Tricyclic Pyrazoles. 4. Synthesis and Biological Evaluation of Analogues of the Robust and Selective CB_2 Cannabinoid Ligand 1-(2′,4′-Dichlorophenyl)-6-methyl-*N*-piperidin-1-yl-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxamide

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New analogues (**2a**−**p**) of the previously reported CB_2 ligands 6-methyl- and 6-chloro-1-(2′,4′-dichlorophenyl)-*N*-piperidin-1-yl-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxamides (**1a**,**b**) have been synthesized and evaluated for cannabinoid receptor affinity. One example, 1-(2′,4′-dichlorophenyl)-6-methyl-*N*-cyclohexylamine-1,4-dihydroindeno[1,2-*c*] pyrazole-3-carboxamide (**2a**) was shown to have single digit nanomolar affinity for cannabinoid CB_2 receptors. Furthermore, compounds **2a** and **2b**, as well as lead structures **1a**,**b**, were also shown to be agonist in an in vitro model based on human promyelocytic leukemia HL-60 cells.

Introduction

The CB_2 receptor, first described in 1993, is a member of the cannabinoid receptor family and possesses, in humans, ∼44% overall homology to the CB_1 receptor,[1−3] with 68% amino acid sequence identity within the transmembrane domains.[2] Unlike the CB_1 receptor, this cannabinoid receptor subtype is rather divergent across human, rat, and mouse, as indicated by the following amino acid identity percentage: 93% between rat and mouse; 81% between rat and human.[3]

CB_2 receptors, as well as the CB_1, belong to the large family of rhodospin-like family of G-protein-coupled receptors (GPCRs), which control different multiple intracellular signal transduction pathways; that is, CB_1 and CB_2 receptor agonists inhibit forskolin-stimulated adenylyl cyclase by activation of a pertussis toxin-sensitive G-protein; CB_2 receptor agonists can produce stimulation of cAMP formation; Gi/o MAP kinase is activated both in cultured human promyelocytic HL-60 and in CHO cells possessing endogenous or expressing recombinant CB_2 receptors, respectively.[4]

CB_2 receptors are expressed in peripheral tissue (tonsils, thymus, spleen, pancreas), peripheral nerve terminals, and skin tumor cells,[4−6] while CB_1 receptors are localized both in brain (cerebellum, hippocampus, cortex, basal ganglia) and in peripheral tissues and organs (testis, eye, urinary bladder, gastrointestinal tract).[4,5,7−10] Moreover, CB_2 is expressed abundantly in various types of inflammatory cells and immune competent cells altering the maturation of macrophages, eosinophils and natural killer and B lymphocytes.[11−16] According to its predominant expression in immune tissues and lymphocytes, CB_2 receptors appear to specifically mediate both immunosuppressive and immunostimulatory effects.[15−19] Gene expression studies showed that activation of the CB_2 receptor is involved in initialization of the immune cell maturation process. A role of the CB_2 receptor in inhibiting the growth of murine lymphoma and leukemia cells has also been described.[20]

Despite the general opinion that CB_2 receptors are almost exclusively expressed in peripheral tissue, the presence of this cannabinoid receptor subtype in the central nervous system has also directly or indirectly been evidenced, as demonstrated by CB_2 receptor expression in rat microglial cells,[18] adult rat retina,[21] human perivascular microglial cells,[22] and neuritic plaques-associated microglial cells in Alzheimer'disease brain.[23] The correlation between CB_2 receptor and central nervous system has been recently supported by a work based on immunohistochemical and gene expression analysis in rat brain.[24] In fact, a widespread expression of CB_2 receptors in the brain and in hippocampal neuron-specific-enolase-positive neuronal cells has been evidenced in this study.[24] Moreover, a possible direct involvement of neural mechanisms on CB_2 receptor-mediated antihyperalgesia has been proposed by Beltramo et al.[25]

The multifocal expression of CB_2 immunoreactivity in glial and neuronal patterns in different brain regions suggests reevaluation of the possible roles played by this receptor subtype in CNS. Consequently, the relevance of ligands able to selectively interact with CB_2 receptors could be higher than that related only to the known pathophysiological processes involving this cannabinoid receptor subtype (i.e., peripheral antinociception, immunomodulation, inflammation, neurodegeneration, uncontrolled cell proliferation).[26−28] Therefore, in addition to the discovery of new CB_1 selective compounds, with significant applications in different therapeutic areas (i.e., smoking cessation, obesity, sexual disorders, pain, gastrointestinal disorders, and glaucoma),[4] the individuation of new classes of CB_2 active and selective ligands is of great interest, too.

Among the different classes of synthetic compounds assuring CB_2 affinity and selectivity, the relevance of pyrazole derivatives have been consolidated by various studies.[29,30] In this context, we have recently reported the synthesis and the CB_2 affinity of several new 1,4-dihydroindeno[1,2-*c*]pyrazol-based ligands **1**, among which compound **1a**, and to minor extend **1b**, exerted robust affinity and selectivity for CB_2 receptor (Figure 1).[31]

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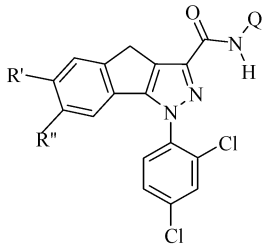
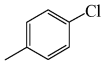
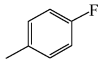
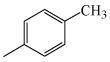
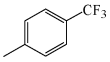
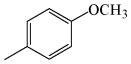
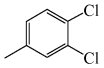
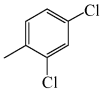
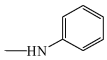
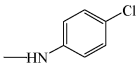
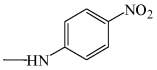
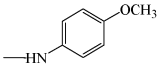
Table 1. Structures and Binding Data of Compounds **2a**−**p**



				Receptor affinity		CB_2 selectivity
Compd 2	**R'**	**R"**	**Q**	**K_iCB_2[a] (nM)**	**K_iCB_1[b] (nM)**	**K_iCB_1/ K_iCB_2**
a	CH_3	H		7.6±0.7	900±45	118:1
b	CH_3	H		32.8±1.7	2409±200	73:1
c	CH_3	H		177±20	>10000	-
d	CH_3	H		61±8	>5000	-
e	CH_3	H		74±14	>5000	-
f	CH_3	H		>5000	>10000	-
g	CH_3	H		114±20	>10000	-
h	CH_3	H		>10000	>10000	-
i	CH_3	H		>5000	2625±898	-
j	CH_3	H		63±8	2275±330	36:1
k	CH_3	H		>5000	>10000	-
l	CH_3	H		2116±197	4500±200	2:1
m	CH_3	H		3000±150	>5000	-
n	CH_3	Cl		25±3	833±144	33:1
o	CH_3	Cl		200±29	916±68	4.6:1
p	Cl	CH_3		85±11	1200±227	14:1
SR144528				0.28±0.04	70±10	250:1
CP55,940				1.11±0.09	1.50±0.12	1.4:1
1a	CH_3	H		0.037±0.003	363±30	9810:1
1b	Cl	H		0.34±0.06	2050±90	6029:1

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

This finding prompted us to investigate new 1,4-dihydroindeno[1,2-*c*]pyrazoles **2a**−**p** (Table 1), which were obtained by modifying the carbamoyl moiety and the aryl substitution of the lead compounds **1a**,**b**.

This study also sought to establish how these compounds modulate CB_2 receptors (intrinsic activity) using an in vitro model based on human promyelocytic leukemia HL-60 cells.

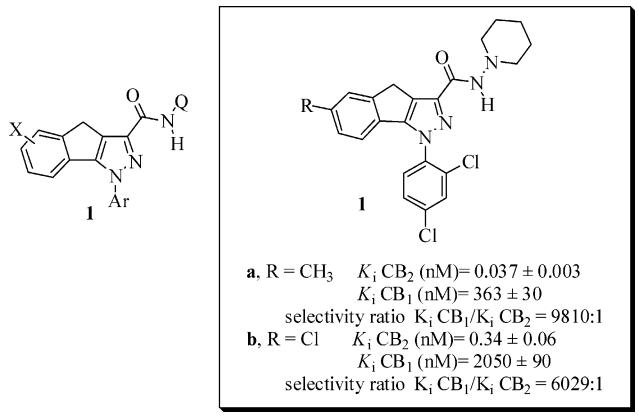
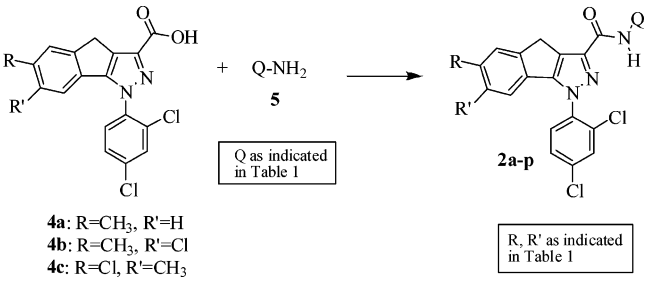


Figure 1.

Scheme 1. Synthesis of Compounds **2a**−**p**



Chemistry

The compounds described in this report were prepared as shown in Scheme 1.

Target compounds **2a**−**p** were obtained by amidation of the appropriate amine or hydrazine **5** and the necessary 1-(2′,4′-dichlorophenyl)-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxylic acid (**4a**,[31] **4b**, or **4c**), using thionyl chloride (method A) or 1-hydroxybenzotriazole (BtOH) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC; method B).

The required unknown tricyclic acids (**4b**,**c**) were synthesized as reported in the Scheme 2.

The 1,3-diketoesters **7b** and **7c**, as a tautomeric equilibrium shifted toward the alkenylidene structure (**7′**), were prepared from the disubstituted indanones **6b** and **6c**,[32] respectively, and diethyl oxalate in the presence of sodium ethylate.

Compounds **7b**,**c** and the 2,4-dichlorophenylhydrazine hydrochloride were heated in acetic acid to afford the desired dihydroindenopyrazoles **8b**,**c**.

Saponification of the esters **8b**,**c** furnished acids **4b**,**c**.

The 6-chloro-5-methylindanone (**6b**), so far undescribed in literature, was prepared starting from the 5-methylindanone (**9**),[31] as depicted in Scheme 3.

Briefly, indanone **9** was nitrated with potassium nitrate in concentrated sulfuric acid, to give a mixture of isomers **10a** and **10b**, which could be chromatographically separated and characterized via ^{1}H NMR.

Subsequent reduction of the nitro group of **10a** by palladium-catalyzed hydrogenation afforded 6-amino-5-methylindanone **11**, which then underwent diazoniation and final reaction with copper(I) chloride to form indanone **6b**.

Biology

Cannabinoid receptor affinities of the 1,4-dihydroindeno[1,2-*c*]pyrazoles were determined by radioligand binding experiments according to the previously reported procedures by Ruiu[33] and expressed as K_i values. In the CB_2 assays, homogenates of mouse spleen were used as starting material. Mouse brain (minus cerebellum) homogenates were instead employed for CB_1 affinity evaluation. ^{3}H-CP 55 940 was used as radioligand in both cases. The results from the in vitro binding assay were compared with the K_i values of the prototypical cannabinoid ligands **1a** and **1b** and of the reference compounds SR144528 and CP55 940.

The capability of these new derivatives and of the lead compounds to modulate CB_2 receptors has been further investigated through an in vitro model based on human promyelocytic leukemia HL-60 cells. This cell line, selectively expressing the CB_2 but not the CB_1 receptor, represents a reliable platform for the study of the mechanism of action of cannabinoids.[11,15,17,34] In fact, it has been shown that the exposure of HL-60 cells to the cannabinoid nonselective synthetic agonist CP55 940 and the endogenous ligand 2-AG induces a rapid phosphorylation and activation of the extracellular signal-related kinases (ERK 1/2 or p44/p42-MAPK) and that the addition of SR144528, a CB_2 receptor-specific antagonist, abolished the response induced by the agonists, thus indicating that the P-ERK 1/2 activation is CB_2 specific.[12,17,35] Accordingly, the activity of both the new derivatives with the best CB_2 affinity (**2a** and **2b**) and the reference compounds (**1a**, **1b**, and CP55 940) was assayed by the exposure of human promyelocytic leukemia HL-60 cells to the potential CB_2 ligands and the consequent western blot analysis of the cell extract proteins. In the case of P-ERK 1/2 activation, to establish CB_2-specific activity of the compounds, experiments involving a preliminary step characterized by the adding of the CB_2 receptor antagonist SR144528, with the subsequent exposure with the new derivatives, were also carried out.

Results and Discussion

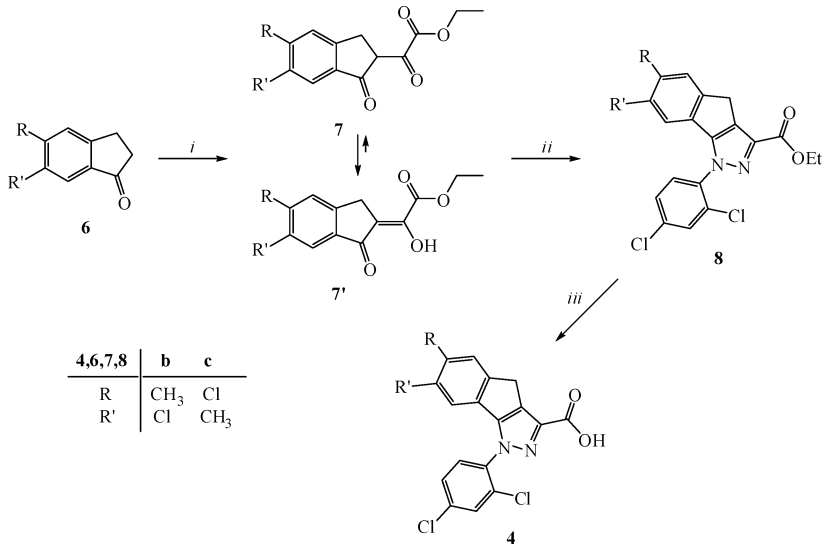
Radioligand Binding Assays. The cannabinoid receptor affinities of the new 1,4-dihydroindeno[1,2-*c*]pyrazole derivatives are shown in Table 1. For comparison, the K_i values of the lead compounds 6-methyl- and 6-chloro-1-(2′,4′-dichlorophenyl)-*N*-piperidin-1-yl-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxamides (**1a**,**b**) and reference CB_2 ligands SR144528 and CP55 940 have been reported. Results are the average of five independent experiments with three replicates at each concentration.

Our initial result showed that isosteric replacement of the carboxamide *N*-piperidinyl moiety of **1a** with the cyclohexyl ring gave the analogue **2a** with nanomolar CB_2 affinity and acceptable subtype receptor selectivity (K_iCB$_2$ = 7.6 nM; K_iCB$_1$/K_iCB$_2$ = 118). Because the bioisosteric analogue **2a** displayed reasonable CB_2 affinity and selectivity, we decided to do a detailed structure−activity relationship (SAR) investigation of **2a** to provide an understanding of the structural features that influence the affinity of this novel compound.

Our initial strategy was to prepare analogues of **2a** by stepwise introduction of various carbamoyl moieties at the pyrazole ring of the *N*-cyclohexyl 1,4-dihydroindeno[1,2-*c*]-pyrazole-3-carboxamide template of **2a**. In addition, analogues of **2a** having a modified aromatic substitution of the tricyclic ring system and carbamoyl unit were also prepared. As exemplified by compound **2b**, replacement of the cyclohexyl ring of the carbamoyl moiety of **2a** with a phenyl group led to a 4-fold affinity decrease (K_iCB$_2$ = 32.8 nM).

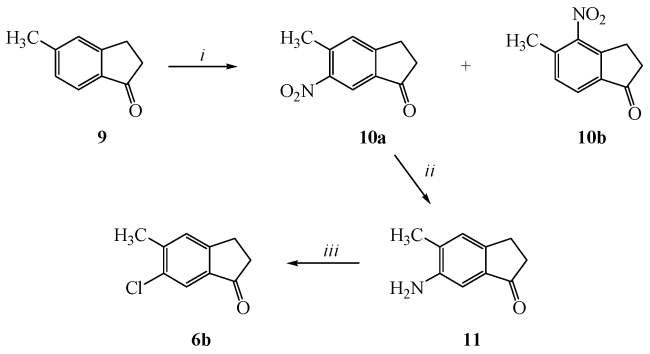
Benzamides with a *p*-substituent as chlorine (**2c**), fluoro (**2d**), methyl (**2e**), and methoxy (**2g**), resulted in a somewhat lower CB_2 affinity as compared to the phenyl derivative **2b**. Moreover, the presence of the strongly electron-withdrawing trifluorom-

Scheme 2



Reagents and conditions: (*i*) Na, dry EtOH, $(COOEt)_2$; (*ii*) 2,4-$Cl_2C_6H_3NHNH_2$·HCl, CH_3CO_2H; (*iii*) KOH, EtOH/H_2O.

Scheme 3



Reagents and conditions: (*i*) H_2SO_4, KNO_3; (*ii*) H_2, Pd/C 10%, EtOH; (*iii*) $NaNO_2$, HCl, CuCl.

ethyl group at the phenyl *para*-position (**2f**), as well as the disubstitution of the same phenyl ring (**2h** and **2i**), had marked unfavorable effect on CB_2 affinity. In fact, in these last mentioned cases, K_i values higher than 5000 were determined for the CB_2 receptor, suggesting a negative influence of the steric effect of these substituents on the carboxamide phenyl group.

Compounds **2j**−**m** were prepared to investigate the influence of several arylhydrazide derivatives on the affinity toward CB_2 receptors. In particular, the phenyl (**2j**), *p*-chlorophenyl (**2k**), *p*-nitrophenyl (**2l**), and *p*-methoxyphenyl (**2m**) hydrazides were assayed. All these compounds showed no significant affinity for CB_2 receptors except **2j**, the unsubstituted phenyl hydrazide, which displayed a moderate CB_2 affinity ($K_iCB_2 = 63$ nM).

Three analogues were prepared to explore alternative substitution on the aryl moiety of the core 1,4-dihydroindeno[1,2-*c*]pyrazole ring system. The 6-methyl-7-chloro-disubstituted analogue **2n** bound the CB_2 receptors with a K_i of 25 nM, 3-fold less potent than **2a**. A more marked reduction of affinity was observed for 6-methyl-7-chloro- and the reversed 6-chloro-7-methyl-disubstituted derivatives **2o** and **2p**, respectively.

Generally, the CB_1 receptor affinities of all the investigated compounds were lower than their CB_2 receptor affinities.

In Vitro CB_2 Activity Evaluation. To assess in vitro function, **2a**,**b** and leads **1a**,**b** were tested for intrinsic activity in CB_2 receptors. Our data confirmed previously reported results[12,17,35] on the effect of the CB_2 receptor agonist CP55 940 toward phosphorylated ERK 1/2 (P-ERK 1/2) expression in HL-60 cells (Figure 2). In particular, through the adopted procedure, CP55 940 induced a time- (data not shown) and dose-related induction of P-ERK 1/2 expression in HL-60 cells (Figure 2A), peaking at the concentration of 100 nM, 10 min after exposure ($+103.0 \pm 8.2\%$ vs vehicle). Moreover, the CB_2 receptor antagonist SR144528, which does not alter the P-ERK 1/2 expression, completely blocked this effect if used at a concentration of 50 nM and added 5 min before the exposure with the CB_2 agonist ($+14.5 \pm 12.4\%$ vs vehicle and $-44.0 \pm 8.5\%$ vs CP55 940; Figure 2B).

As shown in Figure 3, the tested compounds **1a**, **1b**, **2a**, and **2b** significantly increased P-ERK 1/2 expression in HL-60 cells. Time course studies showed that the tested compound reached the maximum effect after 10 min of treatment (data not shown) and, as indicated in Figure 3, the plateau was reached at the concentration of 10 nM ($+83.0 \pm 4.2\%$, $+65.0 \pm 18.5\%$, $+61.3 \pm 12.4\%$, and $+125.0 \pm 35.8\%$ vs vehicle for **1a**, **1b**, **2a**, and **2b**, respectively). The increase of P-ERK 1/2 was comparable with that elicited by CP55 940. The exposure to the CB_2 receptor antagonist SR144528 (50 nM) 5 min before the adding of the tested compounds was able to reverse this effect, as shown in Figure 4. Altogether, our data showed that the tested compounds act as CB_2 receptor agonists and that their effect is specific as it was blocked by a CB_2 receptor antagonist.

Conclusions

In the present study, the preparation of a series of new ligands with affinity for the CB_2 subtype of the cannabinoid receptors is reported.

The results obtained from radioligand binding experiments on compounds **2** suggest that substitution of the cyclohexyl carbamoyl groups of **2a** with a phenyl carbamoyl moiety (**2b**) led to a decrease of affinity. Moreover, substitution of the carbamoyl phenyl ring was less tolerated, however, fluoro (**2d**), methyl (**2e**), and methoxyl (**2g**) derivatives maintained a moderate CB_2 affinity. On the other hand, the introduction of an additional substituent at position 3′ or 2′ of the phenyl carbamoyl group led to a substantial decrease of affinity.

We found that substitution of the carbamoyl moiety with the phenylhydrazide unit at position 3 of the indenopyrazole ring system resulted in a first-order decrease of activity. The presence of substituents on the phenyl ring of phenylhydrazide **2j** was detrimental for the CB_2 affinity.

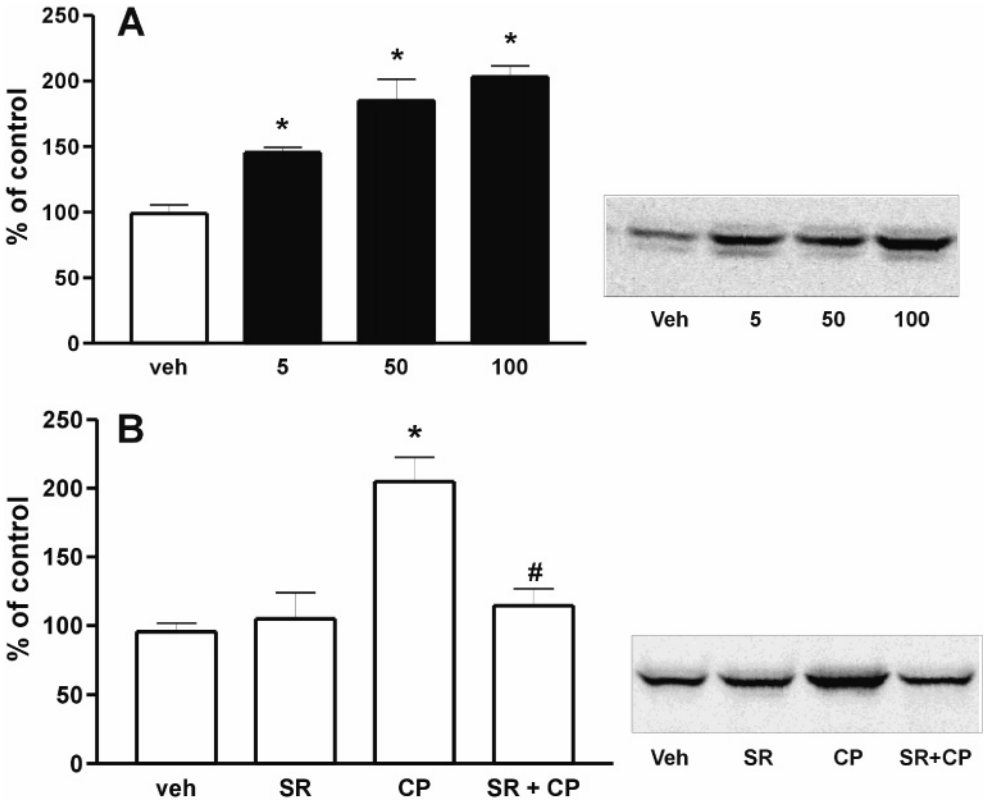


Figure 2. Panel A shows a dose response study of P-ERK 1/2 expression following a 10 min exposure to CB_2 receptor agonist CP55 940 (CP). Doses are expressed in nM. The effect of a 5 min pretreatment with the CB_2 receptor antagonist SR144528 (50 nM; SR) on the agonist-induced P-ERK 1/2 is shown in panel B. On the right are shown representative western blots of the P-ERK expression. Data are expressed as a mean percentage of vehicle ± SEM and are the results of five separate experiments. *$p < 0.05$ vs vehicle; #$p < 0.05$ vs CP55 940.

Finally, the simultaneous introduction of two substituents (CH_3 and Cl) at positions 6 and 7 of the indenopyrazole core gave rise to compounds that retained good to moderate activity.

For the first time, in vitro CB_2 intrinsic activity evaluation assays, based on the determination of P-ERK 1/2 expression increasing in HL-60 cells exposed to the compounds to be assayed, highlighted agonist activity toward CB_2 receptors for two representative terms **2a** and **2b** and for prototype **1a** and **1b**. These results could open interesting pharmacological developments for the potent selective CB_2 agonists **1a** and **1b**, or other analogues, especially for the treatment of immune disorders or, as recently emerged from literature,[36,37] as antinociceptive agents. However, further in vitro and in vivo studies of the more active compounds in this series are needed, in order to define the full range of their pharmacological actions and to demonstrate the therapeutic potential of these molecules.

Experimental Section

General Procedures. 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 ^1H and ^{13}C being observed at 200 and 50 MHz, respectively. Chemical shifts for ^1H and ^{13}C NMR spectra were reported in δ or ppm downfield from TMS [$(CH_3)_4$-Si]. Multiplicities are recorded as s (singlet), br s (broad singlet), d (doublet), t (triplet), q (quartet), qu (quintuplet), dd (doublet of doublets), and m (multiplet). Atmospheric pressure ionization electrospray (API-ES) mass spectra, when reported, were obtained on an 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 an argon atmosphere. Flash chromatography (FC) was performed using Merck silica gel 60 (230−400 mesh ASTM). Thin layer chromatography was performed with Polygram SIL N-HR/HV_{254} precoated plastic sheets (0.2 mm). The pyrazole acid **4a**[31] and the indanone **6c**[32] were prepared according to the previously described literature.

General Procedure I: Synthesis of Carboxamides (2a−i,n) and Carbohydrazides (2j−m,o,p). Method A. A mixture of 1-(2′,4′-dichlorophenyl)-6-methyl-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxilic acid **4a**[31] (1 equiv, 0.69 mmol) and thionyl chloride (3.0 equiv) in toluene (5.22 mL) was refluxed for 3 h. The solvent and the excess of $SOCl_2$ were removed under reduced pressure, and the resulting dark solid in CH_2Cl_2 (2.6 mL) was dropwise added to a solution of requisite amine (1.5 equiv) in CH_2Cl_2 (2.6 mL) at 0 °C. The mixture was warmed to room temperature for 0.5−3 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 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.

Method B. A mixture of the appropriate carboxylic acid **4** (1 equiv, 0.69 mmol), EDC (1.2 equiv), and BtOH (1.2 equiv) in acetonitrile (6.9 mL) was stirred at room temperature for 15 min. A solution of the requisite amine or hydrazine **5** (4 equiv) in acetonitrile (0.7 mL) was dropwise added, and the whole was stirred at room temperature for 2−20 h. The solution was taken up with water, and the precipitate was filtered off and air-dried. The analytically pure product was isolated by trituration with an appropriate solvent, as indicated below.

1-(2′,4′-Dichlorophenyl)-6-methyl-*N*-cyclohexylamine-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxamide (2a). Method A was used to convert **4a** and cyclohexylamine into the title product. The mixture was stirred at room temperature for 3 h and purified by trituration with petroleum ether to afford **2a** (0.17 g, 56.7%) as a

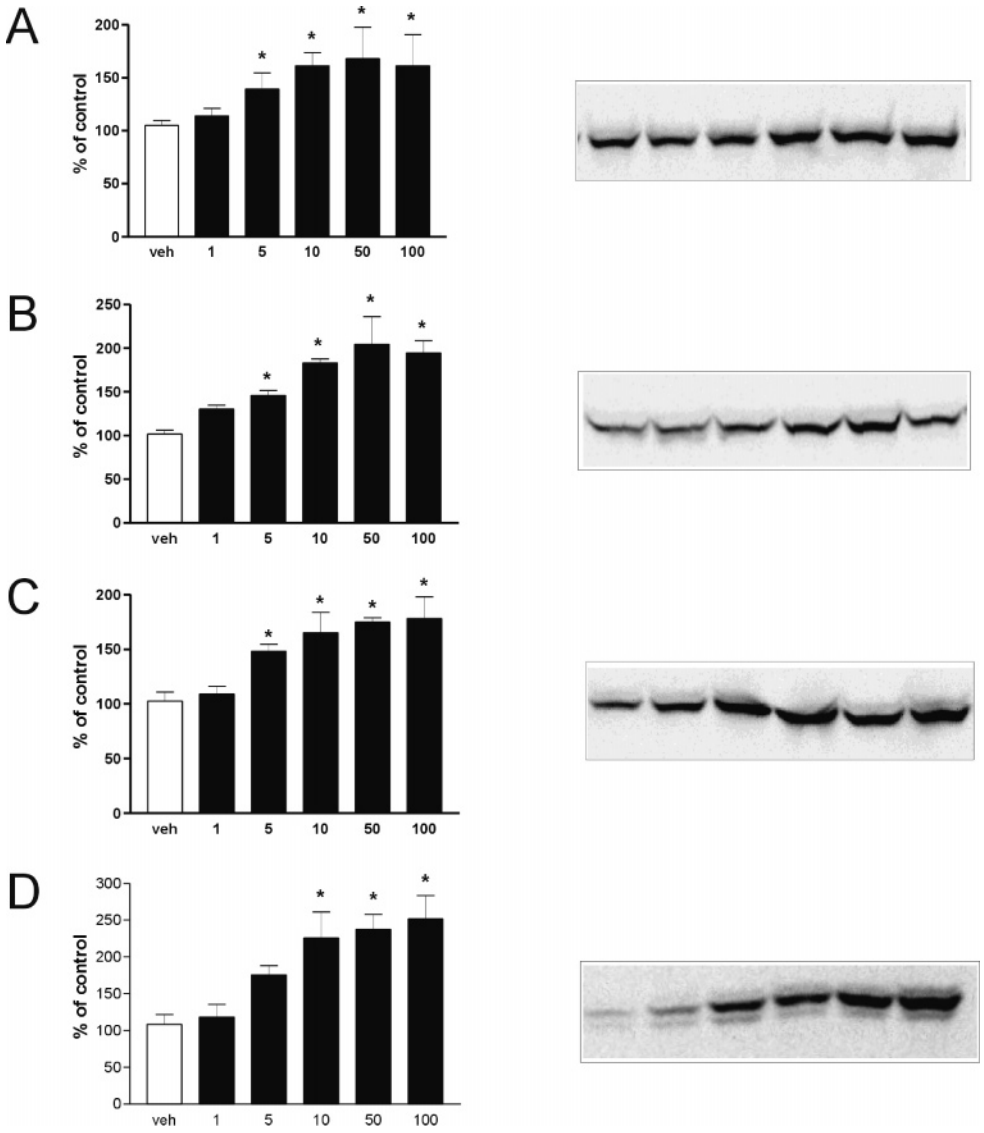


Figure 3. Panels A−D show the effect of different doses (nM) of CB_2 receptor ligands on P-ERK 1/2 expression in HL-60 cells after 10 min of treatment (**1a**, **1b**, **2a**, and **2b**, respectively). On the right side are shown representative western blots of P-ERK expression. Data are expressed as a mean percentage of vehicle ± SEM and are the results of five separate experiments. *$p < 0.05$ vs vehicle; #$p < 0.05$ vs CB_2 ligands (**1a**, **1b**, **2a**, and **2b**, respectively).

cream solid. $R_f = 0.136$ (petroleum ether/EtOAc 9.5:0.5); mp 190.5 °C; IR 1658, 3413; ^{1}H NMR ($CDCl_3$) δ 1.10−1.55 (m, 4H), 1.58−1.85 (m, 4H), 1.97−2.10 (m, 2H), 2.40 (s, 3H), 3.85 (s, 2H), 3.90−4.07 (m, 1H), 6.80 (d, 1H, $J = 7.8$ Hz, NH, exch. with D_2O), 6.88 (d, 1H, $J = 7.8$ Hz), 7.04 (d, 1H, $J = 7.8$ Hz), 7.39 (s, 1H), 7.45 (dd, 1H, $J = 8.4$ Hz, $J = 2.2$ Hz), 7.55 (d, 1H, $J = 8.4$ Hz), 7.66 (d, 1H, $J = 2.2$ Hz); ^{13}C NMR ($CDCl_3$) δ 21.60 (CH_3), 25.00 ($CH_2 \times 2$), 25.60 (CH_2), 29.65 (CH_2), 33.20 ($CH_2 \times 2$), 48.10 (CH), 118.67 (CH), 127.14 (CH), 127.28 (CH), 127.79 (C), 128.14 (CH), 128.67 (C), 129.75 (CH), 130.46 (CH), 131.94 (C), 135.91 (C), 136.09 (C), 137.13 (C), 142.28 (C), 150.05 (C), 152.04 (C), 161.00 (CO). API-ES calcd, 440.36; found, 440.20.

1-(2′,4′-Dichlorophenyl)-6-methyl-*N*-phenyl-1,4-dihydroindeno-[1,2-*c*]pyrazole-3-carboxamide (2b). Method A was used to convert **4a** and aniline into the title product. The mixture was stirred at room temperature for 2 h and purified by FC (petroleum ether/EtOAc 7:3) to afford **2b** (0.18 g, 60.0%) as a cream solid. $R_f = 0.204$ (petroleum ether/EtOAc 9.5:0.5); mp 197.8 °C; IR 1687, 3389; ^{1}H NMR ($CDCl_3$) δ 2.41 (s, 3H), 3.91 (s, 2H), 6.91 (d, 1H, $J = 7.8$ Hz), 7.04−7.20 (m, 2H), 7.30−7.45 (m, 3H), 7.49 (dd, 1H, $J = 8.0$ Hz, $J = 2.2$ Hz), 7.59 (d, 1H, $J = 8.4$ Hz), 7.69−7.34 (m, 3H), 8.73 (br s, 1H, NH, exch. with D_2O); ^{13}C NMR ($CDCl_3$) δ 21.63 (CH_3), 29.69 (CH_2), 118.76 (CH), 119.62 (CH × 2), 124.11 (CH), 127.42 (CH), 128.00 (C), 128.21 (CH), 128.22 (CH), 128.50 (C), 129.01 (CH × 2), 129.68 (CH), 130.56 (CH), 130.90 (C), 132.00 (C), 135.90 (C), 136.20 (C), 137.65 (C), 137.85 (C), 142.00 (C), 149.80 (C), 159.80 (CO); API-ES calcd, 434.32; found, 434.20.

1-(2′,4′-Dichlorophenyl)-6-methyl-*N*-*p*-chlorophenyl-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxamide (2c). Method A was used to convert **4a** and *p*-chloroaniline into the title product. The mixture was stirred at room temperature for 2 h and purified by trituration with petroleum ether to afford **2c** (0.18 g, 57.6%) as a cream solid. $R_f = 0.227$ (petroleum ether/EtOAc 9.5:0.5); mp 211.5 °C; IR 1678, 3374; ^{1}H NMR ($CDCl_3$) δ 2.41 (s, 3H), 3.90 (s, 2H), 6.90 (d, 1H, $J = 7.8$ Hz), 7.06 (d, 1H, $J = 7.8$ Hz), 7.32 (d, 2H, $J = 8.8$ Hz), 7.41 (s, 1H), 7.49 (dd, 1H, $J = 6.8$ Hz, $J = 1.8$ Hz), 7.58 (d, 1H, $J = 8.2$ Hz), 7.67 (d, 2H, $J = 8.8$ Hz), 7.69 (d, 2H, $J = 2.2$ Hz), 8.74 (s, 1H, NH, exch. with D_2O); ^{13}C NMR ($CDCl_3$) δ 21.64 (CH_3), 29.62 (CH_2), 118.79 (CH), 120.78 (CH × 2), 127.22 (CH), 127.47 (CH), 127.95 (C), 128.24 (CH), 128.43 (C), 129.04 (CH × 2), 129.65 (CH), 130.58 (CH), 131.97 (C), 135.84 (C), 136.25(C), 136.40 (C), 137.53 (C), 141.68 (C), 149.91 (C × 2), 152.57 (C), 159.72 (CO); API-ES calcd, 468.76; found, 469.20.

1-(2′,4′-Dichlorophenyl)-6-methyl-*N*-*p*-fluorophenyl-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxamide (2d). Method A was used to convert **4a** and *p*-fluoroaniline into the title product. The mixture was stirred at room temperature for 1 h and purified by trituration with petroleum ether to afford **2d** (0.20 g, 65.8%) as a

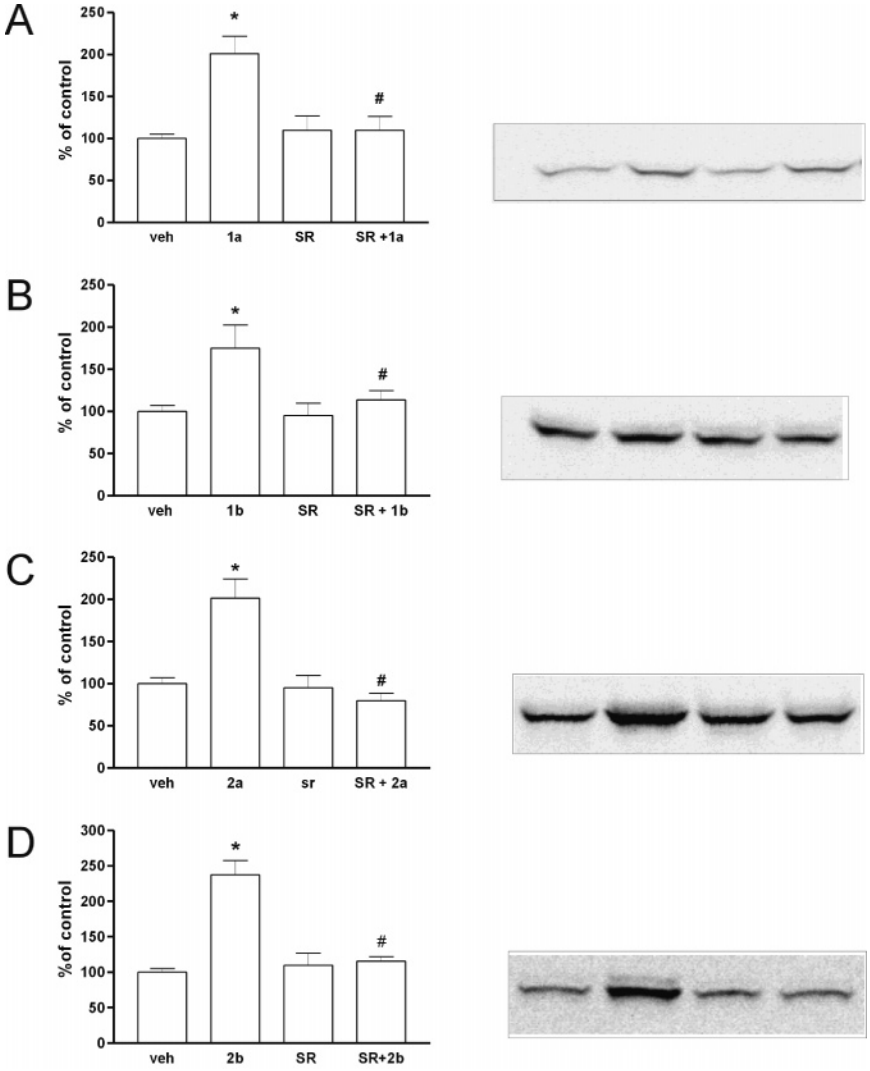


Figure 4. Panels A−D show the CB_2 receptor antagonist SR144528 (SR) inhibition of P-ERK 1/2 activation. CB_2 receptor ligands were used at a concentration of 50 nM. On the right side are shown representative western blots of P-ERK expression. Data are expressed as a mean percentage of vehicle ± SEM and are the results of five separate experiments. *$p < 0.05$ vs vehicle; #$p < 0.05$ vs CB_2 ligands (**1a**, **1b**, **2a**, and **2b**, respectively).

cream solid. $R_f = 0.189$ (petroleum ether/EtOAc 9.5:0.5); mp 206−208 °C; IR 1689, 3373; ^{1}H NMR ($CDCl_3$) δ 2.41 (s, 3H), 3.89 (s, 2H), 6.89 (d, 1H, $J = 7.8$ Hz), 7.00−7.12 (m, 3H), 7.40 (s, 1H), 7.48 (dd, 1H, $J = 8.4$ Hz, $J = 1.8$ Hz), 7.58 (d, 1H, $J = 8.6$ Hz), 7.62−7.75 (m, 3H), 8.73 (s, 1H, NH, exch. with D_2O); ^{13}C NMR ($CDCl_3$) δ 21.60 (CH_3), 29.59 (CH_2), 115.39 (CH), 115.83 (CH), 118.75 (CH), 121.19 (CH), 121.35 (CH), 127.18 (CH), 127.43 (CH), 127.91 (C), 128.20 (CH), 128.42 (CH), 129.62 (C), 130.54 (CH), 131.94 (C), 133.79 (C), 135.83 (C), 136.17 (C), 137.45 (C), 141.75 (C), 149.90 (C), 152.47 (C), 156.79 (C), 159.65 (CO); API-ES calcd, 452.31; found, 452.20.

1-(2′,4′-Dichlorophenyl)-6-methyl-*N*-*p*-methylphenyl-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxamide (2e). Method A was used to convert **4a** and *p*-toluidine into the title product. The mixture was stirred at room temperature for 2 h and purified by trituration with petroleum ether to afford **2e** (0.22 g, 73.0%) as a pink solid. $R_f = 0.212$ (petroleum ether/EtOAc 9.5:0.5); mp 119 °C; IR 1684, 3389; ^{1}H NMR ($CDCl_3$) δ 2.34 (s, 3H), 2.41 (s, 3H), 3.90 (s, 2H), 6.90 (d, 1H, $J = 7.6$ Hz), 7.06 (d, 1H, $J = 7.6$ Hz), 7.16 (d, 2H, $J = 8.4$ Hz), 7.41 (s, 1H), 7.49 (dd, 1H, $J = 8.4$ Hz, $J = 2.2$ Hz), 7.57−7.62 (m, 3H), 7.68 (d, 1H, $J = 2.2$ Hz), 8.69 (br s, 1H, NH, exch. with D_2O); ^{13}C NMR ($CDCl_3$) δ 20.88 (CH_3), 21.61 (CH_3), 29.65 (CH_2), 118.75 (CH), 119.63 (CH × 2), 127.19 (CH), 127.39 (CH), 127.96 (C), 128.20 (CH), 128.53 (C), 129.50 (CH × 2), 129.69 (CH), 130.54 (CH), 131.97 (C), 133.68 (C), 135.25 (C), 135.95 (C), 136.11 (C), 137.37 (C), 142.09 (C), 149.98 (C), 152.40 (C), 159.62 (CO); API-ES calcd, 448.34; found, 448.20.

1-(2′,4′-Dichlorophenyl)-6-methyl-*N*-*p*-trifluoromethylphenyl-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxamide (2f). Method A was used to convert **4a** and *p*-trifluoroaniline into the title product. The mixture was stirred at room temperature for 2.5 h and purified by FC (petroleum ether/EtOAc 9.5:0.5) to afford **2f** (0.06 g, 19.0%) as an orange solid. $R_f = 0.303$ (petroleum ether/EtOAc 9.5:0.5); mp 223 °C; IR 1696, 3373; ^{1}H NMR ($CDCl_3$) δ 2.42 (s, 3H), 3.91 (s, 2H), 6.90 (d, 1H, $J = 7.8$ Hz), 7.07 (d, 1H, $J = 8.0$ Hz), 7.42 (s, 1H), 7.50 (dd, 1H, $J = 8.0$ Hz, $J = 1.8$ Hz), 7.56−7.62 (m, 3H), 7.69 (d, 1H, $J = 2.2$ Hz), 7.84 (d, 2H, $J = 8.6$ Hz), 8.89 (br s, 1H, NH, exch. with D_2O); ^{13}C NMR ($CDCl_3$) δ 21.63 (CH_3), 29.63 (CH_2), 118.82 (CH), 119.16 (CH), 126.19 (CH), 126.26 (CH), 126.34 (CH), 127.23 (CH), 127.52 (CH), 128.02 (C), 128.26 (CH), 128.38 (C), 129.65 (CH), 130.61 (CH), 132.01 (C), 135.80 (C), 136.34 (C), 137.62 (C × 2), 140.88 (C), 141.49 (C), 149.88 (C × 2), 152.72 (C), 159.93 (CO); API-ES calcd, 502.31; found, 502.20.

1-(2′,4′-Dichlorophenyl)-6-methyl-*N*-*p*-methoxyphenyl-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxamide (2g). Method A was used to convert **4a** and *p*-anisidine into the title product. The mixture was stirred at room temperature for 0.5 h and purified by trituration with petroleum ether to afford **2e** (0.12 g, 38.5%) as a gray solid. $R_f = 0.341$ (petroleum ether/EtOAc 8:2); mp 148 °C; IR 1675, 3358; ^{1}H NMR ($CDCl_3$) δ 2.41 (s, 3H), 3.82 (s, 3H), 3.90 (s, 2H), 6.90 (d, 3H), 7.06 (d, 1H, $J = 7.6$ Hz), 7.41 (s, 1H), 7.49 (dd, 1H, $J = 6.2$ Hz, $J = 1.8$ Hz), 7.57−7.65 (m, 3H), 7.69 (d, 1H, $J = 2.2$ Hz), 8.64 (br s, 1H, NH, exch. with D_2O); ^{13}C NMR ($CDCl_3$) δ 21.62 (CH_3), 29.65 (CH_2), 55.48 (CH_3), 114.16 (CH), 118.75 (CH),

121.29 (CH × 2), 127.20 (CH), 127.40 (CH), 127.92 (C), 128.21 (CH), 128.43 (C), 129.68 (CH × 2), 130.55 (CH), 130.97 (C), 131.97 (C), 136.24 (C), 136.40 (C), 137.37 (C), 141.68 (C), 149.99 (C), 152.57 (C), 156.25 (C), 159.44 (CO); API-ES calcd, 464.34; found, 467.20.

1-(2′,4′-Dichlorophenyl)-6-methyl-*N*-*m*, *p*-dichlorophenyl-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxamide (2h). Method B was used to convert **4a** and 2,4-dichloroaniline into the title product. The mixture was stirred at room temperature for 2 h and purified by trituration with petroleum ether to afford **2h** (0.22 g, 62.8%) as a white solid. R_f = 0.204 (petroleum ether/EtOAc 9.5:0.5); mp 186−187 °C; IR 1710, 3347; ^{1}H NMR ($CDCl_3$) δ 2.43 (s, 3H), 3.84 (s, 2H), 6.95−7.14 (m, 3H), 7.40−7.70 (m, 6H), 8.12 (br s, 1H, NH, exch. with D_2O); ^{13}C NMR ($CDCl_3$) δ 20.68 (CH_3), 29.62 (CH_2), 121.71 (CH), 121.98 (CH), 124.68 (CH), 126.21 (CH), 127.71 (CH), 129.45 (CH), 129.86 (CH × 2), 130.10 (CH), 132.40 (C), 132.00 (C), 132.72 (C), 133.14 (C), 133.87 (C), 134.21 (C), 135.61 (C), 142.05 (C), 146.01 (C), 151.27 (C), 152.91 (C), 156.63 (C), 158.03 (CO); API-ES calcd, 503.21; found, 503.10.

1-(2′,4′-Dichlorophenyl)-6-methyl-*N*-*o*, *p*-dichlorophenyl-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxamide (2i). Method A was used to convert **4a** and 3,4-dichloroaniline into the title product. The mixture was stirred at room temperature for 3 h and purified by trituration with petroleum ether to afford **2i** (0.20 g, 58.8%) as a white solid. R_f = 0.166 (petroleum ether/EtOAc 9.5:0.5); mp 259−269 °C; IR 1693, 3390; ^{1}H NMR ($CDCl_3$) δ 2.42 (s, 3H), 3.90 (s, 2H), 6.90 (d, 1H, J = 8.0 Hz), 7.06 (d, 1H, J = 7.8 Hz), 7.35−7.61 (m, 5H), 7.69 (s, 1H), 8.02 (s, 1H), 8.77 (br s, 1H, NH, exch. with D_2O); ^{13}C NMR ($CDCl_3$) δ 20.68 (CH_3), 29.44 (CH_2), 121.71 (CH), 121.98 (CH), 124.68 (CH), 126.21 (CH), 127.71 (CH), 129.45 (CH), 129.86 (CH × 2), 130.10 (CH), 131.40 (C), 132.00 (C), 132.72 (C), 133.14 (C), 133.87 (C), 134.21 (C), 135.61 (C), 142.05 (C), 146.01 (C), 151.27 (C), 151.91 (C), 156.63 (C), 158.03 (CO); API-ES calcd, 503.21; found, 503.10.

1-(2′,4′-Dichlorophenyl)-6-methyl-*N*-phenyl-1,4-dihydroindeno-[1,2-*c*]pyrazole-3-carbohydrazide (2j). Method B was used to convert **4a** and phenylhydrazine into the title product. The mixture was stirred at room temperature for 9 h and purified by trituration with petroleum ether to afford **2j** (0.26 g, 83.1%) as an orange solid. R_f = 0.194 (petroleum ether/EtOAc 8:2); mp 195−196 °C; IR 1685, 3347; ^{1}H NMR ($CDCl_3$) δ 2.40 (s, 3H), 3.84 (s, 2H), 6.21 (d, 1H, J = 4.2 Hz, NH, exch. with D_2O), 6.85−7.10 (m, 5H), 7.20−7.30 (m, 2H), 7.38 (s, 1H), 7.48 (dd, 1H, J = 8.6 Hz, J = 2.2 Hz), 7.58 (d, 1H, J = 8.6 Hz), 7.69 (d, 1H, J = 2.2 Hz), 8.59 (d, 1H, J = 4.2 Hz, NH, exch. with D_2O); ^{13}C NMR ($CDCl_3$) δ 21.61 (CH_3), 29.52 (CH_2), 113.72 (CH × 2), 118.80 (CH), 121.24 (CH), 127.17 (CH), 127.43 (CH), 128.20 (CH), 128.41 (C), 129.17 (CH × 2), 129.59 (CH), 130.58 (CH), 131.86 (C), 135.88 (C), 136.14 (C), 137.44 (C × 2), 140.19 (C), 148.10 (C), 149.86 (C), 152.07 (C), 161.94 (CO); API-ES calcd, 449.33; found, 449.23.

1-(2′,4′-Dichlorophenyl)-6-methyl-*N*-*p*-chlorophenyl-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carbohydrazide (2k). Method B was used to convert **4a** and *p*-chlorophenylhydrazine into the title product. The mixture was stirred at room temperature for 3 h and purified by trituration with petroleum ether to afford **2k** (0.30 g, 90.8%) as a cream solid. R_f = 0.070 (petroleum ether/EtOAc 8:2); mp 245.5 °C; IR 1658, 3370; ^{1}H NMR ($CDCl_3$) δ 2.41 (s, 3H), 3.84 (s, 2H), 6.91 (d, 1H, J = 7.8 Hz), 7.06 (d, 1H, J = 7.8 Hz), 7.35−7.52 (m, 4H), 7.60 (d, 1H, J = 8.2 Hz), 7.68 (d, 1H, J = 2.2 Hz), 7.94 (d, 2H, J = 8.4 Hz), 9.21 (d, 1H, NH, exch. with D_2O), 10.26 (br s, 1H, NH, exch. with D_2O); ^{13}C NMR ($CDCl_3$) δ 21.40 (CH_3), 29.22 (CH_2), 118.56 (CH), 126.81 (CH), 127.18 (CH), 127.71 (C), 127.65 (CH), 128.12 (C), 128.35 (CH × 2), 129.07 (CH × 2), 129.50 (CH), 130.12 (CH), 131.30 (C), 135.64 (C), 135.75 (C), 137.08 (C × 2), 137.84 (C), 139.74 (C), 149.38 (C × 2), 160.02 (CO); API-ES calcd, 483.78; found, 483.68.

1-(2′,4′-Dichlorophenyl)-6-methyl-*N*-*p*-nitrophenyl-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carbohydrazide (2l). Method B was used to convert **4a** and *p*-nitrophenylhydrazine into the title product. The mixture was stirred at room temperature for 9 h and purified by FC (petroleum ether/EtOAc 8:2) to afford **2l** (0.17 g, 49.4%) as a yellowish solid. R_f = 0.10 (petroleum ether/EtOAc 8:2); mp 121.7−122 °C; IR 1682, 3347; ^{1}H NMR ($CDCl_3$) δ 2.41 (s, 3H), 3.84 (s, 2H), 6.52 (br s, 1H, NH, exch. with D_2O), 6.91 (d, 1H, J = 7.8 Hz), 6.97 (d, 1H, J = 9.0 Hz), 7.07 (d, 1H, J = 8.2 Hz), 7.09 (d, 1H, J = 8.2 Hz), 7.39 (s, 1H), 7.50 (dd, 1H, J = 8.2 Hz; J = 2 Hz), 7.58 (d, 1H, J = 8.4 Hz), 7.70 (d, 1H, J = 1.8 Hz), 8.16 (d, 2H, J = 9.0 Hz), 8.65 (br s, 1H, NH, exch. with D_2O); ^{13}C NMR ($CDCl_3$) δ 21.64 (CH_3), 29.49 (CH_2), 112.19 (CH), 118.87 (CH × 2), 125.86 (CH × 2), 127.21 (CH), 127.56 (CH), 128.20 (CH), 128.29 (C × 2), 129.50 (CH), 130.66 (CH), 131.86 (C), 135.70 (C), 136.38 (C), 137.73 (C), 139.52 (C), 141.26 (C), 149.73 (C), 152.35 (C), 153.56 (C), 161.94 (CO); API-ES calcd, 494.33; found, 434.23.

1-(2′,4′-Dichlorophenyl)-6-methyl-*N*-*p*-methoxyphenyl-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carbohydrazide (2m). Method B was used to convert **4a** and *p*-methoxyphenylhydrazine into the title product. The mixture was stirred at room temperature for 9 h and purified by FC (petroleum ether/EtOAc 6:4) to afford **2m** (0.07 g, 20.9%) as an orange solid. R_f = 0.151 (petroleum ether/EtOAc 9.5:0.5); mp 62−63 °C; IR 1670, 3347; ^{1}H NMR ($CDCl_3$) δ 2.40 (s, 3H), 3.75 (s, 3H), 3.84 (s, 2H), 6.10 (br s, 1H, NH, exch. with D_2O), 6.82 (d, 2H, J = 8.6 Hz), 6.89−6.98 (m, 3H), 7.07 (d, 1H, J = 8.2 Hz), 7.39 (s, 1H), 7.49 (dd, 1H, J = 8.2 Hz; J = 2 Hz), 7.56 (d, 1H, J = 8.0 Hz), 7.68 (d, 1H, J = 2.0 Hz), 8.65 (br s, 1H, NH, exch. with D_2O); ^{13}C NMR ($CDCl_3$) δ 21.62 (CH_3), 29.54 (CH_2), 55.63 (CH_3), 114.60 (CH), 115.72 (CH), 118.79 (CH), 127.18 (CH), 127.42 (CH), 128.14 (C), 128.21 (CH), 128.42 (C), 128.82 (CH), 129.59 (CH), 130.57 (CH), 130.90 (CH), 131.86 (C), 135.89 (C), 135.12 (C), 137.43 (C), 140.24 (C), 141.77 (C), 149.85 (C), 152.04 (C), 154.74 (C), 161.97 (CO); API-ES calcd, 479.36; found, 479.26.

7-Chloro-1-(2′,4′-dichlorophenyl)-6-methyl-*N*-cyclohexylamine-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxamide (2n). Method B was used to convert **4b** and cyclohexylamine into the title product using CH_2Cl_2 instead of acetonitrile. The mixture was stirred at room temperature for 20 h and purified by FC (petroleum ether/EtOAc 9:1) to afford **2n** (0.20 g, 63%) as a pale yellow solid. R_f = 0.24 (petroleum ether/EtOAc 9:1); mp 241−243 °C; IR 1658, 3413; ^{1}H NMR ($CDCl_3$) δ 1.07−2.10 (m, 10H), 2.41 (s, 3H), 3.84 (s, 2H), 3.76−4.10 (m, 1H), 6.79 (d, 1H, J = 8.4 Hz, NH, exch. with D_2O), 6.94 (s, 1H), 7.43 (s, 1H), 7.43−7.75 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 20.42 (CH_3), 24.96 (CH_2 × 2), 25.55 (CH_2), 29.34 (CH_2), 33.15 (CH_2 × 2), 48.10 (CH), 119.43 (CH), 128.31 (CH), 128.56 (CH), 128.69 (C), 129.62 (CH), 130.33 (C), 130.61 (CH), 131.76 (C), 132.70 (C), 134.67 (C), 135.73 (C), 136.20 (C), 142.29 (C), 148.09 (C), 150.73 (C), 160.76 (CO); API-ES calcd, 474.81; found, 474.25.

7-Chloro-1-(2′,4′-dichlorophenyl)-6-methyl-*N*-piperidin-1-yl-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxamide (2o). Method B was used to convert **4b** and *N*-aminopiperidine into the title product using CH_2Cl_2 instead of acetonitrile. The mixture was stirred at room temperature for 7 h and purified by FC (petroleum ether/EtOAc 8:2) to afford **2o** (0.21 g, 64%) as a yellow solid. R_f = 0.14 (petroleum ether/EtOAc 8:2); mp 230−232 °C; IR 1685, 3395; ^{1}H NMR ($CDCl_3$) δ 1.33−1.90 (m, 6H), 2.41 (s, 3H), 2.73−3.09 (m, 4H), 3.84 (s, 2H), 6.94 (s, 1H), 7.42 (s, 1H), 7.43−7.76 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 20.42 (CH_3), 23.29 (CH_2), 25.36 (CH_2 × 2), 29.32 (CH_2), 57.07 (CH_2 × 2), 119.45 (CH), 128.32 (CH), 128.56 (CH), 129.17 (C), 129.58 (CH), 130.21 (C), 130.64 (CH), 131.74 (C), 132.71 (C), 134.78 (C), 135.64 (C), 136.26 (C), 141.43 (C), 148.09 (C), 150.64 (C), 158.95 (CO). API-ES calcd, 475.80; found, 476.05.

6-Chloro-1-(2′,4′-dichlorophenyl)-7-methyl-*N*-piperidin-1-yl-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxamide (2p). Method B was used to convert **4c** and *N*-aminopiperidine into the title product using CH_2Cl_2 instead of acetonitrile. The mixture was stirred at room temperature for 20 h and purified by FC (petroleum ether/EtOAc 6:4) to afford **2p** (0.18 g, 54%) as a yellow solid. R_f = 0.25 (petroleum ether/EtOAc 6:4); mp 235−237 °C; IR 1684, 3346; ^{1}H NMR ($CDCl_3$) δ 1.05−1.93 (m, 6H), 2.34 (s, 3H), 2.78−3.05 (m, 4H), 3.85 (s, 2H), 6.80 (s, 1H), 7.40−7.60 (m, 2H), 7.53 (s,

1H), 7.60−7.79 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 20.36 (CH_3), 23.29 (CH_2), 25.36 ($CH_2 \times 2$), 29.31 (CH_2), 57.06 ($CH_2 \times 2$), 120.82 (CH), 126.91 (CH), 128.29 (CH), 129.02 (C), 129.69 (CH), 129.81 (C), 130.55 (CH), 131.85 (C), 133.23 (C), 134.48 (C), 135.77 (C), 136.19 (C), 141.43 (C), 148.48 (C), 150.87 (C), 158.97 (CO); API-ES calcd, 475.80; found, 475.25.

Synthesis of 5-Methyl-6-nitroindanone (10a) and 5-Methyl-4-nitroindanone (10b). To a mixture of 5-methylindanone **9**[31] (20.52 mmol, 1 equiv) in concentrated H_2SO_4 (26 mL) cooled at −5 °C, a solution of KNO_3 (0.9 equiv) in concentrated H_2SO_4 (6.7 mL) was dropwise added in 2 h. The mixture was stirred for 2 h at the same temperature and then poured in ice. The resulting mixture was stirred for 13 h at room temperature, then extracted with Et_2O and washed (H_2O). The organic layers, dried (Na_2SO_4) and concentrated, afforded a crude product that was purified by FC (petroleum ether/EtOAc, 9:1) to afford the analytically pure products, which showed two bands at R_f 0.20 and 0.41. The component at R_f 0.20 (2.31 g, 66%) is a pale yellow solid. IR, ^{1}H NMR spectra, and elemental analysis showed it to be 5-methyl-6-nitroindanone (**10a**); IR 1690; ^{1}H NMR ($CDCl_3$) δ 2.68 (s, 3H), 2.73−2.91 (m, 2H), 3.14−3.31 (m, 2H), 7.47 (s, 1H), 8.31 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 21.13 (CH_3), 25.76 (CH_2), 36.52 (CH_2), 120.08 (CH), 130.74 (CH), 135.94 (C), 139.88 (C), 149.16 (C), 158.55 (C), 204.40 (CO). The component at R_f 0.41 (0.93 g, 26%) is a yellow solid. IR, ^{1}H NMR spectra, and elemental analysis showed it to be 5-methyl-4-nitroindanone (**10b**); IR 1690; ^{1}H NMR ($CDCl_3$) δ 2.59 (s, 3H), 2.70−2.87 (m, 2H), 3.25−3.42 (m, 2H), 7.40 (d, 1H, J = 7.8 Hz), 7.83 (d, 1H, J = 7.8 Hz); ^{13}C NMR ($CDCl_3$) δ 19.56 (CH_3), 24.69 (CH_2), 35.99 (CH_2), 126.59 (CH), 132.02 (CH), 137.45 (C), 138.97 (C), 148.32 (C), 148.33 (C), 204.14 (CO).

Synthesis of 6-Amino-5-methylindan-1-one (11). A solution of 5-methyl-6-nitroindanone **10a** (2.51 g, 13.13 mmol) in 38 mL of EtOH was hydrogenated in a Parr shaker over 0.28 g (0.26 mmol) of 10% Pd/C under a hydrogen pressure of 2 atm at room temperature for 1 h. The mixture was filtered through Celite, and the catalyst was washed with several portions of hot EtOH. The solution was evaporated, and the residue was purified by FC ($CHCl_3$/MeOH 9.8:0.2) to afford a pale yellow solid (1.58 g, 75%). R_f = 0.41 ($CHCl_3$/MeOH 9.8:0.2); mp 186−186.5 °C; IR 1700; ^{1}H NMR ($CDCl_3$) δ 2.26 (s, 3H), 2.60−2.80 (m, 2H), 2.93−3.14 (m, 2H), 3.72 (br s, 2H), 7.00 (s, 1H), 7.17 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 18.43 (CH_3), 24.95 (CH_2), 36.73 (CH_2), 107.53 (CH), 127.95 (CH), 131.42 (C), 141.52 (C), 144.27 (C), 146.03 (C), 207.14 (CO).

Synthesis of 6-Chloro-5-methylindan-1-one (6b). To a mixture of aminoketone **11** (1.86 mmol, 1 equiv) in a 15% solution of HCl (2.50 mL) was cautiously added an aqueous solution of $NaNO_2$ (1.2 equiv, 1 mL), and the whole was stirred at 0 °C. The resulting solution was dropwise added to a mixture of CuCl (3.6 equiv) in concentrated HCl (5.30 mL) at the same temperature. The resulting mixture was stirred for 24 h at room temperature, then poured in water, and extracted with EtOAc. The organic layers, dried (Na_2SO_4) and concentrated, afforded a crude product that was purified by FC (petroleum ether/EtOAc, 8:2), furnishing the analytically pure products as pale yellow solid (0.34 g, quantitative yield). R_f = 0.48 (petroleum ether/EtOAc 8:2); mp 96−98 °C; IR 1690; ^{1}H NMR ($CDCl_3$) δ 2.46 (s, 3H), 2.67−2.73 (m, 2H), 3.05−3.11 (m, 2H), 7.36 (s, 1H), 7.72 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 21.08 (CH_3), 25.27 (CH_2), 36.60 (CH_2), 123.90 (CH), 128.71 (CH), 136.45 (C), 141.50 (C), 143.29 (C), 153.34 (C), 205.18 (CO).

General Procedure II: Synthesis of α,γ-Diketoesters (7b,c). Sodium metal (2.0 equiv) was added in small portion to dry ethanol (1.8 mL) and stirred until all the sodium had reacted. Ethyl oxalate (2.0 eq) was added, followed by dropwise addition of a solution of appropriate ketone **6b**,**c** starting material (1.0 equiv, 1.88 mmol) in dry ethanol (18 mL). The mixture was stirred at room temperature for 24 h and than it was slowly poured into ice and 2 N HCl was added. The precipitate was filtered, washed with cool ethanol, and air-dried to yield the analytically pure diketoester.

Ethyl α-(6-Chloro-5-methyl-1-oxo-2,3-dihydro-1*H*-inden-2-yl)-α-oxo-acetate (7b). General procedure IV was used to convert **6b** into the title product. Compound **7a** (0.51 g, 96.0%) was isolated as a white solid. R_f = 0.21 (petroleum ether/EtOAc 9:1); IR 1680, 1730, 3440; ^{1}H NMR ($CDCl_3$) δ 1.43 (t, 3H, J = 7.2 Hz), 2.49 (s, 3H), 3.92 (s, 2H), 4.42 (q, 2H, J = 7.2 Hz), 7.42 (s, 1H), 7.82 (s, 1H), 13.20 (br s, 1H, OH exchange with D_2O); ^{13}C NMR ($CDCl_3$) δ 14.15 (CH_3), 21.27 (CH_3), 31.01 (CH_2), 62.31 (CH_2), 116.58 (C), 124.08 (CH), 128.22 (CH), 134.58 (C), 136.38 (C), 144.00 (C), 148.71 (C), 153.91 (C), 162.59 (CO), 197.30 (CO).

Ethyl α-(5-Chloro-6-methyl-1-oxo-2,3-dihydro-1*H*-inden-2-yl)-α-oxo-acetate (7c). General procedure IV was used to convert **6c** into the title product. Compound **7c** (0.46 g, 87.0%) was isolated as a white solid. R_f = 0.50 (petroleum ether/EtOAc 7:3); IR 1685, 1725, 3445; ^{1}H NMR ($CDCl_3$/DMSO) δ 1.44 (t, 3H, J = 7.2 Hz), 2.45 (s, 3H), 3.93 (s, 2H), 4.45 (q, 2H, J = 7.2 Hz), 7.54 (s, 1H), 7.71 (s, 1H), 14.50 (br s, 1H, OH exchange with D_2O); ^{13}C NMR ($CDCl_3$) δ 14.15 (CH_3), 21.27 (CH_3), 31.01 (CH_2), 62.31 (CH_2), 116.38 (C), 124.08 (CH), 128.22 (CH), 134.58 (C), 136.38 (C), 144.00 (C), 148.71 (C), 153.75 (C), 162.59 (CO), 197.30 (CO).

General Procedure III: Synthesis of Tricyclic Esters (8b,c). A stirred mixture of diketoester **7b,c** (1.0 equiv, 7.20 mmol) and 2,4-dichlorophenylhydrazine hydrochloride (1.1 equiv) in CH_3-COOH (54 mL) was heated under reflux for 8 h. The reaction was allowed to cool to room temperature, and the precipitate was filtered, washed with water, and air-dried to yield the analytically pure ester.

Ethyl 7-Chloro-1-(2′,4′-dichlorophenyl)-6-methyl-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxylate (8b). General procedure III was used to convert **7b** and 2,4-dichlorophenylhydrazine hydrochloride into the title product **8b** (3.03 g, quantitative yield) as a pale yellow solid. R_f = 0.24 (petroleum ether/EtOAc 9:1); mp 216−218 °C; IR 1725; ^{1}H NMR ($CDCl_3$/DMSO) δ 1.44 (t, 3H, J = 7.0 Hz), 2.41 (s, 3H), 3.80 (s, 2H), 4.44 (q, 2H, J = 7.2 Hz), 6.93 (s, 1H), 7.43−7.75 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 14.46 (CH_3), 20.52 (CH_3), 29.41 (CH_2), 61.25 (CH_2), 120.95 (CH), 126.85 (CH), 128.21 (CH), 129.94 (C), 129.96 (CH), 130.33 (CH), 131.86 (C), 133.26 (C), 134.69 (C), 135.78 (C), 136.34 (C), 139.35 (C), 147.88 (C × 2), 150.76 (C), 162.09 (CO).

Ethyl 6-Chloro-1-(2′,4′-dichlorophenyl)-7-methyl-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxylate (8c). General procedure III was used to convert **7c** and 2,4-dichlorophenylhydrazine hydrochloride into the title product **8c** (1.24 g, 89.0%) as a pale yellow solid. R_f = 0.31 (petroleum ether/EtOAc 9:1); mp 245−246 °C; IR 1720; ^{1}H NMR ($CDCl_3$) δ 1.44 (t, 3H, J = 7.2 Hz), 2.34 (s, 3H), 3.81 (s, 2H), 4.47 (q, 2H, J = 7.2 Hz), 6.93 (s, 1H), 7.43−7.75 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 14.44 (CH_3), 20.37 (CH_3), 29.40 (CH_2), 61.27 (CH_2), 120.95 (CH), 126.85 (CH), 128.21 (CH), 129.94 (C), 129.96 (CH), 130.33 (CH), 131.86 (C), 133.27 (C), 134.69 (C), 135.80 (C), 136.28 (C), 139.35 (C), 147.88 (C × 2), 150.76 (C), 162.09 (CO).

General Procedure IV: Synthesis of Carboxylic Acids (4b,c). A mixture of ester **8b**,**c** (1.0 equiv, 0.5 mmol) and potassium hydroxide in pellets (13 equiv) in EtOH/H_2O 1:1 (6 mL) was heated under reflux for 4 h. The mixture was concentrated and then acidified with 37% HCl. The precipitate was filtered, washed with water, and air-dried to yield the analytically pure acid.

7-Chloro-1-(2′,4′-dichlorophenyl)-6-methyl-1,4-dihydroindeno-[1,2-*c*]pyrazole-3-carboxylic Acid (4b). General procedure II was used to convert **8b** into the title product **4b** (0.20 g, quantitative yield) as a yellow solid. R_f = 0.47 ($CHCl_3$/MeOH 9:1); mp 239−239.5 °C; IR 1690, 3410; ^{1}H NMR ($CDCl_3$/DMSO) δ 2.41 (s, 3H), 3.79 (s, 2H), 6.94 (s, 1H), 7.35−7.75 (m, 4H); ^{13}C NMR ($CDCl_3$/DMSO) δ 19.78 (CH_3), 28.79 (CH_2), 118.79 (CH), 127.70 (CH), 127.93 (CH), 129.31 (CH), 129.68 (CH), 130.81 (C), 132.06 (C), 133.99 (C × 2), 135.12 (C), 135.49 (C), 139.30 (C), 146.97 (C × 2), 149.75 (C), 163.06 (CO).

6-Chloro-1-(2′,4′-dichlorophenyl)-7-methyl-1,4-dihydroindeno-[1,2-*c*]pyrazole-3-carboxylic Acid (4c). General procedure II was used to convert **8c** into the title product **4c** (0.18 g, 93%) as a yellow solid. R_f = 0.28 ($CHCl_3$/MeOH 9:1); mp 229−231 °C; IR 1690, 3410; ^{1}H NMR ($CDCl_3$/DMSO) δ 2.33 (s, 3H), 3.79 (s, 2H), 6.84 (s, 1H), 7.48−7.93 (m, 4H); ^{13}C NMR ($CDCl_3$/DMSO) δ 19.91 (CH_3), 29.00 (CH_2), 120.52 (CH), 126.47 (CH), 128.25 (CH),

129.32 (C), 129.61 (C), 129.84 (CH × 2), 130.90 (C), 132.25 (C), 133.96 (C), 135.35 (C), 135.53 (C), 139.64 (C), 147.66 (C),149.96 (C), 162.98 (CO).

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 p. m.), 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).

Chemicals and Drugs. [^{3}H]-CP-55 940 (specific activity 180 Ci/mmol) was purchased from New England Nuclear (Boston, MA). CP-55 940 was obtained from Tocris Cookson Ltd (Bristol, U.K.). SR144528 was kindly provided by Sanofi−Synthélabo (now Sanofi−Aventis). For binding experiments, drugs were dissolved in dimethyl sulfoxide (DMSO). DMSO concentration in the different assays never exceeded 0.1% (v/v) and was without effect on radioligand binding.

For radioligand binding assays and in vitro CB_2 activity evaluation, if not otherwise specified, chemicals were purchased from Sigma Aldrich (Milano, Italy).

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 1086 × g for 10 min at 4 °C, and the resulting supernatants were centrifuged at 45 000 × g for 30 min.

[^{3}H]-CP-55 940 binding was performed by the method previously described by Ruiu et al.[33] 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). Nonspecific binding was estimated in the presence of 1 μM of CP-55 940. All binding studies were performed in disposable glass tubes pretreated with Sigma-Cote (Sigma Chemical Co., Ltd., Poole, U.K.) to reduce nonspecific 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). 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, U.S.A.) with 4 mL of scintillation fluid (Ultima Gold MV, Packard).

Protein determination was performed by means of Bradford protein assay[38] 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.[39]

In Vitro CB_2 Activity Evaluation. Human promyelocytic leukemia HL-60 cells from the European Collection of Cell Cultures (ECACC, Salisbury, U.K.) were purchased from Sigma-Aldrich (Milano, Italy). Cell lines were grown at 37 °C in humidified 5% CO_2 in RPMI 1640 medium (Gibco-BRL, Gaithersburg, MA) supplemented with 10% heat-inactivated fetal bovine serum FBS (Gibco-BRL), 25 mM HEPES, 2.5 mM sodium pyruvate, and 20 μg/mL gentamicin. Culture medium was added every 2 days and experiments were made at 80% cell confluency. Tested and reference compounds were dissolved in culture medium with 1% DMSO and treatments were made for time course, dose response, and competition studies in a volume of 10 μL/mL of cell suspension.

To verify whether the observed effects were CB_2 receptor specific, a 5 min pretreatment with the CB_2 receptor antagonist SR144528 (50 nM) was performed before the exposure to the compounds that displayed a significant induction of P-ERK (as shown by western blot analysis). The dose of SR144528 was chosen among the doses lacking an intrinsic activity and providing an appropriate block of the CB_2 receptor.[12,30,34,40]

For western blot analysis after appropriate time of exposure, cells were collected by centrifugation at 1000 × g and the resulting pellets were washed in ice-cold PBS buffer by centrifugation at 1000 × g. The cells were then lysed at 4 °C in 50 μL of 20 mM HEPES buffer (pH 7.9) containing NaCl 125 mM; $MgCl_2$ 5 mM; glycerol 12%; ethylenediaminetetracetic acid (EDTA) 0.2 mM; Nonidet P-40 0.1%; dithithreitol (DTT) 5 mM; phenilmethylsulphonil fluoride (PMSF) 0.5 mM; leupeptin 0.5 μg/mL; and pepstatin A 0.7 μg/mL. The extracts were then centrifuged at 10 000 × g (at 4 °C) for 15 min, and the resulting supernatant was collected as total cell extracts. An aliquot was analyzed for protein concentration determination by using protein assay kit II (Bio-Rad Laboratories, Hercules, CA), and the rest was frozen at −80 °C until assayed. Western blot studies were performed as previously described.[41] Briefly, aliquots of cell extracts containing 50 μg of total protein were separated by 10% sodium dodecyl sulfate-polyacrilamide gels (SDS PAGE, Bio-Rad) and transferred to nitrocellulose membranes (Bio-Rad). Blots were blocked with 5% nonfat dry milk in TBST (0.1% Tween 20 inTris borate saline; Bio-Rad) and probed with a specific antibody against Phospho MAPK 1/2 (Cell Signaling Technology, Beverly, MA) with a 1:1000 dilution in 1% BSA TBST overnight. After washing in TBST, blots were probed with horseradish peroxidase conjugated antibodies (Cell Signaling Technology) with a 1:2000 dilution in TBST plus 5% milk, and after washing in TBST, chemiluminescence was detected by West Pico chemiluminescent substrate (Pierce, Rockford, IL). Immunoreactive bands were visualized with a Fuji Las 1000 image analyzer (Raytest Isotopenmessgeräte GmbH, Straubenhartd, Germany), and the optical density of immunoreactive bands was measured using a specific software (AIDA 2.11, Raytest Isotopenmessgeräte GmbH, Straubenhartd, Germany). One-way ANOVA was performed as a statistical analysis using Graph Pad Prism program (San Diego, CA).

Supporting Information Available: Table of elemental analyses of compounds **2a**−**p** are included. This material is available free of charge via the Internet at http://pubs.acs.org.

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