Synthesis and pharmacological evaluation of new biphenylic derivatives as CB_2 receptor ligands

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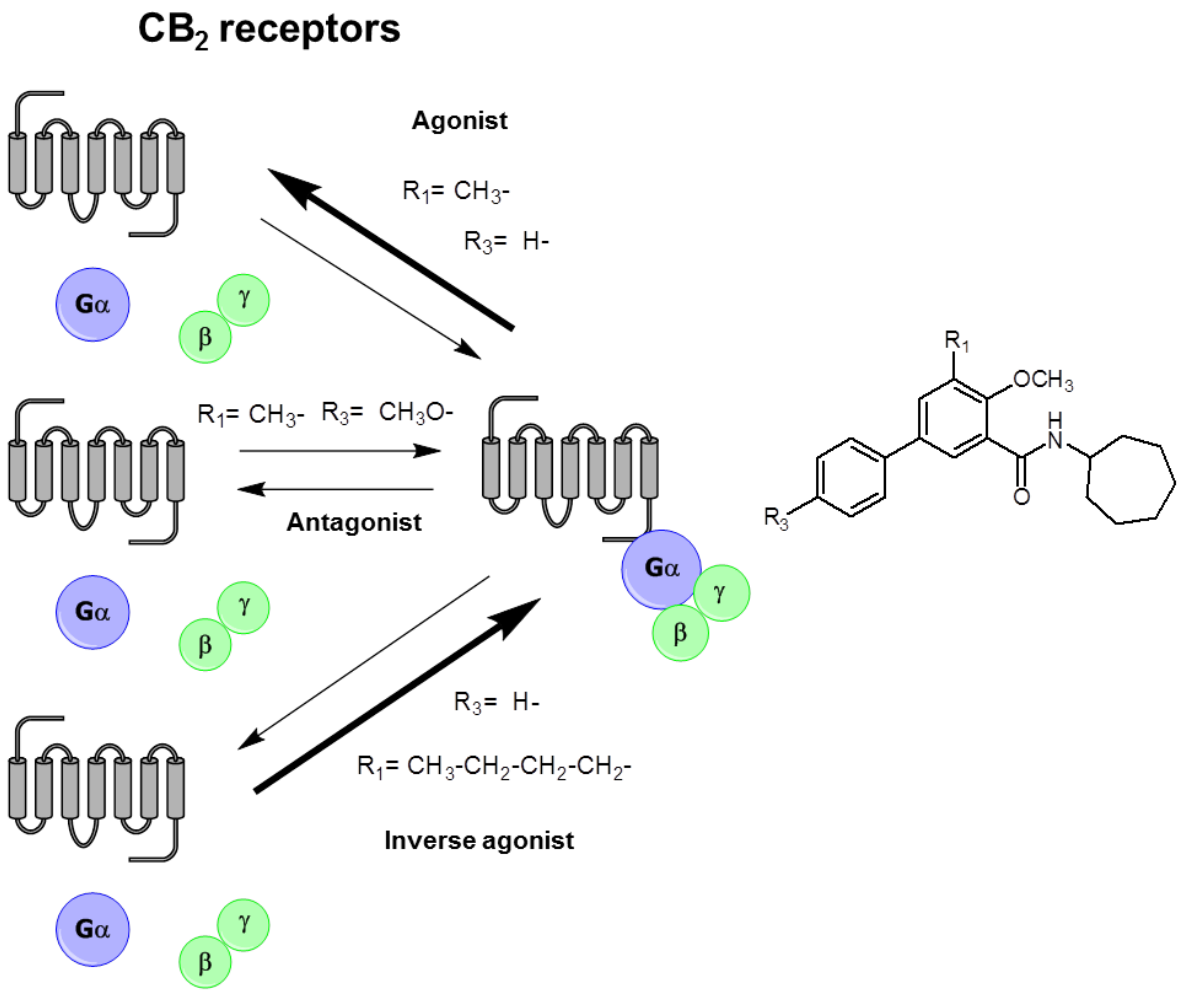
Highlights

- We synthesized 18 biphenylic carboxamides as new CB_2-selective ligands.
- The functional activity is influenced by the substituent at position 4′ and 5.
- The methoxyl group at position 4′ is responsible for neutral antagonist behaviour.

Graphical abstract



Abstract

Targeting type-2 cannabinoid receptor (CB_2) is considered a feasible strategy to develop new drugs for the treatment of diseases like neuropathic pain, chronic inflammation, neurodegenerative disorders and cancer. Such drugs are devoid of the undesired central side effects that are typically mediated by the CB_1 receptor. In this work we synthesized 18 biphenylic carboxamides as new CB_2-selective ligands and evaluated their pharmacological profiles.The functional activity of these compounds is strongly influenced by the nature of the substituent at position 4′ and 5 of the biphenyl scaffold. Position 5 seems to be responsible for the agonist or inverse agonist behaviour independently of the substituent in position 4′, with the exception of the methoxyl group which transforms both full agonists and inverse agonists into neutral antagonists. This study provides a novel complete toolbox of CB_2 functional modulators that derive from the same chemical scaffold. Such probes may be useful to investigate the biological role of CB_2 receptors in cellular assays.

Keywords: *CB_2, cannabinoid receptor, biphenyl-carboxamides, CB_2 agonist, CB_2 antagonist, CB_2 inverse agonist, methylhonokiol, endocannabinoid system*

1. Introduction

The endocannabinoid system (ECS) comprises the two main cannabinoid receptors (CB_1 and CB_2), their endogenous ligands (endocannabinoids) and the enzymes responsible for the biosynthesis and metabolism of endocannabinoids [1, 2].

Over the past two decades, great efforts have been made in order to fully understand the biological role and regulatory functions of CB receptors in pathophysiological conditions.

CB_1 receptors are mainly expressed in the central nervous system, but also in peripheral districts including spleen, heart, reproductive organs, lungs and adipose tissue [1]. CB_2 receptors are widely localized in cells and tissues of the immune system, cardiovascular system, bone, liver, kidney and the gut [3-5]. In healthy brain, CB_2 receptors are almost absent, although their presence in some restricted neuronal populations has been reported [6]. However, CB_2 receptors become significantly expressed in activated microglial cells and astrocytes upon specific conditions such as neuroinflammation [3-5]. In the perpiphery, CB_2 receptors are expressed in different immune cells where they show dynamic trafficking between cytoplasm and cell membrane where they may even form heterodimers with CB_1 receptors [7].

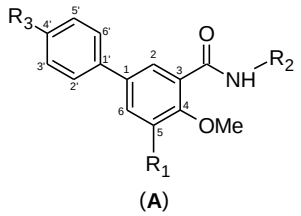
CB_1 and CB_2 receptors have been proposed as potential therapeutic targets for the treatment of several diseases including actute and chronic inflammation, neurodegenerative and eating disorders, neuropathic pain, cancer and osteoporosis [4, 8, 9]. Non-selective CB_1/CB_2 receptor agonists are the active principles of some approved medicines (i. e. Marinol®, Cesamet®, Sativex®). However, non-selective agonists produce adverse effects (i. e. psyichotropic) almost exclusively due to the activation of central CB_1 receptors. The occurrence of these side effects represents the major limitation in the therapeutic use of these compounds, especially for chronic treatment. One of the proposed strategies to avoid the

unwanted consequences of the central CB_1 receptor activation is to selective target CB_2 receptors.

In some therapeutic field such as pain, inflammation and osteoporosis, the potential usefulness of both CB_2-agonists and CB_2-inverse agonists has been reported [10-16], while very few compounds acting as neutral CB_2-antagonists have been described [17, 18].

These considerations strongly support the importance of the synthesis and pharmacological characterization of new CB_2-selective ligands bearing such functional profiles (agonists or inverse agonists or neutral antagonists) in order to delineate a precise structure-activity relationship for this kind of molecules and to deepen the role of the CB_2 receptors in pathophysiological conditions.

We recently described the synthesis, binding properties, functional activity and molecular modelling of a series of biphenylic carboxamides (general structure **A**, Figure 1) as selective CB_2 receptor ligands [19]. Some of these compounds showed CB_2-affinity levels in the nM range. The most potent and selective derivative of that series showed an interesting pharmacological profile as a selective neutral antagonist for CB_2 receptors, thus being a silent ligand when tested alone and dose-dependently reverting the response of a CB_2 agonist (HU-210) and a CB_2 inverse agonist (SR144528) [19].



(**A**)

Figure 1. General structure of biphenylic carboxamides [19].

In an effort to identify additional and more potent or functionally distinct biphenyl derivatives as selective CB_2 receptor ligands, and to develop structure-activity relationships (SAR) for these type of compounds, we engaged on the synthesis and characterization of the

pharmacological properties of a number of biphenyl derivatives (compounds **1a-r**, Table I). To that aim, other combinations of substituents from the ones already mentioned in the previous report were explored and new substituents were also introduced.

In particular, position 5 (R_1) was functionalized with *n*-butyl, benzyl or *p*-fluorobenzyl; in position 4′ (R_3) there was a hydrogen, a fluorine or a methoxy group; finally the substituent on carboxamide moiety (R_2) was a cycloheptyl or a 4-methylcyclohexyl (*cis* or *trans* isomer).

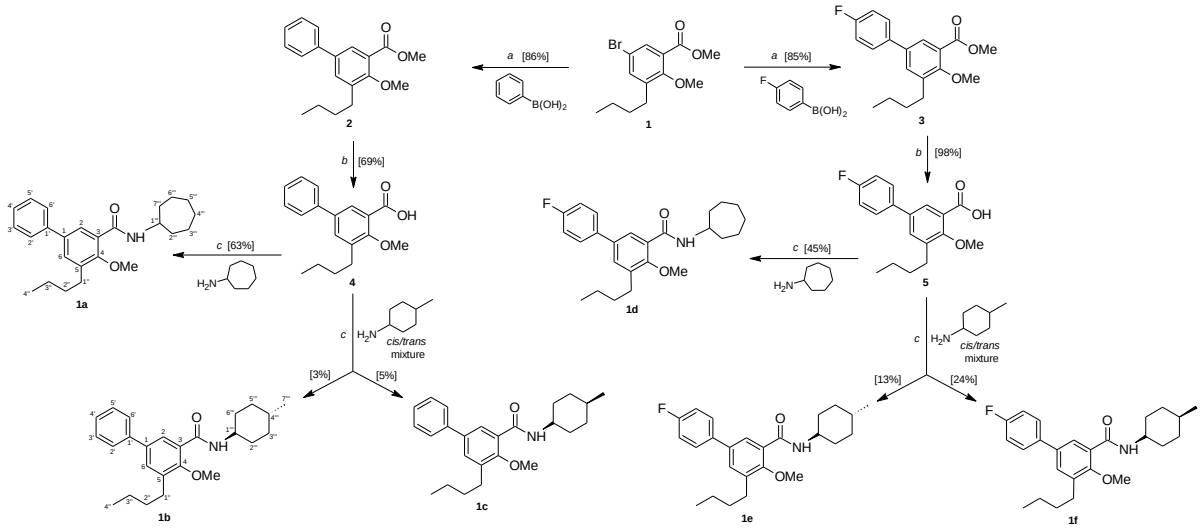
The new compounds were tested on cannabinoid receptors in order to evaluate the binding affinity and selectivity. The displacement assays were performed using 0.5 nM of [^{3}H]CP55,940 and 15 µg of clean membrane preparations obtained from CHO-K1 cells stably transfected with *h*CB_1 and *h*CB_2 receptors, respectively. The most affine ligands were also assessed in functional tests using the [^{35}S]GTPγS assay as previously described [20].

2. Results and discussion

2.1 Chemistry

The synthesis of compounds **1a-f** is shown in Scheme 1. The atom numbering of carbon skeleton (and of substituents) of compounds **1a** and **1b** (as examples for all the final products) is also included in Scheme 1. Compound **1a-f** were synthesized starting from methyl 5-bromo-3-butyl-2-methoxybenzoate (**1**), which was prepared as previously reported by our research group [19] (Scheme 1). Compound **1** was submitted to a Suzuki reaction with the proper arylboronic acid (phenyl, or 4-fluorophenyl boronic acid), aqueous sodium carbonate, palladium acetate and triphenylphosphine in methanol/toluene [21], affording the intermediates **2** and **3** which were then hydrolysed in presence of potassium hydroxide in methanol [22] leading to the corresponding acid derivatives **4** and **5**. Intermediates **4** and **5** were treated with thionyl chloride and subsequently with cycloheptylamine in anhydrous

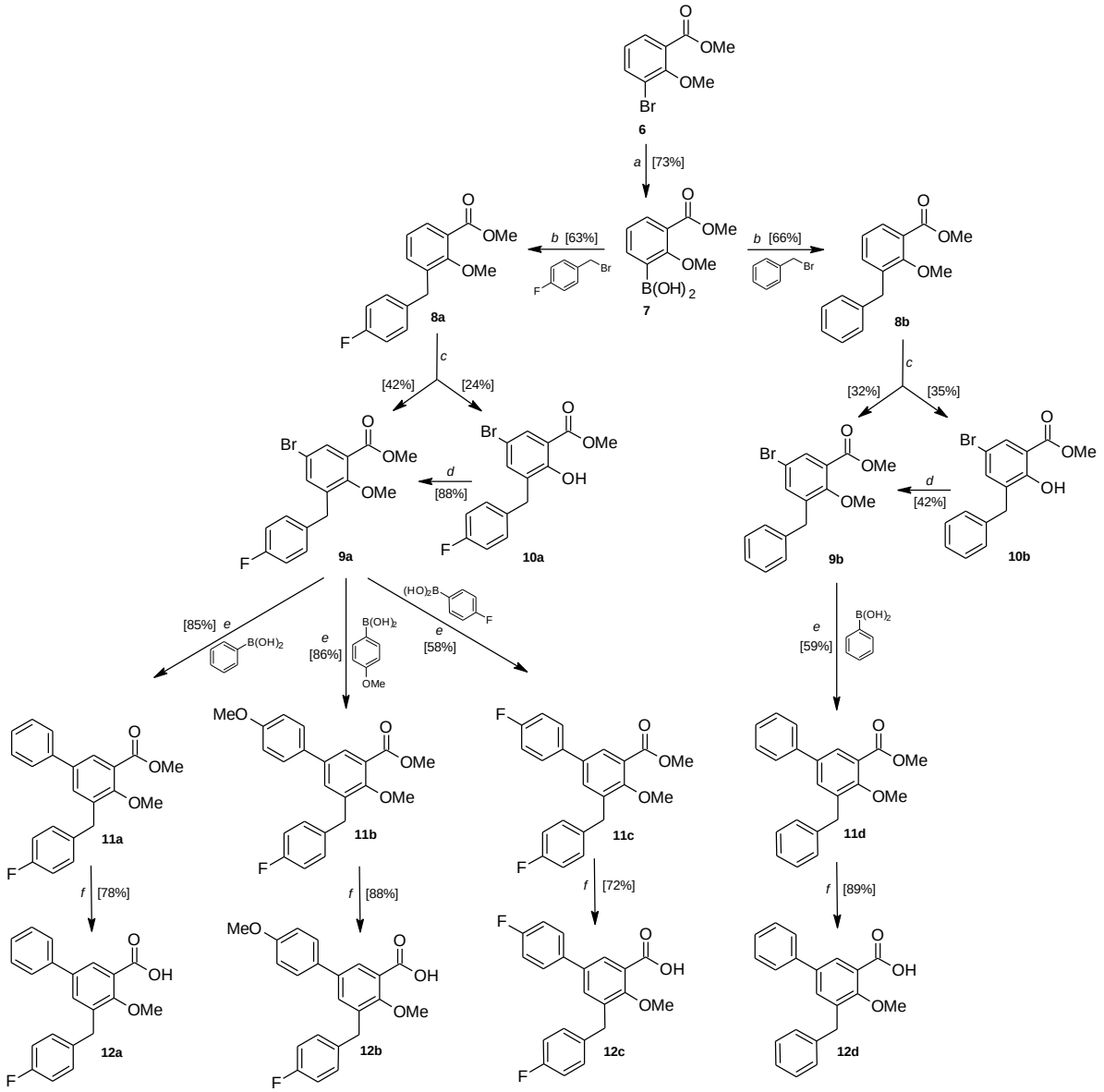
dichloromethane [23], obtaining the desired final products **1a** and **1d**. Treatment of **4** and **5** with thionyl chloride and subsequently with 4-methyl-cyclohexylamine (*cis*/*trans* mixture) in anhydrous dichloromethane [23] followed by chromatographic separation of the *cis*- and *trans*-isomers, allowed to obtain the desired final products **1b-c** and **1e-f**. The two isomers were identified on the basis of the chemical shift of the proton bound to the C1‴ position (see the atom numbering of compound **1b** in Scheme 1). In the *cis* isomer this proton resonates at higher ppm (δ from 4.20 to 4.39 ppm) compared to the same proton in the *trans* isomer (δ from 3.87 to 4.11 ppm), as previously reported [19].



Scheme 1. Reagents and conditions: *a)* Suitable arylboronic acid, $Pd(PPh_3)_4$, aq. Na_2CO_3, toluene/MeOH, microwave (150 °C, 5 bar, 200 W), 10 min.; *b)* KOH, MeOH, reflux, 19 h; *c)* 1) $SOCl_2$, reflux, 30 min.; 2) Suitable cycloalkylamine, CH_2Cl_2, RT, overnight.

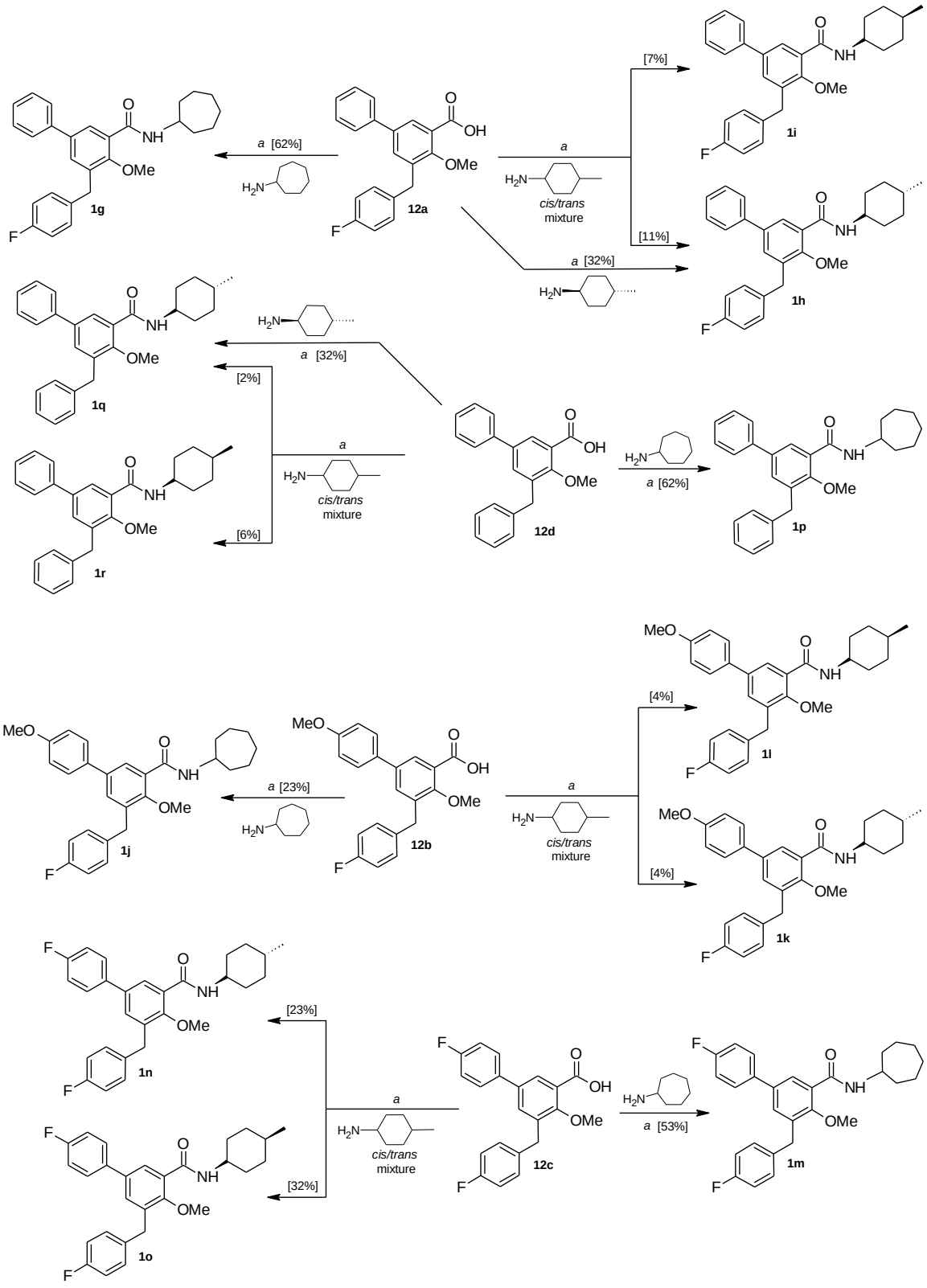
Compounds **12a-d**, direct precursors of final products **1g-r**, were synthesized as shown in Scheme 2, starting from methyl 3-bromo-2-methoxybenzoate (**6**), which was prepared as previously reported by our research group [19]. Compound **6** was converted in the corresponding arylboronic acid **7** through a two steps procedure. **6** was first submitted to a

Miyaura borylation in presence of potassium acetate and bis(diphenylphosphinoferrocene)palladium dichloride ((dppf)$PdCl_2$) in anhydrous 1,4-dioxane [24]. After that, the obtained pinacol ester was hydrolyzed in presence of ammonium acetate and sodium periodate in a mixed solution of acetone and water (1:1) to afford the desired arylboronic acid **7**, which was subjected to a cross-coupling reaction with benzyl bromide or 4-fluorobenzyl bromide in the presence of tetrakis(triphenylphosphine)palladium(0) as catalyst and sodium carbonate as base, in a mixture of anhydrous 1,2-dimethoxyethane and water (2:1) [25] to give derivatives **8a-b**. Subsequently, these compounds were brominated using bromine in chloroform [26], affording the desired compounds **9a-b** and a significant amount of the corresponding demethylated products **10a-b**, which were converted again in the derivatives **9a-b** upon treatment with dimethyl sulphate and sodium hydroxide in the presence of tetrabutylammonium bromide in water/dichloromethane [27]. The subsequent Suzuki reaction with the suitable arylboronic acid (phenyl, 4-fluorophenyl and 4-methoxyphenyl boronic acid), aqueous sodium carbonate, palladium acetate and triphenylphosphine in methanol/toluene [21], afforded the intermediates **11a-d** which were hydrolysed in the presence of potassium hydroxide in methanol [22] leading to the corresponding acid derivatives **12a-d**.



Scheme 2. Reagents and conditions: *a)* 1) Bis(pinacolate)diboron, KOAc, Pd(dppf)Cl_2, anhydrous 1,4-dioxane, 110 °C, 2 h (or microwave: 130 °C, 5 bar, 200 W, 30 min.); 2) NH_4OAc, $NaIO_4$, acetone/H_2O, RT.; b) Suitable aryl bromide, Na_2CO_3, Pd(PPh_3)$_4$, DME/H_2O, 100 °C, 4 h (or microwave: 140 °C, 5 bar, 200 W, 15 min.); *c)* Br_2, $CHCl_3$, RT, overnight; *d)* TBAB, $(CH_3)_2SO_4$, aq. NaOH, CH_2Cl_2, RT, overnight; *e)* Suitable arylboronic acid, Pd(PPh_3)$_4$, aq. Na_2CO_3, toluene/MeOH, microwave (150 °C, 5 bar, 200 W), 10 min.; *f)* KOH, MeOH, reflux, 19 h.

Final products **1g-r** were obtained from intermediates **12a-d** as shown in Scheme 3. **12a-d** were treated with thionyl chloride and subsequently with cycloheptylamine in anhydrous dichloromethane [23], affording the desired final products **1g**, **1j**, **1m** and **1p**. Treatment of **12a** with thionyl chloride and subsequently with 4-methyl-cyclohexylamine (*cis*/*trans* mixture) in anhydrous dichloromethane [23] allowed to obtain the desired final products **1h** (*trans*-isomer) and **1i** (*cis*-isomer) after chromatographic separation. *Trans*-isomer **1h** was also obtained by treatment of **12a** with thionyl chloride and subsequently with *trans*-4-methyl-cyclohexylamine in anhydrous dichloromethane [23]. Treatment of **12d** with thionyl chloride and subsequently with 4-methyl-cyclohexylamine (*cis*/*trans* mixture) in anhydrous dichloromethane [23] allowed to obtain the desired final products **1q** (*trans*-isomer) and **1r** (*cis*-isomer) after chromatographic separation. *Trans*-isomer **1q** was also obtained by treatment of **12d** with thionyl chloride and subsequently with *trans*-4-methyl-cyclohexylamine in anhydrous dichloromethane [23]. Finally, treatment of **12b** and **12c** with thionyl chloride and subsequently with 4-methyl-cyclohexylamine (*cis*/*trans* mixture) in anhydrous dichloromethane [23], followed by chromatographic separation of the *cis*- and *trans*-isomers, allowed to obtain the desired final products **1k**, **1l**, **1n** and **1o**.

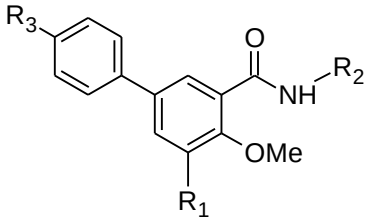


Scheme 3. Reagents and conditions: *a)* 1) $SOCl_2$, reflux, 30 min.; 2) Suitable cycloalkylamine, CH_2Cl_2, RT, overnight.

2.2 Binding to CB_1 and CB_2 receptors

We synthesized 18 biphenyl carboxamides to investigate the impact of different substituents on the binding and activation of CB_2 receptors. First, we assessed the impact of the carboxamide moiety keeping constant the substituents in R_1 and R_3 (Fig. 1, Table I).

Table I. Radioligand binding data of biphenylic derivatives.[a]



				K_i (nM)		
Compound	R_1	R_2	R_3	CB_1	CB_2	S.I.[b]
1a	*n*-butyl	cycloheptyl	H	1859	352	5.3
1b	*n*-butyl	*trans*-4-methylcyclohexyl	H	3608	2390	0.7
1c	*n*-butyl	*cis*-4-methylcyclohexyl	H	549	1262	2.3
1d	*n*-butyl	cycloheptyl	F	6111	166	**36.8**
1e	*n*-butyl	*trans*-4-methylcylclohexyl	F	≥10000	1763	≥5.8
1f	*n*-butyl	*cis*-4-methylcyclohexyl	F	2523	1413	1.8
1g	4-fluorobenzyl	cycloheptyl	H	904.5	84.1	**10.7**
1h	4-fluorobenzyl	*trans*-4-methylcyclohexyl	H	3086	2617	1.2
1i	4-fluorobenzyl	*cis*-4-methylcyclohexyl	H	826	1106	1.3
1k	4-fluorobenzyl	cycloheptyl	OMe	1173	811	1.4
1j	4-fluorobenzyl	*trans*-4-methylcyclohexyl	OMe	≥10000	≥10000	n.d.
1l	4-fluorobenzyl	*cis*-4-methylcyclohexyl	OMe	380	196	2.0

1m	4-fluorobenzyl	cycloheptyl	F	2831	265	**10.7**
1n	4-fluorobenzyl	*trans*-4-methylcyclohexyl	F	1852	1086	1.7
1o	4-fluorobenzyl	*cis*-4-methylcyclohexyl	F	1070	337	3.2
1p	benzyl	cycloheptyl	H	1557	594	2.6
1q	benzyl	*trans*-4-methylcyclohexyl	H	2502	4809	1.9
1r	benzyl	*cis*-4-methylcyclohexyl	H	n.d.	2667	n.d.
1s[c]	methyl	cycloheptyl	H	656	148	4.5
1t[c]	methyl	cycloheptyl	OMe	16542	2742	6.1
1u[c]	*n*-butyl	cycloheptyl	OMe	n.d.	249	n.d.

[a]Binding experiments were carried out using purified membranes generated in-house from CHO-*h*CB_1 and CHO-*h*CB_2 stably transfected cell lines. [^{3}H]CP55940 concentration was 0.5 nM and its K_d value for CB_1 and CB_2 receptor is 0.5 nM and 0.69 nM, respectively. Values represent means and 95% confidence intervals. n.d.: not determined [b]S.I.: selectivity index for CB_2 receptor calculated as $K_i(CB_1)/K_i(CB_2)$ ratio. [c]See Ref. [19].

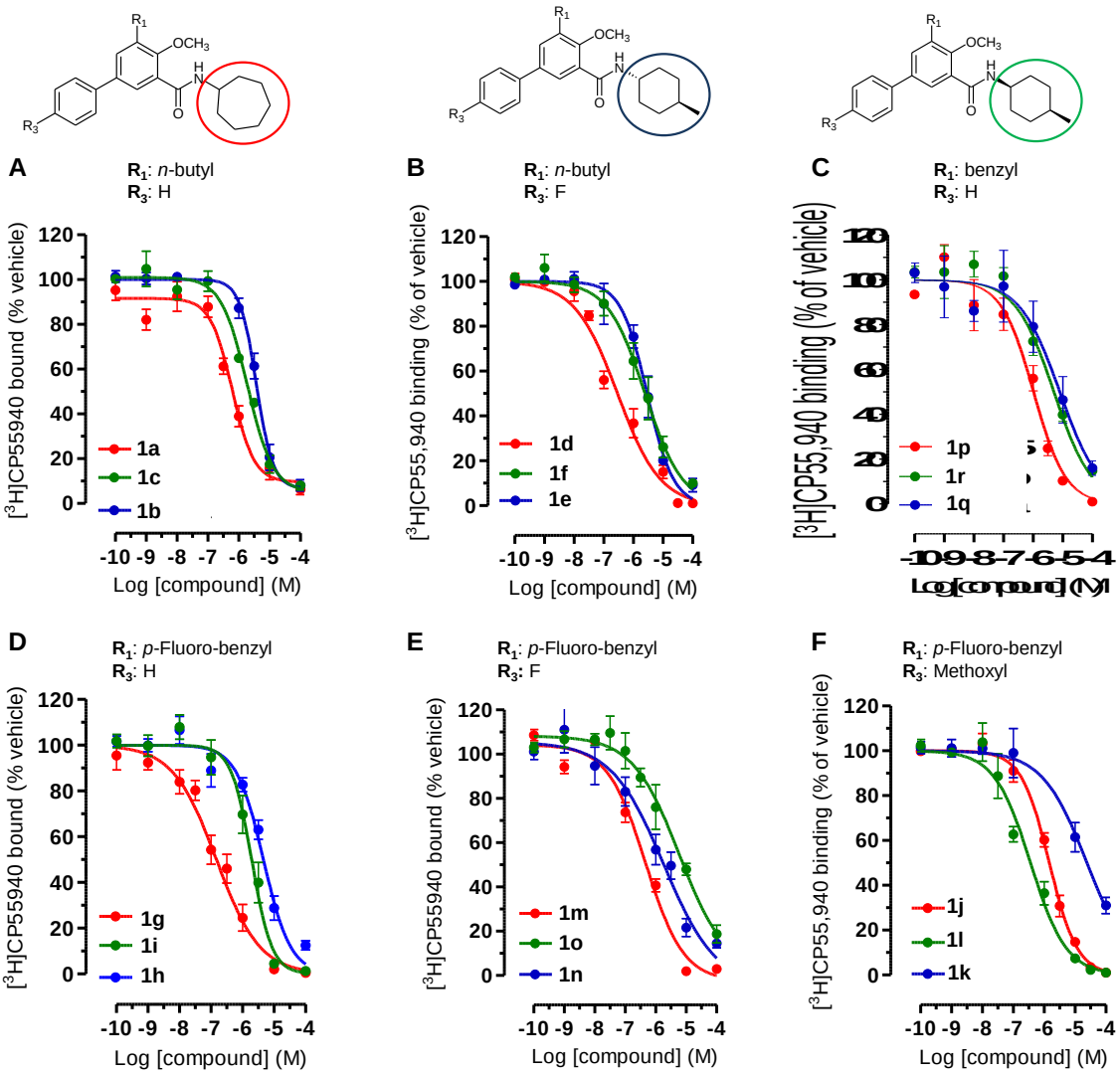


Figure 2. Binding curves to CB_2 receptors. The full concentration-dependent binding curves to CB_2 receptor are reported for different series of analogs. In each panel, the impact of the type of substituent on the carboxamide moiety (R_2) is reported for the cycloheptyl (red), *cis*-4-methylcyclohexyl (green) and *trans*-4-methylcyclohexyl (blue) group. The role of the substituent in R_1 and R_3 was evaluated by using groups with different size and polarity, as reported in the graphs.

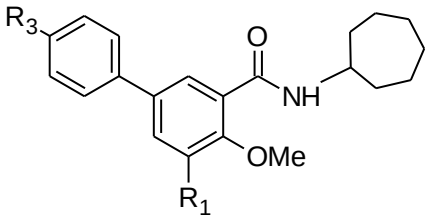
The results show that cycloheptyl carboxamides have higher affinity to CB_2 receptors compared to 4-methylcyclohexyl-bearing molecules without major differences between the *cis* and *trans* isomer (Fig. 2). This is in agreement with the previous molecular modelling study performed on similar biphenyl compounds [19]. Only compound **1l** bearing a *cis*-4-methylcyclohexyl carboxamide showed a similar K_i value to the cycloheptyl derivative (**1j**) and significantly higher potency compared to the *trans* isomer (**1k**) (Table I and Fig. 2F). Compound **1j-l** differ from the other set of compounds (Fig. 2A-E) for the substituent in R_3, which is neither a hydrogen (compounds **1a-c**) nor a fluorine (compounds **1d-f**) but a methoxyl group (Fig. 2F). This might suggest that a bigger substituent in position 4′ could abolish the privileged interaction with the CB_2 receptor binding pocket provided by the cycloheptyl carboxamide compared to the 4-methylcyclohexyl moiety. Then, we investigated the impact of different substituents in R_1 and R_3 on CB_1 and CB_2 receptors binding. In agreement with the previous docking study, the results here reported confirmed that the presence of a *n*-butyl chain in position 5 accounts for a higher selectivity to CB_2 over CB_1 receptors. Indeed, compound **1d** showed the highest selectivity for CB_2 receptors (36-folds). Furthermore, our data also indicate that by replacing the linear alkyl chain with an aromatic group the selectivity towards CB_2 receptors is not only retained (e.g. **1d** vs **1m**) but rather increased (e.g. by a factor 2 for **1g** vs **1a**).

3.3 Assessment of functional activity at CB_2 receptors

We assessed the functional activity of the biphenyl derivatives at CB_2 receptors using the [^{35}S]GTPγS binding assay (Table II). Based on the binding data, we decided to investigate only the compounds bearing a cycloheptyl carboxamide moiety in combination with different substituents in position 5 and 4′, with the exception of **1l**, which showed a higher affinity compared to corresponding cycloheptyl derivative (**1j**), as described above. Initially, we

investigated the impact of three different groups in R_1 (methyl, *n*-butyl, 4-fluorobenzyl) keeping the position 4′ unsubstituted. The results show that the presence of a methyl group leads to full agonism at CB_2 receptors, while bigger substituents dramatically shift the functional activity towards inverse agonism (**1a** and **1g**, Fig. 3A).

Table II. Effects of different substituents in R_1 and R_3 to the biphenylic scaffold on the functional activation of CB_2 receptors.



Compound	R_1	R_3	CB_2 effects
1a	*n*-butyl	H	Inverse agonist
1d	*n*-butyl	F	Inverse agonist
1u	*n*-butyl	Methoxyl	Antagonist
1g	4-Fluorobenzyl	H	Inverse agonist
1m	4-Fluorobenzyl	F	Inverse agonist
1j	4-Fluorobenzyl	Methoxyl	Antagonist
1p	Benzyl	H	Inverse Agonist
1s[*]	Methyl	H	Agonist
1v[*]	Methyl	F	Agonist
1t[*]	Methyl	Methoxyl	Antagonist

[*]See Ref. [19].

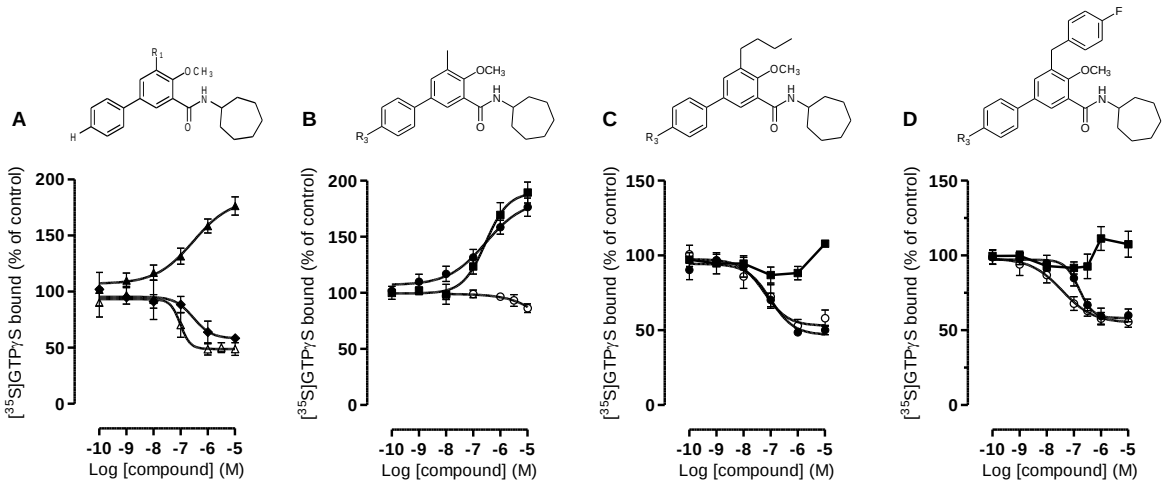


Figure 3. Functional activity at CB_2 receptors. Concentration-dependent curves of CB_2 receptor-mediated G-protein activation ([^{35}S]GTPγS binding). A) The effect of methyl (triangles solid), *n*-butyl (open triangels) and benzyl group (solid diamond) in R_1 was evaluated in molecules bearing a hydrogen in R_3. In panels B-D the impact of different substituents in R_3 is reported. Hydrogen (solid circles), fluorine (open circles) and methoxyl (solid square) groups were tested in molecules bearing a methyl (B), *n*-butyl (C) and 4-fluorobenzyl (D) group in R_1.

Then, we carried out further experiments to assess the impact of different substituents in R_3 keeping fixed R_1. In Fig. 3B-D the [^{35}S]GTPγS binding curves obtained with molecules bearing a methyl (Fig. 3B), *n*-butyl (Fig. 3C) and 4-fluorobenzyl (Fig. 3D) group in R_1 are showed. The combination of a methyl group in R_1 and a hydrogen, (compound **1s** [19]) or a fluorine (compound **1v** [19]) in R_3 generates full agonism at CB_2 receptors, while the presence of a methoxyl group (compound **1t** [19]) turns the molecule into a neutral antagonist which does not shift the receptor population neither towards the active (i.e. agonist) nor the inactive (i.e. inverse agonist) conformation (Fig. 3B). When the methyl group in R_1 is replaced by a bigger group such as a *n*-butyl or 4-fluorobenzyl group, the molecules bearing a small substituent in R_3 behave as inverse agonists at CB_2 receptors, but again turning into

neutral antagonists when a methoxyl group is present (Fig. 3C-D). As summarized in Table II, our results indicate that the presence of a methoxyl group in R_3 generates neutral antagonists at CB_2 receptors independently of the type of substituent present in R_1. Nonetheless, when a hydrogen or fluorine is present in R_3, it is the type of the substituent present in R_1 to dictate the functional activity of the molecule at CB_2 receptors. In particular, the presence of a methyl group generates full agonism, while a linear alkyl chain or an aromatic ring is responsible for inverse agonism. These results indicate that by introducing little modifications of the size and position of the substituents on the biphenyl scaffold, we could generate molecules that behave as full agonists, inverse agonists and neutral antagonists at CB_2 receptor. As hypothesized in the previous docking study, the methoxyl group in R_3 could perfectly fill the TM3-TM5 cleft establishing a T-shape interaction with $W^{5.43}$ [19]. Our current results also suggest that molecules bearing the methoxyl group do not perturb the endogenous balance among the different conformational states of CB_2 receptors, thus behaving as neutral antagonists. On the other side, when a smaller substituent (i.e. hydrogen and fluorine) is present in R_3, the molecule preferentially stabilizes the active (e.g. **1s**) or the inactive (e.g. **1a**, **1g**) conformation, thus behaving as an agonist or an inverse agonist, respectively. In line with these results, we recently reported that the 1,2-dihydro-2-oxopiridine-3-carboxamides bearing a phenyl or a *p*-methoxyphenyl group in position 5 behave as inverse agonists or a neutral antagonists, respectively, while the unsubstituted compound behaves as a full agonist [28]. In our first investigation of the biphenyl carboxamide scaffold, we showed that the 5-*n*-butyl-4,4′-dimethoxy-*N*-cycloheptylbiphenyl-3-carboxamide (compound **1u** [19]) possesses a strong affinity to CB_2 receptors (K_i = 11.5 nM) and a high selectivity over CB_1 (130 fold) behaving as a neutral antagonist in functional assays [19]. In competition experiments, the antagonistic effects of 5-*n*-butyl-4,4′-dimethoxy-*N*-cycloheptylbiphenyl-3-carboxamide (compound **1u** [19]) against 1 nM of HU-210 became

evident at concentrations that are 1000 to 10000 times higher than the full agonist (i.e. 1-10 μM). Increasing concentrations of the antagonist induced a right-shift of the agonist curve without apparently changing the maximal response. Nonetheless, the derived Schild plot indicates a non-competitive antagonism (slope = 0.65) which might likely derive from a slightly different accommodation of the agonist and antagonist on the binding pocket rather than an irreversible interaction of the antagonist with CB_2 receptors. In addition, the antagonistic effect of **1u** started to occur at 1-10 μM which is 100-1000 higher than the measured binding affinity. In our current study we included **1u** as a reference compound and we could confirm its neutral antagonist behaviour up to 10 μM. On the other hand, we obtained a lower binding affinity to CB_2 receptors (K_i = 249 nM, Table I) compared to the previously reported K_i value (K_i = 11.5 nM) [19]. Our current binding data are more in line with the antagonistic effects previously showed by **1u** in [^{35}S]GTPγS assays.

In our study, we further evaluated the effect of the new neutral antagonist **1l** against the structurally-related full agonist **1s**. As shown in Fig. 4A, increasing concentrations of the antagonist determined a right shift of the [^{35}S]GTPγS binding curve of **1s** without affecting the maximal response. The slope obtained from the linear regression analysis reported in the Schild plot (Fig. 4B, slope = 1.039 ± 0.025) do not differ from the unity, thus confirming a competitive antagonism. Altogether, our current study provides new insights into the functional SAR of the biphenyl carboxamides as CB_2 ligands, indicating that the type of substituent in position 5 and 4′ are crucially involved in the functional effect of the molecule at CB_2 receptors. Our data also suggest the presence of a subordinate relationship between these two positions, with R_1 being responsible for the agonist or inverse agonist behaviour independently of the substituent in R_3 with the exception of the methoxyl group which transforms both full agonists and inverse agonists into neutral antagonists. Overall, our data provide further information about the SAR of biphenyl carboxamides as CB_2 receptor ligands

and indicate important features which are responsible for the functional switch between agonist, inverse agonist and neutral antagonist at CB_2 receptor (see Table II for summary).

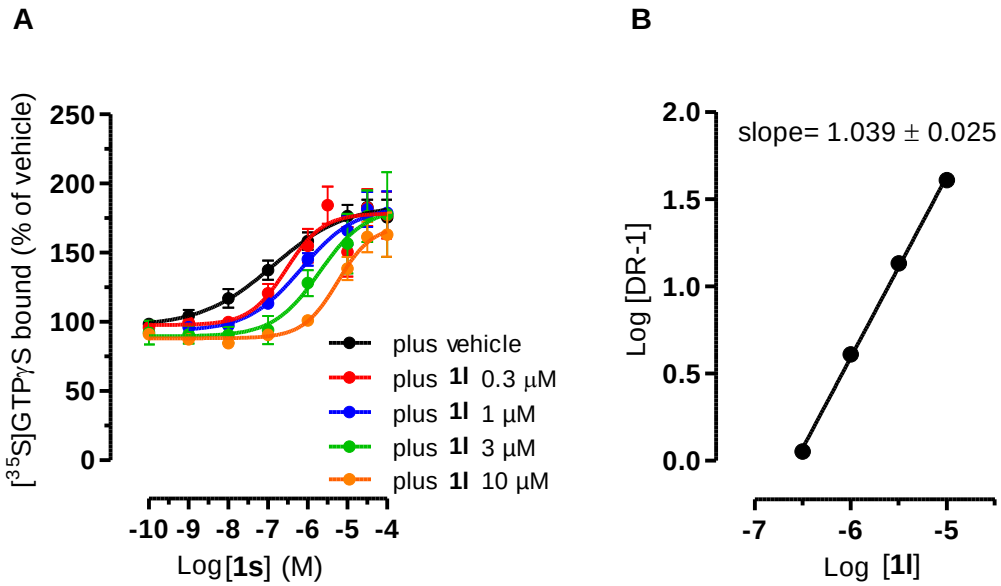
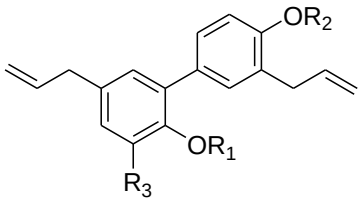


Figure 4. Compound 1l comepetitively antagonized the 1s-induced CB_2 receptor activation. A) Compound **1l** dose-dependently antagonizes the **1s**-induced CB_2 receptor activation without attenuating the maximal agonist response. B) The slope calculated from the Schild-plot (1.039 ± 0.025) is not statistically different from the unity (unpaired *t*-test) indicating that the **1l** and **1s** competitively interact with CB_2 receptors. The results represent the average and S.D. calculated from least three independent experiments performed in duplicates

We recently reported another class of natural and natural-derived biphenyl compounds as CB_2 ligands which showed different functional behaviours [12, 29]. Interestingly, some of the compounds bear one or two alkyl ethers on the two phenyl rings similarly to the biphenyl carboxamides here described. In order to further evaluate the role of the methoxyl group in determining the antagonist behaviour we evaluated few more compounds of this class of molecules (Table III).

Table III. Cannabinoid receptor pharmacology of additional natural and natural-derived biphenyl compounds.



Compound	R_1	R_2	R_3	K_i CB_1 (nM)	K_i CB_2 (nM)	CB_2 effects
Honokiol	H	H	H	[‡]6.46 ± 3.54	[‡]5.61 ± 2.02	Antagonist/ inverse agonist
4-*O′*-Methylhonokiol	H	Methyl	H	[*]2400 ± 600	[*]188 ± 116	Agonist
2a	Methyl	H	H	[*]790 ± 96	[*]114 ± 96	Agonist
2b	Methyl	Methyl	H	n.d.	1375 ± 319	Agonist
2c	H	*n*-butyl	H	615 ± 186	103 ± 71	Antagonist
2d	*n*-butyl	H	H	[*]1120 ± 106	[*]325 ± 115	Protean agonist
2e	*n*-butyl	*n*-butyl	H	n.d.	1783 ± 401	Antagonist
2f	H	Methyl	CH_3CONH	n.d.	>10000	-
2g	H	Methyl	$C_{11}H_{23}CONH$	n.d.	>10000	-
2h	H	Methyl	$C_{17}H_{35}CONH$	n.d.	>10000	-

[‡]See Ref. [25].
[*]See Ref. [24].

The natural compound 4′-*O*-methylhonokiol (MH) and its structural isomer **2a** bear a methoxyl and a hydroxyl group on the two phenyl rings and both behave as selective and moderately potent agonists at CB_2 receptors (Table III). Compound **2b**, which has two methoxyl groups, retains the agonist activity despite significantly losing the binding affinity (by a factor 10). A longer alkyl chain (*n*-butyl) does not affect the binding affinity but meaningfully modifies the functional activity at CB_2 receptors. Compound **2c** and **2d** which bear a *n*-butyl group instead of a methoxyl group compared to MH and **2a**, behave as protean agonist and neutral antagonist, respectively. The double *n*-butyl ether (**2e**) showed a 6-10 fold lower affinity to CB_2 receptors compared to the mono-ether derivatives, similarly to **2b**, but unlike the latter compound, **2e** behaves as a neutral antagonist (Table III). Honokiol, which bears two hydroxyl groups is a weak ligand at CB_2 receptors (K_i value = 2-5 μM) [12, 30]. The presence of a carboxamide in R_3 completely abolishes the binding to CB_2 receptors (compound **2f-h**, Table III). Although the small number of compounds, the combination of binding and functional data suggest that the presence of one alkyl ether is necessary for generating moderately potent and selective CB_2 ligands, while the presence of two ethers has detrimental effects leading to a significant loss in binding affinity to CB_2 receptors. The length of the alkyl chain, rather than its position on the biphenyl scaffold appears to be responsible for the functional effects indicating different tridimensional interactions for these molecules within the binding pocket of CB_2 receptors compared to the biphenyl carboxamides. Assuming a certain similarity in the interaction with CB_2 receptors between the two classes of biphenyl compounds and based on the docking study on biphenyl carboxamides, we might speculate that the methoxyl group of MH cannot reach the cleft between the TM3-TM5 as reported for **1u** thus leading the molecule to behave as a CB_2 agonist. On the other hand, the longer alkyl chain of **2b** could protrude into the cleft and acting as a neutral antagonist, similarly to the proposed docking for **1u**.

Recently, Smoum *et al.*, [31] reported two enantiomer agonists of the CB_2 cannabinoid receptor, HU-308 and HU-433, with paradoxical pharmacological properties. HU-308 exhibited stronger receptor binding but weaker potency while HU-433 showed opposite features (i.e. lower binding affinity and higher potency) in several *in vitro* assays for osteoblast proliferation and osteoclast differentiation and in mouse models for rescuing the ovariectomy-induced bone loss and ear inflammation. A molecular-modelling analysis suggested some small differences in possible binding conformations of the two enantiomers within the CB_2 receptor binding pocket which seem to be responsible for the striking differences in binding affinity and potency [31]. This indicates that very similar chemical structures may have significant different orientations relative to the same binding site, leading to different biological properties, as we described here for some biphenyl carboxamides.

3. Conclusion

In conclusion, our study provides new insights into the functional SAR of the biphenyl carboxamides as CB_2 ligands indicating that the type of substituent in position 5 and 4′ are crucially involved in the functional activity of these molecules at CB_2 receptors. Our data also suggest the presence of a subordinate relationship between these two positions, with position 5 being responsible for the agonist or inverse agonist behaviour, independently of the substituent in position 4′ with the exception of the methoxyl group which transforms both full agonists and inverse agonists into neutral antagonists. Our new compounds provide a complete toolbox of CB_2 functional modulators (i.e. full agonists, inverse agonists and neutral antagonists) derived from the same chemical scaffold and apparently acting on the same or an overlapping binding site. One of the major confounding factors in studying CB_2 pharmacology is related to the constitutive activity of these receptors. This includes the widespread use of antagonists at concentrations which exhibit inverse agonist activity (i.e.

AM630 and SR144528), thus biasing the experimental read-outs. The biphenyl carboxamides shown here would enable researchers with a valuable set of tool compounds which could be applied to unambiguously investigate the biological roles of CB_2 receptors in cellular systems and potentially in pathophysiological conditions *ex-vivo* and *in vivo*.

4. Experimental

4.1 Chemistry

Commercially available reagents were purchased from Sigma Aldrich or Alfa Aesar, and used without purification. ^{1}H NMR spectra were recorded on a Varian Gemini 200 spectrometer (operating at 200 MHz) or on a Bruker AVANCE III™ 400 spectrometer (operating at 400 MHz). ^{13}C NMR spectra were recorded on a Bruker AVANCE III™ 400 spectrometer. Chemical shift (δ) are reported in parts per million related to the residual solvent signal, while coupling constants (*J*) are expressed in Hertz (Hz). Microwave-assisted reactions were run in a Biotage® microwave synthesizer. All of the final products undergoing biological testing were >96% pure as demonstrated by analysis carried out with a Varian Prostar HPLC system equipped with an PDA Detector at 260 nm (column Luna C18 (2) 5μ (150 mm × 4.6 mm)), gradient A/B 70/30 to 90/10 in 20 min, A consisting of methanol, B consisting of buffer ammonium acetate (pH = 4, 10 mM), flow rate of 0.6 mL/min, room temperature). High-resolution mass spectra (HRMS) were recorded on a LTQ Orbitrap XL (Thermo Fisher Scientific) with nanoESI in the positive ion mode (700 - 800 V on the emitter). Evaporation was carried out under vacuum using a rotating evaporator. Silica gel flash chromatography was performed using silica gel 60 Å (0.040-0.063 mm; MERK). Reactions was monitored by TLC on Merck aluminium silica gel (60 F254) plates that were visualized under a UV lamp (λ = 254 nm).

*4.1.1. General procedure for the synthesis of derivatives **2-3** and **11a-d**.*

A microwave sealed-tube was charged, under nitrogen flux, with a solution of the corresponding aryl-halide **1** or **9a-b** (0.57 mmol) in anhydrous toluene (1.3 mL), tetrakis(triphenylphosphine)palladium(0) (0.02 mmol), sodium carbonate (1.14 mmol) in water (1.2 mL) and the proper boronic acid (0.86 mmol) in methanol (1.2 mL). The system was sealed and heated at 150 °C in a microwave reactor for 10 min (5 bar, 200 W). After cooling, the reaction mixture was diluted with water and extracted with ethyl acetate. The organic phases were combined, dried over anhydrous Na_2SO_4, filtered, concentrated and purified.

*4.1.1.1 Methyl 5-butyl-4-methoxy-[1,1'-biphenyl]-3-carboxylate (**2**)*

A microwave sealed-tube was charged, under nitrogen flux, with a solution of methyl 5-bromo-3-butyl-2-methoxybenzoate **1** (171.7 mg, 0.57 mmol) in anhydrous toluene (1.3 mL), tetrakis(triphenylphosphine)palladium(0) (23.1 mg, 0.02 mmol), a solution of sodium carbonate (120.8 mg, 1.14 mmol) in water (1.2 mL) and a solution of phenylboronic acid (104.9 mg, 0.86 mmol) in methanol (1.2 mL). The system was sealed and heated at 150 °C in a microwave reactor for 10 min (5 bar, 200 W). After cooling, the reaction mixture was diluted with water and extracted with ethyl acetate. The organic phases were combined, dried over anhydrous Na_2SO_4, filtered, concentrated in vacuo. The crude thus obtained was purified by flash column chromatography (*n*-hexane/EtOAc 9:1), affording of pure intermediate **2** (146.2 mg, 0.49 mmol). Yield: 86%. ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 7.88 (d, 1H, J = 2.4 Hz, H2), 7.60-7.54 (m, 3H, H6, H2′, H6′), 7.48-7.30 (m, 3H, H3′, H4′, H5′), 3.95 (s, 3H, OCH_3), 3.87 (s, 3H, COOCH_3), 2.72 (t, 2H, J = 7.8 Hz, CH_2CH$_2$CH$_2$CH$_3$), 1.72-1.56 (m, 2H, CH$_2$CH_2CH$_2$CH$_3$), 1.51-1.32 (m, 2H, CH$_2$CH$_2$CH_2CH$_3$), 0.95 (t, 3H, J = 7.1 Hz, CH$_2$CH$_2$CH$_2$CH_3).

*4.1.1.2 Methyl 5-butyl-4'-fluoro-4-methoxy-[1,1'-biphenyl]-3-carboxylate (**3**)*

Prepared from aryl-halide **1** (171.7 mg, 0.57 mmol) using 4-fluorophenylboronic acid. Purification by flash column chromatography (*n*-hexane/EtOAc 9:1). **3** (152.9 mg, 0.48 mmol). Yield: 85%. ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 7.82 (d, 1H, *J* = 2.6 Hz, H2), 7.57-7.47 (m, 3H, H6, H2′, H6′), 7.18-7.05 (m, 2H, H3′, H5′), 3.94 (s, 3H, OCH_3), 3.86 (s, 3H, COOCH_3), 2.71 (t, 2H, *J* = 7.7 Hz, CH_2CH$_2$CH$_2$CH$_3$), 1.71-1.55 (m, 2H, CH$_2$CH_2CH$_2$CH$_3$), 1.50-1.31 (m, 2H, CH$_2$CH$_2$CH_2CH$_3$), 0.95 (t, 3H, *J* = 7.2 Hz, CH$_2$CH$_2$CH$_2$CH_3).

*4.1.1.3 Methyl 5-(4-fluorobenzyl)-4-methoxy-[1,1'-biphenyl]-3-carboxylate (**11a**)*

Prepared from aryl-halide **9a** (201.3 mg, 0.57 mmol) using phenylboronic acid. Purification by flash column chromatography (*n*-hexane/EtOAc 9:1). **11a** (169.7 mg, 0.48 mmol). Yield: 85%. ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 7.92 (d, 1H, *J* = 2.4 Hz, H2), 7.56-7.33 (m, 6H, H6, H2′, H3′, H4′, H5′, H6′), 7.23-7.15 (m, 2H, H2″, H6″), 7.02-6.90 (m, 2H, H3″, H5″), 4.07 (s, 2H, benzylic CH_2), 3.95 (s, 3H, OCH_3), 3.77 (s, 3H, COOCH_3).

*4.1.1.4 Methyl 5-(4-fluorobenzyl)-4,4'-dimethoxy-[1,1'-biphenyl]-3-carboxylate (**11b**)*

Prepared from aryl-halide **9a** (201.3 mg, 0.57 mmol) using 4-methoxyphenylboronic acid. Purification by flash column chromatography (*n*-hexane/EtOAc 9:1). **11b** (125.5 mg, 0.33 mmol). Yield: 58%. ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 7.88 (d, 1H, *J* = 2.5 Hz, H2), 7.49-7.41 (m, 3H, H6, H2′, H6′), 7.21-7.14 (m, 2H, H2″, H6″), 7.01-6.91 (m, 4H, H3′, H5′, H3″, H5″), 4.05 (s, 2H, benzylic CH_2), 3.94 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.75 (s, 3H, COOCH_3).

*4.1.1.5 Methyl 4'-fluoro-5-(4-fluorobenzyl)-4-methoxy-[1,1'-biphenyl]-3-carboxylate (**11c**)*

Prepared from aryl-halide **9a** (201.3 mg, 0.57 mmol) using 4-fluorophenylboronic acid. Purification by flash column chromatography (*n*-hexane/EtOAc 9:1). **11c** (180.5 mg, 0.49 mmol). Yield: 86%. ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 7.87 (d, 1H, *J* = 2.6 Hz, H2), 7.51-7.43 (m, 3H, H6, H2′, H6′), 7.22-7.04 (m, 4H, H3′, H5′, H2″, H6″), 7.03-6.93 (m, 2H, H3″, H5″), 4.06 (s, 2H, benzylic CH_2), 3.95 (s, 3H, OCH_3), 3.76 (s, 3H, COOCH_3).

*4.1.1.6 Methyl 5-benzyl-4-methoxy-[1,1'-biphenyl]-3-carboxylate (**11d**)*

Prepared from aryl-halide **9b** (191.0 mg, 0.57 mmol) using phenylboronic acid. Purification by flash column chromatography (*n*-hexane/EtOAc 9:1). **11d** (111.7 mg, 0.336 mmol). Yield: 59%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.92 (d, 1H, *J* = 2.4 Hz, H2), 7.53-7.50 (m, 3H, H6, H2′, H6′), 7.45-7.38 (m, 2H, H3′, H5′), 7.36-7.17 (m, 6H, H4′, H2″, H3″, H4″, H5″, H6″), 4.11 (s, 2H, benzylic CH_2), 3.95 (s, 3H, OCH_3), 3.77 (s, 3H, COOCH_3).

*4.1.2. General procedure for the synthesis of derivatives **4-5** and **12a-d**.*

The suitable ester **2-3** or **11a-d** (1.96 mmol) was dissolved in methanol (90 mL) followed by addition of solid potassium hydroxide (19.6 mmol). The resulting suspension was stirred until completed dissolution of the solids. Then, the mixture was heated at reflux for 19 h. The reaction was allowed to cool to room temperature, and methanol was removed under vacuum to afford a yellow oil that was partitioned between water and ethyl acetate. After separation of the two phases, the aqueous layer was acidified to pH = 2 with 0.6 N hydrochloric acid solution, to obtain a white precipitate. The precipitate was repeatedly extracted with ethyl acetate, and the combined organic fractions were dried over anhydrous Na_2SO_4, filtered and evaporated under vacuum to afford the desired acid derivative, that was used in the next step without further purification.

*4.1.2.1 5-butyl-4-methoxy-[1,1′-biphenyl]-3-carboxylic acid (**4**)*

Methyl 5-butyl-4-methoxy-[1,1′-biphenyl]-3-carboxylate **2** (584.8 mg, 1.96 mmol) was dissolved in methanol (90.0 mL) followed by addition of solid potassium hydroxide (1.10 g, 19.6 mmol). The resulting suspension was stirred until completed dissolution of the solids. Then, the mixture was heated at reflux for 19 h. The reaction was allowed to cool to room temperature, and methanol was removed under vacuum to afford a yellow oil that was partitioned between water and ethyl acetate. After separation of the two phases, the aqueous layer was acidified to pH = 2 with 0.6 N hydrochloric acid solution, to obtain a white precipitate. The precipitate was repeatedly extracted with ethyl acetate, and the combined organic fractions were dried over anhydrous Na_2SO_4, filtered and evaporated under vacuum to afford the acid derivative **4** (386.7 mg, 1.36 mmol) that was used in the next step without further purification. Yield: 69%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.20 (d, 1H, *J* = 2.4 Hz, H2), 7.67 (d, 1H, *J* = 2.4 Hz, H6), 7.61-7.56 (m, 2H, H2′, H6′), 7.50-7.36 (m, 3H, H3′, H4′, H5′), 3.96 (s, 1H, OC*H*$_3$), 2.75 (t, 2H, *J* = 7.8 Hz, C*H*$_2$CH$_2$CH$_2$CH$_3$), 1.77-1.61 (m, 2H, CH$_2$C*H*$_2$CH$_2$CH$_3$), 1.53-1.34 (m, 2H, CH$_2$CH$_2$C*H*$_2$CH$_3$), 0.97 (t, 3H, *J* = 7.2 Hz, CH$_2$CH$_2$CH$_2$C*H*$_3$).

*4.1.2.2 5-butyl-4'-fluoro-4-methoxy-[1,1'-biphenyl]-3-carboxylic acid (**5**)*

Prepared from ester **3** (620.1 mg, 1.96 mmol). **5** (580.5 mg, 1.92 mmol). Yield: 98%. ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 8.11 (d, 1H, *J* = 2.6 Hz, H2), 7.60 (d, 1H, *J* = 2.4 Hz, H6), 7.58-7.48 (m, 2H, H2′, H6′), 7.21-7.07 (m, 2H, H3′, H5′), 3.95 (s, 3H, OC*H*$_3$), 2.74 (t, 2H, *J* = 7.8 Hz, C*H*$_2$CH$_2$CH$_2$CH$_3$), 1.76-1.58 (m, 2H, CH$_2$C*H*$_2$CH$_2$CH$_3$), 1.53-1.32 (m, 2H, CH$_2$CH$_2$C*H*$_2$CH$_3$), 0.97 (t, 3H, *J* = 7.2 Hz, CH$_2$CH$_2$CH$_2$C*H*$_3$).

*4.1.2.3 5-(4-fluorobenzyl)-4-methoxy-[1,1'-biphenyl]-3-carboxylic acid (**12a**)*

Prepared from ester **11a** (686.7 mg, 1.96 mmol). **12a** (514.6 mg, 1.53 mmol). Yield: 78%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.22 (d, 1H, J = 2.4 Hz, H2), 7.56 (d, 1H, J = 2.5 Hz, H6), 7.54-7.50 (m, 2H, H3′, H5′), 7.46-7.40 (m, 2H, H2′, H6′), 7.39-7.32 (m 1H, H4′), 7.22-7.15 (m, 2H, H2″, H6″), 7.04-6.96 (m, 2H, H3″, H5″), 4.11 (s, 2H, benzylic CH_2), 3.89 (s, 3H, OCH_3).

*4.1.2.4 5-(4-fluorobenzyl)-4,4'-dimethoxy-[1,1'-biphenyl]-3-carboxylic acid (**12b**)*

Prepared from ester **11b** (745.6 mg, 1.96 mmol). **12b** (630.2 mg, 1.72 mmol). Yield: 88%. ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 8.15 (d, 1H, J = 2.6 Hz, H2), 7.50 (d, 1H, J = 2.6 Hz, H6), 7.46 (AA′XX′, 2H, J_{AX} = 9.0 Hz, $J_{AA'XX'}$ = 2.5 Hz, H2′, H6′), 7.22-7.14 (m, 2H, H2″, H6″), 6.95 (AA′XX′, 2H, J_{AX} = 9.0 Hz, $J_{AA'XX'}$ = 2.1 Hz, H3′, H5′), 7.04-6.94 (m, 2H, H3″, H5″), 4.08 (s, 2H, benzyilic CH_2), 3.86 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3).

*4.1.2.5. 4'-fluoro-5-(4-fluorobenzyl)-4-methoxy-[1,1'-biphenyl]-3-carboxylic acid (**12c**)*

Prepared from ester **11c** (722.0 mg, 1.96 mmol). **12c** (499.6 mg, 1.41 mmol). Yield: 72%. ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 8.15 (d, 1H, J = 2.4 Hz, H2), 7.51-7.42 (m, 3H, H6, H2′, H6′), 7.22-6.95 (m, 6H, H3′, H5′, H2″, H6″, H3″, H5″), 4.10 (s, 2H, benzylic CH_2), 3.88 (s, 3H, OCH_3).

*4.1.2.6. 5-benzyl-4-methoxy-[1,1'-biphenyl]-3-carboxylic acid (**12d**)*

Prepared from ester **11d** (651.5 mg, 1.96 mmol). **12d** (553.9 mg, 1.74 mmol). Yield: 89%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.16 (d, J = 2.4 Hz, H2), 7.58 (d, 1H, J = 2.4 Hz, H6), 7.54-7.50 (m, 2H, H2′, H6′), 7.44-7.38 (m, 2H, H3′, H5′), 7.37-7.27 (m, 3H, H4′, H3″, H5″), 7.25-7.19 (m, 3H, H2″, H4″, H6″), 4.13 (s, 2H, benzylic CH_2), 3.85 (s, 3H, OCH_3).

*4.1.3. General procedure for the synthesis of carboxamides (**1a-r**)*

The suitable acid **4-5** or **12a-d** (0.15 mmol) was suspended in thionyl chloride (1.5 mmol) and heated at reflux for 30 minutes. Excess of thionyl chloride was removed by evaporation under nitrogen atmosphere and the obtained acid chloride was treated with the proper amine (0.34 mmol) dissolved in the minimum amount of dichloromethane. The resulting mixture was stirred at room temperature overnight, diluted with dichloromethane and washed with 0.6 N hydrochloric acid solution and then with a saturated aqueous sodium bicarbonate solution. The organic layer was dried over anhydrous Na_2SO_4, filtered, concentrated and purified.

*4.1.3.1. 5-butyl-N-cycloheptyl-4-methoxy-[1,1'-biphenyl]-3-carboxamide (**1a**)*

5-Butyl-4-methoxy-[1,1′-biphenyl]-3-carboxylic acid **4** (42.6 mg, 0.15 mmol) was suspended in thionyl chloride (0.11 mL, 1.5 mmol) and heated at reflux for 30 minutes. Excess of thionyl chloride was removed by evaporation under nitrogen atmosphere and the obtained acid chloride was treated with cycloheptylamine (0.04 mL, 0.34 mmol) dissolved in the minimum amount of dichloromethane. The resulting mixture was stirred at room temperature overnight, diluted with dichloromethane and washed with 0.6 N hydrochloric acid solution and then with a saturated aqueous sodium bicarbonate solution. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. Purification by flash column chromatography (*n*-hexane/EtOAc 8:2) afforded pure **1a** (36.0 mg, 0.095 mmol). Yield: 63%. ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 8.10 (d, 1H, *J* = 2.4 Hz, H2), 7.67 (bd, 1H, exchangeable, N*H*), 7.62-7.57 (m, 2H, H2′, H6′), 7.52 (d, 1H, *J* = 2.6 Hz, H6), 7.47-7.31 (m, 3H, H3′, H4′, H5′), 4.34-4.17 (m, 1H, NH-C*H*), 3.79 (s, 3H, OCH_3), 2.71 (t, 2H, *J* = 7.8 Hz, CH_2CH$_2$CH$_2$CH$_3$), 2.12-2.02 (m, 2H, CH$_2$CH_2CH$_2$CH$_3$), 1.73-1.50 (m, 12H, cycloheptyl), 1.49-1.34 (m, 2H, CH$_2$CH$_2$CH_2CH$_3$), 0.97 (t, 3H, *J* = 7.2 Hz, CH$_2$CH$_2$CH$_2$CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 14.00 (C4″), 22.83 (C3″), 24.23 (C3‴, C6‴), 28.20 (C4‴, C5‴),

29.46 (C1″), 33.03 (C2″), 35.17 (C2‴, C7‴), 50.44 (C1‴), 62.21 (O*C*H$_3$), 127.04 (C2′, C6′), 127.33 (C3), 127.70 (C5), 127.88 (C2), 128.76 (C3′, C5′), 131.55 (C4′), 136.68 (C6), 137.55 (C1), 140.16 (C1′), 155.52 (C4), 164.48 (*C*=O). ESI-HRMS *m/z*: 380.2574; calc. for $C_{25}H_{34}O_2N$: 380.2584 ($M + H^+$).

*4.1.3.2. 5-butyl-4-methoxy-N-(trans-4-methylcyclohexyl)-[1,1'-biphenyl]-3-carboxamide (**1b**) and 5-butyl-4-methoxy-N-(cis-4-methylcyclohexyl)-[1,1'-biphenyl]-3-carboxamide (**1c**)*

Prepared from carboxylic derivative **4** (42.6 mg, 0.15 mmol) using 4-methylcyclohexylamine (*cis/trans* mixture). Purification by flash column chromatography (*n*-hexane/EtOAc 8:2) allowed the separation of *trans* (**1b**) and *cis* (**1c**). **1b** (1.7 mg, 0.0045 mmol). Yield: 3%. ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 8.08 (d, 1H, *J* = 2.4 Hz, H2), 7.62-7.31 (m, 6H, H6, H2′, H3′, H4′, H5′, H6′), 4.05-3.89 (m, 1H, NH-C*H*), 3.78 (s, 3H, OC*H*$_3$), 2.70 (t, 2H, *J* = 7.3 Hz, C*H*$_2$CH$_2$CH$_2$CH$_3$), 2.13-2.05 (m, 2H, CH$_2$C*H*$_2$CH$_2$CH$_3$), 1.79-1.09 (m, 11H, CH$_2$CH$_2$C*H*$_2$CH$_3$ + cyclohexyl), 0.97 (t, 3H, *J* = 7.1 Hz, CH$_2$CH$_2$CH$_2$C*H*$_3$), 0.92 (d, 3H, *J* = 6.2 Hz, CH-C*H*$_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 14.10 (C4″), 22.35 (C7‴), 22.93 (C3″), 29.60 (C1″), 32.21 (C4‴), 33.15 (C3‴, C5‴), 33.36 (C2″), 34.05 (C2‴, C6‴), 48.63 (C1‴), 62.28 (O*C*H$_3$), 127.18 (C2′, C6′), 127.45 (C3), 127.88 (C5), 128.00 (C2), 128.88 (C3′, C5′), 131.70 (C4′), 136.80 (C6), 137.69 (C1), 140.29 (C1′), 155.60 (C4), 165.00 (*C*=O). ESI-HRMS *m/z*: 380.2574; calc. for $C_{25}H_{34}O_2N$: 380.2584 ($M + H^+$). **1c** (2.85 mg, 0.0075 mmol). Yield: 5%. ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 8.12 (d, 1H, *J* = 2.4 Hz, H2), 7.96 (bd, 1H, exchangeable, N*H*), 7.62-7.56 (m, 2H, H2′, H6′), 7.53 (d, 1H, *J* = 2.4 Hz, H6), 7.46-7.33 (m, 3H, H3′, H4′, H5′), 4.36-4.26 (m, 1H, NH-C*H*), 3.82 (s, 3H, OC*H*$_3$), 2.72 (t, 2H, *J* = 7.3 Hz, C*H*$_2$CH$_2$CH$_2$CH$_3$), 1.86-1.38 (m, 13H, CH$_2$C*H*$_2$C*H*$_2$CH$_3$ + cyclohexyl), 0.98 (t, 3H, *J* = 7.1 Hz, CH$_2$CH$_2$CH$_2$C*H*$_3$), 0.96 (d, 3H, *J* = 6.6 Hz, CH-C*H*$_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ

(ppm): 14.12 (C4″), 21.72 (C7‴), 22.98 (C3″), 29.50 (C1″), 29.85 (C4‴), 30.42 (C3‴, C5‴), 31.03 (C2″), 33.11 (C2‴, C6‴), 45.47 (C1‴), 62.50 (O*C*H$_3$), 127.19 (C2′, C6′), 127.46 (C3), 127.64 (C5), 128.04 (C2), 128.88 (C3′, C5′), 131.76 (C4′), 136.80 (C6), 137.73 (C1), 140.29 (C1′), 155.71 (C4), 164.90 (*C*=O). ESI-HRMS *m/z*: 380.2579; calc. for $C_{25}H_{34}O_2N$: 380.2584 ($M + H^+$).

*4.1.3.3. 5-butyl-N-cycloheptyl-4'-fluoro-4-methoxy-[1,1'-biphenyl]-3-carboxamide (**1d**)*

Prepared from carboxylic derivative **5** (43.4 mg, 0.15 mmol) using cycloheptylamine. Purification by flash column chromatography (*n*-hexane/EtOAc 8:2). **1d** (26.8 mg, 0.0675 mmol). Yield: 45%. ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 8.04 (d, 1H, *J* = 2.4 Hz, H2), 7.68 (bd, 1H, exchangeable, N*H*), 7.58-7.49 (m, 2H, H2′, H6′), 7.46 (d, 1H, *J* = 2.4 Hz, H6), 7.17-7.04 (m, 2H, H3′, H5′), 4.25-4.17 (m, 1H, NH-C*H*), 3.78 (s, 3H, OC*H*$_3$), 2.70 (t, 2H, *J* = 7.3 Hz, C*H*$_2$CH$_2$CH$_2$CH$_3$), 2.08-2.01 (m, 2H, CH$_2$C*H*$_2$CH$_2$CH$_3$), 1.73-1.34 (m, 14H, CH$_2$CH$_2$C*H*$_2$CH$_3$ + cycloheptyl), 0.97 (t, 3H, *J* = 7.2 Hz, CH$_2$CH$_2$CH$_2$C*H*$_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 14.03 (C4″), 22.88 (C3″), 24.25 (C3‴, C6‴), 28.24 (C4‴, C5‴), 29.50 (C1″), 33.08 (C2″), 35.19 (C2‴, C7‴), 50.50 (C1‴), 62.27 (O*C*H$_3$), 115.65 (d, *J* = 21.4 Hz, C3′, C5′), 127.76 (C2), 127.81(C5),, 128.65 (d, *J* = 8.0 Hz, C2′, C6′), 131.43 (C6), 136.33 (d, *J* = 3.1 Hz, C1′), 136.62 (C3), 136.87 (C1), 155.54 (C4), 162.54 (d, *J* = 246.4 Hz, C4′), 164.41 (*C*=O). ESI-HRMS *m/z*: 398.2478; calc. for $C_{25}H_{33}O_2NF$: 398.2490 ($M + H^+$).

*4.1.3.4. 5-butyl-4'-fluoro-4-methoxy-N-(trans-4-methylcyclohexyl)-[1,1'-biphenyl]-3-carboxamide (**1e**) and 5-butyl-4'-fluoro-4-methoxy-N-(cis-4-methylcyclohexyl)-[1,1'-biphenyl]-3-carboxamide (**1f**)*

Prepared from carboxylic derivative **5** (43.4 mg, 0.15 mmol) using 4-methylcyclohexylamine (*cis/trans* mixture). Purification by flash column chromatography (*n*-hexane/EtOAc 8:2)

allowed the separation of *trans* (**1e**) and *cis* (**1f**). **1e** (7.75 mg, 0.0195 mmol). Yield: 13%. ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 8.02 (d, 1H, *J* = 2.4 Hz, H2), 7.58-7.44 (m, 3H, H2′, H6′, N*H*), 7.45 (d, 1H, *J* = 2.4 Hz, H6), 7.16-7.05 (m, 2H, H3′, H5′), 4.05-3.87 (m, 1H, CH-N*H*), 3.78 (s, 3H, OC*H*$_3$), 2.69 (t, 2H, *J* = 7.5 Hz, C*H*$_2$CH$_2$CH$_2$CH$_3$), 2.13-2.04 (m, 2H, CH$_2$C*H*$_2$CH$_2$CH$_3$), 1.79-1.15 (m, 11H, CH$_2$CH$_2$C*H*$_2$CH$_3$ + cyclohexyl), 0.96 (t, 3H, *J* = 7.3 Hz, CH$_2$CH$_2$CH$_2$C*H*$_3$), 0.92 (d, 3H, *J* = 6.2 Hz, CH-C*H*$_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 14.10 (C4″), 22.35 (C7‴), 22.94 (C3″), 29.60 (C1″), 32.21 (C4‴), 33.16 (C2″), 33.34 (C3‴, C5‴), 34.05 (C2‴, C6‴), 48.68 (C1‴), 62.29 (OC*H*$_3$), 115.75 (d, *J* = 21.3 Hz, C3′, C5′), 127.88 (C2), 127.90 (C5), 128.75 (d, *J* = 8.0 Hz C2′, C6′), 131.53 (C6), 136.43 (d, *J* = 3.5 Hz, C1′) 136.74 (C3), 136.94 (C1), 155.59 (C4), 162.65 (d, *J* = 246.4 Hz, C4′), 164.90 (*C*=O). ESI-HRMS *m/z*: 398.2477; calc. for $C_{25}H_{33}O_2NF$: 398.2490 (M + H^+). **1f** (14.3 mg, 0.036 mmol). Yield: 24%. ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 8.07 (d, 1H, *J* = 2.4 Hz, H2), 7.96 (bd, 1H, exchangeable, N*H*), 7.58-7.51 (m, 2H, H2′, H6′), 7.47 (d, 1H, *J* = 2.4 Hz, H6), 7.16-7.06 (m, 2H, H3′, H5′), 4.38-4.27 (m, 1H, C*H*-NH), 3.81 (s, 3H, OC*H*$_3$), 2.71 (t, 2H, *J* = 7.5 Hz, C*H*$_2$CH$_2$CH$_2$CH$_3$), 1.85-1.72 (m, 13H, CH$_2$C*H*$_2$C*H*$_2$CH$_3$ + cyclohexyl), 0.98 (t, 3H, *J* = 7.5 Hz, CH$_2$CH$_2$CH$_2$C*H*$_3$), 0.96 (d, 3H, *J* = 6.6 Hz, CH-C*H*$_3$).^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 14.11 (C4″), 22.35 (C7‴), 22.98 (C3″), 29.60 (C1″), 32.20 (C4‴), 33.17 (C2″), 33.33 (C3‴, C5‴), 34.03 (C2‴, C6‴), 48.66 (C1‴), 62.51 (OCH_3), 115.75 (d, *J* = 21.9 Hz C3′, C5′), 127.67 (C2), 127.93 (C5), 128.75 (d, *J* = 8.0 Hz C2′, C6′), 131.58 (C6), 136.41 (d, *J* = 3.5 Hz C1′), 136.76 (C3), 136.94 (C1), 155.69 (C4), 162.64 (d, *J* = 246.4 Hz, C4′), 164.77 (*C*=O). ESI-HRMS *m/z*: 398.2478; calc. for $C_{25}H_{33}O_2NF$: 398.2490 (M + H^+).

*4.1.3.5. N-cycloheptyl-5-(4-fluorobenzyl)-4-methoxy-[1,1'-biphenyl]-3-carboxamide (**1g**)*

Prepared from carboxylic derivative **12a** (50.4 mg, 0.15 mmol) using cycloheptylamine. Purification by flash column chromatography (*n*-hexane/EtOAc 8:2). **1g** (40.1 mg, 0.093 mmol). Yield: 62%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.12 (d, 1H, *J* = 2.5 Hz, H2), 7.56-7.51 (m, 2H, H2′, H6′), 7.51 (bs, 1H, N*H*), 7.43-7.37 (m, 3H, H3′, H4′, H5′), 7.34-7.30 (m, 1H, H6), 7.20-7.15 (m, 2H, H2″, H6″), 7.02-6.95 (m, 2H, H3″, H5″), 4.25-4.18 (m, 1H, NHC*H*), 4.06 (s, 2H, benzylic CH_2), 3.70 (s, 3H, OCH_3), 2.09-2.01 (m, 2H, cycloheptyl), 1.75-1.50 (m, 10H, cycloheptyl). ^{13}C NMR (100 MHz, CDCl3) δ (ppm): 24.23 (C3‴, C6‴), 28.19 (C4‴, C5‴), 35.04 (benzylic), 35.16 (C2‴, C7‴), 50.58 (C1‴), 62.28 (O*C*H_3), 115.41 (d, *J* = 21.3 Hz, C3″, C5″), 127.03 (C2′, C6′), 127.51 (C2), 128.13 (C5), 128.70 (C4′), 128.84 (C3′, C5′), 130.28 (d, *J* = 8.1 Hz, C2″, C6″), 132.23 (C6), 134.84 (C1), 135.99 (d, *J* = 3.7 Hz, C1″), 137.79 (C3), 139.81 (C1′), 155.49 (C4), 161.56 (d, *J* = 244.8 Hz, C4″), 164.34 (*C*=O). ESI-HRMS *m/z*: 432.2319; calc. for $C_{28}H_{31}O_2NF$: 432.2333 ($M + H^+$).

*4.1.3.6. 5-(4-fluorobenzyl)-4-methoxy-N-(trans-4-methylcyclohexyl)-[1,1'-biphenyl]-3-carboxamide (**1h**)*

Prepared from carboxylic acid **12a** (50.4 mg, 0.15 mmol) using *trans*-4-methylcyclohexylamine. Purification by flash column chromatography (*n*-hexane/EtOAc 8:2). **1h** (20.7 mg, 0.048 mmol). Yield: 32%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.11 (d, 1H, *J* = 2.5 Hz, H2), 7.55-7.52 (m, 2H, H2′, H6′), 7.43-7.38 (m, 3H, H3′, H4′, H5′), 7.35-7.30 (m, 2H, H6 + N*H*), 7.20-7.14 (m, 2H, H2″, H6″), 7.01-6.95 (m, 2H, H3″, H5″), 4.05 (s, 2H, benzylic CH_2), 4.00-3.93 (m, 1H, NHC*H*), 3.70 (s, 3H, OCH_3), 2.15-2.05 (m, 2H, cyclohexyl), 1.80-1.65 (m, 2H, cyclohexyl), 1.40-1.18 (m, 5H, cyclohexyl), 0.92 (d, 3H, *J* = 6.4 Hz, CH-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 22.33 (C7‴), 32.17 (C3‴, C5‴) , 33.29 (C4‴), 34.01 (C2‴, C6‴), 35.16 (benzylic), 48.70 (C1‴), 62.29 (O*C*H_3), 115.50 (d, *J* = 21.3 Hz, C3″, C5″), 127.13 (C2′, C6′), 127.59 (C2), 128.23 (C5), 128.79 (C4′), 128.91 (C3′,

C5′), 130.32 (d, *J* = 7.9 Hz, C2″, C6″), 132.33 (C6), 134.88 (C1), 136.07 (d, *J* = 3.7 Hz, C1″), 137.90 (C3), 139.90 (C1′), 155.53 (C4), 161.64 (d, *J* = 244.5 Hz, C4″), 164.80 (*C*=O). ESI-HRMS *m/z*: 432.2320; calc. for $C_{28}H_{31}O_2NF$: 432.2333 ($M + H^+$).

*4.1.3.7. 5-(4-fluorobenzyl)-4-methoxy-N-(cis-4-methylcyclohexyl)-[1,1'-biphenyl]-3-carboxamide (**1i**)*

Prepared from carboxylic derivative **12a** (50.4 mg, 0.15 mmol) using 4-methylcyclohexylamine (*cis/trans* mixture). Purification by flash column chromatography (*n*-hexane/EtOAc 8:2) allowed the separation of the two isomers affording the *cis* (**1i**) pure (4.5 mg, 0.0105 mmol, yield: 7%) and *trans* (**1h**) pure (7.1 mg, 0.0165 mmol, yield: 11%). **1i**: ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.17 (d, 1H, *J* = 2.5 Hz, H2), 7.82 (bs, 1H, N*H*), 7.56-7.52 (m, 2H, H2′, H6′), 7.44-7.38 (m, 3H, H3′, H4′, H5′), 7.35-7.29 (m, 1H, H6), 7.22-7.16 (m, 2H, H2″, H6″), 7.03-6.96 (m, 2H, H3″, H5″), 4.35-4.28 (m, 1H, NHC*H*), 4.09 (s, 2H, benzylic CH_2), 3.73 (s, 3H, OCH_3), 1.88-1.75 (m, 2H, cyclohexyl), 1.75-1.61 (m, 5H, cyclohexyl), 1.31-1.17 (m, 2H, cyclohexyl), 0.96 (d, 3H, *J* = 6.4 Hz, CH-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 21.63 (C7‴), 29.78 (C3‴, C5‴), 30.41 (C4‴), 30.93 (C2‴, C6‴), 35.03 (benzylic), 45.60 (C1‴), 62.54 (O*C*H_3), 115.54 (d, *J* = 21.3 Hz C3″, C5″), 127.15 (C2′, C6′), 127.61 (C2), 128.10 (C5), 128.84 (C4′), 128.92 (C3′, C5′), 130.42 (d, *J* = 8.1 Hz C2″, C6″), 132.41 (C6), 134.92 (C1), 136.03 (d, *J* = 2.9 Hz C1″), 137.94 (C3), 139.93 (C1′), 155.65 (C4), 161.69 (d, *J* = 244.4 Hz, C4″), 164.71 (*C*=O). ESI-HRMS *m/z*: 432.2322; calc. for $C_{28}H_{31}O_2NF$: 432.2333 ($M + H^+$).

*4.1.3.8. N-cycloheptyl-5-(4-fluorobenzyl)-4,4'-dimethoxy-[1,1'-biphenyl]-3-carboxamide (**1j**)*

Prepared from carboxylic derivative **12b** (54.9 mg, 0.15 mmol) using cycloheptylamine. Purification by flash column chromatography (*n*-hexane/EtOAc 9:1). **1j** (15.9 mg, 0.0345 mmol). Yield: 23%. ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 8.09 (d, 1H, *J* = 2.4 Hz, H2), 7.54 (bd, 1H, N*H*), 7.47 (AA′XX′, 2H, J_{AX} = 9.0 Hz, $J_{AA'XX'}$ = 2.6 Hz, H2′, H6′), 7.38 (d, 1H, *J* = 2.6 Hz, H6), 7.21-7.12 (m, 2H, H2″, H6″), 7.04-6.94 (m, 2H, H3″, H5″), 6.94 (AA′XX′, 2H, J_{AX} = 9.0 Hz, $J_{AA'XX'}$ = 2.6 Hz, H3′, H5′), 4.31-4.16 (m, 1H, NHC*H*), 4.05 (s, 2H, benzylic CH_2), 3.83 (s, 3H, OCH_3), 3.69 (s, 3H, OCH_3), 2.11-2.01 (m, 2H, cycloheptyl), 1.69-1.54 (m, 10H, cycloheptyl). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 24.33 (C3‴, C6‴), 28.29 (C4‴, C5‴), 35.13 (benzylic), 35.26 (C2‴, C7‴), 50.63 (C1‴), 55.48 (O*C*H$_3$), 62.35 (O*C*H$_3$), 114.38 (C3′, C5′), 115.49 (d, *J* = 21.3 Hz, C3″, C5″), 128.13 (C2), 128.16 (C2′, C6′), 128.30 (C5), 130.35 (d, *J* = 8.0 Hz C2″, C6″), 131.86 (C6), 132.44 (C1), 134.78 (C1′), 136.13 (d, *J* = 2.8 Hz C1″), 137.52 (C3), 155.09 (C4), 159.45 (C4′), 161.64 (d, *J* = 244.3 Hz, C4″), 164.48 (*C*=O). ESI-HRMS *m/z* : 462.2424; calc. for $C_{29}H_{33}O_3NF$: 462.2439 ($M + H^+$).

*4.1.3.9. 5-(4-fluorobenzyl)-4,4'-dimethoxy-N-(trans-4-methylcyclohexyl)-[1,1'-biphenyl]-3-carboxamide (**1k**) and 5-(4-fluorobenzyl)-4,4'-dimethoxy-N-(cis-4-methylcyclohexyl)-[1,1'-biphenyl]-3-carboxamide (**1l**)*

Prepared from carboxylic derivative **12b** (54.9 mg, 0.15 mmol) using 4-methylcyclohexylamine (*cis/trans* mixture). Purification by flash column chromatography (*n*-hexane/EtOAc 8:2) allowed the separation of *trans* (**1k**) and *cis* (**1l**). **1k** (2.8 mg, 0.006 mmol). Yield: 4%. ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 8.07 (d, 1H, *J* = 2.4 Hz, H2), 7.47 (AA′XX′, 2H, J_{AX} = 8.6 Hz, $J_{AA'XX'}$ = 2.6 Hz, H2′, H6′), 7.38 (d, 1H, *J* = 2.6 Hz, H6), 7.22-7.13 (m, 2H, H2″, H6″), 7.03-6.90 (m, 4H, H3′, H5′, H3″, H5″), 4.11-3.94 (m, 1H, NHC*H*), 4.04 (s, 2H, benzylic CH_2), 3.83 (s, 3H, OCH_3), 3.68 (s, 3H, OCH_3), 2.16-2.04 (m, 2H, cyclohexyl), 1.81-1.03 (m, 7H, cyclohexyl), 0.92 (d, 3H, *J* = 6.2 Hz, CH-CH_3). ^{13}C NMR

(100 MHz, $CDCl_3$) δ (ppm): 21.85 (C7‴), 31.19 (C3‴, C5‴), 32.31 (C4‴), 33.03 (C2‴, C6‴), 34.18 (benzylic), 47.71 (C1‴), 54.50 (O*C*H_3), 61.29 (O*C*H_3), 113.39 (C3′, C5′), 114.50 (d, *J* = 21.3 Hz, C3″, C5″), 127.13 (C1), 127.18 (C2′, C6′), 129.36 (d, *J* = 8.0 Hz, C2″, C6″), 130.90 (C2), 131.45 (C5), 133.79 (C6), 133.97 (C1′), 135.15 (d, *J* = 3.6 Hz, C1″), 136.55 (C3), 154.07 (C4), 158.45 (C4′), 160.64 (d, *J* = 242.3 Hz, C4″), 163.90 (*C*=O). ESI-HRMS *m/z*: 462.2428; calc. for $C_{29}H_{33}O_3NF$: 462.2439 ($M + H^+$). **1l** (2.8 mg, 0.006 mmol). Yield: 4%. ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 8.11 (d, 1H, *J* = 2.4 Hz, H2), 7.83 (bd, 1H, exchangeable, N*H*), 7.47 (AA′XX′, 2H, J_{AX} =8.8 Hz, $J_{AA'XX'}$ = 2.4 Hz, H2′, H6′), 7.37 (d, 1H, *J* = 2.4 Hz, H6), 7.22-7.15 (m, 2H, H2″, H6″), 7.02-6.91 (m, 4H, H3′, H5′, H3″, H5″), 4.39-4.22 (m, 1H, NHC*H*), 4.07 (s, 2H, benzylic CH_2), 3.83 (s, 3H, OCH_3), 3.71 (s, 3H, OCH_3), 1.85-1.62 (m, 7H, cyclohexyl), 1.34-1.12 (m, 2H, cyclohexyl), 0.95 (d, 3H, *J* = 6.4 Hz, CH-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 22.50 (C7‴), 29.93 (C3‴, C5‴), 32.33 (C4‴), 33.45 (C2‴, C6‴), 35.32 (benzylic), 48.85 (C1‴), 55.64 (O*C*H_3), 62.43 (O*C*H_3), 114.53 (C3′, C5′), 115.66 (d, *J* = 21.3 Hz C3″, C5″), 128.27 (C2′, C6′), 128.32 (C2), 128.45 (C1), 130.47 (d, *J* = 8.0 Hz C2″, C6″), 132.04 (C5), 132.59 (C6), 134.93 (C1′), 136.29 (d, *J* = 3.6 Hz C1″), 137.68 (C3), 155.21 (C4), 159.59 (C4′), 161.78 (d, *J* = 242.3 Hz, C4″), 165.04 (*C*=O). ESI-HRMS *m/z*: 462.2427; calc. for $C_{29}H_{33}O_3NF$: 462.2439 ($M + H^+$).

*4.1.3.10. N-cycloheptyl-4'-fluoro-5-(4-fluorobenzyl)-4-methoxy-[1,1'-biphenyl]-3-carboxamide (**1m**)*

Prepared from carboxylic derivative **12c** (53.1 mg, 0.15 mmol) using cycloheptylamine. Purification by flash column chromatography (*n*-hexane/EtOAc 8:2). **1m** (35.7 mg, 0.0795 mmol). Yield: 53%. ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 8.07 (d, 1H, *J* = 2.4 Hz, H2), 7.58-7.43 (m, 3H, H2′, H6′ + N*H*), 7.36 (d, 1H, *J* = 2.4 Hz, H6), 7.21-6.93 (m, 6H, H3′, H5′, H2″, H3″, H5″, H6″), 4.31-4.14 (m, 1H, NHC*H*), 4.05 (s, 2H, benzylic CH_2), 3.69 (s, 3H,

OC*H*$_3$), 1.78-1.47 (m, 12H, cycloheptyl). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 24.27 (C3‴, C6‴), 28.23 (C4‴, C5‴), 35.06 (benzylic), 35.20 (C2‴, C7‴), 50.63 (C1‴), 62.33 (O*C*H$_3$), 115.49 (d, *J* = 21.3 Hz, C3″, C5″), 115.75 (d, *J* = 21.3 Hz, C3′, C5′), 128.23 (C2), 128.65 (C5), 128.67 (d, *J* = 8.0 Hz, C2′, C6′), 130.31 (d, *J* = 8.0 Hz, C2″, C6″), 132.08 (C6), 135.01 (C1), 135.96 (d, *J* = 4.0 Hz, C1″), 136.00 (d, *J* = 4.0 Hz, C1′), 136.88 (C3), 155.52 (C4), 161.62 (d, *J* = 245.0 Hz, C4″), 162.63 (d, *J* = 247.0 Hz, C4′), 164.24 (*C*=O). ESI-HRMS *m/z*: 450.2223; calc. for $C_{28}H_{30}O_2NF_2$: 450.2239 ($M + H^+$).

*4.1.3.11. 4'-fluoro-5-(4-fluorobenzyl)-4-methoxy-N-(trans-4-methylcyclohexyl)-[1,1'-biphenyl]-3-carboxamide (**1n**) and 4'-fluoro-5-(4-fluorobenzyl)-4-methoxy-N-(cis-4-methylcyclohexyl)-[1,1'-biphenyl]-3-carboxamide (**1o**)*

Prepared from carboxylic derivative **12c** (53.1 mg, 0.15 mmol) using 4-methylcyclohexylamine (*cis/trans* mixture). Purification by flash column chromatography (*n*-hexane/EtOAc 8:2) allowed the separation of *trans* (**1n**) and *cis* (**1o**). **1n** (15.5 mg, 0.0345 mmol). Yield: 23%. ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 8.05 (d, 1H, *J* = 2.4 Hz, H2), 7.52-7.41 (m, 2H, H2′, H6′), 7.36 (d, 1H, J = 2.4 Hz, H6), 7.35 (bd, 1H, N*H*), 7.21-6.92 (m, 6H, H3′, H5′, H2″, H3″, H5″, H6″), 4.11-3.85 (m, 1H, NHC*H*), 4.04 (s, 1H, benzylic C*H*$_2$), 3.69 (s, 3H, OC*H*$_3$), 2.12-2.00 (m, 2H, cyclohexyl), 1.80-1.65 (m, 2H, cyclohexyl), 1.40-1.05 (m, 5H, cyclohexyl), 0.92 (d, 1H, *J* = 6.2 Hz, CH-C*H*$_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 22.31 (C7‴), 32.15 (C4‴), 33.26 (C3‴, C5‴), 33.99 (C2‴, C6‴), 35.13 (benzylic), 48.72 (C1‴), 62.28 (O*C*H$_3$), 115.51 (d, *J* = 21.3 Hz, C3″, C5″), 115.78 (d, *J* = 21.5 Hz C3′, C5′), 128.28 (C2), 128.66 (C5), 128.70 (d, *J* = 8.1 Hz, C2′, C6′), 130.30 (d, *J* = 8.1 Hz C2″, C6″), 132.12 (C6), 135.02 (C1), 135.98 (d, *J* = 2.9 Hz, C1″), 136.04 (d, *J* = 2.9 Hz, C1′), 136.91 (C3), 155.51 (C4), 162.13 (d, *J* = 246.8 Hz, C4″), 162.67 (d, *J* = 246.5 Hz, C4′), 164.68 (*C*=O). ESI-HRMS *m/z*: 450.2227; calc. for $C_{28}H_{30}O_2NF_2$: 450.2239 ($M + H^+$). **1o** (21.6 mg,

0.048 mmol). Yield: 32%. ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 8.10 (d, 1H, *J* = 2.4 Hz, H2), 7.81 (bd, 1H exchangeable, N*H*), 7.52-7.44 (m, 2H, H2′, H6′), 7.36 (d, 1H, *J* = 2.4 Hz, H6), 7.24-6.94 (m, 6H, H3′, H5′, H2″, H3″, H5″, H6″), 4.39-4.20 (m, 1H, NHC*H*), 4.07 (s, 2H, benzylic CH_2), 3.72 (s, 3H, OCH_3), 1.90-1.51 (m, 7H, cyclohexyl), 1.35-1.10 (m, 2H, cyclohexyl), 0.96 (d, 3H, *J* = 6.4 Hz, CH-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 21.62 (C7‴), 29.76 (C3‴, C5‴), 30.40 (C2‴, C6‴), 30.90 (C4‴), 34.99 (benzylic), 45.62 (C1‴), 62.55 (O*C*H_3), 115.56 (d, *J* = 21.3 Hz C3″, C5″), 115.80 (d, *J* = 21.8 Hz C3′, C5′), 128.12 (C2), 128.71 (C5), 128.72 (d, *J* = 8.1 Hz C2′, C6′), 130.40 (d, *J* = 7.3 Hz C2″, C6″), 132.20 (C6), 135.05 (C1), 135.94 (d, *J* = 2.9 Hz C1″), 136.04 (d, *J* = 2.9 Hz C1′), 136.96 (C3), 155.63 (C4), 161.69 (d, *J* = 244.8 Hz, C4″), 162.70 (d, *J* = 247.2 Hz, C4′), 164.58 (*C*=O). ESI-HRMS *m/z*: 450.2231; calc. for $C_{28}H_{30}O_2NF_2$: 450.2239 ($M + H^+$).

*4.1.3.12. 5-benzyl-N-cycloheptyl-4-methoxy-[1,1'-biphenyl]-3-carboxamide (**1p**)*

Prepared from carboxylic derivative **12d** (47.7 mg, 0.15 mmol) using cycloheptylamine. Purification by flash column chromatography (*n*-hexane/EtOAc 8:2). **1p** (38.5 mg, 0.093 mmol). Yield: 62%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.12 (d, 1H, *J* = 2.5 Hz, H2), 7.58-7.51 (m, 3H, H2′, H6′ + N*H*), 7.46 (d, 1H, *J* = 2.5 Hz, H6), 7.44-7.37 (m, 2H, H3′, H5′), 7.34-7.27 (m, 3H, H4′, H3″, H5″), 7.25-7.19 (m, 3H, H2″, H4″, H6″), 4.30-4.15 (m, 1H, NHC*H*), 4.10 (s, 2H, benzylic CH_2), 3.70 (s, 3H, OCH_3), 2.08-1.97 (m, 2H, cycloheptyl), 1.75-1.52 (m, 10H, cycloheptyl). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 24.34 (C3‴, C6‴), 28.30 (C4‴, C5‴), 35.29 (C2‴, C7‴), 35.90 (benzylic), 50.64 (C1‴), 62.39 (O*C*H_3), 127.16 (C2′, C6′), 127.54 (C2), 128.14 (C5), 128.71 (C4″) 128.73 (C3″, C5″), 128.90 (C2″, C6″), 128.94 (C4′), 128.99 (C3′, C5′), 132.55 (C6), 135.04 (C1), 137.82 (C3), 140.02 (C1″), 140.43 (C1′), 155.68 (C4), 164.49 (*C*=O). ESI-HRMS *m/z*: 414.2423; calc. for $C_{28}H_{32}O_2N$: 414.2428 ($M + H^+$).

*4.1.3.13. 5-benzyl-4-methoxy-N-(trans-4-methylcyclohexyl)-[1,1'-biphenyl]-3-carboxamide (**1q**)*

Prepared from carboxylic acid **12d** (47.7 mg, 0.15 mmol) using *trans*-4-methylcyclohexylamine. Purification by flash column chromatography (*n*-hexane/EtOAc 8:2). **1q** (19.8 mg, 0.048 mmol). Yield: 32%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.12 (d, 1H, *J* = 2.4 Hz, H2), 7.57-7.52 (m, 2H, H2′, H6′), 7.46 (d, 1H, *J* = 2.8 Hz, H6), 7.42-7.36 (m, 2H, H3′, H5′), 7.34-7.28 (m, 3H, H4′, H2″, H6″), 7.25-7.19 (m, 3H, H3″, H4″, H5″), 4.10 (s, 2H, benzylic C*H*$_2$), 4.03-3.94 (m, 1H, NHC*H*), 3.69 (s, 3H, OC*H*$_3$), 2.11-2.07 (m, 2H, cyclohexyl), 1.78-1.74 (m, 2H, cyclohexl), 1.42-1.18 (m, 5H, cyclohexyl), 0.92 (d, 3H, *J* = 6.8 Hz, CH-C*H*$_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 22.30 (C7‴), 32.14 (C4‴), 33.26 (C3‴, C5‴), 34.00 (C2‴, C6‴), 35.90 (benzylic), 48.66 (C1‴), 62.25 (O*C*H$_3$), 127.11 (C2′, C6′), 127.48 (C2), 128.11 (C5), 128.64 (C4′, C4″), 128.67 (C3″, C5″), 128.84 (C3′, C5′), 128.91 (C2″, C6″), 132.49 (C6), 134.99 (C1), 137.75 (C3), 139.97 (C1″), 140.39 (C1′), 155.61 (C4), 164.84 (*C*=O). ESI-HRMS *m/z*: 414.2416; calc. for $C_{28}H_{32}O_2N$: 414.2428 ($M + H^+$).

*4.1.3.14. 5-benzyl-4-methoxy-N-(cis-4-methylcyclohexyl)-[1,1'-biphenyl]-3-carboxamide (**1r**)*

Prepared from carboxylic derivative **12d** (47.7 mg, 0.15 mmol) using 4-methylcyclohexylamine (*cis/trans* mixture). Purification by flash column chromatography (*n*-hexane/EtOAc 8:2) allowed the separation of the two isomers affording the *cis* (**1r**) pure (3.7 mg, 0.009 mmol, yield: 6%) and the *trans* (**1q**) pure (1.23 mg, 0.003 mmol, yield: 2%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.16 (d, 1H, *J* = 2.5 Hz, H2), 7.85 (bd, 1H, N*H*), 7.56-7.53 (m, 2H, H2′, H6′), 7.46 (d, 1H, *J* = 2.5 Hz, H6), 7.43-7.36 (m, 2H, H3′, H5′), 7.34-7.27 (m,

3H, H4′, H2″, H6″), 7.25-7.19 (m, 3H, H3″, H4″, H5″), 4.35-4.27 (m, 1H, NHC*H*), 4.12 (s, 2H, benzylic C*H*$_2$), 3.73 (s, 3H, OC*H*$_3$), 1.84-1.79 (m, 2H, cyclohexyl), 1.74-1.63 (m, 5H, cyclohexyl), 1.35-1.25 (m, 2H, cyclohexyl), 0.96 (d, 3H, J = 6.4 Hz, CH-C*H*$_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 21.63 (C7‴), 29.78 (C3‴, C5‴), 30.40 (C2‴, C6‴), 31.00 (C4‴), 35.77 (benzylic), 45.56 (C1‴), 62.55 (O*C*H$_3$), 126.47 (C3′, C5′), 127.16 (C2′, C6′), 127.53 (C2), 127.97 (C5), 128.70 (C4′), 128.73 (C3″, C5″), 128.88 (C4″), 129.03 (C2″, C6″), 132.62 (C6), 135.04 (C1), 137.82 (C3), 140.01 (C1″), 140.38 (C1′), 155.75 (C4), 164.77 (*C*=O). ESI-HRMS *m/z*: 414.2421; calc. for $C_{28}H_{32}O_2N$: 414.2428 ($M + H^+$).

4.1.4. 2-methoxy-3-(methoxycarbonyl)phenylboronic acid (7)

To a solution of methyl-3-bromo-2-methoxylbenzoate **6** (1.5 g, 6.12 mmol) in 13 mL of anhydrous 1,4-dioxane were subsequently added bis(pinacolate)diboron (2.3 g, 9.18 mmol), potassium acetate (1.5 g, 15.3 mmol), followed by bis(diphenylphospinoferrocene)palladium dichloride (134.6 mg, 0.184 mmol). The resulting mixture was heated to 110 °C in an oil bath for 2 hours or to 130 °C in a microwave reactor for 30 min (5 bar, 200 W). Afterwards, the solvent was removed under reduced pressure to afford the crude pinacol ester that was used in the following step without further purification. Then, ammonium acetate (1.4 g, 18.36 mmol) and sodium periodate (3.9 g, 18.36 mmol) were added to a solution of the crude pinacol ester in a mixed solution of acetone (11.0 mL) and water (11.0 mL). The resulting mixture was stirred at r. t. until complete conversion of the starting material was observed by TLC. The precipitate was then filtered off, and the filtrate was concentrated under reduce pressure. The residue was extracted with ethyl acetate, and the combined organic extracts were dried over anhydrous Na_2SO_4, filtered and evaporated. The crude product was finally purified by silica gel column chromatography (*n*-hexane/ethyl acetate 6:4) to give 2-methoxy-3-(methoxycarbonyl)phenylboronic acid **7** (938.6 mg, 4.47 mmol). Yield: 73%. ^{1}H

NMR (400 MHz, $CDCl_3$) δ (ppm): 8.04 (dd, 1H, *J* = 7.4 Hz, *J* = 1.9 Hz, H4), 7.96 (dd, 1H, *J* = 7.8 Hz, *J* = 1.9 Hz, H6), 7.24 (t, 1H, *J* = 7.5 Hz, H5), 6.15 (bs, 2H, $B(OH)_2$), 3.94 (s, 3H, OCH_3), 3.92 (s, 3H, $COOCH_3$).

*4.1.5. General procedure for the synthesis of derivatives **8a-b**.*

A dried sealed-tube was charged, under nitrogen flux, with the proper benzyl bromide (0.95 mmol), sodium carbonate (6.45 mmol), the boronic acid **7** (0.95 mmol), tetrakis(triphenylphosphine)palladium (0.06 mmol), anhydrous 1,2-dimethoxyethane (12.3 mL) and water (6.1 mL). The tube was sealed and heated at 100 °C in an oil bath for 4 h or at 140 °C in a microwave reactor for 15 min (5 bar, 200 W). After cooling, the resulting mixture was diluted in water, and the aqueous layer was extracted with ethyl acetate. Then the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered, evaporated and purified.

*4.1.5.1. Methyl 3-(4-fluorobenzyl)-2-methoxybenzoate (**8a**)*

A dried sealed-tube was charged, under nitrogen flux, with 4-fluorobenzyl bromide (179.6 mg, 0.95 mmol), sodium carbonate (683.7 mg, 6.45 mmol), the boronic acid **7** (199.5 mg, 0.95 mmol), tetrakis(triphenylphosphine)palladium (69.3 mg, 0.06 mmol), anhydrous 1,2-dimethoxyethane (12.3 mL) and water (6.1 mL). The tube was sealed and heated at 100 °C in an oil bath for 4 h. After cooling, the resulting mixture was diluted in water, and the aqueous layer was extracted with ethyl acetate. Then the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and evaporated in vacuo. Purification by flash column chromatography (*n*-hexane/EtOAc 9:1) afforded pure **8a** (164.1 mg, 0.6 mmol). Yield: 63%. ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 7.70 (dd, 1H, *J* = 7.7 Hz, *J* = 1.8 Hz, H6), 7.27 (dd, 1H, *J* = 7.5 Hz, *J* = 1.8 Hz, H4), 7.19-7.08 (m, 2H, H2′, H6′), 7.08 (t, 1H, *J* = 7.5 Hz, H5),

7.03-6.88 (m, 2H, H3′, H5′), 4.01 (s, 2H, benzylic C*H*$_2$), 3.92 (s, 3H, OC*H*$_3$), 3.73 (s, 3H, COOC*H*$_3$).

*4.1.5.2. Methyl 3-benzyl-2-methoxybenzoate (**8b**)*

Prepared from boronic acid **7** (199.5 mg, 0.95 mmol) using benzyl bromide. Purification by flash column chromatography (*n*-hexane/EtOAc 9:1). **8b** (160.7 mg, 0.627 mmol). Yield: 66%. ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 7.71 (dd, 1H, *J* = 7.7 Hz, *J* = 1.8 Hz, H6), 7.36-7.16 (m, 6H, H4, H2′, H3′, H4′, H5′, H6′), 7.09 (t, 1H, *J* = 7.6 Hz, H5), 4.07 (s, 2H, benzylic C*H*$_2$), 3.94 (s, 3H, OC*H*$_3$), 3.75 (s, 3H, COOC*H*$_3$).

*4.1.6. General procedure for the synthesis of derivatives **9a-b** and **10a-b**.*

Compound **8a** or **8b** (0.46 mmol) was dissolved in 0.8 mL of chloroform. Then a solution of bromine (0.46 mmol) in chloroform (0.5 mL) was added at room temperature, and the resulting mixture was left under stirring overnight. After washing once with a saturated sodium thiosulphate aqueous solution, the organic layer was dried over anhydrous Na_2SO_4, filtered, evaporated under vacuum and purified.

*4.1.6.1. Methyl 5-bromo-3-(4-fluorobenzyl)-2-methoxybenzoate (**9a**) and methyl 5-bromo-3-(4-fluorobenzyl)-2-hydroxybenzoate (**10a**)*

Compound **8a** (126.2 mg, 0.46 mmol) was dissolved in 0.8 mL of chloroform. Then a solution of bromine (0.02 mL, 0.46 mmol) in chloroform (0.5 mL) was added at room temperature, and the resulting mixture was left under stirring overnight. After washing once with a saturated sodium thiosulphate aqueous solution, the organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated under vacuum. Purification by flash column chromatography (*n*-hexane/EtOAc 9:1) allowed the separation of **9a** and of its demethylated

derivative **10a**. **9a** (68.2 mg, 0.193 mmol). Yield: 42%. ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 7.82 (d, 1H, *J* = 2.2 Hz, H6), 7.36 (d, 1H, *J* = 2.6 Hz, H4), 7.18-7.10 (m, 2H, H2′, H6′), 7.02-6.94 (m, 2H, H3′, H5′), 3.96 (s, 2H, benzylic C*H*$_2$), 3.92 (s, 3H, OC*H*$_3$), 3.72 (s, 3H, COOC*H*$_3$). **10a** (37.3 mg, 0.110 mmol). Yield: 24%. ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 11.05 (s, 1H, O*H*), 7.85 (d, 1H, *J* = 2.6 Hz, H6), 7.32 (d, 1H, *J* = 2.6 Hz, H4), 7.22-7.16 (m, 2H, H2′, H6′), 7.02-6.92 (m, 2H, H3′, H5′), 3.95 (s, 2H, benzylic C*H*$_2$), 3.95 (s, 3H, OC*H*$_3$).

*4.1.6.2. Methyl 3-benzyl-5-bromo-2-methoxybenzoate (**9b**) and methyl 3-benzyl-5-bromo-2-hydroxybenzoate (**10b**)*

Prepared from derivative **8b** (117.9 mg, 0.46 mmol). Purification by flash column chromatography (*n*-hexane/EtOAc 9:1) allowed the separation of **9b** and of its demethylated derivative **10b**. **9b** (49.3 mg, 0.147 mmol). Yield: 32%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.81 (d, 1H, *J* = 2.6 Hz, H6), 7.38 (d, 1H, *J* = 2.6 Hz, H4), 7.33-7.27 (m, 2H, H3′, H4′, H5′), 7.24-7.16 (m, 3H, H4′, H2′, H6′), 4.00 (s, 2H, benzylic C*H*$_2$), 3.91 (s, 3H, OC*H*$_3$), 3.72 (s, 3H, COOC*H*$_3$). **10b** (51.7 mg, 0.161 mmol). Yield: 35%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 11.04 (s, 1H, O*H*), 7.84 (d, 1H, *J* = 2.5 Hz, H6), 7.34 (d, 1H, *J* = 2.5 Hz, H4), 7.32-7.27 (m, 2H, H3′, H5′), 7.25-7.18 (m, 3H, H4′, H2′, H6′), 3.98 (s, 2H, benzylic C*H*$_2$), 3.94 (s, 3H, OC*H*$_3$).

*4.1.7. General procedure for the conversion of derivatives **10a-b** into **9a-b**.*

To a solution of compound **10a** (274.7 mg, 0.81 mmol) or **10b** (260.1 mg, 0.81 mmol) in dichloromethane (2.8 mL) was added tetrabutylammonium bromide (26.1 mg, 0.081 mmol), followed by a solution of sodium hydroxide (96.0 mg, 2.4 mmol) in water (1.4 mL) and dimethyl sulphate (201.8 mg, 1.60 mmol). The resulting mixture was left under stirring at room temperature, overnight. Then the reaction was quenched by adding solid ammonium

chloride, and the pH was adjusted to 5-6 by adding a 10% hydrochloric acid aqueous solution. Then the two phases were separated, and the aqueous phase repeatedly extracted with dichloromethane. The combined organic layers were dried over anhydrous Na_2SO_4, filtered and evaporated under vacuum. The crude product was then purified by silica gel column chromatography (*n*-hexan/EtOAc 9:1) affording pure **9a** (251.8 mg, 0.713 mmol, yield: 88%) from **10a** and pure **9b** (114.0 mg, 0.34 mmol, yield: 42%) form **10b**.

4.2 CB_1 and CB_2 receptor binding assays

Receptor binding experiments were performed with membrane preparations as previously reported [32]. Briefly, clean membranes expressing *h*CB_1 or *h*CB_2 were re-suspended in binding buffer (50 mM Tris-HCl, 2.5 mM EDTA, 5 mM $MgCl_2$, 0.5 % fatty acid-free bovine serum albumin (BSA), pH 7.4) and incubated with vehicle or compounds and 0.5 nM of [^{3}H]CP55,940 for 2 h at 30 °C. Non-specific binding was determined in the presence of 10 μM of WIN55,512. After incubation, membranes were filtered through a pre-soaked 96-well microplate bonded with GF/B filters under vacuum and washed twelve times with 150 μL of ice-cold binding buffer. The radioactivity was measured and the results expressed as [^{3}H]CP55,940 binding.

4.3 Functional activity at CB_2 receptor

Assays were performed as previously described [33]. Briefly, *h*CB_2-expressing membranes (5 μg) were diluted in binding buffer (50 mM Tris-HCl, 3 mM $MgCl_2$, 0.2 mM EDTA, and 100 mM NaCl at pH 7.4 plus 0.5% fatty acid-free BSA) in the presence of 10 μM of GDP and 0.1 nM of [^{35}S]GTPγS. The mixture was kept on ice until the binding reaction was started by adding the vehicle or compounds. Non-specific binding was measured in the presence of 10 μM of GTPγS. The tubes were incubated at 30 °C for 90 min. The reaction was stopped by

rapid filtration through a 96-well microplate bonded with GF/B filters previously pre-soaked with washing buffer (50 mM of Tris-HCl pH 7.4 plus 0.1% fatty acid-free BSA). The filters were washed six times with 180 μL of washing buffer under vacuum. The radioactivity was measured, and the results were expressed as [^{35}S]GTPγS binding.

Acknowledgements

We are grateful to the University of Pisa (Progetti di Ricerca di Ateneo, PRA) for funding.

References

[1] C. Howlett, F. Barth, T. I. Bonner, G. Cabral, P. Casellas, W. A. Devane, C. C. Felder, M. Herkenham, K. Mackie, B. R. Martin, R. Mechoulam, R. G. Pertwee, Pharmacol. Rev. 54 (2002) 161-202.

[2] P. Pacher, S. Batkai, G. Kunos, Pharmacol. Rev. 58 (2006) 389-462.

[3] P. Pacher, R. Mechoulam, Prog. Lipid Res. 50 (2011) 193-211.

[4] R. G. Pertwee, Br. J. Pharmacol. 156 (2009) 397-411.

[5] M. D. Van Sickle, M. Duncan, P. J. Kingsley, A. Mouihate, P. Urbani, K. Mackie, N. Stella, A. Makriyannis, D. Piomelli, J. S. Davison, L. J. Marnett, V. Di Marzo, Q. J. Pittman, K. D. Patel, K. A. Sharkey, Science 310 (2005) 329-32.

[6] E. S. Onaivi, H. Ishiguro, S. Gu, Q.-R.Liu, J. Psychopharmacol. (London, United Kingdom) 26 (2012), 92-103.

[7] J. Kleyer, S. Nicolussi, P. Taylor, D. Simonelli, E. Furger, P. Anderle, J. Gertsch, Biochem. Pharmacol. 15 (2012) 1393-1412.

[8] D. Baker, G. Pryce, G. Giovannoni, A. J. Thompson, Lancet Neurol. 2 (2003) 291-8.

[9] P. Yang, L. Wang, X. Xie, Future Med. Chem. 4 (2012) 187-204.

[10] O. Ofek, M. Karsak, N. Leclerc, M. Fogel, B. Frenkel, K. Wright, J. Tam, M. Attar-Namdar, V. Kram, E. Shohami, R. Mechoulam, A. Zimmer, I. Bab, PNAS 103 (2006) 696-701.

[11] A. I. Idris, R. J. van 't Hof, I. R. Greig, S. A. Ridge, D. Baker, R. A. Ross, S. H. Ralston, Nature Med. 11 (2005) 774-779.

[12] W. Schuehly, J. M. Viveros Paredes, J. Kleyer, A. Huefner, S. Anavi-Goffer, S. Raduner, K. H. Altmann, J. Gertsch, Chem. Biol. 18 (2011) 1053-1064.

[13] F. Vincenzi, M. Targa, C. Corciulo, M. A. Tabrizi, S. Merighi, S. Gessi, G. Saponaro, P. G. Baraldi, P. A. Borea, K. Varani, Pain 154 (2013) 864-873.

[14] S. G. Kinsey, A. Mahadevan, B. Zhao, H. Sun, P. S. Naidu, R. K. Razdan, D. E. Selley, M. Imad Damaj, A. H. Lichtman, Neuropharmacology 60 (2011) 244-251.

[15] C. A. Lunn, E. P. Reich, J. S. Fine, B. Lavey, J. A. Kozlowski, R. W. Hipkin, D. J. Lundell, L. Bober, Br. J. Pharmacol. 153 (2008) 226-239.

[16] V. Di Marzo, Nat. Rev. Drug. Discov. 7 (2008) 438-455.

[17] J. R. Savinainen, T. Kokkola, O. M. Salo, A. Poso, T. Järvinen, J. T. Laitinen, Br. J. Pharmacol. 145 (2005) 636-645.

[18] G. G. Muccioli, Chem. Biodivers. 4 (2007) 1805-1827.

[19] S. Bertini, T. Parkkari, J. R. Savinainen, C. Arena, G. Saccomanni, S. Saguto, A. Ligresti, M. Allarà, A. Bruno, L. Marinelli, V. Di Marzo, E. Novellino, C. Manera, M. Macchia, Eur. J. Med. Chem. 90 (2015) 526-536.

[20] A. Chicca, D. Caprioglio, A. Minassi, V. Petrucci, G. Appendino, O. Taglialatela-Scafati, J. Gertsch, ACS Chem. Biol. 9 (2014) 1499-1507.

[21] S.-K. Khim, M. Dai , X. Zhang , L. Chen , L. Pettus , K. Thakkar, A. G. Schultz, J. Org. Chem. 69 (2004) 7728-7733.

[22] M. D. Cullen , B.-L. Deng , T. L. Hartman , K. M. Watson , R. W. Buckheit, C. Pannecouque , E. De Clercq, M. Cushman, J. Med. Chem. 50 (2007) 4854-4867.

[23] D. Shabashov, J. R. Molina Maldonado, O. Daugulis, J. Org. Chem. 73 (2008) 7818-7821.

[24] B. R. Kusuma, L. B. Peterson, H. Zhao, G. Vielhauer, J. Holzbeierlein, B.S. Blagg, J. Med. Chem. 18 (2011) 6234-53.

[25] N. Henry, C. Enguehard-Gueiffier, I. Thery, A. Gueiffier, Eur. J. Org. Chem. (2008) 4824-4827.

[26] N. Gisch, J. Balzarini, C. Meier, J. Med. Chem. 50 (2007) 1658-1667.

[27] B.-L. Deng, T. L. Hartman, R. W. Buckheit, C. Pannecouque, E. De Clercq, P. E. Fanwick, M. Cushman, J. Med. Chem. 48 (2005) 6140-6155.

[28] V. Lucchesi, T. Parkkari, J. R. Savinainen, A. M. Malfitano, M. Allarà, S. Bertini, F. Castelli, S. Del Carlo, C. Laezza, A. Ligresti, G. Saccomanni, M. Bifulco, V. Di Marzo, M. Macchia, C. Manera, Eur. J. Med. Chem. 74 (2014) 524-532.

[29] A. Chicca, M. S. Gachet, V. Petrucci, W. Schuehly, R.-P. Charles, J. Gertsch, J. Neuroinflammation 12 (2015) 1-16.

[30] V. Rempel, A. Fuchs, S. Hinz, T. Karcz, M. Lehr, U. Koetter, C. E. Müller, ACS Med. Chem. Lett. 4 (2013) 41-45.

[31] R. Smoum, S. Baraghithy, M. Chourasia, A. Breuer, N. Mussai, M. Attar-Namdar, N. M. Kogan, B. Raphael, D. Bolognini, M. G. Cascio, P. Marinid, R. G. Pertwee, A. Shurkia, R. Mechoulam, I. Bab, PNAS, 112 (2015**)** 8774-8779.

[32] A. Chicca, J. Marazzi, J. Gertsch, Br. J. Pharmacol. 167 (2002) 1596-1608.

[33] O. Rosati, F. Messina, A. Pelosi, M. Curini, V. Petrucci, J. Gertsch, A. Chicca, Eur. J. Med. Chem. 85 (2014) 77-86.