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Original article

Rational design, synthesis and biological evaluation of new 1,5-diarylpyrazole derivatives as CB_1 receptor antagonists, structurally related to rimonabant

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Abstract

Among cannabinoid type-1 (CB_1) receptor antagonists, those developed around the 1,5-diarylpyrazole scaffold of rimonabant (Acomplia™) are the most extensively investigated. In recent years, many SAR and QSAR reports on this topic have been published, focusing on the substitution and orientation of the N1 and C5 aryl functionalities and on the substituents at the 3-carboxamide position. In this context, the purpose of our study was to design and synthesize a set of 1-(2,4-dichlorophenyl)-5-arylpyrazoles strictly related to rimonabant, but with the hydrazide/amide group shifted from position 3 to position 4 of the pyrazole scaffold. The synthesized compounds were evaluated in vitro for their affinity on human CB_1 and CB_2 (cannabinoid type-2) receptors. Computational studies, performed both in the design step and after biological assays, contributed to rationalize the obtained results in terms of specific molecular interactions between antagonists and the human CB_1 receptor.

Keywords: 1,5-Diarylpyrazoles; CB1 antagonists; Rimonabant analogues; Synthesis; Computational studies

1. Introduction

Despite the fact that medicinal and cognitive effects of Marijuana (*Cannabis sativa*) have been known for thousands of years, only recent studies have provided convincing information on the biological mediation of its effects. Currently, two subtypes of cannabinoid receptor (namely, CB_1 and CB_2) have been cloned and pharmacologically characterized. Both CB_1 and CB_2 belong to the G-protein-coupled receptor family (GPCRs); CB_1 are mainly located within the central nervous system (CNS) and at presynaptic nerve terminals and they are present also within the periphery in gut, eye, testis and bladder. CB_2 receptors are mainly associated with immune system cells (e.g., B-cells, natural killer cells and monocytes) [1], however, they have recently been found in CNS tissues [2].

The development of CB_1 receptor ligands represents undoubtedly an interesting field in medicinal chemistry. In fact, CB_1 agonists showed utility as appetite stimulants, antiemetics, analgesics, tumour growth inhibitors and neuroprotectants [3], while CB_1 antagonists proved to be effective for the treatment of obesity, metabolic disorders [4,5] and drug abuse, including alcoholism, nicotine dependence and heroine addiction [6]. The 1,5-diarylpyrazole derivative rimonabant (SR141716, Acomplia™, Fig. 1) is the first therapeutically relevant CB_1 receptor antagonist [7,8], just launched by Sanofi-Aventis in the European market as an antiobesity drug. Moreover, recent investigation has shown the ability of rimonabant to improve memory acquisition and consolidation in mouse [9] and to prolong spatial memory duration, in combination with donepezil, in cognitive deficits associated with neurodegenerative disorders in rat [10]. A combination of a CB_1 antagonist with a dopaminergic neurotransmission enhancer has been recently patented for the treatment of Parkinson disease [11]. Full pharmacological potential of rimonabant has been discussed in a recent review [12].

Since its discovery, rimonabant has been extensively studied to increase its affinity and selectivity toward *h*CB_1

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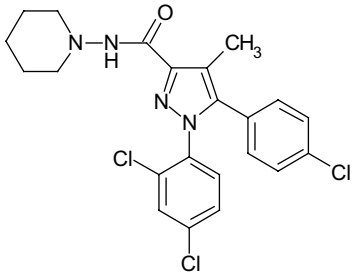


Fig. 1. Chemical structure of rimonabant.

receptor. Among the different modifications made on the parent compound, some groups focused on various substitutions on the 1,5-diphenylpyrazole scaffold [13–19], while others operated more heavily on the rimonabant structure with isosteric replacements of the pyrazole or phenyl rings [20–24].

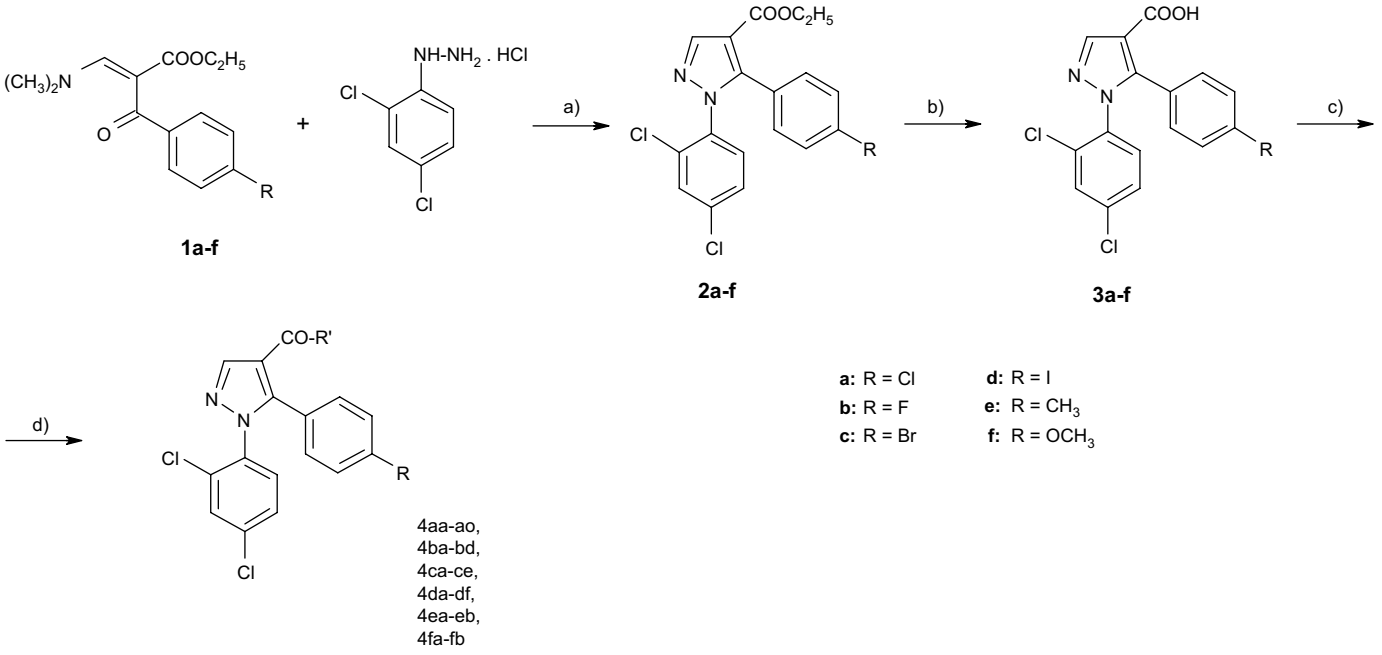
One of the less explored variations of the parent compound concerns the position of the carboxamide group, in which all derivatives have been maintained at position 3, with the exception of the compounds reported in a patent [25] deposited during the development of our research project. Our expertise in the pyrazole chemistry [26,27] prompted us to design and synthesize a series of 1,5-diarylpyrazoles bearing at 4 position different carboxamide/hydrazide groups (compounds **4**, Scheme 1). Our strategy was focused on the variation of the 4-substituent keeping constant the 2,4-dichlorophenyl ring at position 1 and applying limited variations on the phenyl ring at position 5 (*para*-substitution with groups with different electronic and steric properties, as halogens, methyl, methoxy). To support and guide the synthetic efforts, *in silico* docking studies were performed. The biological results of the synthesized compounds provided indirect validation of the calculated docking models.

2. Chemistry

Compounds **4** were obtained through the four-step procedure previously reported (Scheme 1) ([26,27] and literature cited therein). Dimethylaminomethylene intermediates **1a–1c**,**1e** and **f** were known [26,28]; **1d** was prepared by treatment of ethyl 4-iodobenzoylacetate with *N*,*N*-dimethylformamide dimethylacetal, as already reported [26]. Enaminones **1** were condensed with 2,4-dichlorophenylhydrazine to afford pyrazoles **2** as single products, as assessed by ^{1}H NMR spectra. The regioselective outcome of the cyclization reaction can be ascribed to the different nucleophilic properties of the NHAr and NH_2 groups of the 2,4-dichlorophenylhydrazine. Thus, the highly nucleophilic primary amine group exclusively reacted with the highly electrophilic extra-chain carbon atom of intermediates **1a–1f** to form pyrazoles **2**. Only compound **2a** was known [27]. Esters **2** were hydrolyzed in presence of KOH in 95% ethanol to lead to the carboxylic acids **3** which were then converted to the corresponding acid chlorides by treatment with thionyl chloride. The acid chlorides obtained in this way were reacted with a number of primary and secondary amines and hydrazines, in the presence of triethylamine, to give the desired carboxamides **4**.

3. Molecular modeling

Modeling studies, performed on rimonabant and its 1,5-diarylpyrazole analogues [4] predicted that the antagonists bind to human CB_1 (hCB_1) receptor in a hydrophobic region located within the transmembrane (TM) core region in the crevice formed by TM3, TM5, TM6 and TM7 [29]. The optimal interaction between rimonabant and CB_1 would be amenable to the double hydrogen bond involving the inhibitor amide



Scheme 1. Synthesis of target rimonabant analogues: (a) absolute EtOH; (b) KOH, EtOH 95%; (c) $SOCl_2$, toluene; (d) hydrazines or amines, Et_3N, CH_2Cl_2.

function and the key residues Lys192 and Ser383 [30]. In particular, the interaction with Lys192 is considered essential for the antagonistic affinity [31], exerting a stabilizing effect on the Lys192-Asp366 salt bridge at the intracellular end of TM3 and TM6 helices. Rimonabant–CB_1 complex would be further stabilized, according to our binding model, by a number of hydrophobic contacts between the piperidine ring and Phe174, Val196, Met384 and Leu387 residues. Moreover, the two phenyl rings are both involved in π–π stacking interactions with Tyr275, Trp279, Trp356 and Phe379 side-chains.

In order to explore any residue and ligand feature involved in the CB_1 antagonism and to analyze the effects of the shift of the amidic substitution from position 3 (rimonabant) to position 4 on receptor affinity, a two step computational protocol was performed on 1,5-diarylpyrazoles **4**.

A three-dimensional model of the *h*CB_1 receptor was built by homology modeling techniques, starting from the three-dimensional structure coordinate file of bovine rhodopsine (PDB entry 1F88) which is the common template for protein belonging to Class A of GPCRs [32]. Briefly, the amino acid sequence of *h*CB_1 was aligned with that of bovine rhodopsine on the basis of the highly conserved amino acid residues, according to the previously reported procedure [33] (seeSection 6). Then rimonabant (assumed as template molecule) was docked into the putative binding site of the *h*CB_1 receptor by means of the flexible docking module implemented in MOE [34]. The results obtained in this first step were in agreement with site-directed mutagenesis data and with other computational studies reported in the literature (Fig. 2) [4,35]. This evidence indirectly validated our docking protocol which was therefore used for the *in silico* screening of a virtual library created by an exhaustive combination of intermediates **3** and a set of commercially available amines/hydrazines. The compounds with the highest predicted *h*CB_1 affinities, according to the binding energy evaluation performed by MOE, were selected and synthesized (compounds **4**).

4. Results and discussion

All compounds **4** were preliminary screened by Cerep (Poitiers, France) at 10 μM concentration in a competitive binding experiment toward *h*CB_1 and *h*CB_2 receptors, expressed in membranes of Chinese Hamster Ovarian (CHO) cells. [^{3}H]-CP 55940 (0.5 nM) and [^{3}H]-WIN 55212-2 (0.8 nM) were used as radioligands in these experiments for *h*CB_1 and *h*CB_2, respectively. Table 1 summarizes the results expressed as the percentages of the radioligand displacement for derivatives **4**. In the biological assays, almost all the tested compounds displayed significant competitive binding (radioligand displacement >50% at 10 μM concentration) but a weak selectivity toward the *h*CB_1 receptor.

Compound **4aa** is the closest rimonabant analogue and showed a competitive binding of 79% and 37% against *h*CB_1 and *h*CB_2, respectively. Secondary *N*-cycloalkyl amides **4af**–**4ak** showed similar or slightly increased affinity toward CB_1 receptor, in comparison with **4aa** and the other hydrazides **4ab**–**4ae**. The *N*,*N*-disubstitution of the carboxamide function (**4al**–**4an**) resulted in a reduction of the binding affinity and selectivity for *h*CB_1 receptor. The insertion of an ethylene spacer between the piperidin-1-yl substituent and the carboxamide nitrogen atom caused a reduction of both *h*CB_1 and *h*CB_2 receptors affinities (compare **4aa** and **4ao**).

Cl/F (**4ba**–**4bd**) or Cl/CH_3 (**4ea**, **4eb**) replacement at the *para*-position of 5-phenyl ring lowered affinity for both receptors, while 4-anisoyl derivatives **4fa**, **4fb** showed increased binding affinity and selectivity for *h*CB_2 receptor. In general, bromo

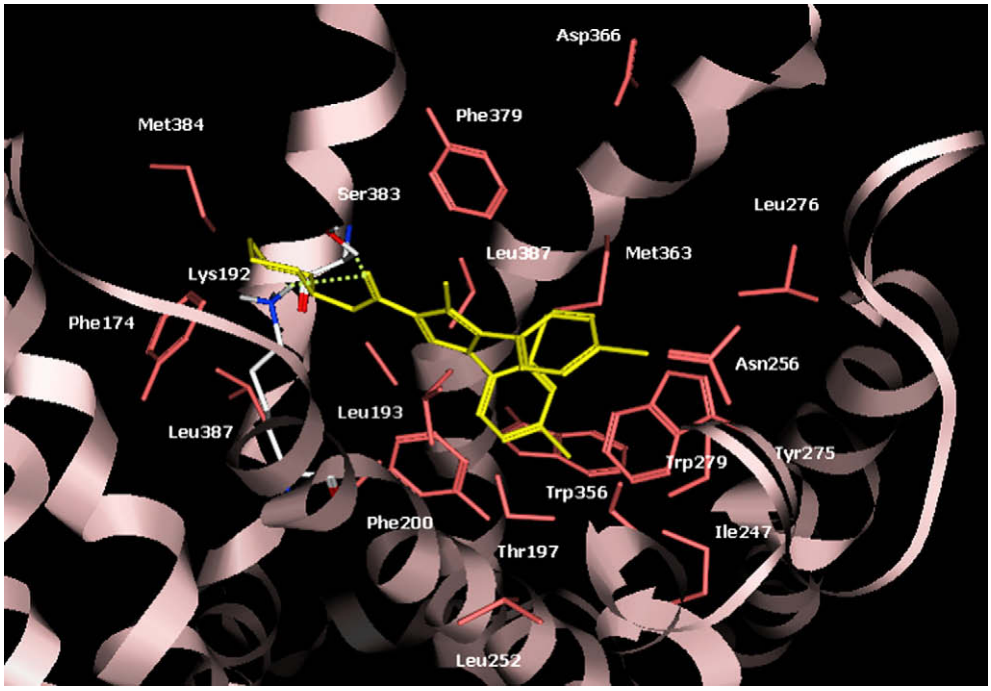
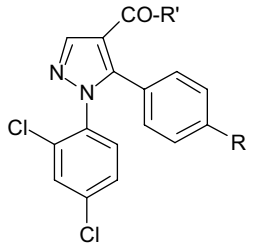


Fig. 2. Binding mode of rimonabant (yellow) inside *h*CB_1 receptor; H-bonds are displayed as dashed lines.

Table 1
Binding affinities of compounds **4** toward hCB$_1$ and hCB$_2$ receptors



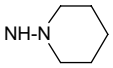
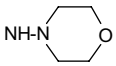
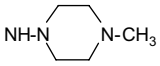
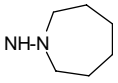
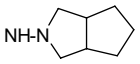
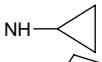
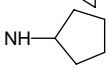
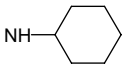
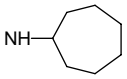
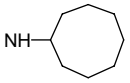
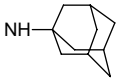
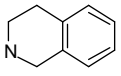
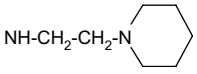
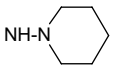
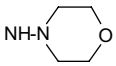
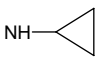
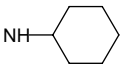
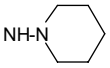
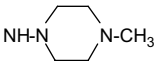
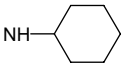
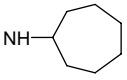
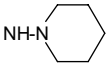
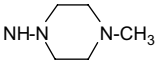
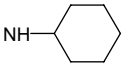
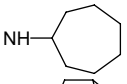
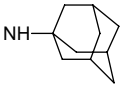
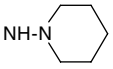
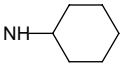
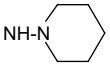
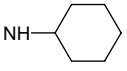
Compd.	R	R′	Radioligand displacement [%] at 10 μM	
			hCB$_1$ Receptor[a]	hCB$_2$ Receptor[b]
4aa	Cl		79	37
4ab	Cl		65	12
4ac	Cl		61 ($K_i = 16$ μM)	−23
4ad	Cl		75	64
4ae	Cl		86	56
4af	Cl		87 ($K_i = 0.85$ μM)	30 ($K_i = 21$ μM)
4ag	Cl		82	61
4ah	Cl		68	52
4ai	Cl		78	50
4aj	Cl		84	44
4ak	Cl		72	22
4al	Cl		49	43
4am	Cl		59	45
4an	Cl		58	69
4ao	Cl		39	14
4ba	F		43	7
4bb	F		30	−9
4bc	F		62	7

Table 1 (*continued*)

Compd.	R	R′	Radioligand displacement [%] at 10 μM	
			hCB$_1$ Receptor[a]	hCB$_2$ Receptor[b]
4bd	F		69	18
4ca	Br		74	55
4cb	Br		67 ($K_i = 2.6$ μM)	−12 ($K_i = 30$ μM)
4cc	Br		84 ($K_i = 1.0$ μM)	43
4cd	Br		81	44
4ce	Br		88 ($K_i = 1.1$ μM)	60
4da	I		94 ($K_i = 1.3$ μM)	84 ($K_i = 1.9$ μM)
4db	I		78	42
4dc	I		91 ($K_i = 0.21$ μM)	60
4dd	I		64	59
4de	I		90 ($K_i = 1.1$ μM)	61
4df	I		76	44
4ea	CH_3		29	27
4eb	CH_3		48	46
4fa	OCH_3		58	70
4fb	OCH_3		64	76
Rimonabant[c]			$K_i = 0.025$ μM	$K_i = 1.58$ μM

[a] Data reported as percentage ($n = 2$) of displacement of [^{3}H]-CP 55940 (0.5 nM) from hCB$_1$ receptor, at an inhibitor concentration of 10 μM. In brackets the K_i value, when calculated.

[b] Data reported as percentage ($n = 2$) of displacement of [^{3}H]-WIN 55212-2 (0.8 nM) from hCB$_2$ receptor, at an inhibitor concentration of 10 μM. In brackets the K_i value, when calculated.

[c] Ref. [22].

and iodo *para*-substituted compounds **4ca**–**4ce** and **4da**–**4df** showed a slightly higher binding affinity than their corresponding chlorinated analogues toward both receptor subtypes. For a number of pyrazole analogues endowed with the highest selectivity/potency toward hCB$_1$ (as assessed by their radioligand displacements), the affinity constants K_i were evaluated (Table 1). Derivatives **4ac**, **4cb**, **4cc**, **4ce**, **4da** and **4de** were endowed with micromolar activity (K_i range: 1.0–16 μM) whereas **4af** showed submicromolar K_i value (0.85 μM). Compound **4dc** was identified as the most active compound of the screened set ($K_i = 0.21$ μM) but resulted in about 10-fold less active than rimonabant ($K_i = 5.6$ nM [7], $K_i = 25$ nM [22]).

The K_i values for hCB$_2$ of **4af**, **4cb** and **4da** confirmed the weak selectivity of the synthesized derivatives toward

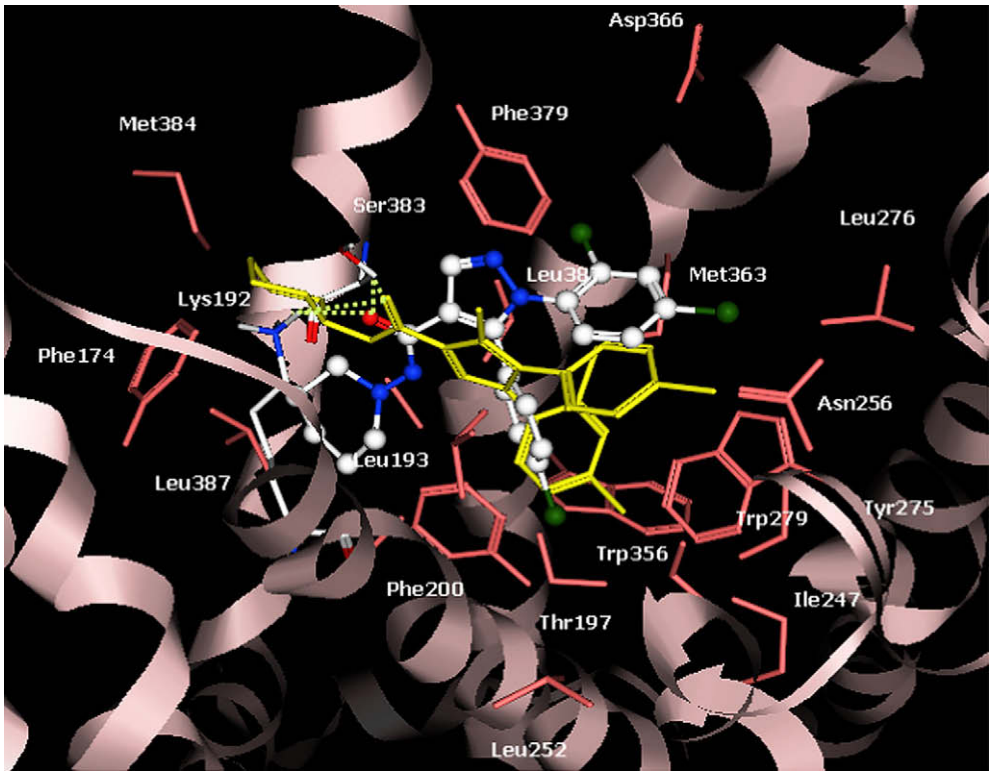


Fig. 3. Binding mode of rimonabant (yellow) and its most structurally related analogue **4aa** (coloured by atom type), inside *h*CB_1 receptor; H-bonds are displayed as dashed lines.

cannabinoid receptor type-2, as anticipated by the radioligand displacement percentages.

As assessed by computational studies, all compounds **4** share a common binding pose, comparable with that of rimonabant. In Fig. 3, as an example, the binding of the closest rimonabant analogue **4aa** is reported. The amidic carbonyl is engaged in a double hydrogen bond with Lys192 and Ser393 (as observed in rimonaband–CB_1 binding model) while the two aromatic rings are involved in π–π stacking interactions with Trp279, Trp356 and Phe379. Differently from rimonabant, the **4aa** piperidinyl substituent does not interact with Phe174, Val196, Met384 and Leu387. The loss of these anchoring points inside the receptor could rationalize the lower affinity of pyrazoles **4** in comparison with the parent compound.

Thus, according to our calculations, the double hydrogen bond with Lys192 and Ser383 seems to be essential for the proper positioning of ligand within the receptor binding site. However, this interaction does not appear to be sufficient to switch the K_i of the CB_1 antagonist toward nanomolar values. This result seems to be achieved only by a contemporary hydrophobic interaction which some specific residues in the surrounding part of the protein, suggesting that an accurate balance between the hydrophilic and hydrophobic substituents on the molecule is extremely important in the case of *h*CB_1 antagonists.

5. Conclusions

For rimonabant and its 3-substituted analogues, various reports have highlighted three principal structural requirements for displaying a potent and selective CB_1 receptor antagonistic activity: (a) a 2,4-dichlorophenyl substituent at position 1, (b) a *para*-substituted phenyl ring at position 5 and (c) a carboxamide group at position 3 of the pyrazole ring [14–20]. Taking into account these indications we evaluated *in silico* the *h*CB_1 binding affinity of a set of 1,5-diarylpyrazole-4-carboxamides and the best ranked derivatives were synthesized.

The preliminary biological results for compounds **4** suggested that the shift of the carboxamidic function from position 3 to position 4 of the pyrazole ring led to CB_1 ligands endowed with micromolar or submicromolar affinity. Docking studies carried out on rimonabant and its analogues **4**, confirmed the structural requirements for CB_1 interaction and further highlighted the importance of a double hydrogen bond with Lys192 and Ser383 in order to achieve a high affinity toward the receptor. This information will provide the basis for the synthesis of new 4-substituted 1,5-diarylpyrazoles endowed with improved affinity and selectivity toward *h*CB_1 or *h*CB_2 receptor.

6. Experimental section

6.1. Chemistry

All chemicals and solvents used were commercially available and of analytical grade or were prepared according to the procedure described in the literature [26,27]. All compounds were tested for purity by thin layer chromatography (TLC) on silica gel plates (60 F_{254}, Merck; ethyl acetate/

petroleum ether 1:1 as eluant), visualizing with ultraviolet light. Melting points were determined with a Fisher–Johns apparatus and are uncorrected. IR spectra were registered on a Perkin–Elmer 398 spectrophotometer and are expressed in cm^{-1}. ^1H NMR spectra were registered on a Varian Gemini 200 (200 MHz) spectrometer; chemical shifts are reported as δ values (ppm) relative to TMS as internal standard; coupling constants (J) are expressed in hertz (Hz). The following NMR abbreviations are used: br (broad), s (singlet), d (doublet), t (triplet), q (quartet), sex (sextet), m (multiplet), ex (exchangeable with D_2O), ar (aromatic proton), pip (piperidine), pyr (pyrazole). Analyses indicated by the symbols of the elements (C, H, N) were performed using a Carlo Erba Elemental Analyzer Model EA 1110 and were within ±0.4% of the theoretical values.

6.1.1. Ethyl 3-(dimethylamino)-2-(4-iodobenzoyl)-acrylate (**1d**)

A solution of ethyl 4-iodobenzoylacetate (6.36 g, 20 mmol) in *N*,*N*-dimethylformamide dimethyl acetal (2.86 g, 24 mmol) was refluxed for 1 h. The excess acetal was distilled off under reduced pressure and the residue was chromatographed on Florisil, using ethyl ether as eluant. The crude product was then recrystallized from ethyl ether/petroleum ether (5.06 g, 68% yield); mp 89–90 °C. IR ($CHCl_3$): $\nu = 1682$ (CO, ester), 1628 (CO, ketone) and 1586 cm^{-1} (aromatic C–C); ^1H NMR ($CDCl_3$): $\delta = 0.85$ (t, $J = 7.0$ Hz, 3H, ethyl CH_3), 2.90 (br s, 6H, $N(CH_3)_2$), 3.89 (q, $J = 7.0$ Hz, 2H, ethyl CH_2), 7.40 (d, $J = 8.0$ Hz, 2H, ar), 7.67 (d, $J = 8.0$ Hz, 2H, ar), 7.72 ppm (s, 1H, =CH). Anal. $C_{14}H_{16}INO_3$ (C, H, N).

6.1.2. General procedure for the preparation of compounds **2b–2f**

2,4-Dichlorophenylhydrazine hydrochloride (2.35 g, 11 mmol) was added to a stirred solution of the proper ethyl 3-(dimethylamino)-2-aroyl-acrylate **1** (10 mmol) in absolute ethanol (50 ml). The mixture was refluxed for 2 h and evaporated under reduced pressure. The residue was dissolved in chloroform and the organic solution was washed with water, dried (magnesium sulphate), filtered and evaporated under reduced pressure. The crude product was purified by recrystallization from proper solvent.

6.1.2.1. Ethyl 1-(2,4-dichlorophenyl)-5-(4-fluorophenyl)-1H-pyrazole-4-carboxylate (**2b**).

Yield 3.25 g (86%); mp 78–79 °C (from petroleum ether). IR ($CHCl_3$): $\nu = 1712$ cm^{-1} (CO); ^1H NMR ($CDCl_3$): $\delta = 1.26$ (t, $J = 7.0$ Hz, 3H, CH_3), 4.25 (q, $J = 7.0$ Hz, 2H, CH_2), 6.95–7.10 (m, 2H, ar), 7.20–7.35 (m, 4H, ar), 7.44 (s, 1H, ar), 8.23 ppm (s, 1H, pyr H-3). Anal. $C_{18}H_{13}Cl_2FN_2O_2$ (C, H, N).

6.1.2.2. Ethyl 5-(4-bromophenyl)-1-(2,4-dichlorophenyl)-1H-pyrazole-4-carboxylate (**2c**).

Yield 4.05 g (92%); mp 121–122 °C (from petroleum ether). IR ($CHCl_3$): $\nu = 1714$ cm^{-1} (CO); ^1H NMR ($CDCl_3$): $\delta = 1.27$ (t, $J = 7.2$ Hz, 3H, CH_3), 4.25 (q, $J = 7.2$ Hz, 2H, CH_2), 7.15 (d, $J = 8.6$ Hz, 2H, ar), 7.20–7.35 (m, 2H, ar), 7.43 (s, 1H, ar), 7.45 (d, $J = 8.6$ Hz, 2H, ar), 8.22 ppm (s, 1H, pyr H-3). Anal. $C_{18}H_{13}BrCl_2N_2O_2$ (C, H, N).

6.1.2.3. Ethyl 1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-1H-pyrazole-4-carboxylate (**2d**).

Yield 4.42 g (91%); mp 156–157 °C (from ethyl ether). IR ($CHCl_3$): $\nu = 1713$ cm^{-1} (CO); ^1H NMR ($CDCl_3$): $\delta = 1.19$ (t, $J = 7.2$ Hz, 3H, CH_3), 4.16 (q, $J = 7.2$ Hz, 2H, CH_2), 6.93 (d, $J = 8.6$ Hz, 2H, ar), 7.15–7.25 (m, 2H, ar), 7.35 (s, 1H, ar), 7.57 (d, $J = 8.6$ Hz, 2H, ar), 8.13 ppm (s, 1H, pyr H-3). Anal. $C_{18}H_{13}Cl_2IN_2O_2$ (C, H, N).

6.1.2.4. Ethyl 1-(2,4-dichlorophenyl)-5-(4-methylphenyl)-1H-pyrazole-4-carboxylate (**2e**).

Yield 2.85 g (76%); mp 88–89 °C (from petroleum ether). IR ($CHCl_3$): $\nu = 1712$ cm^{-1} (CO); ^1H NMR ($CDCl_3$): $\delta = 1.27$ (t, $J = 7.2$ Hz, 3H, ethyl CH_3), 2.33 (s, 3H, CH_3-Ar), 4.25 (q, $J = 7.2$ Hz, 2H, CH_2), 7.05–7.30 (m, 6H, ar), 7.43 (s, 1H, ar), 8.22 ppm (s, 1H, pyr H-3). Anal. $C_{19}H_{16}Cl_2N_2O_2$ (C, H, N).

6.1.2.5. Ethyl 1-(2,4-dichlorophenyl)-5-(4-methoxyphenyl)-1H-pyrazole-4-carboxylate (**2f**).

Yield 3.36 g (86%); mp 71–72 °C (from ethyl ether/petroleum ether). IR ($CHCl_3$): $\nu = 1709$ cm^{-1} (CO); ^1H NMR ($CDCl_3$): $\delta = 1.19$ (t, $J = 7.0$ Hz, 3H, ethyl CH_3), 3.71 (s, 3H, CH_3–O), 4.17 (q, $J = 7.0$ Hz, 2H, CH_2), 7.74 (d, $J = 8.8$ Hz, 2H, ar), 7.10–7.20 (m, 4H, ar), 7.35 (s, 1H, ar), 8.13 ppm (s, 1H, pyr H-3). Anal. $C_{19}H_{16}Cl_2N_2O_3$ (C, H, N).

6.1.3. General procedure for the synthesis of compounds **3a–3f**

Potassium hydroxide (1.68 g, 30 mmol) dissolved in 95% ethanol (10 ml) was added to a solution of the proper ester **2** (10 mmol) in the same solvent (30 ml). The mixture was refluxed under stirring for 5 h, the solvent was evaporated under reduced pressure and the residue was dissolved with water (50 ml). The aqueous solution was acidified with 6 N hydrochloric acid (pH ~ 1) and the solid which separated was extracted with chloroform. The organic layer was separated, washed with water, dried (magnesium sulphate) and evaporated under reduced pressure to yield a white solid which was then recrystallized from suitable solvent.

6.1.3.1. 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-1H-pyrazole-4-carboxylic acid (**3a**).

Yield 3.60 g (98%); mp 205–206 °C (from ethyl ether/petroleum ether). IR ($CHCl_3$): $\nu = 3200–2500$ (OH) and 1690 cm^{-1} (CO); ^1H NMR ($CDCl_3$): $\delta = 7.15–7.35$ (m, 6H, ar), 7.44 (s, 1H, ar), 8.28 (s, 1H, pyr H-3), ~11.9 ppm (br s, 1H, CO_2H, ex). Anal. $C_{16}H_9Cl_3N_2O_2$ (C, H, N).

6.1.3.2. 1-(2,4-Dichlorophenyl)-5-(4-fluorophenyl)-1H-pyrazole-4-carboxylic acid (**3b**).

Yield 3.30 g (94%); double mp 158–159 and 171–172 °C (from ethyl ether/petroleum ether). IR ($CHCl_3$): $\nu = 3200–2500$ (OH) and 1688 cm^{-1} (CO); ^1H NMR ($CDCl_3$): $\delta = 6.95–7.10$ (m, 2H, ar), 7.20–7.35 (m,

4H, ar), 7.44 (s, 1H, ar), 8.28 (s, 1H, pyr H-3), ~10.6 ppm (br s, 1H, CO_2H, ex). Anal. $C_{16}H_9Cl_2FN_2O_2$ (C, H, N).

*6.1.3.3. 5-(4-Bromophenyl)-1-(2,4-dichlorophenyl)-1H-pyrazole-4-carboxylic acid (**3c**).* Yield 3.87 g (94%); mp 214–215 °C (from ethyl ether/petroleum ether). IR ($CHCl_3$): $\nu = 3200-2500$ (OH) and 1691 cm^{-1} (CO); ^1H NMR ($CDCl_3$): $\delta = 7.15$ (d, $J = 8.4$ Hz, 2H, ar), 7.20–7.40 (m, 2H, ar), 7.44 (s, 1H, ar), 7.46 (d, $J = 8.4$ Hz, 2H, ar), 8.27 (s, 1H, pyr H-3), ~9.3 ppm (br s, 1H, CO_2H, ex). Anal. $C_{16}H_9BrCl_2N_2O_2$ (C, H, N).

*6.1.3.4. 1-(2,4-Dichlorophenyl)-5-(4-iodophenyl)-1H-pyrazole-4-carboxylic acid (**3d**).* Yield 4.40 g (96%); mp 232–233 °C (from ethyl ether). IR ($CHCl_3$): $\nu = 3200-2500$ (OH) and 1690 cm^{-1} (CO); ^1H NMR ($CDCl_3$): $\delta = 6.93$ (d, $J = 8.4$ Hz, 2H, ar), 7.10–7.30 (m, 2H, ar), 7.36 (s, 1H, ar), 7.57 (d, $J = 8.4$ Hz, 2H, ar), 8.19 (s, 1H, pyr H-3), ~10.2 ppm (br s, 1H, CO_2H, ex). Anal. $C_{16}H_9Cl_2IN_2O_2$ (C, H, N).

*6.1.3.5. 1-(2,4-Dichlorophenyl)-5-(4-methylphenyl)-1H-pyrazole-4-carboxylic acid (**3e**).* Yield 3.36 g (97%); mp 228–229 °C (from 95% ethanol). IR ($CHCl_3$): $\nu = 3200-2500$ (OH), 1691 cm^{-1} (CO); ^1H NMR ($CDCl_3$): $\delta = 2.34$ (s, 3H, CH_3), 7.05–7.30 (m, 6H, ar), 7.43 (s, 1H, ar), 8.27 (s, 1H, pyr H-3), ~10.5 ppm (br s, 1H, CO_2H, ex). Anal. $C_{17}H_{12}Cl_2N_2O_2$ (C, H, N).

*6.1.3.6. 1-(2,4-Dichlorophenyl)-5-(4-methoxyphenyl)-1H-pyrazole-4-carboxylic acid (**3f**).* Yield 3.27 g (90%); mp 190–191 °C (from ethyl ether). IR ($CHCl_3$): $\nu = 3200-2500$ (OH) and 1689 cm^{-1} (CO); ^1H NMR ($CDCl_3$): $\delta = 3.72$ (s, 3H, CH_3), 6.74 (d, $J = 8.0$ Hz, 2H, ar), 7.12 (d, $J = 8.0$ Hz, 2H, ar), 7.15–7.25 (m, 2H, ar), 7.35 (s, 1H, ar), 8.18 (s, 1H, pyr H-3), ~10.1 ppm (br s, 1H, CO_2H, ex). Anal. $C_{17}H_{12}Cl_2N_2O_3$ (C, H, N).

*6.1.4. General procedure for the synthesis of compounds **4aa–4ao, 4ba–4bd, 4ca–4ce, 4da–4df, 4ea, 4eb, 4fa, 4fb***

A mixture of the proper carboxylic acid **3** (3 mmol) and thionyl chloride (0.8 ml, 11 mmol) in 20 ml of toluene was refluxed for 3 h and then evaporated to dryness under reduced pressure. The residue was taken up in 20 ml of toluene and the solvent was evaporated again under reduced pressure to give the crude acid chloride, which was dissolved in 15 ml of dry dichloromethane. This solution was added dropwise to a solution of the proper amine/hydrazine (4.5 mmol) and triethylamine (0.45 g, 4.5 mmol) in 10 ml of dichloromethane, cooled to 0 °C. After stirring at room temperature for 16 h, the reaction mixture was added to brine (30 ml) and extracted with dichloromethane. The organic layer was separated, dried (magnesium sulphate) and evaporated under reduced pressure. The residue was purified by chromatography on Florisil using ethyl acetate as eluant, to give a white solid, which was then recrystallized from proper solvent.

*6.1.4.1. 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-N-piperidin-1-yl-1H-pyrazole-4-carboxamide (**4aa**).* Yield 0.98 g (72%); mp 218–219 °C (from 95% ethanol). IR ($CHCl_3$): $\nu = 3415$ and 3326 (NH) and 1668 cm^{-1} (CO); ^1H NMR ($CDCl_3$): $\delta = 1.30-1.45$ (m, 2H, CH_2), 1.55–1.75 (m, 4H, $2CH_2$), 2.60–2.75 (m, 4H, $2CH_2N$), 6.40 (br s, 1H, NH, ex), 7.15–7.40 (m, 6H, ar), 7.44 (s, 1H, ar), 8.15 ppm (s, 1H, pyr H-3). Anal. $C_{21}H_{19}Cl_3N_4O$ (C, H, N).

*6.1.4.2. 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-N-(morpholin-4-yl)-1H-pyrazole-4-carboxamide (**4ab**).* Yield 0.68 g (50%); mp 232–233 °C (from ethyl acetate). IR ($CHCl_3$): $\nu = 3415$ and 3329 (NH) and 1671 cm^{-1} (CO); ^1H NMR ($CDCl_3$): $\delta = 2.65-2.85$ (m, 4H, $2CH_2N$), 3.65–3.85 (m, 4H, $2CH_2O$), 6.67 (s, 1H, NH, ex), 7.10–7.35 (m, 6H, ar), 7.43 (s, 1H, ar), 8.18 ppm (s, 1H, pyr H-3). Anal. $C_{20}H_{17}Cl_3N_4O_2$ (C, H, N).

*6.1.4.3. 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-N-(4-methylpiperazin-1-yl)-1H-pyrazole-4-carboxamide (**4ac**).* Yield 1.03 g (74%); mp 189–190 °C (from ethyl ether). IR ($CHCl_3$): $\nu = 3413$ and 3328 (NH) and 1671 cm^{-1} (CO); ^1H NMR ($CDCl_3$): $\delta = 2.18$ (s, 3H, CH_3), 2.40–2.55 (m, 4H, $2CH_2$), 2.60–2.75 (m, 4H, $2CH_2$), 6.21 (s, 1H, NH, ex), 7.05–7.25 (m, 6H, ar), 7.32 (s, 1H, ar), 8.05 ppm (s, 1H, pyr H-3). Anal. $C_{21}H_{20}Cl_3N_5O$ (C, H, N).

*6.1.4.4. N-Azepan-1-yl-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-1H-pyrazole-4-carboxamide (**4ad**).* Yield 0.70 g (50%); mp 202–203 °C (from ethyl acetate). IR ($CHCl_3$): $\nu = 3413$ and 3351 (NH) and 1668 cm^{-1} (CO); ^1H NMR ($CDCl_3$): $\delta = 1.30-1.65$ (m, 8H, $2CH_2$), 2.65–3.05 (m, 4H, $2CH_2N$), 6.55 (br s, 1H, NH, ex), 7.00–7.35 (m, 6H, ar), 7.37 (s, 1H, ar), 8.09 ppm (s, 1H, pyr H-3). Anal. $C_{22}H_{21}Cl_3N_4O$ (C, H, N).

*6.1.4.5. 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-N-(hexahydrocyclopenta[c]pyrrol-2-(1H)yl)-1H-pyrazole-4-carboxamide (**4ae**).* Yield 0.98 g (69%); mp 192–193 °C (from ethyl acetate). IR ($CHCl_3$): $\nu = 3418$ and 3322 (NH) and 1668 cm^{-1} (CO); ^1H NMR ($CDCl_3$): $\delta = 1.20-1.70$ (m, 6H, $3CH_2$), 2.05–2.25 (m, 1H, CH), 2.30–2.60 (m, 4H, $2CH_2N$), 2.90–3.10 (m, 1H, CH), 5.95 (s, 1H, NH, ex), 7.00–7.30 (m, 6H, ar), 7.37 (s, 1H, ar), 8.04 ppm (s, 1H, pyr H-3). Anal. $C_{23}H_{21}Cl_3N_4O$ (C, H, N).

*6.1.4.6. 5-(4-Chlorophenyl)-N-cyclopropyl-1-(2,4-dichlorophenyl)-1H-pyrazole-4-carboxamide (**4af**).* Yield 1.07 g (88%); mp 210–211 °C (from 95% ethanol). IR ($CHCl_3$): $\nu = 3433$ (NH) and 1659 cm^{-1} (CO); ^1H NMR ($CDCl_3$): $\delta = 0.25-0.40$ (m, 2H, CH_2), 0.60–0.75 (m, 2H, CH_2), 2.60–2.75 (m, 1H, CHN), 5.79 (br s, 1H, NH, ex), 7.10–7.25 (m, 6H, ar), 7.33 (s, 1H, ar), 8.02 ppm (s, 1H, pyr H-3). Anal. $C_{19}H_{14}Cl_3N_3O$ (C, H, N).

*6.1.4.7. 5-(4-Chlorophenyl)-N-cyclopentyl-1-(2,4-dichlorophenyl)-1H-pyrazole-4-carboxamide (**4ag**).* Yield 1.22 g (94%); mp 189–190 °C (from ethyl acetate). IR ($CHCl_3$): $\nu = 3432$ (NH) and 1649 cm^{-1} (CO); ^1H NMR ($CDCl_3$):

$\delta = 1.00-1.25$ (m, 2H, CH_2), 1.30–1.60 (m, 4H, $2CH_2$), 1.70–1.95 (m, 2H, CH_2N), 4.20 (sex, $J = 6.8$ Hz, 1H, CHN), 5.34 (d, $J = 7.8$ Hz, 1H, NH, ex), 7.05–7.30 (m, 6H, ar), 7.34 (s, 1H, ar), 8.06 ppm (s, 1H, pyr H-3). Anal. $C_{21}H_{18}Cl_3N_3O$ (C, H, N).

*6.1.4.8. 5-(4-Chlorophenyl)-N-cyclohexyl-1-(2,4-dichlorophenyl)-1H-pyrazole-4-carboxamide (**4ah**).* Yield 1.28 g (95%); mp 182–183 °C (from 95% ethanol). IR ($CHCl_3$): $\nu = 3429$ (NH) and 1648 cm^{-1} (CO); ^1H NMR ($CDCl_3$): $\delta = 0.90-1.65$ (m, 8H, $4CH_2$), 1.75–1.95 (m, 2H, CH_2), 3.80–4.0 (m, 1H, CHN), 5.38 (d, $J = 7.8$ Hz, 1H, NH, ex), 7.20–7.40 (m, 6H, ar), 7.42 (s, 1H, ar), 8.14 ppm (s, 1H, pyr H-3). Anal. $C_{22}H_{20}Cl_3N_3O$ (C, H, N).

*6.1.4.9. 5-(4-Chlorophenyl)-N-cycloheptyl-1-(2,4-dichlorophenyl)-1H-pyrazole-4-carboxamide (**4ai**).* Yield 1.22 g (88%); mp 173–174 °C (from 95% ethanol). IR ($CHCl_3$): $\nu = 3431$ (NH) and 1648 cm^{-1} (CO); ^1H NMR ($CDCl_3$): $\delta = 1.10-1.55$ (m, 10H, $5CH_2$), 1.65–1.85 (m, 2H, CH_2), 3.90–4.10 (m, 1H, CHN), 5.35 (d, $J = 7.8$ Hz, 1H, NH, ex), 7.10–7.30 (m, 6H, ar), 7.35 (s, 1H, ar), 8.05 ppm (s, 1H, pyr H-3). Anal. $C_{23}H_{22}Cl_3N_3O$ (C, H, N).

*6.1.4.10. 5-(4-Chlorophenyl)-N-cyclooctyl-1-(2,4-dichlorophenyl)-1H-pyrazole-4-carboxamide (**4aj**).* Yield 1.13 g (79%); mp 165–166 °C (from ethyl acetate). IR ($CHCl_3$): $\nu = 3431$ (NH) and 1648 cm^{-1} (CO); ^1H NMR ($CDCl_3$): $\delta = 1.20-1.55$ (m, 12H, $6CH_2$), 1.60–1.80 (m, 2H, CH_2), 3.90–4.10 (m, 1H, CHN), 5.32 (d, $J = 7.8$ Hz, 1H, NH, ex), 7.10–7.30 (m, 6H, ar), 7.35 (s, 1H, ar), 8.07 ppm (s, 1H, pyr H-3). Anal. $C_{24}H_{24}Cl_3N_3O$ (C, H, N).

*6.1.4.11. N-Adamantan-1-yl-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-1H-pyrazole-4-carboxamide (**4ak**).* Yield 1.30 g (87%); double mp 186–187 and 208–209 °C (from 95% ethanol). IR ($CHCl_3$): $\nu = 3422$ (NH) and 1654 cm^{-1} (CO); ^1H NMR ($CDCl_3$): $\delta = 1.50-2.10$ (m, 15 H, $6CH_2 + 3CH$), 5.08 (br s, 1H, NH, ex), 7.0–7.30 (m, 6H, ar), 7.35 (s, 1H, ar), 8.03 ppm (s, 1H, pyr H-3). Anal. $C_{26}H_{24}Cl_3N_3O$ (C, H, N).

*6.1.4.12. 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-(pyrrolidin-1-ylcarbonyl)-1H-pyrazole (**4al**).* Yield 1.13 g (90%); double mp 118–119 and 150–151 °C (from ethyl ether/petroleum ether). IR ($CHCl_3$): $\nu = 1612$ cm^{-1} (CO); ^1H NMR ($CDCl_3$): $\delta = 1.60-1.90$ (m, 4H, $2CH_2$), 3.15 (t, $J = 6.4$ Hz, 2H, CH_2N), 3.48 (t, $J = 6.4$ Hz, 2H, CH_2N), 7.05–7.25 (m, 6H, ar), 7.37 (s, 1H, ar), 7.85 ppm (s, 1H, pyr H-3). Anal. $C_{20}H_{16}Cl_3N_3O$ (C, H, N).

*6.1.4.13. 1-{[5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-1H-pyrazol-4-yl]carbonyl}piperidine (**4am**).* Yield 1.12 g (86%); mp 196–197 °C (from 95% ethanol). IR ($CHCl_3$): $\nu = 1615$ cm^{-1} (CO); ^1H NMR ($CDCl_3$): $\delta = 1.00-1.25$ (m, 2H, CH_2), 1.35–1.60 (m, 4H, $2CH_2$), 3.05–3.25 (m, 2H, CH_2N), 3.45–3.65 (m, 2H, CH_2N), 7.06 (d, $J = 8.8$ Hz, 2H, ar), 7.15–7.30 (m, 4H, ar), 7.40 (s, 1H, ar), 7.78 ppm (s, 1H, pyr H-3). Anal. $C_{21}H_{18}Cl_3N_3O$ (C, H, N).

*6.1.4.14. 2-{[5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-1H-pyrazol-4-yl]carbonyl}-1,2,3,4-tetrahydroisoquinoline (**4an**).* Yield 1.33 g (92%); mp 163–164 °C (from 95% ethanol). IR ($CHCl_3$): $\nu = 1615$ cm^{-1} (CO); ^1H NMR ($CDCl_3$): $\delta = 2.40-2.60$ (m, 1H, CH), 2.70–2.90 (m, 1H, CH), 3.40–3.60 (m, 1H, CH), 3.75–3.95 (m, 1H, CH), 4.25–4.45 (m, 1H, CH), 6.65–6.85 (m, 1H, CH), 6.90–7.30 (m, 10H, ar), 7.40 (s, 1H, ar), 7.85 ppm (s, 1H, pyr H-3). Anal. $C_{25}H_{18}Cl_3N_3O$ (C, H, N).

*6.1.4.15. 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-N-(2-piperidin-1-ylethyl)-1H-pyrazole-4-carboxamide (**4ao**).* Yield 1.30 g (91%); mp 148–149 °C (from 95% ethanol). IR ($CHCl_3$): $\nu = 3394$ (NH) and 1646 cm^{-1} (CO); ^1H NMR ($CDCl_3$): $\delta = 1.20-1.40$ (m, 6H, $3CH_2$), 2.10–2.20 (m, 4H, 2 pip CH_2N), 2.26 (t, $J = 6.0$ Hz, 2H, CH_2N), 3.30 (q, $J = 6.0$ Hz, 2H, CH_2NCO), 6.12 (d, $J = 6.0$ Hz, 1H, NH, ex), 7.10–7.30 (m, 6H, ar), 7.35 (s, 1H, ar), 8.10 ppm (s, 1H, pyr H-3). Anal. $C_{23}H_{23}Cl_3N_4O$ (C, H, N).

*6.1.4.16. 1-(2,4-Dichlorophenyl)-5-(4-fluorophenyl)-N-piperidin-1-yl-1H-pyrazole-4-carboxamide (**4ba**).* Yield 0.80 g (62%); mp 209–210 °C (from ethyl acetate). IR ($CHCl_3$): $\nu = 3415$ and 3326 (NH) and 1669 cm^{-1} (CO); ^1H NMR ($CDCl_3$): $\delta = 1.25-1.45$ (m, 2H, CH_2), 1.55–1.75 (m, 4H, $2CH_2$), 2.55–2.75 (m, 4H, $2CH_2N$), 6.30 (br s, 1H, NH, ex), 6.90–7.10 (m, 2H, ar), 7.15–7.35 (m, 4H, ar), 7.43 (s, 1H, ar), 8.17 ppm (s, 1H, pyr H-3). Anal. $C_{21}H_{19}Cl_2FN_4O$ (C, H, N).

*6.1.4.17. 1-(2,4-Dichlorophenyl)-5-(4-fluorophenyl)-N-morpholin-4-yl-1H-pyrazole-4-carboxamide (**4bb**).* Yield 0.97 g (75%); mp 210–211 °C (from ethyl acetate). IR ($CHCl_3$): $\nu = 3417$ and 3328 (NH) and 1672 cm^{-1} (CO); ^1H NMR ($CDCl_3$): $\delta = 2.65-2.85$ (m, 4H, $2CH_2$ N), 3.65–3.85 (m, 4H, $2CH_2O$), 6.56 (s, 1H, NH, ex), 6.90–7.10 (m, 2H, ar), 7.15–7.35 (m, 4H, ar), 7.42 (s, 1H, ar), 8.17 ppm (s, 1H, pyr H-3). Anal. $C_{20}H_{17}Cl_2FN_4O_2$ (C, H, N).

*6.1.4.18. N-Cyclopropyl-1-(2,4-dichlorophenyl)-5-(4-fluorophenyl)-1H-pyrazole-4-carboxamide (**4bc**).* Yield 1.05 g (90%); mp 177–178 °C (from ethyl ether). IR ($CHCl_3$): $\nu = 3433$ (NH) and 1659 cm^{-1} (CO); ^1H NMR ($CDCl_3$): $\delta = 0.20-0.35$ (m, 2H, CH_2), 0.55–0.75 (m, 2H, CH_2), 2.55–2.75 (m, 1H, CHN), 5.65 (br s, 1H, NH, ex), 6.85–7.0 (m, 2H, ar), 7.05–7.25 (m, 4H, ar), 7.33 (s, 1H, ar), 8.04 ppm (s, 1H, pyr H-3). Anal. $C_{19}H_{14}Cl_2FN_3O$ (C, H, N).

*6.1.4.19. N-Cyclohexyl-1-(2,4-dichlorophenyl)-5-(4-fluorophenyl)-1H-pyrazole-4-carboxamide (**4bd**).* Yield 0.88 g (68%); mp 170–171 °C (from ethyl acetate). IR ($CHCl_3$): $\nu = 3428$ (NH) and 1648 cm^{-1} (CO); ^1H NMR ($CDCl_3$): $\delta = 0.90-1.65$ (m, 8H, $4CH_2$), 1.75–1.95 (m, 2H, CH_2), 3.75–3.95 (m, 1H, CHN), 5.34 (d, $J = 7.8$ Hz, 1H, NH, ex),

7.0–7.40 (m, 6H, ar), 7.43 (s, 1H, ar), 8.16 ppm (s, 1H, pyr H-3). Anal. $C_{22}H_{20}Cl_2FN_3O$ (C, H, N).

*6.1.4.20. 5-(4-Bromophenyl)-1-(2,4-dichlorophenyl)-N-piperidin-1-yl-1H-pyrazole-4-carboxamide (**4ca**).* Yield 0.81 g (55%); mp 219–220 °C (from 95% ethanol). IR ($CHCl_3$): $\nu = 3415$ and 3326 (NH) and 1668 cm^{-1} (CO); ^1H NMR ($CDCl_3$): $\delta = 1.30$–1.50 (m, 2H, CH_2), 1.55–1.80 (m, 2H, $2CH_2$), 2.60–2.85 (m, 4H, $2CH_2N$), 6.38 (br s, 1H, NH, ex), 7.05–7.30 (m, 4H, ar), 7.35–7.60 (m, 3H, ar), 7.44 (s, 1H, ar), 8.12 ppm (s, 1H, pyr H-3). Anal. $C_{21}H_{19}BrCl_2N_4O$ (C, H, N).

*6.1.4.21. 5-(4-Bromophenyl)-1-(2,4-dichlorophenyl)-N-(4-methylpiperazin-1-yl)-1H-pyrazole-4-carboxamide (**4cb**).* Yield 1.60 g (78%); mp 203–204 °C (from ethyl acetate). IR ($CHCl_3$): $\nu = 3417$ and 3328 (NH) and 1671 cm^{-1} (CO); ^1H NMR ($CDCl_3$): $\delta = 2.23$ (s, 3H, CH_3), 2.35–2.55 (m, 4H, $2CH_2$), 2.60–2.80 (m, 4H, $2CH_2$), 6.30 (s, 1H, NH, ex), 7.09 (d, $J = 8.0$ Hz, 2H, ar), 7.15–7.25 (m, 2H, ar), 7.35 (s, 1H, ar), 7.39 (d, $J = 8.0$ Hz, 2H, ar), 8.08 ppm (s, 1H, pyr H-3). Anal. $C_{21}H_{20}BrCl_2N_5O$ (C, H, N).

*6.1.4.22. 5-(4-Bromophenyl)-N-cyclopropyl-1-(2,4-dichlorophenyl)-1H-pyrazole-4-carboxamide (**4cc**).* Yield 1.23 g (91%); mp 220–221 °C (from 95% ethanol). IR ($CHCl_3$): $\nu = 3434$ (NH) and 1659 cm^{-1} (CO); ^1H NMR ($CDCl_3$): $\delta = 0.30$–0.40 (m, 2H, CH_2), 0.65–0.80 (m, 2H, CH_2), 2.60–2.75 (m, 1H, CHN), 5.65 (br s, 1H, NH, ex), 7.00–7.25 (m, 4H, ar), 7.30–7.45 (m, 3H, ar), 8.02 ppm (s, 1H, pyr H-3). Anal. $C_{19}H_{14}BrCl_2N_3O$ (C, H, N).

*6.1.4.23. 5-(4-Bromophenyl)-N-cyclohexyl-1-(2,4-dichlorophenyl)-1H-pyrazole-4-carboxamide (**4cd**).* Yield 1.33 g (90%); mp 206–207 °C (from 95% ethanol). IR ($CHCl_3$): $\nu = 3429$ (NH) and 1648 cm^{-1} (CO); ^1H NMR ($CDCl_3$): $\delta = 0.85$–1.55 (m, 8H, $4CH_2$), 1.70–1.90 (m, 2H, CH_2), 3.70–3.90 (m, 1H, CHN), 5.32 (d, $J = 7.8$ Hz, 1H, NH, ex), 6.96 (d, $J = 8.2$ Hz, 2H, ar), 7.10–7.25 (m, 2H, ar), 7.35 (s, 1H, ar), 7.62 (d, $J = 8.2$ Hz, 2H, ar), 8.04 ppm (s, 1H, pyr H-3). Anal. $C_{22}H_{20}BrCl_2N_3O$ (C, H, N).

*6.1.4.24. 5-(4-Bromophenyl)-N-cycloheptyl-1-(2,4-dichlorophenyl)-1H-pyrazole-4-carboxamide (**4ce**).* Yield 1.24 g (81%); double mp 188–189 and 192–193 °C (from 95% ethanol). IR ($CHCl_3$): $\nu = 3431$ and 3326 (NH) and 1648 cm^{-1} (CO); ^1H NMR ($CDCl_3$): $\delta = 1.10$–1.55 (m, 10H, $5CH_2$), 1.70–1.90 (m, 2H, CH_2), 3.90–4.10 (m, 1H, CHN), 5.35 (d, $J = 7.8$ Hz, 1H, NH, ex), 7.10 (d, $J = 8.6$ Hz, 2H, ar), 7.15–7.25 (m, 2H, ar), 7.35 (s, 1H, ar), 7.42 (d, $J = 8.6$ Hz, 2H, ar), 8.05 ppm (s, 1H, pyr H-3). Anal. $C_{23}H_{22}BrCl_2N_3O$ (C, H, N).

*6.1.4.25. 1-(2,4-Dichlorophenyl)-5-(4-iodophenyl)-N-piperidin-1-yl-1H-pyrazole-4-carboxamide (**4da**).* Yield 1.08 g (67%); mp 229–230 °C (from 95% ethanol). IR ($CHCl_3$): $\nu = 3415$ and 3326 (NH) and 1668 cm^{-1} (CO); ^1H NMR ($CDCl_3$): $\delta = 1.20$–1.40 (m, 2H, CH_2), 1.50–1.70 (m, 4H, $2CH_2$), 2.70–2.95 (m, 4H, $2CH_2N$), 6.55 (br s, 1H, NH, ex), 6.95 (d, $J = 8.4$ Hz, 2H, ar), 7.10–7.25 (m, 2H, ar), 7.35 (s, 1H, ar), 7.60 (d, $J = 8.2$ Hz, 2H, ar), 8.18 ppm (s, 1H, pyr H-3). Anal. $C_{21}H_{19}Cl_2IN_4O$ (C, H, N).

*6.1.4.26. 1-(2,4-Dichlorophenyl)-5-(4-iodophenyl)-N-(4-methylpiperazin-1-yl)-1H-pyrazole-4-carboxamide (**4db**).* Yield 1.22 g (73%); mp 233–234 °C (from 95% ethanol). IR ($CHCl_3$): $\nu = 3415$ and 3327 (NH) and 1671 cm^{-1} (CO); ^1H NMR ($CDCl_3$): $\delta = 2.23$ (s, 3H, CH_3), 2.35–2.55 (m, 4H, $2CH_2$), 2.60–2.80 (m, 4H, $2CH_2$), 6.20 (s, 1H, NH, ex), 6.95 (d, $J = 8.2$ Hz, 2H, ar), 7.10–7.25 (m, 2H, ar), 7.35 (s, 1H, ar), 7.60 (d, $J = 8.2$ Hz, 2H, ar), 8.08 ppm (s, 1H, pyr H-3). Anal. $C_{21}H_{20}Cl_2IN_5O$ (C, H, N).

*6.1.4.27. N-Cyclopropyl-1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-1H-pyrazole-4-carboxamide (**4dc**).* Yield 1.39 g (93%); mp 228–229 °C (from 95% ethanol). IR ($CHCl_3$): $\nu = 3433$ (NH) and 1658 cm^{-1} (CO); ^1H NMR ($CDCl_3$): $\delta = 0.25$–0.40 (m, 2H, CH_2), 0.60–0.75 (m, 2H, CH_2), 2.60–2.75 (m, 1H, CHN), 5.65 (br s, 1H, NH, ex), 6.94 (d, $J = 8.6$ Hz, 2H, ar), 7.10–7.25 (m, 2H, ar), 7.35 (s, 1H, ar), 7.60 (d, $J = 8.6$ Hz, 2H, ar), 8.01 ppm (s, 1H, pyr H-3). Anal. $C_{19}H_{14}Cl_2IN_3O$ (C, H, N).

*6.1.4.28. N-Cyclohexyl-1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-1H-pyrazole-4-carboxamide (**4dd**).* Yield 1.54 g (95%); mp 228–229 °C (from 95% ethanol). IR ($CHCl_3$): $\nu = 3429$ (NH) and 1647 cm^{-1} (CO); ^1H NMR ($CDCl_3$): $\delta = 0.85$–1.60 (m, 8H, $4CH_2$), 1.65–1.85 (m, 2H, CH_2), 3.70–3.90 (m, 1H, CHN), 5.33 (d, $J = 7.8$ Hz, 1H, NH, ex), 7.10 (d, $J = 7.8$ Hz, 2H, ar), 7.15–7.25 (m, 2H, ar), 7.35 (s, 1H, ar), 7.42 (d, $J = 7.8$ Hz, 2H, ar), 8.05 ppm (s, 1H, pyr H-3). Anal. $C_{22}H_{20}Cl_2IN_3O$ (C, H, N).

*6.1.4.29. N-Cycloheptyl-1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-1H-pyrazole-4-carboxamide (**4de**).* Yield 1.63 g (98%); mp 199–200 °C (from 95% ethanol). IR ($CHCl_3$): $\nu = 3431$ (NH) and 1648 cm^{-1} (CO); ^1H NMR ($CDCl_3$): $\delta = 1.10$–1.55 (m, 10H, $5CH_2$), 1.65–1.85 (m, 2H, CH_2), 3.90–4.10 (m, 1H, CHN), 5.34 (d, $J = 7.8$ Hz, 1H, NH, ex), 6.96 (d, $J = 8.6$ Hz, 2H, ar), 7.10–7.25 (m, 2H, ar), 7.35 (s, 1H, ar), 7.63 (d, $J = 8.6$ Hz, 2H, ar), 8.05 ppm (s, 1H, pyr H-3). Anal. $C_{23}H_{22}Cl_2IN_3O$ (C, H, N).

*6.1.4.30. N-Adamantan-1-yl-1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-1H-pyrazole-4-carboxamide (**4df**).* Yield 1.72 g (97%); mp 238–239 °C (from 95% ethanol). IR ($CHCl_3$): $\nu = 3422$ (NH) and 1653 cm^{-1} (CO); ^1H NMR ($CDCl_3$): $\delta = 1.50$–2.10 (m, 15 H, $6CH_2 + 3CH$), 5.08 (br s, 1H, NH, ex), 6.95 (d, $J = 8.4$ Hz, 2H, ar), 7.10–7.25 (m, 2H, ar), 7.35 (s, 1H, ar), 7.62 (d, $J = 8.4$ Hz, 2H, ar), 8.01 ppm (s, 1H, pyr H-3). Anal. $C_{26}H_{24}Cl_2IN_3O$ (C, H, N).

*6.1.4.31. 1-(2,4-Dichlorophenyl)-N-(piperidin-1-yl)-5-(4-methylphenyl)-1H-pyrazole-4-carboxamide (**4ea**).* Yield 0.75 g

(58%); mp 228–229 °C (from 95% ethanol). IR ($CHCl_3$): $\nu = 3410$ and 3323 (NH) and 1666 cm^{-1} (CO); ^1H NMR ($CDCl_3$): $\delta = 1.25$–1.45 (m, 2H, CH_2), 1.55–1.70 (m, 4H, $2CH_2$), 2.37 (s, 3H, CH_3), 2.50–2.65 (m, 4H, $2CH_2N$), 6.24 (br s, 1H, NH, ex), 7.15–7.30 (m, 6H, ar), 7.42 (s, 1H, ar), 8.22 ppm (s, 1H, pyr H-3). Anal. $C_{22}H_{22}Cl_2N_4O$ (C, H, N).

*6.1.4.32. N-Cyclohexyl-1-(2,4-dichlorophenyl)-5-(4-methylphenyl)-1H-pyrazole-4-carboxamide (**4eb**).* Yield 1.10 g (86%); mp 183–184 °C (from ethyl acetate/petroleum ether). IR ($CHCl_3$): $\nu = 3421$ and 3326 (NH) and 1646 cm^{-1} (CO); ^1H NMR ($CDCl_3$): $\delta = 0.80$–1.55 (m, 8H, $4CH_2$), 1.70–1.90 (m, 2H, CH_2), 2.36 (s, 3H, CH_3), 3.75–3.95 (m, 1H, CHN), 5.38 (d, $J = 7.8$ Hz, 1H, NH, ex), 7.20–7.30 (m, 6H, ar), 7.42 (s, 1H, ar), 8.21 ppm (s, 1H, pyr H-3). Anal. $C_{23}H_{23}Cl_2N_3O$ (C, H, N).

*6.1.4.33. 1-(2,4-Dichlorophenyl)-5-(4-methoxyphenyl)-N-(piperidin-1-yl)-1H-pyrazole-4-carboxamide (**4fa**).* Yield 0.80 g (60%); mp 199–200 °C (from 95% ethanol). IR ($CHCl_3$): $\nu = 3409$ and 3322 (NH) and 1669 cm^{-1} (CO); ^1H NMR ($CDCl_3$): $\delta = 1.15$–1.25 (m, 4H, CH_2), 1.45–1.65 (m, 4H, $2CH_2$), 2.45–2.65 (m, 4H, $2CH_2N$), 6.24 (br s, 1H, NH, ex), 6.78 (d, $J = 8.6$ Hz, 2H, ar), 7.0–7.20 (m, 4H, ar), 7.32 (s, 1H, ar), 8.12 ppm (s, 1H, pyr H-3). Anal. $C_{22}H_{22}Cl_2N_4O_2$ (C, H, N).

*6.1.4.34. N-Cyclohexyl-1-(2,4-dichlorophenyl)-5-(4-methoxyphenyl)-1H-pyrazole-4-carboxamide (**4fb**).* Yield 0.96 g (72%); mp 194–195 °C (from 95% ethanol). IR ($CHCl_3$): $\nu = 3420$ (NH) and 1645 cm^{-1} (CO); ^1H NMR ($CDCl_3$): $\delta = 0.75$–1.50 (m, 8H, $4CH_2$), 1.60–1.80 (m, 2H, $2CH_2$), 3.74 (s, 3H, CH_3), 3.70–3.90 (m, 1H, CHN), 5.30 (d, $J = 7.8$ Hz, 1H, NH, ex), 6.80 (d, $J = 8.6$ Hz, 2H, ar), 7.05–7.20 (m, 4H, ar), 7.33 (s, 1H, ar), 8.12 ppm (s, 1H, pyr H-3). Anal. $C_{23}H_{23}Cl_2N_3O_2$ (C, H, N).

6.2. Computational methods

The studied compounds were sketched and energy minimized within MOE (CCG) [34]. The theoretical three-dimensional model of the hCB$_1$ receptor was built by homology modeling using bovine rhodopsine coordinates [PDB code 1F88] according to the procedure already described [33]. Briefly, the amino acid sequence of the CB$_1$ receptor was retrieved from the SWISSPROT database. The sequence alignment was performed with CLUSTALW, results were in agreement with those obtained by Shim et al. [29]. Secondary structure predictions were carried out using PSIPRED V2.0. Fold recognition and sequence to structure alignment was performed using THREADER. The three-dimensional structure coordinate file of bovine rhodopsin was obtained from the Protein Data Bank. Only the seven TM receptor domains were modelled, since the exact conformation of the extracellular loops is not computable without X ray data of the specific receptor structure. The final structure was subjected to 5000 steps of energy minimisation using the AMBER force field. Subsequently, a 100 ps molecular dynamics simulation at 300°K (step length: 0.001 ps) involving only the side-chain atoms was carried out. The structure was then subjected to another 5000 steps of energy minimisation. Structural evaluation of the overall model was accomplished using the program PROCHECK. Docking studies were subsequently performed, according the following protocol. In a first step, a blind docking procedure was applied on rimonabant so as to identify its putative binding site inside the protein, then results were compared with site-directed mutagenesis data and with computational results available in literature. In a second step, the validated docked procedure was applied to identify the more promising hCB$_1$ ligands out of a virtual library obtained by the combinatorial combination of intermediates **3** and a number of amines/hydrazines available on the market. The docking poses were evaluated in terms of total estimated binding energy, internal strain energy of the ligand, van der Waals and electrostatic interaction energies and for each ligand, 20 best docked conformations, according to the MOE score, were saved and analysed.

All calculations were carried out on a standard personal computer running under Windows XP.

6.3. Biology

Competitive binding experiments toward the human CB$_1$ and CB$_2$ cannabinoid receptors, expressed in membranes of Chinese Hamster Ovarian (CHO) cells, were performed by Cerep (Poitiers, France) at 10 μM concentrations. [^{3}H]-CP 55940 (0.5 nM) and [^{3}H]-WIN 55212-2 (0.8 nM) were used as radioligands for the hCB$_1$ and hCB$_2$ receptors, respectively. Further details of the methodologies for each assay can be found at the following URL: http://www.cerep.fr. The specific ligand binding to the receptors is defined as the difference between the total binding and the non-specific binding determined in the presence of an excess of unlabelled ligand. The results are expressed as a percentage of control specific binding obtained in the presence of the test compounds. The affinity constants (K_i) were calculated from the Cheng–Prusoff equation ($K_i = IC_{50}/(1 + (L/K_D))$, where L = concentration of radioligand in the assay, and K_D = affinity of the radioligand for the receptor).

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