Development of novel oxazolo[5,4-*d*]pyrimidines as competitive CB_2 neutral antagonists [1] based on scaffold hopping

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Abstract: A series of novel oxazolo[5,4-*d*]pyrimidines was designed via a scaffold hopping strategy and synthesized through a newly developed approach. All these compounds were evaluated for their biological activity toward CB_1/CB_2 cannabinoid receptors, their metabolic stability in mice liver microsomes and their cytotoxicity against several cell lines. Eight compounds have been identified as CB_2 ligands with K_i values less than 1 μM. It is noteworthy that 2-(2-chlorophenyl)-5-methyl-7-(4-methylpiperazin-1-yl) oxazolo[5,4-*d*]pyrimidine **47**

Abbreviations: Boc, *tert*-butyloxycarbonyl; cAMP, cyclic adenosine monophosphate; CHO, Chinese hamster ovary cells; CHO-CB_2, Chinese hamster ovary cells expressing CB_2 receptors; CHO-WT, Chinese hamster ovary cells wild type; DIEA, *N*,*N*-diisopropylethylamine; ECS, endocannabinoid system; KHMDS, potassium bis(trimethylsilyl)amide; SAR, structure-activity relationship; TFA, trifluoroacetic acid.

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and 2-(2-chlorophenyl)-7-(4-ethylpiperazin-1-yl)- 5-methyloxazolo[5,4-*d*]pyrimidine **48** showed CB_2 binding affinity in the nanomolar range and significant selectivity over CB_1 receptors. Interestingly, functionality studies imply that they behave as competitive neutral antagonists. Moreover, all tested compounds are devoid of cytotoxicity toward several cell lines, including Chinese hamster ovary cells (CHO) and human colorectal adenocarcinoma cells HT29.

Key words: Cannabinoid, CB_2 receptor, neutral antagonist, oxazolo[5,4-*d*]pyrimidine

1. Introduction

The endocannabinoid system (ECS), present in mammals and comprising two primary G protein-coupled receptors, is linked with endogenous lipid mediators and relevant enzymes responsible for the synthesis and degradation of lipid mediators [2]. Due to its functions in the regulation of pain, inflammation, motor behavior, emotion, appetite and addiction, the ECS has been regarded as an ideal target with broad therapeutic potential [2-4]. The two G protein-coupled cannabinoid CB_1 and CB_2 receptors, are predominantly expressed in the central nervous system (e.g. brain) and immune cells (e.g. macrophages), respectively [3, 5-7]. CB_1 receptor antagonists have shown potential in the treatment of addiction and obesity. It has been demonstrated that the administration of a CB_1 antagonist (e.g., rimonabant) brings about a reduction of drug-seeking behaviors and weight loss [8-11]. By contrast, CB_2 receptor agonists lead to down regulation of inflammation and pain by restoring the equilibrium between pro- and anti-inflammatory cytokines [3, 5, 7, 12]. Moreover, several studies

have shown that CB_2 inverse agonists can regulate bone proliferation and relieve inflammation through modulating the migration of immune cells [13-15]. However, research on CB_2 neutral antagonists is rare, and their therapeutic potential remains unknown. In some cases, CB_2 neutral antagonists can be considered as ideal tools for investigating the biological function of receptors due to their ability to combine with receptors without altering corresponding biomolecular levels [16, 17]. In addition, targeting CB_1 receptors rather than CB_2 receptors has been demonstrated to induce tetrahydrocannabinol-like side effects (e.g., hypomotility, hypothermia, catalepsy) [18]. Thus, the development of selective CB_2 ligands has been considered as an interesting therapeutic strategy.

Over the course of the last few decades, the development of CB_2 ligands has significantly progressed. Numerous selective CB_2 ligands have shown therapeutic potential in a wide range of *in vivo* models, including pain, inflammation, cancer, immune disorders, neurodegenerative diseases and bone disorders [14, 19-26]. Specifically, more than ten CB_2 selective agonists have been reported to be under evaluation in clinical trials for the treatment of inflammation and pain [12]. For instance, compound **1** (GRC10693, Figure 1), developed by Glenmark Pharmaceuticals, successfully completed Phase I trials in 2009 [27]. It was intended to be used for pain remission, including neuropathic pain and inflammatory pain. Moreover, Eli Lilly and Co. disclosed a series of purines as selective CB_2 agonists [28-31]. Of note, compound **2** (LY2828360, Figure 1), derived from the lead compound **3** (Figure 1), has been recognized as a potent and efficacious analgesic

agent in a rat model of osteoarthritis related chronic pain at a 1 mg/kg oral dose [30]. Accordingly, clinical trials for the treatment of osteoarthritic knee pain were performed [32]. Additionally, it was reported that compound **4** (AM630, Figure 2), one of the most potent CB_2 inverse agonist identified to date, can attenuate osteoporosis [25, 33, 34]. Two other notable CB_2 inverse agonists, **5** (Sch225336, Figure 2) and **6** (Sch414319, Figure 2), have been shown to reduce inflammation through modulating the recruitment of immune cells [13, 15].

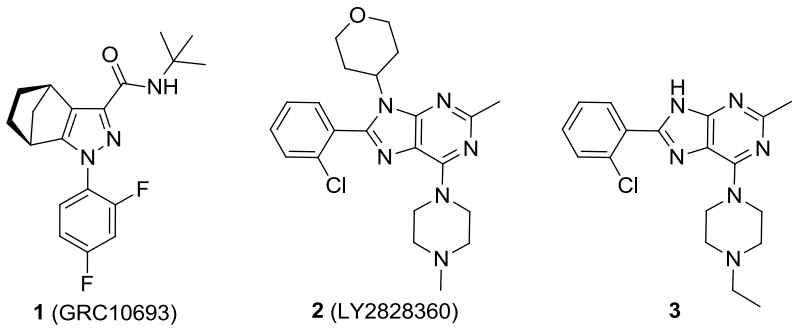


Figure 1. Structures of representative CB_2 agonists evaluated in clinical trials.

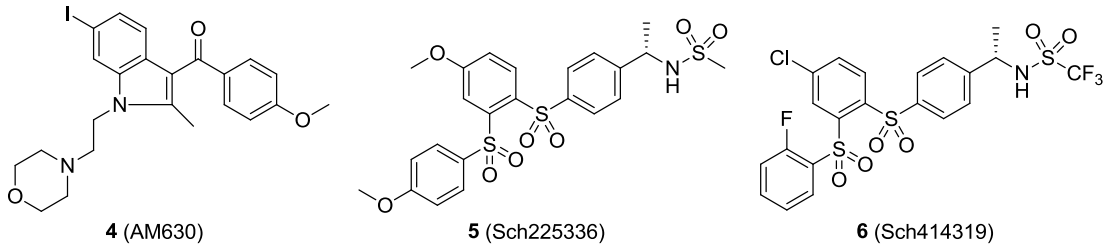


Figure 2. Representative CB_2 inverse agonists.

Scaffold hopping is a useful and powerful strategy for drug discovery. It has been widely used for the design of potential bioactive molecules [35-37]. In general, scaffold hopping incorporate heterocycle replacement, ring opening and closure, pseudopeptides and peptidomimetics, and topology/shape-based scaffold hopping [36].

Herein, we applied the heterocycle replacement-based scaffold hopping strategy toward compound **3**, a CB_2 agonist developed by Eli Lilly and Co [30]. Oxazolo[5,4-*d*]pyrimidine is a versatile scaffold used for the design of bioactive ligands against enzymes or receptors [38-40]. Hence, we replaced the purine core by this scaffold, with the intention of observing its influence on bioactivity toward CB_2 receptors (Figure 3). Afterwards, we carried out pharmacomodulations around the oxazolopyrimidine core (Figure 4). The newly synthesized compounds were tested for their CB_2 vs CB_1 affinity in a binding assay, their efficacy toward CB_2, and their metabolic stability in mice liver microsomes.

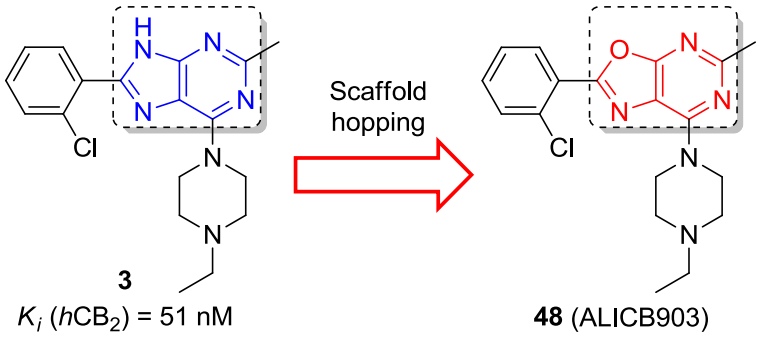


Figure 3. Heterocycle replacement-based scaffold hopping toward compound **3**.

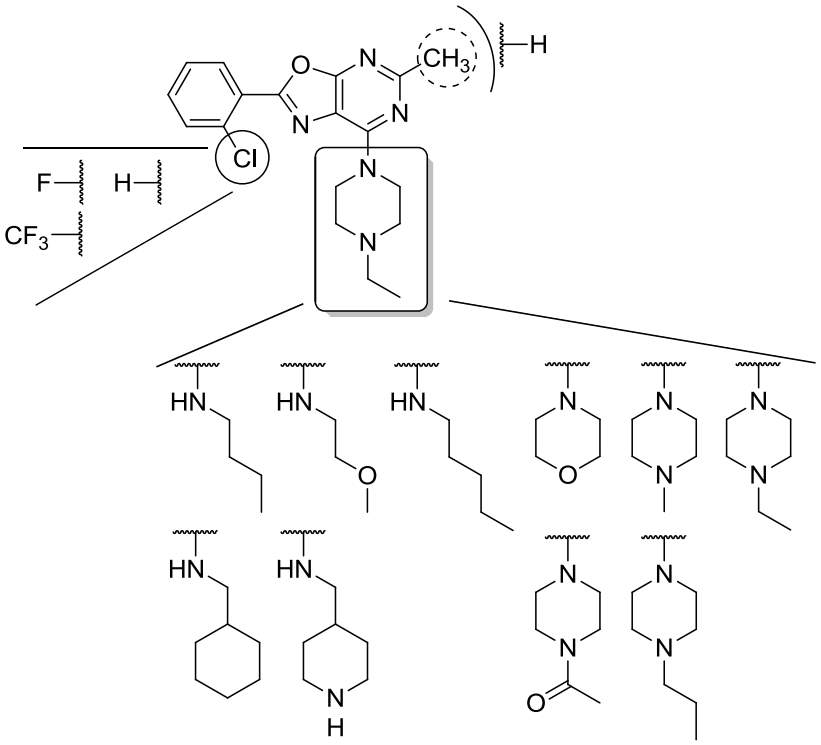


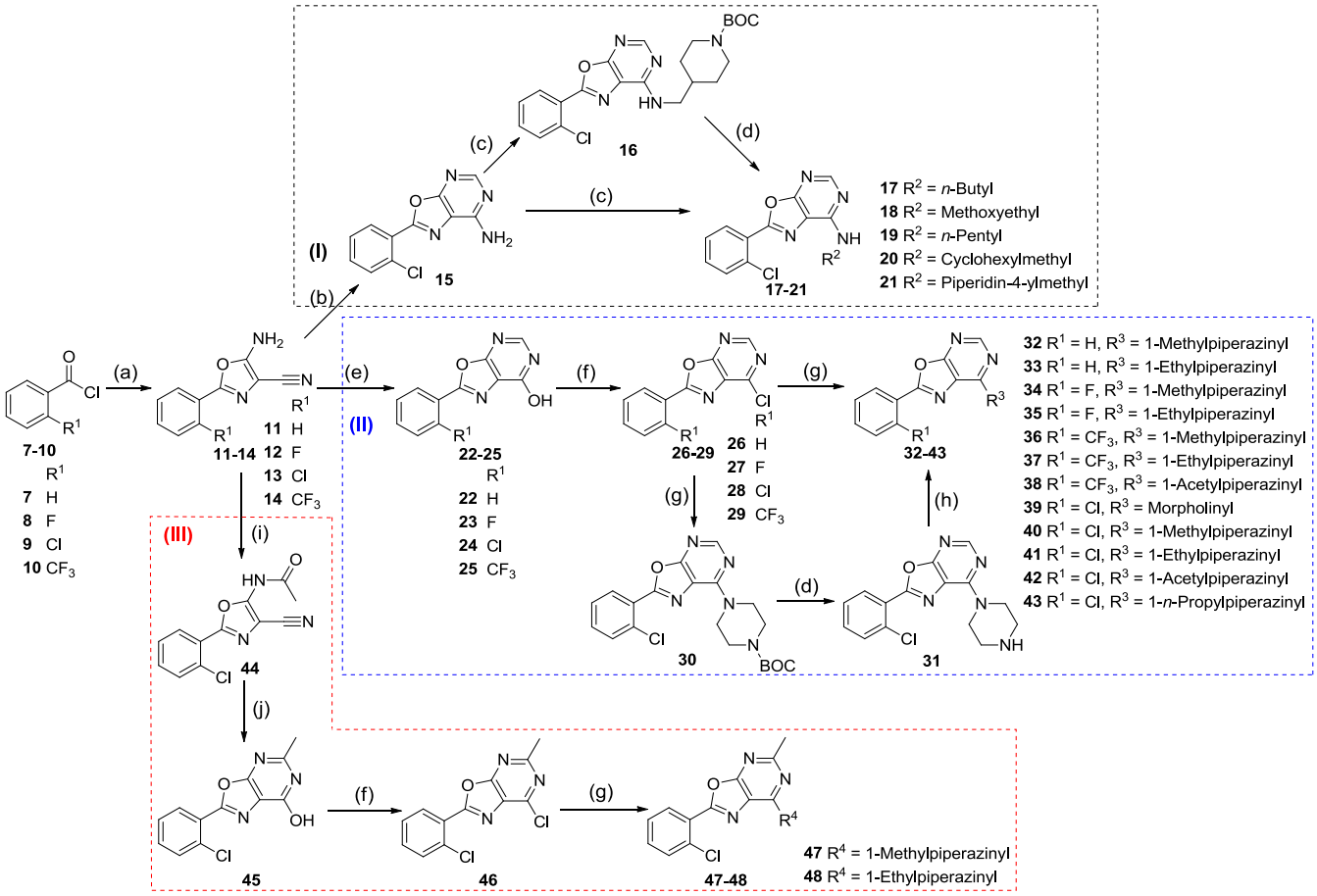
Figure 4. Structural modifications of substituents on the oxazolo[5,4-*d*]pyrimidine core.

2. Results and discussion

2.1. Chemistry

Compounds **11-14** were prepared by a cyclisation reaction between benzoyl chloride or ortho-halogenated benzoyl chloride derivatives and aminomalononitrile *p*-toluenesulfonate (75-90% yields). Starting from these compounds, target compounds were obtained through three different synthetic pathways (Scheme 1): (I) 5-amino-2-(2-chlorophenyl)oxazole-4-carbonitrile (**13**) reacted with formamidine acetate to generate 2-(2-chlorophenyl)oxazolo[5,4-*d*]pyrimidin-7-amine **15** (86% yield), which was further involved in a nucleophilic substitution using 15 wt. % potassium bis(trimethylsilyl)amide (KHMDS) solution in toluene and brominated derivatives to either give target compounds **17**-**20** (37-52% yields) directly, or to

afford a *tert*-butyloxycarbonyl (Boc)-protected precursor **16** (43% yield). Deprotection of the Boc group of **16** using trifluoroacetic acid (TFA) followed by neutralisation yielded compound **21** (99% yield). (II) Alternatively, compounds **11**-**14** were cyclized using formic acid to give 2-phenyloxazolo[5,4-*d*]pyrimidin-7-ones **22**-**25** (33-46% yields), respectively. Afterwards, $POCl_3$-dependent chlorination converted these 2-phenyloxazolo[5,4-*d*]pyrimidin-7-ones into compounds **26**-**29** (72-85% yields). The chloride group of **26**-**29** was substituted by a piperazine or morpholine derivative to either give target compounds **32**-**42** (98-99% yields) directly, or to afford Boc-protected compound **30** (98% yield). Compound **43** (98% yield) was obtained after deprotection of the piperazine group of compound **30** using TFA followed by a nucleophilic substitution on bromopropane. (III) Additionally, compound **13** was acetylated to afford **44** (78% yield) in the presence of acetyl chloride and *N*,*N*-diisopropylethylamine (DIPEA). A solution of **44** in 30% NaOH and 35% H_2O_2 was refluxed to give **45** (69% yield), which was further chlorinated by $POCl_3$ (77% yield) and substituted with piperazine derivatives to produce compounds **47**-**48** (98-99% yields).



Scheme 1. Synthetic routes to prepare compounds **17-21**, **32-43**, and **47-48**. (a) aminomalononitrile p-toluenesulfonate, NMP, microwave, 80 °C, 25 min, 75-90%. (b) formamidine acetate, NMP, 100 °C, 3 h, 86%. (c) KHMDS, 15 wt. % in toluene, brominated derivative, DMF, room temperature (rt), 3 h, 37-52%. (d) TFA, CH_2Cl_2, rt, 3 h, 99%. (e) formic acid, reflux, overnight, 31-37%. (f) $POCl_3$, DIPEA, toluene, rt, 2 h, 72-85%. (g) piperazine derivative or morpholine, Cs_2CO_3, DMF, 100 °C, 30 min, 98-99%. (h) bromopropane, Cs_2CO_3, DMF, 100 °C, 30 min, 99%. (i) acetyl chloride, DIPEA, NMP, 45 °C, 6 h, 78%. (j) 30% NaOH and 35% H_2O_2 in water, reflux, 2 h, 61%.

2.2. *In vitro* binding assays and cytotoxicity

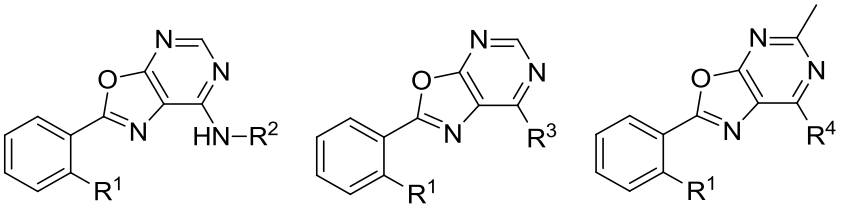
The affinities of each synthesized compound for CB receptors were determined

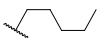
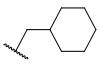
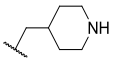
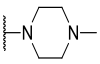
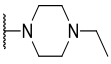
by a competitive radioligand displacement assay using the dual CB_1/CB_2 ligand [^{3}H]-CP55,940 [22, 41]. All compounds were first screened at the concentration of 1 μM. The binding affinities (K_i) were calculated using the Cheng-Prusoff equation based on the experimental IC_{50} values for compounds exhibiting a competitive binding superior to 50% for CB_2. K_d values of CP-55,940 towards CB_1 and CB_2 are 0.62 and 0.85 nM, respectively.

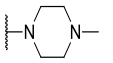
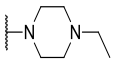
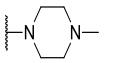
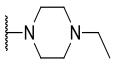
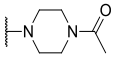
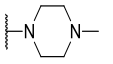
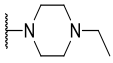
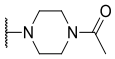
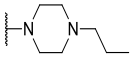
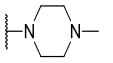
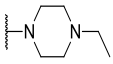
As illustrated in Table 1, compounds **47** and **48** bearing a chlorine at the ortho position of the phenyl ring, a C5-substituted methyl group, and a C7-substituted piperazine moiety, possess the best affinities toward CB_2 receptors, in the double-digit nanomolar range, with good selectivity over CB_1 receptors (SI > 12). The removal of the C5-substituted methyl group of **47** and **48** (K_i = 27.5 nM and 23 nM, respectively) leads to a sharp decrease in CB_2 binding affinities (i.e. compounds **40** and **41**, K_i = 350 and 329 nM, respectively). These results imply that the methyl group at the C5 position plays a crucial role in binding affinity for CB_2, probably due to hydrophobic interactions with the receptor. In general, C7-substituted piperazine derivatives (**40**-**42**, K_i values range from 350 nM to 678 nM) are preferred over morpholine (**39**, K_i > 1000 nM) or secondary amine substituents (**17**-**21**, K_i > 1000 nM). Of note, compounds bearing an *N*-ethylpiperazine at the C7 position (**37**, **41**, and **48**, K_i = 95.5 nM, 329 nM, and 23 nM, respectively) possess slightly superior CB_2 binding affinities than the corresponding compounds bearing a *N*-methylpiperazine (**36**, **40**, and **47**, K_i = 155 nM, 350 nM, and 27.5 nM, respectively) and are approximately 2-3 times more potent CB_2 ligands than compounds bearing a *N*-acetylpiperazine substituent (**38** and

42, K_i = 254 nM and 678 nM, respectively). Specifically, extending the carbon chain on the nitrogen at the C7 position of **41** (K_i = 329 nM) from 2C to 3C (**43**, K_i > 1000 nM) leads to almost complete loss of CB_2 binding affinity. Moreover, large *ortho* substituents (such as CF_3, **36-38**, K_i values range from 95.5 nM to 254 nM, and Cl, **40-42**, K_i values range from 329 nM to 678 nM) on the phenyl ring contribute to better CB_2 binding affinities in comparison with small groups (such as F, **34-35**, K_i > 1000 nM, and H, **32-33**, K_i > 1000 nM). This observation might be attributed to the fact that larger groups make stronger hydrophobic interactions with the CB_2 receptor.

Table 1. Affinities (K_i values) of compounds **17-21**, **32-43**, **47-48**, and reference compounds **3**, **4**, WIN 55,212-2 toward *h*CB_2 and *h*CB_1 cannabinoid receptors, selectivity ratios *h*CB_2 versus *h*CB_1, and cytotoxicity on CHO-WT, CHO-CB_2, and HT29 Cells.



Compounds	R^1	R^2	Binding assays (nM)[a]		CB_1/CB_2	Cytotoxicity assays % inhibition at 10 μM		
			K_i *h*CB_2	K_i *h*CB_1	ratio	CHO-WT	CHO-CB_2	HT29
17	Cl		> 1000	> 1000	N.D.[b]	0	18	0
18	Cl		> 1000	> 1000	N.D.[b]	0	0	0
19	Cl		> 1000	> 1000	N.D.[b]	6	13	10
20	Cl		> 1000	> 1000	N.D.[b]	0	10	0
21	Cl		> 1000	> 1000	N.D.[b]	0	9	41
	R^1	R^3						
32	H		> 1000	> 1000	N.D.[b]	0	0	0
33	H		> 1000	> 1000	N.D.[b]	0	0	0

34	F		> 1000	> 1000	N.D.[b]	8	7	0
35	F		> 1000	> 1000	N.D.[b]	10	8	24
36	CF_3		155 ± 8	> 1000	> 6.4	3	0	0
37	CF_3		95.5 ± 11.5	> 1000	> 10.4	10	5	1
38	CF_3		254 ± 26	> 1000	> 3.9	12	1	12
39	Cl		> 1000	> 1000	N.D.[b]	0	10	5
40	Cl		350 ± 33	> 1000	> 2.8	0	0	8
41	Cl		329 ± 61	> 1000	> 3.0	1	9	4
42	Cl		678 ± 84	> 1000	> 1.4	23	11	0
43	Cl		> 1000	> 1000	N.D.[b]	0	2	0
	R^1	**R^4**						
47	Cl		27.5 ± 0.5	> 1000	> 36.3	0	4	24
48	Cl		23 ± 4	293	12.7	0	16	16
3 (agonist)			51 ± 9	> 1000	> 19.6	30	0	0
4 (inverse agonist)			91 ± 11	> 1000	> 11.0	0	0	0
WIN55212-2 (agonist)			3.01 ± 0.99					

[a] Data represent the mean ± SEM of at least three experiments performed in duplicate or triplicate.

[b] N.D. means not determined.

In summary, the structure-activity relationship (SAR) studies indicate that piperazine moieties at the C7 position favor CB_2 binding affinities of the molecules. Notably, lipophilic piperazine moieties (i.e., methylpiperazine and ethylpiperazine) are preferred for CB_2 affinity rather than a hydrophilic piperazine moiety (i.e., acetylpiperazine). However, there seems to be steric limitations due to a sudden

decrease in CB_2 binding affinity caused by extending the *N*-substituted alkyl chain from 2C to 3C. Moreover, large *ortho* substituents on the phenyl ring are beneficial for CB_2 binding affinities. The introduction of a methyl group at the C5 position seems to significantly improve the CB_2 binding affinities of the molecules (Figure 5). It is noteworthy that the two most potent compounds **47** and **48** (K_i = 27.5 nM and 23 nM, respectively) manifest CB_2 binding affinities twice as potent as the reference compound **3** (K_i = 51 nM). This result implies that oxazolo[5,4-*d*]pyrimidine-based compounds can act as effective bioisosteres of purines in terms of CB_2 binding. Additionally, compounds **47** (SI > 36) and **48** (SI = 12.7) are selective CB_2 ligands over CB_1 receptors.

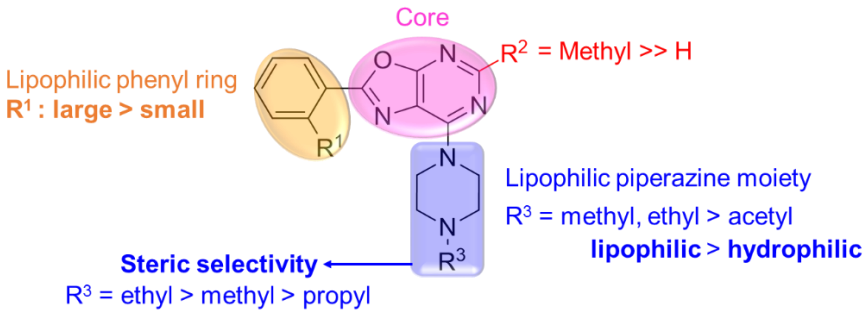


Figure 5. Structure-affinity relationships of oxazolo[5,4-*d*]pyrimidine-based CB_2 ligands.

The cytotoxicity of these compounds was determined at 10 μM using a cell proliferation assay on Chinese hamster ovary cells wild type (CHO-WT), Chinese hamster ovary cells expressing CB_2 receptors (CHO-CB_2), and human colorectal adenocarcinoma cells HT29. This test is based on a colorimetric method, which measures the activity of cellular enzymes that reduce the tetrazolium dye (MTS, yellow) to its insoluble formazan, giving a purple color. This assay measures cellular

metabolic activity via NADPH-dependent cellular oxidoreductase enzymes and reflects, under defined conditions, the number of viable cells [5, 42]. No significant cytotoxicity over 72h was observed for these new compounds.

2.3. Cyclic adenosine monophosphate (cAMP) assays

Eight compounds displaying potent CB_2 affinity were further studied for their functionality by cAMP assays in CHO-CB_2 cells. Cells were treated with forskolin (3 μM) in order to activate adenylyl cyclase and thereby producing cAMP [43-45]. CB_2 ligands can be classified as agonists, neutral antagonists, or inverse agonists regarding to their functional effects on cAMP formation. Agonists inhibit forskolin-induced cAMP production, whereas inverse agonists promote cAMP production. On the contrary, neutral antagonists do not significantly alter cAMP levels [46, 47]. As shown in Table 2, the maximum efficacy (E_{max}) of a compound represents the maximum response at 1 μM and is expressed as the percentage of forskolin-induced cAMP production [5]. Despite their potent binding affinities toward CB_2 receptors, these compounds did not significantly affect CB_2-mediated regulation of cAMP accumulation (E_{max} values range from 88% to 131%), compared with forskolin (100%). Data in Figure 6 further demonstrated that compounds **47** and **48** showed competitive antagonist properties. These two compounds remarkably antagonized CB_2 agonist (0.1 μM WIN 55,212-2)-induced inhibition of cAMP formation at 1 μM and almost completely restored forskolin-induced cAMP levels at 10 μM. IC_{50} values of these two compounds are 0.93 μM (**47**) and 0.12 μM (**48**), respectively.

Interestingly, although replacement of the purine core by oxazolo[5,4-*d*]pyrimidine does not alter the binding affinity of the molecule toward CB_2, it appears to alter the biological response from a CB_2 agonist to competitive neutral antagonist.

Table 2. Maximum efficacy (E_{max}) of compounds **36**-**38**, **40**-**42**, **47**-**48**, and reference compounds **3**, **4**, WIN 55,212-2 at 1 μM.

Compounds	CB_2 cAMP assays E_{max} (%)[a]
36	129 ± 3
37	117 ± 15
38	107 ± 16
40	131 ± 13
41	120 ± 8
42	117 ± 7
47	94 ± 2
48	88 ± 7
forskolin	100
3 (agonist)	71 ± 3
4 (inverse agonist)	863 ± 28
WIN55212-2 (agonist)	64 ± 12

[a] Data represent the mean ± SEM of at least three independent experiments performed in duplicate or triplicate. E_{max} values are expressed as the percentage of forskolin-induced cAMP production. Forskolin (3 μM) effect was set to 100%.

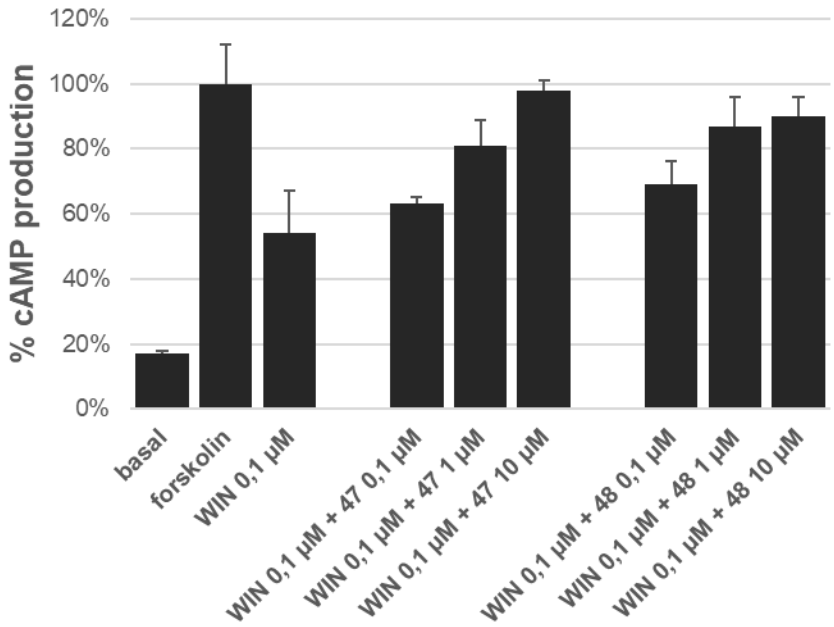


Figure 6. Inhibition of forskolin-stimulated cAMP formation by WIN 55,212-2 (0.1 μM) and antagonism by **47** and **48** (0.1-10 μM) in CB_2-CHO cells. Forskolin (3 μM) effect was set to 100%.

Data represent the mean ± SEM of at least three experiments performed in duplicate.

2.4. Metabolic stability in mice liver microsomes

These eight potent CB_2 ligands have also been examined for their in vitro metabolic stability in the presence of mice liver microsomes (Figure 7, Table 3). It was found that almost all of compounds **36**, **37**, **40** and **41** were rapidly eliminated under 5 min ($t_{1/2} \approx 1$ min). Compounds **47** and **48** showed lower intrinsic clearance ($t_{1/2} = 4.1$ and 8.1 min respectively) than compounds **40** and **41** ($t_{1/2} = 1.3$ and 1.2 min respectively). This observation implies that a C5-substituted methyl group confers higher metabolic stability to the molecules[48], although the clearance values are still rather high. In addition, the replacement of an *N*-alkyl group by an *N*-acetyl group on the piperazine moiety (**38** and **42**, $t_{1/2} = 12.5$ and 12.2 min respectively) has been deemed to endow the molecules with significantly improved metabolic stability. Although these eight newly synthesized compounds are less metabolically stable than compounds **3** in mice liver microsomes, the study of their structure-stability relationships may be helpful to further improve the metabolic stability of oxazolo[5,4-*d*]pyrimidine derivatives.

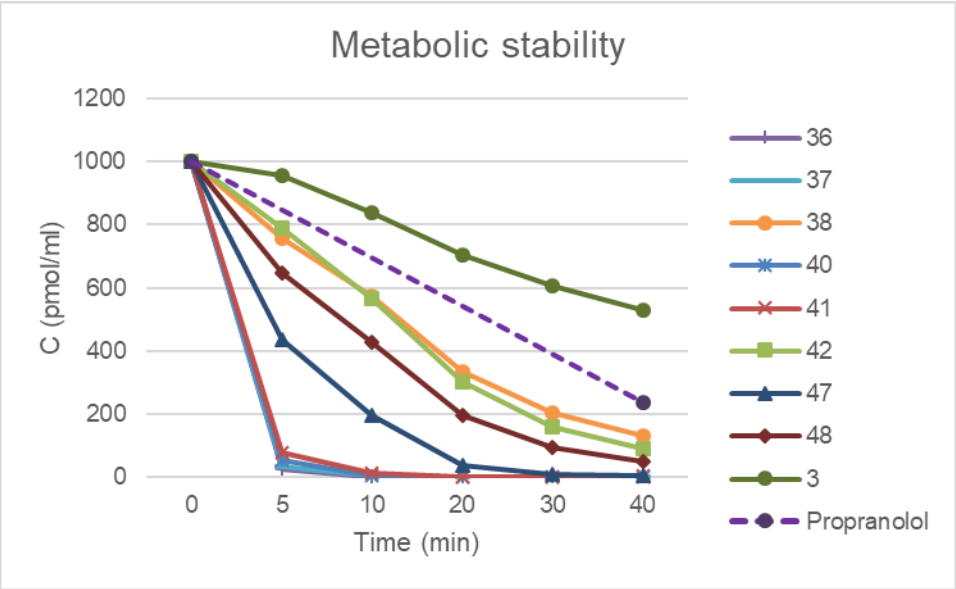


Figure 7. Metabolic stability of compounds **36**-**38**, **40**-**42**, **47**, **48**, **3**, and propranolol.

Table 3. Cl_{int} and $t_{1/2}$ of compounds **36**-**38**, **40**-**42**, **47**, **48**, and **3** in the presence of mice liver microsomes.

Compounds	Cl_{int} (µL/min/mg)[a]	$t_{1/2}$ (min)
36	1257	0.9
37	1236	1.0
38	174	12.5
40	1178	1.3
41	1101	1.2
42	194	12.2
47	502	4.1
48	263	8.1
3	54	44

[a] Cl_{int} = dose/AUC, expressed as µL/min/mg proteins.

3. Conclusion and perspective

A novel series of oxazolo[5,4-*d*]pyrimidines has been synthesized. Some of them display potent affinity toward CB_2 at nanomolar concentrations and significant selectivity over CB_1. Especially, compounds **47** and **48** have been identified as competitive neutral antagonists. Our studies indicate that oxazolo[5,4-*d*]pyrimidine-based compounds can act as effective bioisosteres of

purines in CB_2 binding. Interestingly, such a replacement changes the biological response from a CB_2 agonist to a competitive neutral antagonist.

There are many reports on CB_2 agonists and inverse agonists but few in the case of neutral antagonists. During the last few years, several clinical trials of CB_2 agonists have been launched. The therapeutic potential of CB_2 inverse agonists has also progressed. To the best of our knowledge, research on neutral antagonists is rare. Indeed, the first reported antagonist SR144528 was then identified to behave as an inverse agonist [16, 49]. Some purported "antagonists" are actually inverse agonists [50-54]. The real first reported CB_2 neutral antagonist may be WIN55212-3 (Figure 8), an enantiomer of WIN55212-2, but it showed weak affinity toward CB_2 (K_i > 1 μM). Another reported CB_2 neutral antagonist is XIE35-1001 (Figure 8) [55]. However, data in a cAMP assay or [^{35}S]-GTPγS assay are missing. Recently, Bertini and coworkers described a non-competitive CB_2 neutral antagonist (**49**, Figure 8) [56], which significantly reversed CB_2 agonist (1 nM HU-210) responses at 10 μM but did not alter maximal responses of the agonist (0.1 μM HU-210) even at 20 μM in a [^{35}S]-GTPγS assay. On the contrary, our two neutral antagonists **47** and **48** remarkably reversed CB_2 agonist (0.1 μM WIN 55,212-2)-induced inhibition of cAMP formation at 1 μM. This observation makes compounds **47** and **48** useful tools for studying the function of CB_2 receptors at the cellular level.

In this context, we identified that oxazolo[5,4-*d*]pyrimidine could be a useful scaffold for the development of new selective neutral antagonists toward CB_2.

Moreover, although CB_2 inverse antagonists have shown therapeutic potential in bone-dependent disorders and inflammation through somehow modulating the migration of immune cells, relevant mechanisms remain unclear. The discovery of new CB_2 neutral antagonists with high affinity might be helpful for elucidating how CB_2 inverse agonists regulate bone proliferation and the migration of immune cells. Notably, compounds **47** and **48**, which display nanomolar potency and behave as competitive neutral antagonists toward CB_2, may serve as lead compounds for further studies. Furthermore, we will continue the structural optimization of these oxazolepyrimidines to improve their metabolic stability so that they can be suitable for systemic applications.

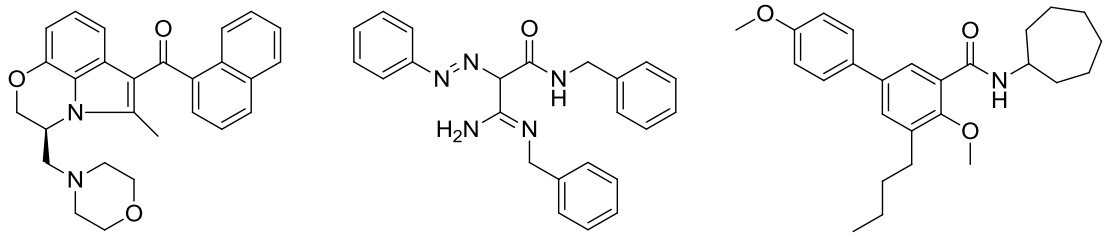


Figure 8. Structures of representative CB_2 neutral antagonists.

4. Experimental section

4.1. Chemistry

4.1.1. General procedures

All reactions involving air or moisture-sensitive compounds were performed under argon atmosphere. Solvents and reagents were purchased from commercial suppliers and were used without further purification. The thin layer chromatography

was performed using silica gel plates (Polygram Sil G/UV254, thickness: 0.2 mm, ref. 805023 Macherey Nagel). The revelation was made under UV (253 and 366 nm). The purifications by column chromatography were performed using silica gel 60, granulometry 40-63 μm (ref. 9385.5000 Merck). The microwave reactions were performed using a CEM DiscoverSP. Melting points were recorded on a Büchi melting point apparatus and are uncorrected. They are expressed in degrees Celsius. NMR spectra were recorded at 300 (^{1}H) and 75 (^{13}C) MHz on a Bruker AC300P Fourier transform spectrometer. Chemical shifts (δ) are reported relative to tetramethylsilane (TMS) as the internal standards in the NMR laboratory of Physical Chemistry of University Lille 2. Spectra were recorded at ambient temperature. Each signal is identified by its chemical shift δ expressed in parts per million (ppm). Coupling constant (*J*) is reported in Hertz (Hz). All target compounds were characterized by LC-MS and HRMS. The high performance liquid chromatograph (ODS column, mobile phase: $H_2O/CH_3CN/HCOOH$ using gradient method) is coupled to a UV detector and a type of $APCI^+$ (atmospheric pressure chemical ionization) mass detector. HRMS analyzes were performed under ESI (electrospray ionization) using a TOF analyzer in V mode with a mass resolution of 9000. The spectra were recorded at the Centre Universitaire des Mesures Analytique of University Lille 2 using Thermo Electron Surveyor MSQ mass spectrometer. The purity of all compounds was determined by HPLC using a Chromazing column (4.6 x 150 mm, 5 μm, 100 Å, mobile phase: $CH_3CN/H_2O/HCOOH$ = 100/25/1) and a WATERS 600 pump chromatograph equipped with a WATERS 2487 dual absorption

wavelength UV detector (λ = 254 nm and 366 nm). Retention time was obtained with flow rates of 1 mL/min. The acquisition time is 20 min.

4.1.2. Synthesis of 5-amino-2-(2-substituted phenyl)oxazole-4-carbonitriles (**11**-**14**)

A solution of benzoyl chloride or ortho halogenated benzoyl chloride (4.8 mmol, 1.2 equiv) and aminomalononitrile p-toluenesulfonate (4 mmol, 1 equiv) in 15 mL NMP was stirred at room temperature for 10 min, and then heated by microwave irradiation at 80 °C for 25 min. At the end of the reaction, after cooling, the solution was diluted with 100 mL saturated brine and neutralized (pH ~8) to give a white precipitate, which was further recrystallized in CH_2Cl_2 to afford compounds **11**-**14**. These compounds were used for the next reaction.

4.1.3. Synthesis of 2-(2-chlorophenyl)oxazolo[5,4-*d*]pyrimidin-7-amine (**15**)

A solution of compound **13** (2.5 mmol, 1 equiv) and formamidine acetate (3 mmol, 1.2 equiv) in 15 mL NMP was stirred and heated at 100 °C for 3 h. At the end of the reaction, after cooling, the solution was diluted with 100 mL saturated brine to give a white precipitate, which was recrystallized in CH_2Cl_2 to afford compound **15**. This compound was used for the next reaction.

4.1.4. Synthesis of 2-(2-chlorophenyl)-*N*-alkyloxazolo[5,4-*d*]pyrimidin-7-amines (**16**-**20**)

To a solution of compound **15** (0.25 mmol, 0.1 equiv) and brominated derivative (0.2 mmol, 0.8 equiv) in 15 mL DMF, cooled to 0 °C, 15 wt. % KHMDS (0.3 mmol,

1.2 equiv) solution in toluene was added dropwise. Afterwards, the mixture solution was recovered to room temperature and stirred for 3 h. At the end of the reaction, the solution was diluted with 100 mL saturated brine, and then extracted with 80 mL ethyl acetate. The organic phase was washed twice with 50 mL distilled water and dried over $MgSO_4$. The solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography using CH_2Cl_2/ethyl acetate (8: 2 v/v) as eluent to give compounds **16**-**20**.

4.1.4.1. *N*-Butyl-2-(2-chlorophenyl)oxazolo[5,4-*d*]pyrimidin-7-amine (**17**)

The title compound was obtained as a white solid, mp 104-105 °C, purity (HPLC): 98.8%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.46 – 8.19 (m, 2H), 8.09 (d, J = 7.8 Hz, 1H), 7.72 (dd, J = 7.9, 1.6 Hz, 1H), 7.70 – 7.52 (m, 2H), 3.90 – 3.71 (m, 0.5H), 3.62 – 3.41 (m, 1.5H), 1.73 – 1.50 (m, 2H), 1.48 – 1.27 (m, 2H), 0.92 (t, J = 7.3 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 164.1 (C), 156.2 (C), 155.5 (C), 155.2 (CH_A), 154.8 (CH_B), 133.3 (CH), 132.4 (C), 132.2 (CH), 131.7 (CH), 128.2 (CH), 125.8 (C), 116.1 (C), 42.7 (CH_{2B}), 40.3 (CH_{2A}), 32.5 (CH_{2B}), 31.4 (CH_{2A}), 20.1 (CH_2), 14.2 (CH_3). HRMS (ESI) calcd. for $C_{15}H_{15}ON_4Cl$ ($[M+H]^+$) 303.10072, found 303.10004.

4.1.4.2. 2-(2-Chlorophenyl)-*N*-(2-methoxyethyl)oxazolo[5,4-*d*]pyrimidin-7-amine (**18**)

The title compound was obtained as a white solid, mp 124-125 °C, purity (HPLC): 95.1%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.43 – 8.20 (m, 2H), 8.10 (dd, J =

7.6, 2.0 Hz, 1H), 7.72 (dd, *J* = 7.8, 1.6 Hz, 1H), 7.70 – 7.52 (m, 2H), 4.09 – 3.88 (m, 0.5H), 3.76 – 3.64 (m, 1.5H), 3.63 – 3.51 (m, 2H), 3.28 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 164.1 (C), 156.3 (C), 155.5 (C), 155.1 (CH), 133.3 (CH), 132.4 (C), 132.2 (CH), 131.7 (CH), 128.2 (CH), 125.7 (C), 116.3 (C), 71.4 (CH_{2B}), 70.5 (CH_{2A}), 58.4 (CH_3), 42.6 (CH_{2B}), 40.1 (CH_{2A}). HRMS (ESI) calcd. for $C_{14}H_{13}O_2N_4Cl$ ($[M+H]^+$) 305.07998, found 305.07927.

4.1.4.3. 2-(2-Chlorophenyl)-*N*-pentyloxazolo[5,4-*d*]pyrimidin-7-amine (**19**)

The title compound was obtained as a white solid, mp 122-123 °C, purity (HPLC): 98.4%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.43 – 8.19 (m, 2H), 8.08 (dd, *J* = 7.5, 1.9 Hz, 1H), 7.70 (dd, *J* = 7.9, 1.5 Hz, 1H), 7.68 – 7.50 (m, 2H), 3.86 – 3.68 (m, 0.5H), 3.58 – 3.41 (m, 1.5H), 1.72 – 1.49 (m, 2H), 1.38 – 1.25 (m, 4H), 0.86 (t, *J* = 6.7 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 164.1 (C), 156.2 (C), 155.5 (C), 155.2 (CH_A), 154.8 (CH_B), 133.3 (CH), 132.4 (C), 132.2 (CH), 131.7 (CH), 128.2 (CH), 125.8 (C), 116.1 (C), 43.0 (CH_{2B}), 40.8 (CH_{2A}), 30.0 (CH_{2B}), 29.0 (CH_{2A} × 2), 22.4 (CH_2), 14.4 (CH_3). HRMS (ESI) calcd. for $C_{16}H_{17}ON_4Cl$ ($[M+H]^+$) 317.11637, found 317.11586.

4.1.4.4. 2-(2-Chlorophenyl)-*N*-(cyclohexylmethyl)oxazolo[5,4-*d*]pyrimidin-7-amine (**20**)

The title compound was obtained as a solid, mp 140-141 °C, purity (HPLC): 97.6%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.45 – 8.20 (m, 2H), 8.08 (d, *J* = 7.7 Hz, 1H), 7.76 – 7.66 (m, 1H), 7.69 – 7.51 (m, 2H), 3.70 – 3.57 (m, 0.5H), 3.36 (t, *J* = 6.4

Hz, 1.5H), 1.83 – 1.55 (m, 6H), 1.28 – 1.11 (m, 3H), 1.06 – 0.86 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 164.1 (C), 156.2 (C), 155.7 (C), 155.1 (CH_A), 154.8 (CH_B), 133.3 (CH), 132.4 (C), 132.3 (CH), 131.7 (CH), 128.2 (CH), 125.8 (C), 116.1 (C), 48.9 (CH_{2B}), 46.7 (CH_{2A}), 38.6 (CH_B), 37.6 (CH_A), 30.9 (CH_2 × 2), 26.6 (CH_2), 25.8 (CH_2 × 2). HRMS (ESI) calcd. for $C_{18}H_{20}ON_4Cl$ ($[M+H]^+$) 343.13202, found 343.13139.

4.1.5. Synthesis of 2-(2-chlorophenyl)-*N*-(piperidin-4-ylmethyl)oxazolo[5,4-*d*] pyrimidin-7-amine (**21**)

To a solution of compound **16** (0.2 mmol, 1 equiv) in 50 mL CH_2Cl_2 was added TFA (2 mmol, 10 equiv). The mixture was stirred at room temperature for 3 h. At the end of the reaction, the solvent was removed under reduced pressure and the residue was dissolved in water and the solution was neutralised to pH 9 using 30% NaOH, then the solution was extracted with ethyl acetate. The organic layer was washed with brine and distilled water successively, dried over $MgSO_4$ and evaporated under reduced pressure to give compound **21**. The title compound was obtained as a white solid, mp > 270 °C, purity (HPLC): 96.8%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.53 – 8.19 (m, 2H), 8.09 (d, J = 7.6 Hz, 1H), 7.71 (dd, J = 7.8, 1.6 Hz, 1H), 7.70 – 7.51 (m, 2H), 3.71 – 3.61 (m, 0.5H), 3.44 – 3.33 (m, 1.5H), 3.00 – 2.84 (m, 2H), 2.46 – 2.31 (m, 2H), 1.88 – 1.52 (m, 3H), 1.20 – 0.92 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 164.1 (C), 156.2 (C), 155.7 (C), 155.1 (CH_A), 154.8 (CH_B), 133.3 (CH), 132.4 (C), 132.2 (CH), 131.7 (CH), 128.2 (CH), 125.8 (C), 116.1 (C), 48.9 (CH_{2B}), 46.7 (CH_{2A}),

46.3 (CH_2 × 2), 37.7 (CH_B), 36.6 (CH_A), 31.5 (CH_2), 31.2 (CH_2). HRMS (ESI) calcd. for $C_{17}H_{19}ON_5Cl$ ($[M+H]^+$) 344.12726, found 344.12650.

4.1.6. Synthesis of 2-(2-substituted phenyl)oxazolo[5,4-*d*]pyrimidin-7-ols (**22**-**25**)

To a solution of compound **11**, **12**, **13**, or **14** (1 mmol) in 20 mL formic acid was refluxed overnight. At the end of the reaction, after cooling, formic acid was removed under reduced pressure. The residue was purified by silica gel column chromatography using CH_2Cl_2/ethyl acetate (6: 4 v/v) as eluent to give compound **22**, **23**, **24**, or **25**, respectively.

4.1.7. Synthesis of 7-chloro-2-(2-substituted phenyl)oxazolo[5,4-*d*]pyrimidines (**26**-**29**)

To a solution of compound **22**, **23**, **24**, or **25** (0.5 mmol, 1 equiv) in 30 mL toluene were added DIPEA (0.75 mmol, 1.5 equiv) and $POCl_3$ (1.5 mmol, 3 equiv) successively at 0 °C. The mixture was stirred for 2 h. At the end of the reaction, the solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography using CH_2Cl_2/ethyl acetate (95: 5 v/v) as eluent to give compound **26**, **27**, **28**, or **29**, respectively.

4.1.8. Synthesis of 2-(2-substituted phenyl)-7-(4-substituted piperazinyl or morpholine)oxazolo[5,4-*d*]pyrimidines (**30**, **32**-**42**)

To a solution of compound **26**, **27**, **28**, or **29** (0.15 mmol, 1 equiv) in 15 mL DMF were added Cs_2CO_3 (0.3 mmol, 2 equiv) and piperazine derivative/morpholine

(0.18 mmol, 1.2 equiv). The mixture was stirred and heated at 100 °C for 30 min. At the end of the reaction, the solution was diluted with 50 mL saturated brine, and then extracted twice with 30 mL ethyl acetate. The organic phase was washed twice with 50 mL distilled water and dried over $MgSO_4$. The solvent was evaporated under reduced pressure to give pure target compound directly or crude compound, which was purified by silica gel column chromatography using ethyl acetate/methanol (98: 2 v/v) as eluent.

4.1.8.1. 7-(4-Methylpiperazin-1-yl)-2-phenyloxazolo[5,4-*d*]pyrimidine (**32**)

The title compound was obtained as a yellow solid, mp 136-137 °C, purity (HPLC): 99.3%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.36 (s, 1H), 8.24 – 8.13 (m, 2H), 7.56 – 7.46 (m, 3H), 4.55 – 4.06 (m, 4H), 2.64 – 2.52 (m, 4H), 2.38 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.5 (C), 157.5 (C), 154.1 (C), 153.7 (CH), 131.6 (CH), 128.9 (CH × 2), 127.3 (CH × 2), 126.5 (C), 116.9 (C), 55.0 (CH_2 × 2), 46.2 (CH_3), 45.1 (CH_2 × 2). HRMS (ESI) calcd. for $C_{16}H_{18}ON_5$ ($[M+H]^+$) 296.15059, found 296.14967.

4.1.8.2. 7-(4-Ethylpiperazin-1-yl)-2-phenyloxazolo[5,4-*d*]pyrimidine (**33**)

The title compound was obtained as a yellow solid, mp 120-121 °C, purity (HPLC): 99.3%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.36 (s, 1H), 8.25 – 8.12 (m, 2H), 7.56 – 7.47 (m, 3H), 4.57 – 4.00 (m, 4H), 2.70 – 2.57 (m, 4H), 2.50 (q, J = 7.1 Hz, 2H), 1.15 (t, J = 7.1 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.5 (C), 157.5 (C), 154.1 (C), 153.7 (CH), 131.5 (CH), 128.9 (CH × 2), 127.3 (CH × 2), 126.5 (C), 116.8

(C), 52.8 (CH_2 × 2), 52.4 (CH_2), 44.9 (CH_2 × 2), 11.9 (CH_3). HRMS (ESI) calcd. for $C_{17}H_{20}ON_5$ ($[M+H]^+$) 310.16624, found 310.16501.

4.1.8.3. 2-(2-Fluorophenyl)-7-(4-methylpiperazin-1-yl)oxazolo[5,4-*d*]pyrimidine (**34**)

The title compound was obtained as a yellow solid, mp 142-143 °C, purity (HPLC): 98.1%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.38 (s, 1H), 8.19 – 8.11 (m, 1H), 7.56 – 7.46 (m, 1H), 7.33 – 7.27 (m, 1H), 7.25 – 7.19 (m, 1H), 4.54 – 4.09 (m, 4H), 2.57 (t, *J* = 5.0 Hz, 4H), 2.37 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.3 (C), 160.67 (C, d, *J* = 259.8 Hz), 154.2 (C), 154.0 (CH), 153.8 (C, d, *J* = 5.7 Hz), 133.1 (CH, d, *J* = 8.5 Hz), 130.1 (CH), 124.5 (CH, d, *J* = 3.8 Hz), 117.1 (CH, d, *J* = 21.0 Hz), 116.6 (C), 115.0 (C, d, *J* = 10.6 Hz), 55.0 (CH_2 × 2), 46.2 (CH_3), 45.1 (CH_2 × 2). HRMS (ESI) calcd. for $C_{16}H_{17}ON_5F$ ($[M+H]^+$) 314.14116, found 314.14037.

4.1.8.4. 7-(4-Ethylpiperazin-1-yl)-2-(2-fluorophenyl)oxazolo[5,4-*d*]pyrimidine (**35**)

The title compound was obtained as a yellow solid, mp 127-128 °C, purity (HPLC): 99.4%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.38 (s, 1H), 8.19 – 8.11 (m, 1H), 7.56 – 7.46 (m, 1H), 7.33 – 7.27 (m, 1H), 7.25 – 7.19 (m, 1H), 4.54 – 4.07 (m, 4H), 2.61 (t, *J* = 5.1 Hz, 4H), 2.50 (q, *J* = 7.2 Hz, 2H), 1.15 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.3 (C), 160.7 (C, d, *J* = 259.9 Hz), 154.1 (C), 154.0 (CH), 153.8 (C, d, *J* = 5.6 Hz), 133.1 (CH, d, *J* = 8.5 Hz), 130.1 (CH), 124.5 (CH, d, *J* = 3.9 Hz), 117.1 (CH, d, *J* = 21.0 Hz), 116.6 (C), 115.0 (C, d, *J* = 10.4 Hz), 52.8 (CH_2 × 2), 52.4 (CH_2), 45.1 (CH_2 × 2), 11.9 (CH_3). HRMS (ESI) calcd. for $C_{17}H_{19}ON_5F$ ($[M+H]^+$) 328.15681, found 328.15533.

4.1.8.5.

7-(4-Methylpiperazin-1-yl)-2-(2-(trifluoromethyl)phenyl)oxazolo[5,4-*d*]pyrimidine (**36**)

The title compound was obtained as a yellow solid, mp 137-138 °C, purity (HPLC): 99.3%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.39 (s, 1H), 8.19 – 8.09 (m, 1H), 7.93 – 7.83 (m, 1H), 7.77 – 7.59 (m, 2H), 4.57 – 3.99 (m, 4H), 2.58 (t, J = 5.0 Hz, 4H), 2.37 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.6 (C), 154.7 (C), 154.3 (CH, C), 132.0 (CH), 131.6 (CH), 131.1 (CH), 129.0 (C, q, J = 32.2 Hz), 127.4 (CH, q, J = 5.8 Hz), 125.3 (C), 123.3 (CF_3, q, J = 273.7 Hz), 116.6 (C), 54.9 (CH_2 × 2), 46.1 (CH_3), 45.1 (CH_2 × 2). HRMS (ESI) calcd. for $C_{17}H_{17}ON_5F_3$ ($[M+H]^+$) 364.13797, found 364.13656.

4.1.8.6.

7-(4-Ethylpiperazin-1-yl)-2-(2-(trifluoromethyl)phenyl)oxazolo[5,4-*d*]pyrimidine (**37**)

The title compound was obtained as a yellow solid, mp 104-105 °C, purity (HPLC): 98.1%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.39 (s, 1H), 8.18 (d, J = 7.3 Hz, 1H), 8.02 (d, J = 7.3 Hz, 1H), 7.96 – 7.80 (m, 2H), 4.51 – 3.86 (m, 4H), 2.59 – 2.50 (m, 4H), 2.38 (q, J = 7.1 Hz, 2H), 1.03 (t, J = 7.1 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.6 (C), 154.7 (C), 154.3 (CH, C), 132.0 (CH), 131.6 (CH), 131.1 (CH), 129.0 (C, q, J = 32.2 Hz), 127.4 (CH, q, J = 5.8 Hz), 125.3 (C), 123.3 (CF_3, q, J = 271.9 Hz), 116.6 (C), 52.7 (CH_2 × 2), 52.4 (CH_2), 45.2 (CH_2 × 2), 11.9 (CH_3). HRMS (ESI) calcd. for $C_{18}H_{19}ON_5F_3$ ($[M+H]^+$) 378.15362, found 378.15199.

4.1.8.7.

1-(4-(2-(2-(Trifluoromethyl)phenyl)oxazolo[5,4-*d*]pyrimidin-7-yl)piperazin-1-yl)etha none (**38**)

The title compound was obtained as a yellow solid, mp 145-146 °C, purity (HPLC): 96.3%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.42 (s, 1H), 8.19 (d, J = 6.7 Hz, 1H), 8.07 – 7.98 (m, 1H), 7.96 – 7.83 (m, 2H), 4.45 – 3.89 (m, 4H), 3.71 – 3.55 (m, 4H), 2.07 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 169.0 (C=O), 165.7 (C), 155.1 (C), 154.6 (CH), 154.2 (C), 133.6 (CH), 132.7 (CH), 132.6 (CH), 127.7 (C, q, J = 31.9 Hz), 127.9 (CH, q, J = 5.5 Hz), 124.7 (C), 123.9 (CF_3, q, J = 273.3 Hz), 116.2 (C), 45.7 (CH_2), 44.9 (CH_2 × 2), 41.0 (CH_2), 21.7 (CH_3). HRMS (ESI) calcd. for $C_{18}H_{17}O_2N_5F_3$ ($[M+H]^+$) 392.13289, found 392.13206.

4.1.8.8. 2-(2-Chlorophenyl)-7-morpholinooxazolo[5,4-*d*]pyrimidine (**39**)

The title compound was obtained as a yellow solid, mp 155-156 °C, purity (HPLC): 98.2%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.40 (s, 1H), 8.12 (dd, J = 7.6, 1.9 Hz, 1H), 7.71 (dd, J = 7.9, 1.4 Hz, 1H), 7.67 – 7.53 (m, 2H), 4.50 – 3.88 (m, 4H), 3.76 (t, J = 4.8 Hz, 4H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 165.4 (C), 155.2 (C), 154.5 (CH), 154.2 (C), 133.5 (CH), 132.5 (C), 132.1 (CH), 131.9 (CH), 128.3 (CH), 125.1 (C), 116.1 (C), 66.5 (CH_2 × 2), 45.8 (CH_2 × 2). HRMS (ESI) calcd. for $C_{15}H_{14}O_2N_4Cl$ ($[M+H]^+$) 317.07998, found 317.07944.

4.1.8.9. 2-(2-Chlorophenyl)-7-(4-methylpiperazin-1-yl)oxazolo[5,4-*d*]pyrimidine (**40**)

The title compound was obtained as a yellow solid, mp 135-136 °C, purity (HPLC): 99.9%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.37 (s, 1H), 8.14 – 8.07 (m, 1H), 7.74 – 7.67 (m, 1H), 7.66 – 7.53 (m, 2H), 4.47 – 3.87 (m, 4H), 2.45 (t, J = 5.0 Hz, 4H), 2.22 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 165.4 (C), 155.0 (C), 154.5 (CH), 154.1 (C), 133.4 (CH), 132.4 (C), 132.1 (CH), 131.8 (CH), 128.3 (CH), 125.1 (C), 116.0 (C), 54.8 (CH_2 × 2), 46.1 (CH_3), 45.1 (CH_2 × 2). HRMS (ESI) calcd. for $C_{16}H_{17}ON_5Cl$ ($[M+H]^+$) 330.11161, found 330.11082.

4.1.8.10. 2-(2-Chlorophenyl)-7-(4-ethylpiperazin-1-yl)oxazolo[5,4-*d*]pyrimidine (**41**)

The title compound was obtained as a yellow solid, mp 115-116 °C, purity (HPLC): 99.0%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.38 (s, 1H), 8.14 – 8.08 (m, 1H), 7.74 – 7.68 (m, 1H), 7.67 – 7.54 (m, 2H), 4.52 – 3.89 (m, 4H), 2.75 – 2.50 (m, 4H), 2.47 – 2.32 (m, 2H), 1.05 (t, J = 7.2 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 165.4 (C), 155.1 (C), 154.5 (CH), 154.1 (C), 133.4 (CH), 132.5 (C), 132.1 (CH), 131.9 (CH), 128.3 (CH), 125.1 (C), 116.0 (C), 52.5 (CH_2), 51.9 (CH_2 × 2), 44.8 (CH_2 × 2), 12.2 (CH_3). HRMS (ESI) calcd. for $C_{17}H_{19}ON_5Cl$ ($[M+H]^+$) 344.12726, found 344.12641.

4.1.8.11.

1-(4-(2-(2-Chlorophenyl)oxazolo[5,4-*d*]pyrimidin-7-yl)piperazin-1-yl)ethanone (**42**)

The title compound was obtained as a yellow solid, mp 189-190 °C, purity (HPLC): 97.3%. ^{1}H NMR (300 MHz, $CDCl_3$) δ ^{1}H NMR 8.43 (s, 1H), 8.17 – 8.07 (m, 1H), 7.62 – 7.53 (m, 1H), 7.53 – 7.37 (m, 2H), 4.49 – 4.12 (m, 4H), 3.80 (t, J = 5.4 Hz, 2H), 3.65 (t, J = 5.2 Hz, 2H), 2.19 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.3 (C=O),

165.3 (C), 155.6 (C), 154.1 (C, CH), 133.5 (C), 132.2 (CH), 131.7 (CH), 131.3 (CH), 127.1 (CH), 125.0 (C), 116.7 (C), 46.2 (CH_2), 45.1 (CH_2 × 2), 41.4 (CH_2), 21.5 (CH_3). HRMS (ESI) calcd. for $C_{17}H_{17}O_2N_5Cl$ ($[M+H]^+$) 358.10653, found 358.10581.

4.1.9. Synthesis of 2-(2-chlorophenyl)-7-(piperazin-1-yl)oxazolo[5,4-*d*]pyrimidine (**31**)

The synthesis of **31** started from compound **30**. The synthetic procedure is the same as in part 4.1.5. It was used for the next step directly.

4.1.10. Synthesis of 2-(2-chlorophenyl)-7-(4-propylpiperazin-1-yl)oxazolo[5,4-*d*] pyrimidine (**43**)

To a solution of compound **31** (0.15 mmol, 1 equiv) in 15 mL DMF were added Cs_2CO_3 (0.3 mmol, 2 equiv) and bromopropane (0.18 mmol, 1.2 equiv). The mixture was stirred and heated at 100 °C for 30 min. At the end of the reaction, after cooling, the solution was diluted with 50 mL saturated brine, and then extracted twice with 30 mL ethyl acetate. The organic phase was washed twice with 50 mL distilled water and dried over $MgSO_4$. The solvent was evaporated under reduced pressure to give target compound **43**. The title compound is yellow solid, mp 136-137 °C, purity (HPLC): 95.5%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.38 (s, 1H), 8.14 – 8.07 (m, 1H), 7.59 – 7.53 (m, 1H), 7.48 – 7.38 (m, 2H), 4.68 – 3.89 (m, 4H), 2.68 – 2.52 (m, 4H), 2.38 (t, J = 7.7 Hz, 2H), 1.64 – 1.50 (m, 2H), 0.94 (t, J = 7.4 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.3 (C), 155.1 (C), 154.2 (C), 154.1 (CH), 133.4 (C), 131.9 (CH), 131.6 (CH), 131.3 (CH), 127.0 (CH), 125.3 (C), 116.5 (C), 60.6 (CH_2), 53.2 (CH_2 × 2), 45.0 (CH_2

× 2), 19.9 (CH_2), 11.9 (CH_3). HRMS (ESI) calcd. for $C_{18}H_{21}ON_5Cl$ ($[M+H]^+$) 358.14291, found 358.14179.

4.1.11. Synthesis of *N*-(2-(2-chlorophenyl)-4-cyanooxazol-5-yl)acetamide (**44**)

To solution of compound **13** (1 mmol, 1 equiv) in 30 mL NMP was added DIPEA (4 mmol, 4 equiv) and acetyl chloride (4 mmol, 4 equiv) dropwise, successively. The mixture was stirred and heated at 45 °C for 6 h. At the end of the reaction, after cooling, the solution was diluted with 200 mL saturated brine, and then extracted twice with 100 mL ethyl acetate. The organic phase was washed twice with 150 mL distilled water and dried over $MgSO_4$. The solvent was evaporated under reduced pressure. The crude product was used for the next step without purification.

4.1.12. Synthesis of 2-(2-chlorophenyl)-5-methyloxazolo[5,4-*d*]pyrimidin-7-ol (**45**)

A solution of crude **44** (0.5 mmol) in 30 mL 30% NaOH and 10 mL 35% H_2O_2 (aqueous solution) was refluxed for 2 h. At the end of the reaction, the solution was acidified using 3 N HCl to pH 2 and a white flocculent precipitate appeared. The mixture was extracted twice with 30 mL ethyl acetate. The organic phase was washed twice with 50 mL distilled water and dried over $MgSO_4$. The solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography using CH_2Cl_2/ethyl acetate (6: 4 v/v) as eluent to give compound **45**.

4.1.13. Synthesis of 7-chloro-2-(2-chlorophenyl)-5-methyloxazolo[5,4-*d*]pyrimidine (**46**)

The synthesis of **46** started from compound **45**. The synthetic procedure is the same as in part 4.1.7.

4.1.14. Synthesis of 2-(2-chlorophenyl)-5-methyl-7-(4-substituted piperazin-1-yl) oxazolo [5,4-*d*]pyrimidines (**47**-**48**)

The synthesis of compounds **47**-**48** started from compound **46**. The synthetic procedure is the same as in part 4.1.8.

4.1.14.1.

2-(2-Chlorophenyl)-5-methyl-7-(4-methylpiperazin-1-yl)oxazolo[5,4-*d*]pyrimidine (**47**)

The title compound was obtained as a yellow solid, mp 151-152 °C, purity (HPLC): 99.5%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.13 – 8.05 (m, 1H), 7.58 – 7.51 (m, 1H), 7.46 – 7.37 (m, 2H), 4.68 – 4.07 (m, 4H), 2.81 – 2.62 (m, 4H), 2.59 (s, 3H), 2.44 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.0 (C), 164.1 (C), 154.7 (C), 153.7 (C), 133.3 (C), 131.7 (CH), 131.6 (CH), 131.2 (CH), 127.0 (CH), 125.4 (C), 114.3 (C), 54.8 (CH_2 × 2), 45.9 (CH_3), 44.7 (CH_2 × 2), 26.1 (CH_3). HRMS (ESI) calcd. for $C_{17}H_{19}ON_5Cl$ ($[M+H]^+$) 344.12726, found 344.12607.

4.1.14.2.

2-(2-Chlorophenyl)-7-(4-ethylpiperazin-1-yl)-5-methyloxazolo[5,4-*d*]pyrimidine (**48**)

The title compound was obtained as a yellow solid, mp 139-140 °C, purity (HPLC): 99.2%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.06 (d, J = 7.7 Hz, 1H), 7.68 (d, J

= 7.9, 1.6 Hz, 1H), 7.63 – 7.51 (m, 2H), 4.43 – 3.85 (m, 4H), 3.44 – 3.38 (m, 2H), 3.30 – 3.22 (m, 2H), 2.47 (s, 3H), 2.37 (q, *J* = 7.2 Hz, 2H), 1.03 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.0 (C), 164.3 (C), 155.4 (C), 153.2 (C), 133.5 (C), 132.0 (CH), 131.7 (CH), 131.2 (CH), 127.1 (CH), 125.1 (C), 114.6 (C), 52.6 (CH_2 × 2), 51.6 (CH_2), 42.5 (CH_2 × 2), 26.1 (CH_3), 9.7 (CH_3). HRMS (ESI) calcd. for $C_{18}H_{21}ON_5Cl$ ($[M+H]^+$) 358.14291, found 358.14184.

4.2. Biological evaluation

4.2.1. Competition binding assay

Stock solutions of the compounds were prepared in DMSO and further diluted with the binding buffer to the desired concentration. Briefly, [^{3}H]-CP-55,940 (0.5 nM), nonselective human CB_1 and CB_2 cannabinoid receptor, were added to 6 μg of membranes from CB_1- or CB_2-overexpressing CHO cells in binding buffer (50 mM Tris-HCl, 5 mM $MgCl_2$, 2.5 mM EDTA, 0.5 mg/mL BSA, pH 7.4). After 90 min at 30 °C, the incubation was stopped and the solutions were rapidly filtered over a UniFilter-96 GF/C glass fiber plate, presoaked in PEI (0.05%) on a Filtermate UniFilter 96-Harvester (PerkinElmer), and washed 10 times with ice-cold 50 mM Tris-HCl pH 7.4. The radioactivity on the filters was measured using a TopCount NXT microplate scintillation counter (PerkinElmer) using 30 μL of MicroScint 40 (PerkinElmer). Assays were performed at least in three independent experiments in duplicate or triplicate. The nonspecific binding was determined in the presence of 5 μM (R)-(+)-WIN 55,212-2 (Sigma).

4.2.2. Cell culture and proliferation assay

CHO-WT, CHO-CB_2, and HT29 cells were grown at 37 °C in a humidified atmosphere containing 5% CO_2, in HAM F12 medium or DMEM-GlutaMAX medium (for HT29 cells, Life Technologies) supplemented with 10% fetal bovine serum, penicillin (100 IU/mL), and streptomycin (100 µg/mL). In the cell proliferation assay, cells were plated in triplicate on 96-well plates (3 × 103 cells per well) and incubated for 24 h. Cells were then incubated in culture medium that contained 10 µM test compounds. After 72 h, cell growth was estimated by the colorimetric MTS test.

4.2.3. Cell-based HTRF cAMP assay

Cellular cAMP levels were measured using reagents supplied by Cisbio International (HTRF dynamic 2 cAMP kit). Briefly, CHO-CB_2 cells were harvested and were collected by centrifugation for 5 min at 1200 rpm. The cells were then resuspended in an appropriate final volume of culture medium and incubated with the phosphodiesterase inhibitor IBMX (0.5 mM). Cells were incubated for 15 min with the compounds at room temperature in a 384-well plate (2000 cells per well) before the addition of forskolin (3 µM) for 30 min at room temperature. The dye d2-conjugated cAMP and Europium cryptate-conjugated anti-cAMP antibodies were added to the assay plate, according to manufacturer's instructions. After 1 hour incubation at room temperature, the plate was read on a Spectramax microplate reader (Molecular Devices) with excitation wavelength at 340 nm and emission wavelengths

at 665 nm and 620 nm. Assays were performed at least in three independent experiments in duplicate or triplicate.

4.2.4. Metabolic stability in mice liver microsomes

The 1 µM compounds tested and referenced were incubated in duplicate (0.5 mL reaction volume) with male mice (CD-1) liver microsomes (20 mg/mL, BD Gentest™) at 37 °C in 50 mM phosphate buffer (5 mM $MgCl_2$, 1 mM NADP, 0.4 U/mL glucose-6-phosphate dehydrogenase, and 5 mM glucose-6-phosphate, pH 7.4). For the estimation of CL_{int}: sampling of a 50 µL aliquot at 0, 5, 10, 20, 30 and 40 min, and the reaction was stopped by 4 volumes of acetonitrile containing the internal standard (200 nM compound **40** or 200 nM compound **42**). After centrifugation at 10000 g, 10 min, 4 °C, the supernatants were kept at 4 °C to be analyzed immediately (or placed at -80 °C if the analysis was postponed). Controls (t_0 and t_{final}) in triplicate: incubation with microsomes denatured by acetonitrile containing the internal standard. Propranolol was used as a positive control. Analysis was determined by UPLC-MS/MS (Waters* Acquity I-Class / Xevo TQD) using a column (Waters* Acquity BEH C18, 50 x 2.1 mm, 1.7 µm; mobile phase: A: 10 mM ammonium ethanoate, B: CH_3CN 0.1% HCOOH). Retention time was obtained with flow rates of 600 µL/min. The acquisition time is 4 min.

Conflict of interest

None of the authors have a conflict of interest to declare.

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Appendix A. Supplementary data

Representative ^{1}H, ^{13}C, and HSQC spectra.

References

[1] All CB_2 neutral antagonists mentioned in this context are defined as ligands that do not significant alter forskolin-induced cAMP production or G protein activation in cAMP assay or [^{35}S]-GTPγS assay, performed in CHO-CB_2 cells that have not been pretreated with other CB_2 ligands.

[2] O. Aizpurua-Olaizola, I. Elezgarai, I. Rico-Barrio, I. Zarandona, N. Etxebarria, A. Usobiaga, Targeting the endocannabinoid system: future therapeutic strategies, Drug Discovery Today 22 (2017) 105-110.

[3] N. Leleu-Chavain, P. Desreumaux, P. Chavatte, R. Millet, Therapeutical potential of CB_2 receptors in immune-related diseases, Curr. Mol. Pharmacol. 6 (2013) 183-203.

[4] W. Tuo, N. Leleu-Chavain, J. Spencer, S. Sansook, R. Millet, P. Chavatte, Therapeutic potential of fatty acid amide hydrolase, monoacylglycerol lipase, and N-acylethanolamine acid amidase inhibitors, J. Med. Chem. 60 (2017) 4-46.

[5] S. Sansook, W. Tuo, L. Lemaire, A. Tourteau, A. Barczyk, X. Dezitter, F. Klupsch, N. Leleu-Chavain, G. J. Tizzard, S. J. Coles, R. Millet, J. Spencer, Synthesis of bioorganometallic nanomolar-potent CB_2 agonists containing a ferrocene unit, Organometallics 35 (2016) 3361-3368.

[6] A. Zimmer, A. M. Zimmer, A. G. Hohmann, M. Herkenham, T. I. Bonner, Increased mortality, hypoactivity, and hypoalgesia in cannabinoid CB_1 receptor knockout mice, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 5780-5785.

[7] N. Leleu-Chavain, M. Body-Malapel, J. Spencer, P. Chavatte, P. Desreumaux, R. Millet, Recent advances in the development of selective CB_2 agonists as promising anti-inflammatory agents, Curr. Med. Chem. 19 (2012) 3457-3474.

[8] L. F. Van Gaal, A. M. Rissanen, A. J. Scheen, O. Ziegler, S. Rössner, Effects of the cannabinoid-1 receptor blocker rimonabant on weight reduction and cardiovascular risk factors in overweight patients: 1-year experience from the RIO-Europe study, The Lancet 365 (2005) 1389-1397.

[9] M. A. Huestis, S. J. Boyd, S. J. Heishman, K. L. Preston, D. Bonnet, G. Le Fur, D. A. Gorelick, Single and multiple doses of rimonabant antagonize acute effects of smoked cannabis in male cannabis users, Psychopharmacology (Berl) 194 (2007) 505-515.

[10] R. S. Padwal, S. R. Majumdar, Drug treatments for obesity: orlistat, sibutramine, and rimonabant, The

Lancet 369 (2007) 71-77.

[11] R. Maldonado, O. Valverde, F. Berrendero, Involvement of the endocannabinoid system in drug addiction, Trends Neurosci. 29 (2006) 225-232.

[12] M. Aghazadeh Tabrizi, P. G. Baraldi, P. A. Borea, K. Varani, Medicinal chemistry, pharmacology, and potential therapeutic benefits of cannabinoid CB_2 receptor agonists, Chem. Rev. 116 (2016) 519-560.

[13] C. A. Lunn, E. P. Reich, J. S. Fine, B. Lavey, J. A. Kozlowski, R. W. Hipkin, D. J. Lundell, L. Bober, Biology and therapeutic potential of cannabinoid CB_2 receptor inverse agonists, Br. J. Pharmacol. 153 (2008) 226-239.

[14] P. Yang, K. Z. Myint, Q. Tong, R. Feng, H. Cao, A. A. Almehizia, M. H. Alqarni, L. Wang, P. Bartlow, Y. Gao, J. Gertsch, J. Teramachi, N. Kurihara, G. D. Roodman, T. Cheng, X. Q. Xie, Lead discovery, chemistry optimization, and biological evaluation studies of novel biamide derivatives as CB_2 receptor inverse agonists and osteoclast inhibitors, J. Med. Chem. 55 (2012) 9973-9987.

[15] C. A. Lunn, J. S. Fine, A. Rojas-Triana, J. V. Jackson, X. Fan, T. T. Kung, W. Gonsiorek, M. A. Schwarz, B. Lavey, J. A. Kozlowski, S. K. Narula, D. J. Lundell, R. W. Hipkin, L. A. Bober, A novel cannabinoid peripheral cannabinoid receptor-selective inverse agonist blocks leukocyte recruitment in vivo, J. Pharmacol. Exp. Ther. 316 (2006) 780-788.

[16] M. Rinaldi-Carmona, F. Barth, J. Millan, J. M. Derocq, P. Casellas, C. Congy, D. Oustric, M. Sarran, M. Bouaboula, B. Calandra, M. Portier, D. Shire, J. C. Brelière, G. L. Le Fur, SR 144528, the first potent and selective antagonist of the CB_2 cannabinoid receptor, J. Pharmacol. Exp. Ther. 284 (1998) 644-650.

[17] J. R. Savinainen, T. Kokkola, O. M. Salo, A. Poso, T. Järvinen, J. T. Laitinen, Identification of WIN55212-3 as a competitive neutral antagonist of the human cannabinoid CB_2 receptor, Br. J. Pharmacol. 145 (2005) 636-645.

[18] J. C. Ashton, M. Glass, The cannabinoid CB_2 receptor as a target for inflammation-dependent neurodegeneration, Curr. Neuropharmacol. 5 (2007) 73-80.

[19] F. Vincenzi, M. Targa, C. Corciulo, M. A. Tabrizi, S. Merighi, S. Gessi, G. Saponaro, P. G. Baraldi, P. A. Borea, K. Varani, Antinociceptive effects of the selective CB_2 agonist MT178 in inflammatory and chronic rodent pain models, Pain 154 (2013) 864-873.

[20] S. G. Kinsey, A. Mahadevan, B. Zhao, H. Sun, P. S. Naidu, R. K. Razdan, D. E. Selley, M. Imad Damaj, A. H. Lichtman, The CB_2 cannabinoid receptor-selective agonist O-3223 reduces pain and inflammation without apparent cannabinoid behavioral effects, Neuropharmacology 60 (2011) 244-251.

[21] J. El Bakali, P. Gilleron, M. Body-Malapel, R. Mansouri, G. G. Muccioli, M. Djouina, A. Barczyk, F. Klupsch, V. Andrzejak, E. Lipka, C. Furman, D. M. Lambert, P. Chavatte, P. Desreumaux, R. Millet, 4-Oxo-1,4-dihydropyridines as selective CB_2 cannabinoid receptor ligands part 2: discovery of new agonists endowed with protective effect against experimental colitis, J. Med. Chem. 55 (2012) 8948-8952.

[22] A. Tourteau, V. Andrzejak, M. Body-Malapel, L. Lemaire, A. Lemoine, R. Mansouri, M. Djouina, N. Renault, J. El Bakali, P. Desreumaux, G. G. Muccioli, D. M. Lambert, P. Chavatte, B. Rigo, N. Leleu-Chavain, R. Millet, 3-Carboxamido-5-aryl-isoxazoles as new CB_2 agonists for the treatment of colitis, Bioorg. Med. Chem. 21 (2013) 5383-5394.

[23] Z. Qamri, A. Preet, M. W. Nasser, C. E. Bass, G. Leone, S. H. Barsky, R. K. Ganju, Synthetic cannabinoid receptor agonists inhibit tumor growth and metastasis of breast cancer, Mol. Cancer Ther. 8 (2009) 3117-3129.

[24] E. Aso, I. Ferrer, CB_2 cannabinoid receptor as potential target against alzheimer's disease, Front. Neurosci. 10 (2016) 243.

[25] D. C. Geng, Y. Z. Xu, H. L. Yang, X. S. Zhu, G. M. Zhu, X. B. Wang, Inhibition of titanium particle-induced inflammatory osteolysis through inactivation of cannabinoid receptor 2 by AM630, J. Biomed. Mater. Res. A 95 (2010) 321-326.

[26] P. Yang, L. Wang, R. Feng, A. A. Almehizia, Q. Tong, K. Z. Myint, Q. Ouyang, M. H. Alqarni, L. Wang, X. Q. Xie, Novel triaryl sulfonamide derivatives as selective cannabinoid receptor 2 inverse agonist and osteoclast inhibitors: discovery, optimization and biological evaluation, J. Med. Chem. 56 (2013) 2045-2058.

[27] Glenmark's molecule for neuropathic pain, osteoarthritis - GRC 10693, successfully completes phase I trials. http://www.evaluategroup.com/Universal/View.aspx?type=Story&id=183092 (accessed 15.08.2017).

[28] S. P. Hollinshead, P. C. Astles, M. G. Chambers, M. P. Johnson, J. Palmer, M. W. Tidwell, Discovery and optimization of novel purines as potent and selective CB_2 agonists, Bioorg. Med. Chem. Lett. 22 (2012) 4962-4966.

[29] A. C. DeBaillie, C. D. Jones, M. E. Laurila, N. A. Magnus, M. A. Staszak, The development of practical synthetic routes to a CB_2 agonist: efficient construction of a densely substituted purine core, Org. Process Res. Dev. 17 (2013) 231-238.

[30] S. P. Hollinshead, M. W. Tidwell, J. Palmer, R. Guidetti, A. Sanderson, M. P. Johnson, M. G. Chambers, J. Oskins, R. Stratford, P. C. Astles, Selective cannabinoid receptor type 2 (CB_2) agonists: optimization of a series of purines leading to the identification of a clinical candidate for the treatment of osteoarthritic pain, J. Med. Chem. 56 (2013) 5722-5733.

[31] R. Guidetti, P. C. Astles, A. J. Sanderson, S. P. Hollinshead, M. P. Johnson, M. G. Chambers, The SAR development of substituted purine derivatives as selective CB_2 agonists for the treatment of chronic pain, Bioorg. Med. Chem. Lett. 24 (2014) 5572-5575.

[32] Eli Lilly and Company. A study of LY2828360 in patients with osteoarthritic knee pain. https://clinicaltrials.gov/ct2/show/NCT01319929 (accessed 15.08.2017).

[33] R. A. Ross, H. C. Brockie, L. A. Stevenson, V. L. Murphy, F. Templeton, A. Makriyannis, R. G. Pertwee, Agonist-inverse agonist characterization at CB_1 and CB_2 cannabinoid receptors of L759633, L759656 and AM630, Br. J. Pharmacol. 26 (1999) 665-672.

[34] R. Pertwee, G. Griffin, S. Fernando, X. Li, A. Hill, A. Makriyannis, AM630, a competitive cannabinoid receptor antagonist, Life sci. 56 (1995) 1949-1955.

[35] G. Hessler, K. H. Baringhaus, The scaffold hopping potential of pharmacophores, Drug Discovery Today: Technol. 7 (2011) e263-e269.

[36] H. Sun, G. Tawa, A. Wallqvist, Classification of scaffold-hopping approaches, Drug discovery today 17 (2012) 310-324.

[37] Y. Hu, D. Stumpfe, J. Bajorath, Recent Advances in Scaffold Hopping, J. Med. Chem. 60 (2017) 1238-1246.

[38] M. Bauser, G. Delapierre, M. Hauswald, T. Flessner, D. D'Urso, A. Hermann, B. Beyreuther, J. De Vry, P. Spreyer, E. Reissmüller, H. Meier, Discovery and optimization of 2-aryl oxazolo-pyrimidines as adenosine kinase inhibitors using liquid phase parallel synthesis, Bioorg. Med. Chem. Lett. 14 (2004) 1997-2000.

[39] M. H. Holschbach, D. Bier, S. Stüsgen, W. Wutz, W. Sihver, H. H. Coenen, R. A. Olsson, Synthesis and evaluation of 7-amino-2-(2 (3)-furyl)-5-phenylethylamino-oxazolo [5, 4-d] pyrimidines as

potential A 2A adenosine receptor antagonists for positron emission tomography (PET), Eur. J. Med. Chem. 41 (2006) 7-15.

[40] F. Ye, Y. Wang, S. Nian, Y. Wang, D. Chen, S. Yu, S. Wang, Synthesis and evaluation of biological and antitumor activities of 5,7-dimethyl- oxazolo[5,4-d]pyrimidine-4,6(5H,7H)-dione derivatives as novel inhibitors of FGFR1, J. Enzyme Inhib. Med. Chem. 30 (2015) 961-966.

[41] S. J. Govaerts, E. Hermans, D. M. Lambert, Comparison of cannabinoid ligands affinities and efficacies in murine tissues and in transfected cells expressing human recombinant cannabinoid receptors, Eur. J. Pharm. Sci. 23 (2004) 233-243.

[42] W. Tuo, N. Leleu-Chavain, A. Barczyk, N. Renault, L. Lemaire, P. Chavatte, R. Millet, Design, synthesis and biological evaluation of potent FAAH inhibitors, Bioorg. Med. Chem. Lett. 26 (2016) 2701-2705.

[43] B. Horváth, L. Magid, P. Mukhopadhyay, S. Bátkai, M. Rajesh, O. Park, G. Tanchian, R. Y. Gao, C. E. Goodfellow, M. Glass, R. Mechoulam, P. Pacher, A new cannabinoid CB_2 receptor agonist HU-910 attenuates oxidative stress, inflammation and cell death associated with hepatic ischaemia/reperfusion injury, Br. J. Pharmacol. 165 (2012) 2462-2478.

[44] P. Marini, M. G. Cascio, R. G. Pertwee, The cyclic AMP assay using human cannabinoid CB_2 receptor-transfected cells, Methods Mol. Biol. 1412 (2016) 85-93.

[45] P. Marini, M. G. Cascio, A. King, R. G. Pertwee, R. A. Ross, Characterization of cannabinoid receptor ligands in tissues natively expressing cannabinoid CB_2 receptors, Br. J. Pharmacol. 169 (2013) 887-899.

[46] J. L. Shoemaker, M. B. Ruckle, P. R. Mayeux, P. L. Prather, Agonist-directed trafficking of response by endocannabinoids acting at CB2 receptors, J. Pharmacolo. Exp. Ther. 315 (2005) 828-838.

[47] D. G. Demuth, A. Molleman, Cannabinoid signalling, Life Sci. 78 (2006) 549-563.

[48] E. J. Barreiro, A. E. Kümmerle, C. A. M. Fraga, The methylation effect in medicinal chemistry, Chem. Rev. 111 (2011) 5215-5246.

[49] M. Portier, M. Rinaldi-Carmona, F. Pecceu, T. Combes, C. Poinot-Chazel, B. Calandra, F. Barth, G. Le Fur, P. Casellas, SR 144528, an antagonist for the peripheral cannabinoid receptor that behaves as an inverse agonist, J. Pharmacol. Exp. Ther. 288 (1999) 582-589.

[50] E. Kotsikorou, F. Navas III, M. J. Roche, A. F. Gilliam, B. F. Thomas, H. H. Seltzman, P. Kumar, Z. H. Song, D. P. Hurst, D. L. Lynch, P. H. Reggio, The importance of hydrogen bonding and aromatic stacking to the affinity and efficacy of cannabinoid receptor CB_2 antagonist, 5-(4-chloro-3-methylphenyl)-1-[(4-methylphenyl)methyl]-*N*-[(1*S*,2*S*,4*R*)-1,3,3-trimethylbicyclo[2.2.1] hept-2-yl]-1H-pyrazole-3-carboxamide (SR144528), J. Med. Chem. 56 (2013) 6593-6612.

[51] G. Ragusa, M. Gómez-Cañas, P. Morales, D. P. Hurst, F. Deligia, R. Pazos, G. A. Pinna, J. Fernández-Ruiz, P. Goya, P. H. Reggio, N. Jagerovic, M. García-Arencibia, G. Murineddu, Synthesis, pharmacological evaluation and docking studies of pyrrole structure-based CB_2 receptor antagonists, Eur. J. Med. Chem. 101 (2015) 651-667.

[52] P. Morales, L. Hernandez-Folgado, P. Goya, N. Jagerovic, Cannabinoid receptor 2 (CB_2) agonists and antagonists: a patent update, Expert Opin. Ther. Pat. 26 (2016) 843-856.

[53] V. Deiana, M. Gómez-Cañas, M. R. Pazos, J. Fernández-Ruiz, B. Asproni, E. Cichero, P. Fossa, E. Muñoz, F. Deligia, G. Murineddu, M. García-Arencibia, G. A. Pinna, Tricyclic pyrazoles. Part 8. Synthesis, biological evaluation and modelling of tricyclic pyrazole carboxamides as potential CB_2 receptor ligands with antagonist/inverse agonist properties, Eur. J. Med. Chem. 112 (2016) 66-80.

[54] A. Dore, B. Asproni, A. Scampuddu, S. Gessi, G. Murineddu, E. Cichero, P. Fossa, S. Merighi, S.

Bencivenni, G. A. Pinna, Synthesis, molecular modeling and SAR study of novel pyrazolo[5,1-*f*][1,6]naphthyridines as CB_2 receptor antagonists/inverse agonists, Bioorg. Med. Chem. 24 (2016) 5291-5301.

[55] Z. Feng, M. H. Alqarni, P. Yang, Q. Tong, A. Chowdhury, L. Wang, X. Q. Xie, Modeling, molecular dynamics simulation, and mutation validation for structure of cannabinoid receptor 2 based on known crystal structures of GPCRs, J. Chem. Inf. Model. 54 (2014) 2483-2499.

[56] 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, Synthesis, biological activity and molecular modeling of new biphenylic carboxamides as potent and selective CB_2 receptor ligands, Eur. J. Med. Chem. 90 (2015) 526-536.

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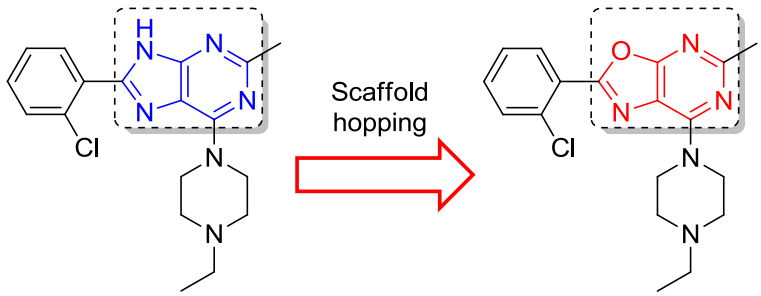

3
K_i (hCB$_2$) = 51 nM
CB$_2$ agonist

48 (ALICB903)
K_i (hCB$_2$) = 23 nM
CB$_2$ neutral antagonist