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ABSTRACT

In the last few years, cannabinoid type-2 receptor (CB_2R) selective ligands have shown a great potential as novel therapeutic drugs in several diseases. With the aim of discovering new selective cannabinoid ligands, a series of pyridazinone-4-carboxamides was designed and synthesized, and the new derivatives tested for their affinity toward the *h*CB_1R and *h*CB_2R. The 6-(4-chloro-3-methylphenyl)-2-(4-fluorobenzyl)-*N*-(*cis*-4-methylcyclohexyl)-3-oxo-2,3-dihydropyridazine-4-carboxamide (**9**) displayed high CB_2-affinity (K_iCB_2 = 2.0 ± 0.81 nM) and a notable selectivity (K_iCB_1/K_iCB_2 > 2000). In addition, **9** and other active new synthesized entities have demonstrated to behave as CB_2R inverse agonists in [^{35}S]-GTPγS binding assay. ADME predictions of the newly synthesized CB_2R ligands suggest a favourable pharmacokinetic profile. Docking studies disclosed the specific pattern of interactions of these derivatives. Our results support that pyridazinone-4-carboxamides represent a new promising scaffold for the development of potent and selective CB_2R ligands.

Keywords: scaffold hopping, synthesis, cannabinoid receptors, CB_2 antagonism, ADME model, docking studies

1. Introduction

At the beginning of 1960, with the increase in recreational use of marijuana, the scientific community started to study the pharmacological mechanisms underlying the psychotropic action of cannabis plant. The identification of the main psychotropic component of *Cannabis sativa*, the (−)-*trans*-Δ^9-tetrahydrocannabinol (Δ^9-THC) [1], and the discovery of its molecular targets [2,3], the so-called cannabinoid receptors, along with their respective endocannabinoids and the enzymes responsible for their regulation [4-6], led to the identification of a complex intercellular communicating system, named endocannabinoid system involved in numerous physiological and physiopathological processes in the body.

Among the two types of G-protein coupled receptors (GPCRs) [7], cannabinoid receptors type-1 (CB_1Rs) and type-2 (CB_2Rs), the former are expressed predominantly in the central nervous system (CNS), in which they are implicated in the control of many neurobiological processes, including nociception, control of movement, emesis, learning and cognitive function [8], whereas the latter are expressed mainly in cells of the immune system such as monocytes, macrophages, B-cells, and T-cells, in which they modulate cytokine release and immune cell migration [9]. Nevertheless, further investigations revealed that CB_2R are also expressed in many other types of cells and tissues, including activated microglia and infiltrated macrophages in the brain [10], vascular endothelial cells, cardiomyocytes, and bone cells, as well as tissues of the gastrointestinal tract [11]. In fact, CB_1R and CB_2R are often co-expressed in the same peripheral tissues and cells [12], and even they may form heteromers enabling synergies at their receptor signalling [13].

As CB_1R is probably involved in most of side effects of cannabinoid treatments in the CNS [14], researchers focused their attention to the development of CB_2R selective ligands, either as agonists or as antagonist/inverse agonists. These ligands would lack of undesirable psychoactive side-

effects due to the limited distribution at the CNS of CB_2R, although such side-effects could appear in the immune system (e.g. immunosuppression). The preclinical evidences accumulated in the last years support that CB_2R selective ligands could be used for the treatment of various pathologies including some CNS-related disorders [15,16]. Activation of CB_2R has shown to be an interesting approach for pain, inflammation, arthritis, neuroprotection, addictions, and cancer. However, none of the CB_2 agonists tested in clinical phases has reached the market yet, probably due to their lack of efficacy [17]. Among the CB_2 ligands developed by academic and pharmaceutical researchers, only few showed inverse agonism/antaginism activity at CB_2R. Thus, it is interesting to discover new CB_2 antagonist scaffolds.

Recently, we have synthesized a series of pyrrole-analogues of the robust CB_2R antagonist SR144528 typified by derivative **1** (**Fig. 1**), which showed high CB_2R affinity (K_i = 5.7 ± 0.7 nM) and selectivity (K_iCB_1/K_iCB_2 = 258) [18]. The same derivative **1** was also reported by Reggio's group (K_iCB_2 = 15.3 nM) as a tool for molecular modelling studies focused on the hydrogen bonding, hydrophobic, and aromatic stacking interactions in the CB_2R binding pocket of the inactive state of the receptor [19].

INSERT FIG. 1

The structure-activity relationship (SAR) studies on CB_2R pyrazole- and pyrrole-based ligands developed in our group [18,20-24] allowed us to determine the key features to CB_2R affinity: a pivotal aromatic ring containing one or more nitrogen atoms bearing a *N*-benzyl group, a carboxamide moiety and a lipophilic aromatic ring. Keeping in mind these pharmacophoric elements and pushed by our interest in pyridazinone derivatives [25,26], we postulated that the introduction of those pharmacophoric elements in the dihydropyridazinone core might provide CB_2R ligands endowed of receptor selectivity and biological activity.

In the present study, we report the synthesis of the new pyridazinone derivatives **2-19, 39** (**Table 1**), preliminary pharmacological data from receptor binding and functionality assays, and docking studies.

2. Result and discussion

2.1. Chemistry

The 3-oxo-6-phenyl-2,3-dihydropyridazines **2–19** were obtained following the synthetic route depicted in **scheme 1**. The condensation of the bromide **21** or **22** with diethyl malonate in presence of sodium hydride, gave the corresponding diester **23** and **24** [27], which reacted with hydrazine monohydrate in ethanol to give the dihydropyridazinones **25** and **26** [27]. Their bromine-assisted oxidation in acetic acid provided compounds **27** and **28** [27]. Heating pyridazinones with the appropriate benzyl halide in presence of K_2CO_3 gave the *N*-alkylated pyridazinones **29–31**. Compared to conventional heating, ultrasonic irradiation led to an improved yield when pentylchloride was used instead of benzyl halides obtaining derivatives **32-33**. Then, hydrolysis of esters **29–33** afforded the carboxylic acids **34-38** that were treated with EDC and HOBt followed by the appropriate amines to give the desired compounds **2-19**.

INSERT SCHEME. 1

The tricyclic derivative **39** was synthesized according to the synthetic pathway illustrated in **scheme 2**. Commercial 1-indanone **40** was treated under heating with diethylketomalonate to obtain the diester **41** [28], that reacted with hydrazine dihydrochloride in ethanol to form the ring system **42** [28]. Then, *N*-alkylation of **43** under ultrasound irradiation with 4-fluorobenzylbromide in the presence of K_2CO_3 gave **43**. Saponification of **43** afforded the acid **44**, whose activation with EDC and HOBt, followed by reaction with cycloheptylamine yielded the desired tricyclic pyridazinone **3**.

INSERT SCHEME. 2

2.2. CB_1R and CB_2R affinities

With the exception of compounds **3**, **4** and **14**, which proved to be insoluble under the test conditions, the affinity and selectivity of all the new pyridazinone-4-carboxamides for cannabinoid receptors were determined by radioligand competition binding assay. Their ability to displace [^{3}H]CP, 55-940 from human cannabinoid CB_1R or CB_2R genes transfected into HEK293 EBNA cells were evaluated. They were first evaluated into a preliminary screening at a concentration of 4 μM. Only those compounds able to displace the [^{3}H]CP,55-940 to CB_1R or CB_2R more than 70% in such preliminary screening were subjected to a secondary evaluation consisting in a complete dose-response curve to determine the inhibition constants (K_i values). The CB_2R and CB_1R K_i values of the new synthesized compounds are reported in **Table 1**. For comparison, the K_i values of the reference cannabinoid ligands HU-308 (selective for the CB_2R) and WIN55,212-2 (it binds CB_2R but also CB_1R) in our receptor binding analysis procedure have been also included in **Table 1**.

INSERT TABLE. 1

Firstly, we evaluated the effect of the modulation of the C4 carboxamide moiety on the 6-(4-chloro-3-methylphenyl)-2-(4-methylbenzyl)-3-oxo-2,3-dihydropyridazine scaffold: among the three selected derivatives **2-4**, only compound **2**, bearing a cyclohexyl group, could be tested due to insolubility problems. The CB_2R binding data of **2** showed moderate CB_2R affinity (K_i = 240 ± 190 nM). With the aim to improve the solubility of these templates, we planned the synthesis of three analogues of **2-4** with a 4-F-benzyl group in N_2 position, compounds **5-7**. The modification of the N_2-benzyl group in the dihydropiridazinone ring led to an improve of CB_2R affinity in the carboxamides bearing a cyclohexyl (**5**), cycloheptyl (**6**) and 1-adamantyl (**7**) group with K_i values of 4.7 ± 2.1 nM, 4.9

± 2.6 nM and 3.4 ± 3.0 nM, respectively, and a good selectivity (K_iCB$_1$/K_iCB$_2$ > 851, 816 and 1176, respectively). Encouraged by these results, we synthesized compound **8**, bearing a fenchyl group in the amide portion, which showed an increase in CB$_2$R affinity (K_i = 2.6 ± 0.47 nM) with higher CB$_2$R selectivity (K_iCB$_1$/K_iCB$_2$ > 1538) compared to **7**. Then, we modulated the carboxamide portion by introduction of simplified cyclic group as *cis*-4-methyl-cyclohexyl (**9**), its *trans* isomer (**10**) and ethylmorpholine derivative (**11**), leading to the best compound of this new series of derivatives, carboxamide **9**, endowed with the high CB$_2$R affinity (K_i = 2.0 ± 0.81 nM) and selectivity (K_iCB$_1$/K_iCB$_2$ > 2000) in the whole series, whereas the ethylmorpholine group (**11**) was unfavourable for the receptor affinity.

In light of the results obtained with these modifications in the N_2 and C4 positions in compounds **5-10**, it was of interest to further determine the influence of the elimination of 4-Cl,3-CH$_3$ substituents in the C6 phenyl ring in the 3-oxo-2,3-dihydropyridazine scaffold. The selectivity for CB$_2$R drastically changed with this modification. All derivatives (**12-16**) showed mixed CB$_1$R and CB$_2$R affinities, greater for the CB$_2$R, always in the low-medium nanomolar range (<100 nM), and lower for the CB$_1$R, always in the submicromolar range (1000-100 nM).

N_2-Alkyl substitution of the pyridazine ring was also analysed. Interestingly, the resulting compounds (**17–19**) maintained high affinity for the CB$_2$R. However, they lost CB$_2$R selectivity showing moderate affinity for CB$_1$R, in particular compound **17** with a K_i in the nM range (K_iCB$_2$ =7.6 ± 4.0 nM, K_iCB$_1$ = 25 ± 8.6 nM).

Also in this series, as already noted in previously described [18], the constraining of the flexible 6-(4-chloro-3-methylphenyl)-2-(4-methylbenzyl)-3-oxo-2,3-dihydropyridazine pharmacophore drastically changed the binding behaviour, resulting the 3-oxo-3,5-dihydro-2H-indeno[1,2-*c*]pyridazine **39** with not affinity for the CBRs (K_i > 4000 nM). Therefore, the presence of the methylene bridge,

modifying pharmacophoric conformation of the pyridazine ring, was unfavourable particularly for CB_2R binding.

2.3. CB_1R/CB_2R-stimulated GTPγS binding

Six compounds (**5, 8, 9, 15, 17** and **18**) were selected for further characterization based on their affinity for the CB_2R. We investigated their functional activity at this receptor, and also of the reference compound SR144528, by conducting GTPγS binding assays that demonstrated that, as happens with SR144528, the six compounds behaved as inverse agonists of the CB_2R with IC_{50} and Emax values indicated in **Fig. 2**. The mean curve of one of these compounds (**5**) at the GTPγS binding bioassay, together with the curve corresponding to SR144528, is shown in **Fig. 2**. The functional potency (IC_{50}) of all the tested compounds, except **17,** was much better than SR144528 (**Fig. 2**).

INSERT FIG. 2

2.4. ADME properties in silico

ADME properties of the newly synthesized CB_2R ligands and of well-known cannabinoids such as SR144528 and HU308 have been calculated *in silico* by using a set of 34 physicochemical descriptors implemented in QikProp (Maestro Software). *In-silico* ADME approaches can be used to help bias drug discovery processes at an early stage, in a lower cost and time manner [30].

The predicted data for the novel pyridazinone derivatives indicated that Lipinski and Jorgensen pharmacokinetics rules are followed [31,32]. As displayed in **Table 2**, the prediction of blood-brain barrier (BBB) partition coefficient, human oral absorption, bioavailability, and gut-blood barrier permeability suggests a favourable drug-like profile for these new compounds, at least similar to the

reference CB_2 ligands SR144528 and HU308. Like most cannabinoids, pyridazinones are lipophilic and they show aqueous solubility values that fall outside the optimum range of values for 95% of known drugs [33,34]. Even though all of these compounds are hydrophobic, curiously, only compounds **3**, **4** and, **14** could not be tested in binding assays due to solubility deficiency. This could be due to the crystalline form in which these compounds solidified.

The only compound that is predicted to have a better biodisponibility than the reference cannabinoids SR144528 and HU308 with improved aqueous solubility and decreased unspecific human serum binding is the ethylmorpholine analogue (**11**). However, this compound (**11**) showed moderate affinity for CB_2 (K_i = 311±63 nM). Finally, the novel compounds **2-10**, **12-19** and **39** have similar ADME predicted data than most of cannabinoids. Therefore, selection of the best candidate (**9**) for future studies was realized based on binding and functionality data. The next step for compound **9** to be considered as suitable drug-like cannabinoid will be the acquisition of experimental ADME data.

INSERT TABLE. 2

2.5. Molecular docking

In this work, molecular docking studies were performed in order to rationalize the pharmacological data describing the affinity and selectivity profile of the newly synthesized CB_2R inverse agonists. The computational studies were developed relying on a a ligand-based homology model of the human CB_2R we previously built, taking into account the molecular structure of the SR144528 and of related analogues [35].

Briefly, the derived pattern of contacts between the inverse agonists and the biological target proved to include H-bonds at least with S165 for weaker compounds, or both the T114 and S165

residues for the most potent analogues, as recently reported in literature [36]. In particular, the 4-chloro-3-methyl-phenyl and the benzyl group of SR144528 were engaged in Van der Waals interactions and π–π stacking with two hydrophobic crevices, delimited by L195, Y190, W194 and I110, L169, respectively. In this way, the fenchyl substituent of SR144528 was oriented toward L160, V164, F197 and F202.

Interestingly, the relevance of this kind of contacts was also confirmed by docking studies we recently performed within a series of in house potent inverse agonists bearing a tricyclic pyrazole carboxamide core [37].

In this context, the newly synthesized derivatives here proposed are characterized by a comparable binding mode, moving the benzyl moiety toward Y190 and W194 whereas the phenyl ring is engaged in Van der Waals contacts with L181. Moreover, the carboxamide function is stabilized by H-bonds contacts with the key residues T114 and S165 (**Fig. 3**).

INSERT FIG. 3

In particular, as shown in **Fig. 4**, the *cis*-methyl-cyclohexyl carboxamide portion of compound **15** is able to mimic the monoterpene moiety and the pyrazole ring of the refererence compound SR144528, whereas the phenyl ring and the pyridazinone core partially play the same role of the SR144528 4-chloro-3-methyl-phenyl substituent. On the other hand, the benzyl group of the new inverse agonist is engaged in π–π stacking with Y190 and W194, moving quite far from the area occupied by the related benzyl of SR144528.

INSERT FIG. 4

Interestingly, all the inverse agonists here disclosed show a similar affinity profile toward the *h*CB_2R (K_i = 2.0–7.4 nM) whereas they are characterized by a different selectivity trend over the CB_1R.

In details, those compounds bearing a disubstitued phenyl ring rather than the related analogues bearing an unsubstituted phenyl ring are more promising selectivity profile to CB_2R over CB_1R.

Concerning this issue, with the aim at exploring any pattern of contacts that could turn in CB_1R binding, we also performed docking studies involving the CB_1R. This kind of analysis was applied starting from the *h*CB_1R homology model we previously described [38], further refined taking into account the X-ray data of the human β_2-adrenoreceptor [39].

Several computational studies we already carried out within a number of different series of CB_1R antagonists allowed us to reveal that three different receptor sub cavities would be necessary involved in receptor-ligand interaction [40]. In particular, the first one revolves around the residues K192 and S383, which are involved in H-bonds with the most potent antagonists, whilst the second and third cavities are placed in the area including F278, I280, M363 and F200, F289, Y292, W356, respectively.

Interestingly, according to our calculations only those compounds bearing an unsubstituted phenyl ring linked to the pyridazinone core are able to occupy the three aforementioned cavities. As shown in **Fig. 5**, the carboxamide moiety of compound **15** displays H-bond with the key residue K192, moving the Q group toward F170 and I175. In addition, the benzyl group and the phenyl moiety display hydrophobic contacts with F200, F289 and F278, I280, V285 and M363, respectively.

INSERT FIG. 5

On the contrary, the related substituted analogues do not fit the CB_1R binding site. Indeed, the 3-methyl, 4-chloro derivatives are expected to clash with the I280, V285 and M363 amino acids.

On this basis, the final computational data prove to be in good agreement with the pharmacological results providing a further validation of the overall method.

3. Conclusions

Using a scaffold hopping approach on pyrazole CB_2R ligands, we designed novel 3-oxo-6-phenyl-2,3-dihydropyridazine-4-carboxamides as a new interesting scaffold for the development of potent CB_2R ligands. SAR studies derived from compound **2** led us to synthesize the best derivative of this series, compound **9** with nanomolar affinity and high selectivity for the CB_2R. The functionality at CB_2R determined by an *in vitro* assay based on [^{35}S]-GTPγS binding showed that compound **9** and other representative compounds behave as CB_2R inverse agonists as evidenced by docking studies. Pyridazinone **9** presents the highest potency at this receptor with an IC_{50} value of 20.7 nM. Therefore, it can be concluded that compound **9** has better affinity and selectivity than other frequently-used inverse agonists for this receptor such as AM630 [41] or JTE-907 [42] and similar affinity to SR144528 but resulting to be more selective than this reference compound [43,44]. *In-silico* ADME calculations indicate that these novel compounds can be considered as suitable drug-like cannabinoid candidates. Then, compound **9** and related derivatives may serve as experimental tools for investigating CB_2R-mediated effects in cellular and tissue models as well as in *in vivo* studies. They may also have interest for a further therapeutic development aimed at selectively inhibiting the CB_2R-mediated activity in certain pathologies, e.g. immunodeficiency, bone disorders, cerebral malaria, in which an excess of CB_2R-dependent endocannabinoid activity has been associated with the progression of the disease and/or with the occurrence of specific symptoms [45].

4. Experimental section

4.1. General procedures

All reactions involving air or moisture-sensitive compounds were performed under nitrogen atmosphere. Solvents and reagents were obtained from commercial suppliers and were used without further purification. The starting ketones **40** and **20**, bromoketone **22** and amines for the synthesis of final compounds were purchased from Sigma-Aldrich®: fenchylamine [46] was synthesized according to the literature procedure. Ultrasound irradiation experiments were carried out in Bandelin® Sonorex ultrasonic bath RK-100H. Flash column chromatography was performed automatically on Flash-master (Biotage®) with pre-packed Biotage® SNAP silica gel cartridges or manually on silica gel (Kieselgel 60, 0.040−0.063 mm, Merck®). Thin layer chromatography (TLC) was performed with Polygram SIL N-HR/HV_{254} pre-coated plastic sheets (0.2 mm) on aluminum sheets (Kieselgel 60 F254, Merck®). Melting points were obtained on a Köfler melting point apparatus and are uncorrected. IR spectra were recorded as nujol mulls on NaCl plates with a Jasco FT/IR 460 plus spectrophotometer and are expressed in ν (cm^{-1}). NMR experiments were run on a Bruker Avance III Nanoboy 400 system (400.13 MHz for ^{1}H, and 100.62 MHz for ^{13}C). Spectra were acquired using deuterated chloroform (chloroform-*d*) or deuterated dimethylsulfoxide (DMSO-d_6) as solvents. Chemical shifts (δ) for ^{1}H- and ^{13}C-NMR spectra are reported in parts per million (ppm) using the residual non-deuterated solvent resonance as the internal standard (for chloroform-d: 7.26 ppm, ^{1}H and 77.16 ppm, ^{13}C; for DMSO-d_6: 2.50 ppm, ^{1}H, 39.52 ppm, ^{13}C). Data are reported as follows: chemical shift (sorted in descending order), multiplicity (s for singlet, br s for broad singlet, d for doublet, dd for double doublet, t for triplet, q for quadruplet, m for multiplet), integration and coupling constants (J) in Hertz (Hz). LC/MS analyses were run on an Agilent 1100 LC/MSD system consisting of a single quadrupole detector (SQD) mass spectrometer (MS) equipped with an electrospray ionization (ESI) interface and a photodiode array (PDA) detector; PDA range was 120−550 nm. ESI in positive mode was applied; mobile phases: (A) ACN in H_2O (8:2). Analyses were performed with a flow rate of 0.9 mL/min; and a

troom temperature All final compounds displayed ≥ 95% purity as determined by elemental analysis on a Perkin-Elmer 240-B analyser, for C, H, and N.

*4.1.1. 2-Bromo-1-(4-chloro-3-methylphenyl)ethan-1-one (**21**)*

Bromine (3,02 mL, 59,3 mmol, 1 eq) was added dropwise to a stirred solution of commercial ketone **20** (10 g, 59,3 mmol, 1 eq) in glacial AcOH (65 mL), the resulting solution was stirred at room temperature for 8 h. Then ice-water was poured into reaction flask and the resulting precipitate was filtered off, washed with H_2O, and air-dried to give **21** as a white solid (14,6 g, 95%). R_f =0,71 (petroleum ether/EtOAc 9:1); mp 60-61 °C. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.61 (s, 1H), 7.49 (d, *J* = 8.2 Hz, 1H), 7.38 (d, *J* = 8.3 Hz, 1H), 4.56 (s, 2H), 2.40 (s, 3H).

*4.1.2. General Procedure for the preparation of ketodiester derivatives **23** and **24** [27]*

Diethylmalonate (1.83 mL, 12 mmol, 1.2 eq) was treated with NaH in mineral oil (0.480 g, 20 mmol, 2 eq) in anhydrous THF (25 mL) under N_2 flow at 0 °C for 30 min. Then a solution of bromoketone **21** or **22** (10 mmol, 1eq) in anhydrous THF (7 mL) was added dropwise and the resulting mixture was stirred at room temperature until complete conversion on starting materials (TLC). Then the solution diluted with EtOAc was poured into a separatory funnel, and washed with saturated NH_4Cl solution and brine. The organic solution was dried (Na_2SO_4) and the solvent removed under reduced pressure. The resulting residue was purified by column flash chromatography using the appropriate eluents.

*4.1.3. Diethyl 2-(2-(4-chloro-3-methylphenyl)-2-oxoethyl)malonate (**23**)*

Mixture of **21**, diethylmalonate and NaH was stirred at room temperature for 3 h. Crude of reaction was purified by flash chromatography (petroleum ether/EtOAc 95:5) to afford **23** as a light yellow oil (1,79 g, 55%), R_f = 0.43 (petroleum ether/EtOAc 9:1); ^{1}H NMR (400 MHz, Chloroform-*d*) δ

7.61 (s, 1H), 7.49 (d, *J* = 8.2 Hz, 1H), 7.38 (d, *J* = 8.3 Hz, 1H), 4.28 – 4.19 (m, 4H), 4.09 – 4.01 (m, 1H), 3.63 (d, *J* = 7.1 Hz, 2H), 2.40 (s, 3H), 1.34 – 1.22 (m, 9H).

*4.1.4. Diethyl 2-(2-oxo-2-phenylethyl)malonate (**24**) [27]*

A mixture of **22**, diethylmalonate and NaH was stirred at room temperature for 1 h. Crude of reaction was purified by flash chromatography (petroleum ether/EtOAc 9:1) to afford **24** [27] as a light yellow oil (1,67 g, 60%), R_f = 0.48 (petroleum ether/EtOAc 8:2); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.99 (d, *J* = 7.7 Hz, 2H), 7.58 (t, *J* = 7.3 Hz, 1H), 7.47 (t, *J* = 7.4 Hz, 2H), 4.29 – 4.19 (m, 4H), 4.09 – 4.03 (m, 1H), 3.63 (d, *J* = 7.1 Hz, 2H), 1.34 – 1.22 (m, 9H).

*4.1.5. General procedure for the preparation of dihidropyridazinone derivatives **25** and **26** [27]*

A solution of diketone **23** or **24** [27] (10 mmol, 1 eq) and hydrazine monohydrate 64-65% (0,48 mL, 10 mmol, 1 eq) in absolute ethanol (50 mL), was heated to reflux and strirred for 24 h. Then the solution was allowed to stand at room temperature and the resulting precipitate was isolated by filtration in vacuum and purified by column flash chromatography using the appropriate eluents.

*4.1.6. Ethyl 5-(4-chloro-3-methylphenyl)-2-oxo-1,2,3,4-tetrahydropyridine-3-carboxylate (**25**)*

From diketone **23** was obtained a solid which purified by flash chromatography (petroleum ether/EtOAc 7:3) gave **25** as a pale yellow solid (1,01 g, 35%). R_f = 0.32 (petroleum ether/EtOAc 7:3); mp 164-165 °C; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.90 (s, 1H, NH, exch with D_2O), 7.61 (s, 1H), 7.49 (d, *J* = 8.2 Hz, 1H), 7.38 (d, *J* = 8.3 Hz, 1H), 4.25 (q, *J* = 7.0 Hz, 2H), 3.60 (t, *J* = 7.6 Hz, 1H), 3.41 (dd, *J* = 16.9, 8.2 Hz, 1H), 3.07 (dd, *J* = 17.0, 6.9 Hz, 1H), 2.41 (s, 3H), 1.28 (t, *J* = 7.2 Hz, 3H).

*4.1.7. Ethyl 3-oxo-6-phenyl-2,3,4,5-tetrahydropyridazine-4-carboxylate (**26**) [27]*

From diketone **24** was obtained a solid which purified by flash chromatography (petroleum ether/EtOAc 1:1) gave **26** [27] as a white solid (1,2 g, 50%). R_f = 0.32 (petroleum ether/EtOAc 1:1);

mp 156-158 °C (158 °C);[26] ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.68 (s, 1H, NH, exch with D_2O), 7.81 – 7.67 (m, 2H), 7.50 – 7.37 (m, 3H), 4.26 (q, *J* = 7.1 Hz, 2H), 3.61 (t, *J* = 7.7 Hz, 1H), 3.45 (dd, *J* = 16.9, 8.4 Hz, 1H), 3.12 (dd, *J* = 16.9, 7.0 Hz, 1H), 1.28 (t, *J* = 7.1 Hz, 3H).

*4.1.8. General procedure for the preparation of pyridazinones **27** and **28** [27]*

To a solution of dihydropyridazinone **25** or **26** [27] (10 mmol, 1 eq) in glacial AcOH (65 mL) was added dropwise a solution of bromine (1 mL, 20 eq) in glacial AcOH (15 mL). The resulting mixture was stirred at room temperature for 6 h. Then the solution was poured into ice-water, and aqueous solution was extracted in a separatory funnel with EtOAc. Afterward the collected organic phases were washed with H_2O, saturated $NaHCO_3$ solution and brine. The organic solution dried (Na_2SO_4) and concentrated under reduced pressure yielding a residue which was purified by column flash chromatography using the appropriate eluents.

*4.1.9. Ethyl 6-(4-chloro-3-methylphenyl)-3-oxo-2,3-dihydropyridazine-4-carboxylate (**27**)*

General procedure for the preparation of pyridazinones was used to oxidize **25.** Mixture of reaction was purified by flash chromatography (petroleum ether/EtOAc 4:6) to afford **27** as a pale yellow solid (1.57 g, 55%). R_f = 0.33 (petroleum ether/EtOAc 1:1); mp 153-154 °C; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.27 (s, 1H), 7.70 (s, 1H), 7.57 (d, *J* = 8.4 Hz, 1H), 7.44 (d, *J* = 8.3 Hz, 1H), 4.47 (q, *J* = 7.1 Hz, 2H), 2.45 (s, 3H), 1.44 (t, *J* = 7.1 Hz, 3H).

*4.1.10. Ethyl 3-oxo-6-phenyl-2,3-dihydropyridazine-4-carboxylate (**28**) [27]*

General procedure for the preparation of pyridazinones was used to oxidize **26**. Mixture of reaction was purified by flash chromatography (petroleum ether/EtOAc 1:1) to afford **28** [27] as a pale yellow solid (0.87 g, 36%). R_f = 0.38 (petroleum ether/EtOAc 1:1); mp 153-154 °C (150 °C);[26] ^{1}H

NMR (400 MHz, Chloroform-*d*) δ 8.32 (s, 1H), 7.82 (d, *J* = 7.3 Hz, 2H), 7.53 – 7.45 (m, 3H), 4.47 (q, *J* = 7.1 Hz, 2H), 1.43 (t, *J* = 7.1 Hz, 3H).

4.1.11. General procedures for the preparation of N-alkyl pyridazinone derivatives ***29-33***

Method A. K_2CO_3 (8.5 mmol, 2.5 eq) and appropriate benzyl-halide (6.8 mmol, 2 eq) were added to a solution of pyridazinones **27** or **28** (3.4 mmol, 1 eq) in anhydrous DMF (17 mL). The resulting mixture was stirred at 60 °C until complete reaction of starting materials (TLC). Then after cooling, H_2O was added into a reaction flask, and the aqueous solution was extracted with EtOAc in a separatory funnel. The collected organic phases were washed with H_2O, LiCl 5% solution and brine, then was dried (Na_2SO_4) and concentrated under reduced pressure. The obtained residues were purified by column flash chromatography using the appropriate eluents.

Method B. A solution of potassium carbonate (10.2 mmol, 3 eq), pentyl chloride (10.2 mmol, 3 eq) and pyridazinones **27** or **28** (3.4 mmol, 1 eq) in anhydrous DMF (15 mL) was exposed to ultrasound irradiation for 2 h at room temperature. Then the mixture of reaction was poured into ice-water, filtered off, and extracted with EtOAc in a separatory funnel. The collected organic phases were washed with H_2O, LiCl 5% solution and brine, then dried (Na_2SO_4) and concentrated under reduced pressure. No further purification was required.

*4.1.12. Ethyl 6-(4-chloro-3-methylphenyl)-2-(4-methylbenzyl)-3-oxo-2,3-dihydropyridazine-4-carboxylate (**29**)*

Method A was used to convert pyridazinone **27** and methylbenzylchloride into the title product. Mixture of reaction was stirred at 60 °C for 4 h and purification by flash chromatography (petroleum ether/EtOAc 8:2) yielded **29** as a yellow oil (1.21 g, 89.6%), R_f = 0.36 (petroleum ether/EtOAc 7:3); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.15 (s, 1H), 7.65 (s, 1H), 7.56 (d, *J* = 8.3 Hz, 1H), 7.47 – 7.36 (m, 3H), 7.14 (d, *J* = 7.8 Hz, 2H), 5.40 (s, 2H), 4.43 (q, *J* = 7.1 Hz, 2H), 2.45 (s, 3H), 2.32 (s, 3H), 1.40 (t,

J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 163.72 (C), 156.37 (C), 142.60 (C), 137.95 (C), 136.86 (C), 136.00 (C), 132.76 (C), 132.67 (C), 131.68 (CH), 130.36 (C), 129.69 (CH), 129.30 (CH x2), 129.13 (CH x2), 128.16 (CH), 124.48 (CH), 62.32 (CH_2), 56.17 (CH_2), 21.17 (CH_3), 20.26 (CH_3), 14.22 (CH_3).

*4.1.13. Ethyl 6-(4-chloro-3-methylphenyl)-2-(4-fluorobenzyl)-3-oxo-2,3-dihydropyridazine-4-carboxylate (**30**)*

Method A was used to convert pyridazinone **27** and 4-fluorobenzyl bromide into the title product. Mixture of reaction was stirred at 60 °C for 3 h and purification by flash chromatography (petroleum ether/EtOAc 8:2) yielded **30** as a light yellow solid (1.2 g, 87%), R_f = 0.47 (petroleum ether/EtOAc 7:3); mp 124-125 °C; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.16 (s, 1H), 7.65 (s, 1H), 7.60 – 7.48 (m, 3H), 7.43 (d, *J* = 8.3 Hz, 1H), 7.02 (t, *J* = 8.6 Hz, 2H), 5.40 (s, 2H), 4.43 (q, *J* = 7.1 Hz, 2H), 2.46 (s, 3H), 1.41 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 163.87 (C), 163.56 (C), 161.42 (C), 156.35 (C), 142.87 (C), 136.94 (C), 136.15 (C), 132.54 (C), 131.87 (CH), 131.09 (CH), 131.01 (CH), 130.43 (C), 129.74 (CH), 128.16 (CH), 124.48 (CH), 115.64 (CH), 115.43 (CH), 62.39 (CH_2), 55.72 (CH_2), 20.27 (CH_3), 14.22 (CH_3).

*4.1.14. Ethyl 2-(4-fluorobenzyl)-3-oxo-6-phenyl-2,3-dihydropyridazine-4-carboxylate (**31**)*

Method A was used to convert pyridazinone **28** and 4-fluorobenzyl bromide into the title product. Mixture of reaction was stirred at 60 °C for 2 h and purification by flash chromatography (petroleum ether/EtOAc 7:3) yielded **31** as a brown solid (1.12 g, 93%), R_f = 0.40 (petroleum ether/EtOAc 7:3); mp 113-115 °C; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.21 (s, 1H), 7.80 (d, *J* = 7.0 Hz, 2H), 7.57 – 7.44 (m, 5H), 7.01 (t, *J* = 8.7 Hz, 2H), 5.41 (s, 2H), 4.43 (q, *J* = 7.1 Hz, 2H), 1.41 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 163.86 (C), 163.59 (C), 161.41 (C), 156.46 (C),

143.74 (C), 134.08 (C), 132.09, 131.50 (C), 131.13 (CH), 131.04 (CH), 130.37 (CH), 129.75 (CH), 129.07 (CH x2), 125.82 (CH x2) ,115.62 (CH), 115.40 (CH), 62.32 (CH_2), 55.65 (CH_2), 14.22 (CH_3).

*4.1.15. Ethyl 6-(4-chloro-3-methylphenyl)-3-oxo-2-pentyl-2,3-dihydropyridazine-4-carboxylate (**32**)*

Using method B from pyridazinone **27** and pentylchloride was obtained **32** as a yellow oil (1.0 g, 85%), R_f = 0.36 (petroleum ether/EtOAc 8:2); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.16 (s, 1H), 7.67 (s, 1H), 7.56 (d, *J* = 8.2 Hz, 1H), 7.43 (d, *J* = 8.3 Hz, 1H), 4.45 (q, *J* = 7.1 Hz, 2H), 4.29 (t, *J* = 7.4 Hz, 2H), 2.46 (s, 3H), 1.95 – 1.82 (m, 2H), 1.49 – 1.34 (m, 7H), 0.91 (t, *J* = 6.2 Hz, 3H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 163.94 (C), 156.54 (C), 142.41 (C), 136.88 (C), 135.92 (C), 132.79 (C), 131.41 (CH), 129.97 (C), 129.68 (CH), 128.11 (CH), 124.41 (CH), 62.32 (CH_2), 53.02 (CH_2), 28.79 (CH_2), 28.10 (CH_2), 20.24 (CH_3), 14.22 (CH_3), 13.95 (CH_3).

*4.1.16. Ethyl 3-oxo-2-pentyl-6-phenyl-2,3-dihydropyridazine-4-carboxylate (**33**)*

Using method B from pyridazinone **28** and pentylchloride was obtained **33** as a yellow oil (0.92 g, 88%), R_f = 0.31 (petroleum ether/EtOAc 8:2); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.21 (s, 1H), 7.81 (d, *J* = 7.6 Hz, 2H), 7.47 (q, *J* = 9.1, 7.8 Hz, 3H), 4.44 (q, *J* = 7.1 Hz, 2H), 4.30 (t, *J* = 7.4 Hz, 2H), 1.96 – 1.83 (m, 2H), 1.47 – 1.30 (m, 7H), 0.91 (t, *J* = 6.4 Hz, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 163.97 (C), 156.65 (C), 143.29 (C), 134.35 (C), 131.64 (CH), 129.93 (C), 129.54 (CH), 129.03 (CH x2), 125.76 (CH x2), 62.24 (CH_2), 52.98 (CH_2), 28.80 (CH_2), 28.09 (CH_2), 22.32 (CH_2), 14.23 (CH_3), 13.95 (CH_3).

*4.1.17. General procedure for the preparation of pyridazinone carboxilic acid derivatives **34-38***

A solution of NaOH 2 M (50 mL) was added to a stirred solution of pyridazinone ester **29-33** (3 mmol) in EtOH (50 mL). The resulting mixture was heated to reflux and stirred for 8 h. Then the

solution was cooled at room temperature and acidified with HCl *6N* .The resulting precipitate was filtered under vacuum, washed with H_2O and air-dried to yield the analytically pure acid.

*4.1.18. 6-(4-Chloro-3-methylphenyl)-2-(4-methylbenzyl)-3-oxo-2,3-dihydropyridazine-4-carboxylic acid (**34**)*

Hydrolysis of ester **29** allowed to obtain acid **34** as a white solid (0.80 g, 73%), R_f = 0.49 ($CHCl_3$/MeOH 9:1); mp 233-235 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.90 (s, 2H), 7.72 (d, *J* = 8.3 Hz, 1H), 7.50 (d, *J* = 8.6 Hz, 1H), 7.26 (d, *J* = 7.4 Hz, 2H), 7.15 (d, *J* = 7.2 Hz, 2H), 5.29 (s, 2H), 2.39 (s, 3H), 2.27 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.09 (C), 157.76 (C), 142.96 (C), 138.69 (C), 136.81 (C), 136.07 (C), 134.35 (C), 133.58 (C), 133.23 (C), 129.33 (CH), 128.99 (CH x2), 128.44 (CH), 128.07 (CH x2), 126.08 (CH), 124.85 (CH), 54.68 (CH_2), 20.66 (CH_3), 19.57 (CH_3).

*4.1.19. 6-(4-Chloro-3-methylphenyl)-2-(4-fluorobenzyl)-3-oxo-2,3-dihydropyridazine-4-carboxylic acid (**35**).*

Hydrolysis of ester **30** allowed to obtain acid **35** as a white solid (0.95 g, 85%), R_f = 0.52 ($CHCl_3$/MeOH 9:1); mp 209-210 °C; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.62 (s, 1H), 7.70 (s, 1H), 7.65 – 7.45 (m, 3H), 7.07 (t, *J* = 8.6 Hz, 2H), 5.48 (s, 2H), 2.47 (s, 3H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 164.18 (C), 163.03 (C), 161.44 (C), 146.08 (C), 141.56 (C), 138.37 (C), 137.33 (C), 133.36 (CH), 131.58 (CH), 131.17 (CH), 131.08 (C), 130.04 (CH), 128.49 (CH), 126.45 (C), 124.78 (CH), 115.81 (CH), 56.19 (CH_2), 20.28 (CH_3).

*4.1.20. 2-(4-Fluorobenzyl)-3-oxo-6-phenyl-2,3-dihydropyridazine-4-carboxylic acid (**36**)*

Hydrolysis of ester **31** allowed to obtain acid **36** as a pale yellow solid (0.93 g, 94.5%), R_f = 0.20 ($CHCl_3$/MeOH 9:1); mp 151-152 °C; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.66 (s, 1H), 7.85 (d, *J* = 4.6 Hz, 2H), 7.64 – 7.38 (m, 5H), 7.06 (t, *J* = 8.4 Hz, 2H), 5.49 (s, 2H); ^{13}C NMR (101 MHz,

Chloroform-*d*) δ 164.16 (C), 163.17 (C), 161.48 (C), 146.93 (C), 133.54 (CH), 133.18 (C), 131.19 (CH), 131.10 (CH), 130.60 (CH), 130.24 (C), 129.31 (CH x2), 126.55 (C), 126.17 (CH x2), 116.00 (CH), 115.78 (CH), 56.11 (CH_2).

*4.1.21. 6-(4-Chloro-3-methylphenyl)-3-oxo-2-pentyl-2,3-dihydropyridazine-4-carboxylic acid (**37**)*

Hydrolysis of ester **32** allowed to obtain acid **37** as a pink solid (0.95 g, 94%), R_f = 0.59 ($CHCl_3$/MeOH 9:1); mp 128-129 °C; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.64 (s, 1H), 7.72 (s, 1H), 7.63 (d, *J* = 8.0 Hz, 1H), 7.47 (d, *J* = 7.8 Hz, 1H), 4.37 (t, *J* = 7.6 Hz, 2H), 2.47 (s, 3H), 1.93 (s, 2H), 1.41-1.33 (m, 4H), 0.93 (t, *J* = 6.2 Hz, 3H). ^{13}C NMR (101 MHz Chloroform-*d*) δ 163.43 (C), 161.68 (C), 145.74 (C), 137.26 (C), 136.97 (C), 132.88 (CH), 131.78 (C), 129.98 (CH), 128.47 (CH), 125.92 (C), 124.75 (CH), 53.40 (CH_2), 28.63 (CH_2), 28.11 (CH_2), 22.23 (CH_2), 20.26 (CH_3), 13.90 (CH_3).

*4.1.22. 3-Oxo-2-pentyl-6-phenyl-2,3-dihydropyridazine-4-carboxylic acid (**38**)*

Hydrolysis of ester **33** allowed to obtain acid **38** as a pink solid (0.71 g, 82%), R_f = 0.57 ($CHCl_3$/MeOH 9:1); mp 89-91 °C; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.68 (s, 1H), 7.87 (s, 2H), 7.51 (s, 3H), 4.39 (t, *J* = 7.2 Hz, 2H), 1.94 (s, 2H), 1.41-1.33 (m, 4H), 0.93 (t, *J* = 6.2 Hz, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 163.49 (C), 161.75 (C), 146.63 (C), 133.37 (C), 133.08 (CH), 130.45 (CH), 129.27 (CH x2), 126.12 (CH x2), 125.87 (C), 53.35 (CH_2), 28.63 (CH_2), 28.09 (CH_2), 22.24 (CH_2), 13.89 (CH_3).

*4.1.23. Diethyl 2-hydroxy-2-(1-oxo-2,3-dihydro-1H-inden-2-yl)malonate (**41**) [28]*

A mixture of 1-indanone **40** (2 g, 15.1 mmol, 1 eq) and diethylketomalonate (2.76 mL, 18.1 mmol, 1.2 eq) was heated at 100 °C overnight. After cooling at room temperature, the residue was purified by flash chromatography (petroleum ether/EtOAc 8:2) to obtain **41** as a pale yellow solid (4.3 g, 93%), R_f = 0.32 (petroleum ether/EtOAc 8:2); mp 75-77 °C (79-80 °C) [28]; ^{1}H NMR (400 MHz,

Chloroform-*d*) δ 7.71 (d, *J* = 7.6 Hz, 1H), 7.59 (t, *J* = 7.4 Hz, 1H), 7.46 (d, *J* = 7.6 Hz, 1H), 7.35 (t, *J* = 7.4 Hz, 1H), 4.50 – 4.37 (m, 2H), 4.29 (dd, *J* = 10.9, 7.1 Hz, 2H), 3.79 – 3.69 (m, 1H), 3.43 (dd, *J* = 17.2, 8.3 Hz, 1H), 3.20 (dd, *J* = 17.2, 4.8 Hz, 1H), 1.36 (t, *J* = 7.1 Hz, 3H), 1.31 (t, *J* = 7.1 Hz, 3H).

*4.1.24. Ethyl 3-oxo-3,5-dihydro-2H-indeno[1,2-c]pyridazine-4-carboxylate (**42**) [28]*

A solution of diester **41** (4.3 g, 14 mmol, 1 eq) and hydrazine dihydrochloride (1.46 g, 14 mmol, 1 eq) in absolute ethanol (85 mL) was heated to reflux and stirred for 24 h. After cooling at room temperature the solvent was removed under reduced pressure, and the residue was diluted with saturated $KHCO_3$ solution and H_2O, then the aqueous solution was extracted in a separatory funnel with CH_2Cl_2. The collected organic phases dried (Na_2SO_4) and concentrated under reduced pressure gave a residue which purified by flash chromatography (petroleum ether/EtOAc 4:6) yelded **42** as a white solid (1.5 g, 40%), R_f = 0.39 (petroleum ether/EtOAc 4:6); mp 228-230 °C; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 11.17 (br s, 1H, NH, exch with D_2O), 7.86 (d, *J* = 6.9 Hz, 1H), 7.66 – 7.33 (m, 3H), 4.49 (q, *J* = 7.1 Hz, 2H), 4.18 (s, 2H), 1.45 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 163.32 (C), 158.11 (C), 150.89 (C), 150.45 (C), 142.67 (C), 135.21 (C), 130.66 (CH), 128.13 (CH), 125.83 (C), 125.40 (CH), 121.18 (CH), 62.02 (CH_2), 35.32 (CH_2), 14.31 (CH_3).

*4.1.25. Ethyl 2-(4-fluorobenzyl)-3-oxo-3,5-dihydro-2H-indeno[1,2-c]pyridazine-4-carboxylate (**43**)*

A mixture of pyridazinone **42** (0.80 g, 3.12 mmol, 1 eq), potassium carbonate (1.3 g, 9.36 mmol, 3 eq) and 4-fluorobenzyl bromide (1.16 mL, 9.36 mmol, 3 eq) in anhydrous DMF (9 mL) was exposed to ultrasound irradiation for 4 h at 30 °C. Then the suspension was poured into a separatory funnel, diluted with EtOAc and washed with H_2O and LiCl 5% solution (x5). The organic solution dried (Na_2SO_4) and concentrated under reduced pressure gave 0.7 g of a yellow oil which was used for further reaction without purification.

*4.1.26. 2-(4-Fluorobenzyl)-3-oxo-3,5-dihydro-2H-indeno[1,2-c]pyridazine-4-carboxylic acid (**44**)*

To a solution of ester **43** (0.7 g, 1.9 mmol) in EtOH (30 ml) was added a solution of NaOH 2 M (30 mL) and the resulting mixture was refluxed for 6 h. After cooling at room temperature the solution was acidified with HCl 6N. The precipitate was filtered, washed with H_2O and air-dried to yield the analytically pure acid **44** as a brown solid (0.3 g, 46%), R_f = 0.36 ($CHCl_3$/MeOH 98:2); mp 80-82 °C; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.69 (d, *J* = 7.7 Hz, 1H), 7.60 (t, *J* = 7.4 Hz, 1H), 7.48 (d, *J* = 7.6 Hz, 1H), 7.37 (t, *J* = 7.4 Hz, 1H), 7.00 – 6.88 (m, 4H), 5.12 (s, 2H), 4.14 (d, *J* = 13.1 Hz, 1H), 3.26 (d, *J* = 13.1 Hz, 1H).

*4.1.27. General procedure for the synthesis of carboxamides **2-19,39***

A mixture of HOBt (0.15 g, 1.05 mmol, 1.5 eq), EDC (0.27 g, 1,4 mmol, 2 eq) and acids **34-38** and **44** (0,7 mmol, 1 eq) in CH_2Cl_2 (6 mL) was stirred at room temperature for 30 min. Afterward a solution of appropriate amine in CH_2Cl_2 (6 mL) was added dropwise at 0 °C. The resulting mixture was allowed to stand at room temperature, and then stirred for 12-24 h. The solution was then poured into a separatory funnel and H_2O was added. The aqueous phase was separated and extracted with CH_2Cl_2. The collected organic phases were washed with H_2O and brine, dried (Na_2SO_4) and concentrated under reduced pressure. The analytically pure product was isolated by column flash chromatography purification using the appropriate eluents.

*4.1.28. 6-(4-Chloro-3-methylphenyl)-N-cyclohexyl-2-(4-methylbenzyl)-3-oxo-2,3-dihydropyridazine-4-carboxamide (**2**)*

General procedure for the synthesis of carboxamides was used to convert acid **34** and cyclohexylamine into the title product. The mixture of reaction was stirred for 12 h, then the crude of reaction was purified by flash chromatography (petroleum ether/EtOAc 8:2) to afford **2** (91 mg, 29%) as a white solid. R_f = 0.24 (petroleum ether/EtOAc 95:5); mp 133-135 °C; IR 1677 (C=O), 3260 (NH); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.60 (d, J = 8.1 Hz, 1H, NH, exch with D_2O), 8.64 (s, 1H), 7.73

(s, 1H), 7.64 (d, *J* = 8.4 Hz, 1H), 7.43 (d, *J* = 8.3 Hz, 1H), 7.37 (d, *J* = 7.7 Hz, 2H), 7.17 (d, *J* = 7.7 Hz, 2H), 5.44 (s, 2H), 4.03 – 3.91 (m, 1H), 2.45 (s, 3H), 2.33 (s, 3H), 2.02 – 1.91 (m, 2H), 1.83 – 1.70 (m, 2H), 1.59 (d, *J* = 15.2 Hz, 2H), 1.49 – 1.21 (m, 4H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 160.64 (C), 159.74 (C), 144.40 (C), 138.08 (C), 136.85 (C), 136.16 (C), 132.70 (C), 132.48 (C), 131.31 (CH), 129.96 (C), 129.70 (CH), 129.41 (CH x2), 128.67 (CH x2), 128.43 (CH), 124.70 (CH), 56.19 (CH_2), 48.60 (CH), 32.68 (CH_2 x2), 25.61 (CH_2), 24.69 (CH_2 x2), 21.19 (CH_3), 20.23 (CH_3); MS (ESI): $C_{26}H_{28}ClN_3O_2$ requires m/z 449.1, found 450.1 $[M + 1]^+$; Anal. calcd for $C_{26}H_{28}ClN_3O_2$: C, 69.40; H, 6.27; N, 9.34; Found: C, 69.42; H, 6.23; N, 9.36.

*4.1.29. 6-(4-Chloro-3-methylphenyl)-N-cycloheptyl-2-(4-methylbenzyl)-3-oxo-2,3-dihydropyridazine-4-carboxamide (**3**)*

General procedure for the synthesis of carboxamides was used to convert acid **34** and cycloheptylamine into the title product. The mixture of reaction was stirred for 16 h, then the crude of reaction was purified by flash chromatography (petroleum ether/EtOAc 8:2) to afford **3** (78 mg, 24%) as a pale yellow solid. R_f = 0.22 (petroleum ether/EtOAc 95:5); IR 1682 (C=O), 3257 (NH); mp 110-112 °C; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.65 (d, *J* = 8.0 Hz, 1H, NH, exch with D_2O), 8.64 (s, 1H), 7.72 (s, 1H), 7.64 (d, *J* = 8.4 Hz, 1H), 7.43 (d, *J* = 8.3 Hz, 1H), 7.37 (d, *J* = 7.7 Hz, 2H), 7.16 (d, *J* = 7.7 Hz, 2H), 5.43 (s, 2H), 4.27 – 4.04 (m, 1H), 2.45 (s, 3H), 2.33 (s, 3H), 2.09 – 1.91 (m, 2H), 1.75 – 1.49 (m, 10H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 160.41 (C), 159.73 (C), 144.38 (C), 138.07 (C), 136.85 (C), 136.15 (C), 132.70 (C), 132.49 (C), 131.26 (CH), 129.96 (C), 129.71 (CH), 129.40 (CH x2), 128.68 (CH x2), 128.42 (CH), 124.68 (CH), 56.15 (CH_2), 50.91 (CH), 34.72 (CH_2 x2), 28.07 (CH_2 x2), 24.16 (CH_2 x2), 21.19 (CH_3), 20.23 (CH_3); MS (ESI): $C_{27}H_{30}ClN_3O_2$ requires m/z 463.2, found 464.4 $[M + 1]^+$; Anal. calcd for $C_{27}H_{30}ClN_3O_2$: C, 69.89; H, 6.52; N, 9.06; Found: C, 69.86; H, 6.54; N, 9.03.

*4.1.30. N-(Adamantan-1-yl)-6-(4-chloro-3-methylphenyl)-2-(4-methylbenzyl)-3-oxo-2,3-dihydropyridazine-4-carboxamide (**4**)*

General procedure for the synthesis of carboxamides was used to convert acid **34** and 1-adamantylamine into the title product. The mixture of reaction was stirred for 18 h, then the crude of reaction was purified by flash chromatography (petroleum ether/EtOAc 9:1) to afford **4** (84 mg, 24%) as a white solid. R_f = 0.39 (petroleum ether/EtOAc 95:5); IR 1680 (C=O), 3264 (NH); mp 169-170 °C; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.50 (br s, 1H, NH, exch with D_2O), 8.63 (s, 1H), 7.72 (s, 1H), 7.63 (d, *J* = 8.4 Hz, 1H), 7.42 (d, *J* = 8.3 Hz, 1H), 7.36 (d, *J* = 7.7 Hz, 2H), 7.16 (d, *J* = 7.6 Hz, 2H), 5.43 (s, 2H), 2.43 (s, 3H), 2.33 (s, 3H), 2.23 – 2.04 (m, 9H), 1.83 – 1.55 (m, 6H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 159.13 (C), 158.83 (C), 143.32 (C), 137.01 (C), 135.80 (C), 135.09 (C), 131.71 (C), 131.49 (C), 129.90 (CH), 129.77 (C), 128.68 (CH), 128.36 (CH x2), 127.66 (CH x2), 127.36 (CH), 123.58 (CH), 55.11 (CH_2), 51.35 (C), 40.30 (CH_2 x3), 35.40 (CH_2 x3), 28.40 (CH x3), 20.17 (CH_3), 19.19 (CH_3); MS (ESI): $C_{30}H_{32}ClN_3O_2$ requires m/z 501.2, found 502.2 $[M + 1]^+$; Anal. calcd for $C_{30}H_{32}ClN_3O_2$: C, 71.77; H, 6.42; N, 8.37; Found: C, 71.80; H, 6.41; N, 8.35.

*4.1.31. 6-(4-Chloro-3-methylphenyl)-2-(4-fluorobenzyl)-N-(cyclohexyl)-3-oxo-2,3-dihydropyridazine-4-carboxamide (**5**)*

General procedure for the synthesis of carboxamides was used to convert acid **35** and cyclohexylamine into the title product. The mixture of reaction was stirred for 18 h, then the crude of reaction was purified by flash chromatography (petroleum ether/EtOAc 8:2) to afford **5** (91 mg, 29%) as a white solid. R_f = 0.72 (petroleum ether/EtOAc 8:2); mp 151-152 °C; IR 1684 (C=O), 3259 (NH); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.56 (d, J = 8.1 Hz, 1H, NH, exch with D_2O), 8.65 (s, 1H), 7.72 (d, J = 2.3 Hz, 1H), 7.63 (d, J = 8.3 Hz, 1H), 7.52 – 7.38 (m, 3H), 7.04 (t, J = 8.6 Hz, 2H), 5.43 (s, 2H), 4.04 – 3.91 (m, 1H), 2.45 (s, 3H), 2.01 – 1.92 (m, 2H), 1.79 – 1.59 (m, 3H), 1.51 – 1.21 (m, 5H). ^{13}C

NMR (101 MHz, Chloroform-*d*) δ 163.88 (C), 161.43 (C), 160.51 (C), 159.70 (C), 144.62 (C), 136.91 (C), 136.28 (C), 132.56 (C), 131.47 (CH), 130.72 (CH), 130.64 (CH), 130.06 (C), 129.74 (CH), 128.41 (CH), 124.69 (CH), 115.76 (CH), 115.55 (CH), 55.64 (CH_2), 48.60 (CH) 32.67 (CH_2 x 2), 25.60 (CH_2), 24.66 (CH_2 x2), 20.23 (CH_3); MS (ESI): $C_{25}H_{25}ClFN_3O_2$ requires m/z 453.2, found 454.4 $[M + 1]^+$; Anal. calcd for $C_{25}H_{25}ClFN_3O_2$: C, 66.15; H, 5.55; N, 9.26; Found: C, 66.19; H, 5.54; N, 9.22.

*4.1.32. 6-(4-Chloro-3-methylphenyl)-2-(4-fluorobenzyl)-N-(cycloheptyl)-3-oxo-2,3-dihydropyridazine-4-carboxamide (**6**)*

General procedure for the synthesis of carboxamides was used to convert acid **35** and cycloheptylamine into the title product. The mixture of reaction was stirred for 18 h, then the crude of reaction was purified by flash chromatography (petroleum ether/EtOAc 8:2) to afford **6** (144 mg, 30%) as a white solid. R_f = 0.70 (petroleum ether/EtOAc 8:2); mp 140-141 °C; IR 1680 (C=O), 3270 (NH); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.61 (d, J = 8.0 Hz, 1H, NH, exch with D_2O), 8.65 (s, 1H), 7.71 (s, 1H), 7.62 (d, J = 8.4 Hz, 1H), 7.52 – 7.41 (m, 3H), 7.04 (t, J = 8.6 Hz, 2H), 5.43 (s, 2H), 4.22 – 4.07 (m, 1H), 2.45 (s, 3H), 2.08 – 1.92 (m, 2H), 1.74 – 1.50 (m, 10H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 163.89 (C), 161.43 (C), 160.29 (C), 159.71 (C), 144.62 (C), 136.92 (C), 136.29 (C), 132.57 (C), 131.44 (CH), 130.73 (CH), 130.65 (CH), 130.08 (C), 129.75 (CH), 128.41 (CH), 124.67 (CH), 115.76 (CH), 115.55 (CH), 55.61 (CH_2), 50.91 (CH), 34.71 (CH_2 x2), 28.07 (CH_2 x2), 24.14 (CH_2 x2), 20.24 (CH_3); MS (ESI): $C_{26}H_{27}ClFN_3O_2$ requires m/z 467.2, found 468.3 $[M + 1]^+$; Anal. calcd for $C_{26}H_{27}ClFN_3O_2$: C, 66.73; H, 5.82; N, 8.98; Found: C, 66.75; H, 5.81; N, 8.95.

*4.1.33. N-(Adamantan-1-yl)-6-(4-chloro-3-methylphenyl)-2-(4-fluorobenzyl)-3-oxo-2,3-dihydropyridazine-4-carboxamide (**7**)*

General procedure for the synthesis of carboxamides was used to convert acid **35** and 1-adamantylamine into the title product. The mixture of reaction was stirred for 18 h, then the crude of

reaction was purified by flash chromatography (petroleum ether/EtOAc 8:2) to afford **7** (110 mg, 30%) as a white solid. R_f = 0.73 (petroleum ether/EtOAc 8:2); mp 130-132 °C; IR 1685 (C=O), 3262 (NH); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.45 (br s, 1H, NH, exch with D_2O), 8.64 (s, 1H), 7.71 (s, 1H), 7.62 (d, *J* = 8.3 Hz, 1H), 7.52 – 7.40 (m, 3H), 7.04 (t, *J* = 8.3 Hz, 2H), 5.42 (s, 2H), 2.44 (s, 3H), 2.23 – 2.08 (m, 9H), 1.83 – 1.67 (m, 6H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 163.87 (C=O), 161.42 (C=O), 160.01 (C), 159.83 (C), 144.59 (C), 136.90 (C), 136.25 (C), 132.60 (C), 131.11 (CH), 130.91 (C), 130.72 (CH), 130.64 (CH), 129.75 (CH), 128.37 (CH), 124.59 (CH), 115.74 (CH), 115.53 (CH), 55.60 (CH_2), 52.41 (C), 41.33(CH_2 x3), 36.41(CH_2 x3), 29.42(CH x3), 20.22(CH_3); MS (ESI): $C_{29}H_{29}ClFN_3O_2$ requires m/z 505.2, found 506.2 $[M + 1]^+$; Anal. calcd for $C_{29}H_{29}ClFN_3O_2$: C, 68.84; H, 5.78; N, 8.30; Found: C, 68.88; H, 5.77; N, 8.31.

*4.1.34. 6-(4-Chloro-3-methylphenyl)-2-(4-fluorobenzyl)-N-((2S)-fenchyl)-3-oxo-2,3-dihydropyridazine-4-carboxamide (**8**)*

General procedure for the synthesis of carboxamides was used to convert acid **35** and N-(1)-(S)-fenchylamine into the title product. The mixture of reaction was stirred for 18 h, then the crude of reaction was purified by flash chromatography (petroleum ether/EtOAc 8:2) to afford **8** (174 mg, 49%) as a white solid. R_f = 0.23 (petroleum ether/EtOAc 95:5); mp 143-145 °C; IR 1683 (C=O), 3269 (NH); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.83 (d, J = 9.2 Hz, 1H, NH, exch with D_2O), 8.66 (s, 1H), 7.70 (s, 1H), 7.62 (d, *J* = 8.2 Hz, 1H), 7.55 – 7.46 (m, 2H), 7.43 (d, *J* = 8.3 Hz, 1H), 7.05 (t, *J* = 8.6 Hz, 2H), 5.46 (s, 2H), 3.81 (d, *J* = 8.8 Hz, 1H), 2.44 (s, 3H), 1.85 – 1.76 (m, 2H), 1.76 – 1.69 (m, 1H), 1.63 – 1.47 (m, 2H), 1.33 – 1.20 (m, 2H), 1.17 (s, 3H), 1.09 (s, 3H), 0.86 (s, 3H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 162.88 (C), 161.22 (C), 160.42 (C), 158.84 (C), 143.56 (C), 135.87 (C), 135.23 (C), 131.58 (C), 130.35 (CH), 129.92 (CH), 129.83 (CH), 128.85 (C), 128.73 (CH), 127.36 (CH), 123.61 (CH), 114.70 (CH), 114.48 (CH), 63.86 (CH), 54.23 (CH_2), 47.86 (C), 47.28 (CH), 41.72 (CH_2), 38.78

(C), 29.96 (CH_3), 26.65 (CH_2), 25.02 (CH_2), 20.58 (CH_3), 19.20 (CH_3), 18.77 (CH_3); MS (ESI): $C_{29}H_{31}ClFN_3O_2$ requires m/z 507.2, found 508.3 $[M + 1]^+$; Anal. calcd for $C_{29}H_{31}ClFN_3O_2$: C, 68.56; H, 6.15; N, 8.27; Found: C, 68.54; H, 6.18; N, 8.23.

*4.1.35. 6-(4-Chloro-3-methylphenyl)-2-(4-fluorobenzyl)-N-(cis-4-methylcyclohexyl)-3-oxo-2,3-dihydropyridazine-4-carboxamide (**9**) and 6-(4-Chloro-3-methylphenyl)-2-(4-fluorobenzyl)-N-(trans-4-methylcyclohexyl)-3-oxo-2,3-dihydropyridazine-4-carboxamide (**10**)*

Compound **9** and **10** were obtained from acid **35** using the general procedure for the synthesis of carboxamides. The mixture of reaction was stirred for 20 h, then *cis*/*trans* isomers were separated by flash chromatography (petroleum ether/EtOAc 9:1). **9** (65 mg, 27 %) white solid; R_f = 0.62 (petroleum ether/EtOAc 8:2); IR 1682 (C=O), 3271 (NH); mp 125-126 °C; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.85 (d, *J* = 7.8 Hz, 1H, NH, exch with D_2O), 8.65 (s, 1H), 7.71 (s, 1H), 7.63 (d, *J* = 8.4 Hz, 1H), 7.57 – 7.36 (m, 3H), 7.05 (t, *J* = 8.4 Hz, 2H), 5.45 (s, 2H), 4.28 – 4.16 (m, 1H), 2.45 (s, 3H), 1.88 – 1.76 (m, 2H), 1.70 – 1.51 (m, 4H), 1.36 – 1.19 (m, 3H), 0.98 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 163.89 (C), 161.44 (C), 160.64 (C), 159.79 (C), 144.58 (C), 136.91 (C), 136.27 (C), 132.59 (C), 131.32 (CH), 130.82 (CH), 130.73 (CH), 130.10 (C), 129.75 (CH), 128.41 (CH), 124.67 (CH), 115.74 (CH), 115.52 (CH), 55.42 (CH_2), 45.93 (CH), 30.98 (CH), 30.08 (CH_2 x 2), 29.45 (CH_2 x 2), 21.49 (CH_3), 20.23 (CH_3); MS (ESI): $C_{26}H_{27}ClFN_3O_2$ requires m/z 457.5, found 458.5 $[M + 1]^+$; Anal. calcd for $C_{26}H_{27}ClFN_3O_2$: C, 66.73; H, 5.82; N, 8.98; Found: C, 66.70; H, 5.84; N, 8.90. **10** (26 mg, 11 %) white solid; R_f = 0.72 (petroleum ether/EtOAc 8:2); IR 1680 (C=O), 3267 (NH); mp 119-121 °C; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.46 (d, *J* = 8.1 Hz, 1H, NH, exch with D_2O), 8.65 (s, 1H), 7.72 (s, 1H), 7.63 (d, *J* = 8.4 Hz, 1H), 7.52 – 7.41 (m, 3H), 7.04 (t, *J* = 7.9 Hz, 2H), 5.43 (s, 2H), 3.87 (d, *J* = 10.5 Hz, 1H), 2.45 (s, 3H), 2.04 (d, *J* = 12.3 Hz, 2H), 1.75 (d, *J* = 13.3 Hz, 2H), 1.43 – 1.22 (m, 3H), 1.18 – 1.04 (m, 2H), 0.91 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (101 MHz,

Chloroform-*d*) δ 162.87 (C), 160.41 (C), 159.63 (C), 158.67 (C), 143.63 (C), 135.91 (C), 135.29 (C), 131.54 (C), 130.49 (CH), 129.67 (CH), 129.58 (CH), 129.02 (C), 128.74 (CH), 127.41 (CH), 123.68 (CH), 114.75 (CH), 114.54 (CH), 54.68 (CH_2), 48.05 (CH_2), 32.78 (CH), 31.77 (CH_2 x 2), 30.91 (CH_2 x 2), 21.17 (CH_3), 19.22 (CH_3). MS (ESI): $C_{26}H_{27}ClFN_3O_2$ requires m/z 457.5, found 458.5 $[M + 1]^+$; Anal. calcd for $C_{26}H_{27}ClFN_3O_2$: C, 66.73; H, 5.82; N, 8.98; Found: C, 66.71; H, 5.80; N, 8.91.

*4.1.36. 6-(4-Chloro-3-methylphenyl)-2-(4-fluorobenzyl)-N-(2-morpholinoethyl)-3-oxo-2,3-dihydropyridazine-4-carboxamide (**11**)*

General procedure for the synthesis of carboxamides was used to convert acid **35** and 4-(2-aminoethyl)morpholine into the title product. The mixture of reaction was stirred for 18 h, then the crude of reaction was purified by flash chromatography ($CHCl_3$/MeOH 98:2) to afford **11** (105 mg, 31%) as a yellow solid. R_f = 0.57 ($CHCl_3$/MeOH 98:2); mp 142-144 °C; IR 1674 (C=O), 3255 (NH); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.79 (br s, 1H, NH, exch with D_2O), 8.64 (s, 1H), 7.72 (s, 1H), 7.63 (d, *J* = 8.3 Hz, 1H), 7.52 – 7.39 (m, 3H), 7.04 (t, *J* = 8.4 Hz, 2H), 5.44 (s, 2H), 3.75 (t, *J* = 4.6 Hz, 4H), 3.58 (q, *J* = 6.2 Hz, 2H), 2.60 (t, *J* = 6.5 Hz, 2H), 2.52 (t, *J* = 4.5 Hz, 4H), 2.45 (s, 3H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 163.90 (C), 161.70 (C), 161.45 (C), 159.55 (C), 144.51 (C), 136.94 (C), 136.32 (C), 132.52 (C), 131.47 (CH), 131.22 (C), 130.83 (CH), 130.75 (CH), 129.77 (CH), 128.39 (CH), 124.66 (CH), 115.75 (CH), 115.53 (CH), 67.03 (CH_2 x2), 57.02 (CH_2), 55.76 (CH_2), 53.50 (CH_2 x2), 36.81 (CH_2), 20.24 (CH_3); MS (ESI): $C_{25}H_{26}ClFN_4O_3$ requires m/z 484.1, found 485.0 $[M + 1]^+$; Anal. calcd for $C_{25}H_{26}ClFN_4O_3$: C, 61.92; H, 5.40; N, 11.55; Found: C, 61.93; H, 5.40; N, 11.56.

*4.1.37. 2-(4-Fluorobenzyl)-N-(cyclohexyl)-3-oxo-6-phenyl-2,3-dihydropyridazine-4-carboxamide (**12**)*

General procedure for the synthesis of carboxamides was used to convert acid **36** and cyclohexylamine into the title product. The mixture of reaction was stirred for 12 h, then the crude of reaction was purified by flash chromatography (petroleum ether/EtOAc 7:3) to afford **12** (68 mg, 24%)

as an orange solid. R_f = 0.75 (petroleum ether/EtOAc 7:3); mp 118-120 °C; IR 1680 (C=O), 3268 (NH); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.58 (d, *J* = 8.1 Hz, 1H, NH, exch with D_2O), 8.70 (s, 1H), 7.87 (d, *J* = 7.2 Hz, 2H), 7.66 – 7.35 (m, 5H), 7.04 (t, *J* = 8.5 Hz, 2H), 5.44 (s, 2H), 4.17 – 3.78 (m, 1H), 2.06 – 1.92 (m, 2H), 1.88 – 1.70 (m, 2H), 1.69 – 1.56 (m, 2H), 1.54 – 1.32 (m, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 162.85 (C), 160.40 (C), 159.59 (C), 158.78 (C), 144.48 (C), 133.11 (C), 130.71 (CH), 129.73 (CH), 129.64 (CH), 129.00 (C), 128.84 (CH), 128.03 (CH x2), 125.03 (CH x2), 114.72 (CH), 114.51 (CH), 54.55 (CH_2), 47.55 (CH), 31.68 (CH_2 x4), 28.68 (CH_2), 24.59 (CH_2 x2), 23.65 (CH_2 x3); MS (ESI): $C_{24}H_{24}FN_3O_2$ requires m/z 405.2, found 406.1 $[M + 1]^+$; Anal. calcd for $C_{24}H_{24}FN_3O_2$: C, 71.09; H, 5.97; N, 10.36. Found: C, 71.10; H, 5.95; N, 10.37.

*4.1.38. 2-(4-Fluorobenzyl)-N-(cycloheptyl)-3-oxo-6-phenyl-2,3-dihydropyridazine-4-carboxamide (**13**)*

General procedure for the synthesis of carboxamides was used to convert acid **36** and cycloheptylamine into the title product. The mixture of reaction was stirred for 18 h, then the crude of reaction was purified by flash chromatography (petroleum ether/EtOAc 8:2) to afford **13** (114 mg, 39%) as an orange solid. R_f = 0.56 (petroleum ether/EtOAc 8:2); mp 131-133 °C; IR 1682 (C=O), 3253 (NH); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.64 (d, *J* = 7.7 Hz, 1H, NH, exch with D_2O), 8.70 (s, 1H), 7.86 (d, *J* = 7.5 Hz, 2H), 7.59 – 7.37 (m, 5H), 7.04 (t, *J* = 8.5 Hz, 2H), 5.44 (s, 2H), 4.34 – 3.99 (m, 1H), 2.12 – 1.92 (m, 2H), 1.81 – 1.49 (m, 10H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 163.88 (C), 161.42 (C), 160.37 (C), 159.80 (C), 145.49 (C), 134.13 (C), 131.67 (CH), 130.76 (CH), 130.68 (CH), 130.04 (C), 129.86 (CH), 129.06 (CH x2), 126.04 (CH x2), 115.74 (CH), 115.53 (CH), 55.54 (CH_2), 50.88 (CH), 34.74 (CH_2 x2), 28.07 (CH_2 x2), 24.16 (CH_2 x2); MS (ESI): $C_{25}H_{26}FN_3O_2$ requires m/z 419.2, found 420.2 $[M + 1]^+$; Anal. calcd for $C_{24}H_{24}FN_3O_2$: C, 71.58; H, 6.25; N, 10.02. Found: C, 71.59; H, 6.24; N, 10.05.

*4.1.39. N-(Adamantan-1-yl)-2-(4-fluorobenzyl)-3-oxo-6-phenyl-2,3-dihydropyridazine-4-carboxamide (**14**)*

General procedure for the synthesis of carboxamides was used to convert acid **36** and 1-adamantylamine into the title product. The mixture of reaction was stirred for 20 h, then the crude of reaction was purified by flash chromatography (petroleum ether/EtOAc 8:2) to afford **14** (130 mg, 41%) as a white solid. R_f = 0.29 (petroleum ether/EtOAc 95:5); mp 201-203 °C; IR 1681 (C=O), 3269 (NH); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.49 (br s, 1H, NH, exch with D_2O), 8.68 (s, 1H), 7.86 (d, *J* = 8.1 Hz, 2H), 7.54 – 7.39 (m, 5H), 7.04 (t, *J* = 8.6 Hz, 2H), 5.44 (s, 2H), 2.21 – 2.06 (m, 9H), 1.80 – 1.66 (m, 6H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 162.84 (C), 160.38 (C), 159.12 (C), 159.08 (C), 158.89 (C), 144.43 (C), 133.14 (C), 130.31 (CH), 129.84 (C), 129.73 (CH), 129.65 (CH), 128.80 (CH), 128.03 (CH x2), 124.95 (CH x2), 114.70 (CH), 114.48 (CH), 54.51 (CH_2), 51.34 (C), 40.32 (CH_2 x3), 35.39 (CH_2 x3), 28.40 (CH x 3); MS (ESI): $C_{28}H_{28}FN_3O_2$ requires m/z 457.5, found 458.5 $[M + 1]^+$; Anal. calcd for $C_{28}H_{28}FN_3O_2$: C, 73.50; H, 6.17; N, 9.18. Found: C, 73.47; H, 6.19; N, 9.22.

*4.1.40. 6-Phenyl-2-(4-fluorobenzyl)-N-(cis-4-methylcyclohexyl)-3-oxo-6-phenyl-2,3-dihydropyridazine-4-carboxamide (**15**) and 6-Phenyl-2-(4-fluorobenzyl)-N-(trans-4-methylcyclohexyl)-3-oxo-6-phenyl-2,3-dihydropyridazine-4-carboxamide (**16**)*

Compound **15** and **16** were obtained from acid **36** using the general procedure for the synthesis of carboxamides. The mixture of reaction was stirred for 12 h, then *cis*/*trans* isomers were separated by flash chromatography (petroleum ether/EtOAc 85:15). **15** (136 mg, 46%) white solid R_f = 0.46 (petroleum ether/EtOAc 8:2); mp 129-131 °C; IR 1679 (C=O), 3264 (NH); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.87 (d, *J* = 7.7 Hz, 1H), 8.71 (s, 1H), 7.86 (d, *J* = 7.2 Hz, 2H), 7.55 – 7.46 (m, 5H), 7.05 (t, *J* = 8.2 Hz, 2H), 5.46 (s, 2H), 4.30 – 4.15 (m, 1H), 1.87 – 1.77 (m, 2H), 1.72 – 1.57 (m, 4H), 1.35 – 1.13 (m, 3H), 0.98 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (101 MHz, Chloroform) δ 163.88 (C), 161.42

(C), 160.73 (C), 159.87 (C), 145.44 (C), 134.15 (C), 131.56 (CH), 130.85 (CH), 130.77 (CH), 130.05 (C), 129.84 (CH), 129.05 (CH x2), 126.04 (CH x2), 115.71 (CH), 115.50 (CH), 55.35 (CH_2), 45.90 (CH), 30.98 (CH), 30.10 (CH_2 x2), 29.47 (CH_2 x2), 21.47 (CH_3); MS (ESI): $C_{25}H_{26}FN_3O_2$ requires m/z 419.2, found 420.3 $[M + 1]^+$; Anal. calcd for $C_{25}H_{26}FN_3O_2$: C, 71.58; H, 6.25; N, 10.02. Found: C, 71.60; H, 6.21; N, 10.01. **16** (68 mg, 23%), pale yellow solid R_f = 0.55 (petroleum ether/EtOAc 8:2); mp 122-124 °C; IR 1678 (C=O), 3265 (NH); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.48 (d, *J* = 8.0 Hz, 1H, NH, exch with D_2O), 8.70 (s, 1H), 7.87 (d, *J* = 7.5 Hz, 2H), 7.55 – 7.44 (m, 5H), 7.04 (t, *J* = 8.5 Hz, 2H), 5.44 (s, 2H), 3.94 – 3.81 (m, 1H), 2.05 (d, *J* = 12.3 Hz, 2H), 1.75 (d, *J* = 13.0 Hz, 2H), 1.46 – 1.23 (m, 4H), 1.19 – 1.03 (m, 1H), 0.90 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 163.88 (C), 161.43 (C), 160.73 (C), 159.87 (C), 145.44 (C), 134.15 (C), 131.56 (CH), 130.85(CH), 130.77 (CH), 130.05 (C), 129.84(CH), 129.05(CH x2), 126.04 (CH x2), 115.71 (CH), 115.50 (CH), 55.35 (CH_2), 49.03 (CH), 33.82 (CH_2 x2), 32.81 (CH_2 x2), 31.94 (CH), 22.20 (CH_3); MS (ESI): $C_{25}H_{26}FN_3O_2$ requires m/z 419.20, found 420.4 $[M + 1]^+$; Anal. calcd for $C_{25}H_{26}FN_3O_2$: C, 71.58; H, 4.53; N, 10.02; Found: C, 71.55; H, 6.22; N, 10.06.

*4.1.41. N-(Adamantan-1-yl)-6-(4-chloro-3-methylphenyl)-3-oxo-2-pentyl-2,3-dihydropyridazine-4-carboxamide (**17**)*

General procedure for the synthesis of carboxamides was used to convert acid **37** and 1-adamantylamine into the title product. The mixture of reaction was stirred for 18 h, then the crude of reaction was purified by flash chromatography (petroleum ether/EtOAc 9:1) to afford **17** (140 mg, 42%) as a white yellow. R_f = 0.69 (petroleum ether/EtOAc 9:1); mp 127-128 °C; IR 1676 (C=O), 3259 (NH); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.58 (br s, 1H, NH, exch with D_2O), 8.63 (s, 1H), 7.72 (s, 1H), 7.62 (d, *J* = 8.3 Hz, 1H), 7.42 (d, *J* = 8.3 Hz, 1H), 4.30 (t, *J* = 7.5 Hz, 2H), 2.43 (s, 3H), 2.19 – 2.10 (m, 9H), 1.93 – 1.85 (m, 2H), 1.79 – 1.67 (m, 6H), 0.92 (t, *J* = 6.4 Hz, 3H); 13C NMR (101 MHz,

Chloroform-*d*) δ 160.31 (C), 160.02 (C), 144.22 (C), 136.83 (C), 136.04 (C), 132.83 (C), 130.69 (CH), 130.33 (C), 129.70 (CH), 128.33 (CH), 124.55 (CH), 53.06 (CH_2), 52.34 (C), 41.36 (CH_2 x3), 36.42 (CH_2 x3), 29.43 (CH x3), 28.79 (CH_2), 28.18 (CH_2), 22.33 (CH_2), 20.20 (CH_3), 13.96 (CH_3); MS (ESI): $C_{27}H_{34}ClN_3O_2$ requires m/z 467.2, found 468.2 $[M + 1]^+$; Anal. calcd for $C_{27}H_{34}ClN_3O_2$: C, 69.29; H, 7.32; N, 8.98; Found: C, 69.27; H, 7.36; N, 8.99.

*4.1.42. N-(Adamantan-1-yl)-3-oxo-2-pentyl-6-phenyl-2,3-dihydropyridazine-4-carboxamide (**18**)*

General procedure for the synthesis of carboxamides was used to convert acid **38** and 1-adamantylamine into the title product. The mixture of reaction was stirred for 18 h, then the crude of reaction was purified by flash chromatography (petroleum ether/EtOAc 9:1) to afford **18** (180 mg, 61%) as a pale yellow. R_f = 0.37 (petroleum ether/EtOAc 95:5); mp 106-108 °C; IR 1680 (C=O), 3269 (NH); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.62 (br s, 1H, NH, exch with D_2O), 8.68 (s, 1H), 7.86 (d, *J* = 7.3 Hz, 2H), 7.55 – 7.38 (m, 3H), 4.31 (t, *J* = 7.5 Hz, 2H), 2.21 – 2.09 (m, 9H), 1.91 (q, *J* = 7.4 Hz, 2H), 1.78 – 1.68 (m, 6H), 1.43 – 1.37 (m, 4H), 0.92 (t, *J* = 5.9 Hz, 3H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 159.38 (C), 159.10 (C), 144.10 (C), 133.38 (C), 129.91 (CH), 129.28 (C), 128.62 (CH), 127.99 (CH x2), 124.91 (CH x2), 52.01 (CH_2), 51.28 (C), 40.36 (CH_2 x3), 35.41 (CH_2 x3), 28.42 (CH x3), 27.78 (CH_2), 27.15 (CH_2), 21.31 (CH_2), 12.93 (CH_3); MS (ESI): $C_{26}H_{33}N_3O_2$ requires m/z 419.3, found 420.4 $[M + 1]^+$; Anal. calcd for $C_{26}H_{33}N_3O_2$: C, 74.43; H, 7.93; N, 10.02; Found: C, 74.44; H, 7.95; N, 9.99.

*4.1.43. N-Cycloheptyl-3-oxo-2-pentyl-6-phenyl-2,3-dihydropyridazine-4-carboxamide (**19**)*

General procedure for the synthesis of carboxamides was used to convert acid **38** and cycloheptylamine into the title product. The mixture of reaction was stirred for 12 h, then the crude of reaction was purified by flash chromatography (petroleum ether/EtOAc 8:2) to afford **19** (150 mg, 56%) as a pale yellow solid. R_f = 0.33 (petroleum ether/EtOAc 9:1); mp 62-63 °C; IR 1674 (C=O),

3262 (NH); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.78 (d, *J* = 8.1 Hz, 1H, NH, exch with D_2O), 8.70 (s, 1H), 7.87 (d, *J* = 7.3 Hz, 2H), 7.53 – 7.42 (m, 3H), 4.32 (t, *J* = 7.5 Hz, 2H), 4.24 – 4.12 (m, 1H), 2.01 (m, 2H), 1.90 (q, *J* = 7.3 Hz, 2H), 1.75 – 1.52 (m, 10H), 1.48 – 1.35 (m, 4H), 0.93 (t, *J* = 6.1 Hz, 3H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 160.67 (C), 160.00 (C), 145.14 (C), 134.38 (C), 131.25 (CH), 129.68 (CH), 129.46 (C), 129.02 (CH x2), 126.00 (CH x2), 53.00 (CH_2), 50.76 (CH), 34.74 (CH_2 x3), 28.81 (CH_2), 28.12 (CH_2 x2), 24.12 (CH_2 x2), 22.34 (CH_2), 13.96 (CH_3); MS (ESI): $C_{23}H_{31}N_3O_2$ requires m/z 381.2, found 382.2 $[M + 1]^+$; Anal. calcd for $C_{23}H_{31}N_3O_2$: C, 72.41; H, 8.19; N, 11.01; Found: C, 72.39; H, 8.17; N, 11.05.

*4.1.44. N-Cycloheptyl-2-(4-fluorobenzyl)-3-oxo-3,5-dihydro-2H-indeno[1,2-c]pyridazine-4-carboxamide (**39**)*

General procedure for the synthesis of carboxamides was used to convert acid **44** and cycloheptylamine into the title product. The mixture of reaction was stirred for 24 h, then the crude of reaction was purified by flash chromatography (petroleum ether/EtOAc 95:5) to afford **39** (140 mg, 42%) as a yellow. R_f = 0.36 (petroleum ether/EtOAc 95:5); mp 84-86 °C; IR 1681 (C=O), 3270 (NH); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.64 (d, *J* = 7.6 Hz, 1H, NH, exch with D_2O), 7.69 (d, *J* = 7.7 Hz, 1H), 7.60 (t, *J* = 7.5 Hz, 1H), 7.48 (d, *J* = 7.6 Hz, 1H), 7.37 (t, *J* = 7.4 Hz, 1H), 6.97 (d, *J* = 6.9 Hz, 4H), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.64 (d, *J* = 7.6 Hz, 1H), 7.69 (d, *J* = 7.7 Hz, 1H), 7.60 (t, *J* = 7.5 Hz, 1H), 7.48 (d, *J* = 7.6 Hz, 1H), 7.37 (t, *J* = 7.4 Hz, 1H), 6.97 (d, *J* = 6.9 Hz, 4H), 5.21 (s, 2H), 4.45 (d, *J* = 13.2 Hz, 1H), 4.33 – 4.23 (m, 1H), 3.36 (d, *J* = 13.1 Hz, 1H), 2.21 – 2.08 (m, 2H), 1.80 – 1.51 (m, 10H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 160.08 (C), 159.28 (C), 153.42 (C), 149.88 (C), 135.11 (C), 130.64 (CH), 129.01 (C), 128.93 (CH), 128.31 (CH), 127.55 (CH), 124.20 (CH), 120.43 (CH), 115.59 (CH), 115.38 (CH), 55.50 (CH_2), 51.04 (CH), 42.53 (CH_2), 34.89 (CH_2 x3), 28.03 (CH_2

x3), 24.20 (CH_2 x3); MS (ESI): $C_{26}H_{26}FN_3O_2$ requires m/z 431.2, found 432.2 $[M + 1]^+$; Anal. calcd for $C_{26}H_{26}FN_3O_2$: C, 72.37; H, 6.07; N, 9.74; Found: C, 72.40; H, 6.04; N, 9.71.

4.2. Radioligand binding assays for CB_1R and CB_2R

Receptor binding studies were performed using membranes from transfected cells with human CB_1R or CB_2R genes (RBHCB1M400UA and RBXCB2M400UA) purchased by PerkinElmer Life and Analytical Sciences (Boston, MA). The assay was developed in binding buffer (50 mM TrisCl, 5 mM $MgCl_2 \cdot H_2O$, 2.5 mM EDTA, 0.5 mg/mL BSA, pH 7.4 for CB_1R binding; 50 mM TrisCl, 5 mM $MgCl_2 \cdot H_2O$, 2.5 mM EGTA, 1 mg/mL BSA, pH 7.5 for CB_2R binding) in a final volume of 200 μL. Fractions of the final membrane suspension (about 8 μg/well for the CB_1R assay and 1.33 μg/well for the CB_1R assay) were incubated in the corresponding buffer with 0.1 nM [^{3}H]CP,55-940 (131.8 Ci/mmol) for CB_1R and 0.15 nM [^{3}H]CP,55-940 (131.8 Ci/mmol) for CB_2R, in the presence of 4 μM for the first screening, or several concentrations of each compound (10^{-5}–10^{-12} M) for the second screening, during 90 min at 30 °C. Nonspecific binding was determined in the presence of 10 μM WIN 55,212-2, and 100% binding of the radioligand to the membrane was determined by its incubation with membrane without any compound. The 96-well plates and tubes necessary for the experiment were previously siliconized with Sigmacote® (Sigma-Aldrich) to minimize receptor binding loss due to materials adsorption. The reaction was terminated by rapid vacuum filtration with a filter mate Harvester apparatus (Perkin-Elmer) through Filtermat A GF/C filters pre-soaked in 0.05% polyethylenimine (PEI). After filtering, the filter was washed nine times with ice-cold binding buffer and dried, and a melt-on scintillation sheet (Meltilex A, PerkinElmer) was melted onto it. Then bound radioactivity was measured with a 1450 LSC & Luminiscence counter Wallac MicroBeta TriLux (Perkin-Elmer). For all binding experiments, competition binding curves were analyzed by using an iterative curve-fitting procedure GraphPad Prism version 6.01 (GraphPad Software Inc., San Diego,

CA, USA) and K_i values are expressed as mean ± SEM of at least three experiments performed in triplicate for each point.

4.3. [^{35}S]-GTPγS binding analysis

[^{35}S]-GTPγS binding analyses were carried out for compounds **5**, **8**, **9**, **15**, **17** and **18** using CB_2R-containing membranes (HTS020M2, Eurofins Discovery Services). To this end, membranes (5 μg/well) were permeabilized by addition of saponin (Sigma-Aldrich), then mixed with 0.3 nM [^{35}S]-GTPγS (Perkin-Elmer) and 10 μM GDP (Sigma-Aldrich) in 20 mM HEPES (Sigma-Aldrich) buffer containing 100 mM NaCl (Merck) and 10 mM $MgCl_2$ (Merck), at pH 7.4. Increasing concentrations of compounds **5**, **8**, **9**, **15**, **17** and **18** (from 10^{-12} to 10^{-5} M) were added in a final volume of 100 μl and incubated for 30 min at 30 °C. The non-specific signal was measured with 10 μM GTPγS (Sigma-Aldrich). All 96-well plates and the tubes necessary for the experiment were previously siliconized with Sigmacote® (Sigma-Aldrich). The reaction was terminated by rapid vacuum filtration with a filter mate Harvester apparatus (Perkin-Elmer) through Filtermat A GF/C filters. The filters were washed nine times with ice-cold filtration buffer (10 mM sodium phosphate, pH 7.4), and bound radioactivity was measured with a 1450 LSC & Luminiscence counter Wallac MicroBeta TriLux (Perkin-Elmer). [^{35}S]-GTPγS binding data were analyzed to determine the IC50 values by using an iterative curve-fitting procedure with the GraphPad Prism version 6.01 (GraphPad Software Inc., San Diego, CA, USA). IC50 values are expressed as mean ± SEM of at least three experiments performed in triplicate for each point.

4.4. Calculation of in silico ADME parameters

A set of 34 physicochemical descriptors was computed using QikProp version 3.5 integrated in Maestro (Schrödinger, LLC, New York, USA). The QikProp descriptors are shown in Table 2. The 3D conformations used in the calculation of QikProp descriptors were generated using the program Spartan

'08 (Wave function, Inc., Irvine CA) as follows: The structure of each molecule was built from the fragment library available in the program. Then, *ab initio* energy minimizations of each structure at the Hartree−Fock 6-31G* level were performed. A conformational search was next implemented using Molecular Mechanics, followed by a minimization of the energy of each conformer calculated at the Hartree−Fock 6-31G* level. The global minimum energy conformer of each compound was used as input for ADME studies with QikProp.

4.5. Molecular docking

All the compounds were built, parameterized (Gasteiger-Huckel method) and energy minimized within MOE using MMFF94 forcefield [MOE: Chemical Computing Group Inc. Montreal. H3A 2R7 Canada. http://www.chemcomp.com]. Docking studies around the CB_2R were applied starting from the ligand-based human CB_2 homology model we previously built (that include three GPCRs crystal structures, PDB codes: 2RH1, 3EML and 1F88) [36] on the basis of that of the *h*CB_2-WIN-55,212 complex, derived and optimized by means of molecular dynamic simulations [47]. On the other hand, the CB_1 homology model was defined taking into account the X-ray data of the human β_2-adrenoreceptor as reference template [39].

In agreement with mutagenesis data, the *h*CB_2 inverse agonist binding site was defined taking into account any residues placed at 5Å distance far from S165 [48] whilst the putative binding site of the *h*CB_1 antagonist was described using the Site Finder module implemented in MOE and also verifying a good agreement with the information coming from mutagenesis data, following a procedure we already described about other GPCR homology modelling studies [49,50].

In the case of CB_1R, the putative antagonist binding site and the related docking studies proved to involve the key residue K192, as described in literature [51].

Successively, flexible molecular docking calculations were performed by means of the Surflex docking module implemented in Sybyl-X1.0 [Sybyl-X 1.0 Tripos Inc 1699 South Hanley Road. St Louis. Missouri. 63144. USA 25]. Surflex-Dock uses an empirically derived scoring function based on the binding affinities of X-ray protein-ligand complexes.

The Surflex-Dock scoring function is a weighted sum of non-linear functions involving van der Waals surface distances between the appropriate pairs of exposed protein and ligand atoms, including hydrophobic, polar, repulsive, entropic and solvation and crash terms represented in terms of a total score conferred to any calculated conformer.

Then, the best docking geometry (according to the SurFlex scoring functions) was refined by ligand/protein complex energy minimization (CHARMM27) using the MOE software. Finally, the protein-ligand complex stability was successfully assessed running a short ~1 ps run of molecular dynamics (MD) at constant temperature, followed by an all-atom energy minimization (LowModeMD implemented in MOE software). In this way, an exhaustive conformational analysis of the ligand-receptor binding site complex was explored, as we already discussed about other case studies for a preliminary evaluation of the derived docking poses [52,53].

Appendix A. Supplementary data

^{1}H-NMR and ^{13}C-NMR spectra of final compounds **2**-**19** and **39** are available.

Conflict of interest

None of the authors have conflict of interest to declare

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Captions to Figures and Schemes

Fig. 1. Structural design of pyridazinone compounds **2-19**, **39**.

Scheme 1. Reagents and conditions: a) Br_2, AcOH, rt, 8 h; b) diethylmalonate, NaH, THF, 0 °C-rt, 1-3 h; c) $NH_2NH_2xH_2O$, $EtOH_.$, reflux, 24 h; d) Br_2, AcOH, rt, 6 h; e) K_2CO_3, DMF, R^1X, 60 °C, 2-4 h, or K_2CO_3, $DMF_,$))), pentylchloride, rt, 2 h; f) NaOH 2 M, EtOH, reflux, 8 h; g) HOBt, EDC, NH_2-Q, DCM, 0 °C-rt, 12-18 h.

SCHEME 2. Reagents and conditions: a) Diethylketomalonate, 100 °C, 12 h; b) $NH_2NH_2.2HCl$, $EtOH_{abs}$, reflux, 24 h; c) K_2CO_3, $DMF_{anhyd.,}$))), 4-fluorobenzyl bromide, 30 °C, 4 h; d) NaOH 2 M, EtOH, reflux, 6 h; e) HOBt, EDC, cycloheptylamine, DCM, 0 °C-rt, 24 h.

Table 1. Structures and binding data[a] for compounds **2-19,39**.

[a]The K_i values of the new compounds for the CB_1R and CB_2R were determined using RBHCB1M400UA and RBXCB2M400UA membranes, respectively, and [^{3}H]CP,55-940 as radioligand. K_i values were obtained from three independent experiments carried out in triplicate and are expressed as mean ± SEM. ND[b]: not determined due to poor solubility. ND[c], not determined for its well-known CB_2R selectivity previously described in literature [29].

Fig. 2. IC_{50} and Emax values corresponding to compounds **5, 8, 9, 15, 17** and **18**, and the reference compound SR144528, in the GTPγS binding bioassay (obtained from at least three independent experiments carried out in triplicate). The mean curves of the different assays for compound **5** and SR144528 are also shown.

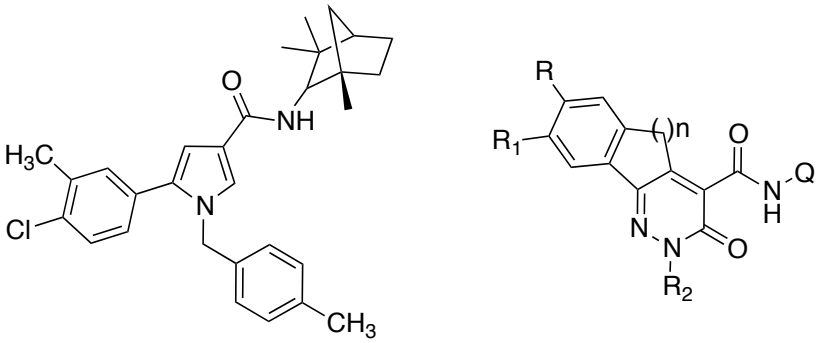
Table 2. Physicochemical Descriptors Calculated for Compounds **2-19, 39** by QikProp 3.5 Integrated in Maestro (Schrödinger, LLC, New York).

[a]Predicted octanol/water partition coefficient [-2.0/6.5]; [b]Predicted log of the brain/blood partition coefficient [-3.0/1.2]; [c]Prediction of binding to human serum albumin [-1.5/1.5]; [d]Apparent Caco-2 cell permeability in nm/s (intestinal drug permeability) [<25 poor, >500 excellent]; [e]Human Oral Absorption in GI [<25% is poor]; [range of 95% of drugs].

Fig. 3. Docking poses of compounds **8** (C atom: yellow), **9** (C atom: dark green) and **15** (C atom: deep magenta), within the *h*CB_2R inverse agonist binding site. The most important residues are labelled.

Fig. 4. Docking poses of compounds **15** (C atom: deep magenta) and of SR144528 (C atom: cyan) within the *h*CB_2R inverse agonist binding site. The most important residues are labelled.

Fig. 5. Docking poses of compounds **13** (C atom: pink) and **15** (C atom: cyan) within the *h*CB_1R antagonist binding site. The most important residues are labelled.



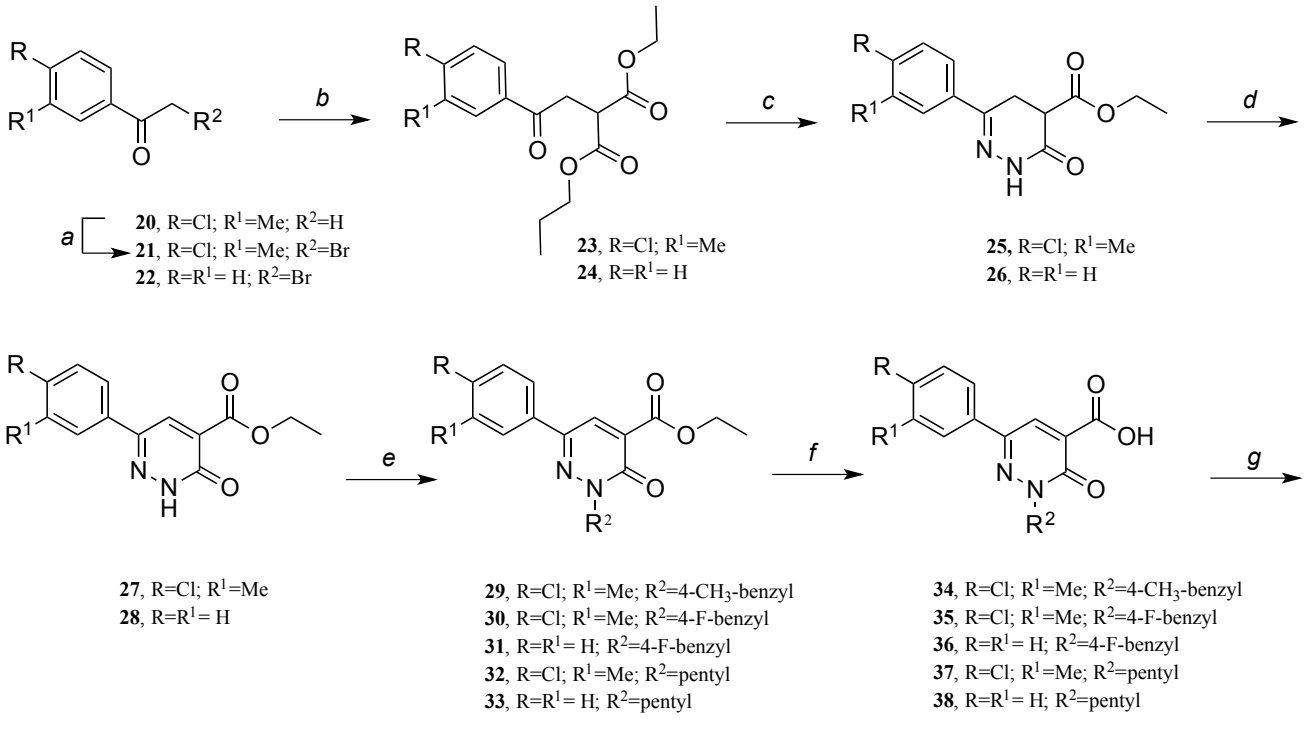
1

K_iCB_2 = 5.7 ± 0.7 nM
K_iCB_1 = 1470 ± 179 nM
selectivity K_iCB_1/K_iCB_2 = 258

2-19, 39

R, R1, R2, n and Q
as reported in Table 1

Fig. 1. Structural design of pyridazinone compounds **2-19**, **39**.



20, R=Cl; R^1=Me; R^2=H
21, R=Cl; R^1=Me; R^2=Br
22, R=R^1= H; R^2=Br

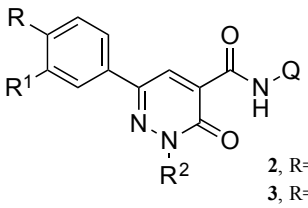
23, R=Cl; R^1=Me
24, R=R^1= H

25, R=Cl; R^1=Me
26, R=R^1= H

27, R=Cl; R^1=Me
28, R=R^1= H

29, R=Cl; R^1=Me; R^2=4-CH_3-benzyl
30, R=Cl; R^1=Me; R^2=4-F-benzyl
31, R=R^1= H; R^2=4-F-benzyl
32, R=Cl; R^1=Me; R^2=pentyl
33, R=R^1= H; R^2=pentyl

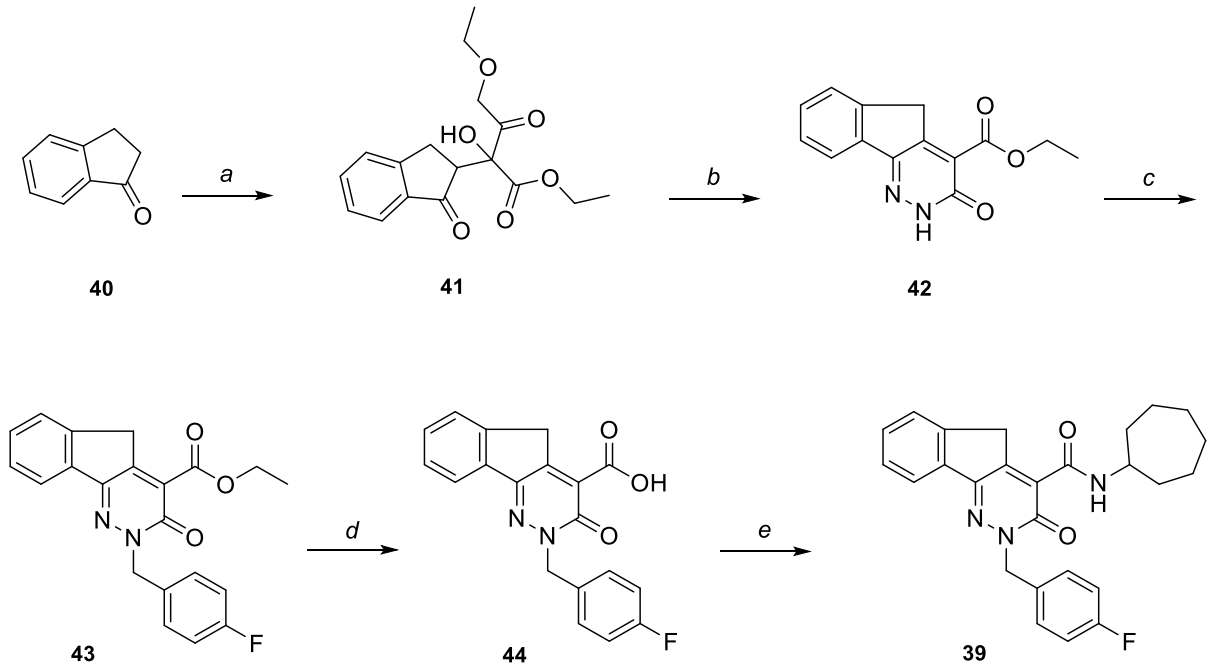
34, R=Cl; R^1=Me; R^2=4-CH_3-benzyl
35, R=Cl; R^1=Me; R^2=4-F-benzyl
36, R=R^1= H; R^2=4-F-benzyl
37, R=Cl; R^1=Me; R^2=pentyl
38, R=R^1= H; R^2=pentyl



2-19

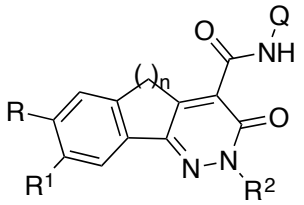
2, R=Cl; R^1=Me; R^2=4-CH_3-benzyl; Q=cyclohexyl
3, R=Cl; R^1=Me; R^2=4-CH_3-benzyl; Q=cycloheptyl
4, R=Cl; R^1=Me; R^2=4-CH_3-benzyl; Q=1-adamantyl
5, R=Cl; R^1=Me; R^2=4-F-benzyl; Q=cyclohexyl
6, R=Cl; R^1=Me; R^2=4-F-benzyl; Q=cyclohepty
7, R=Cl; R^1=Me; R^2=4-F-benzyl; Q=1-adamantyl
8, R=Cl; R^1=Me; R^2=4-F-benzyl; Q=fenchyl
9, R=Cl; R^1=Me; R^2=4-F-benzyl; Q=*cis*-4-methyl-cyclohexyl
10, R=Cl; R^1=Me; R^2=4-F-benzyl; Q=*trans-4*-methyl-cyclohexyl
11, R=Cl; R^1=Me; R^2=4-F-benzyl; Q=ethylmorpholine
12, R=R^1= H; R^2=4-F-benzyl; Q=cyclohexyl
13, R=R^1= H; R^2=4-F-benzyl; Q=cycloheptyl
14, R=R^1= H; R^2=4-F-benzyl; Q=1-adamantyl
15,, R=R^1= H; R^2=4-F-benzyl; Q=*cis*-4-methyl-cyclohexyl
16, R=R^1= H; R^2=4-F-benzyl; Q=*trans*-4-methyl-cyclohexyl
17, R=Cl; R^1=Me; R^2=pentyl; Q=1-Adamantyl
18, R=R^1= H; R^2=pentyl; Q=1-Adamantyl
19, R=R^1= H; R^2=pentyl; Q=cycloheptyl

Scheme 1. Reagents and conditions: a) Br_2, AcOH, rt, 8 h; b) diethylmalonate, NaH, THF, 0 °C-rt, 1-3 h; c) $NH_2NH_2xH_2O$, EtOH, reflux, 24 h; d) Br_2, AcOH, rt, 6 h; e) K_2CO_3, DMF, R^1X, 60 °C, 2-4 h, or K_2CO_3, DMF,))), pentylchloride, rt, 2 h; f) NaOH 2 M, EtOH, reflux, 8 h; g) HOBt, EDC, NH_2-Q, DCM, 0 °C-rt, 12-18 h.



SCHEME 2. Reagents and conditions: a) Diethylketomalonate, 100 °C, 12 h; b) $NH_2NH_2.2HCl$, $EtOH_{abs}$, reflux, 24 h; c) K_2CO_3, $DMF_{anhyd.}$,))), 4-fluorobenzyl bromide, 30 °C, 4 h; d) NaOH 2 M, EtOH, reflux, 6 h; e) HOBt, EDC, cycloheptylamine, DCM, 0 °C-rt, 24 h.

Table 1. Structures and binding data[a] for compounds **2-19,39**.



Compd	R	R^1	R^2	n	Q	K_iCB_1 (nM)[a]	K_iCB_2 (nM)	K_iCB_1/K_iCB_2
2	Cl	CH_3	4-CH_3-benzyl	0	cyclohexyl	> 4000	240 ± 190	>16.6
3	Cl	CH_3	4-CH_3-benzyl	0	cycloheptyl	ND[b]	ND[b]	--
4	Cl	CH_3	4-CH_3-benzyl	0	1-adamantyl	ND[b]	ND[b]	--
5	Cl	CH_3	4-F-benzyl	0	cyclohexyl	> 4000	4.7 ± 2.1	>851
6	Cl	CH_3	4-F-benzyl	0	cycloheptyl	> 4000	4.9 ± 2.6	>816
7	Cl	CH_3	4-F-benzyl	0	1-adamantyl	> 4000	3.4 ± 3.0	>1176
8	Cl	CH_3	4-F-benzyl	0	fenchyl	> 4000	2.6 ± 0.47	>1538
9	Cl	CH_3	4-F-benzyl	0	*cis*-4-methyl-cyclohexyl	> 4000	2.0 ± 0.81	>2000
10	Cl	CH_3	4-F-benzyl	0	*trans*-4-methyl-cyclohexyl	> 4000	9.0 ± 0.80	>444
11	Cl	CH_3	4-F-benzyl	0	ethylmorpholine	> 4000	311 ± 63	>12.9
12	H	H	4-F-benzyl	0	cyclohexyl	467 ± 124	44 ± 10	11
13	H	H	4-F-benzyl	0	cycloheptyl	33 ± 9.4	16 ± 2.3	2
14	H	H	4-F-benzyl	0	1-adamantyl	ND[b]	ND[b]	--
15	H	H	4-F-benzyl	0	*cis*-4-methyl-cyclohexyl	115 ± 29.4	5.7 ± 3.7	20
16	H	H	4-F-benzyl	0	*trans*-4-methyl-cyclohexyl	322 ± 118	66 ± 23	5
17	Cl	CH_3	pentyl	0	1-adamantyl	25 ± 8.6	7.6 ± 4.0	3.3

18	H	H	pentyl	0	1-adamantyl	245 ± 31	4.4 ± 0,74	55.7
19	H	H	pentyl	0	cycloheptyl	365 ± 168	13 ± 5.9	28
39	H	H	4-F-benzyl	1	cycloheptyl	> 4000	> 4000	--
HU-308						ND[c]	11.2 ± 0.78	--
WIN55,212-2						28.8 ± 4.1	3.7 ± 0.12	7.8

[a]The K_i values of the new compounds for the CB_1R and CB_2R were determined using RBHCB1M400UA and RBXCB2M400UA membranes, respectively, and [^{3}H]CP,55-940 as radioligand. K_i values were obtained from three independent experiments carried out in triplicate and are expressed as mean ± SEM. ND[b]: not determined due to poor solubility. ND[c], not determined for its well-known CB_2R selectivity previously described in literature [29].

Compound	IC_{50} (nM)	Emax (%)
5	25.3 ± 10.0	-60.7 ± 6.9
8	51.2 ± 25.5	-58.7 ± 8.6
9	20.7 ± 9.0	-43.8 ± 8.0
15	35.2 ± 8.6	-65.7 ± 7.5
17	295.5 ± 218.6	-53.0 ± 2.9
18	54.6 ± 28.5	-64.7 ± 3.1
SR144528	96.2 ± 2.5	-63.2 ± 3.2

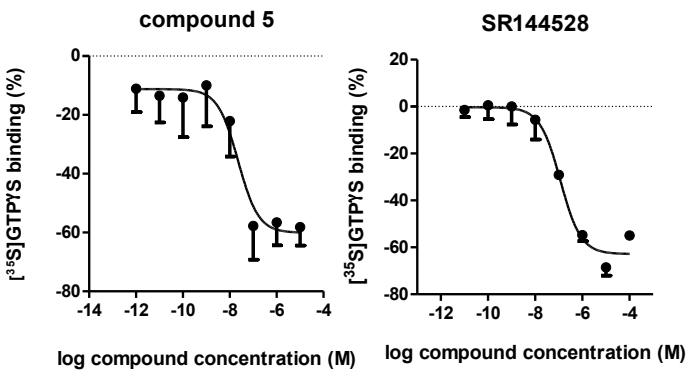


Fig. 2. IC_{50} and Emax values corresponding to compounds **5, 8, 9, 15, 17** and **18**, and the reference compound SR144528, in the GTPγS binding bioassay (obtained from at least three independent experiments carried out in triplicate). The mean curves of the different assays for compound **5** and SR144528 are also shown.

Table 2. Physicochemical Descriptors Calculated for Compounds **2-19, 39** by QikProp 3.5 Integrated in Maestro (Schrödinger, LLC, New York).

Compd	QPlogP[a]	QlogBB[b]	QPlogKhsa[c]	QPPCaco[d]	%Human oral absorption GI[e]
2	7.16	-0.29	1.60	2590	100
3	7.34	-0.24	1.76	2744	100
4	7.86	-0.27	1.97	2673	100
5	6.94	-0.19	1.53	2375	100
6	7.25	-0.10	1.63	2767	100
7	7.76	-0.14	1.86	2680	100
8	8.01	0.03	1.93	3657	100
9	7.30	-0.20	1.67	2379	100
10	7.01	-0.15	1.46	2681	100
11	4.96	-0.07	0.63	453	100
12	6.22	-0.34	1.25	2348	100
13	6.52	-0.22	1.35	2927	100
14	6.99	-0.26	1.57	2699	100
15	6.53	-0.33	1.38	2345	100
16	6.53	-0.34	1.39	2345	100
17	7.38	-0.47	1.77	2329	100
18	6.60	-0.59	1.48	2330	100
19	6.13	-0.53	1.26	2562	100
39	6.59	-0.03	1.41	3678	100
HU308	7.01	-0.52	1.62	3738	100

SR144528	7.61	0.11	1.91	4606	100

[a]Predicted octanol/water partition coefficient [-2.0/6.5]; [b]Predicted log of the brain/blood partition coefficient [-3.0/1.2]; [c]Prediction of binding to human serum albumin [-1.5/1.5]; [d]Apparent Caco-2 cell permeability in nm/s (intestinal drug permeability) [<25 poor, >500 excellent]; [e]Human Oral Absorption in GI [<25% is poor]; [range of 95% of drugs].

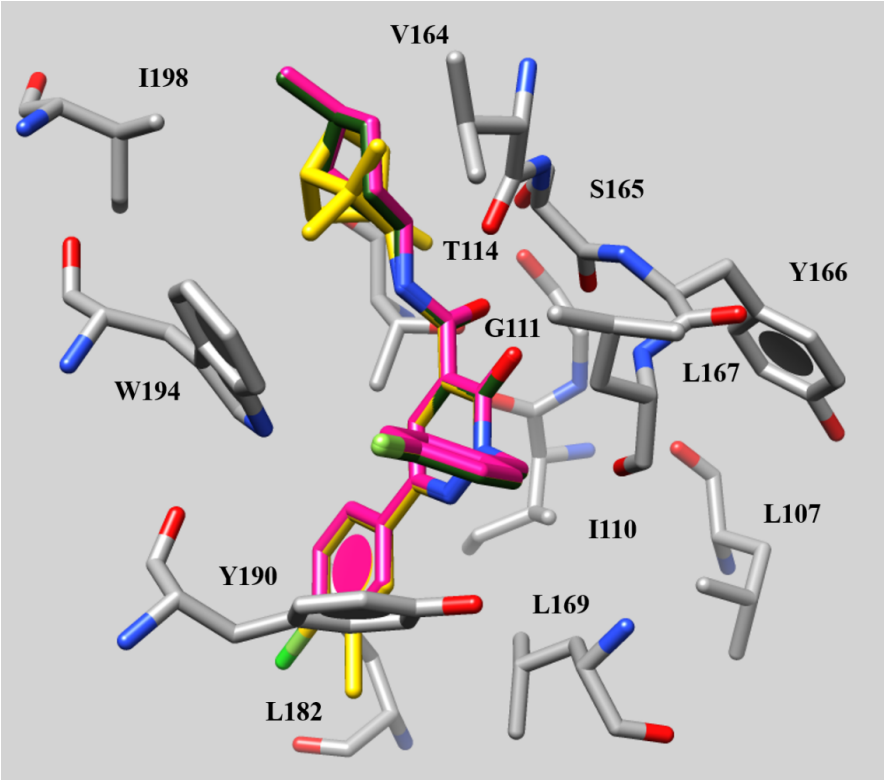


Fig. 3. Docking poses of compounds **8** (C atom: yellow), **9** (C atom: dark green) and **15** (C atom: deep magenta), within the *h*CB_2R inverse agonist binding site. The most important residues are labelled.

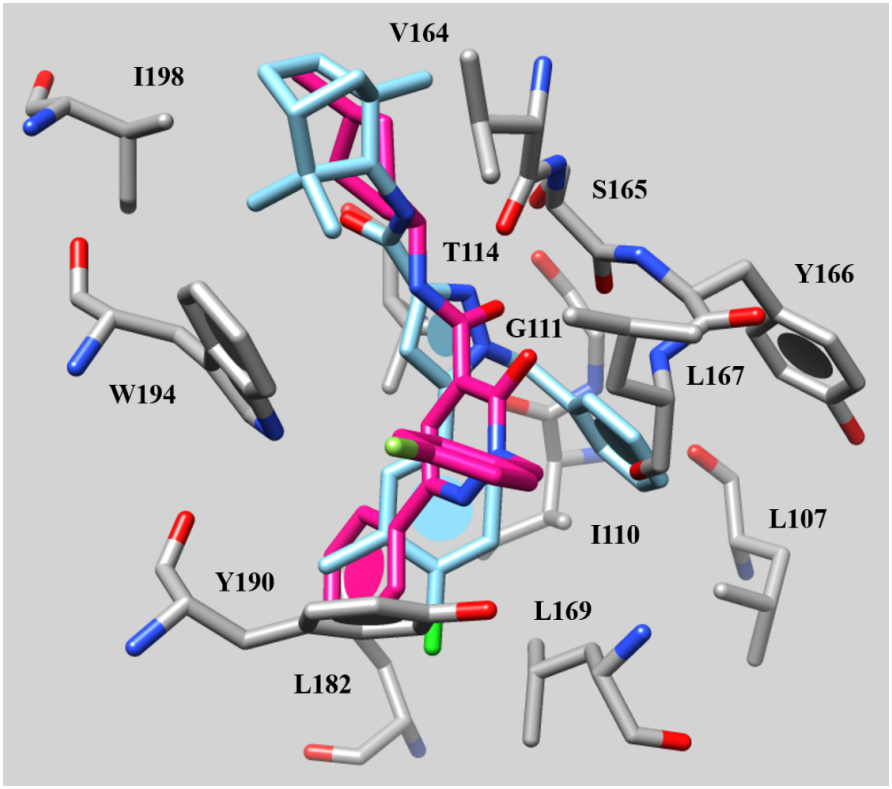


Fig. 4. Docking poses of compounds **15** (C atom: deep magenta) and of SR144528 (C atom: cyan) within the *h*CB_2R inverse agonist binding site. The most important residues are labelled.

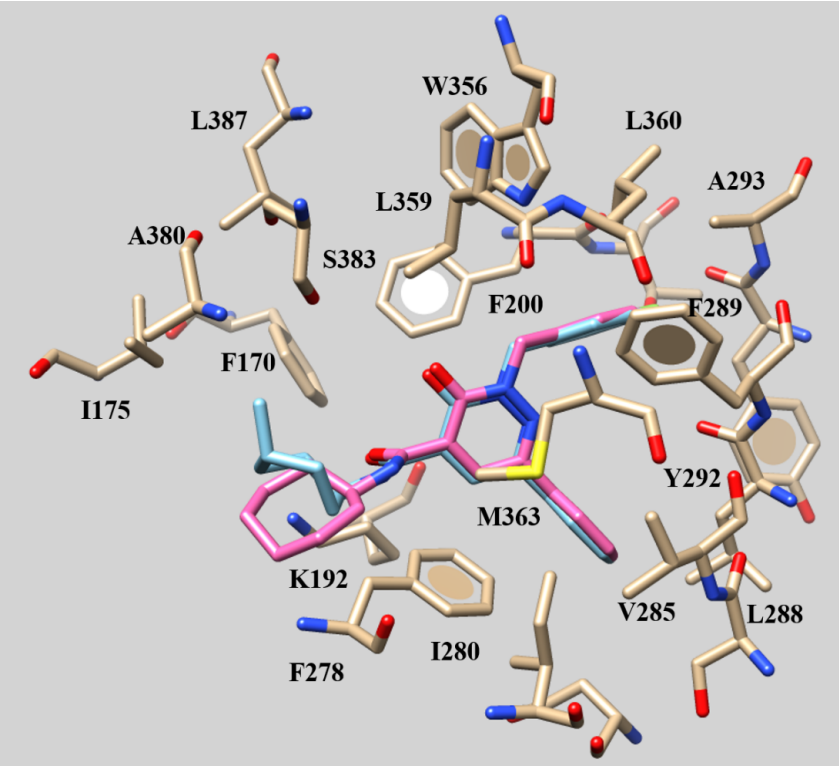


Fig. 5. Docking poses of compounds **13** (C atom: pink) and **15** (C atom: cyan) within the *h*CB_1R antagonist binding site. The most important residues are labelled.