cannabinoid receptor/TRPA1 channel agonist.

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[a] Abbreviations: ES, endocannabinoid system; CB, cannabinoid; MAGL, monoacylglycerol lipase; FAAH, fatty acid amide hydrolase; TRPA1, transient potential ankirin type-1 receptor; THC, (-)-Δ^9-tetrahydrocannabinol; eCBs, endocannabinoids; AEA, anandamide; 2-AG, 2-arachidonoyl-glycerol; COX-2, cyclooxygenase type 2; 2-AGE, 2-arachidonyl-glycerol ether; NAM, *N*-arachidonylmaleimide; EDC, *N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide; HOBt, 1-hydroxybenzotriazole; DMF, N,N-dimethylformamide; HBTU, *N*,*N*,*N'*,*N'*-tetramethyl-*O*-(1*H*-benzotriazol-1yl)uranium hexafluorophosphate; DIPEA, *N*,*N*-diisopropylethylamine; THF, tetrahydrofuran; DEAD, diethyl azodicarboxylate; CNS, central nervous system; TRPV1, transient potential vanilloid type-1 receptor.

Abstract – In our ongoing program aimed at deeply investigating the endocannabinoid system (ES), a set of new alkyl-resorcinol derivatives was prepared focusing on the nature and the importance of the carboxamide functionality. Binding studies on CB_1 and CB_2 receptors, monoacylglycerol lipase (MAGL) and fatty acid amide hydrolase (FAAH) showed that some of the newly developed compounds behaved as very potent cannabinoid receptor ligands (K_i in the nanomolar range) while, however, none of them was able to inhibit MAGL and/or FAAH. Derivative **11** was a potent CB_1 and CB_2 ligand, with K_i values similar to WIN 55,212, exhibiting a CB_1 and CB_2 agonist profile in vitro. In the formalin test of peripheral acute and inflammatory pain in mice, this compound showed a weak and delayed antinociceptive effect against the second phase of the nocifensive response, exhibiting, interestingly, a quite potent transient receptor potential ankyrin type-1 (TRPA1) channel agonist activity. Moreover, derivative **14**, characterized by lower affinity but higher CB_2 selectivity than **11**, proved to behave as a weak CB_2 competitive inverse agonist.

Keywords - cannabinoid ligands, transient receptor potential ankyrin type-1 channel, structure-affinity relationships, alkyl-resorcinol derivatives, anandamide, endocannabinoids, lipid modulators

1. Introduction

The exciting and intensive research on the cannabinoid field started with the identification and chemical synthesis of (-)-Δ^9-tetrahydrocannabinol (THC) and continued with the discovery of an innate signaling system, the endocannabinoid system (ES), characterized by at least two cannabinoid (CB) receptors [1], their endogenous ligands, i.e. endocannabinoids (eCBs), namely anandamide (AEA) [2] and 2-arachidonoyl-glycerol (2-AG) [3], and the enzymes involved in the synthesis and degradation of eCBs [4]. Constitutive cannabinoid-type 1 (CB_1) receptors [5] are found in most tissues and are mainly abundant in the brain where their presynaptic activation reduces the release of both excitatory, i.e. glutamate, and inhibitory, i.e. γ-aminobutirric acid, neurotransmitters [6]. Cannabinoid-type 2 (CB_2) receptors [7,8] are present only in few areas of brain but are most abundant in immune cells and appear to be up-regulated under several pathological conditions [9]. AEA and 2-AG are produced from membrane phosphoglycerides via calcium-sensitive biosynthetic pathways and their pharmacological actions are terminated by re-uptake and metabolism by various enzymes, including fatty acid amide hydrolase (FAAH) [10], monoacylglycerol lipase (MAGL) [11] and cyclooxygenase type 2 (COX-2) [12].

Currently, thanks to the extraordinary achievements in understanding this endogenous signalling system, it is established that the ES is a regulatory and very complex apparatus exerting a homeostatic function, potentially involved in many (patho)physiological conditions [9], such as control of food intake, emesis, obesity and metabolic disorders, neuronal plasticity, mnemonic processes, neuropsychiatric disorders, pain and inflammation. In fact, because of the lipophilic nature of its mediators, it normally acts in a local- and time-depending way and, in the brain, as a "retrograde" neuromodulatory system. Moreover, whereas the biosynthetic precursors of eCBs seem to be ubiquitous in cellular membranes, it is the mutual set of endocannabinoid biosynthesizing enzymes and cannabinoid receptors expressed in "normal" or "dysregulated" conditions that determines levels, specificity and duration of eCB effects [13,14]. As our knowledge of the molecular architecture and biology of this complex system grows, new strategies

for the design and chemical synthesis of compounds with different and/or multiple pharmacological targets are developed, hopefully with the aim to furnishing potential therapeutic agents.

Our initial efforts at designing metabolically stable and potent hybrids, which included the pharmacophoric requirements of both THC and AEA [15-17], culminated in the synthesis of a new class of cannabinoid receptor ligands, chemically characterized by an alkyl-resorcinol nucleus (Chart 1) carrying an amidic head [18]. In previous papers [19,20], the most important chemical features of this novel pharmacophoric model were described, with structure-affinity relationship studies focusing on the lipophilic alkyl resorcinol tail and the length of the alkyloxy chain, linking the aromatic portion to the amide.

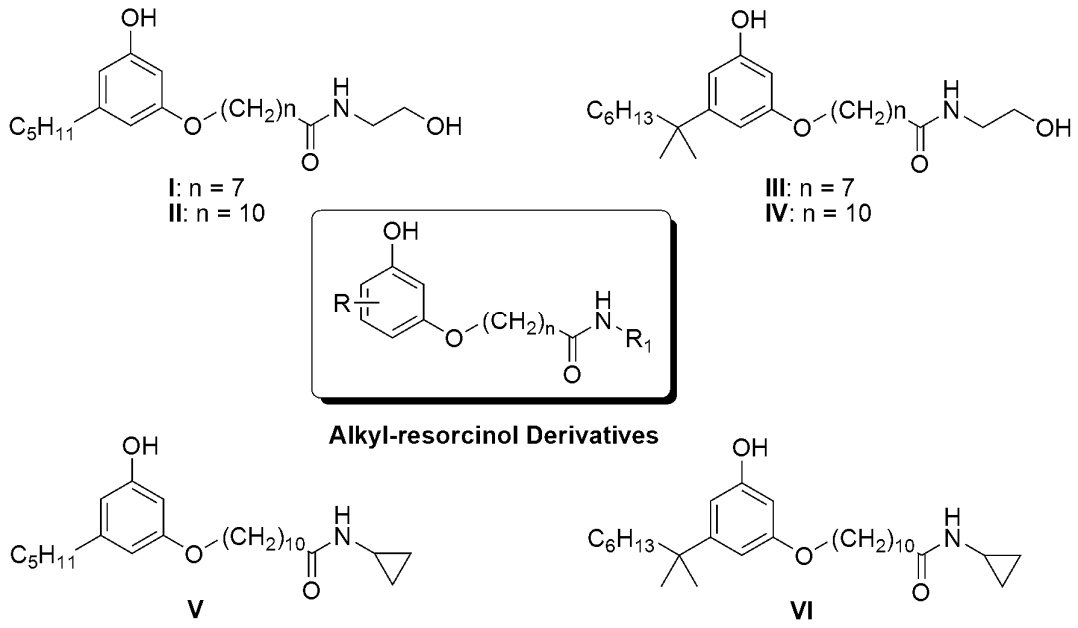


Chart 1. Chemical structure of reference compounds: alkyl-resorcinol derivatives **I-VI**

As an extension of our work, a number of new alkyl-resorcinol derivatives were synthesized and evaluated in order to deeply investigate how the structural requirements of the amide fragment can affect the ability of these compounds to bind cannabinoid receptors, by (a) introducing amines with different electronic properties and size, (b) reversing the positions of the carbonyl and NH groups, thereby producing cyclopropanecarboxamides, and, (c) removing the carbonyl function to obtain

amine analogues (Chart 2), while maintaining a free phenolic hydroxyl group and a linear alkyloxy chain with a range of methylene groups optimal for the cannabinoid receptor interaction [18-20]. Indeed, numerous reports have described several head-analogues of AEA [21-23] highlighting how in the endogenous ligand the structural features of the head group are rather stringent. On the other hand, because of the high degree of stereochemical selectivity observed in AEA-analogues [24], we have considered here the introduction of a (*R*)-methyl or hydroxymethyl group in the 1'-position of the ethanolamine moiety as examples of aliphatic amides with a moderate sterical hindrance in proximity of the amide bond. Besides, the replacement of the classical cyclopropyl portion by allyl, trifluoroethyl, dimethylamino, phenyl, benzyl and piperidinyl groups has been also carried out in order to obtain derivatives with a lipophilic aliphatic or aromatic amide head, ranging from small to relatively large size and having different electron density properties. Additionally, to explore the steric tolerance of the space around the amide function, a very bulky amine, i.e. adamantyl amine, has been introduced. In light of the higher metabolic stability and similar binding affinity displayed by retro-AEA [25] compared to AEA, the synthesis of new alkyl-resorcinol cyclopropanecarboxamides has been pursued without altering the distance between the NH group and the aromatic nucleus because in our previous studies alkyl-resorcinol cyclopropylamides with an alkyloxy chain of 11 carbon atoms proved to be potent cannabinoid receptor ligands [18-20]. Despite the fact that the carbonyl function seems to play a critical role in eCB activity [26], noladin, also known as 2-arachidonyl-glycerol ether (2-AGE) [27], has been proposed as "putative" eCB, acting as full agonist at the CB_1, and much less so, CB_2 receptors [28-30]. Accordingly, the cannabinoid recognition properties of derivatives lacking the carbonyl group have been also investigated.

The manipulation of the ethanolamine moiety of AEA seemed to be a logical and very efficient approach to discover and identify FAAH and/or MAGL inhibitors [31]. Among a wide range of chemical structures characterizing these indirect cannabinoid receptor agonists, the first designed MAGL inhibitor was an AEA derivative, i.e. *N*-arachidonylmaleimide (NAM) [32], which showed

a 20-fold selectivity over FAAH. More recently, the screening of different cysteine traps has led to the identification of isothiazolinone-based compounds as potent MAGL inhibitors [33], suggesting the formation of a reversible disulfide adduct as a probable inhibition mechanism.

Moreover, many classes of these inhibitors are also characterized, like the set of compounds here described, by the presence of an aliphatic chain bearing a terminal aryl ring rather tolerant to the chemical decoration. Thus, we have planned to modulate the cannabinergic properties of these alkyl-resorcinol derivatives, shifting their capability of interaction from CB_1 and CB_2 receptors to catabolic enzymes, by transforming the amide moiety in the phthalimido functionality (Chart 2).

To date, the functional and chemical relationship between ES and TRP channels is well documented [34,35]. Specifically, within the superfamily of the TRP channels, TRPA1 and TRPV1, which are co-expressed in primary sensory neurons [36], appear to play a key role as a biological sensors in several physiological and pathophysiological conditions, such as neurogenic inflammation and pain, overlapping only in part with those in which the cannabinoid receptors participate. Starting from the endocannabinoid AEA, that is presently thought to be also an endovanilloid, the recognition of some similar chemical features, i.e. a lipophilic moiety, a polar head and a connecting functionality, within both classes of TRP and endocannabinoid system modulators led to the discovery and/or identification of endogenous, natural and synthetic TRP and CB ligands [37]. Considering that these alkyl-resorcinol derivatives share the above pharmacophore requirements, herein, the functional activity at CB_1 and CB_2 receptors, the antinociceptive effect in mice, the interaction with both transient potential vanilloid type-1 (TRPV1) and ankyrin type-1 (TRPA1) receptors, and the in vitro cytotoxicity profile of the most interesting compound is also reported.

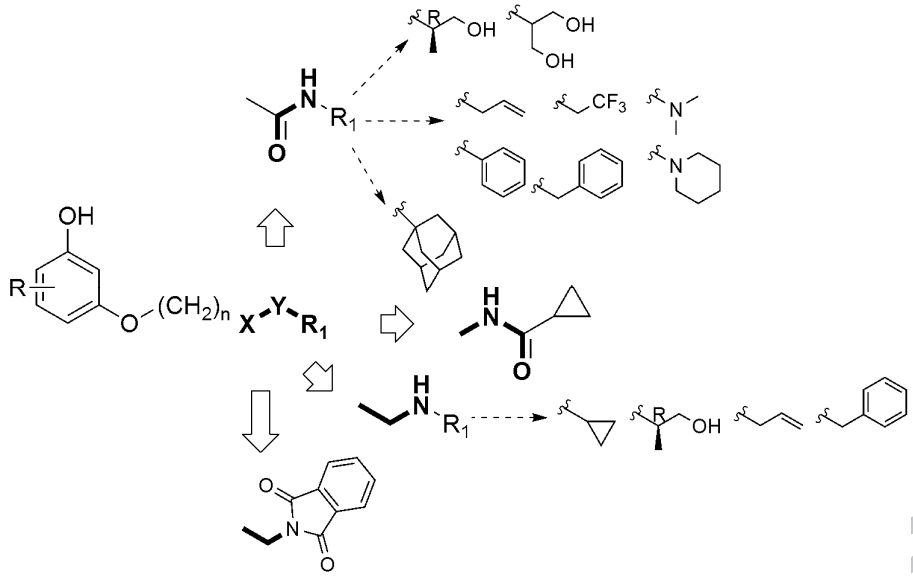
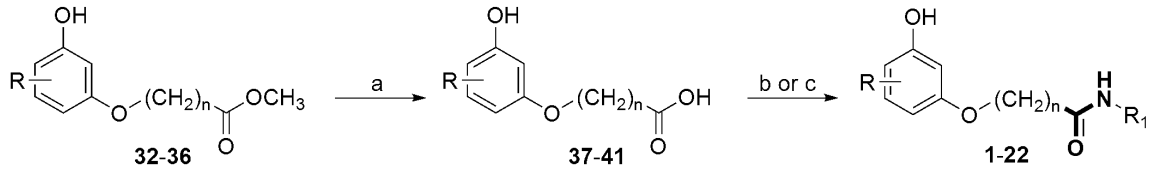


Chart 2. General chemical structures of new synthesized alkyl-resorcinol compounds

2. Results and Discussion

2.1. Chemistry

As depicted in Scheme 1, acids **37**-**41**, obtained from hydrolysis of methyl esters **32**-**36** with methanolic/aqueous sodium hydroxide solution, were coupled in dichloromethane as solvent with the appropriate commercial amines in the presence of *N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide (EDC) hydrochloride and 1-hydroxybenzotriazole (HOBt) to give amides **1**-**20** in excellent yields. Using this method, final hydrazides **21** and **22** were obtained only in small amounts; thus, acid **37** was reacted in dry *N*,*N*-dimethylformamide (DMF) with 1,1-dimethylhydrazine and 1-aminopiperidine, respectively, in the presence of HOBt, *N*,*N*,*N'*,*N'*-tetramethyl-*O*-(1*H*-benzotriazol-1yl)uronium hexafluorophosphate (HBTU) and *N*,*N*-diisopropylethylamine (DIPEA), to provide the expected compounds in acceptable yields (Scheme 1). Esters **32**-**36** were prepared following the synthetic pathway previously reported [18-20].

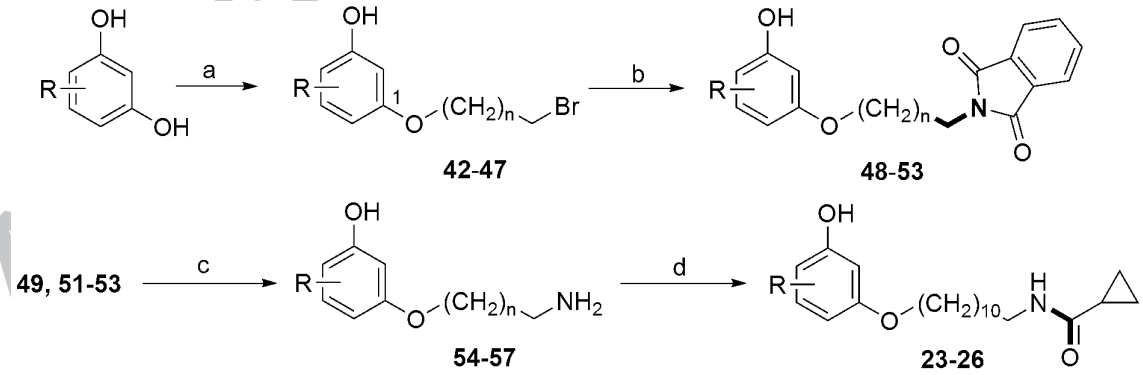


32,37: R = 5-pentyl, n = 10 [29-31]; **33,38**: R = 5-(2-methyloctan-2-yl), n = 7 [31]; **34,39**: R = 5-(2-methyloctan-2-yl), n = 10 [31]; **35,40**: R = 2-hexyl, n = 10 [30]; **36,41**: R = 4-hexyl, n = 10 [30]; **1-22** see Table 1.

Scheme 1. Synthesis of amides **1**-**22**. Reagents and reaction conditions. a: MeOH/NaOH aqueous, reflux, 3h, 85-90%; b: R_1NH_2, HOBt, EDC, DCM, room temp., overnight, 75-90% (**1**-**20**); c: 1,1-dimethylhydrazine or 1-aminopiperidine, HOBt, HBTU, DIPEA, dry DMF, room temp, overnight, 55-60% (**21**, **22**).

For the synthesis of retro-amides we planned a synthetic approach which involved as key intermediates the phthalimido derivatives designed as enzymatic inhibitors (Scheme 2). Olivetol, 5-(2-methyloctan-2-yl)resorcinol and 4-hexylresorcinol, respectively, dissolved in dry tetrahydrofuran (THF), were subjected to Mitsunobu reaction with the appropriate bromoalcohols in the presence of triphenylphosphine and diethyl azodicarboxylate (DEAD), furnishing bromoethers **42**-**47** in satisfactory yields. Then, compounds **48**-**53** were achieved by displacement of bromine atom with potassium phthalimide, at 70-80 °C, in dry DMF.

Hydrazinolysis of *N*-isoindoline-1,3-diones **49** and **51**-**53** in refluxing ethanol [38] afforded corresponding amines **54**-**57** that were coupled with the cyclopropanecarboxylic acid without further purification, following the procedure previously described to give final amides **23**-**26**.



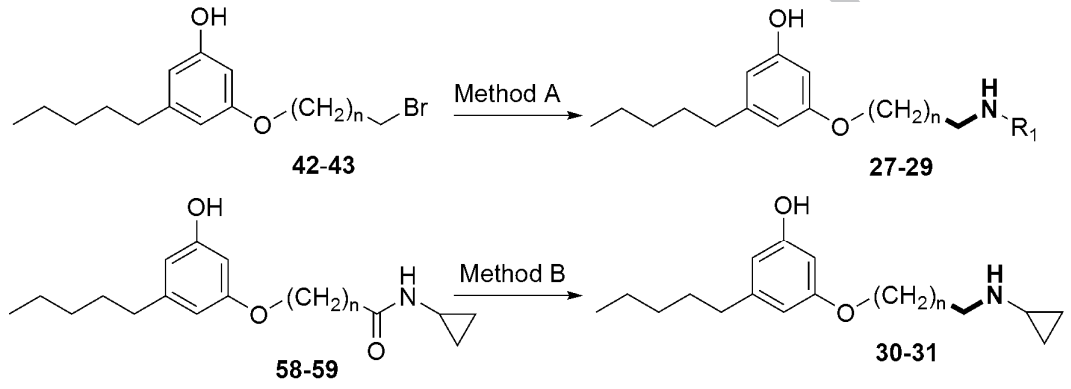
42,48: R = 5-pentyl, n = 8; **43**,**49,54**: R = 5-pentyl, n = 10; **44,50**: R = 5-(2-methyloctan-2-yl), n = 8; **45,51,55**: R = 5-(2-methyloctan-2-yl), n = 10; **46,52,56**: R = 2-hexyl, n = 10; **47,53,57**: R = 4-hexyl, n = 10; **23**-**26**: see Table 1; **48**-**53**: see Table 2.

Scheme 2. Synthesis of phthalimido derivatives **48**-**53** and cyclopropanecarboxamides **23**-**26**. Reagents and reaction conditions. a: bromoalcohol, triphenylphosphine, DEAD, dry THF, room

temp., overnight, 35-60%; b: potassium phthalimide, dry DMF, 70-80 °C, overnight, 70-80%; c: hydrazine, absolute ethanol, 60-80 °C, 5h, 90-95% yield; d: cyclopropanecarboxylic acid, HOBt, EDC, DCM, room temp., overnight, 55-65%.

By heating the bromoethers **42** and **43** with (*R*)-(-)-2-amino-1-propanol, allylamine and benzylamine, the final amine derivatives **27**-**29** were obtained in good yields (Scheme 3, method A). Conversely, being cyclopropylamine a low boiling liquid reagent, the synthesis of the cyclopropylamine analogues **30**-**31** was carried out by reduction of the corresponding amides with litium aluminium hydride in dry THF (Scheme 3, method B).

Amides **58** and **59** were prepared following the synthetic pathway previously reported [18,19].



42: R = 5-pentyl, n = 8; **43**: R = 5-pentyl, n = 10; **58**: R = 5-pentyl, n = 10 [29]; **59**: R = 5-pentyl, n = 11 [30]; **27**-**31**: see Table 1.

Scheme 3. Synthesis of amine derivatives **27**-**31**. Reagents and reaction conditions. Method A: (R)-(-)-2-amino-1-propanol, allylamine, benzylamine, heating, 4-5h, 75-85%; Method B: $LiAlH_4$, dry THF, 50-60 °C, 3h, 70-75%.

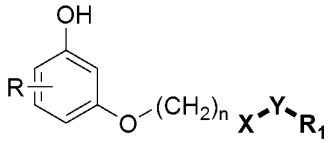
2.2. In vitro CB_1 and CB_2 receptor binding assay

Alkyl-resorcinol derivatives **1**-**31** were evaluated as ligands for the human recombinant CB receptors hCB_1 and hCB_2 and were compared to reference compounds AEA, WIN 55,212-2 and HU-210 and previous lead compounds **I**-**VI** [18-20] (Table 1).

The binding affinities (K_i values) for the receptor were evaluated using membranes from HEK cells transfected with either the hCB_1 or hCB_2 receptor and [^{3}H]-CP-55,940 as high affinity ligand with

K_d values of 0.18 nM for CB_1 receptor and 0.31 nM for CB_2 receptor [39]. Displacement curves were derived after incubation of the drugs with [^{3}H]-CP-55,940 at 0.14 nM for CB_1 and 0.31 nM for the CB_2 binding assay. The K_i values were calculated from the IC_{50} values according to the Cheng-Prusoff equation [40]. Analysis of the binding assay results of the compounds described here confirmed the validity of the alkyl-resorcinol scaffold as a pharmacophore and allows to assess with more accuracy the structure-affinity relationships regarding the amide functionality. In agreement with literature data for the AEA analogues [41], in the series of amides **1**-**22**, introduction of a methyl group at C-1' position to afford chiral compounds **1**-**4** resulted in an increase of affinity compared to the analogous ethanol-amides **I**-**IV**. Compounds **1** and **3** behaved as CB_1 receptor ligands (K_i of 12.8 and 17.2 nM, respectively) with higher affinity than AEA (K_i = 72.0 nM) and the same affinity as WIN 55,121-2 (K_i = 21.0 nM), although not very selective over CB_2 receptors (K_i of 119.3 and 50.2 nM, respectively), while compound **4** was the most potent CB_2 receptor ligand with highest affinity (K_i = 21.2 nM) in this subfamily of derivatives. A further increase of the bulkiness and hydrophilic properties of the substituent at C-1' position with the introduction of a hydrogen-bonding hydroxymethyl group, i.e. compounds **5**-**9**, caused a considerable loss of affinity for both cannabinoid receptors. This drop in cannabinoid receptor binding properties may indicate a stringent steric and hydrophobic requirement in this position. Previous studies [27] actually suggested the existence of a hydrophobic subsite for the anandamide head group in both CB_1 and CB_2 receptors, capable of accommodating relatively bulky substituents. In agreement with data from literature, the gradual enhancement of the head group size within these new derivatives resulted in a parallel loss of cannabinoid receptor affinity.

Table 1. Structure, CB_1 and CB_2 Receptor Affinity (K_i Values), and Selectivity of Alkyl-Resorcinol Derivatives **1**-**31** and Reference Compounds **I**-**VI**, AEA, WIN 55,212-2 and HU-210.[a]



1-31

Compd	R	n	X-Y	R_1	K_i (nM)[b] hCB_1	K_i (nM)[b] hCB_2	SI[c] CB_2 vs CB_1	SI[d] CB_1 vs CB_2
1	5-*n*. C_5H_{11}	7	CONH	(R)-$CH(CH_3)CH_2OH$	**12.8**	119.3	0.11	9.32
2	5-*n*.C_5H_{11}	10	CONH	(R)-$CH(CH_3)CH_2OH$	264.4	97.2	2.72	0.37
3	5-$C(CH_3)_2C_6H_{13}$	7	CONH	(R)-$CH(CH_3)CH_2OH$	**17.2**	50.2	0.34	2.92
4	5-$C(CH_3)_2C_6H_{13}$	10	CONH	(R)-$CH(CH_3)CH_2OH$	132.2	**21.2**	6.24	0.16
5	5-*n*. C_5H_{11}	10	CONH	$CH(CH_2OH)CH_2OH$	>10000	540.0	>18.52	<0.05
6	5-$C(CH_3)_2C_6H_{13}$	7	CONH	$CH(CH_2OH)CH_2OH$	177.8	814.8	0.22	4.60
7	5-$C(CH_3)_2C_6H_{13}$	10	CONH	$CH(CH_2OH)CH_2OH$	1890	230.0	8.22	0.12
8	2-*n*.C_6H_{13}	10	CONH	$CH(CH_2OH)CH_2OH$	>10000	2370	>4.22	<0.24
9	4-*n*.C_6H_{13}	10	CONH	$CH(CH_2OH)CH_2OH$	>10000	>10000	-	-
10	5-*n*.C_5H_{11}	10	CONH	$CH_2CH{=}CH_2$	136.3	**16.4**	8.31	0.12
11	5-$C(CH_3)_2C_6H_{13}$	7	CONH	$CH_2CH{=}CH_2$	**7.22**	**7.36**	0.98	1.02
12	5-*n*.C_5H_{11}	10	CONH	CH_2CF_3	438.0	96.30	4.55	0.22
13	5-$C(CH_3)_2C_6H_{13}$	7	CONH	CH_2CF_3	397.0	**27.9**	14.23	0.07
14	5-*n*.C_5H_{11}	10	CONH	C_6H_5	>10000	127.8	**>78.25**	**<0.01**
15	5-*n*.C_5H_{11}	10	CONH	$CH_2C_6H_5$	>10000	202.3	**>49.50**	**<0.02**
16	5-*n*.C_5H_{11}	10	CONH	1-adamantyl	>10000	>10000	-	-
17	5-$C(CH_3)_2C_6H_{13}$	7	CONH	1-adamantyl	>10000	>10000	-	-
18	5-$C(CH_3)_2C_6H_{13}$	10	CONH	1-adamantyl	>10000	>10000	-	-
19	2-*n*.C_6H_{13}	10	CONH	1-adamantyl	>10000	>10000	-	-
20	4-*n*.C_6H_{13}	10	CONH	1-adamantyl	>10000	>10000	-	-
21	5-*n*.C_5H_{11}	10	CONH	$N(CH_3)_2$	845.5	182.6	4.63	0.22
22	5-*n*.C_5H_{11}	10	CONH	c-$N(CH_2)_5$	1782	677.8	2.63	0.38
23	5-*n*.C_5H_{11}	11	NHCO	c-C_3H_5	2770	460.0	6.02	0.17

24	5-$C(CH_3)_2C_6H_{13}$	11	NHCO	c-C_3H_5	2070	650.0	3.18	0.31
25	2-*n*.C_6H_{13}	11	NHCO	c-C_3H_5	900.0	250.0	3.60	0.28
26	4-*n*.C_6H_{13}	11	NHCO	c-C_3H_5	>10000	>10000	-	-
27	5-*n*.C_5H_{11}	8	CH_2NH	(R)-$CH(CH_3)CH_2OH$	1060	1520	0.70	1.43
28	5-*n*.C_5H_{11}	10	CH_2NH	$CH_2CH{=}CH_2$	1140	1850	0.62	1.62
29	5-*n*.C_5H_{11}	10	CH_2NH	$CH_2C_6H_5$	1240	830.0	1.49	0.67
30	5-*n*.C_5H_{11}	10	CH_2NH	c-C_3H_5	2080	1890	1.10	0.91
31	5-*n*.C_5H_{11}	11	CH_2NH	c-C_3H_5	830.0	>10000	<0.08	>12.05
I[e]	5-*n*.C_5H_{11}	7	CONH	CH_2CH_2OH	130.0	390.0		
II[f]	5-*n*.C_5H_{11}	10	CONH	CH_2CH_2OH	800.0	160.0		
III[g]	5-$C(CH_3)_2C_6H_{13}$	7	CONH	CH_2CH_2OH	56.0	160.0		
IV[g]	5-$C(CH_3)_2C_6H_{13}$	10	CONH	CH_2CH_2OH	310.0	30.0		
V[f]	5-*n*.C_5H_{11}	10	CONH	c-C_3H_5	5.20	13.0		
VI[g]	5-$C(CH_3)_2C_6H_{13}$	10	CONH	c-C_3H_5	21.0	7.9		
AEA					72.0	-		
WIN 55,212-2					21.0	2.1		
HU-210					-	0.15		

[a] Data are mean values for at least three separate experiments. Reference compounds were tested under the same conditions in this study and anandamide was tested in the presence of PMSF (100nM). Binding affinity constants of the most potent compounds (K_i < 50 nM) are in bold as well as the most selective compounds for CB_1 and CB_2. Standard errors of means (SEM) are not shown for the sake of clarity and were never higher than 5% of the mean.
[b] K_i values (nM) for CB_1 and CB_2 binding assays are defined in experimental section.
[c] SI: CB_2 selectivity index was calculated as $K_i(CB_1)/K_i(CB_2)$ ratio.
[d] SI: CB_1 selectivity index was calculated as $K_i(CB_2)/K_i(CB_1)$ ratio.
[e] Lit. [19]; in the paper **I** is denoted as compound **10**.
[f] Lit. [18]; in the paper **II** and **V** are denoted as compound **24** and **25**, respectively.
[g] Lit. [20]; in the paper **III**, **IV** and **VI** are denoted as compound **25**, **26** and **33**, respectively.

While a relatively hindered substituent, as the phenyl, benzyl and 1-piperidinyl group (compounds **14**, **15** and **22**, respectively), was still tolerated, the spherical and bulky 1-adamantyl nucleus afforded derivatives completely devoid of ability to bind both cannabinoid receptors (compounds **16**-**20**). Intriguingly, the CB_2 selectivity-enhancing effect of the aromatic group (SI, calculated as $K_i(CB_1)/K_i(CB_2)$ > 78 and > 49 for compounds **14** and **15**, respectively), although providing compounds with lower affinity, might suggest a higher steric tolerance of the CB_2 binding site and a

possible π-π interaction. We also explored how the electron density of the head group fragment could affect the affinity of these derivatives. The introduction of a double bond, i.e. allyl-amides **10** and **11**, gave analogues with 2-fold higher affinity than similar ethanol-amides, their K_i values reaching the low nanomolar range. Particularly, compound **11**, which contains the 5-(1,1'-dimethylheptyl)resorcinol nucleus, proved to be the most potent CB_1 (K_i = 7.22 nM) and CB_2 (K_i = 7.36 nM) ligand among the alkyl-resorcinol derivatives reported herein. Conversely, replacement of the ethanolamine in the amide head with the 2',2',2'-trifluoroethylamine, in compounds **12** and **13**, or the *N*,*N*-dimethylhydrazine, in compound **21**, did not influence significantly the K_i values. In agreement with our previous studies, current data showed that the 1,1-dimethylheptyl series gave the most potent compounds (i.e. compounds **3**, **11** and **13** with the linker of 8 carbon atoms and compound **4** with the linker of 11 carbon atoms). On the other hand, the binding properties of derivatives having the olivetol nucleus, where an alkyloxy chain of 10 methylene groups was found optimal for cannabinoid receptor affinity [18], appeared to be more affected by the nature of the amide moiety.

The reversal of the carbonyl and NH groups in the amide functionality (cyclopropanecarboxamides **23**-**26**) as well as the replacement of carbonyl moiety with a methylene group (amine-analogues **27**-**31**) afforded very weak ligands (affinity values in the micromolar range) for both receptor subtypes, independently of their aromatic structure and the length of the connecting alkyloxy chain. In fact, in the "inverse amides" the presence of both the cyclopropane moiety and of an alkyloxy chain of 11 carbon atoms, characteristics which conferred the best cannabinoid receptor interaction in the alkyl-resorcinol family [18-20], is not enough to provide the optimal ligand recognition properties. Similarly, all the amino derivatives **27**-**31** did not show good binding affinities, in face of the 5-pentylresorcinol nucleus (olivetol backbone), a variable but appropriate chain length, and head residues usually providing a high cannabinoid receptor affinity (i.e. cyclopropyl and allyl groups) or subtype selectivity (i.e. 1-hydroxypropn-2-yl and benzyl groups). Specifically, compounds **23** and

30 are the retro-amide and amine analogues, respectively, of the reference amide **V**, which showed affinity values in the nanomolar range (see table 1).

Taken together, these results suggest that the carbonyl functionality is essential for interaction with the cannabinoid receptor and even relatively small variations in its position might result in a considerable impairment of such interaction. Moreover, according with our previous results, the resorcinol scaffold afforded very potent compounds when the saturated alkyl tail was placed at the 5 position in the aromatic nucleus, symmetrically to both hydroxy groups.

2.3. In vitro MAGL and FAAH interaction

Despite their different catalytic triad, FAAH and MAGL are known to be inhibited by various non-selective hydrolase inhibitors which possess a hydrophobic fragment, covalently binding groups, and a heterocyclic subunit. The series of *N*-isoindoline-1,3-diones **48**-**53** and the two hydrazide derivatives **21** and **22** were tested for their ability to inhibit MAGL and/or FAAH activity but unexpectedly, no inhibitor of MAGL and/or FAAH activity could be found (see the supplementary data). Although derivatives **48**-**53** and **21**, **22** shared chemical features reportedly relevant for interaction with both enzymes, still they proved to be devoid of any inhibitory activity. Perhaps, the length of the alkyl spacer between the imido or the hydrazido functionality and the phenyl moiety could be a key parameter in the interaction with these enzymes, whereas, in the phthalimido series, the steric hindrance of the heterocyclic head could be detrimental to the inhibitory activity.

2.4. Functional Activity of compounds **11** and **14** at CB_1 and CB_2 receptors

When tested in the [^{3}H]CP-55,940 displacement binding assay, compound **11** exhibited the highest affinity for both CB_1 (K_i = 7.22nM) and CB_2 (K_i = 7.36nM) receptors, while compound **14** showed the best selectivity for CB_2 (K_i = 127.8nM, SI, calculated as $K_i(CB_1)/K_i(CB_2)$, > 78) receptor (Table 1). With the aim to investigate whether these two compounds are able to activate or block cannabinoid CB_1 and/or CB_2 receptors, we performed [^{35}S]GTPγS binding assays using membranes obtained from hCB_1-CHO and hCB_2-CHO cells. The ability of compound **11** to stimulate [^{35}S]GTPγS binding was first investigated using hCB_1-CHO cell membranes (for the 95%

confidence intervals of the mean values see the supplementary data). We found that the compound was able to induce a strong stimulation of [^{35}S]GTPγS binding to hCB$_1$-CHO cell membranes (Figure 1a) although its potency (EC_{50} = 138.40 nM) was significantly lower than that of the well-known CB$_1$/CB$_2$ receptor full agonist CP-55,940 (EC_{50} = 1.43 nM). In particular, compound **11** behaved as a full agonist since its E_{max} value expressed as percentage of stimulation of [^{35}S]GTPγS binding above basal (E_{max} = 60.58%) was not significantly different from that of CP-55,940 (E_{max} = 72.10%).

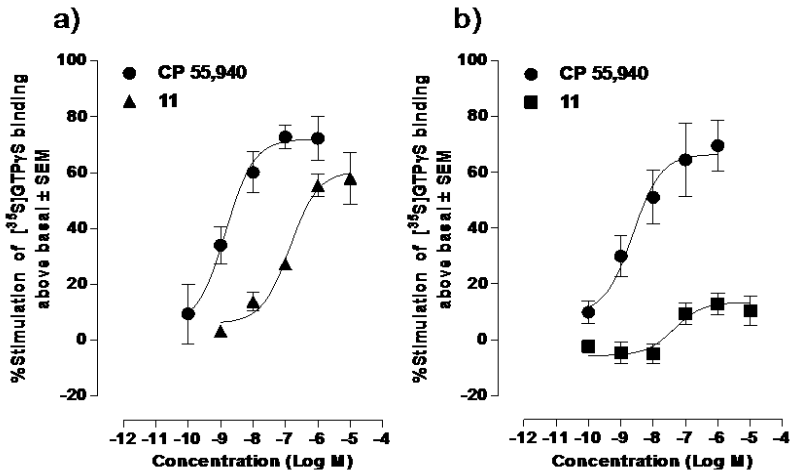


Figure 1. a) The effect of CP-55,940 and **11** on [^{35}S]GTPγS binding to hCB$_1$-CHO cell membranes (n=6). b) The effect of CP-55,940 and **11** on [^{35}S]GTPγS binding to hCB$_2$-CHO cell membranes (n=6). Each symbol represents the mean percentage change in binding ± SEM.

When tested in hCB$_2$-CHO cell membranes, compound **11** produced only slight stimulation in the [^{35}S]GTPγS binding assay (Figure 1b), albeit with a potency (EC_{50} = 44.70 nM) that was not statistically different than that of CP-55,940 (EC_{50} = 2.37 nM). Compound **11** behaved as a partial agonist in hCB$_2$-CHO cells, since its E_{max} value (13.44%) was significantly lower than that of CP-55,940 (E_{max} = 66.52%).

Because of the K_i values obtained for compound **14** (Table 1), this ligand was tested only on hCB$_2$-CHO cell membranes. We found that at the lowest concentrations tested (1-100 nM), compound **14** had no detectable effect in the [^{35}S]GTPγS binding assay. Conversely, at higher concentrations it produced a slight but significant inhibition of [^{35}S]GTPγS binding to hCB$_2$-CHO cell membranes

(Figure 2a) with a mean EC_{50} value of 701.20 nM and a mean E_{max} value of -14.41%. Under the same experimental conditions, the well-established CB_1/CB_2 agonist CP-55,940 was able to induce a full stimulation of [^{35}S]GTPγS binding, with a mean EC_{50} value of 6.58 nM. Since compound **14** behaved as a low-efficacy inverse agonist in this assay, its ability to antagonize CP-55,940 was tested, using the highest concentration (1 μM) at which it did not show any significant stimulatory effect in the [^{35}S]GTPγS binding assay.

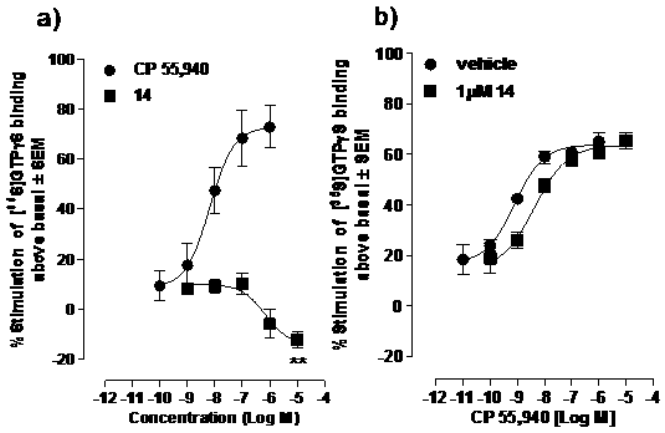


Figure 2. a) The effect of CP-55,940 and **14** on [^{35}S]GTPγS binding to hCB_2-CHO cell membranes (n=6). b) The mean log concentration-response curve of CP-55,940 was constructed in the presence of vehicle (DMSO) or 1μM **14** (n=6). Each symbol represents the mean percentage change in binding of [^{35}S]GTPγS to hCB_2-CHO cell membranes ± SEM. The right-ward shift produced by 1μM **14** (Hill slope: 0.71, 95% confidence interval from 0.24 to 1.18) in the log concentration response curve of CP-55,940 (Hill slope: 0.82, 95% confidence interval from 0.2 to 1.31) did not deviate significantly from parallelism since the 95% confidence intervals of the two Hill slopes overlapped. DMSO, dimethyl sulphoxide. Asterisks denote values that are significantly different from zero ($^{**}P<0.01$; one sample test).

At this concentration compound **14** was able to antagonize CP-55,940-induced stimulation of [^{35}S]GTPγS binding to hCB_2-CHO cell membranes (Figure 2b), as indicated by the right-ward shift in the dose/response curve of the agonist. The mean apparent K_B value (184.00 nM) of compound **14** for its antagonism of CP-55,940 did not differ significantly from its mean K_i value (127.84 nM) determined in the [^{3}H]CP-55,940 displacement binding assay described above (Table 1). The rightward shift induced by compound **14** in the dose/response curve of CP-55,940 (Figure 2b) did not deviate significantly from parallelism, since the 95% confidence interval of the Hill slopes

overlap (Figure 2b). Taken together, these findings support the hypothesis that compound **14** can behave as a weak CB_2 competitive inverse agonist.

2.5. Antinociceptive activity of compound **11** in mice

Based on the results obtained in in vitro assay, we decided to investigate whether compound **11**, like other dual CB_1/CB_2 agonists, displayed any analgesic activity in vivo. To this end, the formalin test of peripheral acute and inflammatory pain in mice was employed. This test is a well-established model of persistent pain in which mice exhibit a transient, biphasic pattern of pain behaviour. The early, short lasting first phase of the nociceptive response is characterized by the activation of C and Aδ fibres and is followed first by a quiescent period and then by a second, prolonged phase of tonic pain, involving an inflammatory reaction in peripheral tissue, an activation of primary afferent nerve fibres [42] and the development of central nervous system (CNS) sensitization [43]. We found that i.p. administration of compound **11** caused a moderate reduction of the second phase of nociceptive behaviour induced by formalin only at the highest doses used (3 and 4 mg/kg, i.p.) (Figure 3) and, unexpectedly, the antinociceptive effect became statistically significant only in the latter part of the second phase (4 mg/kg, i.p., after 40 min) (Figure 3).

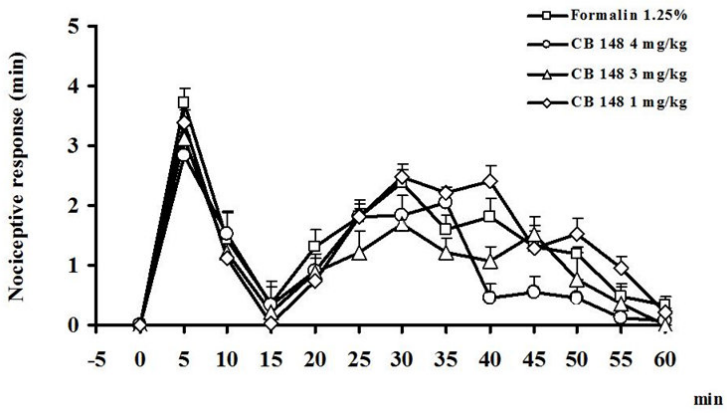


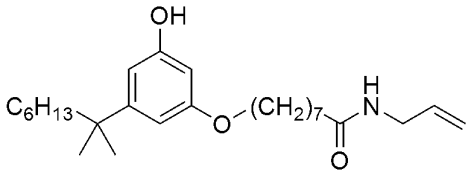
Figure 3. Antinociceptive effect of subcutaneous formalin (1.25%, 30 mL) injections into the hind paws of mice on the time course of nociceptive behaviour. Formalin was injected 10 min after the systemic administration of vehicle (0.9% NaCl, i.p.) or test compound **11**, here denoted as CB148. Recording of nociceptive behaviour began immediately after the injection of formalin (time 0) and was continued for 60 min. Each point represents the total time of the nociceptive responses (mean ± SEM) of 8-10 animals per group, measured every 5 min. Data were analysed using the one-way ANOVA followed by the Bonferroni's test, and statistical significance was taken as $P < 0.05$.

At doses higher than 4 mg/kg, the compound caused abdominal writhing, which prevented us from determining a fully analgesic dose and to investigate this effect in the presence of CB_1/CB_2 antagonists. In any attempt to explain the possible cause of abdominal writhing, which is a typical capsaicin-mediated behaviour, the interaction of compound **11** with both transient potential vanilloid type-1 (TRPV1) and ankyrin type-1 (TRPA1) receptors was studied in HEK293 cells overexpressing either the human recombinant TRPV1 or the rat recombinant TRPA1. The increase in intracellular calcium was used as a measure of these two effects, and values for both EC_{50} (for activation) and IC_{50} (for desensitization of the cell response to either capsaicin, for TRPV1, or allyl isothiocyanate, for TRPA1, after 5 min pre-incubation of compound **11** prior to these typical agonists) were calculated (Table 2). Compound **11** was found to activate both TRP channels, and in particular behaved as a potent TRPA1 agonist endowed with efficacy and potency similar to those of allyl isothiocyanate [44], which produces abdominal pain via activation of this channel [45].

2.6. Cytotoxicity assay

Cytotoxicity assay was performed to establish effects of most potent cannabinoid receptor ligands, i.e. compounds **3** and **11**, on cell viability in vitro. IC_{50} values for the effect on the NIH3T3 cell line for the two compounds were 69 μM and 188 μM, respectively (each value is the mean of six determinations and SEM is ≤ 5%). These data confirmed a low cytotoxic potential for both tested compounds towards NIH3T3 cells.

Table 2. Results of TRPV1 and TRPA1 assays of compound **11**.



TRPV1[b] (efficacy)	TRPV1 EC_{50} (μM)	TRPV1[c] IC_{50} (μM)	TRPA1[d] (efficacy)	TRPA1 EC_{50} (μM)	TRPA1[e] IC_{50} (μM)
18.6 ± 0.4	6.9 ± 0.7	23.9 ± 2.3	51.3 ± 2.5	2.7 ± 0.8	7.4 ± 1.6

[a] Data are means ± SEM of $N = 3$ determinations.
[b] As percent of the effect of ionomycin (4 μM).
[c] Determined against the effect of capsaicin (0.1 μM) after a 5 min pre-incubation with compound **11**.
[d] As percent of the effect of allyl isothiocyanate (100 μM).
[e] Determined against the effect of allyl isothiocyanate (100 μM) after a 5 min pre-incubation with compound **11**.

3. Conclusions

In this study, we have designed and synthesized new cannabinoid receptor ligands based on the alkyl-resorcinol template and carrying an amide functionality as terminal head of its flexible chain. Analysis of the binding assay results was not always easy and often multifaceted; nevertheless, these data showed that affinity was strongly affected by (i) the nature of the amines forming the amide head, (ii) the steric hindrance near the amide bond, and above all (iii) the presence and/or position of the carbonyl functionality. In fact, compounds presenting lipophilic and relatively hindered amide heads furnished the most potent cannabinoid receptor ligands while cyclopropanecarboxamides and amine derivatives, these latter lacking the carbonyl group, exhibited very limited capability of interacting with both cannabinoid receptors. On the other hand, analysis of selectivity trends was more complex and the CB_2 selectivity-enhancing effect of the aromatic group seemed the only parameter able to markedly influence the relative interactions of this series of compounds with either cannabinoid receptor subtype. Unfortunately, our attempt to modulate the cannabinergic properties of this class of compounds by transforming the amide moiety in the phthalimido functionality led to derivatives completely devoid of any enzyme inhibitory activity, underlying the importance of further studies on the elucidation of the chemical determinants for the receptor *versus* enzyme interaction.

In particular, *N*-allyl-8-[3-hydroxy-5-(2-methyloctan-2-yl)phenoxy]octanamide **11** exhibited the lowest K_i values and behaved as a potent CB_1 and CB_2 ligand, with affinities similar to WIN55,212-2 and better than AEA. Moreover, compound **11** behaved as a full agonist and a partial agonist at CB_1 and CB_2 receptors, respectively, endowed with moderate antinociceptive activity on the second phase of the biphasic pattern of formalin-induced pain behaviour. Interestingly, this compound turned out to activate also TRPA1 and TRPV1 channels, which may have decreased its analgesic activity in the formalin test, representing one of the few examples of dual cannabinoid receptor and TRPA1 channel ligand. On the other hand, *N*-phenyl-11-(3-hydroxy-5-pentylphenoxy)undecanamide **14**, although less potent, resulted in a CB_2 selective ligand with a functional profile of competitive inverse agonist.

Overall, the results presented herein confirm that the alkyl-resorcinol nucleus is a valuable scaffold for the design of potent cannabinoid receptor modulators and prompts us to further investigate whether it can represent a suitable lead for the design of new TRP channel ankyrin type-1 ligands.

4. Experimental protocols

4.1. Chemistry

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 two 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 study [31]. Organic solutions were dried over anhydrous sodium sulphate and concentrated with a Büchi rotary evaporator R-110 equipped with 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 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 were expressed as δ (ppm). The chromatography-mass spectrometry (LC-MS) system consisted of an Agilent 1100 series liquid cromatograph 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 solvent degassing unit. Nitrogen (purity 99.995%) was used as nebulizer and drying gas. UV detection was monitored at 254 nm. Mass spectra were acquired in positive or negative mode scanning over the mass range *m*/*z* of 150-1500. The structures of final compounds were unambiguously assessed by ^{1}H NMR, ^{13}C NMR and MS. Optical rotations were measured with a Perkin-Elmer 343 automatic polarimeter in a 0.1 dm tube (*c* = g/mL), using as wavelength the sodium-D line. 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%.

4.1.1. General procedure for the synthesis of final amides **1**-**20** and retro-amides **23**-**26**

To a stirred solution of the appropriate acid (1.0 mmol, acids **37**-**41** or cyclopropanecarboxylic acid) and amine (1.2 mmol, (*R*)-(-)-2-aminopropanol, allylamine, trifluoroethylamine, aniline, benzylamine, adamantylamine or amines **54**-**57**) in dichloromethane, kept at 0 °C in an ice bath, solid HOBt (1.0 mmol) and then a solution in the same solvent of EDC (1.5 mmol) were added. The reaction was allowed to warm to room temperature and stirring was continued for 24 h. The organic solution was washed with 5% aqueous $NaHCO_3$, then with 1M HCl, and dried over anhydrous sodium sulfate. After evaporation of solvent to dryness, the crude product was purified by silica gel column chromatography.

4.1.1.1. (*R*)-8-(3-Hydroxy-5-pentylphenoxy)-*N*-(1-hydroxypropan-2-yl)octanamide **1**

Eluent: $CHCl_3$/MeOH = 47/3. Yield 70% as transparent oil. ^{1}H-NMR ($CDCl_3$): δ 6.25-6.24 (m, 3H), 5.92 (br d, 1H, *J* = 7.2 Hz, disappears on treatment with D_2O), 4.07-3.98 (m, 1H), 3.87 (t, 2H, *J* = 6.5 Hz), 3.64 (dd, 1H, *J* = 3.6 Hz, *J* = 11.0 Hz), 3.49 (dd, 1H, *J* = 5.8 Hz, *J* = 11.0 Hz), 2.46 (t,

2H, *J* = 7.6 Hz), 2.16 (t, 2H, *J* = 7.4 Hz), 1.70-1.58 (mm, 6H), 1.50-1.31 (mm, 10H), 1.14 (d, 3H, *J* = 6.7 Hz), 0.86 (t, 3H, *J* = 6.2 Hz). ^{13}C-NMR ($CDCl_3$): δ 174.43, 160.11, 157.16, 145.59, 108.04, 107.00, 99.29, 67.80, 66.88, 47.73, 36.62, 36.06, 31.53, 30.85, 28.82 (x2), 28.68, 25.59, 25.47, 22.54, 17.00, 14.02. MS *m/z*: 402 $[M+Na]^+$ (100). $[\alpha]_D^{20}$ = + 9.13° (*c* = 0.01204; dichloromethane). Anal. calcd. for $C_{22}H_{37}NO_4$: C, 69.62%; H, 9.83%; N, 3.69%. Found: C, 69.57%; H, 9.86%; N, 3.68%.

5.1.1.2. (*R*)-11-(3-Hydroxy-5-pentylphenoxy)-*N*-(1-hydroxypropan-2-yl)undecanamide **2**

Eluent: $CHCl_3$/MeOH = 48/2. Yield 65% as transparent oil. ^{1}H-NMR ($CDCl_3$): δ 6.25-6.24 (m, 3H), 5.98 (br d, 1H, *J* = 7.1 Hz, disappears on treatment with D_2O), 4.08-3.99 (m, 1H), 3.88 (t, 2H, *J* = 6.4 Hz), 3.64 (dd, 1H, *J* = 3.8 Hz, *J* = 11.0 Hz), 3.50 (dd, 1H, *J* = 5.6 Hz, *J* = 11.1 Hz), 2.46 (t, 2H, *J* = 7.6 Hz), 2.16 (t, 2H, *J* = 7.6 Hz), 1.74-1.68 (m, 2H), 1.63-1.56 (m, 4H), 1.48-1.26 (mm, 16H), 1.14 (d, 3H, *J* = 6.8 Hz), 0.86 (t, 3H, *J* = 6.4 Hz). ^{13}C-NMR ($CDCl_3$): δ 174.50, 160.22, 157.15, 145.51, 107.99, 106.76, 99.45, 67.80, 66.93, 47.73, 36.78, 36.08, 31.53, 30.86, 29.12 (x2), 29.04 (x2), 28.87 (x2), 25.74 (x2), 22.54, 17.01, 14.03. MS *m/z*: 444 $[M+Na]^+$ (100). $[\alpha]_D^{20}$ = + 8.30° (*c* = 0.01265; dichloromethane). Anal. calcd. for $C_{25}H_{43}NO_4$: C, 71.22%; H, 10.28%; N, 3.32%. Found: C, 71.12%; H, 10.30%; N, 3.31%.

5.1.1.3. (*R*)-8-[3-Hydroxy-5-(2-methyloctan-2-yl)phenoxy]-*N*-(1-hydroxypropan-2-yl)octanamide **3**

Eluent: $CHCl_3$/MeOH = 50/2. Yield 68% as transparent oil. ^{1}H-NMR ($CDCl_3$): δ 6.40-6.39 (m, 2H), 6.24 (s, 1H), 5.80 (br d, 1H, *J* = 7.5 Hz, disappears on treatment with D_2O), 4.09-4.03 (m, 1H), 3.89 (t, 2H, *J* = 8.0 Hz), 3.65 (dd, 1H, *J* = 4.0 Hz, *J* = 12.0 Hz), 3.52 (dd, 1H, *J* = 6.2 Hz, *J* = 12.0 Hz), 2.18 (t, 2H, *J* = 7.8 Hz), 1.74-1.71 (m, 2H), 1.64-1.61 (m, 2H), 1.52-1.41 (m, 4H), 1.35-1.34 (m, 4H), 1.24-1.18 (mm, 12H), 1.15 (d, 3H, *J* = 8.2 Hz), 1.04-1.03 (m, 2H), 0.82 (t, 3H, *J* = 6.4Hz). ^{13}C-NMR ($CDCl_3$): δ 174.34, 159.87, 156.81, 152.88, 105.77, 105.42, 98.57, 67.82, 67.07, 47.81, 44.51, 37.80, 36.59, 31.79, 30.03, 28.90 (x2), 28.79 (x2), 28.67, 25.53, 25.43, 24.65, 22.67,

17.02, 14.07. MS *m/z*: 458 $[M+Na]^+$ (100). $[\alpha]_D^{20}$ = + 7.78° (*c* = 0.02700; dichloromethane). Anal. calcd. for $C_{26}H_{45}NO_4$: C, 71.68%; H, 10.41%; N, 3.22%. Found: C, 71.59%; H, 10.44%; N, 3.21%.

4.1.1.4. (*R*)-11-[3-Hydroxy-5-(2-methyloctan-2-yl)phenoxy]-*N*-(1-hydroxypropan-2-yl)undecanamide **4**

Eluent: $CHCl_3$/MeOH = 50/2. Yield 73% as transparent oil. ^{1}H-NMR ($CDCl_3$): δ 6.41-6.38 (m, 2H), 6.24-6.23 (m, 1H), 5.72 (br d, 1H, *J* = 7.8 Hz, disappears on treatment with D_2O), 4.11-4.02 (m, 1H), 3.90 (t, 2H, *J* = 6.0 Hz), 3.65 (dd, 1H, *J* = 4.0 Hz, *J* = 10.0 Hz), 3.52 (dd, 1H, *J* = 6.0 Hz, *J* = 10.0 Hz), 2.17 (t, 2H, *J* = 7.2 Hz), 1.77-1.70 (m, 2H), 1.63-1.60 (m, 2H), 1.53-1.39 (m, 4H), 1.35-1.27 (mm, 6H), 1.24-1.19 (mm, 16H), 1.16 (d, 3H, *J* = 7.8 Hz), 1.04-1.03 (m, 2H), 0.82 (t, 3H, *J* = 6.5Hz). ^{13}C-NMR ($CDCl_3$): δ 174.30, 160.00, 156.71, 152.66, 105.73, 105.16, 98.80, 67.82, 67.23, 47.81, 44.53, 38.01, 36.79, 31.79, 30.03, 28.88 (x2), 28.77 (x2), 28.68 (x2), 25.69 (x2), 25.44 (x2), 24.65, 22.67, 17.04, 14.07. MS *m/z*: 500 $[M+Na]^+$ (100), 478 $[M+H]^+$ (25). $[\alpha]_D^{20}$ = + 6.52° (*c* = 0.03836; dichloromethane). Anal. calcd. for $C_{29}H_{51}NO_4$: C, 72.91%; H, 10.76%; N, 2.93%. Found: C, 72.80%; H, 10.79%; N, 2.92%.

4.1.1.5. *N*-(1,3-Dihydroxypropan-2-yl)-11-(3-hydroxy-5-pentylphenoxy)undecanamide **5**

Eluent: $CHCl_3$/MeOH = 46/4. Yield 70% as white solid, mp = 60-63 °C (K). ^{1}H-NMR ($CDCl_3$): δ 6.28–6.24 (m, 3H), 3.93-3.82 (m, 5H), 3.75 (dd, 2H, *J* = 4.5 Hz, *J* = 11.0 Hz), 2.48 (t, 2H, *J* = 7.6 Hz), 2.22 (t, 2H, *J* = 7.5 Hz), 1.76-1.52 (mm, 6H), 1.49-1.23 (mm, 16H), 0.86 (t, 3H, *J* = 6.6 Hz). ^{13}C-NMR ($CDCl_3$): δ 174.64, 160.43, 156.92, 145.65, 107.96, 106.78, 99.51, 67.80, 63.11 (x2), 52.33, 36.72, 36.08, 31.52, 30.87 (x2), 29.32, 29.19, 29.04 (x2), 28.90, 28.63, 25.60, 22.54, 14.03. MS *m/z*: 460 $[M+Na]^+$ (100). Anal. calcd. for $C_{25}H_{43}NO_5$: C, 68.64%; H, 9.90%; N, 3.20%. Found: C, 68.51%; H, 9.93%; N, 3.19%.

4.1.1.6. *N*-(1,3-Dihydroxypropan-2-yl)-8-[3-hydroxy-5-(2-methyloctan-2-yl)phenoxy]octanamide **6**

Eluent: $CHCl_3$/MeOH = 45/5. Yield 70% as white solid, mp 50-52 °C (K). ^{1}H-NMR ($CDCl_3$): δ 6.42-6.38 (m, 2H), 6.24-6.23 (m, 1H), 3.97-3.94 (m, 1H), 3.91 (t, 2H, *J* = 6.7 Hz), 3.85 (dd, 2H, *J* =

4.2 Hz, *J* = 11.1 Hz), 3.76 (dd, 2H, *J* = 4.2 Hz, *J* = 11.1 Hz), 2.23 (t, 2H, *J* = 7.4 Hz), 1.77-1.58 (m, 4H), 1.53-1.40 (mm, 4H), 1.37-1.35 (m, 4H), 1.24-1.03 (mm, 14H), 0.82 (t, 3H, *J* = 6.9 Hz). ^{13}C-NMR ($CDCl_3$): δ 174.15, 160.04, 158.76, 152.96, 105.34, 104.89, 98.42, 67.87, 63.02 (x2), 52.13, 44.50, 37.83, 35.94, 31.78, 30.02, 29.15, 29.04 (x2), 28.96, 28.84 (x2), 25.73, 24.72, 22.66, 14.06. MS *m/z*: 474 $[M+Na]^+$ (100). Anal. calcd. for $C_{26}H_{45}NO_5$: C, 69.14%; 10.04H, %; N, 3.10%. Found: C, 69.08%; H, 10.02%; N, 3.09%.

4.1.1.7. *N*-(1,3-Dihydroxypropan-2-yl)-11-[3-hydroxy-5-(2-methyloctan-2-yl)phenoxy]undecanamide **7**

Eluent:$CHCl_3$/MeOH = 47/3. Yield 65% as white solid, mp = 56-58 °C (K). ^{1}H-NMR ($CDCl_3$): δ 6.41 (s, 1H), 6.38 (s, 1H), 6.31 (br d, 1H, *J* = 7.1 Hz, disappears on treatment with D_2O), 6.23 (s, 1H), 3.97-3.93 (m, 1H), 3.90 (t, 2H, *J* = 6.4 Hz), 3.84 (dd, 2H, *J* = 4.1 Hz, *J* = 11.1 Hz), 3.74 (dd, 2H, *J* = 4.1 Hz, *J* = 11.1 Hz), 2.20 (t, 2H, *J* = 7.5 Hz), 1.76-1.70 (m, 2H), 1.63-1.60 (m, 4H), 1.52-1.48 (m, 2H), 1.44-1.39 (m, 2H), 1.36-1.27 (mm, 6H), 1.23-1.03 (mm, 16H), 0.82 (t, 3H, *J* = 6.8 Hz). ^{13}C-NMR ($CDCl_3$): δ 174.43, 160.35, 157.96, 152.53, 105.34, 105.13, 97.17, 68.04, 63.15 (x2), 52.48, 44.52, 37.97, 36.15, 31.78, 30.02 (x2), 29.34 (x2), 29.17 (x2), 29.02 (x2), 28.89, 26.09, 25.94, 24.65, 22.86, 14.07. MS *m/z*: 516 $[M+Na]^+$ (100), 494 $[M+H]^+$ (20). Anal. calcd. for $C_{29}H_{51}NO_5$: C, 70.55%; H, 10.41%; N, 2.84%. Found: C, 70.37%; H, 10.45%; 2.83%.

4.1.1.8. *N*-(1,3-Dihydroxypropan-2-yl)-11-(2-hexyl-5-hydroxyphenoxy)undecanamide **8**

Eluent: $CHCl_3$/MeOH = 46/4. Yield 60% as white solid, mp 88-90 °C (K). ^{1}H-NMR ($CDCl_3$): δ 6.92 (d, 1H, *J* = 7.9 Hz), 6.37 (d, 1H, *J* = 2.6 Hz), 6.30 (dd, 1H, *J* = 2.6 Hz, *J* = 7.9 Hz), 3.92-3.83 (m, 5H), 3.77 (dd, 2H, *J* = 4.1 Hz, *J* = 10.8 Hz), 2.49 (t, 2H, *J* = 7.6 Hz), 2.21 (t, 2H, *J* = 7.5 Hz), 1.80-1.73 (m, 2H), 1.69-1.58 (m, 4H), 1.28-1.23 (mm, 18H), 0.86 (t, 3H, *J* = 6.1 Hz). ^{13}C-NMR ($CDCl_3$): δ 173.59, 157.80, 156.83, 130.12, 123.75, 104.36, 99.14, 67.94, 63.27 (x2), 52.36, 36.11, 31.82, 30.14, 29.63, 29.35 (x2), 29.18 (x2), 29.07, 28.85, 28.18, 26.12, 25.43, 22.57, 14.10. MS

m/z: 474 $[M+Na]^+$ (100), 452 $[M+H]^+$ (30). Anal. calcd. for $C_{26}H_{45}NO_5$: C, 69.14%; H, 10.04%; N, 3.10%. Found: C, 69.03%; H, 10.06%; N, 3.11%.

4.1.1.9. *N*-(1,3-Dihydroxypropan-2-yl)-11-(4-hexyl-3-hydroxyphenoxy)undecanamide **9**

Eluent: $CHCl_3$/MeOH = 47/3. Yield 65% as cream solid, mp = 91-93 °C (K). ^{1}H-NMR ($CDCl_3$): δ 6.92 (d, 1H, *J* = 8.1 Hz), 6.40 (d, 1H, *J* = 2.1 Hz), 6.31 (dd, 1H, *J* = 2.1 Hz, *J* = 8.3 Hz), 3.95-3.91 (m, 1H), 3.86 (t, 2H, *J* = 6.4 Hz), 3.61-3.56 (m, 4H), 2.50 (t, 2H, *J* = 7.5 Hz), 2.18 (t, 2H, *J* = 7.4 Hz), 1.73-1.63 (m, 2H), 1.56-1.49 (m, 4H), 1.45-1.28 (mm, 18H), 0.85 (t, 3H, *J* = 6.5 Hz). ^{13}C-NMR ($CDCl_3$): δ 174.27, 158.44, 157.03, 129.96, 120.81, 105.14, 100.24, 68.03, 63.32 (x2), 52.23, 34.14, 36.02, 31.74, 30.05, 29.26 (x2), 29.18, 29.12 (x2), 29.03 (x2), 25.98, 24.83, 22.62, 14.07. MS *m/z*: 474 $[M+Na]^+$ (100), 452 $[M+H]^+$ (15). Anal. calcd. for $C_{26}H_{45}NO_5$: C, 69.14%; H, 10.04%; N, 3.10%. Found: C, 68.97%; H, 10.07%; N, 3.9%.

4.1.1.10. *N*-Allyl-11-(3-hydroxy-5-pentylphenoxy)undecanamide **10**

Eluent: $CHCl_3$. Yield 90% as pale yellow oil. ^{1}H-NMR ($CDCl_3$): δ 6.26 (s, 3H), 5.91-5.74 (m, 1H), 5.70 (br s, 1H, disappears on treatment with D_2O), 5.20-5.09 (m, 2H), 3.90-3.85 (m, 4H), 2.47 (t, 2H, *J* = 7.6 Hz), 2.19 (t, 2H, *J* = 7.6 Hz), 1.75-1.56 (mm, 10H), 1.51-1.26 (mm, 12H), 0.86 (t, 3H, *J* = 6.4 Hz). ^{13}C-NMR ($CDCl_3$): δ 173.62, 160.24, 157.18, 145.46, 134.05, 116.59, 107.97, 106.80, 99.42, 67.80, 42.05, 36.75, 36.07, 31.52, 30.85, 29.18 (x2), 29.08 (x2), 28.94 (x2), 25.74 (x2), 22.54, 14.02. MS *m/z*: 426 $[M+Na]^+$ (100). Anal. calcd. for $C_{25}H_{41}NO_3$: C, 74.40%; H, 10.24%; N, 3.47%. Found: C, 74.29; H, 10.26%; N, 3.46%.

4.1.1.11. *N*-Allyl-8-[3-hydroxy-5-(2-methyloctan-2-yl)phenoxy]octanamide **11**

Eluent: $CHCl_3$/MeOH = 50/2. Yield 85% as pasty yellow oil. ^{1}H-NMR ($CDCl_3$): δ 6.41 (s, 1H), 6.39 (s, 1H), 6.25 (s, 1H), 5.85-5.78 (m, 1H), 5.19-5.11 (m, 2H), 3.91-3.88 (m, 4H), 2.23 (t, 2H, *J* = 6.5 Hz), 1.76-1.65 (m, 4H), 1.52-1.48 (m, 2H), 1.43-1.35 (mm, 6H), 1.24-1.16 (mm, 12H), 1.06-1.03 (m, 2H), 0.82 (t, 3H, *J* = 6.8 Hz). ^{13}C-NMR ($CDCl_3$): δ 173.53, 159.89, 156.73, 152.84, 133.94, 116.72, 105.70, 105.59, 98.51, 67.84, 44.52, 42.14, 37.80, 36.51, 31.79, 30.03 (x2), 29.10

(x2), 28.90 (x2), 25.59 (x2), 24.65, 22.67, 14.07. MS *m/z*: 440 $[M+Na]^+$ (100), 418 $[M+H]^+$ (40). Anal. calcd. for $C_{26}H_{43}NO_3$: C, 74.77%; H, 10.38%; N, 3.35%. Found: C, 74.54%; H, 10.41%; N, 3.34%.

4.1.1.12. *N*-(2,2,2-Trifluoroethyl)-11-(3-hydroxy-5-pentylphenoxy)undecanamide **12**

Eluent: $CHCl_3$. Yield 85% as pale yellow solid, mp = 44-46 °C (K). ^{1}H-NMR ($CDCl_3$): δ 6.52-6.22 (m, 3H), 5.88 (br s, 1H, disappears on treatment with D_2O), 4.10-3.82 (m, 4H), 2.47 (t, 2H, *J* = 7.6 Hz), 2.24 (t, 2H, *J* = 7.4 Hz), 1.75-1.48 (mm, 8H), 1.39-1.26 (mm, 14H), 0.86 (t, 3H, *J* = 6.6 Hz). ^{13}C-NMR ($CDCl_3$): δ 173.68, 160.30, 156.78, 145.62, 127.05 (q, CF_3, *J* = 282 Hz), 107.83, 107.13, 99.31, 67.86, 40.60 (q, CH_2CF_3, *J* = 35 Hz), 36.43, 36.04, 31.51, 30.83, 29.24 (x2), 29.11 (x2), 29.05 (x2), 25.84, 25.41, 22.53, 14.01. MS *m/z*: 468 $[M+Na]^+$ (100), 446 $[M+H]^+$ (20). Anal. calcd. for $C_{24}H_{38}F_3NO_3$: C, 64.70%; H, 8.60%; N, 3.14%. Found: C, 64.59%; H, 8.61%; N, 3.13%.

4.1.1.13. *N*-(2,2,2-Trifluoroethyl)- 8-[3-hydroxy-5-(2-methyloctan-2-yl)phenoxy]octanamide **13**

Eluent: $CHCl_3$. Yield 75% as yellow oil. ^{1}H-NMR ($CDCl_3$): δ 6.41-6.39 (m, 2H), 6.21 (s, 1H), 5.94 (br s, 1H, disappears on treatment with D_2O), 3.98-3.82 (m, 4H), 2.25 (t, 2H, *J* = 7.4 Hz), 1.76-1.62 (m, 4H), 1.54-1.50 (m, 2H), 1.48-1.36 (mm, 6H), 1.21-1.17 (mm, 12H), 1.06-1.02 (m, 2H), 0.82 (t, 3H, *J* = 6.3 Hz). ^{13}C-NMR ($CDCl_3$): δ 173.62, 159.96, 156.54, 152.93, 123.51 (q, CF_3, *J* = 278 Hz), 105.65, 105.59, 98.52, 67.82, 44.50, 40.60 (q, CH_2CF_3, *J* = 34 Hz), 37.81, 36.31, 31.78, 30.02 (x2), 29.04 (x2), 28.88 (x2), 25.74, 25.28, 24.64, 22.66, 14.06. MS *m/z*: 482 $[M+Na]^+$ (100). Anal. calcd. for $C_{25}H_{40}F_3NO_3$: C, 65.33%; H, 8.77%; N, 3.05%. Found: C, 65.20%; H, 8.80%; N, 3.04%.

4.1.1.14. *N*-Phenyl-11-(3-hydroxy-5-pentylphenoxy)undecanamide **14**

Eluent: $CHCl_3$/MeOH = 50/1. Yield 75% as yellow solid, mp = 49-51 °C (K). ^{1}H-NMR ($CDCl_3$): δ 7.49 (d, 2H, *J* = 7.8 Hz), 7.29 (t, 2H, *J* = 7.8 Hz), 7.20 (br s, 1H, disappears on treatment with D_2O), 7.08 (t, 1H, *J* = 7.3 Hz), 6.29 (s, 1H), 6.24-6.23 (m, 2H), 3.88 (t, 2H, *J* = 6.5 Hz), 2.47 (t, 2H, *J* = 7.7 Hz), 2.34 (t, 2H, *J* = 7.5 Hz), 1.75-1.67 (m, 4H), 1.60-1.52 (m, 2H), 1.49-1.29 (mm, 16H), 0.87 (t, 3H, *J* = 6.8 Hz). ^{13}C-NMR ($CDCl_3$): δ 174.05, 160.31, 156.79, 145.82, 129.03 (x2), 124.34,

119.90 (x2), 107.83, 107.15, 99.34, 67.84, 37.87, 36.04, 31.51, 30.83 (x2), 29.32 (x2), 29.12 (x2), 29.01 (x2), 25.82, 25.61, 22.53, 14.01. MS *m/z*: 462 $[M+Na]^+$ (100). Anal. calcd. for $C_{28}H_{41}NO_3$: C, 76.50%; H, 9.40%; N, 3.19%. Found: C, 76.37%; H, 9.42%; N, 3.18%.

4.1.1.15. *N*-Benzyl-11-(3-hydroxy-5-pentylphenoxy)undecanamide **15**

Eluent: $CHCl_3$. Yield 72% as transparent oil. ^{1}H-NMR ($CDCl_3$): δ 7.33-7.29 (m, 2H), 7.27-7.25 (m, 3H), 6.26 (s, 3H), 5.85 (br s, 1H, disappears on treatment with D_2O), 4.42 (d, 2H, *J* = 5.7 Hz), 3.88 (t, 2H, *J* = 6.4 Hz), 2.47 (t, 2H, *J* = 7.7 Hz), 2.20 (t, 2H, *J* = 7.6 Hz), 1.76-1.72 (m, 2H), 1.70-1.64 (m, 2H), 1.58-1.54 (m, 2H), 1.44-1.40 (m, 2H), 1.32-1.26 (mm, 14H), 0.86 (t, 3H, *J* = 6.8 Hz). ^{13}C-NMR ($CDCl_3$): δ 173.56, 160.26, 157.18, 145.49, 128.75 (x2), 127.88 (x2), 127.59, 107.98, 106.84, 99.42, 67.81, 43.74, 36.80, 36.07, 31.53, 30.85 (x2), 29.19 (x2), 29.09 (x2), 28.97 (x2), 25.79, 25.73, 22.54, 14.02. MS *m/z*: 476 $[M+Na]^+$ (100). Anal. calcd. for $C_{29}H_{43}NO_3$: C, 76.78%; H, 9.55%; N, 3.09%. Found: 76.70%; H, 9.58%; N, 3.08%.

4.1.1.16. *N*-(Adamantan-1-yl)-11-(3-hydroxy-5-pentylphenoxy)undecanamide **16**

Eluent: $CHCl_3$/MeOH = 50/0.3. Yield 93% as yellow oil. ^{1}H-NMR ($CDCl_3$): δ 6.28-6.26 (m, 3H), 5.23 (br s, 1H, disappears on treatment with D_2O), 3.88 (t, 2H, *J* = 6.35 Hz), 2.47 (t, 2H, *J* = 7.64 Hz), 2.12-1.99 (mm, 10H), 1.74-1.56 (mm, 13H), 1.39-1.25 (mm, 16H), 0.86 (t, 3H, *J* = 6.36 Hz). ^{13}C-NMR ($CDCl_3$): δ 172.88, 160.24, 157.28, 145.41, 108.00, 106.76, 99.46, 67.76, 51.97, 41.69 (x3), 37.92, 36.35 (x3), 36.08, 31.53, 30.85, 29.45 (x4), 29.10 (x3), 28.88 (x2), 25.83, 25.75, 22.54, 14.02. MS *m/z*: 498 $[M+H]^+$, 520 $[M+23]^+$ (100). Anal. calcd. for $C_{32}H_{51}NO_3$: C, 77.22%; H, 10.33%; N, 2.81%. Found: C, 77.09%; H, 10.35%; N, 2.80%.

4.1.1.17. *N*-(Adamantan-1-yl)-8-[3-hydroxy-5-(2-methyloctan-2-yl)phenoxy]octanamide **17**

Eluent: $CHCl_3$/MeOH = 50/0.3. Yield 72% as transparent oil. ^{1}H-NMR ($CDCl_3$): δ 6.41-6.39 (m, 2H), 6.25-6.23 (m, 1H), 5.13 (br s, 1H, disappears on treatment with D_2O), 3.89 (t, 2H, *J* = 6.72 Hz), 2.12- 1.98 (mm, 10H), 1.76-1.65 (mm, 10H), 1.59-1.34 (mm, 9H), 1.21-1.03 (mm, 14H), 0.82 (t, 3H, *J* = 6.39 Hz). ^{13}C-NMR ($CDCl_3$): δ 172.83, 159.88, 157.15, 152.63, 105.79, 105.28, 98.59,

67.84, 51.99, 44.54, 41.67 (x3), 37.80 (x2), 36.33 (x3), 31.79, 30.04 (x2), 29.44 (x3), 29.07 (x3), 28.90 (x2), 25.74, 24.65, 22.67, 14.07. MS *m/z*: 512 $[M+H]^+$, 534 $[M+23]^+$ (100). Anal. calcd. for $C_{33}H_{53}NO_3$: C, 77.45%; H, 10.44%; N, 2.74%. Found: C, 77.35%; H, 10.47%; N, 2.73%.

4.1.1.18. *N*-(Adamantan-1-yl)-11-[3-hydroxy-5-(2-methyloctan-2-yl)phenoxy]undecanamide **18**

Eluent: $CHCl_3$/MeOH = 50/0.3. Yield 71% as pale yellow oil. ^{1}H-NMR ($CDCl_3$): δ 6.40 (s, 2H), 6.28-6.27 (m, 1H), 5.19 (br s, 1H, disappears on treatment with D_2O), 3.89 (t, 2H, *J* = 6.46 Hz), 2.12-1.99 (mm, 10H), 1.76-1.65 (mm, 10H), 1.57-1.37 (mm, 9H), 1.26-1.03 (mm, 20H), 0.82 (t, 3H, *J* = 6.43 Hz). ^{13}C-NMR ($CDCl_3$): δ 172.83, 159.97, 156.95, 105.77, 105.10, 98.79, 67.78, 51.95, 44.54, 41.69 (x3), 37.92, 37.79, 36.35 (x3), 31.78, 30.04 (x2), 29.44 (x4), 29.10 (x4), 28.92 (x2), 25.78 (x2), 24.65, 22.67, 14.07. MS *m/z*: 554 $[M+H]^+$, 573 $[M+23]^+$ (100). Anal. calcd. for $C_{36}H_{59}NO_3$: C, 78.07%; H, 10.74%; N, 2.53%. Found: C, 77.89%; H, 10.76%; N, 2.54%.

4.1.1.19. *N*-(Adamantan-1-yl)-11-(2-hexyl-5-hydroxyphenoxy)undecanamide **19**

Eluent: $CHCl_3$/MeOH = 50/0.5. Yield 76% as transparent oil. ^{1}H-NMR ($CDCl_3$): δ 6.88 (d, 1H, *J* = 8.00 Hz), 6.36 (s, 1H), 6.31 (d, 1H, *J* = 8.15 Hz), 5.15 (br s, 1H, disappears on treatment with D_2O), 3.85 (t, 2H, *J* = 6.26 Hz), 2.46 (t, 2H, *J* = 7.48 Hz), 2.08-1.96 (mm, 11H), 1.75-1.62 (mm, 8H), 1.54-1.41 (mm, 6H), 1.36-1.24 (mm, 16H), 0.83 (t, 3H, *J* = 6.00 Hz). ^{13}C-NMR ($CDCl_3$): δ 172.71, 157.81, 155.13, 129.97, 123.35, 106.39, 99.73, 67.70, 51.89, 41.71 (x3), 37.91, 36.36 (x3), 31.79, 30.19, 29.66 (x4), 29.45 (x3), 29.20, 29.09 (x3), 25.93, 25.86, 22.65, 14.13. MS *m/z*: 512 $[M+H]^+$, 534 $[M+23]^+$ (100). Anal. calcd. for $C_{33}H_{53}NO_3$: C, 77.45%; H, 10.44%; N, 2.74%. Found: C, 77.38%; H, 10.45%; N, 2.73%.

4.1.1.20. *N*-(Adamantan-1-yl)-11-(4-hexyl-3-hydroxyphenoxy)undecanamide **20**

Eluent: $CHCl_3$/MeOH = 50/0.3. Yield 77% as white solid, mp 78.0 °C (K). ^{1}H-NMR ($CDCl_3$): δ 6.95 (d, 1H, *J* = 8.05 Hz), 6.43 (d, 1H, *J* = 2.16 Hz), 6.36 (dd, 1H, *J* = 2.08 Hz, *J* = 8.15 Hz), 5.16 (br s, 1H, disappears on treatment with D_2O), 3.88 (t, 2H, *J* = 6.70 Hz), 2.51 (t, 2H, *J* = 7.51 Hz), 2.11-1.99 (mm, 11H), 1.75-1.65 (mm, 8H), 1.58-1.45 (mm, 6H), 1.41-1.27 (mm, 16H), 0.86 (t, 3H, *J* = 6.42 Hz). ^{13}C-NMR ($CDCl_3$): δ 172.73, 158.32, 154.90, 130.16, 121.07, 105.79, 102.45, 67.83,

51.93, 41.69 (x3), 37.90, 36.35 (x3), 31.80, 30.05, 29.46 (x4), 29.22, 29.07 (x3), 28.94 (x2), 28.75, 25.79, 25.68, 22.65, 14.10. MS *m/z*: 512 $[M+H]^+$, 534 $[M+23]^+$ (100). Anal. calcd. for $C_{33}H_{53}NO_3$: C, 77.45%; H, 10.44%; N, 2.74%. Found: C, 77.32%; H, 10.47%; N, 2.73%.

4.1.1.21. *N*-[11-(3-Hydroxy-5-pentylphenoxy)undecyl]cyclopropanecarboxamide **23**

This compound was prepared from amine **54** and cyclopropanecarboxylic acid. Eluent: $CHCl_3$/MeOH = 50/0.2. Yield 60% as pasty yellow solid, mp < 30 °C (K). ^{1}H-NMR ($CDCl_3$): δ 6.26-6.24 (m, 3H), 5.98 (br t, 1H, disappears on treatment with D_2O), 3.87 (t, 2H, *J* = 6.41 Hz), 3.27-3.18 (m, 2H), 2.46 (t, 2H, *J* = 7.58 Hz), 1.75-1.63 (m, 3H), 1.59-1.38 (mm, 8H), 1.31-1.25 (mm, 14H), 0.99-0.92 (m, 2H), 0.86 (t, 3H, *J* = 6.48 Hz), 0.74-0.69 (m, 2H). ^{13}C-NMR ($CDCl_3$): δ 174.08, 160.21, 157.39, 145.36, 107.97, 106.77, 99.27, 67.80, 39.99, 36.06, 31.53, 30.84, 29.66, 29.31 (x2), 29.26 (x2), 29.18 (x2), 26.85, 25.91, 22.55, 14.82, 14.02, 7.12 (x2). MS *m/z*: 417 $[M+H]^+$ (20), 440 $[M+23]^+$ (100). Anal. calcd. for $C_{26}H_{43}NO_3$: C, 74.77%; H, 10.38%; N, 3.35%. Found: C, 74.56%; H, 10.41%; N, 3.34%.

4.1.1.22. *N*-[11-[3-Hydroxy-5-(2-methyloctan-2-yl)phenoxy]undecyl]cyclopropanecarboxamide **24**

This compound was prepared from amine **55** and cyclopropanecarboxylic acid. Eluent: $CHCl_3$/MeOH = 50/0.2. Yield 65% as transparent oil. ^{1}H-NMR ($CDCl_3$): δ 6.41 (d, 1H, *J* = 1.70 Hz), 6.38 (s, 1H), 6.24 (d, 1H, *J* = 1.70 Hz), 5.97 (br t, 1H, disappears on treatment with D_2O), 3.87 (t, 2H, *J* = 6.43 Hz), 3.28-3.19 (m, 2H), 2.03-1.66 (m, 3H), 1.54-1.41 (m, 6H), 1.38-1.32 (mm, 8H), 1.25-1.04 (mm, 18H), 0.98-0.93 (m, 2H), 0.82 (t, 3H, *J* = 6.32 Hz), 0.75-0.69 (m, 2H). ^{13}C-NMR ($CDCl_3$): δ 174.17, 159.92, 157.20, 152.56, 105.76, 105.01, 98.59, 67.80, 44.53, 40.02, 37.76, 31.79, 30.05 (x2), 29.64 (x2), 29.32 (x2), 29.21 (x2), 28.92 (x2), 26.87, 25.96, 24.65, 22.68, 14.80, 14.08, 7.14 (x2). MS *m/z*: 474 $[M+H]^+$ (20), 496 $[M+23]^+$ (100). Anal. calcd. for $C_{30}H_{51}NO_3$: C, 76.06%; H, 10.85%; N, 2.96%. Found: C, 75.92%; H, 10.88%; N, 2.95%.

4.1.1.23. *N*-[11-(2-Hexyl-5-hydroxyphenoxy)undecyl]cyclopropanecarboxamide **25**

This compound was prepared from amine **56** and cyclopropanecarboxylic acid. Eluent: $CHCl_3$/MeOH = 50/0.2. Yield 55 % as pasty yellow solid, mp < 30 °C (K). ^{1}H-NMR ($CDCl_3$): δ

6.89 (d, 1H, *J* = 7.93 Hz), 6.38 (d, 1H, *J* = 1.93 Hz), 6.32 (dd, 1H, *J* = 1.96 Hz, *J* = 7.94 Hz), 5.80 (br t, 1H, disappears on treatment with D_2O), 3.86 (t, 2H, *J* = 6.22 Hz), 3.28-3.18 (m, 2H), 2.48 (t, 2H, *J* = 7.57 Hz), 1.78-1.68 (m, 3H), 1.55-1.40 (mm, 8H), 1.33-1.21 (mm, 16H), 1.00-0.93 (m, 2H), 0.86 (t, 3H, *J* = 6.20 Hz), 0.75-0.68 (m, 2H). ^{13}C-NMR ($CDCl_3$): δ 174.15, 157.73, 155.55, 129.90, 122.87, 106.47, 99.77, 67.66, 40.01, 31.81 (x2), 30.25, 29.67 (x2), 29.45 (x3), 29.26 (x3), 26.92, 26.06, 22.66, 14.80, 14.13, 7.13 (x2). MS *m/z*: 432 $[M+H]^+$ (20), 454 $[M+23]^+$ (100). Anal. calcd. for $C_{27}H_{45}NO_3$: C, 75.13%; H, 10.51%; N, 3.24%. Found: C, 75.02%; H, 10.52%; N, 3.25%.

4.1.1.24 *N*-[11-(4-Hexyl-3-hydroxyphenoxy)undecyl]cyclopropanecarboxamide **26**

This compound was prepared from amine **57** and cyclopropanecarboxylic acid. Eluent: $CHCl_3$/MeOH = 50/0.2. Yield 55 % as pale yellow solid, mp = 87-90 °C (K). ^{1}H-NMR ($CDCl_3$): δ 6.96 (d, 1H, *J* = 7.93 Hz), 6.39-6.32 (m, 2H), 5.75 (br t, 1H, disappears on treatment with D_2O), 3.87 (t, 2H, *J* = 6.24 Hz), 3.31-3.20 (m, 2H), 2.49 (t, 2H, *J* = 7.61 Hz), 1.81-1.68 (m, 3H), 1.62-1.40 (mm, 8H), 1.35-1.21 (mm, 16H), 0.99-0.93 (m, 2H), 0.86 (t, 3H, *J* = 6.18 Hz), 0.74-0.67 (m, 2H). ^{13}C-NMR ($CDCl_3$): δ 173.75, 158.37, 154.66, 130.24, 120.88, 106.02, 102.28, 67.94, 39.88, 31.78 (x2), 30.03, 29.69 (x2), 29.32 (x3), 29.17 (x2), 29.00, 26.76, 25.88, 22.64, 14.85, 14.10, 7.05 (x2). MS *m/z*: 432 $[M+H]^+$ (20), 454 $[M+23]^+$ (100). Anal. calcd. for $C_{27}H_{45}NO_3$: C, 75.13%; H, 10.51%; N, 3.24%. Found: C, 75.03%; H, 10.54%; N, 3.23%.

4.1.2. General procedure for the synthesis of final amides **21** and **22**

To a solution of the acid **37** (1.0 mmol) in dry DMF, under nitrogen atmosphere and continuous stirring, HOBt (1.0 mmol), HBTU (2.0 mmol), DIPEA (1.5 mmol) and the appropriate amine (1.2 mmol, *N*,*N*-dimethylhydrazine or 1-aminopiperidine) were added to the solution, and the reaction mixture was stirred at room temperature for 30 min. Further DIPEA (1.5 mmol) was thereafter added, and the reaction mixture was stirred at room temperature overnight. The reaction mixture was poured into water and extracted with ethyl acetate. The organic layers were washed with saturated ammonium chloride solution and with brine, dried over anhydrous sodium sulphate and evaporated to dryness. The crude residue was purified by silica gel column chromatography.

4.1.2.1. 11-(3-Hydroxy-5-pentylphenoxy)-*N'*,*N'*-dimethylundecanehydrazide **21**

Eluent: $CHCl_3$/MeOH = 50/2. Yield 50% as light white solid, mp = 72-75 °C (K). ^{1}H-NMR ($CDCl_3$): δ 6.26-6.23 (m, 3H), 3.88 (t, 2H, *J* = 6.3 Hz), 2.69 (s, 3H), 2.48 (s, 3H), 2.46 (t, 2H, *J* = 7.8 Hz), 2.11 (t, 2H, *J* = 7.5 Hz), 1.75-1.68 (m, 4H), 1.64-1.56 (m, 4H), 1.42-1.25 (mm, 14H), 0.86 (t, 3H, *J* = 6.4 Hz). ^{13}C-NMR ($CDCl_3$): δ 177.01, 160.26, 157.13, 145.47, 107.97, 106.94, 99.45, 67.83, 48.62, 47.51, 36.06, 35.09, 31.91, 31.52, 30.85, 29.37, 29.07 (x2), 25.76, 25.58, 24.85, 22.54, 18.74, 14.02. MS *m/z*: 429 $[M+Na]^+$ (100), 407 $[M+H]^+$ (40). Anal. calcd. for $C_{24}H_{42}N_2O_3$: C, 70.89%; H, 10.41%; N, 6.89%. Found: C, 70.76%; H, 10.43%; N, 6.87%.

4.1.2.2. 11-(3-Hydroxy-5-pentylphenoxy)-*N*-(piperidin-1-yl)-undecanamide **22**

Eluent: $CHCl_3$/MeOH = 50/2. Yield 55% as pale yellow solid, mp = 69-70 °C (K). ^{1}H-NMR ($CDCl_3$): δ 6.25 (s, 3H), 3.88 (t, 2H, *J* = 6.4 Hz), 2.73 (t, 2H, *J* = 5.1 Hz), 2.50-2.41 (m, 4H), 2.08 (t, 2H, *J* = 7.5 Hz), 1.75-1.48 (mm, 10H), 1.42-1.26 (mm, 18H), 0.86 (t, 3H, *J* = 6.5 Hz). ^{13}C-NMR ($CDCl_3$): δ 176.93, 160.15, 157.15, 145.47, 107.98, 106.81, 99.45, 67.83, 58.11, 57.14, 36.07, 35.22, 32.08, 31.52, 30.86, 29.35 (x2), 29.18, 29.07 (x2), 25.60 (x2), 25.12, 24.92, 23.00, 22.54, 14.08. MS *m/z*: 469 $[M+Na]^+$ (100), 447 $[M+H]^+$ (10). Anal. calcd. for $C_{27}H_{46}N_2O_3$: C, 72.60%; H, 10.38%; N, 6.27%. Found: C, 72.49%; H, 10.41%; N, 6.26%.

4.1.3. General procedure for the synthesis of bromoethers **42**-**47**

To a solution of the appropriate alkyl-resorcinol (1.0 mmol, olivetol, 5-(2-methyloctan-2-yl)resorcinol or 4-hexylresorcinol) in dry THF was added triphenylphosphine (1.1 mmol), under stirring and nitrogen atmosphere. The reaction was cooled to 0 °C and a solution of DEAD (1.1 mmol) in dry THF was subsequently added dropwise. The reaction was allowed to equilibrate to room temperature and the yellow coloured solution was stirred overnight. After concentration of THF, the crude material was diluted with ethyl acetate and washed first with saturated ammonium chloride solution and then brine. The organic layer was dried, filtered and concentrated to give a crude product purified by flash chromatography on silica gel.

4.1.3.1. 3-(9-Bromononyloxy)-5-pentylphenol **42**

Eluent: $CHCl_3$. Yield 55% as yellow oil. ^{1}H-NMR ($CDCl_3$): δ 6.31 (s, 1H), 6.23-6.22 (m, 2H), 5.34 (s, 1H), 3.88 (t, 2H, *J* = 6.4 Hz), 3.39 (t, 2H, *J* = 6.7 Hz), 2.50 (t, 2H, *J* = 7.7 Hz), 1.88-1.81 (m, 2H), 1.77-1.67 (m, 2H), 1.64-1.53 (m, 2H), 1.49-1.25 (mm, 14H), 0.87 (t, 3H, *J* = 6.6 Hz). MS *m/z*: 386 $[M+H]^+$ (100).

4.1.3.2. 3-(11-Bromoundecyloxy)-5-pentylphenol **43**

Eluent: $CHCl_3$. Yield 60% as yellow oil. ^{1}H-NMR ($CDCl_3$): δ 6.30 (s, 1H), 6.25-6.23 (m, 2H), 6.14 (s, 1H), 3.88 (t, 2H, *J* = 6.5 Hz), 3.39 (t, 2H, *J* = 6.9 Hz), 2.47 (t, 2H, *J* = 7.7 Hz), 1.91-1.80 (m, 2H), 1.77-1.67 (m, 2H), 1.60-1.53 (m, 2H), 1.41-1.21 (mm, 18H), 0.87 (t, 3H, *J* = 6.6 Hz). MS *m/z*: 414 $[M+H]^+$ (100).

4.1.3.3. 3-(9-Bromononyloxy)-5-(2-methyloctan-2-yl)phenol **44**

Eluent: $CHCl_3$. Yield 60% as yellow oil. ^{1}H-NMR ($CDCl_3$): δ 6.43 (s, 1H), 6.37 (s, 1H), 6.22 (s, 1H), 4.22 (br s, 1H, disappears on treatment with D_2O), 3.88 (t, 2H, *J* = 6.6 Hz), 3.39 (t, 2H, *J* = 6.7 Hz), 1.90-1.67 (m, 4H), 1.54-1.32 (mm, 14H), 1.20-1.02 (mm, 12H), 0.82 (t, 3H, *J* = 6.4 Hz). MS *m/z*: 442 $[M+H]^+$ (100).

4.1.3.4. 3-(11-Bromoundecyloxy)-5-(2-methyloctan-2-yl)phenol **45**

Eluent: $CHCl_3$. Yield 60% as yellow oil. ^{1}H-NMR ($CDCl_3$): δ 6.43 (s, 1H), 6.39 (s, 1H), 6.21 (s, 1H), 5.93 (br s, 1H, disappears on treatment with D_2O), 3.88 (t, 2H, *J* = 6.4 Hz), 3.38 (t, 2H, *J* = 6.8 Hz), 1.84-1.74 (m, 4H), 1.69-1.21 (mm, 16H), 1.20-0.98 (mm, 14H), 0.83 (t, 3H, *J* = 6.4 Hz). MS *m/z*: 492 $[M+Na]^+$ (100).

4.1.3.5. 3-(11-Bromoundecyloxy)-4-hexylphenol **46**

Eluent: $CHCl_3$. Yield 60% as yellow oil. ^{1}H-NMR ($CDCl_3$): δ 6.92 (d, 1H, *J* = 8.0 Hz), 6.35 (d, 1H, *J* = 2.0 Hz), 6.29 (dd, 1H, *J* = 2.0 Hz, *J* = 8.0 Hz), 4.66 (s, 1H), 3.88 (t, 2H, *J* = 6.3 Hz), 3.39 (t, 2H, *J* = 6.7 Hz), 2.50 (t, 2H, *J* = 7.5 Hz), 1.91-1.70 (m, 4H), 1.61-1.45 (m, 4H), 1.41-1.29 (mm, 18H), 0.87 ((t, 3H, *J* = 6.4 Hz). MS *m/z*: 428 $[M+H]^+$ (100).

4.1.3.6. 5-(11-Bromoundecyloxy)-2-hexylphenol **47**

Eluent: $CHCl_3$. Yield 40% as pasty white solid. mp = 69-71 °C (K). ^{1}H-NMR ($CDCl_3$): δ 6.95 (d, 1H, *J* = 8.9 Hz), 6.40-6.35 (m, 2H), 6.04 (s, 1H), 3.86 (t, 2H, *J* = 6.4 Hz), 3.38 (t, 2H, *J* = 6.8 Hz), 2.51 (t, 2H, *J* = 7.5 Hz), 1.87-1.79 (m, 2H), 1.76-1.65 (m, 2H), 1.58-1.51 (m, 4H), 1.48-1.27 (mm, 18H), 0.86 (t, 3H, *J* = 6.5 Hz). MS *m/z*: 428 $[M+H]^+$ (100).

4.1.4. General procedure for the synthesis of *N*-isoindoline-1,3-diones **48**-**53**

To a solution of the appropriate bromoether (1.0 mmol) in dry DMF, under stirring and nitrogen atmosphere, solid potassium phthalimide (10.0 mmol) was added and the reaction mixture heated at 80 °C overnight. The reaction mixture was allowed to equilibrate to room temperature and then was diluted with distilled water; the milky white aqueous layer was extracted with chloroform and the collected organic layers were dried and filtered. After evaporation of solvent, the crude product was purified by flash chromatography on silica gel.

4.1.4.1. 2-(9-(3-Hydroxy-5-pentylphenoxy)nonyl)isoindoline-1,3-dione **48**

Eluent: $CHCl_3$/MeOH = 50/1. Yield 70% as yellow oil. ^{1}H-NMR ($CDCl_3$): δ 7.84-7.78 (m, 2H), 7.72-7.66 (m, 2H), 6.28-6.24 (m, 3H), 5.67 (br s, 1H, disappears on treatment with D_2O), 3.87 (t, 2H, *J* = 6.6 Hz), 3.66 (t, 2H, *J* = 7.3 Hz), 2.47 (t, 2H, *J* = 7.7 Hz), 1.75-1.56 (mm, 8H), 1.52-1.24 (mm, 12), 0.86 (t, 3H, *J* = 6.6 Hz). ^{13}C-NMR ($CDCl_3$): δ 168.69 (x2), 160.30, 156.72, 145.62, 133.94 (x2), 133.12 (x2), 123.24 (x2), 107.80, 107.20, 99.32, 67.94, 38.13, 36.04, 31.51, 30.83, 29.24, 29.10 (x2), 29.00, 28.58, 26.74, 25.94, 22.54, 14.02. MS *m/z*: 474 $[M+Na]^+$ (100). Anal. Calcd. for $C_{28}H_{37}NO_4$: C, 74.47%; H, 8.26%; N, 3.10%. Found: C, 74.34%; H, 8.29%; N, 3.09%.

4.1.4.2. 2-(11-(3-Hydroxy-5-pentylphenoxy)undecyl)isoindoline-1,3-dione **49**

Eluent: $CHCl_3$. Yield 80% as pasty yellow solid. mp < 30 °C (K). ^{1}H-NMR ($CDCl_3$): δ 7.85-7.79 (m, 2H), 7.73-7.66 (m, 2H), 6.29 (s, 1H), 6.23-6.22 (m, 2H), 5.01 (br s, 1H, disappears on treatment with D_2O), 3.88 (t, 2H, *J* = 6.4 Hz), 3.66 (t, 2H, *J* = 7.1 Hz), 2.48 (t, 2H, *J* = 7.7 Hz), 1.79-1.53 (mm, 8H), 1.49-1.26 (mm, 16H), 0.86 (t, 3H, *J* = 6.4 Hz). ^{13}C-NMR ($CDCl_3$): δ 167.56 (x2), 160.45, 155.98, 145.24, 133.54 (x2), 133.09 (x2), 123.24 (x2), 107.42, 107.15, 99.64, 67.72, 38.10, 36.34, 31.49, 30.73, 29.14, 29.11 (x3), 29.03 (x2), 28.53, 26.47, 25.73, 22.49, 14.06. MS *m/z*: 502

$[M+Na]^+$ (100). Anal. Calcd. for $C_{30}H_{41}NO_4$: C, 75.12%; H, 8.62%; N, 2.92%. Found: C, 75.05%; H, 8.65%; N, 2.91%.

4.1.4.3. 2-(9-(3-Hydroxy-5-(2-methyloctan-2-yl)phenoxy)nonyl)isoindoline-1,3-dione **50**

Eluent: $CHCl_3$/MeOH = 50/1. Yield 75% as yellow oil. ^{1}H-NMR ($CDCl_3$): δ 7.87-7.79 (m, 2H), 7.73-7.66 (m, 2H), 6.43 (s,1H), 6.38 (s, 1H), 6.22 (s, 1H), 5.18 (br s, 1H, disappears on treatment with D_2O), 3.88 (t, 2H, *J* = 6.5 Hz), 3.67 (t, 2H, *J* = 7.3 Hz), 1.80-1.62 (m, 4H), 1.55-1.47 (m, 4H), 1.41-1.31 (mm, 10H), 1.21-1.02 (mm, 12H), 0.82 (t, 3H, *J* = 6.4 Hz). ^{13}C-NMR ($CDCl_3$): δ 168.60 (x2), 156.42, 152.94, 138.20, 133.91 (x2), 132.09 (x2), 123.22 (x2), 105.72, 105.62, 98.63, 67.95, 44.51, 38.11, 37.83, 31.78, 30.02 (x2), 29.10 (x3), 28.90 (x2), 28.57, 26.72, 25.93, 24.64, 22.66, 14.06. MS *m/z*: 530 $[M+Na]^+$ (100). Anal. calcd. for $C_{32}H_{45}NO_4$: C, 75.70%; H, 8.93%; N, 2.76%. Found: C, 75.59%; H, 8.95%; N, 2.75%.

4.1.4.4. 2-(11-(3-Hydroxy-5-(2-methyloctan-2-yl)phenoxy)undecyl)isoindoline-1,3-dione **51**

Eluent: $CHCl_3$. Yield 70% as yellow oil. ^{1}H-NMR ($CDCl_3$): δ 7.86-7.80 (m, 2H), 7.72-7.68 (m, 2H), 6.45 (s,1H), 6.39 (s, 1H), 6.23 (s, 1H), 5.08 (br s, 1H, disappears on treatment with D_2O), 3.90 (t, 2H, *J* = 6.5 Hz), 3.67 (t, 2H, *J* = 7.1 Hz), 1.78-1.67 (m, 6H), 1.56-1.48 (m, 4H), 1.28-1.04 (mm, 24H), 0.83 (t, 3H, *J* = 6.4 Hz). ^{13}C-NMR ($CDCl_3$): δ 168.46 (x2), 156.53, 152.89, 138.32, 133.78 (x2), 132.04 (x2), 123.25 (x2), 105.62, 105.35, 98.44, 67.68, 44.50, 38.21, 37.81, 31.80, 30.05 (x2), 29.33 (x2), 29.12 (x3), 28.94 (x2), 28.60, 26.70, 25.87, 24.67, 22.71, 14.04. MS *m/z*: 558 $[M+Na]^+$ (100). Anal. Calcd. for $C_{34}H_{49}NO_4$: C, 76.22%; H, 9.22%; N, 2.61%. Found: C, 76.10%; H, 9.24%; N, 2.60%.

4.1.4.5. 2-(11-(2-Hexyl-5-hydroxyphenoxy)undecyl)isoindoline-1,3-dione **52**

Eluent: $CHCl_3$. Yield 70% as pasty yellow solid. mp 45-48 °C (K). ^{1}H-NMR ($CDCl_3$): δ 7.85-7.80 (m, 2H), 7.70-7.66 (m, 2H), 6.92 (d, 1H, *J* = 8.0 Hz), 6.35-6.28 (m, 2H), 4.82 (br s, 1H, disappears on treatment with D_2O), 3.88 (t, 2H, *J* = 6.3 Hz), 3.66 (t, 2H, *J* = 7.2 Hz), 2.49 (t, 2H, *J* = 7.5 Hz), 1.78-1.58 (mm, 6H), 1.56-1.39 (m, 4H), 1.38-1.27 (mm, 16H), 0.85 (t, 3H, *J* = 6.5 Hz). ^{13}C-NMR ($CDCl_3$): δ 168.57 (x2), 157.88, 154.82, 133.87 (x2), 132.18 (x2), 129.98, 123.90, 123.18 (x2),

106.21, 99.59, 67.77, 38.11, 31.78, 30.18, 29.66 (x2), 29.47 (x2), 29.41 (x2), 29.24 (x2), 28.59, 26.86, 26.09, 22.65, 14.11. MS *m/z*: 516 $[M+Na]^+$ (100). Anal. Calcd. for $C_{31}H_{43}NO_4$: C, 75.42%; H, 8.78%; N, 2.84%. Found: C, 75.23%; H, 8.79%; N, 2.85%.

4.1.4.6. 2-(11-(4-Hexyl-3-hydroxyphenoxy)undecyl)isoindoline-1,3-dione **53**

Eluent: $CHCl_3$. Yield 70% as yellow solid. mp 83-85 °C (K). ^{1}H-NMR ($CDCl_3$): δ 7.85-7.80 (m, 2H), 7.72-7.65 (m, 2H), 6.96 (d, 1H, *J* = 8.0 Hz), 6.42-6.37 (m, 2H), 5.21 (br s, 1H, disappears on treatment with D_2O), 3.87 (t, 2H, *J* = 6.4 Hz), 3.67 (t, 2H, *J* = 7.3 Hz), 2.51 (t, 2H, *J* = 7.5 Hz), 1.75-1.49 (mm, 6H), 1.43-1.26 (mm, H), 0.86 (t, 3H, *J* = 6.2 Hz). ^{13}C-NMR ($CDCl_3$): δ 168.60 (x2), 158.43, 154.28, 133.88 (x2), 132.17 (x2), 130.38, 123.21 (x2), 120.66, 106.42, 102.25, 68.04, 38.13, 31.77, 30.04, 29.32 (x3), 29.20 (x3), 28.57 (x3), 26.80, 25.99, 22.64, 14.09. MS *m/z*: 516 $[M+Na]^+$ (100). Anal. Calcd. for $C_{31}H_{43}NO_4$: C, 75.42%; H, 8.78%; N, 2.84%. Found: C, 75.30%; H, 8.80%; N, 2.83%.

4.1.5. General procedure for the synthesis of amines **54**-**57**

The appropriate N-isoindolin-1,3-dione (1.0 mmol) was stirred and heated under reflux with hydrazine hydrate (2.5 mmol) in ethanol (15 mL) for 5 h. After disappearance of starting material, heating was stopped and the reaction mixture was allowed to get room temperature. The phthalhydrazide formed on cooling was filtered off and the filtrate evaporated to dryness. The crude product was subjected to the successive reaction without further purification.

4.1.6. General procedure for the synthesis of final amines **27**-**29**. Method A

Each bromoether (1.0 mmol, **42** or **43**) was dissolved, under nitrogen atmosphere and stirring, in the appropriate amine (3-4 mL) and warmed at 100-110 °C ((*R*)-(-)-2-amino-1-propanol and benzylamine) or 50 °C (allylamine) for 4-5 h. After cooling, the resulting mixture was diluted with distilled water and extracted with chloroform; the collected organic layers were washed with brine, dried, filtered and concentrated. The raw material was purified by flash chromatography on silica gel furnishing title compound.

4.1.6.1. (*R*)-3-(9-(1-Hydroxypropan-2-ylamino)nonyloxy)-5-pentylphenol **27**

Eluent: $CHCl_3$/MeOH = 43/7. Yield 65% as yellow oil. ^{1}H-NMR ($CDCl_3$): δ 6.24-6.21 (m, 3H), 4.19 (br s, 2H, disappears on treatment with D_2O), 3.88 (t, 2H, *J* = 6.3 Hz), 3.67-3.56 (m, 2H), 3.35 (dd, 1H, *J* = 7.6 Hz, *J* = 10.6 Hz), 2.72 (t, 2H, *J* = 7.1 Hz), 2.47 (t, 2H, *J* = 7.6 Hz), 1.75-1.65 (m, 2H), 1.59-1.44 (m, 6H), 1.40-1.28 (mm, 12H), 1.07 (d, 3H, *J* = 6.4 Hz), 0.86 (t, 3H, *J* = 6.4 Hz). ^{13}C-NMR ($CDCl_3$): δ 160.21, 157.20, 145.55, 108.01, 106.60, 99.38, 67.75, 62.87, 50.02, 47.91, 36.13, 31.62, 30.74, 30.43, 29.88, 29.32, 29.11, 28.81, 27.25, 25.87, 22.52, 17.04, 14.02. MS *m/z*: 380 $[M+H]^+$ (100). Anal. calcd. for $C_{23}H_{41}NO_3$: C, 72.78%; H, 10.89%; N, 3.69%. Found: C, 72.64%; H, 10.90%; N, 3.68%.

4.1.6.2. 3-(11-(Allylamino)undecyloxy)-5-pentylphenol **28**

Eluent: $CHCl_3$/MeOH = 47/3. Yield 90% as yellow oil. ^{1}H-NMR ($CDCl_3$): δ 6.25-6.18 (m, 3H), 5.97-5.87 (m, 1H), 5.22-5.12 (m, 2H), 3.96 (br s, 1H, disappears on treatment with D_2O), 3.89 (t, 2H, *J* = 6.4 Hz), 3.30 (d, 2H, *J* = 6.2 Hz), 2.65 (t, 2H, *J* = 7.4 Hz), 2.47 (t, 2H, *J* = 7.7 Hz), 1.74-1.69 (m, 2H), 1.58-1.51 (m, 4H), 1.43-1.40 (m, 2H), 1.30-1.25 (mm, 16H), 0.86 (t, 3H, *J* = 6.8 Hz). ^{13}C-NMR ($CDCl_3$): δ 160.18, 157.29, 145.42, 130.69, 121.39, 108.05, 106.62, 99.40, 67.71, 50.51, 47.49, 36.06, 31.51, 30.87, 29.45, 29.12, 29.09, 29.05, 29.00, 28.86, 27.21, 26.86, 25.78, 22.53, 14.01. MS *m/z*: 390 $[M+H]^+$ (100). Anal. calcd. for $C_{25}H_{43}NO_2$: C, 77.07%; H, 11.12%; N, 3.60%. Found: C, 76.96%; H, 11.15%; N, 3.59%.

4.1.6.3. 3-(11-(Benzylamino)undecyloxy)-5-pentylphenol **29**

Eluent: $CHCl_3$/MeOH = 50/4. Yield 80% as pasty yellow solid. mp = 45-47 °C (K). ^{1}H-NMR ($CDCl_3$): δ 7.33-7.27 (m, 5H), 6.26 (s, 1H), 6.19-6.17 (m, 2H), 4.05 (br s, 1H, disappears on treatment with D_2O), 3.87 (t, 2H, *J* = 6.4 Hz), 3.81 (s, 2H), 2.64 (t, 2H, *J* = 7.3 Hz), 2.46 (t, 2H, *J* = 7.6 Hz), 1.76-1.63 (m, 2H), 1.56-1.45 (m, 4H), 1.40-1.25 (mm, 18H), 0.87 (t, 3H, *J* = 6.6 Hz). ^{13}C-NMR ($CDCl_3$): δ 160.21, 157.33, 145.46, 139.04, 128.53 (x2), 128.45 (x2), 127.26, 108.15, 106.55, 99.48, 67.74, 53.65, 49.04, 36.07, 31.52, 30.86, 29.41, 29.30 (x2), 29.13 (x2), 29.01, 28.86, 27.17,

25.85, 22.55, 14.03. MS *m/z*: 440 $[M+H]^+$ (100). Anal. calcd. for $C_{29}H_{45}NO_2$: C, 79.22%; H, 10.32%; N, 3.19%. Found: C, 79.10%; H, 10.35%; N, 3.20%.

4.1.7. 11-(3-Hydroxy-5-pentyl-phenoxy)-undecanoic acid cyclopropylamide **58**

This compound was prepared as we previously reported [29].

4.1.8. 12-(3-Hydroxy-5-pentyl-phenoxy)-dodecanoic acid cyclopropylamide **59**

This compound was prepared as we previously reported [30].

4.1.9. General procedure for the synthesis of final amines **30** and **31**. Method B

Each amide (1.0 mmol, **58** or **59**) was dissolved, under nitrogen atmosphere and stirring, in dry THF and the solution cooled at 0 °C in an ice bath. Lithium aluminium hydride was added in portion (5.0 mmol) and the suspension was heated at 50-60 °C for 3 h. After disappearance of starting amide, the reaction was first allowed to equilibrate to room temperature and then cooled at 0 °C, adding ice until complete destruction of hydride. The reaction mixture was extracted with ethyl acetate and the organic layer were dried, filtered and concentrated to dryness. The raw residue was purified by flash chromatography on silica gel furnishing title compound.

4.1.9.1. 3-(11-(Cyclopropylamino)undecyloxy)-5-pentylphenol **30**

Eluent: $CHCl_3$/MeOH = 45/5. Yield 55% as yellow oil. ^{1}H-NMR ($CDCl_3$): δ 6.26-6.22 (m, 2H), 6.18-6.16 (m, 1H), 3.90 (t, 2H, *J* = 6.4 Hz), 3.60 (br s, 1H, disappears on treatment with D_2O), 2.72 (t, 2H, *J* = 7.3 Hz), 2.47 (t, 2H, *J* = 7.6 Hz), 2.19-2.14 (m, 1H), 1.76-1.65 (m, 4H), 1.60-1.38 (mm, 6H), 1.32-1.26 (mm, 14H), 0.87 (t, 3H, *J* = 6.6 Hz), 0.47 (d, 4H, *J* = 5.3 Hz) ^{13}C-NMR ($CDCl_3$): δ 160.20, 157.19, 145.50, 108.09, 106.56, 99.42, 67.76, 49.54, 36.07, 31.51, 30.85, 30.46, 29.87, 29.28, 29.24, 29.12 (x2), 28.99, 28.84, 27.20, 25.83, 22.54, 14.02, 5.70 (x2). MS *m/z*: 390 $[M+H]^+$ (100). Anal. calcd. for $C_{25}H_{43}NO_2$: C, 77.07%; H, 11.12%, N, 3.60%. Found: C, 76.98%; H, 11.15%; N, 3.59%.

4.1.9.2. 3-(12-(Cyclopropylamino)dodecyloxy)-5-pentylphenol **31**

Eluent: $CHCl_3$/MeOH = 50/5. Yield 50% as pasty yellow solid. mp < 30 °C (K). ^{1}H-NMR ($CDCl_3$): δ 6.26-6.17 (m, 2H), 6.16-6.15 (m, 1H), 3.89 (t, 2H, *J* = 6.3 Hz), 3.70 (br s, 1H, disappears on

treatment with D_2O), 2.73 (t, 2H, J = 7.4 Hz), 2.48 (t, 2H, J = 7.6 Hz), 2.22-2.18 (m, 1H), 1.76-1.66 (m, 4H), 1.60-1.38 (mm, 8H), 1.32-1.25 (mm, 14H), 0.87 (t, 3H, J = 6.6 Hz), 0.47 (d, 4H, J = 5.2 Hz) ^{13}C-NMR ($CDCl_3$): δ 160.18, 157.23, 146.01, 107.85, 106.61, 99.35, 67.74, 49.52, 36.11, 31.62, 30.85, 30.43, 29.89 (x2), 29.26, 29.24 (x2), 29.09, 28.87, 28.81, 27.22, 25.86, 22.52, 14.04, 5.70 (x2). MS *m/z*: 404 $[M+H]^+$ (100). Anal. calcd. for $C_{26}H_{45}NO_2$: C, 77.37%; H, 11.24%; N, 3.47%. Found: C, 77.28%; H, 11.27%; N, 3.46%.

4.2. CB_1 and CB_2 receptor binding assays

For both receptor binding assays, the new compounds were tested as previously described [39] and binding affinities of reference compounds were evaluated in parallel with compounds **1**-**31**. K_i means concentration of the competing ligand that will bind to half of the binding sites at equilibrium, in absence of radioligand or other competitors. IC_{50} means concentration of competitor that competes for half of the specific binding (a measure of the competitor's potency at interacting with the receptor against the radioligand). K_i is calculated from EC_{50} using Cheng-Prusoff equation.

4.3. TRPV1 and TRPA1 channel assays

HEK293 (human embryonic kidney) cells stably over-expressing recombinant rat TRPA1, or human TRPV1 were grown on 100 mm diameter Petri dishes as mono-layers in minimum essential medium (EMEM) supplemented with nonessential amino acids, 10% foetal bovine serum, and 2 mM glutamine, and maintained at 5% CO_2 at 37 °C. Stable expression of each channel was checked by quantitative PCR (data not shown). The effect of the substances on intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) was determined by using Fluo-4, a selective intracellular fluorescent probe for Ca^{2+}. On the day of the experiment, cells were loaded for 1 h at room temperature with the methyl ester Fluo-4-AM (4 μM; Invitrogen) in EMEM without foetal bovine serum, then were washed twice in tyroide's buffer (145 mM NaCl, 2.5 mM KCl, 1.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 10 mM D-glucose, and 10 mM HEPES, pH 7.4), resuspended in the same buffer, and transferred to the quartz cuvette of the spectrofluorimeter (Perkin-Elmer LS50B; Perkin-Elmer Life and Analitycal Sciences, Waltham, MA, USA) under continuous stirring. $[Ca^{2+}]_i$ was determined before and after

the addition of various concentrations of test compounds by measuring cell fluorescence (λ_{EX} = 488 nm, λ_{EM} = 516 nm) at 25 °C. Curve fitting (sigmoidal dose-response variable slope) and parameter estimation were performed with GraphPad Prism® (GraphPad Software Inc., San Diego, CA). Potency was expressed as the concentration of test substance exerting half-maximal agonist effect (i.e., half-maximal increases in $[Ca^{2+}]_i$) (EC_{50}). In the case of TRPV1 assay, the efficacy of the agonists was first determined by normalizing their effect to the maximum Ca^{2+} influx effect on $[Ca^{2+}]_i$ observed with application of 4 μM ionomycin (Sigma). The values of the effect on $[Ca^{2+}]_i$ in wild type HEK293 (i.e., not transfected with any construct) cells were taken as baseline and subtracted from the values obtained from transfected cells. The effects of TRPA1 agonists are expressed as a percentage of the effect obtained with 100 μM allyl isothiocyanate (AITC). Antagonist/desensitizing behaviour was evaluated against capsaicin (0.1 μM) for TRPV1 and AITC (100 μM) for TRPA1, by adding the test compounds in the quartz cuvette 5 min before stimulation of cells with aginists. Data are expressed as the concentration exerting a half-maximal inhibition of agonist-induced $[Ca^{2+}]_i$ elevation (IC_{50}), which was calculated again using GraphPad Prism® software. The effect on $[Ca^{2+}]_i$ exerted by agonist alone was taken as 100%. Dose response curves were fitted by sigmoidal regression with variable slope. Statistical analysis of the data was performed by analysis of variance at each point using ANOVA followed by the Bonferroni's test.

4.6. Pharmacological and biological studies

The experimental procedure used here was in accordance with the Italian law on the "Protection of animals used for experimental and other scientific reasons".

4.4.1. [^{35}S]GTPγS assays

Binding assays with [^{35}S]GTPγS were performed with hCB_1-CHO or hCB_2-CHO cell membranes [46]. Both the hCB_1 and hCB_2 receptor-transfected cells were removed from flasks by scraping and then frozen as a pellet at -20°C until required. Before use in a radioligand binding assay, cells were defrosted, diluted in Tris buffer (50 mM Tris-HCl and 50 mM Tris-base) and homogenized with a 1

mL hand-held homogenizer. Protein assays were performed using a Bio-Rad DC Kit (Hercules, CA, USA). The method used for measuring agonist-stimulated binding of [^{35}S]GTPγS was based on previously described methods [47]. The assays were carried out with GTPγS binding buffer (50 mM Tris-HCl, 50 mM Tris–base, 100 mM NaCl, 5 mM $MgCl_2$, 1 mM dithiothreitol, 1 mM EDTA and 0.1%BSA, pH 7.4) 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. Non-specific 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 the addition of ice-cold Tris-binding buffer and vacuum filtration using a 24-well sampling manifold (Brandel Cell Harvester, Alpha Biotech Ltd, London, UK) and Whatman GF/B glass-fibre filters that have been pre-soaked in wash buffer at 4ºC for 24 h. Each reaction tube was washed three times with 4 mL aliquot of buffer. The filters were oven-dried for 60 min and then placed in 5 mL of scintillation fluid (Ultima Gold XR, Packard, Perkin Elmer Ltd, Saxon Way Bar Hill, Cambridge, UK). Radioactivity was quantified by liquid scintillation spectrometry. Nonspecific binding were measured in the presence of 30 µM GTSγS. Compounds under investigation were added to the incubations in 1µL of dimethyl sulphoxide (DMSO); vehicle control contained DMSO alone. In all the [^{35}S]GTPγS-binding assays, we used 0.1 nM [^{35}S]GTPγS, 30 µM GDP and 50 µg (cell membranes) protein per well. In each experiment, the percent increases in [^{35}S]GTPγS binding in response to ligands was calculated using the DMSO-treated membranes as the control.

Values have been expressed as means and variability as SEM or as 95% confidence intervals (CIs). The EC_{50} values and maximal compound-induced increases in [^{35}S]GTPγS binding were determined by fitting the data to a sigmoidal log concentration-response curve using nonlinear regression (GraphPad Prism 5.0®, GraphPad Software, San Diego, CA, USA). Analysis was by one-way ANOVA and Newman–Keuls multiple comparison tests, unless otherwise stated. A P-value of <0.05 was considered significant.

4.4.2. Evaluation of antinociceptive activity in mice

Animal care and all animal procedures were in compliance with Italian (D.L. 116/92) and EEC (O.J. of EC L358/1 18/12/1986) regulations on the protection of laboratory animals. Guidelines of the International Association for the Study of Pain were also followed. Male Swiss-Webster mice (40-45 g) were used in these experiments; they were housed at constant temperature (21±1 °C) and relative humidity (60%) under a regular light/dark schedule (light 7.00-19.00). Food and water were always available. Mice received formalin (1.25% in saline, 30 mL) in the dorsal surface of one side of the hindpaw. Each mouse was randomly assigned to one of the experimental groups and placed in a Plexigas cage and allowed to move freely for 15-20 min. A mirror was placed at a 451 angle under the cage to allow full view of the hindpaws. Lifting, favouring, licking, shaking and flinching of the injected paw were recorded as nociceptive responses. These were measured every 5 min and expressed as their total duration in min (mean±s.e.mean) within each of the 5 min bins. The same observed, unaware of the treatments, scored all behavioural responses. Groups of 8-10 animals per treatment were used with each animal being used for one treatment only. Recording of nocifensive behaviour commenced immediately after formalin injection and was continued for 60 min. The version of the formalin test [48] that we used is based on the fact that correlational analyses showed that no single behaviour measure can be a strong predictor of formalin or drug concentrations on spontaneous behaviour. Therefore, we considered that a simple sum of time spent licking and elevating the paw, or the weighted pain score was better than any single behavioural measure (lifting, favouring, licking, shaking and flinching; r ranging from 0.75 to 0.86) [49].

4.4.3. Cell cultures and cytotoxicity assay

4.4.3.1. Materials

Dulbecco's Modified Eagle's Medium (DMEM), trypsin solution, and all the solvents used for cell culture were purchased from Lonza (Switzerland). Mouse immortalized fibroblasts NIH3T3 were purchased from American Type Culture Collection (USA).

4.4.3.2. Methods

NIH3T3 cells were utilised for citotoxicity experiments. NIH3T3 were maintained in DMEM at 37 °C in a humidified atmosphere containing 5% CO_2. The culture media were supplemented with 10% foetal calf serum (FCS), 1% L-glutamine-penicillin-streptomycin solution, and 1% MEM Non-Essential Amino Acid Solution. Once at confluence, cells were washed with PBS 0.1M, taken up with trypsin-EDTA solution and then centrifuged at 1000 rpm for 5 min. The pellet was re-suspended in medium solution (dilution 1:15) and cells were seeded. After 24 h of incubation, the following samples, i.e. compounds **3** and **11** respectively, dissolved in DMSO, were added to the cells. Concentrations ranging from 5 to 200 μM were tested. Each concentration was tested in six replicate. Cell viability after 24 h of incubation with the different compounds was evaluated by Neutral Red Uptake (Sigma-Aldrich, Switzerland) by the procedure previously reported [50]. Briefly, the following solutions were prepared in order to determine the percentage of viable cells:

1. Neutral Red (NR) Stock Solution: 0.33 g NR Dye powder in 100 mL sterile H_2O;
2. NR Medium: 1.0 mL NR Stock solution + 99.0 mL Routine Culture Medium pre-warmed to 37 °C;
3. NR Desorb solution: 1% Glacial acetic acid solution + 50% Ethanol + 49% H_2O.

At the end of the incubation the routine culture medium was removed from each well, and cells were carefully rinsed with 1.0 mL of pre-warmed D-PBS. Multiwells were then gently blotted with paper towels. 1.0 mL of NR Medium was added to each well and further incubated at 37 °C, 95% humidity, 5.0% CO_2 for 3 h. The cells were checked during the NR incubation for NR crystal formation. After incubation, the NR Medium was removed; cells were carefully rinsed with 1.0 mL of pre-warmed D-PBS. Then, the PBS was decanted and blotted from the wells and exactly 1.0 mL of NR Desorb solution was added to each sample. Multiwells were then put on a shaker for 20-45 min to extract NR from the cells and form a homogeneous solution. During this step the samples were covered in order to protect them from light. After 5 min from the plate shaker removal the absorbance was read at 540 nm by a UV/visible spectrophotometer (Lambda 25, Perkin Elmer).

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Chart 1. Chemical structure of reference compounds: alkyl-resorcinol derivatives **I**-**VI**

Chart 2. General chemical structures of new synthesized alkyl-resorcinol compounds

Scheme 1. Synthesis of amides **1**-**22**. Reagents and reaction conditions. a: MeOH/NaOH aqueous, reflux, 3h, 85-90%; b: R_1NH_2, HOBt, EDC, DCM, room temp., overnight, 75-90% (**1**-**20**); c: 1,1-dimethylhydrazine or 1-aminopiperidine, HOBt, HBTU, DIPEA, dry DMF, room temp, overnight, 55-60% (**21**, **22**).

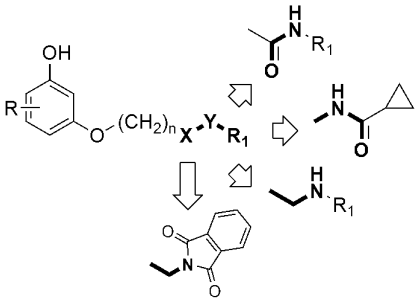
Scheme 2. Synthesis of phthalimido derivatives **48**-**53** and retro-amides **23**-**26**. Reagents and reaction conditions. a: bromoalcohol, triphenylphosphine, DEAD, dry THF, room temp., overnight, 35-60%; b: potassium phthalimide, dry DMF, 70-80 °C, overnight, 70-80%; c: hydrazine, absolute ethanol, 60-80 °C, 5h, 90-95% yield; d: cyclopropanecarboxylic acid, HOBt, EDC, DCM, room temp., overnight, 55-65%.

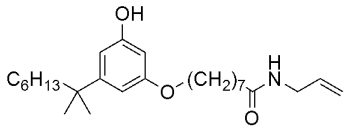
Scheme 3. Synthesis of amine derivatives **27**-**31**. Reagents and reaction conditions. Method A: (R)-(-)-2-amino-1-propanol, allylamine, benzylamine, heating, 4-5h, 75-85%; Method B: $LiAlH_4$, dry THF, 50-60 °C, 3h, 70-75%.

Figure 1. a) The effect of CP-55,940 and **11** on [^{35}S]GTPγS binding to hCB_1-CHO cell membranes (n=6). b) The effect of CP-55,940 and **11** on [^{35}S]GTPγS binding to hCB_2-CHO cell membranes (n=6). Each symbol represents the mean percentage change in binding ± SEM.

Figure 2. a) The effect of CP-55,940 and **14** on [^{35}S]GTPγS binding to hCB_2-CHO cell membranes (n=6). b) The mean log concentration-response curve of CP-55,940 was constructed in the presence of vehicle (DMSO) or 1μM **14** (n=6). Each symbol represents the mean percentage change in binding of [^{35}S]GTPγS to hCB_2-CHO cell membranes ± SEM. The right-ward shift produced by 1μM **14** (Hill slope: 0.71, 95% confidence interval from 0.24 to 1.18) in the log concentration response curve of CP-55,940 (Hill slope: 0.82, 95% confidence interval from 0.2 to 1.31) did not deviate significantly from parallelism since the 95% confidence intervals of the two Hill slopes overlapped. DMSO, dimethyl sulphoxide. Asterisks denote values that are significantly different from zero ($^{**}P<0.01$; one sample test).

Figure 3. Antinociceptive effect of subcutaneous formalin (1.25%, 30 mL) injections into the hind paws of mice on the time course of nociceptive behaviour. Formalin was injected 10 min after the systemic administration of vehicle (0.9% NaCl, i.p.) or test compound **11**, here denoted as CB148. Recording of nociceptive behaviour began immediately after the injection of formalin (time 0) and was continued for 60 min. Each point represents the total time of the nociceptive responses (mean ± SEM) of 8-10 animals per group, measured every 5 min. Data were analysed using the one-way ANOVA followed by the Bonferroni's test, and statistical significance was taken as $P < 0.05$.





Compound **11**
CB1 full agonist, CB2 partial agonist
TRPA1 agonist