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Bioorganic & Medicinal Chemistry 13 (2005) 3309–3320
Bioorganic & Medicinal Chemistry

Tricyclic pyrazoles. Part 2: Synthesis and biological evaluation of novel 4,5-dihydro-1*H*-benzo[*g*]indazole-based ligands for cannabinoid receptors

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Received 25 October 2004; accepted 8 February 2005
Available online 19 March 2005

Abstract—A series of 4,5-dihydro-1*H*-benzo[*g*]indazole-3-carboxamides (**2a**–**k**) as analogues of the previously reported CB_2 ligands 6-chloro- and 6-methyl-1-(2′,4′-dichlorophenyl)-*N*-piperidin-1-yl-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxamides (**1a**,**b**) was synthesized and their affinity and selectivity towards CB_1 and CB_2 receptors were evaluated. Several of the new compounds (**2a**,**b**,**c**,**d** and **i**) exhibited CB_1 affinity in the nanomolar range with moderate or negligible affinity towards CB_2 receptors. Compounds **2a** and **c** increased intestinal propulsion in mouse. Their pro-kinetic effects were reversed by the reference CB agonist CP-55,940. Consequently, in vivo CB_1 antagonistic activity was highlighted for these compounds.

1. Introduction

Cannabinoid (CB) receptors are classified as a receptor family consisting of CB_1 and CB_2 subtypes, both of which have distinct anatomical distribution and ligand binding profiles. CB_1 cannabinoid receptors are present in the central nervous system (CNS), with highest densities in the hippocampus, cerebellum and striatum[1,2] and, to a lesser extent, in several peripheral tissues including enteric nervous system,[3] testis, urinary bladder, vas deferens.[4] Moreover, CB_1 cannabinoid receptor mRNA and protein are expressed in rat and human eye,[5] and in rat thyroid.[6] CB_2 cannabinoid receptors appear to be predominantly located in peripheral tissues as spleen, tonsils, immune cells.[4,7,8] Both receptors belong to the GPCR superfamily of 7-transmembrane receptors that negatively regulate adenylate cyclase[9a] and they were first cloned in 1990 and 1993, respectively.[9b,c]

The physiological role of CB receptors is not yet completely understood, although they seem to be involved in certain pathophysiological processes[10,11] such as asthma, pain, appetite modulation, multiple sclerosis, vomiting, immune and inflammatory diseases. In particular, selective CB_1 receptor ligands might produce potentially beneficial therapeutic effects including prevention of weight gain (treatment of obesity), mediated by interaction between CB_1 receptors, involved in the control of appetite. A pharmacological modulation of the cannabinoid system might provide new therapeutics for the treatment of various diseases and disorders such as anorexia, emesis, pain, neurodegenerative diseases, glaucoma and immune system disorders.[4,12–15]

At present, different cannabinoid binders have been identified; they can be classified into different chemical families (exocannabinoids): tricyclic cannabinols[9d] (classical cannabinoids whose structure is based on the dibenzopyran template of Δ^9-THC), bicyclic cannabinols[9e,f] (nonclassical cannabinoids typified by CP-55,940), indoles, pyrroles and indenes[9g] (typified by WIN-55,212-2), eicosanoid compounds (typified by arachodonilethanolamide, anandamide)[9h] and

Keywords: Cannabinoids; 4,5-Dihydro-1*H*-benzo[*g*]indazoles; 1-(2′,4′-Dichlorophenyl)-7-iodo-*N*-piperidin-1-yl-4,5-dihydro-1*H*-benzo[*g*]indazole-3-carboxamide; Selective CB_1 receptor ligands.
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doi:10.1016/j.bmc.2005.02.032

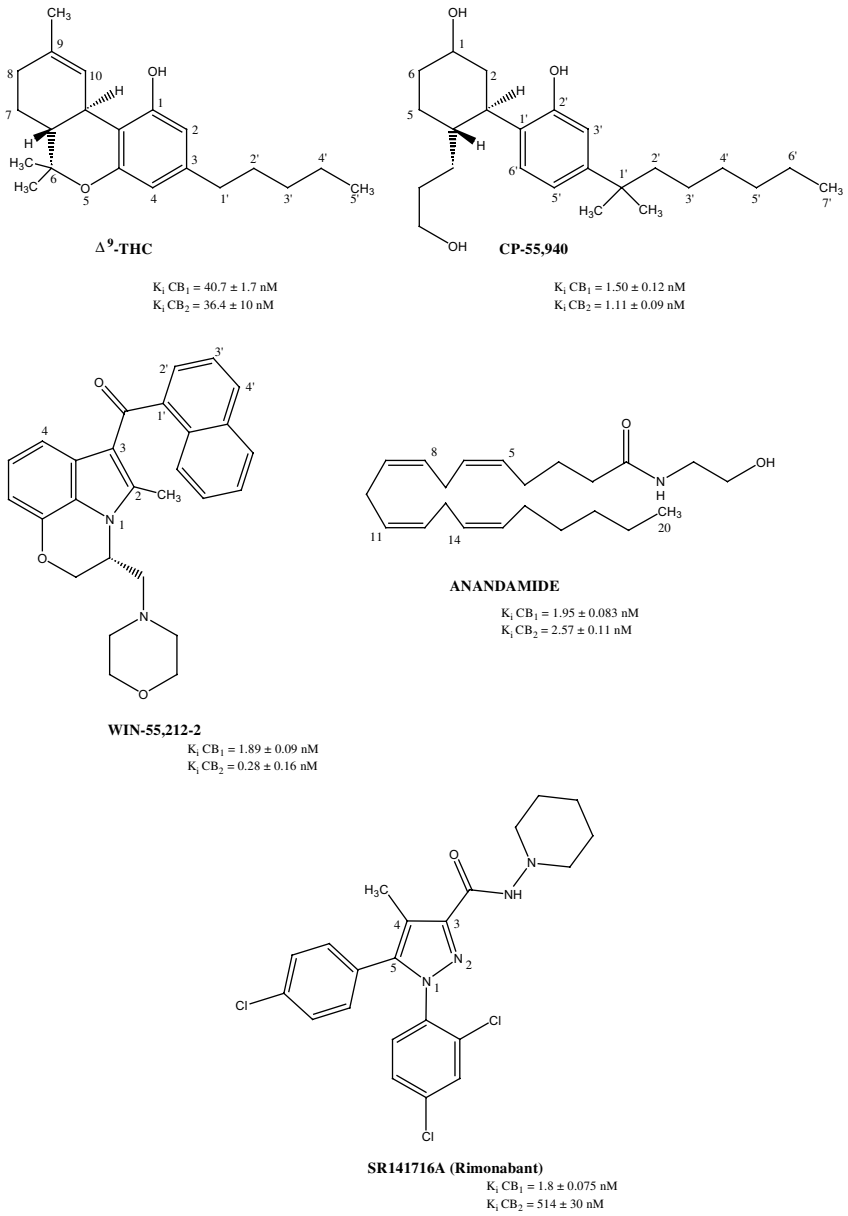


Figure 1. Cannabinoid receptor binder structures and their receptor affinities expressed as K_i.

diarylpyrazoles.[9i] SR 141716A (Rimonabant), the prototypical example of this last class of compounds, is characterized by high affinity and significant selectivity for CB_1 receptors (Fig. 1).

In part 1 of this series we have already reported the design and synthesis of novel 1,4-dihydroindeno[1,2-*c*]pyrazoles **1**, derived from the reference diarylpyrazolic CB_1 ligand SR141716A.[16] Among these compounds, we have disclosed high affinity and selective CB_2 receptor ligands. In particular, compounds **1a** and **b** showed CB_2 affinities (K_i) of 0.34–0.037 nM with K_iCB_1/K_iCB_2 of 6029 and 9810, respectively (Fig. 2).

To further extend the structure affinity studies, our intention was to synthesize homologous 4,5-dihydro-1*H*-benzo[*g*]indazoles **2**. Thereby, we focused on those compounds bearing residues and groups that were expected to show high CB receptor affinity and selectivity.

In this paper, we have reported the preparation and in vitro evaluation of a series of novel substituted 4,5-dihydro-1*H*-benzo[*g*]indazole-3-carboxamides **2** (Fig. 2 and Table 1).

In addition, the in vivo evaluation of cannabinoid activity profile of the new compounds on gastrointestinal transit in mouse was reported (*upper gut transit test*).

1.1. Chemistry

Compounds found in Table 1 were synthesized via the general route depicted in Scheme 1.

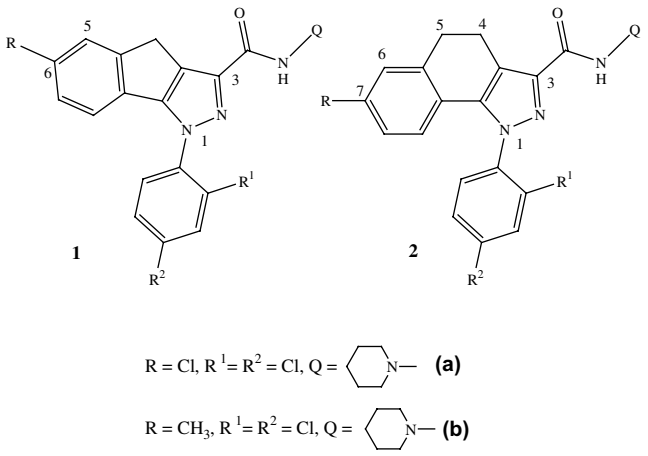


Figure 2. Homologous tricyclic pyrazolic CB-receptor ligands.

Addition of 1 equiv of the requisite benzocyclanone **3** to diethyloxalate in ethanol at room temperature in the presence of 2 equiv of base, afforded Claisen condensation product **4** in which a single carbonyl group partially tautomerized.

Subsequent reaction of 1 equiv of this mixture with 1.15 equiv of the appropriate hydrazine hydrochloride at reflux in ethanol afforded tricyclic benzo[*g*]indazol ester **5**. The final substituent Q was introduced by basic hydrolysis of the ester to corresponding acid **6** followed by coupling reaction of the chloride with an excess of the appropriate amine.

1.2. Biology

Affinities at CB_1 and CB_2 receptors for compounds **2** were assessed by competition of [^{3}H]-CP-55,940 in mouse brain (minus cerebellum) and spleen homogenates, respectively. Radioligand binding experimental procedures previously reported by Ruiu et al. were adopted.[17] The results from in vitro assay were compared with the K_i values of **1a** and **b**, and to the literature K_i values of the reference compounds SR141716A (Rimonabant) and SR144528, a selective ligand of CB_2 receptors (Table 1).[16]

According to the previous studies on SR141716A, the cannabinoid functional profiles of synthesized compounds **2** were evaluated through the estimation of their capabilities to interact with cannabinoid receptors occurring in the mientheric plexus (Auerbach's plexus).[3] In fact, CB_1 cannabinoid receptors are significantly present in the enteral system of various mammalian species (i.e., mankind and mouse), and their function appears to be related to the modulation of the gastrointestinal motility.[3] Several are the effects of this action, including the slowing down of the gastrointestinal transit, and the inhibition of the acetylcholine release in the junctions of smooth musculature. The association of CB_1 receptors with cholinergic neurones, accordingly with functional studies, suggests that cannabinoid agonists act on intestinal motility by suppression of acetylcholine release from myenteric nerves endings. Even intestinal secretion and acid gastric secretion might be influenced via an identical mechanism. The antagonist/reverse agonist of CB_1 receptors SR141716A, has shown to be able not only to forestall such actions induced by cannabinoid agonists, but also to induce an increase of the gastrointestinal motility by itself. This effect could be easily shown through the *upper gastrointestinal transit test*,[18] from the determination of the intestinal length (gastrointestinal transit: GIT) travelled by a non-absorbable marker in consequence of the active compound administration.

Thus the synthesized novel substituted 4,5-dihydro-1*H*-benzo[*g*]indazole-3-carboxamides **2** were in vivo evaluated by the *upper gastrointestinal transit test*, determining both the ability of the compounds to produce a dose-dependent effect (dose–response curve) and their specificity at cannabinoid receptors. The latter was experimentally determined by means of a high affinity CB agonist administered in combination with the compound to be tested. The studies were carried out according to the previously reported procedures,[3b] using CP-55,940, already employed in the binding studies, as reference CB agonist agent.

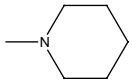
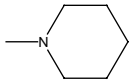
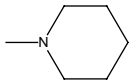
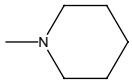
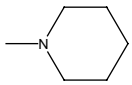
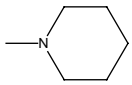
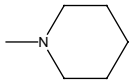
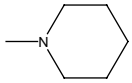
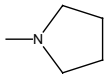
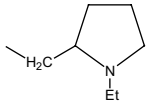
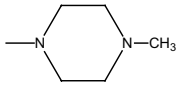
2. Results and discussion

Cannabinoid receptor binding affinity and selectivity are greatly influenced by the size of the central placed carbocyclic ring of the studied series (Table 1). Compounds **2a** and **b** (six membered central ring) had the same substitutions on the phenyl ring of the lead CB_2 selective structures **1a** and **b** bearing a 4,5-dihydro-1*H*-benzo[*g*]indazole core. Both **2a** and **b** compounds showed high CB_1 and relatively low CB_2 receptor affinities. Thus, increasing the carbocyclic central ring size of the tricyclic 1,4-dihydroindeno[1,2-*c*]pyrazoles by one methylene unit leading structures with 4,5-dihydro-1*H*-benzo[*g*]indazole core, primarily involved a marked loss of affinity for CB_2 receptor, a concurrent significant increase in CB_1 affinity, and a consequent loss of CB_2 selectivity.

Considering the 1-(2′,4′-dichlorophenyl)-*N*-piperidin-1-yl-4,5-dihydro-1*H*-benzo[g]indazole-3-carboxamides **2a**–**e**, all the C_7 substituted compounds (**2a**–**d**) had the highest CB_1 affinity (K_i CB_1 from 4.11 nM for the 7-iodo-substituted compound to 14.8 nM for the chloro-analogue). Among this subclass, the selectivity for CB_1 receptors increased in the order: Cl < CH_3 < Br < I. In particular, the iodo-substituted compound **2d** showed a CB_1 selectivity K_i CB_2/K_i CB_1 of 262.

The C_7 unsubstituted 1-(2′,4′-dichlorophenyl)-*N*-piperidin-1-yl-4,5-dihydro-1*H*-benzo[g]indazole-3-carboxamide **2e** had much lower affinity for CB_1 receptors as compared to **2a**–**d**, with CB_2 receptor affinity (K_i CB_2 = 295 nM) similar to the substituted analogues **2a**–**c**.

Table 1. Structures and binding data of compounds **2**

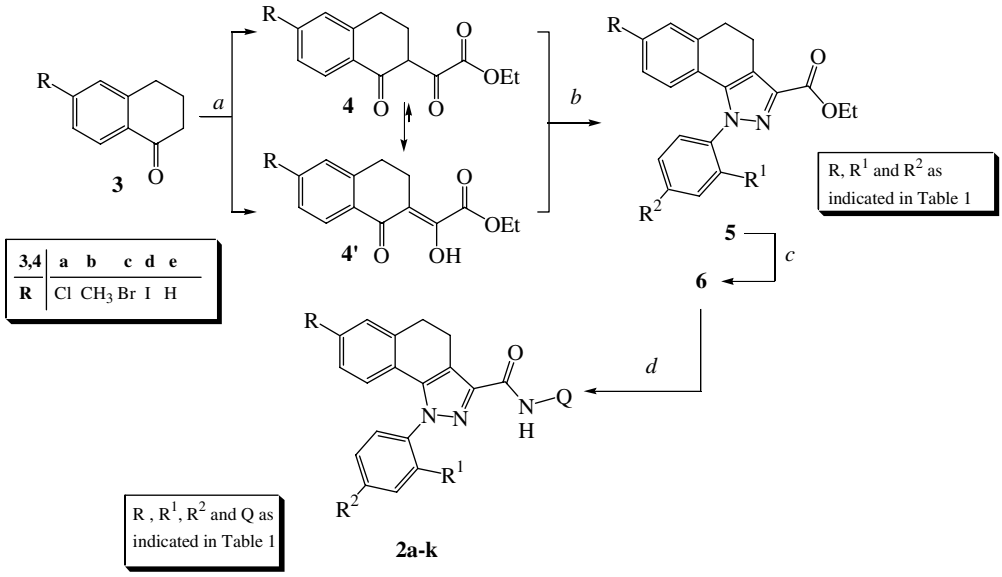
Compound	R	R^1	R^2	Q	Receptor affinity		
					K_iCB_1 (nM)[a]	K_iCB_2 (nM)[b]	K_iCB_2/K_iCB_1
2a	Cl	Cl	Cl		14.8 ± 0.43	227 ± 5	15.3:1
2b	CH_3	Cl	Cl		5.88 ± 0.30	287 ± 10	48.8:1
2c	Br	Cl	Cl		4.87 ± 0.18	367 ± 15	75.3:1
2d	I	Cl	Cl		4.11 ± 0.22	1079 ± 44	262.5:1
2e	H	Cl	Cl		602 ± 89	295 ± 31	0.49:1
2f	Cl	H	Cl		142 ± 15	550 ± 25	3.87:1
2g	Cl	H	H		418 ± 8	1375 ± 120	3.2:1
2h	Cl	H	OCH_3		157 ± 20	1875 ± 125	11.9:1
2i	Cl	Cl	Cl		49 ± 3	387 ± 12	7.9:1
2j	Cl	Cl	Cl		>5000	3875 ± 125	1:1
2k	Cl	Cl	Cl		>5000	>5000	1:1
1a[16]					2050 ± 90	0.34 ± 0.06	0.00016:1
1b[16]					363 ± 30	0.037 ± 0.003	0.0001:1
SR141716A (Rimonabant)					1.8 ± 0.075	514 ± 30	285.6:1
SR144528					70 ± 10	0.28 ± 0.04	0.004:1

[a] Affinity of compounds for the CB_1 receptor was evaluated using mouse brain (minus cerebellum) homogenate and [^{3}H]-CP 55,940.
[b] Affinity of compounds for the CB_2 receptor was assayed using mouse spleen homogenate and [^{3}H]-CP-55,940. K_i values were obtained from five independent experiments carry out in triplicate and are expressed as mean ± standard error.

When the 2′,4′-dichloro substitution pattern on the N_1-phenyl ring of **2a** was modified, some impact on CB_1 and CB_2 receptor affinity was observed. The removal of the C_2′–Cl atom from the C_2′,C_4′–Cl_2 phenyl moiety of **2a**, leading to **2f**, involved a 10-fold loss of CB_1 affinity, and a lower reduction of CB_2 affinity. A consequent worse CB_1 selectivity was determined. The C_2′, C_4′ removal of both chlorine atoms, as illustrated by compound **2g**, led to a considerable decrease in affinity at CB_1 receptors, and to a further reduction of CB_2 affinity (CB_1 selectivity analogous to **2f**). Next, a methoxy in position C_4′, as for compound **2h**, conferred only modest CB_1 affinity (K_i CB_1 = 157 nM). The effect on CB_1 affinity of a methoxylic group in position C_4′ is similar to that of a chlorine atom in the same position (K_i CB_1 **2h** vs K_i CB_1 **2f**). However, highest CB_1 selectivity was determined in the presence of a –OCH_3 group.

The importance of the piperidine ring of the lead compound **2a** for binding and selectivity was confirmed testing compounds **2i**–**k**. Replacing the piperidine ring, only with the pyrrolidinyl moiety **2i** an acceptable CB_1 receptor affinity was maintained. However, the compound **2i** resulted 3-fold lower potent than **2a**. The effect of the substitution of the pyrazole-3-carboxamidic group was also highlighted on CB_2 affinity.

The functional profiles of some of the compounds **2** (**2a**, **c**, **g**, **h** and **i**) were evaluated by the 'upper gastrointestinal transit' assay (Table 2).



Scheme 1. Reagents and conditions: (a) Na, dry EtOH, $(COOEt)_2$; (b) $ArNHNH_2$·HCl, EtOH; (c) KOH, MeOH; (d) $SOCl_2$, C_6H_5–CH_3, CH_2Cl_2, TEA,Q–NH_2.

Table 2. In vivo functional activity of selected CB_1 ligands

Compound	Doses (mg/kg)	% GI motility increase
2a	⩽1	⩾70
2c	⩽1	⩾70
2g	2–9	60–70
2h	2–9	60–70
2i	2–9	60–70

Compounds that gave motility increase higher than 70% for concentration of 1 mg/kg were subsequently screened at lower different doses.

From this test, compound **2a** was able to induce a dose-dependent GI motility increase, as well as compound **2c** (Fig. 3).

The in vivo effect of the two tested compounds was markedly reversed by the in vivo administration of CP-55,940 (Fig. 4), suggesting for the studied series of benzo[*g*]indazole-3-carboxamides, antagonistic profile for the CB receptors.

Finally, the extent of the gastrointestinal transit of the most significative compounds **2a** and **c** is in reasonable accordance with their binding affinities for CB_1 receptor subtypes; thus, the degree of CB_1 selectivity seen in the binding assay is maintained in the GI-based environment. The efficacy of the compounds **2a** and **c** on gastrointestinal transit modulation is comparable to that previously determined with SR141716A.[3b]

In conclusion, in this report we have identified a novel series of 4,5-dihydro-1*H*-benzo[*g*]indazole-based cannabinoid receptor (CB) binders that are selective for CB_1 receptor subtype. The potent and selective analogues in this series have binding affinities of 4.11–

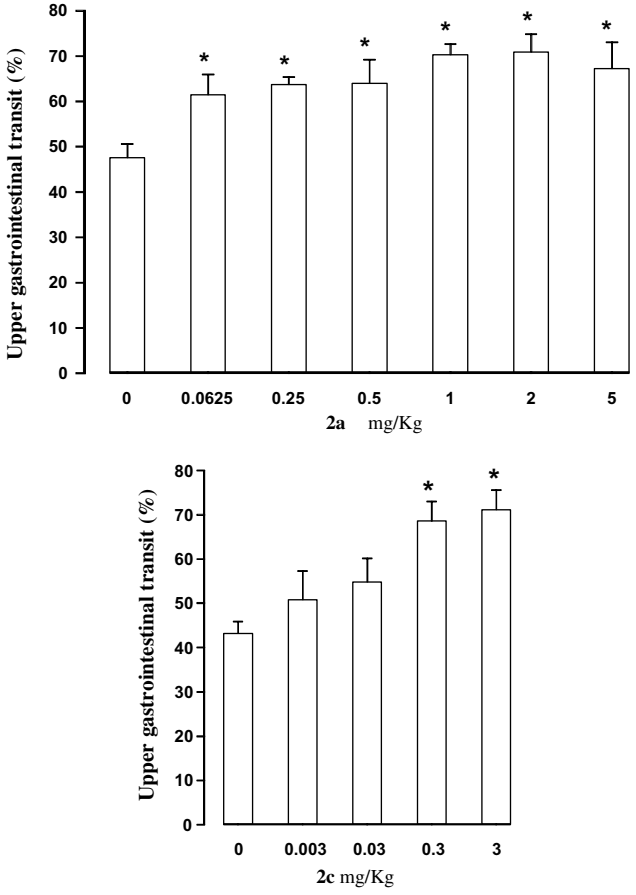


Figure 3. Bar graphs showing concentration–response curves elicited by compounds **2a** and **c** on the gastrointestinal motility at indicated doses respect to the control. Assays were performed as described in the experimental part. Each bar is the mean ± SEM of 9–16 mice. Compound **2a**: one-way ANOVA $F(6,71) = 3.53$, $^{*}P < 0.01$ with respect to vehicle-treated mice (Newman–Keuls test). Compound **2c**: one-way ANOVA $F(4,43) = 6.04$, $^{*}P < 0.001$ with respect to vehicle-treated mice (Newman–Keuls test).

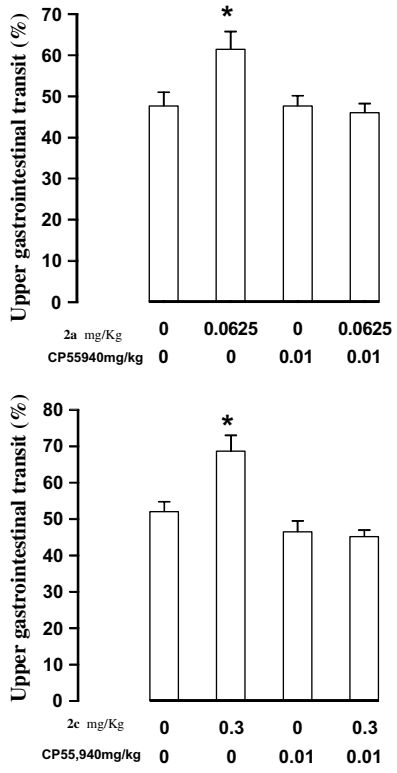


Figure 4. Bar graphs showing the gastrointestinal transit of the marker (carmine): column 1: control; column 2: in the presence of compound **2a** or **c**; column 3: in the presence of CP-55,940; column 4: in the presence of compound **2a** or **c**, in combination with CP-55,940. Assays were performed as described in the experimental part. Each bar is the mean ± SEM of 10–16 mice. Compound **2a**: one-way ANOVA $F(3,46) = 5.10$, $^{*}P < 0.01$ with respect to 0.0625 mg/kg **2a**+0.01 mg/kg CP-55,940 treated mice (Newman–Keuls test). Compound **2c**: one-way ANOVA $F(3,36) = 12.6$, $^{*}P < 0.001$ with respect to 0.3 mg/kg **2b**+0.01 mg/kg CP-55,940 treated mice (Newman–Keuls test).

49 nM at CB_1 receptors and selectivity over CB_2 receptors up to 262-fold. These compounds act as antagonists at CB_1 receptors in a gastrointestinal transit marker assay.

It is evident that structural requirements for CB_1 and or CB_2 receptor binding differ between dihydroindenopyrazole and dihydrobenzo[*g*]indazole-based compounds, thus providing new architectures for a better pharmacophore characterization. From the SAR studies, perhaps, it is not unreasonable to suppose that to achieve high binding affinity to the CB_1 receptors and CB_1 over CB_2 selectivity it is important for the tricyclic system (i.e., dihydrobenzo[*g*]indazole) to be nonplanar and to bear a halogen atom (Cl or Br or I) or a methyl at C_7. Further, the presence of a carboxamide group at tricyclic core C_3 preferably containing a cyclic amine (piperidine or pyrrolidine), would be an additional favourable factor for a better (higher) CB_1 binding affinity (as in compounds **2a**–**d**,**i**).

Consideration of these factors may prove helpful in the designing of new ligands which bind potently and selectively to the CB_1 receptors.

3. Experimental

3.1. Chemistry

3.1.1. General information. Melting points were obtained on a Köfler melting point apparatus and are uncorrected. IR spectra were recorded as thin films (for oils) or nujol mulls (for solids) on NaCl plates with a Perkin Elmer 781 IR spectrophotometer and are expressed in ν (cm^{-1}). All NMR spectra were taken on a Varian XL-200 NMR spectrometer with ^{1}H and ^{13}C being observed at 200 and 50 MHz, respectively. Chemical shifts for ^{1}H and ^{13}C NMR spectra were reported in δ or ppm downfield from TMS [$(CH_3)_4Si$]. Multiplicities are recorded as s (singlet), br s (broad singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), m (multiplet). Atmospheric pressure ionization electrospray (API-ES) mass spectra, when reported, were obtained on a Agilent 1100 series LC/MSD spectrometer. Elemental analyses were performed by Laboratorio di Microanalisi, Dipartimento di Chimica, Università di Sassari, Italy and are within ±0.4% of the calculated values. All reactions involving air or moisture-sensitive compounds were performed under argon atmosphere. Unless otherwise specified, all materials, solvents, reagents and precursor **3e** were obtained from commercial suppliers. Flash chromatography (FC) was performed using Merck silica gel 60 (230–400 mesh ASTM). Thin layer chromatography (TLC) was performed with Polygram® SIL N-HR/HV_{254} precoated plastic sheets (0.2 mm). The starting tetralones **3a**,[19] **b**,[20] **c**,[19] and diketoester **4e**[21] were prepared according to the procedures previously described in the literature.

3.1.2. General procedure I: synthesis of carboxamides. A mixture of the appropriate 4,5-dihydro-1*H*-benzo-[*g*]indazole-3-carboxylic acid **6** (1 equiv, 4.0 mmol) and thionyl chloride (3.0 equiv) in toluene (30 mL) was refluxed for 3–4 h. The solvent and the excess of $SOCl_2$ were removed under reduced pressure and the resulting dark solid in CH_2Cl_2 (15 mL) was dropwise added to a solution of requisite amine or hydrazine (1.5 equiv) and Et_3N (1.5 equiv) in CH_2Cl_2 (15 mL) at 0 °C. The mixture was refluxed for 3–4 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 layers were washed with water, dried over Na_2SO_4 and concentrated under reduced pressure. The analytically pure product was isolated by an appropriate method of purification as indicated below.

3.1.2.1. 7-Chloro-1-(2′,4′-dichlorophenyl)-*N*-piperidin-1-yl-4,5-dihydro-1*H*-benzo[g]indazole-3-carboxamide 2a. General procedure I was used to convert **6a** and *N*-aminopiperidine into the title product. The mixture was refluxed for 3 h and purification by flash chromatography [petroleum ether/EtOAc, 1:1] afforded **2a** (1.58 g, 83.3%) as a white solid. $R_f = 0.68$ (petroleum ether/EtOAc 1:1); mp 223/224 °C (triturated with hexane); IR 1645, 3160; ^{1}H NMR ($CDCl_3$) δ 1.35–1.50 (m, 2H), 1.59–1.85 (m, 8H), 2.85–3.26 (m, 4H), 6.44 (d, 1H), 6.96 (d, 1H), 7.29 (s, 1H), 7.48 (s, 1H), 7.55–

7.65 (m, 3H); Anal. Calcd for $C_{23}H_{21}Cl_3N_4O$: C, 58.06; H, 4.45; Cl, 22.35; N, 11.78. Found: C, 58.18; H, 4.22; Cl, 22.21; N, 12.03.

3.1.2.2. 1-(2′,4′-Dichlorophenyl)-7-methyl-*N*-piperidin-1-yl-4,5-dihydro-1*H*-benzo[*g*]indazole-3-carboxamide 2b. General procedure I was used to convert **6b** and *N*-aminopiperidine into the title product. The mixture was refluxed for 3 h and purification by trituration with petroleum ether afforded **2b** (1.64 g, 89.9%) as a white solid. $R_f = 0.15$ (petroleum ether/EtOAc 8/2); mp 232–234 °C (triturated with petroleum ether); IR 1690, 3290; ^{1}H NMR ($CDCl_3$) δ 1.35–1.50 (m, 2H), 1.69–1.80 (m, 4H), 2.28 (s, 3H), 2,82–2.88 (t, 4H), 2.92–3.25 (m, 4H), 6.41 (d, 1H), 6.81 (d, 1H), 7.11 (s, 1H), 7.46–7.47 (m, 2H), 7.59–7.61 (m, 2H); ^{13}C NMR (DEPT, $CDCl_3$) 19.48 (CH_3), 21.23 (CH_2), 23.76 (CH_2), 25.40 ($CH_2 \times 2$), 30.01 (CH_2), 57.06 ($CH_2 \times 2$), 120.97 (CH), 127.12 (CH), 128.25 (CH), 129.65 (CH), 130.25 (CH), 130.51 (CH), 120.29 (C), 123.17 (C), 133.27 (C), 136.24 (C), 136.97 (C), 137.44 (C), 138.29 (C), 141.16 (C), 142.38 (C), 159.73 (C); API-ES calcd for $C_{24}H_{24}Cl_2N_4O$ 455.4, found 455.1 and Anal. Calcd for $C_{24}H_{24}Cl_2N_4O$: C, 63.30; H, 5.31; Cl, 15.57; N, 12.30. Found: C, 63.18; H, 5.21; Cl, 15.21; N, 12.39.

3.1.2.3. 7-Bromo-1-(2′,4′-dichlorophenyl)-*N*-piperidin-1-yl-4,5-dihydro-1*H*-benzo[*g*]indazole-3-carboxamide 2c. General procedure I was used to convert **6c** and *N*-aminopiperidine into the title product. The mixture was refluxed for 3 h and purification by flash chromatography (petroleum ether/EtOAc, 1:1) afforded **2c** (1.43 g, 68.9%) as a white solid. $R_f = 0.67$ (petroleum ether/EtOAc, 1:1); mp 181–183 °C (triturated with hexane); IR 1645, 3160; ^{1}H NMR ($CDCl_3$) δ 1.33–1.43 (m, 2H), 1.58–1.70 (m, 8H), 2.76–3.08 (m, 4H), 6.30 (d, 1H), 7.08 (d, 1H), 7.39 (m, 2H), 7.53 (m, 3H); Anal. Calcd for $C_{23}H_{21}BrCl_2N_4O$: C, 53.10; H, 4.07; Br, 15.36; Cl, 13.63; N, 10.77. Found: C, 53.18; H, 4.12; Br, 15.22; Cl, 13.52; N, 10.89.

3.1.2.4. 1-(2′,4′-Dichlorophenyl)-7-iodo-*N*-piperidin-1-yl-4,5-dihydro-1*H*-benzo[*g*]indazole-3-carboxamide 2d. General procedure I was used to convert **6d** and *N*-aminopiperidine into the title product. The mixture was refluxed for 3 h and purification by flash chromatography (petroleum ether/EtOAc, 7:3) afforded **2d** (2.05 g, 90.3%) as a yellow solid. $R_f = 0.72$ (petroleum ether/EtOAc, 7:3); mp 240–242 °C (triturated with hexane); IR 1645, 3160; ^{1}H NMR ($CDCl_3$) δ 1.40–1.50 (m, 2H), 1.68–1.85 (m, 4H), 2.80–3.30 (m, 8H), 6.24 (d, 1H), 7.34 (d, 1H), 7.47–7.70 (m, 5H); Anal. Calcd for $C_{23}H_{21}Cl_2IN_4O$: C, 48.70; H, 3.73; Cl, 12.50; I, 22.37; N, 9.88. Found: C, 48.55; H, 3.65; Cl, 12.45; I, 22.09; N, 9.68.

3.1.2.5. 1-(2′,4′-Dichlorophenyl)-*N*-piperidin-1-yl-4,5-dihydro-1*H*-benzo[*g*]indazole-3-carboxamide 2e. General procedure I was used to convert **6e** and *N*-aminopiperidine into the title product. The mixture was refluxed for 3 h and purification by trituration with petroleum ether afforded **2e** (1.34 g, 76.2%) as a white solid. $R_f = 0.49$ (petroleum ether/EtOAc 1/1); mp 237–238 °C (triturated with petroleum ether); IR 1690, 3290; ^{1}H NMR ($CDCl_3$) δ 1.38–1.50 (m, 4H), 1.69–1.85 (m, 6H), 2.83–3.29 (m, 4H), 6.52 (d, 1H), 6.99 (t, 1H), 7.17 (t, 1H), 7.31 (d, 2H), 7.46 (d, 1H), 7.47 (s, 1H), 7.60 (br s, 1H, exch. with D_2O); Anal. Calcd for $C_{23}H_{22}Cl_2N_4O$: C, 62.59; H, 5.02; Cl, 16.07; N, 12.69. Found: C, 62.36; H, 4.88; Cl, 15.87; N, 12.88.

3.1.2.6. 7-Chloro-1-(4′-chlorophenyl)-*N*-piperidin-1-yl-4,5-dihydro-1*H*-benzo[*g*]indazole-3-carboxamide 2f. General procedure I was used to convert **6f** and *N*-aminopiperidine into the title product. The mixture was refluxed for 4 h and purification by crystallization with petroleum ether/EtOAc afforded **2f** (0.97 g, 55%) as a yellow product. $R_f = 0.63$ (petroleum ether/EtOAc 1/1); mp 233–234 °C (crystallized with petroleum ether/EtOAc); IR 1675, 3430; ^{1}H NMR ($CDCl_3$) δ 1.40–1.50 (m, 2H), 1.70–1.85 (m, 4H), 2.83–3.00 (m, 6H), 3.01–3.20 (m, 2H), 6.57 (d, 1H), 6.99 (d, 1H), 7.29 (d, 1H), 7.44 (d, 2H), 7.50 (d, 2H), 7.63 (br s, 1H, exch. with D_2O); Anal. Calcd for $C_{23}H_{22}Cl_2N_4O$: C, 62.59; H, 5.02; Cl, 16.07; N, 12.69. Found: C, 62.48; H, 4.65; Cl, 15.88; N, 12.54.

3.1.2.7. 7-Chloro-1-phenyl-*N*-piperidin-1-yl-4,5-dihydro-1*H*-benzo[*g*]indazole-3-carboxamide 2g. General procedure I was used to convert **6g** and *N*-aminopiperidine into the title product. The mixture was refluxed for 3 h and purification by crystallization with petroleum ether/EtOAc afforded **2g** (1.09 g, 67%) as a yellowish solid. $R_f = 0.53$ (petroleum ether/EtOAc 1/1); mp 191–193 °C (crystallized with petroleum ether/EtOAc); IR 1675, 3340; ^{1}H NMR ($CDCl_3$) δ 1.40–1.48 (m, 2H), 1.63–1.85 (m, 4H), 2.83–3.00 (m, 6H), 3.11–3.20 (m, 2H), 6.62 (d, 1H), 6.95 (d, 1H), 7.27 (d, 2H), 7.50–7.54 (m, 4H), 7.67 (br s, 1H, exch. with D_2O); Anal. Calcd for $C_{23}H_{23}ClN_4O$: C, 67.89; H, 5.70; Cl, 8.71; N, 13.77. Found: C, 67.68; H, 5.74; Cl, 8.62; N, 13.59.

3.1.2.8. 7-Chloro-1-(4′-methoxyphenyl)-*N*-piperidin-1-yl-4,5-dihydro-1*H*-benzo[*g*]indazole-3-carboxamide 2h. General procedure I was used to convert **6h** and *N*-aminopiperidine into the title product. The mixture was refluxed for 3.5 h and purification by flash chromatography (petroleum ether/EtOAc, 1:1) afforded **2h** (1.31 g, 75%) as a yellowish solid. $R_f = 0.40$ (petroleum ether/EtOAc 1/1); mp 166–167 °C (crystallized with petroleum ether/EtOAc); IR 1645, 3180; ^{1}H NMR ($CDCl_3$) δ 1.40–1.48 (m, 2H), 1.69–1.80 (m, 4H), 2.82–3.00 (m, 6H), 3.11–3.20 (m, 2H), 3.90 (s, 3H), 6.63 (d, 1H), 6.93–7.06 (m, 3H), 7.27 (s, 1H), 7.37–7.45 (m, 2H), 7.66 (br s, 1H, exch. with D_2O); Anal. Calcd for $C_{24}H_{25}ClN_4O_2$: C, 65.97; H, 5.77; Cl, 8.11; N, 12.82. Found: C, 65.85; H, 5.96; Cl, 8.19; N, 12.57.

3.1.2.9. 7-Chloro-1-(2′,4′-dichlorophenyl)-*N*-pyrrolidin-1-yl-4,5-dihydro-1*H*-benzo[*g*]indazole-3-carboxamide 2i. General procedure I was used to convert **6a** and *N*-aminopyrrolidine hydrochloride into the title product. Because of an excess of the hydrochloride salt, 3 equiv of Et_3N were used in this reaction. The mixture was refluxed for 3 h and purification by crystallization from EtOH and a few drops of EtOAc afforded **2i** (1.21 g, 65.6%) as a yellowish solid. $R_f = 0.32$ (petroleum ether/EtOAc 1/1); mp 250–251 °C (crystallized with EtOH);

IR 1650, 3190; ^{1}H NMR ($CDCl_3$) δ 1.89–1.98 (m, 4H), 2.94–3.40 (m, 8H), 6.44 (d, 1H), 6.98 (d, 1H), 7.26–7.28 (m, 1H), 7.48 (s, 2H), 7.57 (br s, 1H, exch. with D_2O), 7.61 (s, 1H); Anal. Calcd for $C_{22}H_{19}Cl_3N_4O$: C, 57.22; H, 4.15; Cl, 23.03; N, 12.13. Found: C, 57.01; H, 4.29; Cl, 22.89; N, 12.25.

3.1.2.10. 7-Chloro-1-(2′,4′-dichlorophenyl)-*N*-[(1-ethylpyrrolidin-2-yl)methyl]-4,5-dihydro-1*H*-benzo[*g*]indazole-3-carboxamide 2j. General procedure I was used to convert **6a** and *N*-ethyl-2-aminomethylpyrrolidine into the title product. The mixture was refluxed for 3 h and purification by crystallization from petroleum ether/EtOAc afforded **2j** (1.23 g, 61.1%) as a white solid. R_f = 0.56 ($CHCl_3$/CH_3OH 9/1); mp 111–112 °C (crystallized from petroleum ether/EtOAc); IR 1670, 3380; ^{1}H NMR ($CDCl_3$) δ 1.10 (t, 3H), 1.62–1.98 (m, 4H), 2.10–2.35 (m, 2H), 2.60–2.70 (m, 1H), 2.82–3.30 (m, 7H), 3.65–3.80 (m, 1H), 6.45 (d, 1H), 6.98 (d, 1H), 7.23–7.29 (m, 2H, 1H exch. with D_2O), 7.47 (m, 2H), 7.62 (s, 1H); Anal. Calcd for $C_{25}H_{25}Cl_3N_4O$: C, 59.59; H, 5.00; Cl, 21.11; N, 11.12. Found: C, 59.39; H, 5.11; Cl, 21.02; N, 11.30.

3.1.2.11. 7-Chloro-1-(2′,4′-dichlorophenyl)-*N*-(4-methylpiperazin-1-yl)-4,5-dihydro-1*H*-benzo[*g*]indazole-3-carboxamide 2k. General procedure I was used to convert **6a** and *N*-amino-*N*-methyl piperazine into the title product. The mixture was refluxed for 3 h and purification by crystallization from petroleum ether/EtOAc afforded **2k** (0.97 g, 49.2%) as a yellowish solid. R_f = 0.64 ($CHCl_3$/CH_3OH 9/1); mp 205–206 °C (crystallized with petroleum ether/EtOAc); IR 1670, 3200; ^{1}H NMR ($CDCl_3$) δ 2.32 (s, 3H), 2.63–2.70 (m, 4H), 2.90–3.22 (m, 8H), 6.44 (d, 1H), 6.97 (d, 1H), 7.29 (s, 1H), 7.48 (s, 2H), 7.59 (br s, 1H, exch. with D_2O), 7.62 (s, 1H); Anal. Calcd for $C_{23}H_{22}Cl_3N_5O$: C, 56.28; H, 4.52; Cl, 21.67; N, 14.27. Found: C, 56.15; H, 4.39; Cl, 21.85; N, 14.33.

3.1.3. General procedure II: synthesis of carboxylic acids. To a mixture of ester **5** (1.0 equiv, 5 mmol) in methanol (25 mL) was added a solution of potassium hydroxide (2.0 equiv) in methanol (18 mL). The resulting mixture was heated under reflux overnight. The mixture was allowed to cool to room temperature and then poured into water and acidified with 1 N hydrochloric acid. The precipitate was filtered, washed with water and air-dried to yield the analytically pure acid.

3.1.3.1. 7-Chloro-1-(2′,4′-dichlorophenyl)-4,5-dihydro-1*H*-benzo[*g*]indazole-3-carboxylic acid 6a. General procedure II was used to convert **5a** into the title product **6a** (1.87 g, 95.2%) as a bright pink solid. R_f = 0.54 ($CHCl_3$/MeOH 9:1); mp 247–249 °C; IR 1690, 1710, 3440; ^{1}H NMR ($CDCl_3$) δ 2.94–3.28 (m, 4H), 3.50 (br s, 1H, OH exch. with D_2O), 6.49 (d, 1H), 6.99 (d, 1H), 7.47 (d, 1H), 7.49 (s, 1H), 7.53 (s, 1H), 7.58 (d, 1H). Anal. Calcd for $C_{18}H_{11}Cl_3N_2O_2$: C, 54.92; H, 2.81; Cl, 27.01; N,7.11. Found: C, 54.88; H, 2.93; Cl, 27.31; N,7.01.

3.1.3.2. 1-(2′,4′-Dichlorophenyl)-7-methyl-4,5-dihydro-1*H*-benzo[*g*]indazole-3-carboxylic acid 6b. General procedure II was used to convert **5b** into the title product **6b** (1.64 g, 87.8%) as a white solid. R_f = 0.36 ($CHCl_3$/MeOH 9.5:0.5); mp 266–267 °C; IR 1685; ^{1}H NMR ($CDCl_3$) δ 2.30 (s, 3H), 2.92–3.19 (m, 4H), 4.22 (br s, 1H, OH exch. with D_2O), 6.45 (d, 1H), 6.83 (d, 1H), 7.13 (d, 1H), 7.46 (d, 1H), 7.50 (s, 1H), 7.59 (d, 1H). Anal. Calcd for $C_{19}H_{14}Cl_2N_2O_2$: C, 61.14; H, 3.78; Cl, 18.99; N,7.50. Found: C, 61.32; H, 3.59; Cl, 18.82; N,7.59.

3.1.3.3. 7-Bromo-1-(2′,4′-dichlorophenyl)-4,5-dihydro-1*H*-benzo[*g*]indazole-3-carboxylic acid 6c. General procedure II was used to convert **5c** into the title product **6c** (2.04 g, 93.1%) as a light yellow solid. R_f = 0.54 ($CHCl_3$/MeOH 9:1); mp 248–250 °C; IR 1690, 3350; ^{1}H NMR ($CDCl_3$) δ 2.98–3.22 (m, 4H), 3.50 (br s, 1H, OH exch. with D_2O), 6.42 (d, 1H), 7.17 (d, 1H), 7.47–7.49 (m, 2H), 7.51 (s, 1H), 7.60 (d, 1H). Anal. Calcd for $C_{18}H_{11}BrCl_2N_2O_2$: C, 49.35; H, 2.53; Br, 18.24; Cl, 16.18; N, 7.30. Found: C, 49.21; H, 2.59; Br, 18.36; Cl, 16.10; N, 7.19.

3.1.3.4. 1-(2′,4′-Dichlorophenyl)-7-iodo-4,5-dihydro-1*H*-benzo[*g*]indazole-3-carboxylic acid 6d. General procedure II was used to convert **5d** into the title product **6d** (2.25 g, 93%) as a yellow solid. R_f = 0.69 ($CHCl_3$/MeOH 9:1); mp 264–265 °C; IR 1685, 3400; ^{1}H NMR ($CDCl_3$) δ 2.97–3.20 (m, 4H), 3.50 (br s, 1H, OH exch. with D_2O), 6.29 (d, 1H), 7.37 (d, 1H), 7.47 (d, 1H), 7.52 (s, 1H), 7.57 (d, 1H), 7.66 (s, 1H). Anal. Calcd for $C_{18}H_{11}Cl_2IN_2O_2$: C, 44.56; H, 2.28; Cl, 14.61; I, 26.16; N, 5.77. Found: C, 44.78; H, 2.15; Cl, 14.44; I, 26.00; N, 5.69.

3.1.3.5. 1-(2′,4′-Dichlorophenyl)-4,5-dihydro-1*H*-benzo[*g*]indazole-3-carboxylic acid 6e. General procedure II was used to convert **5e** into the title product **6e** (1.32 g, 75%) as a yellowish solid. R_f = 0.73 ($CHCl_3$/MeOH 8:2); mp 267 °C; IR 1685, 3400; ^{1}H NMR ($CDCl_3$) δ 2.90–3.20 (m, 4H), 4.39 (br s, 1H, OH exch. with D_2O), 6.56 (d, 1H), 7.01 (t, 1H), 7.19 (t, 1H), 7.28 (d, 1H), 7.48 (d, 1H), 7.53 (s, 1H), 7.58 (d, 1H). Anal. Calcd for $C_{18}H_{12}Cl_2N_2O_2$: C, 60.18; H, 3.36; Cl, 19.74; N,7.79. Found: C, 60.33; H, 3.42; Cl, 19.62; N, 7.68.

3.1.3.6. 7-Chloro-1-(4′-chlorophenyl)-4,5-dihydro-1*H*-benzo[*g*]indazole-3-carboxylic acid 6f. General procedure II was used to convert **5f** into the title product **6f** (1.76 g, 98%) as a white solid. R_f = 0.78 ($CHCl_3$/MeOH 8.5:1.5); mp 251 °C; IR 1590, 1710, 3440; ^{1}H NMR ($CDCl_3$) δ 2.90–3.10 (m, 4H), 6.71 (d, 1H), 7.01 (d, 1H), 7.30 (br s, 1H, OH exch. with D_2O), 7.34 (s, 1H), 7.47–7.52 (m, 4H). Anal. Calcd for $C_{18}H_{12}Cl_2N_2O_2$: C, 60.18; H, 3.36; Cl, 19.74; N, 7.79. Found: C, 60.33; H, 3.25; Cl, 19.89; N, 7.68.

3.1.3.7. 7-Chloro-1-phenyl-4,5-dihydro-1*H*-benzo[*g*]-indazole-3-carboxylic acid 6g. General procedure II was used to convert **5g** into the title product **6g** (1.59 g, 98%) as a yellowish solid. R_f = 0.76 ($CHCl_3$/MeOH 8.5:1.5); mp 258 °C; IR 1690; ^{1}H NMR ($CDCl_3$/DMSO) δ 2.91–3.12 (m, 4H), 6.67 (d, 1H), 6.96 (d, 1H), 7.29 (br s, 1H, OH exch. with D_2O), 7.31 (s, 1H), 7.47–7.52 (m, 5H). Anal. Calcd for

$C_{18}H_{13}ClN_2O_2$: C, 66.57; H, 4.03; Cl, 10.92; N, 8.63. Found: C, 66.44; H, 4.15; Cl, 10.88; N, 8.74.

3.1.3.8. 7-Chloro-1-(4′-methoxyphenyl)-4,5-dihydro-1*H*-benzo[*g*]indazole-3-carboxylic acid 6h. General procedure II was used to convert **5h** into the title product **6h** (1.68 g, 95%) as a yellowish solid. $R_f = 0.69$ ($CHCl_3$/MeOH 8.5:1.5); mp 249–250 °C; IR 1695, 3400; ^{1}H NMR ($CDCl_3$) δ 2.96–3.15 (m, 4H), 3.89 (s, 3H), 6.67 (d, 1H), 6.98 (d, 1H), 7.28 (br s, 1H, OH exch. with D_2O), 7.32 (s, 1H), 7.42–7.48 (m, 4H). Anal. Calcd for $C_{19}H_{15}ClN_2O_3$: C, 64.32; H, 4.26; Cl, 9.99; N, 7.90. Found: C, 64.25; H, 4.39; Cl, 10.12; N, 7.76.

3.1.4. General procedure III: synthesis of tricyclic esters. A stirred mixture of diketoester **4** (1.0 equiv, 4 mmol) and the requisite phenyl hydrazine hydrochloride (1.15 equiv) in EtOH (28 mL) was heated under reflux for 1–5 h. The reaction was allowed to cool to room temperature and the insoluble material was collected by filtration and washed with a small volume of ice-cool ethanol. Purification by flash chromatography afforded the analytically pure product.

3.1.4.1. Ethyl 7-chloro-1-(2′,4′-dichlorophenyl)-4,5-dihydro-1*H*-benzo[*g*]indazole-3-carboxylate 5a. General procedure III was used to convert **4a** and 2,4-dichlorophenylhydrazine hydrochloride into the title product. The mixture was heated at reflux for 2.5 h. After cooling at room temperature, the precipitate was filtered off to give **5a** (1.05 g, 62%) as an off-white solid. $R_f = 0.64$ (petroleum ether/EtOAc, 8:2); mp 138–140 °C (triturated with petroleum ether); IR 1710; ^{1}H NMR ($CDCl_3$) δ 1.42 (t, 3H), 2.94–3.25 (m, 4H), 4.45 (q, 2H), 6.47 (d, 1H), 6.97 (d, 1H), 7.30 (s, 1H), 7.43 (d, 1H), 7,50 (s, 1H), 7.56 (d, 1H). Anal. Calcd for $C_{20}H_{15}Cl_3N_2O_2$: C, 56.96; H, 3.59; Cl, 25.22; N, 6.64. Found: C, 56.82; H, 3.44; Cl, 25.39; N, 6.89.

3.1.4.2. Ethyl 1-(2′,4′-dichlorophenyl)-7-methyl-4,5-dihydro-1*H*-benzo[*g*]indazole-3-carboxylate 5b. General procedure III was used to convert **4b** and 2,4-dichlorophenylhydrazine hydrochloride into the title product. The mixture was heated at reflux for 1 h. Purification by flash chromatography (petroleum ether/EtOAc, 9:1) afforded **5b** (0.91 g, 57%) as a white solid. $R_f = 0.27$ (petroleum ether/EtOAc, 9:1); mp 155 °C (triturated with petroleum ether); IR 1725; ^{1}H NMR ($CDCl_3$) δ 1.43 (t, 3H), 2.29 (s, 3H), 2.96–3.04 (m, 4H), 4.45 (q, 2H), 6.46 (d, 1H), 6.82 (d, 1H), 7.12 (s, 1H), 7.44–7.58 (m, 3H). Anal. Calcd for $C_{21}H_{18}Cl_2N_2O_2$: C, 62.85; H, 4.52; Cl, 17.67; N, 6.98. Found: C, 62.51; H, 4.41; Cl, 17.95; N, 7.04.

3.1.4.3. Ethyl 7-bromo-1-(2′,4′-dichlorophenyl)-4,5-dihydro-1*H*-benzo[*g*]indazole-3-carboxylate 5c. General procedure III was used to convert **4c** and 2,4-dichlorophenylhydrazine hydrochloride into the title product. The mixture was heated at reflux for 2 h. After cooling at room temperature,, the precipitate was filtered off to give **5c** (1.14 g, 61%) as a yellowish solid. $R_f = 0.15$ (petroleum ether/EtOAc, 9.5:0.5); mp 147–148 °C (triturated with petroleum ether); IR 1715; ^{1}H NMR ($CDCl_3$) δ 1.42 (t, 3H), 2.96–3.22 (m, 4H), 4.67 (q, 2H), 6.41 (d, 1H), 7.17 (d, 1H), 7.43–7.48 (m, 2H), 7.50 (s, 1H), 7.58 (d, 1H). Anal. Calcd for $C_{20}H_{15}BrCl_2N_2O_2$: C, 51.53; H, 3.24; Br, 17.14; Cl, 15.21; N, 6.01. Found: C, 51.44; H, 3.39; Br, 17.42; Cl, 15.07; N, 6.32.

3.1.4.4. Ethyl 1-(2′,4′-dichlorophenyl)-7-iodo-4,5-dihydro-1*H*-benzo[*g*]indazole-3-carboxylate 5d. General procedure III was used to convert **4d** and 2,4-dichlorophenylhydrazine hydrochloride into the title product. The mixture was heated at reflux for 2 h. Purification by flash chromatography (petroleum ether/EtOAc, 8.5:1.5) afforded **5d** (1.91 g, 93%) as a yellowish solid. $R_f = 0.57$ (petroleum ether/EtOAc, 8.5:1.5); mp 148–150 °C (triturated with petroleum ether); IR 1710; ^{1}H NMR ($CDCl_3$) δ 1.42 (t, 3H), 2.92–3.20 (m, 4H), 4.46 (q, 2H), 6.29 (d, 1H), 7.37 (d, 1H), 7.46 (d, 1H), 7.50 (s, 1H), 7.57 (d, 1H), 7.66 (s, 1H). Anal. Calcd for $C_{20}H_{15}Cl_2IN_2O_2$: C, 46.81; H, 2.95; Cl, 13.82; I, 24.73; N, 5.46. Found: C, 46.67; H, 2.78; Cl, 13.59; I, 24.96; N, 5.62.

3.1.4.5. Ethyl 1-(2′,4′-dichlorophenyl)-4,5-dihydro-1*H*-benzo[*g*]indazole-3-carboxylate 5e. General procedure III was used to convert **4e**[21] and 2,4-dichlorophenylhydrazine hydrochloride into the title product. The mixture was heated at reflux for 3 h. Purification by flash chromatography (petroleum ether/EtOAc, 8.5:1.5) afforded **5e** (1.49 g, 96%) as a pink solid. $R_f = 0.47$ (petroleum ether/EtOAc, 9:1); mp 158 °C (triturated with petroleum ether); IR 1710; ^{1}H NMR ($CDCl_3$) δ 1.43 (t, 3H), 2.95–3.15 (m, 4H), 4.45 (q, 2H), 6.68 (d, 1H), 7.01 (d, 1H), 7.31 (s, 1H), 7.45–7.50 (m, 4H). Anal. Calcd for $C_{20}H_{16}Cl_2N_2O_2$: C, 62.03; H, 4.16; Cl, 18.31; N, 7.23. Found: C, 62.15; H, 4.31; Cl, 18.29; N, 7.42.

3.1.4.6. Ethyl 7-chloro-1-(4′-chlorophenyl)-4,5-dihydro-1*H*-benzo[*g*]indazole-3-carboxylate 5f. General procedure III was used to convert **4a** and 4-chlorophenylhydrazine hydrochloride into the title product. The mixture was heated at reflux for 2.5 h. Purification by crystallization with EtOH afforded **5f** (1.10 g, 71%) as a yellowish solid. $R_f = 0.53$ (petroleum ether/EtOAc, 8:2); mp 137–138 °C (crystallized with EtOH); IR 1715; ^{1}H NMR ($CDCl_3$) δ 1.43 (t, 3H), 2.95–3.15 (m, 4H), 4.45 (q, 2H), 6.68 (d, 1H), 7.01 (d, 1H), 7.31 (s, 1H), 7.45–7.50 (m, 4H). Anal. Calcd for $C_{20}H_{16}Cl_2N_2O_2$: C, 62.03; H, 4.16; Cl, 18.31; N, 7.23. Found: C, 62.32; H, 3.95; Cl, 18.29; N, 7.34.

3.1.4.7. Ethyl 7-chloro-1-phenyl-4,5-dihydro-1*H*-benzo[*g*]indazole-3-carboxylate 5g. General procedure III was used to convert **4a** and phenylhydrazine hydrochloride into the title product. The mixture was heated at reflux for 2.5 h. Purification by crystallization with EtOH afforded **5g** (1.02 g, 72%) as a brown solid. $R_f = 0.43$ (petroleum ether/EtOAc, 9:1); mp 158–160 °C (crystallized with EtOH); IR 1710; ^{1}H NMR ($CDCl_3$) δ 1.42 (t, 3H), 2.94–3.15 (m, 4H), 4.44 (q, 2H), 6.64 (d, 1H), 6.96 (dd, 1H), 7.30 (s, 1H), 7.45–7.55 (m, 5H). Anal. Calcd for $C_{20}H_{17}ClN_2O_2$: C, 68.09; H, 4.86; Cl, 10.05; N, 7.94. Found: C, 68.33; H, 4.59; Cl, 10.25; N, 7.67.

3.1.4.8. Ethyl 7-chloro-1-(4′-methoxyphenyl)-4,5-dihydro-1*H*-benzo[*g*]indazole-3-carboxylate 5h. General procedure III was used to convert **4a** and 4-methoxyphenylhydrazine hydrochloride into the title product. The mixture was heated at reflux for 3 h. Purification by crystallization with EtOH afforded **5h** (0.96 g, 63%) as a pink product. $R_f = 0.33$ (petroleum ether/EtOAc, 8:2); mp 162–163 °C (crystallized with EtOH); IR 1715; ^{1}H NMR ($CDCl_3$) δ 1.43 (t, 3H), 2.96–3.18 (m, 4H), 3.89 (s, 3H), 4.44 (q, 2H), 6.65 (d, 1H), 6.94–7.05 (m, 3H), 7.29 (s, 1H), 7.38–7.42 (dd, 2H). Anal. Calcd for $C_{21}H_{19}ClN_2O_3$: C, 65.88; H, 5.00; Cl, 9.26; N, 7.32. Found: C, 65.72; H, 5.15; Cl, 9.08; N, 7.42.

3.1.5. General procedure IV: synthesis of α,γ-diketoesters. Sodium metal (2.0 equiv) was added in small portion to dry ethanol (5 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 tetralone starting material (1.0 equiv, 6 mmol) in dry ethanol (30 mL). The solution was stirred at room temperature for 2–8 h. The mixture was slowly poured over 2*N* hydrochloride acid and the resulting precipitate was collected by filtration and washed with a small volume of ice-cold ethanol and water. The air-dried residue afforded the analytically pure product.

3.1.5.1. Ethyl α-(6-chloro-1-oxo-1,2,3,4-tetrahydronaphtalen-2-yl)-α-oxo-acetate 4a. General procedure IV was used to convert **3a**[19] into the title product. The mixture was stirred for 3 h at room temperature. Compound **4a** (1.38 g, 82%) was isolated as a yellowish solid. $R_f = 0.64$ (petroleum ether/EtOAc, 8.5:1.5); mp 93–95 °C (triturated with petroleum ether); IR 1610, 1715, 3400; ^{1}H NMR ($CDCl_3$) δ 1.41 (t, 3H), 2.90–3.00 (m, 4H), 4.38 (q, 2H), 7.26 (s, 1H), 7.33 (d, 1H), 7.93 (d, 1H), 15.68 (br s, 1H, OH exch. with D_2O). Anal. Calcd for $C_{14}H_{13}ClO_4$: C, 59.90; H, 4.67; Cl, 12.63. Found: C, 59.79; H, 4.88; Cl, 12.59.

3.1.5.2. Ethyl α-(6-methyl-1-oxo-1,2,3,4-tetrahydronaphtalen-2-yl)-α-oxo-acetate 4b. General procedure IV was used to convert **3b**[20] into the title product. The mixture was stirred for 5 h at room temperature. Compound **4b** (0.66 g, 42%) was isolated as a yellowish solid. $R_f = 0.52$ (petroleum ether/EtOAc, 9:1); mp 39–40 °C (triturated with petroleum ether); IR 1670, 1720, 3400; ^{1}H NMR ($CDCl_3$) δ 1.37 (t, 3H), 2.40 (s, 3H), 2.82–2.92 (m, 4H), 4.38 (q, 2H), 7.06 (s, 1H), 7.16 (d, 1H), 7.90 (d, 1H), 15.88 (br s, 1H, OH exch. with D_2O). Anal. Calcd for $C_{15}H_{16}O_4$: C, 69.22; H, 6.20. Found: C, 69.41; H, 6.15.

3.1.5.3. Ethyl α-(6-bromo-1-oxo-1,2,3,4-tetrahydronaphtalen-2-yl)-α-oxo-acetate 4c. General procedure IV was used to convert **3c**[19] into the title product. The mixture was stirred for 2 h at room temperature. Compound **4c** (0.72 g, 37%) was isolated as a brown solid. $R_f = 0.29$ (petroleum ether/EtOAc, 9.5:1.5); mp 83 °C (triturated with petroleum ether); IR 1715, 3420; ^{1}H NMR ($CDCl_3$) δ 1.41 (t, 3H), 2.89–2.92 (m, 4H), 4.40 (q, 2H), 7.43 (s, 1H), 7.51 (d, 1H), 7.85 (d, 1H), 15.66 (br s, 1H, OH exch. with D_2O). Anal. Calcd for $C_{14}H_{13}BrO_4$: C, 51.71; H, 4.03; Br, 24.57. Found: C, 51.89; H, 4.10; Br, 24.36.

3.1.5.4. Ethyl α-(6-iodo-1-oxo-1,2,3,4-tetrahydronaphtalen-2-yl)-α-oxo-acetate 4d. General procedure IV was used to convert **3d** into the title product. The mixture was stirred for 3 h at room temperature. Compound **4d** (1.47 g, 66%) was isolated as a brown-yellow solid. $R_f = 0.48$ (petroleum ether/EtOAc, 8:2); mp 43 °C (triturated with petroleum ether); IR 1725, 3420; ^{1}H NMR ($CDCl_3$) δ 1.40 (t, 3H), 2.81–3.00 (m, 4H), 4.40 (q, 2H), 7.66–7.72 (m, 3H), 15.64 (br s, 1H, OH exch. with D_2O). Anal. Calcd for $C_{14}H_{13}IO_4$: C, 45.18; H, 3.52; I, 34.10. Found: C, 45.33; H, 3.48; I, 34.00.

3.1.6. Synthesis of 6-Iodo-3,4-dihydro-2*H*-naphtalen-1-one 3d. A solution of $NaNO_2$ (1.2 equiv) in water (1.1 mL) was added dropwise to a stirred solution of the 6-amino-tetralone[22] starting material (1 equiv, 3.1 mmol) in 15% HCl (2.5 mL) at 0 °C. To the resulting solution was added a solution of potassium iodide (1.2 equiv) in water (2 mL) and the whole was stirred at room temperature for 1.5 h and then warmed at 60 °C until gas development ceased. The mixture was allowed to cool to room temperature and then extracted with ether. The combined organic layers were washed with 5% solution of sodium thiosulfate, dried over Na_2SO_4 and concentrated under reduced pressure to afford a crude oil. Purification by flash chromatography (petroleum ether/EtOAc, 9:1) afforded **3d** (0.50 g, 89.3%) as a red oil. $R_f = 0.68$ (petroleum ether/EtOAc, 7:3); IR 1590, 1700; ^{1}H NMR ($CDCl_3$) δ 2.12 (q, 2H), 2.64 (t, 2H), 2.92 (t, 2H), 7.64–7.80 (m, 3H). Anal. Calcd for $C_{10}H_9IO$: C, 44.14; H, 3.33; I, 46.64. Found: C, 44.27; H, 3.22; I, 46.45.

4. Biology

4.1. Animals

Male CD-1 mice (Charles River S.p.A., Calco, LC, Italy), weighing from 20 to 35 g were used. Mice were housed, in plastic cages, under a 12 h artificial light–dark cycle (lights off at 8.00 pm), at a constant temperature (22±2 °C). Water and laboratory rodent chow (MIL Morini, San Polo D'Enza, RE, Italy) were provided ad libitum. All experimental procedures were performed in strict accordance with the E.C. regulation for care and use of experimental animals (EEC No. 86/609).

4.2. Chemicals and drugs

[^{3}H]-CP-55,940 (specific activity 180 Ci/mmol) was purchased from New England Nuclear (Boston, MA, USA). CP-55,940 was obtained from Tocris Cookson Ltd (Bristol UK). For binding experiments, drugs were dissolved in dimethyl-sulfoxide, (DMSO). DMSO concentration in the different assays never exceeded 0.1% (v/v) without any effect on radioligand binding.

In vivo assays were carried out dissolving drugs in a volume of 12.5 mL/kg of saline (0.1% Tween 80). Carmine

(Sigma Chemical Co, St. Louis, MO) was suspended (6% wt/v) in distilled water containing 0.5% methylcellulose and administered by gavage.

4.3. Radioligand binding methods

Mice were killed by cervical dislocation and the brain (minus cerebellum) and spleen were rapidly removed and placed on an ice-cold plate. After thawing, tissues were homogenated in 20 vol. (wt/v) of ice-cold TME buffer (50 mM Tris–HCl, 1 mM EDTA and 3.0 mM $MgCl_2$, pH 7.4). The homogenates were centrifuged at $1,086 \times g$ for 10 min at 4 °C, and the resulting supernatants were centrifuged at $45,000 \times g$ for 30 min.

[^{3}H]-CP-55,940 binding was performed by the method previously described by Ruiu et al.[17] Briefly, the membranes (30–80 μg of protein) were incubated with 0.5–1 nM of [^{3}H]-CP-55,940 for 1 h at 30 °C in a final volume of 0.5 mL of TME buffer containing 5 mg/mL of fatty acid-free bovine serum albumin (BSA). Non-specific binding was estimated in the presence of 1 μM of CP-55,940. All binding studies were performed in disposable glass tubes pre-treated with Sigma-Cote (Sigma Chemical Co. Ltd., Poole, UK), in order to reduce non-specific binding. The reaction was terminated by rapid filtration through Whatman GF/C filters presoaked in 0.5% polyethyleneimine (PEI) using a Brandell 36-sample harvester (Gaithersburg, MD, USA). Filters were washed five times with 4 mL aliquots of ice cold Tris–HCl buffer (pH 7.4) containing 1 mg/mL BSA The filter bound radioactivity was measured in a liquid scintillation counter (Tricarb 2900, Packard, Meridien, USA) with 4 mL of scintillation fluid (Ultima Gold MV, Packard).

Protein determination was performed by means of Bradford protein assay[23] using BSA as a standard according to the protocol of the supplier (Bio-Rad, Milan, Italy).

All experiments were performed in triplicate and results were confirmed in at least five independent experiments. Data from radioligand inhibition experiments were analyzed by nonlinear regression analysis of a Sigmoid Curve using Graph Pad Prism program. IC_{50} values were derived from the calculated curves and converted to K_i values as previously described.[24]

4.4. Gastrointestinal transit (GIT)

GIT in mice was measured by the *upper gastrointestinal transit test* according to procedure validated by Nagakura et al.[18] Different doses of tested compounds were administered ip 30 min before the ig administration of the marker (0.3 mL/mouse of red carmine) to groups of $n = 9$–16 subjects. Twenty minutes later, mice were sacrificed; stomach and small intestine were removed, then the distance travelled by the head of the red marker was measured. The results were expressed as percent of the total length of the small intestine (determined from pyloric sphincter to ileocaecal junction). To evaluate antagonism activity, the reference cannabinoid agonist CP-55,940 (0 and 0.01 mg/kg), was administered ip 10 min after the injection of a chosen dose of tested compound. These doses were chosen on the basis of preliminary experiments. Then the marker was administered ig 20 min after CP-55,940. Finally 20 min later, mice were sacrificed and GIT determined as described.

Data were expressed as group mean ± SEM. Data points were mean values; vertical bars in the figures represented the SEM.

In each experiment, statistical evaluation of the GIT, expressed as percentage of the distance travelled by the head of the marker over the total length of the small intestine, was performed by a one-way analysis of variance (ANOVA), followed by the Newman–Keuls test for post-hoc comparisons.

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