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Original article

# 1,2-Dihydro-2-oxopyridine-3-carboxamides: The C-5 substituent is responsible for functionality switch at CB2 cannabinoid receptor

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ABSTRACT

The relevance of CB2R-mediated therapeutic effects is well-known for the treatment of inflammatory and neuropathic pain and neurodegenerative disorders. In our search for new cannabinoid receptor modulators, we report the optimization of a series of 1,2-dihydro-2-oxopyridine-3-carboxamide derivatives as CB2R ligands. In particular, *N*-cycloheptyl-5-(4-methoxyphenyl)-1-(4-fluorobenzyl)-pyridin-2(1*H*)-on-3-carboxamide (**17**) showed high CB2R affinity ($K_i$ = 1.0 nM), accompanied by interesting $K_i$(CB1R)/$K_i$(CB2R) selectivity ratio (SI = 43.4). Compound **17** was also identified as a potent CB2R neutral antagonist/weak partial inverse agonist. Finally we found that the functionality activity of the series of 1,2-dihydro-2-oxopyridine is controlled by the presence of a substituent in position 5 of the heterocyclic nucleus. In fact when the hydrogen atom in position 5 of the unsubstituted compound **1** was replaced with a phenyl group (compound **18**) the CB2R activity was shifted from agonism to inverse agonism whereas the introduction in the same position of *p*-methoxyphenyl group lead to compound **17** which showed a behavior as CB2R neutral antagonist/weak partial inverse agonist.



## 1. Introduction

The endocannabinoid system (ECS) consists of two cannabinoid receptors, a putative transporter, endogenous ligands, and diverse enzymes involved in the synthesis and degradation of endocannabinoids [1]. An increasing body of evidence implicates a role of the ECS in a variety of physiological and pathophysiological conditions, including immunomodulation, metabolic regulation, bone growth, pain, cancer, and psychiatric disorders [2–8]. Two cannabinoid receptors (CBRs), CB1R and CB2R, have been identified to date as G-protein-coupled receptors (GPCRs) [9]. CB1R is found predominantly in the central nervous system and is thought to be responsible for most of the overt pharmacological effects of cannabinoids [10,11]. Although CB2R was originally identified from macrophages present in the spleen and was initially considered to be expressed primarily by the immune system, it is now well accepted that CB2R is expressed by activated microglia and other macrophages in the brain [12–14]. As CB1R probably mediates most, if not all, of the psychoactive effects of cannabinoids [15], CB2R selective ligands are attractive as therapeutics because they would presumably lack this psychoactivity [16,17]. Several studies have found that selective CB2R agonists may exhibit anti-inflammatory and analgesic properties in animal models [6,18,19], and they could be useful for the treatment of neurodegenerative diseases, including Alzheimer's and Parkinson's disease [1,20], amyotrophic lateral sclerosis [21], and Huntington's disease [22]. Furthermore, CB2R agonists could have cardio-protective effects and be effective in the treatment of cancer, e.g. gliomas [23–26]. Fewer compounds have been described as selective antagonists/inverse agonists. Antagonists/inverse agonists may possess anti-inflammatory activity [27], and may inhibit osteoclast formation and activity *in vitro*

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[28,29]. However, a recent study showed that the CB2R activation enhances osteoclast activity [30]. Moreover, it was also reported that CB2R antagonists may represent a new therapeutic approach for the treatment of obesity-associated inflammation, insulin resistance and non-alcoholic fatty liver disease [31].

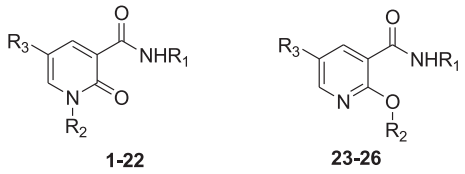
In a research program aimed at obtaining CB2R selective ligands [32–36], we have recently described the synthesis and pharmacological characterization of 1,2-dihydro-2-oxopyridine-3-carboxamide derivatives that exhibited good CB2R affinity and antiproliferative effects in several cancer cell lines [36]. On the basis of our previous results and in an effort to develop structure activity relationship (SAR) for both subtypes CBRs we chose derivative **1** for the development of a new set of 1,2-dihydro-2-oxopyridine-3-carboxamide derivatives **2**–**22** (Table 1). For these compounds the *p*-fluorobenzyl group in *N*1 position and cycloheptyl carboxamide in position 3 of **1** were either retained or replaced by groups with different steric and lipophilic properties, and various substituents at C5 position were introduced. Furthermore, the substituent in *N*-1 position was shifted to the oxygen in position 2 of the heterocyclic nucleus (**23**–**26**, Table 1). Similar analogs were recently reported as CB2R ligands [37–40].

The new compounds were tested on membranes prepared from HEK-293 cells expressing the human CB1R and CB2R, to determine their affinities towards both subtypes of CBRs. Finally the CBRs activity of the reference compound **1** and of the new derivatives **17** and **18** which showed high CB2R affinity and interesting CB2R versus CB1R selectivity, were also investigated by the GTPγS binding assay. The data obtained indicate that the substitution at the position C-5 of the 2-oxopyridine nucleus has a very interesting effect on the activity on the CB2R and permit the identification of the "key" position to be substituted on the 1,2-dihydro-2-oxopyridine scaffold responsible for functionality switch within this series of compounds. Similar results were reported by other authors for pyridine-based compounds [41,42].

## 2. Chemistry

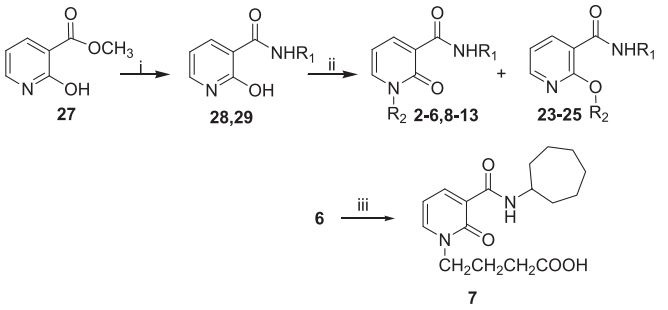
The synthesis of 2-oxopyridine derivatives **2**–**22** and 2-substituted pyridines **23**–**26** was accomplished as depicted in Schemes 1,2. The methyl ester **27** [36], was heated with the 4-methylcyclohexylamine or cycloheptylamine at 150 °C to give the carboxamide derivative **28** and **29** respectively [36] (Scheme 1). *N*-Alkylation of **28** and **29** in anhydrous DMF with the suitable benzyl chloride or alkyl halide or 4-(2-chloroethyl)-morpholine in the presence of NaH afforded the desired 2-oxopyridine derivatives **2**–**6** and **8**–**13**. In the case of the reaction with benzyl chloride or *p*-fluoro-benzyl chloride the *O*-substituted derivatives **23**–**25** were also isolated in according to previously reported [36]. Alkaline hydrolysis of **6** followed by acidification gave the carboxylic acid **7** (Scheme 1).

**Table 1**
Structures and radioligand binding data of 1,2-dihydro-2-oxopyridine- and pyridine-3-carboxamide derivatives.[a]




| | $R_1$ | $R_2$ | $R_3$ | $K_i$ (nM) | | |
|---|---|---|---|---|---|---|
| | | | | $CB_1$[b] | $CB_2$[c] | SI[d] |
| 1 | Cycloheptyl | *p*-Fluorobenzyl | H | 43 | 7.8 | 5.5 |
| 2 | Cycloheptyl | Benzyl | H | 56 ± 2.8 | 8 ± 0.4 | 7 |
| 3 | Cycloheptyl | *p*-Chlorobenzyl | H | 55 ± 2.6 | 4.2 ± 0.2 | 13.1 |
| 4 | Cycloheptyl | *p*-Iodobenzyl | H | 1270 ± 11 | 187 ± 2.4 | 6.8 |
| 5 | Cycloheptyl | *m*-Iodobenzyl | H | 1830 ± 9.5 | 397 ± 2.3 | 4.6 |
| 6 | Cycloheptyl | $(CH_2)_3COOEt$ | H | >10,000 | 1832 ± 5.6 | – |
| 7 | Cycloheptyl | $(CH_2)_3COOH$ | H | >10,000 | >10,000 | – |
| 8 | 4-$CH_3$-cyclohexyl | *p*-Fluorobenzyl | H | 70 ± 2.8 | 4.8 ± 1.8 | 14.6 |
| 9 | 4-$CH_3$-Cyclohexyl | Benzyl | H | 386 ± 8.1 | 53 ± 2.6 | 7.3 |
| 10 | 4-$CH_3$-Cyclohexyl | *n*-Pr | H | >10,000 | 583 ± 2.5 | – |
| 11 | 4-$CH_3$-Cyclohexyl | *n*-Bu | H | 1623 ± 9.6 | 104 ± 2.1 | 15.6 |
| 12 | 4-$CH_3$-Cyclohexyl | $(CH_2)_2OH$ | H | >10,000 | >10,000 | – |
| 13 | 4-$CH_3$-Cyclohexyl | $(CH_2)_3OH$ | H | >10,000 | >10,000 | – |
| 14 | Cycloheptyl | *p*-Fluorobenzyl | Br | 8.8 ± 0.4 | 2.2 ± 0.08 | 4 |
| 15 | Cycloheptyl | Ethylmorpholino | Br | 243 ± 1.6 | 65 ± 2.2 | 3.7 |
| 16 | Cycloheptyl | *n*-Bu | Br | 23 ± 1.1 | 5.1 ± 0.2 | 4.5 |
| 17 | Cycloheptyl | *p*-Fluorobenzyl | *p*-Methoxyphenyl | 63 ± 2.9 | 1.0 ± 0.03 | 63 |
| 18 | Cycloheptyl | *p*-Fluorobenzyl | Phenyl | 35 ± 1.7 | 1.2 ± 0.04 | 29 |
| 19 | Cycloheptyl | *p*-Fluorobenzyl | *p*-Fluorophenyl | 24 ± 0.1 | 2.8 ± 0.1 | 8.6 |
| 20 | Cycloheptyl | Ethylmorpholino | *p*-Fluorophenyl | 2734 ± 13.3 | 63 ± 0.3 | 43.4 |
| 21 | Cycloheptyl | *p*-Fluorobenzyl | *Trans*-phenylvinyl | 24 ± 0.1 | 7.9 ± 0.04 | 3.0 |
| 22 | Cycloheptyl | *p*-Fluorobenzyl | Ethylacrylate | 24.8 ± 0.1 | 13.7 ± 0.07 | 1.8 |
| 23 | Cycloheptyl | Benzyl | H | 1920 ± 9.5 | 481 ± 2.4 | 4 |
| 24 | 4-$CH_3$-cyclohexyl | *p*-Fluorobenzyl | H | 884 ± 4.4 | 80 ± 0.4 | 11 |
| 25 | 4-$CH_3$-cyclohexyl | Benzyl | H | 3093 ± 15.4 | 594 ± 2.8 | 5.2 |
| 26 | Cycloheptyl | *n*-Bu | Br | >10,000 | 396 ± 1.9 | – |
| SR144528 | | | | 437 | 0.6 | |
| JWH 133 | | | | 677 | 3 | |

[a] Data represent mean values for at least three separate experiments performed in duplicate and are expressed as $K_i$ (nM), for CB1R and CB2R binding assays.
[b] Affinity of compounds for CB1R was evaluated using membranes from HEK-293 cells transfected and [$^3$H]CP55,940.
[c] Affinity of compounds for CB2R was evaluated using membranes from HEK-293 cells transfected and [$^3$H]CP-55,940.
[d] Selectivity index for CB2R calculated as $K_i$(CB1R)/$K_i$(CB2R) ratio.




**Scheme 1.** *Reagents and conditions*: (i) $R_1NH_2$, 150 °C, 48 h; (ii) DMF, NaH, $R_2Cl$, r.t. or 70 °C, 24 h; (iii) NaOH 10%, reflux, 2 h, HCl.

As described in Scheme 2, the cycloheptyl carboxamide **29** [36] was refluxed with $Br_2$ and sodium acetate in glacial acetic acid for 8 h to afford 5-bromo-*N*-cycloheptyl-piridin-2(1*H*)-on-3-carboxamide (**30**) which was purified by crystallization from toluene. The treatment of **30** in anhydrous DMF with NaH for 2 h at room temperature or at 70 °C and then with *p*-fluoro-benzyl chloride or 4-(2-chloroethyl)morpholine or *n*-butyl bromide at 70 °C for 24 h afforded crude mixture which was purified by crystallization or by flash chromatography to obtain the desired compounds **14**–**16**. In the case of the reaction of **30** with *n*-butyl bromide, the *O*-substituted derivative **26** was also isolated. The cross-coupling reaction under Suzuki conditions between 5-bromo derivative **30** and the appropriate boronic acid carried out under microwave irradiation afforded 5-substituted carboxamide derivatives **31**–**34** which by treatment with NaH and then with *p*-fluoro-benzyl chloride or 4-(2-chloroethyl)-morpholine, gave the desired compounds **17**–**21**. The 5-ethyl-acrylate derivative **22** was prepared from the *N*-cycloheptyl-5-bromo-1-(4-fluorobenzyl)-pyridin-2(1*H*)-on-3-carboxamide (**14**) with ethylacrylate by Heck vinylation under microwave irradiation according to Scheme 2.

## 3. Results and discussion

### 3.1. CB1 and CB2 receptor affinity

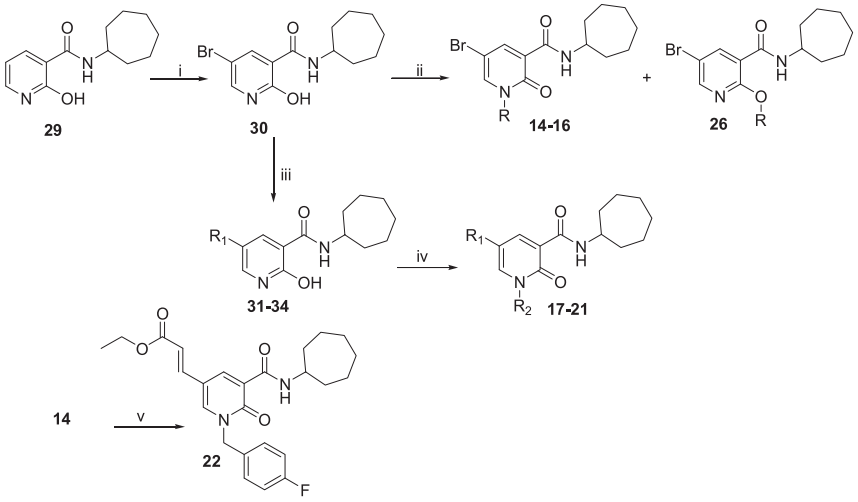
The binding affinities ($K_i$ values) of the compounds **2**–**26** were evaluated by competitive binding assays against [$^3$H]CP-55,940 using human recombinant CB1R and CB2R overexpressed in HEK-293 cells, as previously described [35]. The results are reported in Table 1 with the $K_i$ values of the previously studied *N*-cycloheptyl-1-(p-fluorobenzyl)-1,2-dihydro-2-oxo-pyridine-3-carboxamide (**1**) [36] and the reference compounds SR144528 [43] and JWH 133 [44].

The results indicate that 1,2-dihydro-2-oxopyridine-3-carboxamide derivatives **2**–**22** exhibit higher affinity for the CB2R than for the CB1R. In fact, in no case was the $K_i$(CB1)/$K_i$(CB2) ratio lower than 1. These data are in agreement with those previously reported [36].

Regarding to the structural modifications in the position 1 of the 2-oxo-pyridine nucleus, substitution of the *p*-fluorobenzyl group of the reference compound **1** with benzyl (**2**) or *p*-chlorobenzyl group (**3**) was important in determining the maintenance in the affinity towards both receptor subtypes. On the contrary, the presence of *p*-iodobenzyl (**4**) or *m*-iodobenzyl (**5**) decreases affinity towards both CBRs being more drastic in the case of *m*-iodobenzyl derivative **5**. Furthermore, the substitution of the benzyl group with ethyl butyric ester or butyric acid led to compounds **6** and **7**, respectively, which exhibited very low affinity.

Replacement of the cycloheptylamide with 4-methylcyclohexyl amide in position 3 of the 2-oxo-pyridine nucleus of compound **1** led to compound **8**, which preserved the receptor affinities found for the reference compound. Moreover, if 4-methylcyclo hexylamide in the position 3 was kept and in position 1 the *p*-fluorobenzyl was replaced with benzyl, alkyl or hydroxyalkyl (**9**–**13**), the affinity towards both CBRs decreased. In particular, this decrement was drastic for the hydroxyalkyl derivatives **12** and **13**.

Regarding the introduction of substituent on C-5 of the 2-oxo-pyridine nucleus, the CBRs affinities were different depending on the nature of substituent. In particular, the presence of bromine atom (**14**) caused an increase in affinity against both CBRs, in respect to that of observed for the reference compound **1**. The substitution of the *p*-fluorobenzyl group of **14** with ethylmorpholine reduced receptor affinities, especially against CB1R, as is clear from a comparison of $K_i$ values of compounds **15** with that of **14**. On the contrary, the derivative **16**, characterized by *n*-butyl group in position 1 of the heterocyclic nucleus, possessed receptor affinities comparable to that of compound **14**. The introduction of *trans*-phenylvinyl or ethylacrylate on C5 led to compounds **21** and **22**, respectively, which showed a maintenance (**21**) or a very low




**Scheme 2.** *Reagents and conditions*: (i) glacial AcOH, AcONa, $Br_2$, reflux, 8 h; (ii) DMF, NaH, 1–2 h at r.t. or 70 °C, RCl/RBr, 70 °C, 24 h; (iii) Dioxane, $Ph_3P$, $Pd(OAc)_2$, MeOH, $Na_2CO_3$ 2 M, $R_1B(OH)_2$, microwave, 200 W, 100 psi, 150 °C, 15 min; (iv) DMF, NaH, $R_2Cl/R_2Br$, 70 °C, 24 h; (v) acetonitrile, $Ph_3P$, $Pd(OAc)_2$, $Et_3N$, ethyl-acrylate, microwave, 200 W, 100 psi, 110 °C, 20 min.

decrease (**22**) affinity on CBRs compared to the reference compound **1**. On the contrary, the presence of phenyl (**18**) or *p*-fluorophenyl (**19**) group in the same position led to an increase in affinity against both CBRs which, however, was greater towards CB2R. Also, in the case of compound **19** the substitution of the *p*-fluorobenzyl in position 1 of the 2-oxo-pyridine nucleus with ethylmorpholine reduced the CBRs affinities as can be seen by a comparison of $K_i$ values of compounds **19** with that of **20**. Furthermore, the introduction on C-5 of *p*-methoxyphenyl group led to compound **17** which showed higher CB2R affinity than that of the reference compound **1** ($K_i = 1.0$ nM *vs* $K_i = 7.8$ nM). In particular, derivative **17** was proved to have the highest CB2R affinity and selectivity in this series ($K_i$(CB1)/$K_i$(CB2) = 63).

Finally, the *O*-substituted derivatives (**23**–**26**) showed lower affinity on both CBRs than that of the corresponding *N*1-substituted derivatives. This result is in agreement with the results previously reported [36].

### 3.2. CB1 and CB2 receptor functional activity

CBR-mediated G protein activities of the reference compound **1** and of the new derivatives **17** and **18**, which showed the highest CB2R affinity together with an interesting selectivity, were investigated by the [$^{35}$S]GTPγS binding assay, essentially as previously described [45,46]. The results revealed that compound **1** acts as a potent CB2R agonist (−log EC50 = 8.2 ± 0.3, $n$ = 3, Fig. 1A) when tested in membranes prepared from hCB2-transfected CHO-cells. However, it also showed CB1R activity at rat cerebellar membranes, albeit with ~200-fold lower potency (−log EC50 = 5.8 ± 0.1, $n$ = 3, Fig. 1B). Strikingly, substituent at the position C-5 has a very dramatic effect on the activity of the human CB2R. Indeed, when the C-5 hydrogen of compound **1** was replaced with a phenyl group (compound **18**) the CB2R activity was shifted from agonism to inverse agonism (Fig. 2). Moreover, compound **18** appeared to be as potent inverse agonist (−log IC50 = 8.8 ± 0.1, $n$ = 3) as a well-established CB2R-inverse agonist SR144528 (−log IC50 = 8.5 ± 0.2). Substitution also increased selectivity over the CB1R as it did not stimulate or antagonize CB1R-mediated G protein-activity at concentrations below 10 μM (evoked non-specific effects at higher concentrations). However, maybe the most interesting compound in the series appeared to be the C-5 *p*-methoxyphenyl derivative **17**. Unlike the reference compound **1**, derivative **17** was not able to evoke any agonist response, but was capable of antagonizing CB2R-agonist HU-210 (1 nM) -stimulated activity in CB2R–CHO-membranes (Fig. 3A). Alone, it showed a weak (partial) inverse agonist property (88 ± 3% basal, $n$ = 3 (SEM)) compared to that of full inverse agonist SR144528 (75 ± 1% basal)

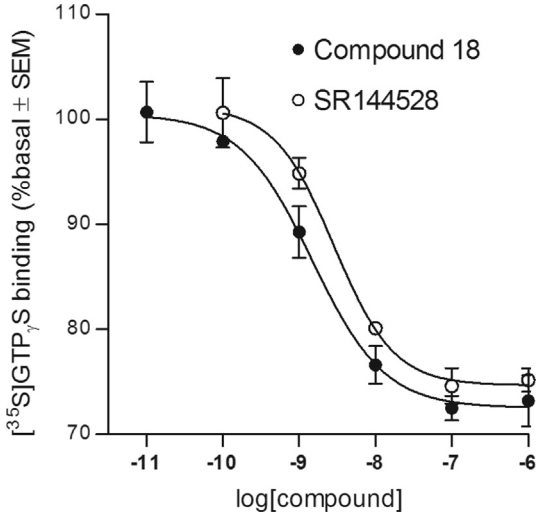



**Fig. 2.** Compound **18** is a more potent compound in inhibiting constitutive CB2R activity in hCB2-CHO-membranes than an established inverse agonist SR144528. The results are from three separate experiments performed in duplicates.

(Fig. 2). However, its ability to reverse the response of the inverse agonist (SR144528, IC50-concentration 3 nM) indicates that would rather be a neutral CB2R antagonist (Fig. 3A). Interestingly, further analysis revealed that **17** is a competitive antagonist in inhibiting HU-210-stimulated activity (pA2 value 7.0) (Fig. 3B), suggesting altogether that it would be a potent competitive CB2R antagonist with properties typical of neutral antagonist and/or weak partial inverse agonist [46]. Furthermore, in rat cerebellar membranes, compound **17** did not stimulate or antagonize CB1R-mediated G protein-activity at concentrations below 10 μM (evoked non-specific effects at higher concentrations) suggesting that it is CB2R-selective compound. All the tested compounds were silent when tested in mock-transfected-cell membranes.

## 4. Conclusion

In this study we have described a new series of 1,2-dihydro-2-oxopyridine-3-carboxamide derivatives with high CB2R affinity and interesting selectivity. The obtained results showed that the nature of substituents at the position 1 of the 2-oxo-pyridine nucleus is crucial for CBRs affinity. Moreover, the introduction of substituents at the position 5 of the heterocyclic nucleus generally increased CB2R affinity. In particular, the *p*-methoxyphenyl

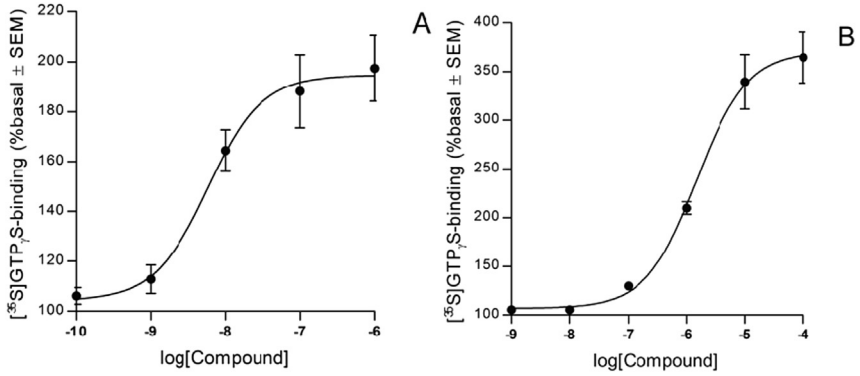



**Fig. 1.** Dose–response curves for the compound **1** at hCB2-CHO cell membranes (A) and at CB1R using rat cerebellar membranes (B). The results are from three separate experiments performed in duplicates.

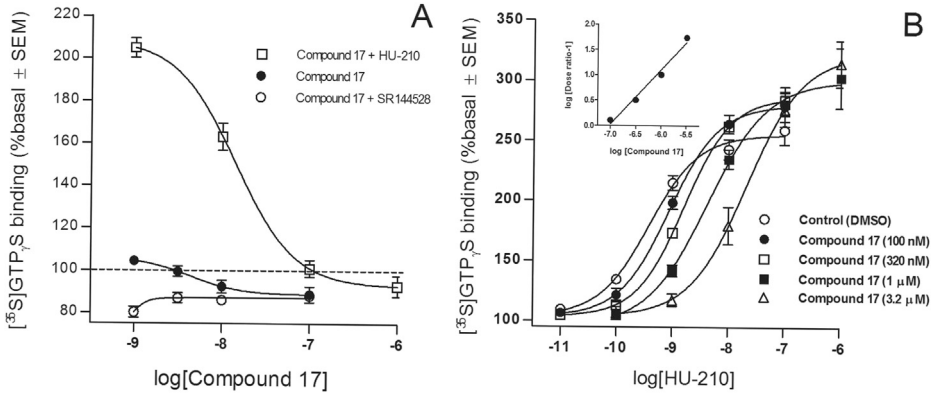



**Fig. 3.** Compound **17** is a silent compound in evoking CB2R-agonist activity in hCB2-CHO-membranes. However, it dose-dependently reversed agonist (HU210, 1 nM (~EC50-value)) and also inverse agonist (SR144528, 3 nM (~IC50-value)) responses to the same final level (A). Derivative **17** competitively antagonizes dose-responses of HU210 at the human CB2R. pA2-value (7.0) for the compound was calculated from the Schild-plot (the slope 1.10 ± 0.1 does not significantly differ from unity ($P < 0.05$)) (B). The results are from least three separate experiments performed in duplicates.

derivative **17** showed high affinity ($K_i$ = 1.0 nM) accompanied by interesting good $K_i$(CB1R)/$K_i$(CB2R) selectivity. Furthermore, we found that the functional activity of this series is controlled by the presence of a substituent at the position 5 of the 2-oxopyridine nucleus. Briefly, a hydrogen atom at this position makes the compound a CB2R agonist (**1**) whereas a corresponding phenyl derivative (**18**) leads to a highly potent inverse CB2R agonist. Importantly, a *p*-methoxyphenyl group at this position makes the compound a competitive CB2R neutral antagonist/weak partial CB2R inverse agonist. All the three compounds described have great potential to serve selective and unique tools for both *in vitro* and *in vivo* studies examining biological functionality of CB2R. Accordingly to recently reported literature [41,42], these results confirm that small structural changes can control functional activity. It will be interesting to study the influence on the CB2R activity of other substituents in the same position (5) and/or in other positions (eg 4 and/or 6) of the 2-oxo-pyridine nucleus and if this is due to steric or electronic effects.

## 5. Experimental

### 5.1. Chemistry

Reagents were purchased from commercial suppliers and used without further purification. Merck silica gel 60 was used for flash chromatography (230–400 mesh). Melting points were determined on a Kofler hot stage apparatus and are uncorrected. IR spectra in Nujol mulls were recorded on an ATI Mattson Genesis Series FTIR spectrometer. $^1$H NMR and $^{13}$C NMR spectra were recorded with a Varian Gemini 200 spectrometer in $\delta$ units from TMS as an internal standard. Microwave-assisted reactions were run in a CEM microwave synthesizer. Mass spectra were performed with a Thermo Quest Finningan GCQ plus. Elemental analyses (C, H, N) were within ±0.4% of theoretical values, and were performed on a Carlo Erba elemental analyzer model 1106 apparatus. The chemical purity of the target compounds was determined under the following conditions: the HPLC system was an LC Workstation Prostar (Varian, Inc., Walnut Creek, CA, USA) consisting of high pressure mixer pump (ProStar, model 230), DAD detector (ProStar, model 330) and a loop of 20 μl. Data were processed by a Star LC Workstation (Varian, Inc.). Chromatographic separation was performed on a Luna $C_{18}$ $ODS_2$ analytical column (150 × 4.6 mm inner diameter, 3 μm particle size, Phenomenex, Torrance, CA, USA) maintained at 25 °C. The mobile phase consisted of acetonitrile:water (70:30 v/v) with 0.6 ml/min flow rate. Wavelengths were set at 220 and 320 nm. The purity of each compound was >96% in either analysis.

#### 5.1.1. *N*-(4-Methylcyclohexyl)-2-hydroxypyridine-3-carboxamide (**28**)

A mixture of methyl 2-hydroxypyridine-3-carboxylate (**27**) (0.590 g, 3.86 mmol) and 4-methylcyclohexylamine (1.32 g, 11.6 mmol) was heated at 150 °C for 48 h. After cooling the reaction mixture was poured into ice and treated with HCl 5% until pH = 4. The precipitated solid was collected by filtration and purified by crystallization from ethyl acetate to give the pure amide **28** (0.89 g, yield 74%); m.p. 248–250 °C; $^1$H NMR (DMSO) $\delta$ 12.47 (br, 1H, OH), 10.01 and 9.87 (2d, $J$ = 7.5 Hz, 1H, NH), 8.30 (m, 1H, Ar), 7.69 (m, 1H, Ar), 6.46 (m, 1H, Ar), 3.75 and 3.54 (2m, 1H, NCH), 1.92–0.83 (m, 12H, cyclohexyl + $CH_3$). Anal. $C_{13}H_{18}N_2O_2$ (C, H, N).

#### 5.1.2. *N*-Cycloheptyl-5-bromo-pyridin-2(1H)-on-3-carboxamide (**30**)

To a suspension of *N*-cyclohepthyl-pyridin-2(1*H*)-on-3-carboxamide (**29**) (1.50 g; 6.40 mmol), sodium acetate (2.0 g; 25.62 mmol) in 15 ml of glacial acid acetic was added dropwise a solution of bromine (0.8 ml; 16.0 mmol) in 10 ml of glacial acetic acid. The mixture was refluxed for 8 h. The crude mixture was treated with an aqueous solution of sodium bisulfate and extracted with dichloromethane. The organic layer was washed with water, dried over anhydrous $Na_2SO_4$ and evaporated to dryness under reduced pressure to obtain a solid residue which was collected by filtration and washed with small portions of isopropyl ether to give pure **30** (1.0 g, yield 50%); mp 185–187 °C (crystallized from toluene); MS *m*/*z* 312 ($M^+$); $^1$H NMR (DMSO) $\delta$ 12.81 (br, 1H, OH), 9.75 (d, $J$ = 6.1 Hz, 1H, NH), 8.27 (d, $J$ = 2.7 Hz, 1H, Ar), 8.00 (d, $J$ = 2.7 Hz, 1H, Ar), 4.01 (m, 1H, CH), 1.81–1.40 (m, 12H, cycloheptyl). Anal. $C_{13}H_{17}BrN_2O_2$ (C, H, N).

#### 5.1.3. *General procedures for the synthesis of compounds **31–34***

A mixture of triphenylphosphine (83.5 mg, 0.32 mmol) and palladium acetate (14.3 mg; 0.06 mmol) in 3 ml of dioxane was stirred under nitrogen at room temperature. After 10 min a solution of *N*-cycloheptyl-5-bromo-pyridin-2(1*H*)-on-3-carboxamide (**30**) (0.200 g; 0.64 mmol) and suitable boronic acid (1.27 mmol) in MeOH (1.5 ml) and $Na_2CO_3$ 2 M (1.3 ml) were added. The reaction mixture was heated by the microwave radiation for 15 min at 150 °C (200 W, 100 psi). The reaction mixture was thereafter cooled

down to room temperature treated with water and extracted with dichloromethane. The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, and evaporated. The residue was purified by flash chromatography on silica gel (**31**, **34**) or by crystallization (**32**, **33**).

*5.1.3.1. N-Cycloheptyl-5-phenyl-pyridin-2(1H)-on-3-carboxamide (**31**).* Yield: 25%; mp 185–190 °C (crystallized from toluene); MS *m/z* 310 ($M^+$); $^1$H NMR (DMSO) $\delta$ 12.80 (br, 1H, OH), 9.92 (d, $J$ = 6.6 Hz, 1H, NH), 8.61 (d, $J$ = 2.7 Hz, 1H, Ar), 8.02 (d, $J$ = 2.7 Hz, 1H, Ar), 7.62 (m, 2H, Ar) 7.41 (m, 3H, Ar), 4.10 (m, 1H, CH), 2.00–1.00 (m, 12H, cycloheptyl). Anal. $C_{19}H_{22}N_2O_2$ (C, H, N).

*5.1.3.2. N-Cycloheptyl-5-(4-methoxyphenyl)-pyridin-2(1H)-on-3-carboxamide (**32**).* Purified by flash chromatography (hexane/ethyl acetate 3:2). Yield: 28%; mp 207–209 °C (crystallized from toluene); MS *m/z* 340 ($M^+$); $^1$H NMR (DMSO) $\delta$ 12.70 (br, 1H, OH), 9.95 (d, $J$ = 6.4 Hz, 1H, NH), 8.56 (d, $J$ = 2.9 Hz, 1H, Ar), 7.93 (d, $J$ = 2.9 Hz, 1H, Ar), 7.52 (d, $J$ = 8.7 Hz, 2H, Ar), 7.00 (d, $J$ = 8.8 Hz, 2H, Ar), 4.06 (m, 1H, CH), 3.78 (s, 3H, $OCH_3$), 2.00–1.00 (m, 12H, cycloheptyl). Anal. $C_{20}H_{24}N_2O_3$ (C, H, N).

*5.1.3.3. N-Cycloheptyl-5-(4-fluorophenyl)-pyridin-2(1H)-on-3-carboxamide (**33**).* Yield: 51%; mp 205–207 °C (crystallized from toluene); MS *m/z* 328 ($M^+$); $^1$H NMR (DMSO) $\delta$ 12.70 (br, 1H, OH), 9.88 (d, $J$ = 7.9 Hz, 1H, NH), 8.57 (d, $J$ = 2.9 Hz, 1H, Ar), 8.01 (d, $J$ = 2.9 Hz, 1H, Ar), 7.65 (m, 2H, Ar), 7.27 (m, 2H, Ar), 4.00 (m, 1H, CH), 2.00–1.30 (m, 12H, cycloheptyl). Anal. $C_{19}H_{21}FN_2O_2$ (C, H, N).

*5.1.3.4. N-Cycloheptyl-5-[(E)-2-phenylethenyl]-pyridin-2(1H)-on-3-carboxamide (**34**).* Purified by flash chromatography (hexane/ethyl acetate 1:1). Yield: 22%; mp 128–130 °C (crystallized from toluene); MS *m/z* 336 ($M^+$); $^1$H NMR (DMSO) $\delta$ 12.35 (br, 1H, OH), 9.86 (d, $J$ = 6.7 Hz, 1H, NH), 8.68 (d, $J$ = 2.5 Hz, 1H, Ar), 7.89 (d, $J$ = 2.5 Hz, 1H, Ar), 7.56 (d, $J$ = 8.0 Hz, 1H, vinyl), 7.36 (m, 2H, Ar) 7.28 (m, 3H, Ar), 7.18 (d, $J$ = 8.0 Hz, 1H, vinyl), 4.07 (m, 1H, CH), 2.00–1.30 (m, 12H, cycloheptyl). Anal. $C_{21}H_{24}N_2O_2$ (C, H, N).

*5.1.4. General procedure for the synthesis of $N_1$- (**2**–**6**, **8**–**21**) and O-substituted pyridin-3-carboxamide derivatives (**23**–**26**)*

An amount of 2.88 mmol of NaH was added to a solution of 0.96 mmol of appropriate pyridin-3-carboxamide **28**–**34** in 12 ml of dry DMF. After 1 h at 70 °C or at room temperature (for compounds **4**–**6**, **15**, **17**, **18** and **21**) 1.92 mmol of the suitable chloride or bromide was added and the mixture was stirred for 24 h at 50 °C or at room temperature (**4**–**6**). After cooling the reaction mixture was concentrated under reduced pressure, treated with water to give a residue which was purified by flash chromatography or by crystallization.

*5.1.4.1. N-Cycloheptyl-1-benzyl-pyridin-2(1H)-on-3-carboxamide (**2**) and N-cycloheptyl-2-benzyloxy-pyridin-2(1H)-on-3-carboxamide (**23**).* Purified by flash chromatography (hexane/ethyl acetate 2:1). **2** as oil. Yield: 38%; MS *m/z* 324 ($M^+$); $^1$H NMR ($CDCl_3$) $\delta$ 9.80 (d, $J$ = 6.4 Hz, 1H, NH), 8.54 (dd, $J$ = 7.1 and 2.2 Hz, 1H, Ar), 7.46 (dd, $J$ = 6.7 and 2.2 Hz, 1H, Ar), 7.36 (m, 5H, Ar), 6.41 (m, 1H, Ar), 5.24 (s, 2H, $CH_2$), 4.16 (m, 1H, CH), 2.03–1.27 (m, 12H, cycloheptyl). $^{13}$C NMR ($CDCl_3$) $\delta$ 162.44, 162.20, 143.73, 140.20, 134.52, 134.23, 130.15, 129.51, 129.44, 122.60, 107.67, 52.77, 51.21, 35.22, 28.18, 24.32. Anal. $C_{20}H_{24}N_2O_2$ (C, H, N). **23** as oil. Yield: 15%; MS *m/z* 324 ($M^+$); $^1$H NMR (DMSO) $\delta$ 8.32 (dd, $J$ = 7.3 and 2.2 Hz, 1H, Ar), 8.13 (dd, $J$ = 6.7 and 2.2 Hz, 1H, Ar), 8.03 (d, $J$ = 7.9 Hz, 1H, NH), 7.45 (m, 5H, Ar), 7.13 (m, 1H, Ar), 5.45 (s, 2H, $CH_2$), 3.85 (m, 1H, CH), 1.92–1.07 (m, 12H, cycloheptyl). Anal. $C_{20}H_{24}N_2O_2$ (C, H, N).

*5.1.4.2. N-Cycloheptyl-1-(4-chlorobenzyl)-pyridin-2(1H)-on-3-carboxamide (**3**).* Yield: 70%; mp 108–110 °C (crystallized from chloroform); MS *m/z* 358 ($M^+$); $^1$H NMR (DMSO) $\delta$ 9.73 (d, $J$ = 6.2 Hz, 1H, NH), 8.34 (dd, $J$ = 7.3 and 2.2 Hz, 1H, Ar), 8.26 (dd, $J$ = 6.6 and 2.2 Hz, 1H, Ar), 7.42 (d, $J$ = 8.3 Hz, 2H, Ar), 7.31 (d, $J$ = 8.3 Hz, 2H, Ar), 6.59 (m, 1H, Ar), 5.22 (s, 2H, $CH_2$), 3.99 (m, 1H, CH), 1.96–1.20 (m, 12H, cycloheptyl). $^{13}$C NMR ($CDCl_3$) $\delta$ 162.44, 162.20, 143.73, 140.20, 134.52, 134.23, 129.51, 129.44, 129.17, 122.60, 107.17, 52.27, 50.71, 35.19, 28.38, 24.51. Anal. $C_{20}H_{23}ClN_2O_2$ (C, H, N).

*5.1.4.3. N-Cycloheptyl-1-(4-iodobenzyl)-pyridin-2(1H)-on-3-carboxamide (**4**).* Yield: 81%; mp 88–90 °C; (crystallized from chloroform); MS *m/z* 450 ($M^+$); $^1$H NMR (DMSO) $\delta$ 9.73 (d, $J$ = 6.8 Hz, 1H, NH), 8.33 (dd, $J$ = 7.1 and 1.9 Hz, 1H, Ar), 8.16 (dd, $J$ = 6.7 and 2.2 Hz, 1H, Ar), 7.72 (d, $J$ = 8.3 Hz, 2H, Ar), 7.31 (d, $J$ = 8.3 Hz, 2H, Ar), 6.58 (m, 1H, Ar), 5.19 (s, 2H, $CH_2$), 4.00 (m, 1H, CH), 1.99–1.15 (m, 12H, cycloheptyl). $^{13}$C NMR (DMSO) $\delta$ 162.44; 162.20; 143.81; 140.26; 138.36; 135.38; 130.00; 122.54; 107.25; 94.30; 52.48; 50.73; 35.20; 28.41; 24.51. Anal. $C_{20}H_{23}IN_2O_2$ (C, H, N).

*5.1.4.4. N-Cycloheptyl-1-(3-iodobenzyl)-pyridin-2(1H)-on-3-carboxamide (**5**).* Purified by flash chromatography (hexane/ethyl acetate 1:4). Yield: 75%; mp 128–130 °C; (crystallized from chloroform); MS *m/z* 450 ($M^+$); $^1$H NMR (DMSO) $\delta$ 9.71 (d, $J$ = 7.8 Hz, 1H, NH), 8.35 (dd, $J$ = 7.3 and 2.2 Hz, 1H, Ar), 8.20 (dd, $J$ = 6.6 and 2.2 Hz, 1H, Ar), 7.67 (m, 2H, Ar), 7.26 (m, 2H, Ar), 6.56 (m, 1H, Ar), 5.20 (s, 2H, $CH_2$), 3.98 (m, 1H, CH), 1.90–1.25 (m, 12H, cycloheptyl). $^{13}$C NMR (DMSO) $\delta$ 162.42; 162.22; 143.80; 140.36; 137.36; 135.38; 134.34; 131.20; 130.45; 124.54; 107.55; 94.20; 52.44; 50.70; 35.21; 28.44; 24.51. Anal. $C_{20}H_{23}IN_2O_2$ (C, H, N).

*5.1.4.5. Ethyl 4-(3-(cycloheptylcarbamoyl)-2-oxopyridin-1(2H)-yl) butanoate (**6**).* Purified by flash chromatography (hexane/ethyl acetate 1:1, v/v) as oil. Yield: 66%; MS *m/z* 348 ($M^+$); $^1$H NMR (DMSO) $\delta$ 9.86 (d, $J$ = 7.8 Hz, 1H, NH), 8.30 (dd, $J$ = 7.0 and 2.2 Hz, 1H, Ar), 7.99 (dd, $J$ = 6.5 and 2.2 Hz, 1H, Ar), 6.51 (m, 1H, Ar), 3.96 (m, 5H, CH + $CH_2$), 2.30 (t, $J$ = 7.2 Hz, 2H, $CH_2$), 1.98–1.47 (m, 14H, cycloheptyl + $CH_2$), 1.13 (t, $J$ = 7.1 Hz, 3H, $CH_3$). $^{13}$C NMR (DMSO) $\delta$ 173.71, 162.42; 161.23; 143.82; 134.34; 131.20; 107.55; 61.43; 50.70; 45.69; 35.21; 31.43; 28.44; 24.51; 23.35; 15.63. Anal. $C_{19}H_{28}N_2O_4$ (C,H, N).

*5.1.4.6. N-(4-Methylcyclohexyl)-1-(4-fluorobenzyl)-pyridin-2(1H)-on-3-carboxamide (**8**) and N-(4-Methylcyclohexyl)-2-(4-fluorobenzyloxy)-pyridin-2(1H)-on-3-carboxamide (**24**).* Purified by flash chromatography (hexane/ethyl acetate 2:1). **8** as oil. Yield: 25%; MS *m/z* 342 ($M^+$); $^1$H NMR ($CDCl_3$) $\delta$ 9.98 and 9.59 (2d, $J$ = 6.9 Hz, 1H, NH), 8.37 (m, 1H, Ar), 8.20 (m, 1H, Ar), 7.36 (m, 2H, Ar), 7.23 (m, 2H, Ar), 6.41 (m, 1H, Ar), 5.24 and 5.21 (2s, 2H, $CH_2$), 4.13 and 3.85 (2m, 1H, CH), 2.03–0.98 (m, 12H, cyclohexyl + $CH_3$). $^{13}$C NMR ($CDCl_3$) $\delta$ 162.89, 162.26, 160.92, 145.58, 134.79, 137.83, 131.33, 128.64, 115.34, 111.71, 50.12, 47.36, 44.89, 34.92, 33.66, 32.54, 31.97, 30.54, 29.78, 22.56, 21.95. Anal. $C_{20}H_{23}FN_2O_2$ (C, H, N). **24** as oil. Yield: 10%; (crystallized from hexane); MS *m/z* 342 ($M^+$); $^1$H NMR (DMSO) $\delta$ 8.37 (m, 2H, NH + Ar), 8.06 (m, 1H, Ar), 7.61 (m, 2H, Ar), 7.18 (m, 3H, Ar), 5.44 and 5.41 (2s, 2H, $CH_2$), 4.03 and 3.85 (2m, 1H, CH), 1.92–0.69 (m, 12H, cyclohexyl + $CH_3$). Anal. $C_{20}H_{23}FN_2O_2$ (C, H, N).

*5.1.4.7. N-(4-Methylcyclohexyl)-1-benzyl-pyridin-2(1H)-on-3-carboxamide (**9**) and N-(4-Methylcyclohexyl)-2-benzyloxy-pyridin-2(1H)-on-3-carboxamide (**25**).* Purified by flash chromatography (hexane/ethyl acetate 2:1). **9** as oil. Yield: 28%; MS *m/z* 324 ($M^+$); $^1$H NMR ($CDCl_3$) $\delta$ 10.02 and 9.69 (2d, $J$ = 6.8 Hz, 1H, NH), 8.36 (m, 1H, Ar), 8.18 (m, 1H, Ar), 7.31 (m, 5H, Ar), 6.52 (m, 1H, Ar), 5,28 and 5.24

(2s, 2H, $CH_2$), 4,15 and 3.75 (2m, 1H, CH), 2.01–0.97 (m, 12H, cyclohexyl + $CH_3$). $^{13}C$ NMR ($CDCl_3$) $\delta$ 162.34, 160.68, 145.49, 141.76, 135.01, 131.83, 128.93, 127.87, 126.43, 111.86, 50.03, 47.28, 44.56, 34.89, 33.38, 32.78, 31.76, 30.34, 29.68, 22.25, 21.82. Anal. $C_{20}H_{24}N_2O_2$ (C, H, N). **25** as oil. Yield: 15%; MS *m/z* 324 ($M^+$); $^1H$ NMR (DMSO) $\delta$ 8.33 (m, 2H, NH + Ar), 8.10 (m, 1H, Ar), 7.43 (m, 2H, Ar), 7.22 (m, 3H, Ar), 7.12 (m, 1H, Ar), 5,47 and 5.44 (2s, 2H, $CH_2$), 4.00 and 3.75 (2m, 1H, CH), 1.98–0.63 (m, 12H, cyclohexyl + $CH_3$). Anal. $C_{20}H_{24}N_2O_2$ (C, H, N).

*5.1.4.8. N-(4-Methylcyclohexyl)-1-propyl-pyridin-2(1H)-on-3-carboxamide (**10**).* Purified by flash chromatography (hexane/ethyl acetate 1:1.5). Yield: 71%; MS *m/z* 276 ($M^+$); $^1H$ NMR ($CDCl_3$) $\delta$ 9.65 and 10.03 (2d, $J$ = 7.5 Hz, 1H, NH), 8.55 (dd, $J$ = 7.1 and 2.2 Hz, 1H, Ar), 7.46 (dd, $J$ = 6.6 and 2.2 Hz, 1H, Ar), 6.40 (m, 1H, Ar), 3.94–4.03 (m, 3H, CH + $CH_2$), 0.92–1.89 (m, 17H, cyclohexyl + $CH_2$ + $CH_3$). $^{13}C$ NMR ($CDCl_3$) $\delta$ 162.66, 160.78, 145.28, 134.72, 131.64, 111.33, 50.12, 47.36, 38.95, 34.79, 33.82, 32.87, 31.67, 30.66, 30.34, 29.91, 24.62, 22.81, 21.75, 14.65. Anal. $C_{16}H_{24}N_2O_2$ (C, H, N).

*5.1.4.9. N-(4-Methylcyclohexyl)-1-butyl-pyridin-2(1H)-on-3-carboxamide (**11**).* Purified by flash chromatography (hexane/ethyl acetate 2:1) as oil. Yield: 57%; MS *m/z* 290 ($M^+$); $^1H$ NMR ($CDCl_3$) $\delta$ 9.65 and 10.03 (2d, $J$ = 7.5 Hz, 1H, NH), 8.52 (dd, $J$ = 7.1 and 2.2 Hz, 1H, Ar), 7.48 (dd, $J$ = 6.6 and 2.2 Hz, 1H, Ar), 6.41 (m, 1H, Ar), 3.97–4.15 (m, 3H, CH + $CH_2$), 0.92–2.05 (m, 19H, 4-methycyclohexyl + $2CH_2$ + $CH_3$). $^{13}C$ NMR ($CDCl_3$) $\delta$ 162.48, 160.76, 145.44, 134.73, 131.84, 111.63, 50.01, 47.39, 38.87, 34.79, 33.87, 32.59, 31.73, 30.69, 30.48, 29.85, 29.73, 24.52, 22.68, 21.87, 14.76. Anal. $C_{17}H_{26}N_2O_2$ (C, H, N).

*5.1.4.10. N-(4-Methylcyclohexyl)-1-(2-hydroxyethyl)-pyridin-2(1H)-on-3-carboxamide (**12**).* Purified by flash chromatography (hexane/ethyl acetate 1:2) as oil. Yield: 66%; MS *m/z* 278 ($M^+$); $^1H$ NMR ($CDCl_3$) $\delta$ 9.62 and 10.01 (2d, $J$ = 7.5 Hz, 1H, NH), 8.39 (dd, $J$ = 7.3 and 2.1 Hz, 1H, Ar), 7.58 (dd, $J$ = 6.3 and 2.1 Hz, 1H, Ar), 6.35 (m, 1H, Ar), 3.51–3.99 (m, 3H, $CH_2$ + OH), 4.15–4.23 (m, 3H, CH + $CH_2$), 0.89–2.05 (m, 12H, cyclohexyl + $CH_3$). $^{13}C$ NMR ($CDCl_3$) $\delta$ 162.66, 160.78, 145.28, 134.72, 131.64, 111.33, 61.78, 50.12, 47.36, 41.95, 34.86, 33.79, 32.92, 31.88, 30.49, 29.86, 22.65, 21.88. Anal. $C_{15}H_{22}N_2O_3$ (C, H, N).

*5.1.4.11. N-(4-Methylcyclohexyl)-1-(3-hydroxypropyl)-pyridin-2(1H)-on-3-carboxamide (**13**).* Purified by flash chromatography (toluene/ethyl acetate 1:4) as oil. Yield: 47%; MS *m/z* 292 ($M^+$); $^1H$ NMR ($CDCl_3$) $\delta$ 9.61 and 10.02 (2d, $J$ = 7.5 Hz, 1H, NH), 8.52 (dd, $J$ = 7.3 and 2.2 Hz, 1H, Ar), 7.52 (dd, $J$ = 6.4 and 2.2 Hz, 1H, Ar), 6.38 (m, 1H, Ar), 3.43–3.89 (m, 3H, OH + $CH_2$), 4.01–4.24 (m, 3H, CH + $CH_2$), 0.89–2.08 (m, 14H, cyclohexyl + $CH_2$ + $CH_3$). $^{13}C$ NMR ($CDCl_3$) $\delta$ 162.34, 160.63, 145.54, 134.72, 131.44, 111.63, 62.07, 49.78, 47.41, 40.46, 34.85, 33.98, 32.72, 31.80, 30.63, 30.54, 29.44, 22.74, 21.86. Anal. $C_{16}H_{24}N_2O_3$ (C, H, N).

*5.1.4.12. N-Cycloheptyl-5-bromo-1-(4-fluorobenzyl)-pyridin-2(1H)-on-3-carboxamide (14).* Yield: 90%; mp 166–168 °C; (crystallized from chloroform); MS *m/z* 420 (M+); $^1H$ NMR (DMSO) $\delta$ 9.68 (d, $J$ = 6.2 Hz, 1H, NH), 8.60 (d, $J$ = 2.9 Hz, 1H, Ar), 8.29 (d, $J$ = 2.9 Hz, 1H, Ar), 7.36 (m, 2H, Ar), 7.18 (m, 2H, Ar), 5.18 (s, 2H, CH2), 3.91 (m, 1H, CH), 1.89–1.20 (m, 12H, cycloheptyl). $^{13}C$ NMR (CDCl3) $\delta$ 162.34; 162.10; 161.21; 146.37; 139.82; 135.95; 130.41; 130.24; 123.45; 116.66; 116.22; 99.47; 52.41; 50.87; 35.10; 28.37; 24.43. Anal. $C_{20}H_{22}BrFN_2O_2$ (C, H, N).

*5.1.4.13. N-Cycloheptyl-5-bromo-1-(2-morpholin-4-ylethyl)-pyridin-2(1H)-on-3-carboxamide (**15**).* Purified by flash chromatography (hexane/ethyl acetate 1:3). Yield: 25%; mp 166–168 °C (crystallized from toluene); MS *m/z* 425 ($M^+$); $^1H$ NMR ($CDCl_3$) $\delta$ 9.72 (d, $J$ = 6.2 Hz, 1H, NH), 8.57 (d, $J$ = 2.8 Hz, 1H, Ar), 7.67 (d, $J$ = 2.8 Hz, 1H, Ar), 4.06 (m, 3H, CH and morpholine), 3.70 (m, 4H, morpholine), 2.70 (t, $J$ = 5.9 Hz, 2H, $CH_2$), 2.50 (m, 4H, morpholine), 1.89–1.22 (m, 12H, cycloheptyl). $^{13}C$ NMR ($CDCl_3$) $\delta$ 162.10; 161.20; 139.82; 131.41; 123.44; 99.67; 67.3; 55.30; 52.11; 51.27; 50.76; 35.10; 28.37; 24.43. Anal. $C_{19}H_{28}BrN_3O_3$ (C, H, N).

*5.1.4.14. N-Cycloheptyl-5-bromo-1-butyl-pyridin-2(1H)-on-3-carboxamide (**16**) and N-cycloheptyl-5-bromo-2-butoxy-pyridin-3-carboxamide (**26**).* Purified by flash chromatography (hexane/ethyl acetate 4:1). **16** as oil. Yield: 10%; MS *m/z* 368 ($M^+$); $^1H$ NMR ($CDCl_3$) $\delta$ 9.77 (d, $J$ = 6.3 Hz, 1H, NH), 8.57 (d, $J$ = 2.9 Hz, 1H, Ar), 7.60 (d, $J$ = 2.9 Hz, 1H, Ar), 4.21 (m, 1H, CH), 4.03 (t, $J$ = 7.5 Hz, 2H, $CH_2$), 1.98–1.10 (m, 19H, cycloheptyl + $CH_2$ + $CH_3$). $^{13}C$ NMR ($CDCl_3$) $\delta$ 162.10; 161.20; 139.82; 131.41; 123.44; 99.67; 50.76; 47.34; 35.10; 31.77; 28.36; 24.42; 22.78; 17.23. Anal. $C_{17}H_{25}BrN_2O_2$ (C, H, N). **26** as oil. Yield: 12%, MS *m/z* 368 ($M^+$); $^1H$ NMR ($CDCl_3$) $\delta$ 8.62 (d, $J$ = 2.6 Hz, 1H, Ar), 8.27 (d, $J$ = 2.6 Hz, 1H, Ar), 8.00 (d, $J$ = 6.3 Hz, 1H, NH), 4.47 (t, $J$ = 6.6 Hz, 2H, $CH_2$), 4.20 (m, 1H, CH), 2.00–1.00 (m, 19H, cycloheptyl + $CH_2$ + $CH_3$). Anal. $C_{17}H_{25}BrN_2O_2$ (C, H, N).

*5.1.4.15. N-Cycloheptyl-5-(4-methoxyphenyl)-1-(4-fluorobenzyl)-pyridin-2(1H)-on-3-carboxamide (**17**).* Purified by flash chromatography (hexane/ethyl acetate 5:1) Yield: 34%; mp: 159–161 °C (crystallized from toluene); MS *m/z* 448 ($M^+$); $^1H$ NMR (DMSO) $\delta$ 9.80 (d, $J$ = 6.3 Hz, 1H, NH), 8.59 (s, 2H, Ar), 7.55 (d, $J$ = 8.8 Hz, 2H, Ar), 7.50–7.10 (m, 4H, Ar), 7.04 (d, $J$ = 8.8 Hz, 2H, Ar), 5.29 (s, 2H, $CH_2$), 4.00 (m, 1H, CH), 3.79 (s, 3H, $OCH_3$), 2.00–1.20 (m, 12H, cycloheptyl). $^{13}C$ NMR ($CDCl_3$) $\delta$ 162.49, 161.39, 160.36, 159.65, 143.08, 136.45, 131.66, 131.61, 130.11, 129.95, 128.16, 127.35; 122.08, 121.21, 116.49, 116.06, 114.80, 55.64, 52.45, 50.76; 35.20, 28.42, 24.52. Anal. $C_{27}H_{29}FN_2O_3$ (C, H, N).

*5.1.4.16. N-Cycloheptyl-5-phenyl-1-(4-fluorobenzyl)-pyridin-2(1H)-on-3-carboxamide (**18**).* Purified by flash chromatography (hexane/ethyl acetate 4:1) as oil. Yield: 37%; MS *m/z* 418 ($M^+$); $^1H$ NMR (DMSO) $\delta$ 9.78 (d, $J$ = 7.5 Hz, 1H, NH), 8.66 (d, $J$ = 2.9 Hz, 1H, Ar), 8.63 (d, $J$ = 2.9 Hz, 1H, Ar), 7.63 (m, 2H, Ar), 7.50 (m, 4H, Ar), 7.20 (m, 3H, Ar), 5.31 (s, 2H, $CH_2$), 4.09 (m, 1H, CH), 2.00–1.20 (m, 12H, cycloheptyl). $^{13}C$ NMR ($CDCl_3$) $\delta$ 162.47, 161.19, 160.40, 143.18, 137.15, 131.88, 131.41, 130.01, 129.75, 128.36, 127.65; 122.08, 116.49; 116.36; 52.35, 50.66; 35.20, 28.45, 24.50. Anal. $C_{26}H_{27}FN_2O_2$ (C, H, N).

*5.1.4.17. N-Cycloheptyl-5-(4-fluorophenyl)-1-(4-fluorobenzyl)-pyridin-2(1H)-on-3-carboxamide (**19**).* Purified by flash chromatography (hexane/ethyl acetate 5:1). as oil. Yield: 60%; MS *m/z* 436 ($M^+$); $^1H$ NMR (DMSO) $\delta$ 9.76 (d, $J$ = 7.5 Hz, 1H, NH), 8.65 (d, $J$ = 2.9 Hz, 1H, Ar), 8.63 (d, $J$ = 2.9 Hz, 1H, Ar), 7.67 (m, 2H, Ar), 7.49 (m, 2H, Ar), 7.28 (m, 4H, Ar), 5.29 (s, 2H, $CH_2$), 4.03 (m, 1H, CH), 2.00–1.20 (m, 12H, cycloheptyl). $^{13}C$ NMR ($CDCl_3$) $\delta$ 165.33; 162.36, 161.45, 160.40, 143.06, 136.96, 131.66, 131.86, 131.47, 130.17, 129.66, 128.04, 127.87; 122.28, 120.55, 116.57, 116.15, 115.60, 52.55, 50.82; 35.18, 28.40, 24.49. Anal. $C_{26}H_{26}F_2N_2O_2$ (C, H, N).

*5.1.4.18. N-Cycloheptyl-5-(4-fluorophenyl)-1-(2-morpholin-4-ylethyl)-pyridin-2(1H)-on-3-carboxamide (**20**).* Purified by flash chromatography (hexane/ethyl acetate 2:3). as oil. Yield: 70%; MS *m/z* 441 ($M^+$); $^1H$ NMR (DMSO) $\delta$ 9.79 (d, $J$ = 7.6 Hz, 1H, NH), 8.58 (d, $J$ = 2.9 Hz, 1H, Ar), 8.38 (d, $J$ = 2.9 Hz, 1H, Ar), 7.63 (m, 2H, Ar), 7.33 (m, 2H, Ar), 4.25 (t, $J$ = 6.0 Hz, 2H, $CH_2$), 4.03 (m, 1H, CH), 3.52 (m, 4H, morpholine), 2.58 (m, 6H, morpholine + $CH_2$), 1.85–1.35 (m, 12H, cycloheptyl). $^{13}C$ NMR ($CDCl_3$) $\delta$ 162.54, 161.34, 160.22, 142.79, 138.60, 132.20, 128.86, 127.89, 121.50, 119.53, 116.58, 116.15, 67.22,

57.13, 53.95, 50.67, 47.65; 35.18, 28.42, 24.47. Anal. $C_{25}H_{32}FN_3O_3$ (C, H, N).

*5.1.4.19. N-Cycloheptyl-5-[(E)-2-phenylethenyl]-1-(4-fluorobenzyl)-pyridin-2(1H)-on-3-carboxamide (**21**).* Purified by flash chromatography (hexane/ethyl acetate 3:1). Yield: 26%; mp 135–137 °C (crystallized from hexane); MS *m/z* 444 ($M^+$); $^1$H NMR (DMSO) $\delta$ 9.78 (d, $J$ = 6.7 Hz, 1H, NH), 8.70 (d, $J$ = 2.6 Hz, 1H, Ar), 8.41 (d, $J$ = 2.6 Hz, 1H, Ar), 7.58 (d, 1H, $J$ = 8.0 Hz, vinyl), 7.40 (m, 5H, Ar) 7.20 (m, 5H, Ar + vinyl) 5.26 (s, 2H, $CH_2$), 4.01 (m, 1H, CH), 2.00–1.30 (m, 12H, cycloheptyl). $^{13}$C NMR ($CDCl_3$) $\delta$ 161.83, 161.71, 160.40, 159.87, 141.18, 140.31, 138.58, 131.2, 130.95, 130.25, 128.72, 128.52, 125.45, 122.69, 116.87, 115.80, 52.45, 50.80; 35.12, 28.36, 24.41. Anal. $C_{28}H_{29}FN_2O_2$ (C, H, N).

*5.1.5. Acid 4-(N-cycloheptyl)-pyridin-2(1H)-on-3-(carboxamide) butanoic (**7**)*

A solution of 150 mg (0.43 mmol) of ester **6** in 5 ml of NaOH 10% was heated at 100 °C. After 2 h the reaction mixture was treated with water, acidified with HCl conc. until acid pH and it was extracted with dichloromethane. The organic phase was dried over anhydrous sodium sulfate and evaporated to dryness under reduced pressure to obtain a solid residue which was treated with $Et_2O$ and filtered to give 135 mg the desired compound **7**. Yield: 98%; mp 84–86 °C; MS *m/z* 320 ($M^+$); $^1$H NMR (DMSO) $\delta$ 12.09 (br, 1H, OH), 9.82 (d, $J$ = 7.9 Hz, 1H, NH), 8.30 (dd, $J$ = 7.1 and 2.2 Hz, 1H, Ar), 8.15 (d, $J$ = 6.8 and 2.2 Hz, 1H, Ar), 6.52 (m, 1H, Ar), 4.04 (m, 3H, CH + $CH_2$), 2.24 (t, 2H, $CH_2$), 1.93–1.54 (m, 14H, cycloheptyl + $CH_2$). $^{13}$C NMR (DMSO) $\delta$ 177.71, 162.32, 161.29, 143.72, 134.31, 131.20, 107.50, 50.70, 45.69; 35.22; 31.40; 28.46, 24.51, 23.35. Anal. $C_{17}H_{24}N_2O_4$ (C,H, N).

*5.1.6. (E)-Ethyl-3-[3-(N-cycloheptyl carboxamide)-1-(4-fluorobenzyl)-pyridin-2(1H)-on-5-yl]acrylate (**22**)*

A mixture of triphenylphosphine (31.0 mg; 0.20 mmol) and palladium acetate (5.0 mg; 0.024 mmol) in 1.50 ml acetonitrile was stirred for 10 min under nitrogen. Then *N*-cycloheptyl-5-bromo-1-(4-fluorobenzyl)-pyridin-2(1*H*)-on-3-carboxamide (**14**) (100.0 mg; 0.64 mmol), 1.50 ml of triethylamine, 1.27 ml of ethylacrylate (3.84 mmol) were added. The reaction mixture was heated by the microwave radiation at 110 °C for 20 min (power 200 W, pressure 100 psi, stirring on). The organic layer was evaporated under reduced pressure and the residue was purified by flash chromatography (hexane/ethyl acetate 7:3) to obtain derivative **22**. Yield: 32%; mp 136–139 °C; MS *m/z* 440 ($M^+$); $^1$H NMR (DMSO) $\delta$ 9.55 (d, $J$ = 7.4 Hz, 1H, NH), 8.75 (d, $J$ = 2.5 Hz, 1H, Ar), 8.62 (d, $J$ = 2.5 Hz, 1H, Ar), 7.58 (d, $J$ = 16 Hz, 1H, CH), 7.45 (m, 2H, Ar), 7.20 (m, 2H, Ar), 6.45 (d, $J$ = 16 Hz, 1H, CH), 5.20 (s, 2H, $CH_2$), 4.18 (q, $J$ = 7.0 Hz, 2H, $CH_2$), 3.98 (m, 1H, CH), 2.00–1.28 (m, 12H, cycloheptyl), 1.25 (t, $J$ = 7.0 Hz, 3H, $CH_3$). $^{13}$C NMR ($CDCl_3$) $\delta$ 166.68; 165.44, 161.83, 161.71, 160.40, 143.06, 141.08, 140.31, 138.58, 130.95, 130.25, 122.69, 116.87, 115.80, 60.90, 52.45, 50.80; 35.12, 28.38, 24.43, 14.55. Anal. $C_{25}H_{29}FN_2O_4$ (C, H, N).

*5.2. CB1 and CB2 receptor binding assays*

The new compounds were evaluated in CB1R and CB2R binding assays using membranes from HEK-293 cells transfected with cDNAs encoding the human recombinant CB1R (Bmax) 2.5 pmol/mg protein and human recombinant CB2R (Bmax) 4.7 pmol/mg protein (PerkineElmer, Italy). These membranes were incubated with [$^3$H]CP55,940 [47] (0.14 nM/kd ¼ 0.18 nM and 0.084 nM/kd ¼ 0.31 nM for CB1 and CB2 receptors, respectively) as the high affinity ligand and displaced with 100 nM of WIN55212-2 [48] as the heterologous competitor for nonspecific binding (Ki values 9.2 and 2.1 nM, respectively, for CB1R and CB2R). All compounds were tested following the procedure described by the cell membrane manufacturer. Displacement curves were generated by incubating drugs with [$^3$H]CP55,940 for 90 min at 30 °C. $K_i$ values were calculated by applying the ChengePrusoff equation [49] to the $IC_{50}$ values (obtained by GraphPad) for the displacement of the bound radioligand by increasing concentrations of the test compound. Data are the mean (SEM of at least $n$ = 3 experiments).

*5.3. Functional activity at CB1 and CB2 receptor in vitro*

The [$^{35}$S]GTPγS binding experiments were performed according to the reported procedures [39,40]. The results were calculated using GraphPad Prism 5.0 (California, USA). The GTPγS binding studies were conducted in three independent experiments performed in duplicates. IC50 and EC50 values are presented as mean ± s.e.m.

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