Tricyclic Pyrazoles. Part 8. Synthesis, Biological Evaluation and Modelling of Tricyclic Pyrazole Carboxamides as Potential CB2 Receptor Ligands with Antagonist/Inverse Agonist Properties.

Tricyclic Pyrazoles. Part 8. Synthesis, Biological Evaluation and Modelling of Tricyclic Pyrazole Carboxamides as Potential CB_2 Receptor Ligands with Antagonist/Inverse Agonist Properties

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ABSTRACT

Previous studies have investigated the relevance and structure-activity relationships (SARs) of pyrazole derivatives in relation with cannabinoid receptors, and the series of tricyclic 1,4-dihydroindeno[1,2-*c*]pyrazoles emerged as potent CB_2 receptor ligands. In the present study, novel 1,4-dihydroindeno[1,2-*c*]pyrazole and 1*H*-benzo[*g*]indazole carboxamides containing a cyclopropyl or a cycloexyl substituent were designed and synthesized to evaluate the influence of these structural modifications towards CB_1 and CB_2 receptor affinities. Among these derivatives, compound **15** (6-cyclopropyl-1-(2,4-dichlorophenyl)-*N*-(adamantan-1-yl)-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxamide) showed the highest CB_2 receptor affinity (K_i = 4 nM) and remarkable selectivity (K_iCB_1/K_iCB_2 = 2232), whereas a similar affinity, within the nM range, was seen for the fenchyl derivative (compound **10**: K_i = 6 nM), for the bornyl analogue (compound **14**: K_i = 38 nM) and, to a lesser extent, for the aminopiperidine derivative (compound **6**: K_i = 69 nM). Compounds **10** and **14** were also highly selective for the CB_2 receptor (K_iCB_1/K_iCB_2 > 1000), whereas compound **6** was relatively selective (K_iCB_1/K_iCB_2 = 27). The four compounds were also subjected to GTPγS binding analysis showing antagonist/inverse agonist properties (IC_{50} for compound **14** = 27 nM, for **15** = 51 nM, for **10** = 80 nM and for **6** = 294 nM), and this activity was confirmed for the three more active compounds in a CB_2 receptor-specific *in vitro* bioassay consisting in the quantification of prostaglandin E2 release by LPS-stimulated BV2 cells, in the presence and absence of WIN55,212-2 and/or the investigated compounds. Modelling studies were also conducted with the four compounds, which conformed with the structural requirements stated for the binding of antagonist compounds to the human CB_2 receptor.

Keywords: tricyclic pyrazoles, synthesis, cannabinoid receptors, CB_2 antagonism, molecular modelling

1. Introduction

Derivatives of *Cannabis sativa*, commonly known as marijuana and hashish, have been known due to their medical and recreational properties for hundreds of years.[1] Despite the active constituents responsible for these properties in *Cannabis sativa* being identified in the 60s, in particular Δ^9-tetrahydrocannabinol (Δ^9-THC), the principal psychoactive component of *Cannabis* (see **1** in Figure 1), the biochemical and physiological bases underlying the effects of cannabinoids were identified only in the 90s. These studies derived in the identification of cannabinoid receptors,[2,3] endogenous ligands *N*-arachidonoylethanolamine (anandamide) **2**[4] and 2-arachidonoylglycerol (2-AG) **3**[5] (Figure 1), and the enzymatic machinery for their biosynthesis and hydrolysis,[6] as necessary steps leading to the understanding of the mechanisms by which plant-derived cannabinoids affect our mind and body.

Insert **Figure 1**

To date, two G-protein-coupled seven transmembrane receptors, namely cannabinoid receptor type-1 (CB_1) and cannabinoid receptor type-2 (CB_2), have been identified. CB_1 receptors are mainly expressed in areas of the brain that control movement, motor coordination, sensory perception, learning and memory, reward and emotions, preferentially located on numerous neuronal subpopulations, so they appear to be responsible for most of the central effects of cannabinoids.[7] They are also present outside the central nervous system (CNS) in numerous peripheral tissues.[2] CB_2 receptors are concentrated in cells and tissues of the immune system (e.g. spleen, macrophages, tonsils, B cells and natural killer cells, monocytes, neutrophils and T cells),[3] but they have been recently identified in the brain in healthy conditions (with a more restricted distribution compared to CB_1 receptors) and, in particular, in the damaged brain after different cytotoxic stimuli.[8] Specifically, CB_2 receptors were identified in microglial cells, astrocytes and in certain subpopulations of neurons making this receptor an interesting target for the treatment of neurological diseases.[9]

Recent data indicate that endocannabinoid spectrum is more complex that initially thought, being the transient receptor potential vanilloid-1 channels (TRPV1)[10] or the peroxisome proliferator-activated receptors (PPARs)[11] considered new targets for the action of endocannabinoids and related signaling lipids. Particularly, PPARs are a group of nuclear receptor proteins constituted by different isoforms (α, β/δ and γ), which are involved in regulation of cellular differentiation,[12] energy metabolism[13] and inflammation,[14] so that they may mediate some of the biological effects of endocannabinoids and of some specific plant-derived cannabinoids too.

Given the ubiquitous distribution in the body of endocannabinoids and related lipids, their receptors and the enzymes involved in their metabolism, drugs acting on this modulatory system appear to have therapeutic potential in a number of pathological conditions, including obesity and metabolic syndrome,[15] mood and anxiety disorders,[16] neuropathic pain,[17] multiple sclerosis,[18] neurodegenerative disorders,[19-21] as well as in atherosclerosis,[22] myocardial infarction,[23] cancer,[24] glaucoma[25] and osteoporosis.[26] Thus, in recent years, investigations were aimed to the design of new synthetic molecules targeting endocannabinoid-related receptors and enzymes that provide advantages over the already existing compounds, mainly plant-derived and endogenous cannabinoids, e.g. selectivitity for a specific target, agonist *versus* antagonist/inverse agonist activity, better water solubility, peripherally-restricted action, effects as allosteric modulators, and others.[27]

In our previously published studies,[28-31] we described the preliminary structure-activity relationships (SARs) of different tricyclic compounds typified by a 1,4-dihydroindeno[1,2-*c*] pyrazole and 4,5-dihydro-1*H*-benzo[*g*]indazole structures endowed with interesting cannabinoid binding profiles. Among these, the 1-(2,4-dichlorophenyl)-6-methyl-*N*-piperidin-1-yl-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxamide (compound **4**) showed the best selectivity towards CB_2 receptor compared to CB_1 receptor ($K_iCB_1/K_iCB_2 = 9810$),[30] whereas the 7-iodo-4,5-dihydro-1*H*-benzo[*g*]indazole analogue (compound **5**) exhibited a moderate CB_1 receptor selectivity ($K_iCB_2/K_iCB_1 = 262$)[29] (Figure 2). From these studies, we pointed out that changes in the size and

shape of the tricyclic core of these ligands revealed intriguing effects on biological activity, resulting the endomethylenic bridge in the planar structure of **4** in high affinity and selectivity for the CB_2 receptor. On the other hand, the simple homologation to endoethylenic bridge as in **5** revealed as such not fully planar template shown affinity to CB_1 receptor preferentially.

Insert **Figure 2**

In the present study, we describe the design, synthesis and biological evaluation of different 1,4-dihydroindeno[1,2-*c*]pyrazole and 4,5-dihydro-1*H*-benzo[*g*]indazole carboxamides containing a cyclopropyl or cyclohexyl building block in an attempt to investigate the effects of cycloalkyl moiety on cannabinoid receptor binding and activity. Moreover, here we have synthesized a new series of 7-cyclopropyl-1*H*-benzo[*g*]indazole carboxamides incorporating a fully aromatic scaffold on the basis that planar tricyclic pyrazoles bind preferentially CB_2 receptors. Aliphatic carboxamide groups in position 3 have been selected on the basis of previous cannabinoid pharmacophores.

All compounds were tested in radioligand binding assay towards both CB_1 and CB_2 receptor affinity, and derivatives **6**, **10**, **14**, **15**, exhibiting high affinity and selectivity towards the CB_2 receptor, have been evaluated in GTPγS binding and lipopolysaccharide (LPS)-induced microglial activation assay in order to establish how these ligands behave in relation with the CB_2 receptor (functional activity). Finally, molecular modelling studies were performed with the aim of better understanding of these new derivatives in relation with the structural requirements for the CB_2 receptor binding site.

2. Results and discussion

2.1. Chemistry

The synthetic route to obtain the target 6-cycloalkyl-1-(2,4-dichlorophenyl)-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxamides **6**,**9**,**10**,**13-18** and **22**, 7-cyclopropyl-1-(2,4-dichlorophenyl)-4,5-dihydro-1*H*-benzo[*g*]indazole-3-carboxamides **7,11** and **19** and 7-cyclopropyl-

1-(2,4-dichlorophenyl)-1*H*-benzo[*g*]indazole-3-carboxamides **8,12,20** and **21** is depicted in Scheme 1. All substituents are summarized in Table 1.

The Claisen condensation of the appropriate ketone (**23-25**) with diethyl oxalate in the presence of sodium ethylate afforded the key intermediates 1,3-diketoesters **26-28** as a tautomeric equilibrium shifted towards the hydroxyl-ketoesters (structures not reported). Compounds **26**, **27** or **28** and (2,4-dichlorophenyl)hydrazine hydrochloride were heated in ethanol to afford the corresponding pyrazole esters. Benzo[*g*]indazole ester analogue **32** was synthesized by oxidation of **31** with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in dichloromethane. The ethyl ester derivatives **29-32** were converted by saponification to the corresponding acid derivatives **33-36**. Treatment of acid derivatives with $SOCl_2$ produced the corresponding acid chlorides which were allowed to react with the suitable amines to the desired carboxamides (Scheme 1).

Insert **Scheme 1**

2.2. CB_1/CB_2 receptor binding studies

The CB_1 and CB_2 receptor binding affinities of the new synthesized compounds **6-22** were evaluated by radioligand binding assays carried out by competition with [^{3}H]-CP-55,940 in human CB_1 or CB_2 receptors transfected into HEK293 EBNA cells. The receptor affinities are shown in **Table 1**. For comparison, the K_i values of the lead compounds 6-methyl-1-(2,4-dichlorophenyl)-*N*-piperidin-1-yl-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxamide (**4**), a selective CB_2 receptor ligand,[28] and 7-iodo-1-(2,4-dichlorophenyl)-*N*-piperidin-1-yl-4,5-dihydro-1*H*-benzo[*g*]indazole-3-carboxamide (**5**), a selective CB_1 receptor ligand,[29] are included. Results are the average of three independent experiments with three replicates for each concentration.

Insert **Table 1**

The initial introduction of the cyclopropyl group at the R position of leads **4** and **5** generated compounds **6** and **7**, respectively, with the first (**6**) showing a decrease in cannabinoid receptor affinity (K_iCB_2 = 69 nM; K_iCB_1 = 1852 nM) and in CB_2 selectivity (K_iCB_1/K_iCB_2 = 27) when

compared to lead **4**, whereas the second (**7**) resulted in a better CB_2 receptor binding profile (K_iCB_2 = 143 nM) when compared to compound **5**. To further estimate the influence on the cannabinoid receptor affinity of the addition of the cyclopropyl group in the tricyclic scaffold, we introduced a flattering of the tricyclic core of **7** synthesizing the *1H*-benzo[*g*]indazole analogue **8**: this modification produced a decrease in the affinity for both cannabinoid receptor types (K_iCB_2 = 825 nM; K_iCB_1 > 40000 nM) and also in receptor selectivity (K_iCB_1/K_iCB_2 > 48).

As the introduction of a cyclopropyl ring in the tricyclic core of **4** and **5** resulted in compound **6** with better CB_2 receptor affinity, we evaluated the effect of the homologation of the cyclopropyl ring of **6** to cyclohexyl ring, preparing derivative **9**: this variation led to a 7.33-fold decrease in CB_2 receptor affinity (K_iCB_2 = 509 nM).

In light of the results obtained with these modifications on the central ring of the tricyclic core, compounds **6-9**, it was of interest to further determine the influence of the addition of a fenchyl group at the C3 carboxamide portion, with the aim to evaluate the effects on CB_2 receptor affinity and selectivity of such modification on these four templates. The replacement of the carboxamide *N*-piperidinyl moiety of **6** with the fenchyl residue generated the analogue **10** with increased CB_2 receptor affinity (K_iCB_2 = 6 nM) and improved receptor type selectivity (K_iCB_1/K_iCB_2 > 6944) compared to these values in compound **6**, whereas the same modification in derivatives **7**, **8** and **9**, to give analogues **11**, **12** and **13**, respectively, was detrimental for CB_2 receptor affinity (K_iCB_2 = 5517 nM, K_iCB_2 > 40000 nM and K_iCB_2 >10000 nM, respectively).

Because derivative **10** displayed a reasonable CB_2 receptor affinity and selectivity, we decided to further explore the SAR of this ligand through the introduction of other bulky groups in the carboxamide moiety, which should provide an improved understanding of the structural features that influence the affinity of this novel tricyclic scaffold. We first synthesized a small library of five compounds (**14**, **15**, **16**, **17** and **18**). Compound **14** containing the monoterpene bornylamine-side moiety showed a reduced CB_2 receptor affinity compared to compound **10**, although still within the

nM range (K_iCB_2 = 38 nM). Unlike derivative **14**, the adamantane derivative **15** showed a K_iCB_2 value of 4 nM equivalent to compound **10** and a good selectivity for this receptor type (K_iCB_1/K_iCB_2 > 2232).

To further explore whether improvements in cannabinoid receptor affinity might be obtained by modifying the C3-carboxamide side group, we next synthesized the three analogues of **10**, reported in Table 1, in which we simplified the skeleton of the amine side chain at the C3 carboxamide unit (compounds **16**-**18**). Among these carbocyclic compounds, the 1-aminopyrrolidinyl derivative **16** and the 1-aminomorpholinyl derivative **17** displayed a similar CB_2 receptor affinity (K_iCB_2 = 152 nM and K_iCB_2 = 197 nM, respectively) and selectivity (K_iCB_1/K_iCB_2 of 27.7 and 20.4-fold), whereas the introduction on the piperidine moiety led to **18** with a worsened CB_2 receptor affinity (K_iCB_2 = 413 nM).

In order to investigate the effect of the substitution on the C3 carboxamide portion also in the **7**, **8** and **9** templates, compounds **19**, **20**, **21** and **22** with myrtanyl, *N*-1-pyrrolidinyl and *N*-piperidinyl substituents were also examined. In general, the substitutions on these templates resulted in compounds with very low affinity for both CB_1 and CB_2 receptors.

These results are of interest because they show that the introduction of the cyclopropyl ring generated compounds with good (**6**) or improved (**7**) CB_2 receptor affinity as compared to the parent compounds **4** and **5**, respectively. Among the dihydroindenopyrazole series, the introduction of a fenchyl or a 1-adamantyl group led to an improvement of the CB_2 receptor affinity for compounds **10** and **15**, respectively, both derived from **6**. In contrast, the introduction of a cyclohexyl R substituent on tricyclic system or the homologation of the bridged endoalkyl/-enyl group led to a decreased affinity.

Overall, as previously reported,[28,30,31] the 1,4-dihydroindeno[1,2-*c*]pyrazole core showed preference for CB_2 receptors. The introduction of a cyclopropyl group in all new compounds seems

to play a modest role in lowering the levels of CB_2 receptor affinity as compared to the lead **4**. Nevertheless, these compounds provide further information regarding the structural features responsible for CB_2 affinity and selectivity.

2.3. Determination of the functional activity of selected compounds at the CB_2 receptor

Four compounds (**6**, **10**, **14** and **15**) were selected for further characterization based on their affinity and selectivity for the CB_2 receptor. We investigated their functional activity at this receptor, first by conducting GTPγS binding assays that demonstrated that the four compounds behaved as antagonists/inverse agonists of the CB_2 receptor with values of IC_{50} (nM) of 294.2 ± 127.5, 80.4 ± 17.0, 26.9 ± 2.8 and 50.7 ± 19.2, respectively. These IC_{50} values demonstrate that compound **6** was the less active of the four compounds in agreement with its higher K_i value for the CB_2 receptor (Table 1). Representative curves for each compound at the GTPγS binding bioassay are shown in Figure 3.

Insert **Figure 3**

Next, we confirmed the functional activity of the three most active compounds (**10**, **14** and **15**) following the data obtained in GTPγS binding studies using an in vitro bioassay specific for CB_2 receptor function that measures the effect exerted by ligands of this receptor on the release of prostaglandin E2 (PGE2) by LPS-stimulated BV2 cells.[32] Compounds **10**, **14** and **15** showed no effect on LPS-induced PGE2 release when incubated alone, despite certain trends towards an increase for compound **10** (see Figure 4) which are concordant with its profile as inverse agonist. Co-incubation of these compounds with SR144528, a classic CB_2 receptor antagonist/inverse agonist, did not enhance LPS-induced PGE2 release compared to cells incubated with LPS alone or LPS combined with each of these compounds, except for a small increase in the case of compound **14**. Their antagonist profile was evident in the fact that the three compounds reversed the reduction

caused by WIN55,212-2 in LPS-induced PGE2 release, in particular compound **10** and, to a lesser extent, compounds **14** and **15** (see Figure 4).

Insert **Figure 4**

2.4. Molecular modelling studies

In this work, with the aim of gaining a better understanding of the agonist or antagonist activity trend followed by the in-house library of pyrazole-based analogues, molecular docking studies were also performed. In particular, we focused our attention on the promising agonist **4** and on the antagonists **6**, **10**, **14** and **15**. In the first case, we started our work from the CB_2 receptor homology model we previously described, that allowed us to derive the human CB_2 (hCB_2) receptor model in complex with the reference agonist compound WIN-55,212-2 (hCB_2 = 8.89). Briefly, the derived model displayed a CB_2 agonist recognition site which proved to be delimited by TM3, TM5 and TM6, being in agreement with site-directed mutagenesis data.[33]

In addition, our previously published molecular dynamic simulation[33] revealed a specific pattern of H-bonds responsible of the high affinity of WIN-55,212-2, including S112, N188 and S285.

According to our calculations, compound **4** here disclosed, displays a comparable docking mode revolving around key H-bonds with S112 and S285, through the nitrogen atom at the pyrazole position 3 and (weakly) by means of those of the carboxamide moiety (Figure 5). Consequently, the tricyclic core and the dichloro-phenyl ring were oriented towards two hydrophobic pockets delimited by F197, L192, M265 and by F87, F117, V261 and C288, respectively. On the other hand, the piperidine ring was engaged in Van der Waals contacts with L108 and L182.

Notably, relevant steric requirements at the R and Q substituents resulted to be necessary, in order to guarantee an efficient binding mode. Indeed, bulkier group than the methyl substituent in R could be disfavoured, turning in clash with the L192, F197 and M265 side-chains. Furthermore, the

introduction of a norbornane or an adamantly group in Q is prevented by L108 and L182. Indeed, this information is supported by the different pharmacological profile displayed by **6**, **10**, **14** and **15**, being active as CB_2 antagonists, and probably binding elsewhere.

Insert **Figure 5**

In addition, the presence of a longer connection in X position also proved to be detrimental for the agonist affinity, causing a quite switched docking mode of compounds, lacking the aforementioned key H-bonds. Accordingly, compound **4** showed higher affinity values than the benzo[*g*]indazole analogues.

Concerning the antagonists **6**, **10**, **14** and **15**, their docking mode was investigated taking into account that of the reference CB_2 antagonist SR144528 (hCB_2 = 0.6 nM). As shown in Figure 6, the nitrogen atom at the position 2 of the SR144528 pyrazole moiety displayed one H-bond interaction with the T118 and S165 side chains, while the carbonyl oxygen showed one H-bond with the S165 side chain. The 4-chloro-3-methyl-phenyl at the position 5 of the pyrazole ring established Van der Waals interactions and π-π stacking with V164, L195 and Y190, W194 respectively. The benzyl group at the position 1 of the pyrazole moiety was oriented towards the hydrophobic CB_2 cavity including residues I110 and L169. On the other hand, the norbornane portion was oriented towards L160, V164, F197 and F202. Notably, our results were in agreement with those discussed by Montero and coworkers about the putative antagonist binding site of the hCB_2 receptor.[34]

Insert **Figure 6**

On the contrary, compounds **6**, **10**, **14** and **15** displayed an overturned docking mode in comparison with the previously one described for SR144528, moving the tricyclic core and the phenyl ring towards L160, V164, F197 and F202 while the Q substituent occupied the crevice delimited by

L167, L169 and Y190. Nevertheless, the compounds carboxamide function was able to interact properly with the key residues T118 and S165 and also to display π-π stacking with F197 and F202.

3. Conclusions

In summary, the introduction of a cyclopropyl group and the modulation of the carboxamide moiety in existing CB_2 receptor ligands allowed us to obtain novel CB_2 receptor selective 1,4-dihydroindeno[1,2-*c*]pyrazoles. Based on results of SAR studies around **4**, we generated four CB_2 receptor antagonists/inverse agonists **6**, **10**, **14** and **15** with nanomolar affinity for the CB_2 receptor and high selectivity for this receptor type over the CB_1, in which the presence of bulky amines in the carboxylic portion seems to play a pivotal role in determining the activity of such derivatives.

Moreover, this study further supports the development of new potential chemical entities based on pyrazole based tricyclic condensed scaffold, which may serve as experimental tools for investigating CB_2 receptor-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 CB_2 receptor-mediated activity in certain pathologies, e.g. immunodeficiency, bone disorders, cerebral malaria, in which an excess of CB_2 receptor-dependent endocannabinoid activity has been associated with the progression of the disease and/or with the occurrence of specific symptoms.[35]

4. Experimental

4.1. Chemistry

4.2. General

All reactions involving air or moisture-sensitive compounds were performed under argon atmosphere. Solvents and reagents were obtained from commercial suppliers and were used without

further purification. Flash column chromatography (FC) 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®). The progress of all reactions was monitored by thin layer chromatography (TLC) 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 or films 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 Nanobody 400 MHz spectrometer with ^{1}H and ^{13}C being obseved at 400 and 100.6 MHz, respectively. Spectra were acquired using deuterated chloroform (chloroform-d, $CDCl_3$) as solvent. Chemical shifts for ^{1}H and ^{13}C NMR spectra were reported in δ or ppm downfield from TMS [$(CH_3)_4Si$]. Data are reported as follows: chemical shift (sorted in descending order), multiplicity (s for singlet, bs for broad singlet, d for doublet, t for triplet, q for quadruplet, qu for quintuplet, m for multiplet), integration and coupling constants (J) in Hertz (Hz). All final compounds displayed ≥ 95% purity as determined by elemental analysis on a Perkin-Elmer 240-B analyser, for C, H, and N. The starting indanone **25** was prepared according to the described literature.[36]

*4.1.2. General procedure I: synthesis of cyclopropyl ketones **23** and **24***

To a solution of aryl bromide (0.948 mmol, 1 equiv), boronic acid (1.3 equiv), potassium phosphate (3.5 equiv) and tricyclohexylphosphine (0.1 equiv) in toluene (3.3 mL) and water (0.17 mL) under argon atmosphere was added palladium acetate (0.05 equiv). The mixture was heated to 100 °C for 4 h and then cooled to room temperature. Water was added and the mixture extracted with EtOAc, the combined organics were washed with brine, dried over Na_2SO_4 and concentrated in vacuo. Purification by FC afforded the desired compound.

4.1.2.1. 5-Cyclopropyl-2,3-dihydro-1H-inden-1-one (***23***). General procedure I was used to convert 5-bromo-indanone and cyclopropylboronic acid into the title product. Purification by FC (petroleum

ether/EtOAc, 9:1) afforded **23** (0,147g, 90%) as a light yellow solid. Mp 57-59 °C (EtOAc/petroleum ether). IR (nujol) ν: 1610 (CO). ^{1}H NMR ($CDCl_3$) δ 0.79-0.81 (m, 2H), 1.07-1.11 (m, 2H), 1.95-2.00 (m, 1H), 2.71 (t, 2H, J=2.8 Hz), 3.07 (t, 2H, J=3.1 Hz), 7.05 (d, 1H, J_o=7.8 Hz), 7.14 (s, 1H); 7.64 (d, 1H, J_o=7.8 Hz). ^{13}C NMR (DEPT, $CDCl_3$) 10.61 (CH_2 x 2), 16.13 (CH), 25.59 (CH_2), 34.63 (CH_2), 123.01 (CH), 123.06 (CH), 124.91 (CH), 134.82 (C), 152.44 (C), 155.78 (C), 206.41 (C). Anal. calcd for $C_{12}H_{12}O$: C, 83.69 (84.01); H, 7.02 (6.99). Found: C, 84.01; H, 6.99.

4.1.2.2. 6-Cyclopropyl-3,4-dihydronaphthalen-1(2H)-one (***24***). General procedure I was used to convert 6-bromo-tetralone and cyclopropylboronic acid into the title product. Purification by FC (petroleum ether/EtOAc, 9:1) afforded **24** (0.111g, 63%) as a brown oil. IR (film) ν: 1669 (CO). ^{1}H NMR ($CDCl_3$) δ 0.75-0.80 (m, 2H), 1.01-1.07 (m, 2H), 1.85-1.94 (m, 1H), 2.11 (q, 2H, J=6.4 Hz), 2.62 (t, 2H, J=6.4 Hz), 2.91 (t, 2H, J=6.0 Hz), 6.92 (s, 1H), 6.96 (dd, 1H, J_o=6.8 Hz, J_m=1.6 Hz), 7.92 (d, 1H, J_o=8.0 Hz); ^{13}C NMR (DEPT, $CDCl_3$) 10.28 (CH_2 x 2), 15.77 (CH), 23.36 (CH_2), 29.83 (CH_2), 39.11 (CH_2), 123.72 (CH), 125.48 (CH), 127.39 (CH), 130.32 (C), 144.58 (C), 150.68 (C), 198.06 (C). Anal. calcd for $C_{13}H_{14}O$: C, 83.83; H, 7.58. Found: C, 84.12; H, 7.59.

4.1.3. General procedure II: synthesis of α,γ-diketoesters ***26-28***.

Sodium metal (2.0 equiv) was added in small portion to dry ethanol (2.34 mL) and stirred until all the sodium had reacted. Ethyl oxalate (1.0 equiv) was added, followed by dropwise addition of a solution of appropriate indanone or tetralone starting material (2.78 mmol, 1.0 equiv) in dry ethanol (3.34 mL). The solution was stirred at room temperature for 5 h. The mixture was slowly poured over 2N hydrochloride acid and the aqueous layer was separated and extracted with CH_2Cl_2. The combined organic layer were washed with water, dried over Na_2SO_4 and concentrated under reduced pressure affording the analytically pure product or a crude oil that was purified by FC.

4.1.3.1. Ethyl 2-(5-cyclopropyl-1-oxo-2,3-dihydro-1H-inden-2-yl)-2-oxoacetate (***26***). General procedure II was used to convert **23** into the title product **26** (0.470g, 62%) as a brown solid. Mp 89-91 °C (EtOAc/petroleum ether). IR (nujol) ν: 1609 (CO), 1661 (CO), 1728 (CO). ^{1}H NMR ($CDCl_3$) δ 0.81-0.86 (m, 2H), 1.08-1.15 (m, 2H), 1.43 (t, 3H, J=7.2 Hz), 1.96-2.04 (m, 1H), 3.93 (s, 2H), 4.41 (qu, 2H, J=7.2 Hz), 7.10 (d, 1H, J_o=8.0 Hz), 7.19 (s, 1H), 7.74 (d, 1H, J_o=8.0 Hz). ^{13}C NMR (DEPT, $CDCl_3$) 10.92 (CH_2 x 2), 14.16 (CH_3), 16.41 (CH), 31.35 (CH_2), 62.13 (CH_2), 116.91 (C), 122.61 (CH), 123.90 (CH), 125.33 (CH), 134.78 (C), 151.06 (C x 2), 153.33 (C), 162.85 (C), 198.32 (C). Anal. calcd for $C_{16}H_{16}O_4$: C, 70.57 (70.30); H, 5.92. Found: C, 70.30; H, 5.94.

4.1.3.2. Ethyl 2-(6-cyclopropyl-1-oxo-1,2,3,4-tetrahydronaphthalen-2-yl)-2-oxoacetate (***27***). General procedure II was used to convert **24** into the title product. Purification by FC (petroleum ether/EtOAc, 9:1) afforded **27** (0.645g, 81%) as a orange oil. IR (film) ν: 1605 (CO), 1674 (CO), 1731 (CO). ^{1}H NMR ($CDCl_3$) δ 0.76-0.86 (m, 2H), 1.02-1.11 (m, 2H), 1.40 (t, 3H, J=7.2 Hz), 1.87-1.96 (m, 1H), 2.81-2.86 (m, 2H), 2.88-2.94 (m, 2H), 4.37 (qu, 2H, J=7.2 Hz), 6.90 (s, 1H), 7.01 (dd, 1H, J_o=6.8 Hz, J_m=1.2 Hz), 7.88 (d, 1H, J_o=8.8 Hz). ^{13}C NMR (DEPT, $CDCl_3$) 10.56 (CH_2 x 2), 14.11 (CH_3), 15.99 (CH), 22.69 (CH_2), 28.19 (CH_2), 61.99 (CH_2), 124.20 (CH), 124.83 (CH), 127.14 (CH), 108.92 (C), 128.81 (C), 142.76 (C), 151.42 (C), 163.10 (C), 168.11 (C), 187.63 (C). Anal. calcd for $C_{17}H_{18}O_4$: C, 71.31; H, 6.34. Found: C, 71.03; H, 6.31.

4.1.3.3. Ethyl 2-(5-cyclohexyl-1-oxo-2,3-dihydro-1H-inden-2-yl)-2-oxoacetate (***28***). General procedure II was used to convert **25** into the title product **28** (0.770g, 88%) as a brown solid. Mp 89-90 °C (EtOAc/petroleum ether). IR (nujol) ν: 1608 (CO), 1655 (CO), 1719 (CO). ^{1}H NMR ($CDCl_3$) δ 1.25-1.53 (m, 8H), 1.73-1.95 (m, 5H), 2.56-2.66 (m, 1H), 3.94 (s, 2H), 4.41 (q, 2H, J=4Hz), 7.28 (d, 1H, J_o=7.2Hz), 7.36 (s, 1H), 7.77 (d, 1H, J_o=8Hz). ^{13}C NMR (DEPT, $CDCl_3$) 14.10 (CH_3), 26.29 (CH_2 x 4), 29.35 (CH_2 x 2), 46.46 (CH), 62.17 (CH_2), 116.98 (C), 122.87 (CH), 123.50 (CH), 128.23 (CH), 135.12 (C), 151.06 (C), 153.21 (C), 155.44 (C), 162.88 (C), 198.57 (C). Anal. calcd (found) for $C_{19}H_{22}O_4$: C, 72.59; H, 7.05. Found: C, 72.85; H, 7.02.

*4.1.4. General procedure III: synthesis of tricyclic esters **29-31***

A mixture of diketoester **26-28** (0.80 mmol, 1.0 equiv) and 2,4-dichlorophenylhydrazine hydrochloride (1.15 equiv) in ethanol (5 mL) was heated under reflux for 24 h. The reaction mixture was allowed to cool to room temperature and then poured into water. The precipitate was filtered, washed with water and air-dried to yield the analytically pure product.

4.1.4.1. Ethyl 6-cyclopropyl-1-(2,4-dichlorophenyl)-1,4-dihydroindeno[1,2-c]pyrazole-3-carboxylate (***29***). General procedure III was used to convert **26** into the title product **29** (0.311g, 94%) as a brown solid. Mp 88-91 °C (EtOAc/petroleum ether). IR (nujol) ν: 1724 (CO). ^{1}H NMR ($CDCl_3$) δ 0.69-0.75 (m, 2H), 0.96-1.03 (m, 2H), 1.44 (t, 3H, J=7.2 Hz), 1.91-1.97 (m, 1H), 3.80 (s, 2H), 4.46 (q, 2H, J=7.2 Hz), 6.88 (d, 1H, J_o=8 Hz), 6.96 (dd, 1H, J_o=6.8 Hz, J_m=1.2 Hz), 7.25 (s, 1H), 7.43 (dd, 1H, J_o=6.4 Hz, J_m=2.4 Hz), 7.57 (d, 1H, J_o=8.4 Hz), 7.62 (d, 1H, J_m=2 Hz). ^{13}C NMR (DEPT, $CDCl_3$) 9.60 (CH_2 x 2), 14.45 (CH_3), 15.65 (CH), 29.77 (CH_2), 61.19 (CH_2), 118.90 (CH), 123.47 (CH), 124.43 (CH), 128.11 (CH), 128.70 (C), 129.17 (C), 130.00 (CH), 130.27 (CH), 131.96 (C), 136.06 (C), 136.08 (C), 139.33 (C), 143.69 (C), 149.56 (C), 151.74 (C), 162.36 (C); Anal. calcd for $C_{22}H_{18}Cl_2N_2O_2$: C, 63.93; H, 4.39; N, 6.78. Found: C, 63.98; H, 4.40; N, 6.79.

4.1.4.2. Ethyl 7-cyclopropyl-1-(2,4-dichlorophenyl)-4,5-dihydro-1H-benzo[g]indazole-3-carboxylate (***30***). General procedure III was used to convert **27** into the title product. Purification by FC (petroleum ether/EtOAc, 95:5) afforded **30** (0.198g, 58%) as a pale pink solid. Mp 202-205 °C (EtOAc/petroleum ether). IR (nujol) ν: 1723 (CO). ^{1}H NMR ($CDCl_3$) δ 0.58-0.62 (m, 2H), 0.85-0.91 (m, 2H), 1.35 (t, 3H, J=7.2 Hz), 1.71-1.79 (m, 1H), 2.86-3.14 (m, 4H), 4.37 (qu, 2H, J=7.2 Hz), 6.37 (d, 1H, J_o=8.0 Hz), 6.63 (dd, 1H, J_o=6.0 Hz, J_m=2.0 Hz), 6.93 (d, 1H, J_m=1.6 Hz), 7.35 (dd, 1H, J_o=6.0 Hz, J_m=2.4 Hz), 7.43 (d, 1H, J_o=8.4 Hz), 7.50 (d, 1H, J_m=2.0 Hz). ^{13}C NMR (DEPT, $CDCl_3$) 9.47 (CH_2), 9.48 (CH_2), 14.46 (CH_3), 15.30 (CH), 19.93 (CH_2), 30.12 (CH_2), 60.98 (CH_2), 121.09 (C), 121.19 (CH), 123.17 (C), 123.76 (CH), 126.18 (CH), 128.20 (CH), 130.40 (CH), 130.49 (CH), 133.52 (C), 136.37 (C), 137.13 (C), 137.23 (C), 140.87 (C), 141.18 (C), 144.73 (C),

162.74 (C). Anal. calcd for $C_{23}H_{20}Cl_2N_2O_2$: C, 64.65; H, 4.72; N, 6.56. Found: C, 64.78; H, 4.70; N, 6.53.

4.1.4.3. Ethyl 6-cyclohexyl-1-(2,4-dichlorophenyl)-1,4-dihydroindeno[1,2-c]pyrazole-3-carboxylate (***32***). General procedure III was used to convert **28** into the title product **32** (0.353, 97%) as an orange solid. Mp 175-178 °C (EtOAc/petroleum ether). IR (nujol) ν: 1716 (CO). ^{1}H NMR ($CDCl_3$) δ 1.37-1.49 (m, 8H), 1-87-1.91 (m, 5H); 2.54 (bs, 1H), 3.82 (s, 2H), 4.47 (q, 2H, J=7.2 Hz), 6.92 (d, 1H, J_o=8 Hz), 7.08 (dd, 1H, J_o=8 Hz, J_m=1.2 Hz), 7.42 (s, 1H), 7.43 (dd, 1H, J_o=8.8 Hz, J_m=2.4 Hz), 7.56 (d, 1H, J_o=8.4Hz), 7.63 (d, 1H, J_m=2.4 Hz). ^{13}C NMR (DEPT, $CDCl_3$) 14.46 (CH_3), 26.09 (CH_2), 26.86 (CH_2 x 2), 29.84 (CH_2), 34.56 (CH_2 x 2), 44.77 (CH), 61.19 (CH_2), 118.94 (CH), 124.81 (CH), 125.52 (CH), 128.08 (CH), 129.04 (C), 129.34(C), 129.99 (CH), 130.28 (CH), 132.04 (C), 136.08 (C x 2), 139.33 (C), 147.69 (C), 149.49 (C), 151.81 (C), 162.38 (C). Anal. calcd for $C_{25}H_{24}Cl_2N_2O_2$: C, 65.94; H, 5.31; N, 6.15. Found: 65.71; H, 5.29; N, 6.17.

4.1.5. Synthesis of ethyl 7-cyclopropyl-1-(2,4-dichlorophenyl)-1H-benzo[g]indazole-3-carboxylate (***31***). A stirred mixture of **30** (0.198g, 0.469 mmol, 1 equiv) and DDQ (4.2 equiv) in CH_2Cl_2 (5 mL) was heated under reflux for 10 h. The reaction was allowed to cool to room temperature, taken up with a 3% NH_4OH aqueous solution, dried (Na_2SO_4) and concentrated in vacuum. The residue was purified by FC (petroleum ether/EtOAc, 95:5) affording the analytically pure product **31** (0.148g, 76%) as a pale pink solid. Mp 138-140 °C (EtOAc/petroleum ether). IR (nujol) ν: 1631 (C=C), 1715 (CO). ^{1}H NMR ($CDCl_3$) δ 0.76-0.82 (m, 2H), 1.02-1.08 (m, 2H), 1.51 (t, 3H, J=6.8 Hz), 2.00-2.08 (m, 1H), 4.57 (q, 2H, J=7.2 Hz), 7.08 (dd, 1H, J_o=7.2 Hz, J_m=1.6 Hz), 7.19 (d, 1H, J_o=8.8 Hz), 7.51 (dd, 1H, J_o=6.4 Hz, J_m=2.0 Hz), 7.58 (d, 1H, J_o=8.4 Hz), 7.64 (d, 1H, J_o=8.8 Hz), 7.67-7.69 (m, 2H), 8.24 (d, 1H, J_o=8.8 Hz). ^{13}C NMR (DEPT, $CDCl_3$) 9.64 (CH_2), 9.69 (CH_2), 14.52 (CH_3), 15.56 (CH), 61.35 (CH_2), 118.32 (C), 119.73 (CH), 120.71 (CH), 120.80 (C), 125.05 (CH), 125.36 (CH), 125.50 (CH), 128.41 (CH), 130.50 (CH), 131.07 (CH), 133.47 (C), 134.47 (C), 136.93 (C), 137.64

(C), 138.01 (C), 138.55 (C), 143.23 (C), 162.67 (C). Anal. calcd for $C_{23}H_{18}Cl_2N_2O_2$: C, 64.95; H, 4.27; N, 6.59. Found: C, 65.20; H, 4.28; N, 6.61.

*4.1.6. General procedure IV: synthesis of carboxylic acids **33-36***

To a mixture of the suitable ester **29-32** (0.33 mmol, 1.0 equiv) in ethanol (1.9 mL) was added a solution of potassium hydroxide (2.0 equiv) in ethanol (1.23 mL). The resulting mixture was heated under reflux for 2 h. The mixture was allowed to cool to room temperature and then poured into water and acidified with 1N hydrochloric acid. The precipitate was filtered, washed with water and air-dried to yield the pure acid.

4.1.6.1. 6-Cyclopropyl-1-(2,4-dichlorophenyl)-1,4-dihydroindeno[1,2-c]pyrazole-3-carboxylic acid (***33***). General procedure IV was used to convert **29** into the title product **33** (0.102g, 80%) as a brown solid. Mp 230-231 °C. IR (nujol) ν: 1720 (CO), 3400 (OH). ^{1}H NMR (DMSO) δ 0.65-0.73 (m, 2H), 0.93-1.00 (m, 2H), 1.92-2.00 (m, 1H), 3.76 (s, 2H), 6.81 (d, 1H, J_o=8 Hz), 7.01 (dd, 1H, J_o=6.8 Hz, J_m=1.2 Hz), 7.32 (s, 1H), 7.72 (dd, 1H, J_o=6.4 Hz, J_m=2.4 Hz), 7.80 (d, 1H, J_o=8.4 Hz), 8.04 (d, 1H, J_m=2 Hz), 13.08 (bs, 1H). ^{13}C NMR (DEPT, DMSO) 9.62 (CH_2 x 2), 15.25 (CH), 29.31 (CH_2), 118.32 (CH), 123.34 (CH), 124.31 (CH), 127.96 (C), 128.57 (C), 128.83 (CH), 130.14 (CH), 130.40 (CH), 130.87 (C), 135.25 (C), 135.63 (C), 139.40 (C), 143.18 (C), 149.22 (C), 150.84 (C), 162.89 (C). Anal. calcd for $C_{20}H_{14}Cl_2N_2O_2$: C, 62.35; H, 3.66; N, 7.27. Found: C, 62.46; H, 3.65; N, 7.25.

*4.1.6.2. 7-Cyclopropyl-1-(2,4-dichlorophenyl)-4,5-dihydro-1H-benzo[g]indazole-3-carboxylic acid (**34**)*. General procedure IV was used to convert **30** into the title product **34** (0.092g, 70%) as a white solid. Mp 150-152 °C. IR (nujol) ν: 1713 (CO), 3417 (OH). ^{1}H NMR (DMSO) δ 0.63-0.68 (m, 2H), 0.89-0.95 (m, 2H), 1.79-1.88 (m, 1H), 2.49-2.53 (m, 2H), 2.88-2.95 (m, 2H), 6.34 (d, 1H, J_o=8.0 Hz), 6.78 (dd, 1H, J_o=6.8 Hz, J_m=1.6 Hz), 7.09 (s, 1H), 7.71 (dd, 1H, J_o=6.0 Hz, J_m=2.4 Hz), 7.77 (d, 1H, J_o=8.4 Hz), 8.01 (d, 1H, J_m=2.4 Hz). ^{13}C NMR (DEPT, DMSO) 9.48 (CH_2), 9.50

(CH_2), 14.94 (CH), 19.50 (CH_2), 29.29 (CH_2), 120.08 (C), 120.59 (CH), 122.57 (C), 123.56 (CH), 125.99 (CH), 128.96 (CH), 130.13 (CH), 131.06 (CH), 132.21 (C), 135.55 (C), 136.78 (C), 136.79 (C), 140.35 (C), 140.86 (C), 144.41 (C), 163.36 (C); Anal. calcd for $C_{21}H_{16}Cl_2N_2O_2$: C, 63.17; H, 4.04; N, 7.02. Found: C, 63.25; H, 4.03; N, 7.04.

4.1.6.3. 7-Cyclopropyl-1-(2,4-dichlorophenyl)-1H-benzo[g]indazole-3-carboxylic acid (***35***). General procedure IV was used to convert **31** into the title product **35** (0.117g, 89%) as a pale pink solid. Mp 262-264 °C. IR (nujol) ν: 1623 (CO). ^{1}H NMR (DMSO) δ 0.74-0.85 (m, 2H), 0.98-1.06 (m, 2H), 2.03-2.11 (m, 1H), 7.10 (d, 1H, J_o=8.8 Hz), 7.20 (dd, 1H, J_o=6.8 Hz, J_m=2.0 Hz), 7.75 (d, 1H, J_o=8.8 Hz), 7.82 (dd, 1H, J_o=6.4 Hz, J_m=2.4 Hz), 7.85 (d, 1H, J_m=1.2 Hz), 7.95 (d, 1H, J_o=8.4 Hz), 8.13 (d, 1H, J_m=2.0 Hz), 8.16 (d, 1H, J_o=8.8 Hz). ^{13}C NMR (DEPT, DMSO) 9.83 (CH_2), 9.89 (CH_2), 15.19 (CH), 117.63 (C), 119.32 (CH), 120.03 (C), 120.19 (CH), 124.96 (CH x 2), 125.28 (CH), 129.24 (CH), 130.27 (CH), 131.67 (CH), 132.81 (C), 133.06 (C), 136.17 (C), 137.21 (C), 137.78 (C), 137.91 (C), 143.17 (C), 163.33 (C); Anal. calcd for $C_{21}H_{14}Cl_2N_2O_2$: C, 63.49; H, 3.55; N, 7.05. Found: C, 63.74; H, 3.56; N, 7.07.

4.1.6.4. 6-Cyclohexyl-1-(2,4-dichlorophenyl)-1,4-dihydroindeno[1,2-c]pyrazole-3-carboxylic acid (***36***). General procedure IV was used to convert **32** into the title product **36** (0.121g, 86%) as a brown solid. Mp 250 °C. IR (nujol) ν: 1725 (CO), 3443 (OH). ^{1}H NMR (DMSO) δ 1.39 (s, 4H, J=10.4 Hz), 1.65-1.87 (m, 6H), 2.51 (s, 1H), 3.78 (s, 2H), 6.85 (d, 1H, J_o=8 Hz), 7.14 (d, 1H, J_o=7.6 Hz), 7.45 (s, 1H), 7.73 (dd, 1H, J_o=6.4 Hz, J_m=2 Hz), 7.80 (d, 1H, J_o=8.4 Hz), 8.05 (d, 1H, J_m=2.4 Hz). ^{13}C NMR (DEPT, DMSO) 26.29 (CH_2 x 4), 29.35 (CH_2 x 2), 33.87 (C x 3), 43.91 (CH x 2), 118.42 (CH), 124.76 (CH), 128.71 (C), 130.15 (CH x 2), 130.41 (CH), 130.94 (C), 135.27 (C), 135.65 (C), 146.16 (C), 149.93 (C), 162.92 (C). Anal. calcd for $C_{23}H_{20}Cl_2N_2O_2$: C, 64.65; H, 4.72; N, 6.56. Found: C, 64.41; H, 4.71; N, 6.58.

4.1.7. General procedure V: synthesis of carboxamides and hydrazides ***6-22***

A mixture of the appropriate carboxylic acid **33-36** (0.52 mmol, 1 equiv) and thionyl chloride (3.0 equiv) in toluene (2 mL) was refluxed for 5 h. The solvent and the excess of $SOCl_2$ were removed under reduced pressure and the resulting dark solid was diluted with CH_2Cl_2 (3 mL). To the resulting solution was added the requisite amine or hydrazine (1.5 equiv) and Et_3N (1.5 equiv) at 0 °C. The mixture was warmed to room temperature and stirred for 3 h. The mixture was then poured into a separatory funnel and brine was added. The aqueous layer was separated and extracted with CH_2Cl_2. The combined organic layer were washed (H_2O), dried (Na_2SO_4) and concentrated under reduced pressure. The analytically pure product was isolated by FC.

4.1.7.1. 6-Cyclopropyl-1-(2,4-dichlorophenyl)-N-(piperidin-1-yl)-1,4-dihydroindeno[1,2-c]pyrazole-3-carboxamide (***6***). General procedure V was used to convert **33** and *N*-aminopiperidine into the title product. Purification by FC (petroleum ether/EtOAc, 7:3) afforded **6** (0.151g, 53%) as a brown solid. Mp 214-216 °C (EtOAc/petroleum ether). IR (nujol) ν: 1679 (CO), 3315 (NH). ^{1}H NMR ($CDCl_3$) δ 0.69-0.74 (m, 2H), 0.96-1.02 (m, 2H), 1.39-1.48 (m, 2H), 1.71-1.80 (m, 4H), 1.89-1.99 (m, 1H), 2.84-2.93 (m, 4H), 3.85 (s, 2H), 6.87 (d, 1H, J_o=8.0 Hz), 6.94 (dd, 1H, J_o=6.8 Hz, J_m=0.8 Hz), 7.25 (s, 1H), 7.45 (dd, 1H, J_o=6.0 Hz, J_m=2.4 Hz), 7.53 (d, 1H, J_o=8.4 Hz), 7.63 (s, 1H, NH exch with D_2O), 7.65 (d, 1H, J_m=2.0 Hz). ^{13}C NMR (DEPT, $CDCl_3$) 9.58 (CH_2 x 2), 15.64 (CH), 23.35 (CH_2), 25.42 (CH_2 x 2), 29.69 (CH_2), 57.10 (CH_2 x 2), 118.77 (CH), 123.52 (CH), 124.27 (CH), 128.18 (CH), 128.29 (C), 128.61 (C), 129.72 (CH), 130.51 (CH), 131.93 (C), 135.98 (C), 136.04 (C), 141.46 (C), 143.62 (C), 150.16 (C), 151.84 (C), 159.22 (C). Anal. calcd for $C_{25}H_{24}Cl_2N_4O$: C, 64.24; H, 5.18; N, 11.99. Found: C, 63.98; H, 5.16; N, 11.95.

4.1.7.2. 7-Cyclopropyl-1-(2,4-dichlorophenyl)-N-(piperidin-1-yl)-4,5-dihydro-1H-benzo[g]indazole-3-carboxamide (***7***). General procedure V was used to convert **34** and *N*-aminopiperidine into the title product. Purification by FC (petroleum ether/EtOAc, 7:3) afforded **7** (0.155g, 62%) as a white solid. Mp 209-210 °C (EtOAc/petroleum ether). IR (nujol) ν: 1669 (CO),

3415 (NH). ^{1}H NMR ($CDCl_3$) δ 0.64-0.69 (m, 2H), 0.92-0.98 (m, 2H), 1.38-1.46 (m, 2H), 1.70-1.86 (m, 5H), 2.81-2.80 (m, 4H), 2.91-2.98 (m, 2H), 3.05-3.26 (m, 2H), 6.40 (d, 1H, J_o=8.0 Hz), 6.68 (dd, 1H, J_o=6.0 Hz, J_m=2.0 Hz), 6.99 (d, 1H, J_m=0.8 Hz), 7.44-7.46 (m, 2H), 7.59 (bs, 1H, NH exch. with D_2O), 7.60 (d, 1H, J_m=1.6 Hz). ^{13}C NMR (DEPT, $CDCl_3$) 9.46 (CH_2 x 2), 15.30 (CH), 19.56 (CH_2), 23.36 (CH_2), 25.42 (CH_2 x 2), 30.22 (CH_2), 57.12 (CH_2 x 2), 120.29 (C), 121.13 (CH), 123.22 (C), 123.62 (CH), 126.22 (CH), 128.30 (CH), 130.36 (CH), 130.61 (CH), 133.45 (C), 136.33 (C), 137.10 (C), 137.58 (C), 141.23 (C), 142.50 (C), 144.62 (C), 159.83 (C). Anal. calcd for $C_{26}H_{26}Cl_2N_4O$: C, 64.87; H, 5.44; N, 11.64. Found: C, 64.72; H, 5.43; N, 11.63.

4.1.7.3. 7-Cyclopropyl-1-(2,4-dichlorophenyl)-N-(piperidin-1-yl)-1H-benzo[g]indazole-3-carboxamide (***8***). General procedure V was used to convert **35** and *N*-aminopiperidine into the title product. Purification by FC (petroleum ether/EtOAc, 7:3) afforded **8** (0.087g, 35%) as a white solid. Mp 159-162 °C (EtOAc/petroleum ether). IR (nujol) ν: 1678 (CO), 3452 (NH). ^{1}H NMR ($CDCl_3$) δ 0.76-0.81 (m, 2H), 1.01-1.06 (m, 2H), 1.42-1.49 (m, 2H), 1.75-1.82 (m 4H), 1.99-2.07 (m, 1H), 2.91 (bs, 4H), 7.05 (dd, 1H, J_o=6.8 Hz, J_m=1.6 Hz), 7.15 (d, 1H, J_o=8.4 Hz), 7.53-7.55 (m, 2H), 7.60 (d, 1H, J_o=8.8 Hz), 7.66 (d, 1H, J_m=1.6 Hz), 7.71 (d, 1H, J_m=1.6 Hz), 7.75 (s, 1H, NH exch. with D_2O), 8.40 (d, 1H, J_o=8.8 Hz); ^{13}C NMR (DEPT, $CDCl_3$) 9.61 (CH_2), 9.63 (CH_2), 15.55 (CH), 23.37 (CH_2), 25.43 (CH_2 x 2), 57.23 (CH_2 x 2), 118.22 (C), 120.43 (C), 120.45 (CH), 120.67 (CH), 124.75 (CH), 124.95 (CH), 125.55 (CH), 128.50 (CH), 130.67 (CH), 130.98 (CH), 133.63 (C), 134.44 (C), 136.88 (C), 137.65 (C), 138.57 (C), 139.77 (C), 143.07 (C), 159.70 (C). Anal. calcd for $C_{26}H_{24}Cl_2N_4O$: C, 66.14; H, 5.05; N, 11.69. Found: C, 66.19; H, 5.06; N, 11.70.

4.1.7.4. 6-Cyclohexyl-1-(2,4-dichlorophenyl)-N-(piperidin-1-yl)-1,4-dihydroindeno[1,2-c]pyrazole-3-carboxamide (***9***). General procedure V was used to convert **36** and *N*-aminopiperidine into the title product. Purification by FC (petroleum ether/EtOAc, 75:25) afforded **9** (0.093g, 35%) as a white solid. Mp 172-173 °C (EtOAc/petroleum ether). IR (nujol) ν: 1680 (CO), 3250 (NH). ^{1}H NMR ($CDCl_3$) δ 1.36-1.47 (m, 6H), 1.70-1.94 (m, 10H), 2.54 (bs, 1H), 2.89 (s, 4H), 3.86 (s, 2H),

6.90 (d, 1H, J_o=7.6 Hz), 7.06 (d, 1H, J_o=8.4 Hz), 7.41 (s, 1H), 7.45 (dd, 1H, J_o=6.4 Hz, J_m=2.4 Hz), 7.52 (d, 1H, J_o=8.4 Hz), 7.65 (1H, J_m=2 Hz). ^{13}C NMR (DEPT, $CDCl_3$) 23.29 (CH_2), 25.36 (CH_2 x 2), 26.04 (CH_2), 26.81 (CH_2 x 2), 29.71 (CH_2), 34.50 (CH_2 x 3), 44.71 (CH), 57.02 (CH_2), 118.75 (CH), 124.84 (CH), 125.30 (CH), 128.11 (CH), 128.9 (C), 128.75 (C), 129.66 (C), 130.46 (CH), 130.84 (CH), 135.99 (C), 141.36 (C), 147.58 (C), 150.03 (C x2) 151.86 (C), 159.20 (C). Anal. calcd for $C_{28}H_{30}Cl_2N_4O$: C, 66.01; H, 5.94; N, 11.00. Found: C, 65.97; H, 5.93; N, 10.98.

4.1.7.5. 6-Cyclopropyl-1-(2,4-dichlorophenyl)-N-(1)-(S)-fenchyl-1,4-dihydroindeno[1,2-c]pyrazole-3-carboxamide (***10***). General procedure V was used to convert **33** and *N*-(1)-*(S)*-fenchylamine into the title product. Purification by FC (petroleum ether/EtOAc, 9:1) afforded **10** (0.170g, 63%) as an orange solid. Mp 118-120 °C (EtOAc/petroleum ether). IR (nujol) ν: 1662 (CO), 3412 (NH). ^{1}H NMR ($CDCl_3$) δ 0.69-0.76 (m, 2H), 0.82-0.87 (m, 2H), 0.88 (s, 3H), 0.95-1.02 (m, 2H), 1.12 (s, 3H), 1.19 (s, 3H), 1.37-1.54 (m, 2H), 1.67-1.75 (m, 2H), 1.79 (bs, 1H), 1.89-1.97 (m, 1H), 3.82 (dd, 1H, J=8 Hz, J=1.6 Hz), 3.85 (s, 2H), 6.89 (d, 1H, J_o=8 Hz), 6.95 (dd, 1H, J_o=6.8 Hz, J_m=1.2 Hz), 7.02 (d, 1H, J=9.6 Hz, NH exch con D_2O), 7.25 (s, 1H), 7.46 (dd, 1H, J_o=6 Hz, J_m=2.4 Hz), 7.56 (d, 1H, J_o=8.4 Hz), 7.65 (d, 1H, J_m=2.0 Hz). ^{13}C NMR (DEPT, $CDCl_3$) 9.54 (CH_2 x 2), 15.64 (CH_3), 19.80 (CH_3), 21.33 (CH_3), 26.00 (CH_2), 27.35 (CH_2), 29.70 (CH_2), 30.97 (CH), 39.53 (CH_2), 42.75 (C), 48.20 (CH), 48.68 (C), 63.18 (CH), 118.74 (CH), 123.52 (CH), 124.23 (CH), 127.56 (C), 128.07 (CH), 128.81 (C), 129.74 (CH), 130.49 (CH), 131.87 (C), 135.73 (C), 136.22 (C), 142.14 (C), 143.41 (C), 150.13 (C), 151.85 (C), 162.55 (C). Anal. calcd for $C_{30}H_{31}Cl_2N_3O$: C, 69.23; H, 6.00; N, 8.07. Found: C, 69.20; H, 5.98; N, 8.05.

4.1.7.6. 7-Cyclopropyl-1-(2,4-dichlorophenyl)-N-(1)-(S)-fenchyl-4,5-dihydro-1H-benzo[g]indazole-3-carboxamide (***11***). General procedure V was used to convert **34** and N-(1)-(*S*)-fenchylamine into the title product. Purification by FC (petroleum ether/EtOAc, 9:1) afforded **11** (0.250g, 90%) as a white solid. Mp 114-118 °C (EtOAc/petroleum ether). IR (nujol) ν: 1656 (CO), 3344 (NH). ^{1}H NMR ($CDCl_3$) δ 0.62-0.70 (m, 2H), 0.86 (s, 3H), 0.91-0.98 (m, 2H), 1.11 (s, 3H), 1.13-1.20 (m,

4H), 1.26 (s, 3H), 1.35-1.51 (m, 3H), 1.78-1.87 (m, 1H), 2.90-2.99 (m, 2H), 3.04-3.26 (m, 2H), 3.75-3.83 (m, 1H), 6.43 (d, 1H, J_o=8.0 Hz), 6.68 (dd, 1H, J_o=6.4 Hz, J_m=1.6 Hz), 6.98 (s, 1H), 7.00 (d, 1H, J=8.4 Hz, NH exch. with D_2O), 7.43 (d, 1H, J_o=8.8 Hz), 7.48 (dd, 1H, J_o=4.8 Hz, J_m=3.6 Hz), 7.60 (s, 1H). ^{13}C NMR (DEPT, $CDCl_3$) 9.43 (CH_2 x 2), 15.29 (CH_3), 19.74 (CH_2), 19.80 (CH_3), 21.30 (CH_3), 26.00 (CH_2), 27.34 (CH_2), 30.30 (CH_2), 30.98 (CH), 39.51 (CH_2), 42.75 (C), 48.19 (CH), 48.67 (C), 62.96 (CH), 119.60 (C), 121.18 (CH), 123.58 (CH), 126.15 (CH), 128.14 (CH), 130.46 (CH), 130.55 (CH), 133.41 (C), 133.49 (C), 136.07 (C), 137.29 (C), 137.53 (C), 143.14 (C), 143.16 (C), 144.39 (C), 163.17 (C). Anal. calcd for $C_{31}H_{33}Cl_2N_3O$: C, 69.66; H, 6.22; N, 7.86. Found: C, 69.63; H, 6.21; N, 7.85.

4.1.7.7. 7-Cyclopropyl-1-(2,4-dichlorophenyl)-N-(1)-(S)-fenchyl-1H-benzo[g]indazole-3-carboxamide (***12***). General procedure V was used to convert **35** and *N*-(1)-(*S*)-fenchylamine into the title product. Purification by FC (petroleum ether/EtOAc, 9:1) afforded **12** (0.219g, 79%) as a white solid. Mp 118-120 °C (EtOAc/petroleum ether). IR (nujol) ν: 1665 (CO), 3396 (NH). ^{1}H NMR ($CDCl_3$) δ 0.76-0.81 (m, 2H), 0.89 (s, 3H), 1.02-1.06 (m, 2H), 1.14 (s, 3H), 1.21 (d, 3H, J=3.6 Hz), 1.25-1.30 (m, 2H), 1.40-1.54 (m, 2H), 1.71-1.77 (m, 2H), 1.79-1.82 (m, 1H), 1.99-2.07 (m, 1H), 3.88-3.93 (m, 1H), 7.06 (dd, 1H, J_o=6.8 Hz, J_m=2.0 Hz), 7.13-7.20 (m, 2H, NH exch. with D_2O), 7.53 (dd, 1H, J_o=6.0 Hz, J_m=2.4 Hz), 7.57-7.61 (m, 2H), 7.65 (d, 1H, J_m=1.6 Hz), 7.71 (d, 1H, J_m=2.4 Hz), 8.42 (d, 1H, J_o=8.8 Hz). ^{13}C NMR (DEPT, $CDCl_3$) 9.58 (CH_2), 9.60 (CH_2), 15.55 (CH_3), 19.80 (CH_3), 21.32 (CH_3), 26.04 (CH_2), 27.38 (CH_2), 31.02 (CH), 39.60 (C), 42.79 (CH_2), 48.21 (CH), 48.69 (C), 63.10 (CH), 118.35 (C), 119.97 (C), 120.60 (CH), 120.73 (CH), 124.65 (CH), 124.74 (CH), 125.50 (CH), 128.34 (CH), 130.59 (CH), 131.09 (CH), 133.56 (C), 134.54 (C), 136.68 (C), 137.86 (C), 138.71 (C), 140.43 (C), 142.87 (C), 163.00 (C). Anal. calcd for $C_{31}H_{31}Cl_2N_3O$: C, 69.92; H, 5.87; N, 7.89. Found: C, 69.88; H, 5.86; N, 7.87.

4.1.7.8. 6-Cyclohexyl-1-(2,4-dichlorophenyl)-N-(1)-(S)-fenchyl-1,4-dihydroindeno[1,2-c]pyrazole-3-carboxamide (***13***). General procedure V was used to convert **36** and *N*-(1)-(*S*)-fenchylamine into

the title product. Purification by FC (petroleum ether/EtOAc, 95:5) afforded **13** (0.123g,42%) as a white solid. Mp 192-194 °C (EtOAc/petroleum ether). IR (nujol) ν: 1680 (CO), 3334 (NH). ^{1}H NMR ($CDCl_3$) δ 0.88 (s, 3H), 1.13 (s, 3H), 1.19 (s, 3H), 1.23-1.28 (m, 2H), 1.36-1.49 (m, 6H), 1.69-1.91 (m, 9H), 2.49-2.58 (m, 1H), 3.82 (dd, 1H, J_1=7.6 Hz, J_2=2.0 Hz), 3.86 (s, 2H), 6.93 (d, 1H, J_o=7.6 Hz), 7.02 (d, 1H, J=9.6 Hz, NH exch. with D_2O), 7.07 (dd, 1H, J_o=6.8 Hz, J_m=0.8 Hz), 7.42 (s, 1H), 7.45 (dd, 1H, J_o=6.0 Hz, J_m=2.4 Hz), 7.55 (d, 1H, J_o=8.4 Hz), 7.66 (d, 1H, J_m=2.4 Hz); ^{13}C NMR (DEPT, $CDCl_3$) 19.81 (CH_3), 21.32 (CH_3), 26.00 (CH_2), 26.11 (CH_2), 26.88 (CH_2 x 2), 27.35 (CH_2), 29.76 (CH_2), 30.96 (CH_3), 34.56 (CH_2 x 2), 39.54 (CH_2), 42.75 (C), 44.76 (CH), 48.20 (CH), 48.67 (C), 63.19 (CH), 118.77 (CH), 124.88 (CH), 125.28 (CH), 127.72 (C), 128.04 (CH), 129.13 (C), 129.72 (CH), 130.51 (CH), 131.93 (C), 135.73 (C), 136.23 (C), 142.12 (C), 147.44 (C), 150.05 (C), 151.92 (C), 162.58 (C). Anal. calcd for $C_{33}H_{37}Cl_2N_3O$: C, 70.45; H, 6.63; N, 7.47. Found: C, 70.39; H, 6.62; N, 7.46.

4.1.7.9. 6-Cyclopropyl-1-(2,4-dichlorophenyl)-N-(R)-(+)-bornyl-1,4-dihydroindeno[1,2-c]pyrazole-3-carboxamide (***14***). General procedure V was used to convert **33** and *N*-(*R*)-(+)-bornylamine into the title product. Purification by FC (petroleum ether/EtOAc, 9:1) afforded **14** (0.187g, 69%) as a white solid. Mp 125-128 °C (EtOAc/petroleum ether). IR (nujol) ν: 1662 (CO), 3412 (NH). ^{1}H NMR ($CDCl_3$) δ 0.68-0.73 (m, 2H), 0.85-0.88 (m, 2H), 0.91 (d, 6H, J=4.8 Hz), 0.93-0.98 (m, 2H), 1.01 (s, 3H), 1.20-1.30 (d, 2H), 1.66-1.72 (m, 2H), 1.89-1.97 (m, 1H), 2.37-2.47 (m, 1H), 3.84 (s, 2H), 4.43-4.51 (m, 1H), 6.88 (d, 1H, J_o=7.6 Hz), 6.93-6.98 (m, 2H, NH exch with D_2O), 7.25 (s, 1H), 7.46 (dd, 1H, J_o=6.4 Hz, J_m=2 Hz), 7.56 (d, 1H, J_o=8.4 Hz), 7.66 (d, 1H, J_m=2.4 Hz). ^{13}C NMR (DEPT, $CDCl_3$) 9.54 (CH_2 x 2), 13.87 (CH_3), 15.64 (CH_3), 18.68 (CH_3), 19.88 (CH), 28.11 (CH_2), 28.41 (CH_2), 29.70 (CH_2), 37.48 (CH_2), 45.00 (CH), 48.23 (C), 49.73 (C), 53.59 (CH), 118.75 (CH), 123.51 (CH), 124.28 (CH), 127.77 (C), 128.14 (CH), 128.78 (C), 129.84 (CH), 130.47 (CH), 131.94 (C), 135.88 (C), 136.17 (C), 142.21 (C), 143.48 (C), 150.15 (C), 151.92 (C), 162.10 (C). Anal. calcd for $C_{30}H_{31}Cl_2N_3O$: C, 69.23; H, 6.00; N, 8.07. Found: C, 69.19; H, 5.99; N, 8.05.

4.1.7.10. 6-Cyclopropyl-1-(2,4-dichlorophenyl)-N-(adamantan-1-yl)-1,4-dihydroindeno[1,2-c]pyrazole-3-carboxamide (***15***). General procedure V was used to convert **33** and 1-adamantylamine into the title product. Purification by FC (petroleum ether/EtOAc, 95:5) afforded **15** (0.156g, 58%) as a light brown solid. Mp 130-133 °C (EtOAc/petroleum ether). IR (nujol) ν: 1668 (CO), 3396 (NH). ^{1}H NMR ($CDCl_3$) δ 0.70-0.74 (m, 2H), 0.96-1.01 (m, 2H), 1.68-1.76 (m, 6H), 1.88-1.97 (m, 1H), 2.10-2.18 (m, 9H), 3.83 (s, 2H), 6.69 (s, 1H, NH exch con D_2O), 6.86 (d, 1H, J_o=8 Hz), 6.94 (dd, 1H, J_o=6.8 Hz, J_m=1.2 Hz), 7.24 (s, 1H), 7.44 (dd, 1H, J_o=6.4 Hz, J_m=2 Hz), 7.53 (d, 1H, J_o=8.8 Hz), 7.64 (d, 1H, J_m=2 Hz). ^{13}C NMR (DEPT, $CDCl_3$) 9.53 (CH_2 x 2), 15.64 (CH), 29.53 (CH x 3), 29.72 (CH_2), 36.43 (CH_2 x 3), 41.74 (CH_2 x 3), 51.99 (C), 118.72 (CH), 123.53 (CH), 124.26 (CH), 127.69 (C), 128.14 (CH), 128.77 (C), 129.81 (CH), 130.45 (CH), 131.94 (C), 135.87 (C), 136.13 (C), 142.98 (C), 143.45 (C), 150.20 (C), 151.98 (C), 161.78 (C). $C_{30}H_{29}Cl_2N_3O$: C, 69.50; H, 5.64; N, 8.10. Found: C, 69.48; H, 5.63; N, 8.09.

4.1.7.11. 6-Cyclopropyl-1-(2^1,4^1-dichlorophenyl)-N-(pyrrolidin-1-yl)-1,4-dihydroindeno[1,2-c]pyrazole-3-carboxamide (***16***). General procedure V was used to convert **33** and *N*-aminopyrrolidine hydrochloride into the title product. Purification by FC (petroleum ether/EtOAc, 6:4) afforded **16** (0.080g, 34%) as a brown solid. Mp 202-204 °C (EtOAc/petroleum ether). IR (nujol) ν: 1660 (CO), 3311 (NH). ^{1}H NMR ($CDCl_3$) δ 0.69-0.74 (m, 2H), 0.96-1.02 (m, 2H), 1.87-1.97 (m, 5H), 3.00-3.07 (m, 4H), 3.86 (s, 2H), 6.86 (d, 1H, J_o=8.0 Hz), 6.94 (dd, 1H, J_o=7.2 Hz, J_m=0.8 Hz), 7.25 (s, 1H), 7.45 (dd, 1H, J_o=6.0 Hz, J_m=2.4 Hz), 7.53 (d, 1H, J_o=8.8 Hz), 7.62 (s, 1H, NH exch with D_2O), 7.65 (d, 1H, J_m=2.0 Hz). ^{13}C NMR (DEPT, $CDCl_3$) 9.58 (CH_2 x 2), 15.64 (CH), 22.33 (CH_2 x 2), 29.72 (CH_2), 55.48 (CH_2 x 2), 118.76 (CH), 123.55 (CH), 124.26 (CH), 128.14 (C), 128.18 (CH), 128.57 (C), 129.72 (CH), 130.51 (CH), 131.96 (C), 136.01 (C x 2), 141.33 (C), 143.65 (C), 150.15 (C), 151.87 (C), 160.11 (C). $C_{24}H_{22}Cl_2N_4O$: C, 63.58; H, 4.89; N, 12.36. Found: C, 63.55; H, 4.87; N, 12.34.

4.1.7.12. 6-Cyclopropyl-1-(2[1],4[1]-dichlorophenyl)-N-(morpholin-4-yl)-1,4-dihydroindeno[1,2-c]pyrazole-3-carboxamide (***17***). General procedure V was used to convert **33** and 4-aminomorpholine into the title product. Purification by FC (petroleum ether/EtOAc, 55:45) afforded **17** (0.117g, 48%) as a brown solid. Mp 181-183 °C (EtOAc/petroleum ether). IR (nujol) ν: 1674 (CO), 3418 (NH). ^{1}H NMR ($CDCl_3$) δ 0.67-0.76 (m, 2H), 0.96-1.04 (m, 2H), 1.89-1.94 (m, 1H), 2.98 (t, 4H, J=4.4 Hz), 3.85 (s, 2H), 3.87 (t, 4H, J=4.4 Hz), 6.87 (d, 1H, J_o=8 Hz), 6.95 (dd, 1H, J_o=6.4 Hz, J_m=1.6 Hz), 7.25 (s, 1H), 7.46 (dd, 1H, J_o=6.4 Hz, J_m=2 Hz), 7.53 (d, 1H, J_o=8 Hz), 7.66 (d, 1H, J_m=2 Hz), 7.70 (s, 1H). ^{13}C NMR (DEPT, $CDCl_3$) 9.61 (CH_2 x 2), 15.65 (CH), 29.66 (CH_2), 56.05 (CH_2 x 2), 66.51 (CH_2 x 2), 118.80 (CH), 123.54 (CH), 123.33 (CH), 128.23 (CH), 128.27 (C), 128.51 (C), 129.69 (CH), 130.55 (CH), 131.95 (C), 135.94 (C), 136.12 (C), 141.10 (C), 143.76 (C), 150.09 (C), 151.99 (C), 159.44 (C). $C_{24}H_{22}Cl_2N_4O_2$: C, 61.41; H, 4.72; N, 11.94. Found: C, 61.43; H, 4.71; N, 11.95.

4.1.7.13. 6-Cyclopropyl-1-(2,4-dichlorophenyl)-N-(piperidinyl)-1,4-dihydroindeno[1,2-c]pyrazol-3-carboxamide (***18***). General procedure V was used to convert **33** and piperidine into the title product. Purification by FC (petroleum ether/EtOAc, 85:15) afforded **18** (0.125g, 53%) as a pale orange solid. Mp 170-172 °C (EtOAc/petroleum ether). IR (nujol) ν: 1616 (CO). ^{1}H NMR ($CDCl_3$) δ 0.69-0.74 (m, 2H), 0.96-1.02 (m, 2H), 1.60-1.75 (m, 6H), 1.89-1.97 (m, 1H), 3.75 (bs, 2H), 3.77 (s, 2H), 4.01 (bs, 2H), 6.90 (d, 1H, J_o=7.6 Hz), 6.95 (dd. 1H, J_o=6.4 Hz, J_m=1.6 Hz), 7.23 (s, 1H), 7.43 (dd, 1H, J_o=6.4 Hz, J_m=2.4 Hz), 7.52 (d, 1H, J_o=8.4 Hz), 7.63 (d, 1H, J_m=2 Hz). ^{13}C NMR (DEPT, $CDCl_3$) 9.52 (CH_2 x 2), 15.63 (CH), 24.77 (CH_2), 25.77 (CH_2), 26.83 (CH_2), 29.75 (CH_2), 43.59 (CH_2), 48.12 (CH_2), 118.71 (CH), 123.47 (CH), 124.25 (CH), 128.05 (CH), 128.78 (C), 129.12 (C), 129.60 (CH), 130.51 (CH), 131.84 (C), 135.56 (C), 136.24 (C), 142.83 (C), 143.31 (C), 149.89 (C), 150.57 (C), 162.38 (C). $C_{25}H_{23}Cl_2N_3O$: C, 66.38; H, 5.12; N, 9.29. Found: C, 66.34; H, 5.11; N, 9.28.

4.1.7.14. 7-Cyclopropyl-1-(2[1],4[1]-dichlorophenyl)-N-(1S,1R)-myrtanyl-4,5-dihydro-1H-benzo[g]indazole-3-carboxamide (***19***). General procedure V was used to convert **34** and *N*-(*1S,1R*)-myrtanylamine into the title product. Purification by FC (petroleum ether/EtOAc, 9:1) afforded **19** (0.233g, 84%) as a white solid. Mp 93-96 °C (EtOAc/petroleum ether). IR (nujol) ν: 1666 (CO), 3419 (NH). ^{1}H NMR ($CDCl_3$) δ 0.64-0.67 (m, 2H), 0.81-0.91 (m, 4H), 0.92-0.98 (m, 2H), 1.07 (s, 3H), 1.20 (s, 3H), 1.78-2.20 (m, 6H), 2.27-2.40 (m, 2H), 2.91-2.99 (m, 2H), 3.28-3.55 (m, 2H), 6.41 (d, 1H, J_o=6.41 Hz), 6.68 (dd, 1H, J_o=6.4 Hz, J_m=1.6 Hz), 6.93 (bs, 1H, NH exch. with D_2O), 6.99 (d, 1H, J_m=1.2 Hz), 0.74-0.77 (m, 2H), 7.60 (d, 1H, J_m=2.0 Hz). ^{13}C NMR (DEPT, $CDCl_3$) 9.50 (CH_2 x 2), 15.30 (CH_3), 19.68 (CH_2), 19.91 (CH_2), 23.24 (CH_3), 26.05 (CH_2), 28.00 (CH), 30.26 (CH_2), 33.32 (CH_2), 38.72 (C), 41.40 (CH), 41.52 (CH), 43.68 (CH), 44.60 (CH_2), 119.74 (C), 121.15 (CH), 123.33 (C), 123.62 (CH), 126.20 (CH), 128.28 (CH), 130.37 (CH), 130.60 (CH), 133.48 (C), 136.29 (C), 137.17 (C), 137.54 (C), 141.34 (C), 143.21 (C), 144.55 (C), 162.55 (C). $C_{31}H_{33}Cl_2N_3O$: C, 69.66; H, 6.22; N, 7.86. Found: C, 69.59; H, 6.20; N, 7.84.

4.1.7.15. 7-Cyclopropyl-1-(2,4-dichlorophenyl)-N-(pyrrolidin-1-yl)-1H-benzo[g]indazole-3-carboxamide (***20***). General procedure V was used to convert **35** and *N*-aminopyrrolidine hydrochloride into the title product. Purification by FC (petroleum ether/EtOAc, 6:4) afforded **20** (0.082g, 34%) as a white solid. Mp 210-212 °C (EtOAc/petroleum ether). IR (nujol) ν: 1684 (CO), 3478 (NH). ^{1}H NMR ($CDCl_3$) δ 0.76-0.81 (m, 2H), 1.01-1.06 (m, 2H), 1.89-1.97 (m, 4H), 1.99-2.07 (m, 1H), 3.02-3.10 (m, 4H), 7.06 (dd, 1H, J_o=7.2 Hz, J_m=1.6 Hz), 7.15 (d, 1H, J_o=8.8 Hz), 7.53-7.55 (m, 2H), 7.60 (d, 1H, J_o=8.8 Hz), 7.66 (d, 1H, J_m=1.6 Hz), 7.71 (d, 1H, J_m=1.6 Hz), 7.73 (s, 1H, NH exch. with D_2O), 8.41 (d, 1H, J_o=8.8 Hz). ^{13}C NMR (DEPT, $CDCl_3$) 9.61 (CH_2), 9.63 (CH_2), 15.55 (CH), 22.29 (CH_2 x 2), 55.59 (CH_2 x 2), 118.22 (C), 120.35 (C), 120.47 (CH), 120.67 (CH), 124.77 (CH), 124.94 (CH), 125.54 (CH), 128.50 (CH), 130.67 (CH), 130.96 (CH), 133.64 (C), 134.44 (C), 136.90 (C), 137.64 (C), 138.57 (C), 139.67 (C), 143.09 (C), 160.58 (C). $C_{25}H_{22}Cl_2N_4O$: C, 64.52; H, 4.76; N, 12.04. Found: C, 64.50; H, 4.75; N, 12.02.

4.1.7.16. 7-Cyclopropyl-1-(2,4-dichlorophenyl)-N-(piperidinyl)-1H-benzo[g]indazol-3-carboxamide (***21***). General procedure V was used to convert **35** and piperidine into the title product. Purification by FC (petroleum ether/EtOAc, 9:1) afforded **21** (0.140g, 58%) as a white solid. Mp 92-95 °C (EtOAc/petroleum ether). IR (nujol) ν: 1684 (CO). ^{1}H NMR ($CDCl_3$) δ 0.76-0.81 (m, 2H), 1.02-1.07 (m, 2H), 1.58-1.76 (m, 6H), 1.99-2.07 (m, 1H), 3.75-3.94 (m, 4H), 7.05 (dd, 1H, J_o=6.8 Hz, J_m=1.6 Hz), 7.19 (d, 1H, J_o=8.4 Hz), 7.50 (dd, 1H, J_o=6.4 Hz, J_m=2.0 Hz), 7.53-7.59 (m, 2H), 7.65 (d, 1H, J_m=1.2 Hz), 7.69 (d, 1H, J_m=2.4 Hz), 8.00 (d, 1H, J_o=8.8 Hz). ^{13}C NMR (DEPT, $CDCl_3$) 9.59 (CH_2), 9.61 (CH_2), 15.55 (CH), 24.73 (CH_2), 25.81 (CH_2), 26.81 (CH_2), 43.55 (CH_2), 48.40 (CH_2), 118.25 (C), 119.71 (CH), 120.82 (C), 120.88 (CH), 124.22 (CH), 124.66 (CH), 125.47 (CH), 128.40 (CH), 130.60 (CH), 131.07 (CH), 133.63 (C), 134.48 (C), 136.55 (C), 137.79 (C), 137.84 (C), 141.79 (C), 142.88 (C), 162.59 (C). $C_{26}H_{23}Cl_2N_3O$: C, 67.25; H, 4.99; N, 9.05. Found: C, 67.21; H, 4.98; N, 9.04.

4.1.7.17. 6-Cyclohexyl-1-(2,4-dichlorophenyl)-N-(pyrrolidin-1-yl)-1,4-dihydroindeno[1,2-c]pyrazole-3-carboxamide (***22***). General procedure V was used to convert **36** and *N*-aminopyrrolidine hydrochloride into the title product. Purification by FC (petroleum ether/EtOAc, 6:4) afforded **22** (0.090g, 35%) as a white solid. Mp 213-214 °C (EtOAc/petroleum ether). IR (nujol) ν: 1689 (CO), 3299 (NH). ^{1}H NMR ($CDCl_3$) δ 1.23-1.28 (m, 1H), 1.33-1.49 (m, 4H), 1.70-1.75 (m, 1H), 1.80-1.94 (m, 8H), 2.49-2.58 (m, 1H), 3.00-3.08 (m, 4H), 3.88 (s, 2H), 6.90 (d, 1H, J_o=8.0 Hz), 7.07 (dd, 1H, J_o=6.8 Hz, J_m=1.2 Hz), 7.41 (s, 1H), 7.50 (dd, 1H, J_o=6.4 Hz, J_m=2.4 Hz), 7.52 (d, 1H, J_o=8.4 Hz), 7.63 (s, 1H, NH exch. with D_2O), 7.66 (d, 1H, J_m=2.4 Hz). ^{13}C NMR (DEPT, $CDCl_3$) 22.31 (CH_2 x 2), 26.07 (CH_2), 26.84 (CH_2 x 2), 29.78 (CH_2), 34.53 (CH_2 x 2), 44.74 (CH), 55.47 (CH_2 x 2), 118.77 (CH), 124.88 (CH), 125.32 (CH), 128.14 (CH), 128.27 (C), 128.89 (C), 129.68 (CH), 130.50 (CH), 132.00 (C), 135.99 (C), 136.01 (C), 141.33 (C), 147.62 (C), 150.06 (C), 151.91 (C), 160.09 (C). $C_{27}H_{28}Cl_2N_4O$: C, 65.45; H, 5.70; N, 11.31. Found: C, 65.41; H, 5.68; N, 11.29.

4.2. Biological evaluation

4.2.1. Radioligand binding assays for CB_1 and CB_2 receptors

Membranes purified from cells transfected with human CB_1 or CB_2 receptors (RBHCB1M400UA and RBXCB2M400UA) were supplied by Perkin-Elmer Life and Analytical Sciences (Boston, MA). The protein concentration was 8 μg/well for the CB_1 receptor membranes and 4 μg/well for the CB_2 receptor. The binding buffer was 50 mM TrisCl, 5 mM $MgCl_2$, 2.5 mM EDTA, 0.5 mg/mL BSA (pH = 7.4) for CB_1, and 50 mM TrisCl, 5 mM $MgCl_2$, 2.5 mM EGTA, 1 mg/mL BSA (pH = 7.5) for CB_2. The radioligand was [^{3}H]-CP55940 (PerkinElmer) used at a concentration of membrane K_D x 0.8 nM, and the final incubation volume was 200 μL for CB_1 and 600 μL for CB_2. 96-Well plates and the tubes necessary for the experiment were previously siliconized with Sigmacote (Sigma). Membranes were resuspended in the corresponding buffer and were incubated (90 min at 30 ºC) with the radioligand and the different compounds at a high concentration (40 μM) with the purpose to determine the % of radioligand displacement. Only in those cases in which radioligand displacement at these conditions was greater than 70%, a complete competition curve with different compound concentrations (10^{-4}-10^{-11} M) was carried out to obtain the K_i values. Non-specific binding was determined with 10 μM WIN55212-2 and total radioligand binding by incubation with the membranes in absence of any compound. Filtration was performed by a Harvester® filtermate (Perkin-Elmer) with Filtermat A GF/C filters pretreated with polyethylenimine 0.05%. After filtering, the filter was washed nine times with binding buffer, dried and a melt-on scintillation sheet (Meltilex™ A, Perkin Elmer) was melted onto it. Then, radioactivity was quantified by a liquid scintillation spectrophotometer (Wallac MicroBeta Trilux, Perkin-Elmer). Competition binding data were analyzed by using GraphPad Prism program and K_i values are expressed as mean ± SEM of at least three experiments performed in triplicate for each point.

4.2.2. [^{35}S]-GTPγS binding analysis

[^{35}S]-GTPγS binding analyses were carried out for compounds **6, 10, 14** and **15** using CB_2 receptor-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 the compound under investigation (from 10^{-12} to 10^{-4} M or in a more restricted range depending on solubility) 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 silanized 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 dried, and a melt-on scintillation sheet (Meltilex™ A, Perkin Elmer) was melted onto it. The bound radioactivity was measured with a Luminiscence counter Wallac MicroBeta TriLux (Perkin-Elmer). [^{35}S]-GTPγS binding data were analyzed to determine the IC_{50} values by using an iterative curve-fitting procedure with the GraphPad Prism version 5.02 (GraphPad Software Inc.). IC_{50} values are expressed as mean ± SEM of at least three experiments performed in triplicate for each point.

4.2.3. Determination of CB_2 receptor-mediated functional activity in a cultured cell-based bioassay

The functional activity of the compounds **10, 14** and **15** for the CB_2 receptor was also evaluated in cultured BV-2 cells, a mouse microglial cell line. Cells were plated at a density of $5x10^5$ cells per well in 12-well culture plates previously coated with 15 μg/mL Poly-L-ornithine

(Sigma), and incubated overnight in Dulbecco's Modified Eagle's Medium (DMEM, Lonza) supplemented with 10% fetal bovine serum (FBS, Lonza), 2 mM Ultraglutamine and antibiotics (Lonza) in a humidified atmosphere of 5% CO_2 at 37 ºC. 1 h before treatment, medium was replaced with DMEM supplemented with 1 % FBS, 2 mM Ultraglutamine and antibiotics. Cells were treated for 16 h with 1 μg/mL LPS (from Escherichia coli 055:B5, Sigma), alone or in combination with the investigated compound, used at a concentration 10-fold the K_i obtained in binding studies. 10 μM WIN55,212-2 (Sigma) and 10 μM SR144528 (Santa Cruz Biotechnology) were used as reference compounds because of their capability to either activate or block the CB_2 receptor, respectively. Media were then removed and used for the determination of PG-E2 release using the ELISA kit DetectX ® Prostaglandin E2 (Arbor Assays). Data were assessed by one-way analysis of variance followed by the Student-Newman-Keuls test using the GraphPad Prism software (version 5.02).

4.3. Molecular modelling studies

All the compounds, WIN-55,212-2 and SR144528 were built, parameterized (Gasteiger-Huckel method) and energy minimized within MOE using MMFF94 forcefield.[37] For docking studies, the previously built homology model of the *h*CB_2 receptor was employed,[33] being already deeply studied by us for the development of CB_2 agonists.

In addition, the *h*CB_2 antagonist binding site was here elucidated taking into account the area around (5Å distance) the key residue S165, as highlighted by mutagenesis experiments.[38]

On the basis, flexible docking studies of all the compounds were performed by the Surflex docking module implemented in Sybyl-X1.0.[39] Then, the best docking geometry (selected on the basis of the SurFlex scoring functions) was refined by ligand/protein complex energy minimization (CHARMM27) by means of the MOE software. Finally, the protein-ligand complex stability was

successfully assessed using a short ~1 ps run of molecular dynamics (MD) at constant temperature, followed by an all-atom energy minimization (LowModeMD implemented in MOE software). This kind of module allowed to perform an exhaustive conformational analysis of the ligand-receptor binding site complex, as we already discussed about other case studies, where it proved to be useful for a preliminary evaluation of docking poses.[40]

Conflict of interest

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

Abbreviations

Δ^9-THC: Δ^9-Tetrahydrocannabinol; 2-AG: 2-Arachidonoylglycerol; CB_1: cannabinoid receptor type 1; CB_2: cannabinoid receptor type 2; CNS: Central nervous system; TRPV1: transient receptor potential vanilloid-1 channel; PPAR: peroxisome proliferator-activated receptor; GTPγS, guanosine 5'-*O*-[gamma-thio]triphosphate; LPS: lipopolysaccharide; DDQ: 2,3-dichloro-5,6-dicyano-1,4-benzoquinone; *h*CB_2: human CB_2 receptor; FC: flash chromatography.

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Appendix A. Supplementary data

^{1}H-NMR and ^{13}C-NMR spectra of representative compounds **10**, **11**, **12**, **15** and **22** related to this article are available.

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Captions to Figures and Schemes

Figure 1. Δ^9-THC and the major endogenous ligands for cannabinoid receptors.

Figure 2. Tricyclic cannabinoid receptor ligands 4 and 5 (see details in references 28-30).

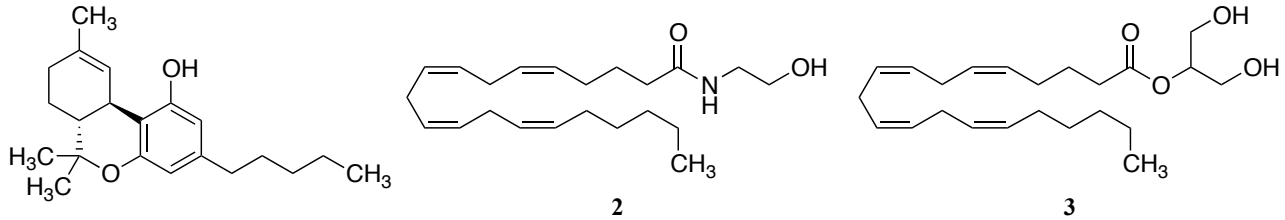
Figure 3. Representative curves for compounds **6**, **10**, **14** and **15** in the GTPγS binding bioassay (obtained from at least three independent experiments carried out in triplicate)

Figure 4. Evaluation of compounds **10**, **14** and **15** for CB_2 receptor activity in an in vitro bioassay based on the analysis of PGE2 release by LPS-stimulated BV2 cells.

Figure 5. WIN-55,212-2 (C atom: tan) and compound **4** (C atom: cyan) docking poses into the hCB_2 agonist binding site. The most important residues are labelled.

Figure 6. SR144528 (C atom: khaki) and compound **15** (C atom: green) docking poses into the hCB_2 antagonist binding site. The most important residues are labelled.

Scheme 1. Reagents and conditions: a) Na, dry EtOH, $(COOEt)_2$; b) 2,4-$Cl_2C_6H_3$-$NHNH_2$·HCl, EtOH; c) DDQ, CH_2Cl_2; d) KOH, EtOH; e) (i) $SOCl_2$, toluene, (ii) CH_2Cl_2, TEA, R-NH_2.

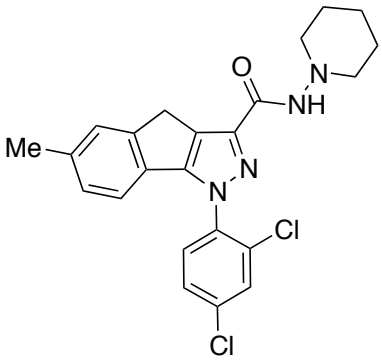


1
(Δ^9-THC)
K_i CB_1= 41 nM
K_i CB_1= 36 nM

2
(Anandamide)
K_i CB_1= 89 nM
K_i CB_1= 371 nM

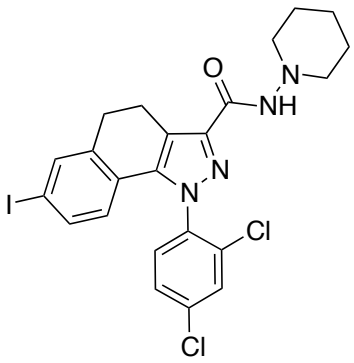
3
(2-AG)
K_i CB_1= 472 nM
K_i CB_1= 1400 nM

Figure 1. Δ^9-THC and the major endogenous ligands for cannabinoid receptors.



4

K_i CB_1= 363 ± 30 nM
K_i CB_2= 0.037 ± 0.003 nM
CB_2 selectivity = 9810

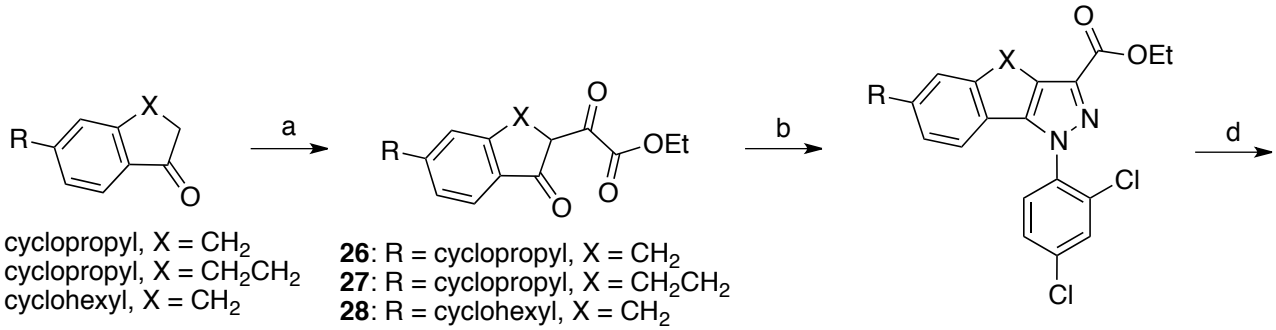


5

K_i CB_1= 4.11 ± 0.22 nM
K_i CB_2 = 1079 ± 44 nM
CB_1 selectivity = 262

Figure 2. Tricyclic cannabinoid receptor ligands **4** and **5** (see details in references 28-30).

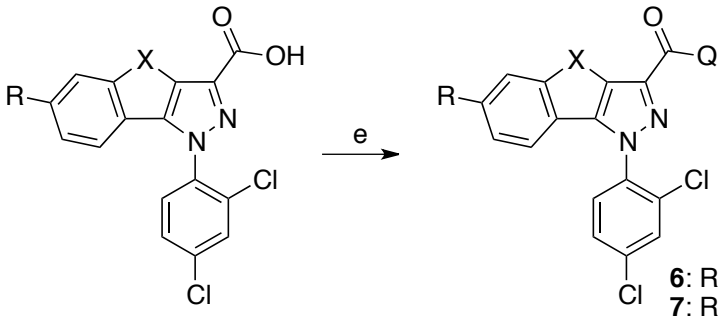
Scheme 1



23: R = cyclopropyl, X = CH_2
24: R = cyclopropyl, X = CH_2CH_2
25: R = cyclohexyl, X = CH_2

26: R = cyclopropyl, X = CH_2
27: R = cyclopropyl, X = CH_2CH_2
28: R = cyclohexyl, X = CH_2

29: R = cyclopropyl, X = CH_2
30: R = cyclopropyl, X = CH_2CH_2
c → **31**: R = cyclopropyl, X = CH=CH
32: R = cyclohexyl, X = CH_2

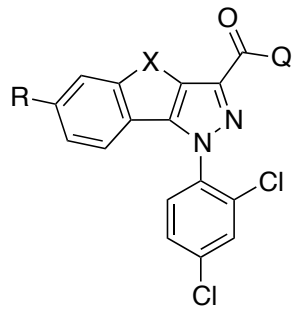


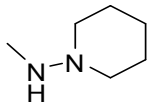
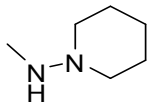
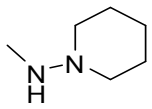
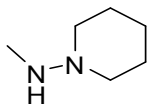
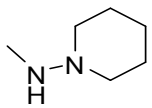
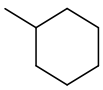
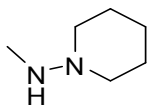
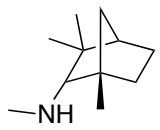
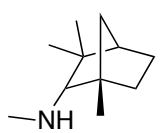
33: R = cyclopropyl, X = CH_2
34: R = cyclopropyl, X = CH_2CH_2
35: R = cyclopropyl, X = CH=CH
36: R = cyclohexyl, X = CH_2

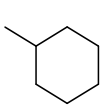
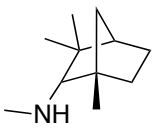
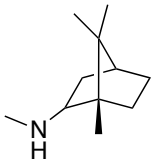
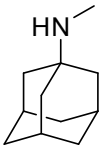
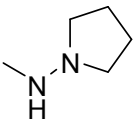
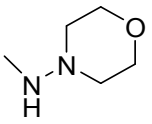
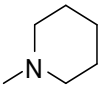
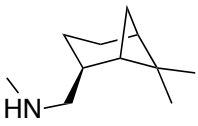
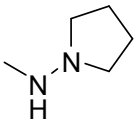
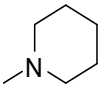
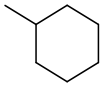
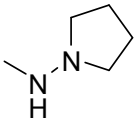
6: R = cyclopropyl, X = CH_2, Q = 1-aminopiperidine
7: R = cyclopropyl, X = CH_2CH_2, Q = 1-aminopiperidine
8: R = cyclopropyl, X = CH=CH, Q = 1-aminopiperidine
9: R = cyclohexyl, X = CH_2, Q = 1-aminopiperidine
10: R = cyclopropyl, X = CH_2, Q = fenchylamine
11: R = cyclopropyl, X = CH_2CH_2, Q = fenchylamine
12: R = cyclopropyl, X = CH=CH, Q = fenchylamine
13: R = cyclohexyl, X = CH_2, Q = fenchylamine
14: R = cyclopropyl, X = CH_2, Q = bornylamine
15: R = cyclopropyl, X = CH_2, Q = 1-adamantylamine
16: R = cyclopropyl, X = CH_2, Q = 1-aminopyrrolidine
17: R = cyclopropyl, X = CH_2, Q = morpholin-4-amine
18: R = cyclopropyl, X = CH_2, Q = piperidine
19: R = cyclopropyl, X = CH_2CH_2, Q = myrtanylamine
20: R = cyclopropyl, X = CH=CH, Q = 1-aminopyrrolidine
21: R = cyclopropyl, X = CH=CH, Q = piperidine
22: R = cyclohexyl, X = CH_2, Q = 1-aminopyrrolidine

Reagents and conditions: a) Na, dry EtOH, $(COOEt)_2$; b) 2,4-$Cl_2C_6H_3$-$NHNH_2$·HCl, EtOH; c) DDQ, CH_2Cl_2; d) KOH, EtOH; e) (i) $SOCl_2$, toluene, (ii) CH_2Cl_2, TEA, R-NH_2.

Table 1. Structures and binding data[a] of compounds **6-22**.



Compd	R	X	Q	K_iCB$_2$ (nM)[a]	K_iCB$_1$ (nM)	K_iCB$_1$/K_iCB$_2$ Selectivity
4[b]	CH_3	-CH_2-		0.037±0.003	363±30	9810
5[b]	I	-CH_2-CH_2-		1079±44	4±0.2	0.0038
6		-CH_2-		69.43±2.76	1852±474	26.7
7·HCl		-CH_2-CH_2-		143±34	> 40000	> 279.6
8		-CH=CH-		825±451	> 40000	> 48.5
9		-CH_2-		509±89	> 10000	> 19.6
10		-CH_2-		6±1	> 40000	> 6944
11		-CH_2-CH_2-		5517±217	> 40000	> 7.25
12·HCl		-CH=CH-		> 40000	> 40000	-

13		-CH_2-		> 10000	> 10000	-
14		-CH_2-		38±10	> 40000	> 1061
15		-CH_2-		4±1	> 10000	> 2232
16		-CH_2-		152±27	4211±645	27.7
17		-CH_2-		198±37	4033±2978	20.4
18		-CH_2-		414±67	> 10000	> 24.2
19		-CH_2-CH_2-		> 10000	> 10000	-
20		-CH=CH-		> 40000	> 40000	-
21		-CH=CH-		> 40000	> 40000	-
22		-CH_2-		515±45	> 10000	> 19.4

[a]Compound affinity for the CB_1 and CB_2 receptors was assayed 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 ± standard error. [b]Binding data for reference compounds **4** and **5** derive from previous published studies using a different binding method[28-30]

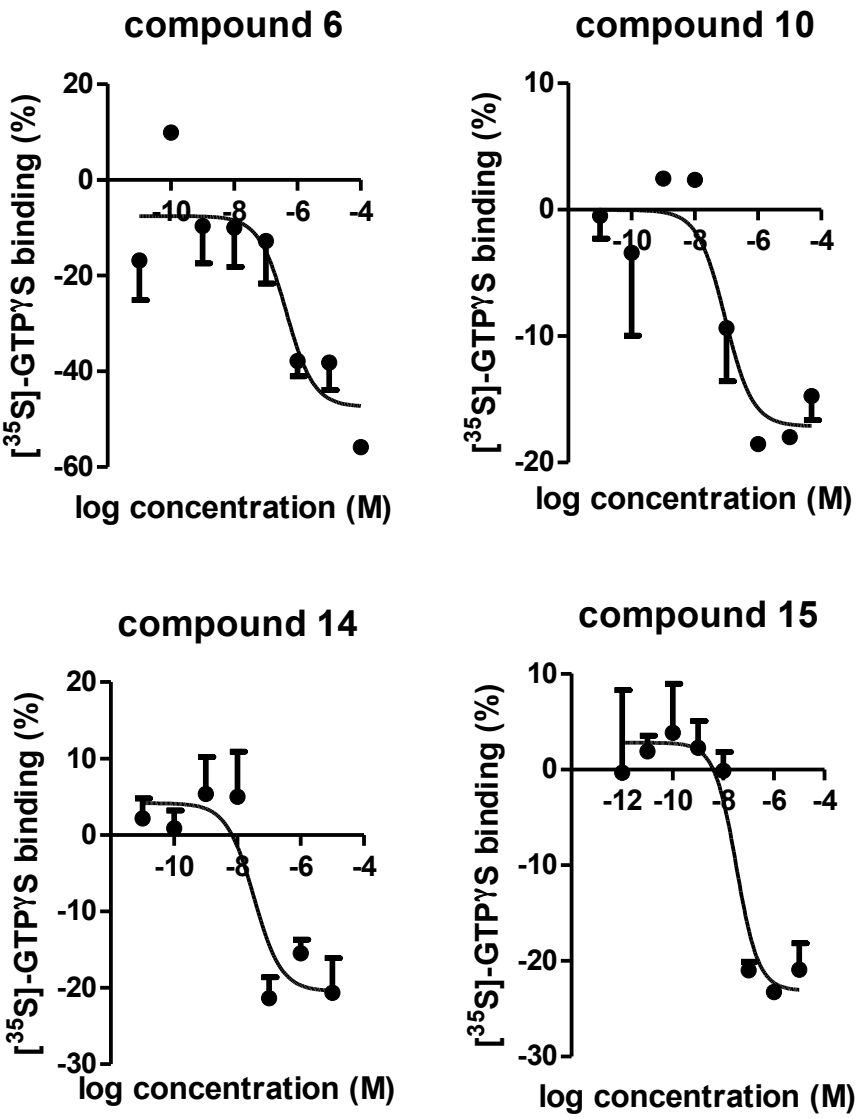


Figure 3. Representative curves for compounds **6**, **10**, **14** and **15** in the GTPγS binding bioassay (obtained from at least three independent experiments carried out in triplicate)

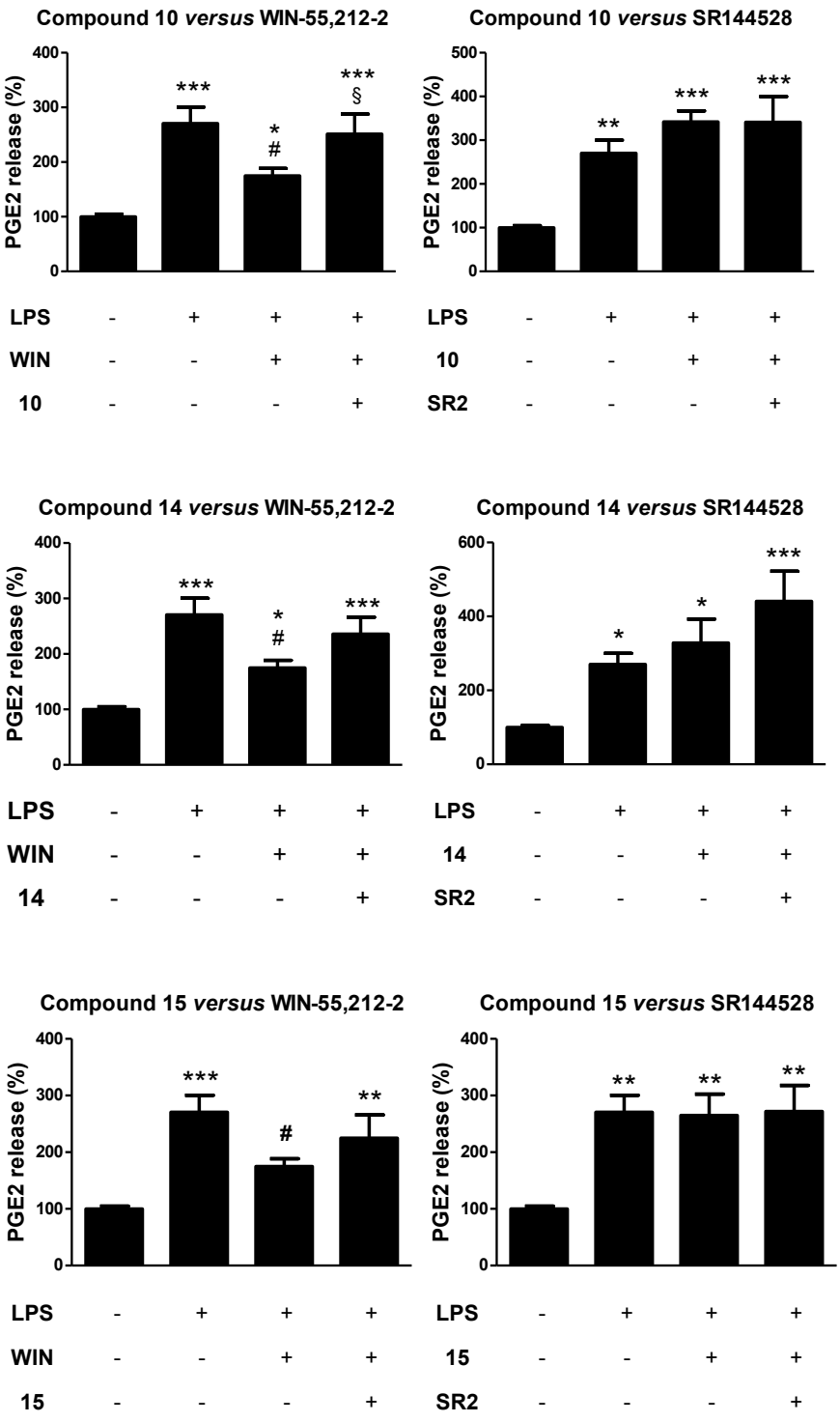


Figure 4. Evaluation of compounds **10**, **14** and **15** for CB_2 receptor activity in an in vitro bioassay based on the analysis of PGE2 release by LPS-stimulated BV2 cells.

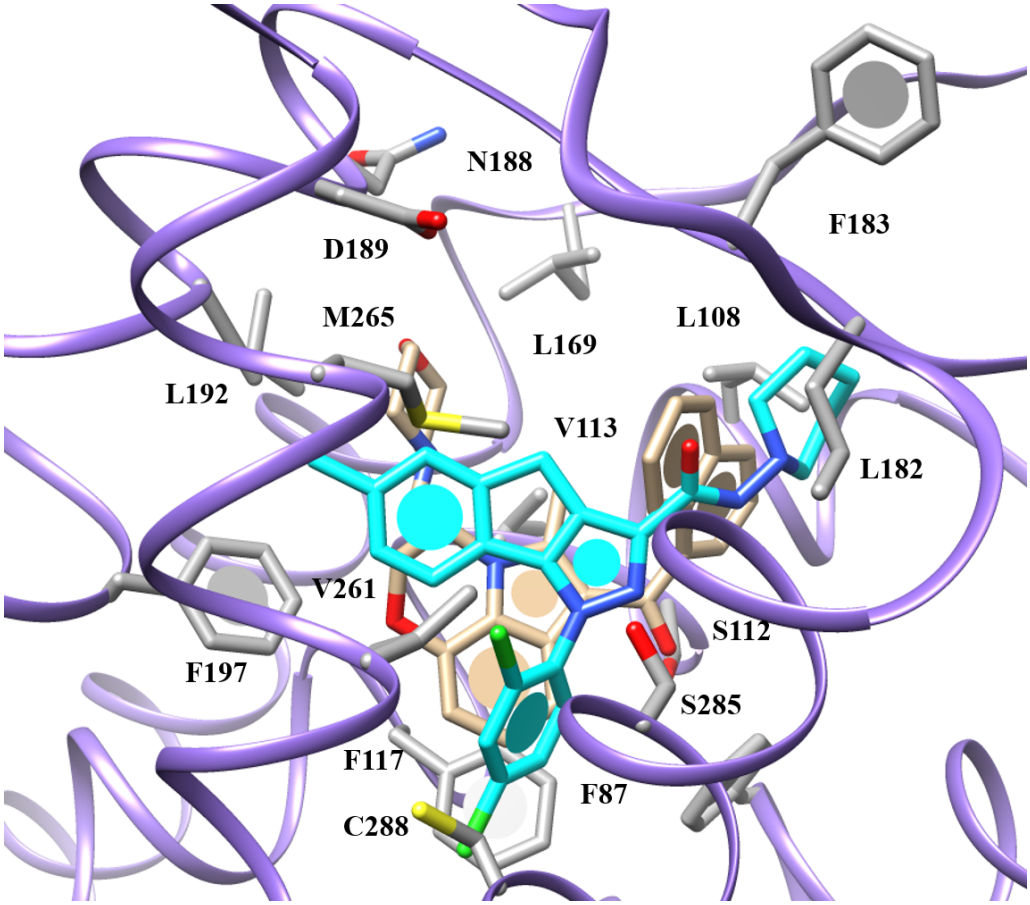


Figure 5. WIN-55,212-2 (C atom: tan) and compound **4** (C atom: cyan) docking poses into the hCB_2 agonist binding site. The most important residues are labelled.

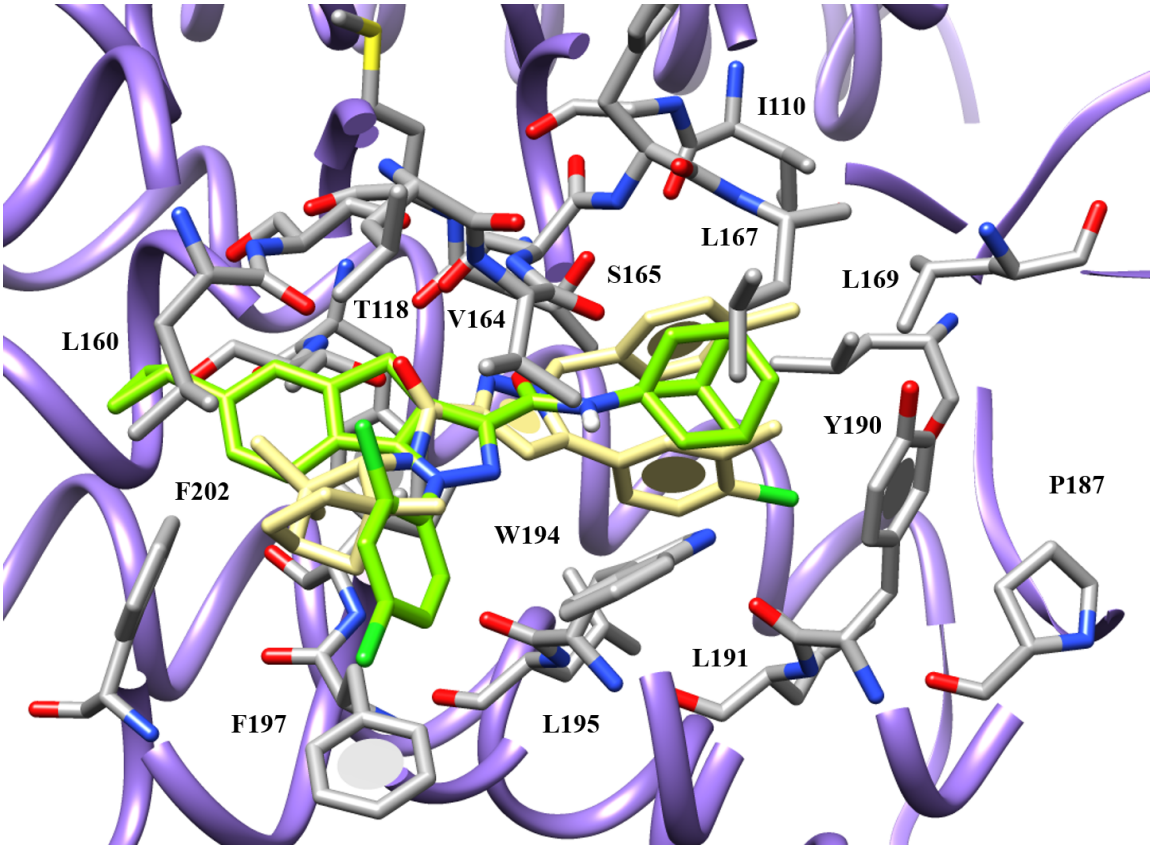


Figure 6. SR144528 (C atom: khaki) and compound **15** (C atom: green) docking poses into the hCB_2 antagonist binding site. The most important residues are labelled.