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Tricyclic Pyrazoles. Part 1: Synthesis and Biological Evaluation of Novel 1,4-Dihydroindeno[1,2-*c*]pyrazol-based Ligands for CB_1 and CB_2 Cannabinoid Receptors

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Abstract—Cannabinoids receptors, cellular elements of the endocannabinoid system, have been the focus of extensive studies because of their potential functional role in several important physiological and pathological processes. To further evaluate the properties of CB receptors, especially CB_1 and CB_2 subtypes, we have designed, using SR141716A as a benchmark, a new series of rigid 1-aryl-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxamides. Compounds **1** were synthesized from substituted 1-aryl-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxylic acids and requisite amines. The various analogues were assayed for binding both to the brain and peripheral cannabinoid receptors (CB_1 and CB_2). Seven of the new compounds displayed very high in vitro CB_2 binding affinities, especially **1a**, **1b**, **1c**, **1e**, **1g**, **1h** and **1j** which showed K_i values of 0.34, 0.225, 0.27, 0.23, 0.385, 0.037 and 0.9 nM, respectively. Compounds **1a**, **1b**, **1c** and **1h** showed the highest selectivity for CB_2 receptor with $K_i(CB_1)$ to $K_i(CB_2)$ ratios of 6029, 5635, 5814 and 9810, respectively. Noticeably, **1h** exhibited the highest affinity and selectivity for CB_2 receptors.
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Introduction

The endogenous cannabinoid system (ECS) in mammals incorporates a variety of cellular elements as potential recognition sites at which a broad group of compounds, termed cannabinoids, interacts. Biological organization of this system includes: (a) two subtypes of G-protein coupled membrane receptors, termed the CB_1[1] receptor (primarily present in the nervous system) and the CB_2[2] receptor (mainly present in the immune system), (b) the endogenous ligands for these receptors anandamide (*N*-arachidonoylethanolamine, AN)[3] and 2-arachidonoylglycerol (2-Ara-Gl)[4] named endocannabinoids and (c) their multiple metabolic pathways for the synthesis degradation[5] and reuptake (only in the case of anandamide)[6–9] (Fig. 1).

Over the past several years a tremendous effort has been focused on studying the physiological functions of endocannabinoid system[10] suggesting that it may play a role in antinociception, brain development, retrograde neuronal communication, memory, appetite, psychomotor control, cardiovascular and immune regulation and cellular proliferation. Recent evidences have indicated that ECS may be a potential therapeutic target for the treatment of diverse patologies[11] including asthma, pain, multiple sclerosis, neurodegenerative, immune and inflammatory diseases.

At present different cannabinoid binders have been identified and can be classified into at least five diverse chemical families (exocannabinoids). These compounds include: tricyclic cannabinols[12] (classical cannabinoids whose structure is based on the dibenzopyran template of Δ^9-THC), bicyclic cannabinols[13,14] (non classical cannabinoids typified by CP-55,940), indoles, pirroles and indenes[15] (typified by WIN-55,212-2), anandamide analogues[16] and diarylpyrazoles[17] (SR 141716A is the prototypical example) (Fig. 2).

These various types of binders, to a different extent, interact with both receptors and the development of

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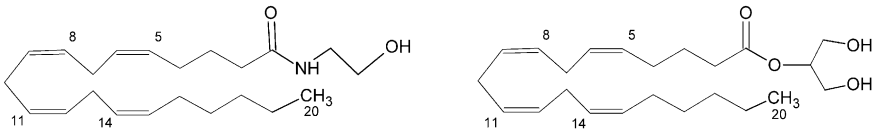


Figure 1. Endogenous cannabinoid receptor ligands and numbering system.

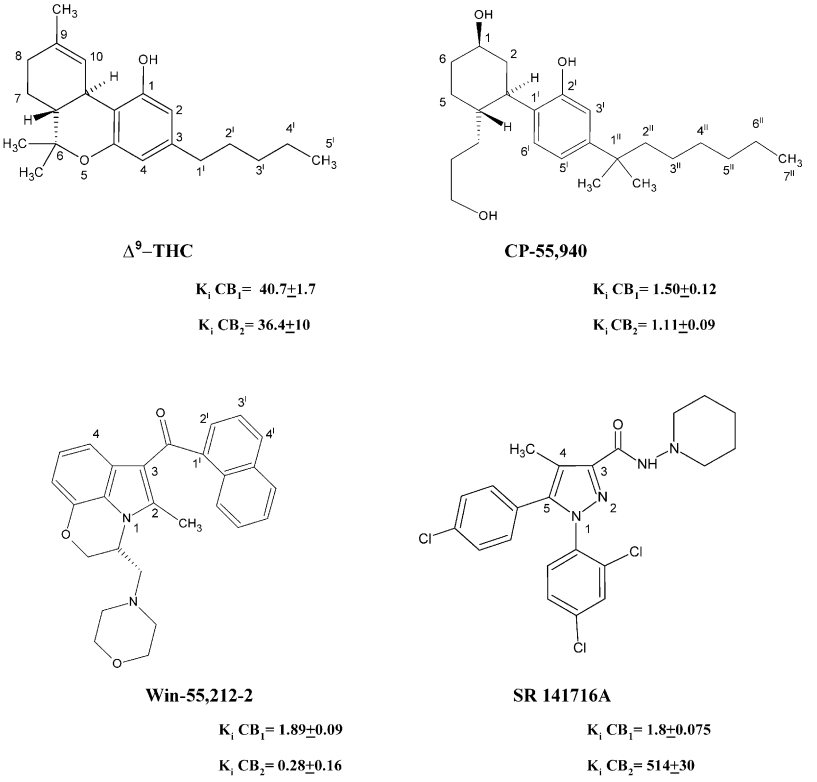


Figure 2. Cannabinoid receptor binder structures and their receptor affinities.

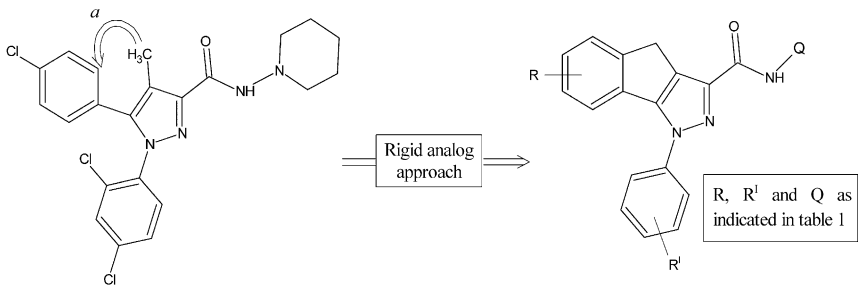
potent and selective ligands for CB_1 and/or CB_2 receptors is of great importance to investigate the involvement of the CB_1 and CB_2 receptors in some (physiological) actions of endocannabinoids (a) in the central nervous system, such as cognition and memory, control of motor function, perception of pain, and (b) some peripheral nerves, where they exert an action in the urogenital, gastrointestinal and cardiovascular systems. The CB_2 receptors are highly expressed in immune cells, B cells and natural killer cells; availability of potent and selective CB_2 receptor ligands would enhance our understanding on the role of this receptor on some effects of cannabinoids such as immunosuppressant and anti-inflammatory.[11d,18]

There has been considerable effort in recent years to determine the relationship of three-dimensional and/or conformational structure with CB_1 and CB_2 cannabinoid affinity, for some selected molecules, belonging to the above mentioned groups.[19] Understanding the preferred structure would provide new leads for cannabinoid ligands with improved biological properties. Our approach to this was to minimize the flexibility of the lead compound through the use of conformationally restricted analogues.[20] It has been proposed that appropriate structural constraints could restrict a pharmacophoric structural element to a sufficiently small region of conformational space thereby permitting the ligand to bind, with high affinity and selectivity, to its designated receptor.[21] In this line, we have designed (Fig. 3) and synthesized (Table 1) an extensive series of rigid SR141716A analogues of general structure **1** and determined in vitro their binding affinities for CB_1 and CB_2 receptors.

Chemistry

Synthesis of title compounds **1** is outlined in Scheme 1.

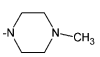
The 1,3-diketoesters **3**, as a tautomeric equilibrium shifted towards the alkenylidene structure (**3′**), were prepared from the indanones **2** and diethyl oxalate in the presence of sodium ethylate. Compounds **3** and the appropriate hydrazines were heated in EtOH to afford the desired 1-arylpyrazoles **4**. The esters **4** were hydrolized and resulting acids **5** treated with $SOCl_2$ to afford the acid chlorides which were allowed to react with the requisite amines to give the desired amides **1a**–**o**,**q**,**r**. Treatment of **1o** with acetone in ethyl acetate gave the ketimine **1p**.



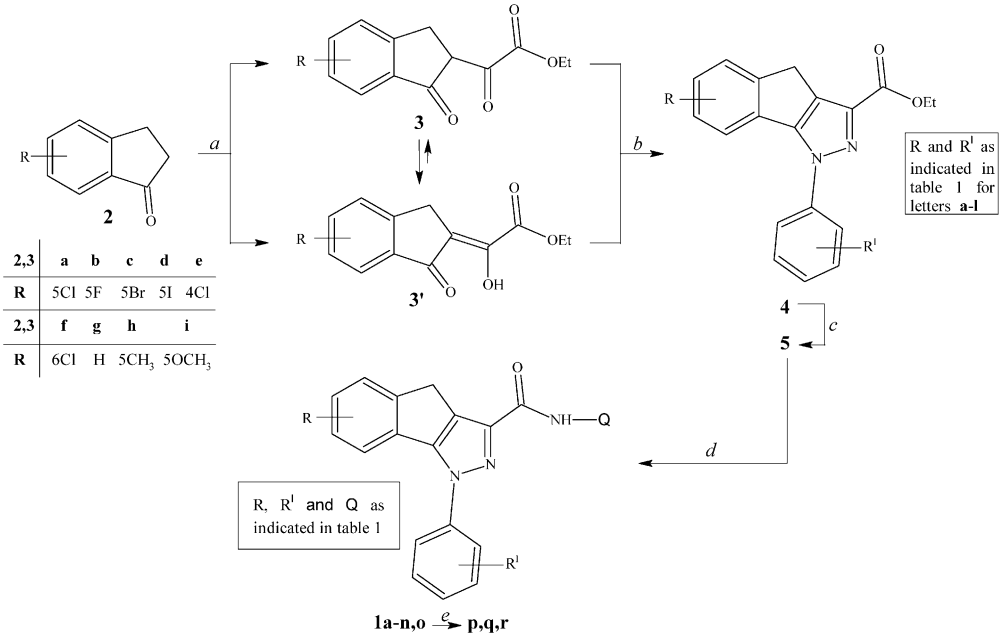
SR141716A **1**

Figure 3. Rigid analogue approach via arrow *a* to obtain 1,4-dihydroindeno[1,2-*c*]pirazole derivatives **1**.

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

Compound **1**	Receptor affinity R	R^I	Q	K_iCB_1(nM)[a]	Selectivity ratio K_iCB_1(nM)[b]	K_iCB_1/K_iCB_2(nM)
a	6Cl	$2^I,4^ICl_2$		2050±90	0.34±0.06	6029:1
b	6F	$2^I,4^ICl_2$		1268±51	0.225±0.02	5635:1
c	6Br	$2^I,4^ICl_2$		1570±15	0.27±0.02	5814:1
d	6I	$2^I,4^ICl_2$		333±0.5	5.5±0.5	60:1
e	5Cl	$2^I,4^ICl_2$		8.25±74	0.23±0.036	3587:1
f	7Cl	$2^I,4^ICl_2$		723±53	6.788±0.47	105:1
g	H	$2^I,4^ICl_2$		1152±65	0.385±0.04	2992:1
h	$6CH_3$	$2^I,4^ICl_2$		363±30	0.037±0.003	9810:1
I	$6OCH_3$	$2^I,4^ICl_2$		399±24	12.3±1	32:1
j	6Cl	4^ICl		1787±85	0.9±0.09	1985:1
k	6Cl	H		>5000	48±5	104:1
l	6Cl	4^IOCH_3		3035±13.5	120±15	25;1
m	6Cl	$2^I,4^ICl_2$		798±48	9.9±0.52	81:1
n	6Cl	$2^I,4^ICl_2$	$-N(CH_3)_2$	1881±119	144±20	13:1
o	6Cl	$2^I,4^ICl_2$	$-NH_2$	2183±123	455±44	5:1
p	6Cl	$2_I,4^ICl_2$		2789±19	978±35	7:1
q	6Cl	$2_I,4_ICl_2$		>5000	>5000	1:1
r	6Cl	$2^I,4^ICl_2$		>5000	>5000	1:1
SR141716A				1.8±0.075	514±30	0.0035:1
SR144528				70±10	0.28±0.04	250:1

[a]Affinnity of compounds for the CB_1 receptor was evaluated using mouse cerebellum membrane and [^{3}H]-CP 55,940.
[b]Affinity of compunds for the CB_2 receptor was assayed using mouse spleen omogenate and [^{3}H]-CP 55,940. K_i vaules were obtained from five indipendent experiments carry out in triplicate are expressed as the mean±standard error.



Scheme 1. (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; (e) $(CH_3)_2CO$, EtOAc.

Biology

Affinities at CB_1 and CB_2 receptor for compounds **1** were assessed by competition of [^{3}H]-CP 55,940 in mouse cerebellum membranes and in mouse spleen omogenate, respectively. For comparison purposes and as reference values, we have also included results obtained for the two prototypical cannabinoid ligands SR141716A and SR144528.

Results and Discussion

Examination of Table 1 reveals that several *N*-piperidin carboxamides containing the planar 1,4-dihydroindeno[1,2-*c*]pyrazole template displayed very high in vitro binding affinity for CB_2 receptors comparable to, or exceeding, that of SR144528 claimed as first highly potent and selective ligand for the CB_2 receptor.[22] Compound **1a**, the ground term of this series of derivatives, possessed high CB_2 receptor affinity and low CB_1 receptor affinity exhibiting higher CB_2 to CB_1 selectivity (6029-fold) than did SR144528 (250-fold). To throw light on the significance of 6-Cl atom in **1a**, the substitution with a variety of substituents, such as F, Br, I, CH_3 and OCH_3 groups, was made. Compounds **1b–d** have a F, Br and I atom, respectively, in place of the chlorine. The fluoro- and bromo-substituted compounds displayed similar CB_2 affinities and selectivities to those of parent compound while the iodo-derivate was 16-fold less potent. Compounds **1e**,**f** contained a C_5 and a C_7 chlorine atom, respectively. Their CB_2 receptor affinities were either slightly increased with C_5 substitution (**1e**) or decreased with C_7 substitution (**1f**) suggesting that the shifting of the Cl atom from C_6 to C_5 was well tolerated. The C_6 unsubstituted compound **1g** maintained a CB_2 receptor affinity that is comparable to that of **1a**. Noticeably the C_6 methyl substituted analogue **1h** had K_i values of 363 nM for CB_1 receptor, 0.037 nM for CB_2 receptor and a CB_2/CB_1 selectivity ratio of 9810. As a result, **1h** had both the highest potency and selectivity among all of the new ligand tested in this study. The 6-OCH_3 substituted analogue **1i** possessed a 36-fold lower CB_2 receptor affinity than **1a** whereas the CB_1 receptor affinity was increased. When the 2^I,4^I-dichloro substitution pattern on the N_1-phenyl ring of **1a** was modified, some impact on CB_2 receptor affinity was observed. While the removal of the C_2,-Cl atom from the 2^I,4^I-Cl_2 phenyl moiety of **1a**, leading to **1j**, had a minimal effect on CB_2 receptor affinity (**1j**: $K_i = 0.9$ nM), the C_2, C_4 removal of both chlorine atoms, as illustrated by compound **1k** ($K_i = 48$ nM), led to a marked decrease in affinity at CB_2 receptors. Next, a methoxy in position C_4, as for compound **1l** conferred only modest CB_2 affinity ($K_i = 120$ nM). The importance of the piperidine ring of the lead compound **1a** for binding and selectivity was confirmed testing compounds **1m–r**. Of the piperidine replacements made, only the pyrrolydinyl derivative **1m** maintained a CB_2 receptor affinity even if 29-fold lower potent than **1a**.

In conclusion, the present study investigated a series of 1,4-dihydroindeno[1,2-*c*]pyrazol-derivatives **1** for their binding affinities for CB_1 and CB_2 receptors. Several compounds in this series exhibited a very high degree of potency and selectivity for CB_2 compared to CB_1. Therefore the synthesis of conformationally restricted analogues of the lead compound SR141716A resulted in either markedly improvement of the CB_2 binding affinity and selectivity. Hence, restriction of the flexible 5-aryl-4-methyl-pyrazole backbone into the rigid and planar 1,4-dihydroindeno[1,2-*c*]pyrazole architecture suggests that this spatial arrangement is a determining factor in the potency of these derivatives as CB_2 receptor ligands. Moreover, substituents such as F, Cl, Br and CH_3 group, introduced at C_6 on the phenyl ring of

the tricyclic system, give the highest increase in affinity and selectivity for CB_2 receptors. Among them, 1-(2^I,4^I-dichlorophenyl)-6-methyl-*N*-piperidin-1-yl-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxamide (**1h**) deserves special attention as being, to our knowledge, the most potent and selective CB_2 ligand describe to date.

Experimental

Chemistry

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}). UV–Vis spectra were recorded as ethanolic solution with a Perkin–Elmer λ 5 spectrophotometer and are the absorption wavelength expressed as λ_{max} in nm followed by log ε in $dm^3 \cdot mol^{-1} \cdot cm^{-1}$. 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 [$(CH_3)_4Si$]. 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 precursors **2a**–**c**,**g**,**i** 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 indanone (**2h**)[23] and diketoester (**3g**)[24] were prepared according to the previously described literature.

General procedure I: synthesis of carboxamides and hydrazides

A mixture of the appropriate 1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxylic acid **5** (1 equiv, 4.0 mmol) and thionyl chloride (3.0 equiv) in toluene (30 mL) was refluxed for 2.5–10 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 warmed to room temperature and stirred for 3 h. The mixture was then poured into a separatory funnel and brine was added. The aqueous layer was separated and extracted with CH_2Cl_2. The combined organic layer were washed 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 below indicated.

6-Chloro-1-(2^I,4^I-dichlorophenyl)-*N*-piperidin-1-yl-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxamide 1a. General procedure I was used to convert **5a** and *N*-aminopiperidine hydrochloride into the title product. The mixture was refluxed for 3 h purification by flash chromatography [petroleum ether/EtOAc, 6:4] afforded **1a** (0.73g, 43%) as a yellow solid. $R_f = 0.41$ (petroleum ether/EtOAc 6:4); mp 189/191 °C (triturated with hexane); IR 1665, 3250; UV λ(log ε) 254.1 (4.42), 269.4 (4.51), 281.2 (4.55), 295.3 (4.38), 303.0 (4.28); ^1H NMR ($CDCl_3$) δ 1.27–1.48 (m, 2H), 1.51–1.80 (m, 4H), 2.82 (t, 4H), 3.82 (s, 2H), 6.83 (d, 1H), 7.13 (d, 1H), 7.43 (dd, 1H), 7.46 (s, 1H), 7.50 (s, 1H), 7.53–7.64 (m, 2H, NH exch. with D_2O); ^{13}C NMR (DEPT, $CDCl_3$) 23.29 (CH_2), 25.36 ($CH_2\times2$), 29.73 (CH_2), 57.12 ($CH_2\times2$), 119.77 (CH), 126.73 (CH), 126.97 (CH), 128.31 (CH), 129.68 (CH), 130.55 (CH), 128.81 (C), 129.62 (C), 131.74 (C), 133.04 (C), 135.67 (C), 136.24 (C), 141.47 (C), 150.78 (C), 151.29 (C), 158.86 (C); API-ES calcd for $C_{22}H_{19}Cl_3N_4O$ 461.8, found 461.7 and Anal. calcd: C, 57.22; H, 4.15; Cl, 23.03; N, 12.13. Found: C, 57.18; H, 4.02; Cl, 23.21; N, 12.33.

1-(2^I,4^I-Dichlorophenyl)-6-fluoro-*N*-piperidin-1-yl-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxamide 1b. General procedure I was used to convert **5b** and *N*-aminopiperidine hydrochloride into the title product. The mixture was refluxed for 4 h purification by crystallization from ethyl acetate/petroleum ether afforded **1b** (0.71g, 40%) as a white solid. $R_f = 0.52$ (petroleum ether/EtOAc 6:4); mp 221/222 °C; IR 1590, 1690, 3260; UV λ(log ε) 245.0 (4.46), 277.6 (4.30), 292.6 (4.16), 300.0 (4.09); ^1H NMR ($CDCl_3$) δ 1.38–1.55 (m, 2H), 1.68–1.88 (m, 4H), 2.88(t, 4H), 3.70 (s, 2H), 6.93 (d,1H), 7.28 (d,1H), 7.43–7.61 (m, 3H), 7.63 (br s, 1H, NH exch. with D_2O), 7.66 (d, 1H); API-ES calcd for $C_{22}H_{19}Cl_2FN_4O$ 445.3, found 445.2, and Anal calcd: C, 59.34; H, 4.30; Cl, 15.92; F, 4.27; N, 12.58. Found: C, 59.06; H, 4.25; Cl, 15.81; F, 4.01; N, 12.77.

6-Bromo-1-(2^I,4^I-dichlorophenyl)-*N*-piperidin-1-yl-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxamide 1c. General procedure I was used to convert **5c** and *N*-aminopiperidine hydrochloride into the title product. The mixture was refluxed for 5 h purification by flash chromatography (petroleum ether/EtOAc, 1:1) afforded **1c** (1.40g, 65%) as a bright yellow solid. $R_f = 0.67$ (petroleum ether/EtOAc 1:1); mp 181/183 °C (methanol); IR 1570, 1665, 3360; UV λ(log ε) 242.4 (4.22), 254.6 (4.27), 267.6 (4.28), 281.2 (4.28), 295.0 (4.18), 303.4 (4.09); ^1H NMR ($CDCl_3$) δ 1.38–1.54 (m, 2H), 1.69–1.92 (m, 4H), 2.88 (t, 4H), 3.89 (s, 2H), 6.84 (d, 1H), 7.37 (dd, 1H), 7.48 (dd, 1H), 7.55 (d, 1H), 7.65 (br s, 1H, NH exch. with D_2O), 7.68 (m, 2H); ^{13}C NMR (DEPT, $CDCl_3$) 23.26 (CH_2), 25.34 ($CH_2\times2$), 29.69 (CH_2), 57.11 ($CH_2\times2$), 120.13 (CH), 128.32 (CH), 129.62 (CH), 129.67 (CH), 129.83 (CH), 130.55 (CH), 121.09 (C), 128.72 (C), 130.01 (C), 131.72 (C), 135.64 (C), 136.26 (C), 141.42 (C), 150.81 (C), 151.48 (C), 158.86 (C); API-ES calcd for $C_{22}H_{19}BrCl_2N_4O$ 506.2, found 506.1 and Anal. calcd: C, 52.20; H, 3.78; Br, 15.78; Cl, 14.01; N, 11.07. Found: C, 52.13; H, 3.90; Br, 15.66; Cl, 14.24; N, 11.01.

1-(2^I,4^I-Dichlorophenyl)-6-iodo-*N*-piperidin-1-yl-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxamide 1d. General procedure I was used to convert **5d** and *N*-aminopiperidine hydrochloride into the title product. The mixture was refluxed for 4 h purification by crystallization from ethyl acetate/petroleum ether afforded **1d** (0.56g, 25%) as a yellow solid. R_f = 0.57 (petroleum ether/EtOAc 1:1); mp 174/176 °C; IR 1570, 1650, 3170; UV λ(log ε) 247.6 (4.45), 250.8 (4.37), 278.6 (4.30), 304.2 (4.20); ^{1}H NMR ($CDCl_3$) δ 1.35–1.55 (m, 2H), 1.73–1.88 (m, 4H), 2.88 (t, 4H), 3.87 (s, 2H), 6.73 (d,1H), 7.43–7.72 (m, 5H, NH exch. with D_2O), 7.91 (s, 1H); ^{13}C NMR (DEPT, $CDCl_3$) 23.28 (CH_2), 25.36 (CH_2×2), 29.52 (CH_2), 57.11 (CH_2×2), 120.45 (CH), 128.30 (CH), 129.65 (CH), 130.54 (CH), 135.42 (CH), 135.72 (CH), 92.43 (C), 128.55 (C), 130.52 (C), 131.71 (C), 135.65 (C), 136.24 (C), 141.41 (C), 150.90 (C), 151.58 (C), 158.84 (C); API-ES calcd for $C_{22}H_{19}Cl_2IN_4O$ 553.2, found 553.1 and Anal. calcd: C, 47.76; H, 3.46; Cl, 12.82; I, 22.94; N, 10.13. Found: C, 47.74; H, 3.22; Cl, 12.91; I, 22.80; N, 10.35.

5-Chloro-1-(2^I,4^I-dichlorophenyl)-*N*-piperidin-1-yl-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxamide 1e. General procedure I was used to convert **5e** and *N*-aminopiperidine hydrochloride into the title product. The mixture was refluxed for 4 h purification by flash chromatography (petroleum ether/EtOAc, 6:4) afforded **1e** (1.13 g, 63%) as a colourless solid. R_f = 0.76 (petroleum ether/EtOAc 1:1); mp 160/162 °C (EtOAc/petroleum ether); IR 1570, 1655, 3200; UV λ(log ε) 246.2 (4.24), 264.4 (4.26), 276.6 (4.25), 289.6 (4.12), 297.0 (3.90); ^{1}H NMR ($CDCl_3$) δ 1.37–1.56 (m, 2H), 1.67–1.90 (m, 4H), 2.90 (t, 4H), 3.92 (s, 2H), 7.69 (d,1H), 7.15- 7.35 (m, 2H), 7.40–7.58 (m, 2H), 7.65 (s, 1H), 7.67 (br s, 1H, NH exch. with D_2O); ^{13}C NMR (DEPT, $CDCl_3$) 23.29 (CH_2), 25.35 (CH_2×2), 29.46 (CH_2), 57.17 (CH_2×2), 117.41 (CH), 127.27 (CH), 128.29 (CH), 128.37 (CH), 129.70 (CH), 130.53 (CH), 128.44 (C), 131.71 (C), 131.85 (C), 132.58 (C), 135.59 (C), 136.27 (C), 141.53 (C), 147.07 (C), 150.98 (C), 158.77 (C); API-ES calcd for $C_{22}H_{19}Cl_3N_4O$ 461.8, found 461.7 and anal. calcd: C, 57.22; H, 4.15; Cl, 23.03; N, 12.13. Found: C, 57.11; H, 4.00; Cl, 23.25 N, 12.34.

7-Chloro-1-(2^I,4^I-dichlorophenyl)-*N*-piperidin-1-yl-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxamide 1f. General procedure I was used to convert **5f** and *N*-aminopiperidine hydrochloride into the title product. The mixture was refluxed for 4 h purification by flash chromatography (petroleum ether/EtOAc, 4:6) afforded **1f** (1.20 g, 64%) as a yellow product. R_f = 0.42 (petroleum ether/EtOAc 1:1); mp 237/238 °C (acetone); IR 1610, 1670, 3280; UV λ(log ε) 248.4 (4.27), 260.6 (4.25), 293.4 (4.14), 300.2 (3.96); ^{1}H NMR ($CDCl_3$) δ 1.52–1.62 (m, 2H), 1.69–1.87 (m, 4H), 2.88 (t, 4H), 3.88 (s, 2H), 6.93 (d,1H), 7.26 (dd, 1H), 7.46 (s, 1H), 7.49–7.60 (m, 2H), 7.62 (br s, 1H, NH exch. with D_2O), 7.69 (d, 1H); ^{13}C NMR (DEPT, $CDCl_3$) 23.29 (CH_2), 25.37 (CH_2×2), 29.55 (CH_2), 57.13 (CH_2×2), 118.64 (CH), 126.97 (CH), 127.28 (CH), 128.33 (CH), 128.70 (CH), 129.05 (CH), 128.94 (C), 131.76 (C), 132.65 (C), 132.96 (C), 135.60 (C), 136.25 (C), 141.50 (C), 148.07 (C), 151.73 (C), 158.80 (C); API-ES calcd for $C_{22}H_{19}Cl_3N_4O$ 461.8, found 461.7 and anal. calcd: C, 57.22; H, 4.15; Cl, 23.03; N, 12.13. Found: C, 57.00; H, 4.35; Cl, 23.05; N, 12.22.

1-(2^I,4^I-Dichlorophenyl)-*N*-piperidin-1-yl-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxamide 1g. General procedure I was used to convert **5g** and *N*-aminopiperidine hydrochloride into the title product. The mixture was refluxed for 3 h purification by flash chromatography (petroleum ether/EtOAc, 1:1) afforded **1g** (0.71 g, 83%) as a colourless solid. R_f = 0.65 (petroleum ether/EtOAc 1:1); mp 228 °C (EtOAc/petroleum ether); IR 1610, 1665, 3290; UV λ(log ε) 245.6 (4.20), 278.0 (4.09), 287.4 (4.02), 296.0 (3.98); ^{1}H NMR ($CDCl_3$) δ 1.34–1.56 (m, 2H), 1.65–1.87 (m, 4H), 2.89 (t, 4H), 3.90 (s, 2H), 6.99 (d, 1H), 7.19–7.39 (m, 2H), 7.44–7.63 (m, 3H), 7.62–7.71 (m, 2H, NH exch. with D_2O); ^{13}C NMR (DEPT, $CDCl_3$) 23.26 (CH_2), 25.33 (CH_2×2), 29.71 (CH_2), 57.06 (CH_2×2), 118.97 (CH), 126.26 (CH), 126.58 (CH), 127.00 (CH), 128.14 (CH), 129.66 (CH), 130.44 (CH), 128.67 (C), 131.03 (C), 131.87 (C), 135.83 (C), 136.01 (C), 141.38 (C), 149.60 (C), 151.69 (C), 159.01 (C); API-ES calcd for $C_{22}H_{20}Cl_2IN_4O$ 427.3, found 427.2 and anal. calcd: C, 61.83; H, 4.72; Cl, 16.59; N, 13.11. Found: C, 61.96; H, 4.52; Cl, 16.44; N, 13.26.

1-(2^I,4^I-Dichlorophenyl)-6-methyl-*N*-piperidin-1-yl-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxamide 1 h. General procedure I was used to convert **5h** and *N*-aminopiperidine hydrochloride into the title product. The mixture was refluxed for 5 h purification by flash chromatography (petroleum ether/EtOAc, 1:1) afforded **1h** (1.2 g, 67%) as a colourless solid. R_f = 0.60 (petroleum ether/EtOAc 1:1); mp 165 °C (EtOAc/petroleum ether); IR 1670, 3250; UV λ(log ε) 250.6 (4.37), 282.2 (4.22), 293.2 (4.14), 301.6 (4.13); ^{1}H NMR ($CDCl_3$) δ 1.37–1.53 (m, 2H), 1.69–1.88 (m, 4H), 2.40(s, 3H), 2.89 (t, 4H), 3.66 (s, 2H), 6.89 (d,1H), 7.04 (d, 1H), 7.38 (s, 1H), 7.47 (dd, 1H), 7.55 (d, 1H), 7.66 (br s, 1H, NH exch. with D_2O), 7.67 (d, 1H); ^{13}C NMR (DEPT, $CDCl_3$) 21.61 (CH_3), 23.31 (CH_2), 25.37 (CH_2×2), 29.61 (CH_2), 57.10 (CH_2×2), 118.70 (CH), 127.13 (CH), 127.29 (CH), 128.16 (CH), 129.69 (CH), 130.47 (CH), 128.26 (C), 128.52 (C), 131.87 (C), 135.95 (C), 137.23 (C), 141.38 (C), 144.74 (C), 150.03 (C), 151.81 (C), 159.15 (C); API-ES calcd for $C_{23}H_{22}Cl_2N_4O$ 441.3, found 441.2 and anal. calcd: C, 62.59; H, 5.02; Cl, 16.07; N, 12.69. Found: C, 62.36; H, 5.22; Cl, 16.27; N, 12.61.

1-(2^I,4^I-Dichlorophenyl)-6-methoxy-*N*-piperidin-1-yl-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxamide 1i. General procedure I was used to convert **5i** and *N*-aminopiperidine hydrochloride into the title product. The mixture was refluxed for 2.5 h purification by flash chromatography (petroleum ether/EtOAc, 3:7) afforded **1i** (0.84 g, 46%) as a colourless solid. R_f = 0.31 (petroleum ether/EtOAc 1:1); mp 238 °C (EtOAc/petroleum ether); IR 1610, 1680, 3330; UV λ(log ε) 254.2 (4.32), 287. (4.10), 301.2 (4.08), 311.4 (4.06); ^{1}H NMR ($CDCl_3$) δ 1.37–1.54 (m, 2H), 1.64–1.88 (m, 4H), 2.88 (t, 4H), 3.84 (s, 3H), 3.87 (s, 2H), 6.77 (dd, 1H), 6.90 (d, 1H), 7.12 (s, 1H), 7.47 (dd, 1H), 7.55 (d, 1H), 7.65 (br s, 1H, NH exch. with D_2O), 7.66 (d, 1H); ^{13}C NMR (DEPT, $CDCl_3$) 55.52 (CH_3), 23.32 (CH_2), 25.39 (CH_2 x 2), 29.93 (CH_2),

57.11 ($CH_2\times2$), 112.13 (CH), 112.55 (CH), 119.69 (CH), 128.18 (CH), 129.71 (CH), 130.48 (CH), 124.28 (C), 127.56 (C), 131.80 (C), 135.91 (C), 135.96 (C), 141.33 (C), 151.64 (C), 151.95 (C), 159.16 (C), 159.27 (C); API-ES calcd for $C_{23}H_{22}Cl_2N_4O_2$ 457.3, found 457.2 and anal. calcd: C, 60.40; H, 4.85; Cl, 15.50; N, 12.25. Found: C, 60.59; H, 4.95; Cl, 15.44; N, 12.11.

6-Chloro-1-(4^{I}-chlorophenyl)-*N*-piperidin-1-yl-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxamide 1j. General procedure I was used to convert **5j** and *N*-aminopiperidine hydrochloride into the title product. The mixture was refluxed for 10 h purification by flash chromatography (petroleum ether/EtOAc, 1:1) afforded **1j** (0.95g, 55%) as a colourless solid. R_f = 0.63 (petroleum ether/EtOAc 1:1); mp 233–234 °C (EtOAc/petroleum ether); IR 1595, 1690, 3290; UV λ(log ε) 255.0 (4.32), 279.8 (4.40), 295.4 (4.27), 304.4 (4.17); ^{1}H NMR ($CDCl_3$) 1.39–1.58 (m, 2H), 1.70–1.89 (m, 4H), 2.90 (t, 4H), 3.88 (s, 2H), 7.22–7.39 (m, 2H), 7.53–7.77 (m, 6H, NH exch. with D_2O); ^{13}C NMR (DEPT, $CDCl_3$) 23.30 (CH_2), 25.39 ($CH_2\times2$), 29.60 (CH_2), 57.14 ($CH_2\times2$), 119.88 (CH), 124.55 (CH×2), 126.94 (CH×2), 129.78 (CH×2), 129.71 (C), 130.16 (C), 133.01 (C), 134.23 (C), 138.03 (C), 141.14 (C), 148.60 (C), 151.48 (C), 158.93 (C); API-ES calcd for $C_{22}H_{20}Cl_2N_4O$ 427.3, found 427.2 and anal. calcd: C, 61.83; H, 4.72; Cl, 16.59; N, 13.11. Found: C, 61.94; H, 4.59; Cl, 16.45; N, 13.16.

6-Chloro-1-phenyl-*N*-piperidin-1-yl-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxamide 1k. General procedure I was used to convert **5k** and *N*-aminopiperidine hydrochloride into the title product. The mixture was refluxed for 7 h purification by flash chromatography (petroleum ether/EtOAc, 1:1) afforded **1k** (1.06 g, 67%) as a colourless solid. R_f = 0.53 (petroleum ether/EtOAc 1:1); mp 191–193 °C (EtOAc/petroleum ether); IR 1595, 1690, 3330; UV λ(log ε) 253.6 (4.30), 270.8 (4.37), 278.6 (4.38), 295.4 (4.23), 304.4 (4.14); ^{1}H NMR ($CDCl_3$) δ 1.39–1.53 (m, 2H), 1.69–1.87 (m, 4H), 2.89 (t, 4H), 3.88 (s, 2H), 7.23 (d, 1H), 7.35 (d, 1H), 7.43–7.78 (m, 7H, NH exch. with D_2O); ^{13}C NMR (DEPT, $CDCl_3$) 23.31 (CH_2), 25.39 ($CH_2\times2$), 29.59 (CH_2), 57.14 ($CH_2\times2$), 119.96 (CH), 123.35 (CH×2), 126.82 (CH×2), 128.51 (CH×2), 129.60 (CH), 129.92 (C), 129.95 (C), 132.79 (C), 139.50 (C), 140.84 (C), 148.56 (C), 151.46 (C), 159.11 (C); API-ES calcd for $C_{22}H_{21}ClN_4O$ 392.9, found 392.8 and anal. calcd: C, 67.26; H, 5.39; Cl, 9.02 N, 14.26. Found: C, 67.40; H, 5.31; Cl, 9.21 N, 14.06.

6-Chloro-1-(4^{I}-methoxyphenyl)-*N*-piperidin-1-yl-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxamide 1l. General procedure I was used to convert **5l** and *N*-aminopiperidine hydrochloride into the title product. The mixture was refluxed for 3 h purification by trituration with petroleum ether containing a few drops of EtOAc afforded **1l** (1.28 g, 75%) as a colourless solid. R_f = 0.40 (petroleum ether/EtOAc 1:1); mp 166–167 °C; IR 1595, 1680, 3330; UV λ(log ε) 254.0 (4.24), 271.8 (4.36), 279.6 (4.39), 295.4 (4.24), 303.8 (4.13); ^{1}H NMR ($CDCl_3$) δ 1.38–1.53 (m, 2H), 1.69–1.91 (m, 4H), 2.89 (t, 4H), 3.87 (s, 2H), 3.91 (s, 3H), 7.05–7.13 (m, 2H), 7.18–7.35 (m, 2H), 7.52–7.64 (m, 3H), 7.71 (br s, 1H, NH exch. with D_2O); ^{13}C NMR (DEPT, $CDCl_3$) 55.67 (CH_3), 23.30 (CH_2), 25.38 ($CH_2\times2$), 29.59 (CH_2), 57.15 ($CH_2\times2$), 114.65 (CH×2), 119.76 (CH), 124.94 (CH×2), 126.79 (CH×2), 129.41 (C), 129.99 (C), 132.59 (C), 132.68 (C), 140.40 (C), 148.67 (C), 151.40 (C), 159.20 (C), 159.65 (C); API-ES calcd for $C_{23}H_{23}ClN_4O_2$ 422.9, found 422.8 and anal. calcd: C, 65.32; H, 5.48; Cl, 8.38; N, 13.25. Found: C, 65.39; H, 5.44; Cl, 8.25; N, 13.14.

6-Chloro-1-(2^{I},4^{I}-dichlorophenyl)-*N*-pyrrolidin-1-yl-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxamide 1m. General procedure I was used to convert **5a** and *N*-aminopyrrolidine hydrochloride into the title product. Because of an excess of the hydrochloride salt, 3 equiv of TEA were used in this reaction. The mixture was refluxed for 3 h purification by crystallization from EtOAc afforded **1m** (1.55 g, 86%) as a colourless solid. R_f = 0.38 (petroleum ether/EtOAc 1:1); mp 213/215 °C; IR 1665, 3200; UV λ(log ε) 255.0 (4.30), 266.8 (4.29), 281.6 (4.27), 294.6 (4.16), 302.8 (4.99); ^{1}H NMR ($CDCl_3$) δ 1.84–2.02 (m, 4H), 2.95–3.12 (m, 4H), 3.91 (s, 2H), 6.90 (d, 1H), 7.22 (dd, 1H), 7.43–7.60 (m, 3H), 7.61 (br s, 1H, NH exch. with D_2O), 7.67 (d, 1H); ^{13}C NMR (DEPT, $CDCl_3$) 22.24 ($CH_2\times2$), 29.76 (CH_2), 55.54 ($CH_2\times2$), 119.75 (CH), 126.72 (CH), 126.96 (CH), 128.29 (CH), 129.66 (CH), 130.54 (CH), 128.64 (C), 129.58 (C), 131.76 (C), 133.03 (C), 135.63 (C), 136.25 (C), 141.35 (C), 150.78 (C), 151.26 (C), 159.74 (C); API-ES calcd for $C_{21}H_{17}Cl_3N_4O$ 447.7, found 447.6 and anal. calcd: C, 56.33; H, 3.83; Cl, 23.75; N, 12.51. Found: C, 56.39; H, 3.73; Cl, 23.56; N, 12.77.

6-Chloro-1-(2^{I},4^{I}-dichlorophenyl)-*N'*,*N'*-dimethyl-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carbohydrazide 1n. General procedure I was used to convert **5a** and dimethyl hydrazine into the title product. The mixture was refluxed for 3 h purification by crystallization from EtOAc afforded **1n** (0.95 g, 67%) as a colourless solid. R_f = 0.43 (petroleum ether/EtOAc 2.5:7.5); mp 214/215 °C; IR 1680, 3200; UV λ(log ε) 255.6 (4.30), 266.4 (4.29), 279.6 (4.28), 294.8 (4.15), 302.8 (4.06); ^{1}H NMR ($CDCl_3$) δ 2.73 (s, 6H), 3.92 (s, 2H), 6.90 (d, 1H), 7.23 (dd, 1H), 7.43–7.60 (m, 3H), 7.62 (br s, 1H, NH exch. with D_2O), 7.67 (d, 1H); ^{13}C NMR (DEPT, $CDCl_3$) 29.76 (CH_2), 47.72 ($CH_3\times2$), 119.74 (CH), 126.71 (CH), 126.97 (CH), 128.30 (CH), 129.65 (CH), 130.54 (CH), 128.61 (C), 129.53 (C), 131.75 (C), 133.05 (C), 135.59 (C), 136.28 (C), 141.24 (C), 150.81 (C), 151.23 (C), 159.21 (C); API-ES calcd for $C_{19}H_{15}Cl_3N_4O$ 421.7, found 421.6 and anal. calcd: C, 54.11; H, 3.59; Cl, 25.22; N, 13.29. Found: C, 54.30; H, 3.38; Cl, 25.33; N, 13.20.

6-Chloro-1-(2^{I},4^{I}-dichlorophenyl)-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carbohydrazide 1o. General procedure I was used to convert **5a** and hydrazine hydrate into the title product. The mixture was refluxed for 3 h purification by trituration with from petroleum ether afforded **1o** (1.40 g, 90%) as a pink solid. R_f = 0.29 ($CHCl_3$/MeOH 8.5:1.5); mp 201/202 °C; IR 1660, 3160, 3340; UV λ(log ε) 253.4 (4.12), 281.6 (4.10), 294.4 (3.98), 302.8 (3.88); ^{1}H NMR ($CDCl_3$) 3.89 (s, 2H), 4.06 (br s, 2H, NH_2 exch. with D_2O), 6.91 (d, 1H), 7.24 (dd, 1H), 7.44–7.54 (m, 2H), 7.55 (s, 1H), 7.66 (d, 1H), 8.03 (br s,

1H, NH exch. with D_2O); ^{13}C NMR (DEPT, $CDCl_3$/DMSO) 28.52 (CH_2), 118.87 (CH), 125.56 (CH), 125.96 (CH), 127.43 (CH), 128.91 (CH), 129.31 (CH), 126.92 (C), 128.57 (C), 130.33 (C), 131.59 (C), 134.56 (C), 135.00 (C), 139.85 (C), 149.38 (C), 149.94 (C), 160.68 (C); API-ES calcd for $C_{17}H_{11}Cl_3N_4O$ 393.6, found 393.5 and anal. calcd: C, 51.87; H, 2.82; Cl, 27.02; N, 14.23. Found: C, 51.83; H, 2.70; Cl, 27.21; N, 14.29.

6-Chloro-1-(2^I,4^I-dichlorophenyl)-*N*-(4-methylpiperazin-1-yl)-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxamide 1q. General procedure I was used to convert **5a** and *N*-amino-*N*-methyl piperazine into the title product. The mixture was refluxed for 3 h purification by crystallization from acetone containing a few drop of EtOAc afforded **1q** (1.42 g, 60%) as a colourless solid. R_f = 0.61 ($CHCl_3$/MeOH 8.5:1.5); mp 172/173 °C; IR 1695, 3300; UV λ(log ε) 255.8 (4.36), 265.6 (4.35), 280.4 (4.33), 282.2 (4.32), 295.0 (4.20), 303.0 (4.11); ^1H NMR ($CDCl_3$) δ 2.33 (s, 3H), 2.60–2.74 (m, 4H), 2.90–3.09 (m, 4H), 3.89 (s, 2H), 6.90 (d, 1H), 7.22 (dd, 1H), 7.45–7.58 (m, 3H), 7.60 (br s, 1H, NH exch. with D_2O), 7.67 (d, 1H); ^{13}C NMR (DEPT, $CDCl_3$) 45.76 (CH_3), 29.74 (CH_2), 54.31 ($CH_2\times2$), 55.58 ($CH_2\times2$), 119.71 (CH), 126.66 (CH), 126.93 (CH), 128.26 (CH), 129.63 (CH), 130.48 (CH), 128.69 (C), 129.48 (C), 131.69 (C), 133.00 (C), 135.48 (C), 136.24 (C), 141.13 (C), 151.10 (C), 151.14 (C), 158.87 (C); API-ES calcd for $C_{22}H_{20}Cl_3N_5O$ 476.8, found 476.7 and anal. calcd: C, 55.42; H, 4.23; Cl, 22.31; N, 14.69. Found: C, 55.26; H, 4.50; Cl, 22.20; N, 14.67.

6-Chloro-1-(2^I,4^I-dichlorophenyl)-*N*-[(1-ethylpyrrolidin-2-yl)methyl]-1,4-dihydroindeno[1,2-*c*] pyrazole-3-carboxamide 1r. General procedure I was used to convert **5a** and *N*-ethyl-2-aminomethylpirrolidine into the title product. The mixture was refluxed for 3 h purification by crystallization from ethyl acetate afforded **1r** (1.54g, 79%) as a colourless solid. R_f = 0.64 ($CHCl_3$/MeOH 8.5:1.5); mp 213–215 °C; IR 1655, 3390; UV λ(log ε) 253.6 (4.46), 265.4 (4.43), 279.4 (4.41), 294.6 (4.28), 303.0 (4.19); ^1H NMR ($CDCl_3$) δ 1.12 (t, 3H), 1.60–2.01 (m, 4H), 2.10–2.40 (m, 2H), 2.63–3.00 (m, 2H), 3.13–3.35 (m, 2H), 3.65–3.80 (m, 1H), 3.89 (s, 2H), 6.91 (d, 1H), 7.18–7.38 (m, 2H, NH exch. with D_2O), 7.48 (dd, 1H), 7.51–7.61 (m, 2H), 7.66 (d, 1H); ^{13}C NMR (DEPT, $CDCl_3$) 13.98 (CH_3), 22.87 (CH_2), 28.58 (CH_2), 29.71 (CH_2), 41.30 (CH_2), 48.54 (CH_2), 53.69 (CH_2), 62.78 (CH), 119.74 (CH), 126.71 (CH), 126.94 (CH), 128.24 (CH), 129.63 (CH), 130.54 (CH), 128.22 (C), 129.77 (C), 131.69 (C), 132.88 (C), 135.80 (C), 136.06 (C), 142.11 (C), 150.74 (C), 151.20 (C), 161.97 (C); API-ES calcd for $C_{24}H_{23}Cl_3N_4O$ 489.8, found 489.7 and anal. calcd: C, 55.85; H, 4.73; Cl, 21.71; N, 11.44. Found: C, 58.68; H, 4.88; Cl, 21.61; N, 11.49.

6-Chloro-1-(2^I,4^I-dichlorophenyl)-*N*'-(1-methylethylidene)-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carbohydrazide 1p. Compound **1o** (0.5 g, 1.27 mmol) was dissolved in ethyl acetate (5 mL) and acetone (2 mL). The reaction mixture was stirred at reflux for 1 h and then cooled to room temperature. The crystallized product was filtered off to give the title compound **1p** (0.46g, 84%). R_f = 0.23 (petroleum ether/EtOAc 1:1); mp 201/202 °C; IR 1730, 3360; UV λ(log ε) 263.6 (4.36), 282.2 (4.27), 294.4 (4.12), 303.0 (4.01); ^1H NMR ($CDCl_3$) δ 1.97 (s, 3H), 2.15 (s, 3H), 3.94 (s, 2H), 6.92 (d, 1H), 7.23 (dd, 1H), 7.51 (dd, 1H), 7.55 (s, 1H), 7.59 (s, 1H), 7.68 (d, 1H), 9.51 (br s, 1H, NH exch. with D_2O); ^{13}C NMR (DEPT, $CDCl_3$) 16.62 (CH_3), 25.55 (CH_3), 29.80 (CH_2), 119.75 (CH), 126.73 (CH), 126.99 (CH), 128.26 (CH), 129.63 (CH), 130.56 (CH), 128.79 (C), 129.53 (C), 131.78 (C), 133.11 (C), 135.62 (C), 136.27 (C), 141.09 (C), 150.97 (C), 151.25 (C), 154.93 (C), 157.29 (C); API-ES calcd for $C_{20}H_{15}Cl_3N_4O$ 433.7, found 433.6 and anal. calcd: C, 55.38; H, 3.49; Cl, 24.52; N, 12.92. Found: C, 55.60; H, 3.32; Cl, 24.39; N, 12.87.

General procedure II: synthesis of carboxylic acids

To a mixture of ester **4** (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 1N hydrochloric acid. The precipitate was filtered, washed with water and air-dried to yield the analytically pure acid.

6-Chloro-1-(2^I,4^I-dichlorophenyl)-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxylic acid 5a. General procedure II was used to convert **4a** into the title product **5a** (1.84 g, 97%) as a bright yellow solid. R_f = 0.60 ($CHCl_3$/MeOH 6:4); mp 263/265 °C; IR 1590, 1710, 3440; ^1H NMR ($CDCl_3$/DMSO) δ 3.43 (br s, 1H, OH exch. with D_2O), 3.86 (s, 2H), 6.94 (d, 1H), 7.24 (d, 1H), 7.47 (dd, 1H), 7.55 (s, 1H), 7.59 (s, 1H), 7.64 (dd, 1H). Anal. calcd for $C_{17}H_9Cl_3N_2O_2$: C, 53.79; H, 2.39; Cl, 28.02; N,7.38. Found: C, 53.65; H, 2.59; Cl, 28.33; N,7.22.

1-(2^I,4^I-Dichlorophenyl)-6-fluoro-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxylic acid 5b. General procedure II was used to convert **4b** into the title product **5b** (1.78 g, 98%) as a colourless solid. R_f = 0.25 (petroleum ether/EtOAc 6:4); mp 274/275 °C; IR 1600, 1700, 3435; ^1H NMR ($CDCl_3$/DMSO) δ 2.68 (br s, 1H, OH exch. with D_2O), 3.86 (s, 2H), 6.95 (d, 1H), 7.27 (d, 1H), 7.47 (d, 1H), 7.56 (s, 1H), 7.64 (m, 2H). Anal. calcd for $C_{17}H_9Cl_2FN_2O_2$: C, 56.22; H, 2.50; Cl, 19.52F, 5.23; N, 7.71. Found: C, 56.10; H, 2.32; Cl, 19.72; F, 5. 32; N, 7.66.

6-Bromo-1-(2^I,4^I-dichlorophenyl)-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxylic acid 5c. General procedure II was used to convert **4c** into the title product **5c** (1.80 g, 96%) as a lighter brown solid. R_f = 0.49 ($CHCl_3$/MeOH, 9:1); mp 253/254 °C; IR 1590, 1720, 3400; ^1H NMR (DMSO/TFA) δ 3.58 (br s, 1H, OH exch. with D_2O), 3.85 (s, 2H), 6.90 (d, 1H), 7.25 (s, 1H), 7.43 (dd, 1H), 7.62 (dd, 1H), 7.70–7.87 (m, 2H). Anal. calcd for $C_{17}H_9BrCl_2N_2O_2$: C, 48.15; H, 2.14; Br, 18.84; Cl, 16.72 N, 6.61. Found: C, 48.33; H, 2.21; Br, 18.77; Cl, 16.70 N, 6.43.

1-(2^I,4^I-Dichlorophenyl)-6-iodo-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxylic acid 5d. General procedure II was used to convert **4d** into the title product. Because of

the low solubility of **4d** in methanol, a mixture of tetrahydrofuran/dioxane/ethanol (28/28/18.5 mL) was used. Compound **5d** (2.17 g, 92%) was isolated as a yellow solid. R_f = 0.38 (petroleum ether/EtOAc, 1:1); mp 292 °C; IR 1590, 1710, 3380; ^{1}H NMR (DMSO) δ 3.67 (br s, 1H, OH exch. with D_2O), 3.83 (s, 2H), 6.77 (d, 1H), 7.60–7.94 (m, 4H), 8.03 (d, 1H). Anal. calcd for $C_{17}H_9Cl_2IN_2O_2$: C, 43.34; H, 1.93; Cl, 15.05; I, 26.94, 16.72 N, 5.95. Found: C, 43.38; H, 1.76; Cl, 15.35; I, 27.03, N, 5.75.

5-Chloro-1-(2^I,4^I-dichlorophenyl)-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxylic acid 5e. General procedure II was used to convert **4e** into the title product **5e** (1.83 g, 96%) as a cream solid. R_f = 0.61 ($CHCl_3$/MeOH, 9:1); mp 247/249 °C; IR 1610, 1720, 3420; ^{1}H NMR ($CDCl_3$/DMSO) δ 3.87 (s, 2H), 4.25 (br s, 1H, OH exch. with D_2O), 6.93 (d, 1H), 7.18–7.34 (m, 2H), 7.49 (s, 1H), 7,57–7.72 (m, 2H). Anal. calcd for $C_{17}H_9Cl_3N_2O_2$: C, 53.79; H, 2.39; Cl, 28.02; N,7.38. Found: C, 53.68; H, 2.52; Cl, 28.35; N,7.20.

7-Chloro-1-(2^I,4^I-dichlorophenyl)-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxylic acid 5f. General procedure II was used to convert **4f** into the title product **5f** (1.74 g, 92%) as a cream solid. R_f = 0.38 ($CHCl_3$/MeOH, 9:1); mp > 300 °C dec.; IR 1610, 1700, 3410; ^{1}H NMR ($CDCl_3$/DMSO) δ 3.68 (br s, 1H, OH exch. with D_2O), 3.83 (s, 2H), 6.93 (d, 1H), 7.27 (dd, 1H), 7.46–7.73 (m, 4H). Anal. calcd for $C_{17}H_9Cl_3N_2O_2$: C, 53.79; H, 2.39; Cl, 28.02; N,7.38. Found: C, 53.78; H, 2.33; Cl, 28.25; N,7.22.

1-(2^I,4^I-Dichlorophenyl)-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxylic acid 5g. General procedure II was used to convert **4g** into the title product **5g** (1.32 g, 75%) as a cream solid. R_f = 0.48 ($CHCl_3$/MeOH, 8/2); mp 271–272 °C; IR 1705, 3350; ^{1}H NMR ($CDCl_3$/DMSO) δ 3.49(br s, 1H, OH exch. with D_2O), 3.82 (s, 2H), 6.99 (d, 1H), 7.20–7.39 (m, 3H), 7.58 (d, 1H), 7.66 (s, 1H), 7.73 (d, 1H). Anal. calcd for $C_{17}H_{10}Cl_2N_2O_2$: C, 59.15; H, 2.92; Cl, 20.54; N, 8.12. Found: C, 59.33; H, 2.81; Cl, 20.42; N, 8.23.

1-(2^I,4^I-Dichlorophenyl)-6-methyl-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxylic acid 5h. General procedure II was used to convert **4h** into the title product **5h** (1.78 g, 98%) as a cream solid. R_f = 0.49 ($CHCl_3$/MeOH, 9/1); mp 267 °C; IR 1710, 3440; ^{1}H NMR ($CDCl_3$/DMSO) δ 2.40 (s, 3H), 2.74 (br s, 1H, OH exch. with D_2O), 3.81 (s, 2H), 6.91 (d, 1H), 7.07 (d, 1H), 7.38 (s, 1H), 7.45 (dd, 1H), 7.58 (s, 1H), 7.63 (d, 1H). Anal. calcd for $C_{18}H_{12}Cl_2N_2O_2$: C, 60.19; H, 3.37; Cl, 19.74; N, 7.80. Found: C, 60.02; H, 3.17; Cl, 19.60; N, 7.96.

1-(2^I,4^I-Dichlorophenyl)-6-methoxy-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxylic acid 5i. General procedure II was used to convert **4i** into the title product **5i** (1.61 g, 86%) as a white solid. R_f = 0.45 ($CHCl_3$/MeOH, 9/1); mp 274–275 °C; IR 1710, 3400; ^{1}H NMR ($CDCl_3$/DMSO) δ 3.00 (br s, 1H, OH exch. with D_2O), 3.82 (s, 2H), 3.85 (s, 3H), 6.79 (dd, 1H), 6.92 (d, 1H), 7.12 (s, 1H), 7.45 (dd, 1H), 7.59 (s, 1H), 7.64 (d, 1H). Anal. calcd for $C_{18}H_{12}Cl_2N_2O_3$: C, 57.62; H, 3.22; Cl, 18.90; N, 7.47. Found: C, 57.53; H, 3.17; Cl, 18.80; N, 7.66.

6-Chloro-1-(4^I-chlorophenyl)-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxylic acid 5j. General procedure II was used to convert **4j** into the title product **5j** (1.69 g, 98%) as a white solid. R_f = 0.76 ($CHCl_3$/MeOH, 8.5/1.5); mp 258 °C; IR 1720, 3440; ^{1}H NMR ($CDCl_3$/DMSO) δ 3.83 (s, 2H), 3.99 (br s, 1H, OH exch. with D_2O), 7.23–7.44 (m, 3H), 7.52–7.66 (m, 3H), 7.71 (d,1H). Anal. calcd for $C_{17}H_{10}Cl_2N_2O_2$: C, 59.15; H, 2.92; Cl, 20.54; N, 8.12. Found: C, 59.39; H, 3.04; Cl, 20.33; N, 8.26.

6-Chloro-1-phenyl-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxylic acid 5k. General procedure II was used to convert **4k** into the title product **5k** (1.50 g, 97%) as a colourless solid. R_f = 0.73 ($CHCl_3$/MeOH, 8.5/1.5); mp 247–248 °C; IR 1720, 3400; ^{1}H NMR ($CDCl_3$/DMSO) δ 3.84 (s, 2H), 7.21–7.47 (m, 2H), 7.48–7.63 (m, 6H, OH exch. with D_2O), 7.75 (d, 1H). Anal. calcd for $C_{17}H_{11}ClN_2O_2$: C, 65.71; H, 3.57; Cl, 11.41; N, 9.02. Found: C, 65.80; H, 3.41; Cl, 11.27; N, 9.22.

6-Chloro-1-(4^I-methoxyphenyl)-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxylic acid 5l. General procedure II was used to convert **4l** into the title product. Because of the low solubility of **4l** in methanol, a mixture of methanol/tetrahydrofuran (45/12 mL) was used. Compound **5l** (1.56 g, 92%) was isolated as a yellow solid. R_f = 0.52 ($CHCl_3$/MeOH, 8.5/1.5); mp 245–247 °C; IR 1710, 3410; ^{1}H NMR ($CDCl_3$/DMSO) δ 3.82 (s, 2H), 3.91 (s, 3H), 6.51 (br s, 1H, OH exch. with D_2O), 7.07 (d, 2H), 7.22–7.40 (m, 2H), 7.53 (s, 1H), 7.63 (d, 2H). Anal. calcd for $C_{18}H_{13}ClN_2O_2$: 63.44; H, 3.85; Cl, 10.40; N, 8.22. Found: 63.54; H, 3.66; Cl, 10.61; N, 8.11.

General procedure III: synthesis of tricyclic esters

To a stirred mixture of diketoester **3** (1.0 equiv, 4 mmol) and the requisite phenyl hydrazine hydrochloride (1.15 equiv) in EtOH (28 mL) was heated under reflux for 2–5 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.

Ethyl 6-chloro-1-(2^I,4^I-dichlorophenyl)-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxylate 4a. General procedure III was used to convert **3a** and 2,4-dichlorophenylhydrazine hydrochloride into the title product. The mixture was heated at reflux for 5 h. Purification by flash chromatography (petroleum ether/EtOAc, 8.5/1.5) afforded **4a** (1.12 g, 80%) as a off-white solid. R_f = 0.54 (petroleum ether/EtOAc, 8:2); mp 210–212 °C (triturated with petroleum ether); IR 1710; ^{1}H NMR ($CDCl_3$) δ 1.45 (t, 3H), 3.85 (s, 2H), 4.41–4.54 (q, 2H), 6.92 (d, 1H), 7.24 (d, 1H), 7.46 (dd, 1H), 7.55 (s, 1H), 7.57 (s, 1H), 7.63 (dd, 1H). Anal. calcd for $C_{19}H_{13}Cl_3N_2O_2$: C, 55.98; H, 3.21; Cl, 26.09; N, 6.87. Found: C, 55.77; H, 3.20; Cl, 26.39; N, 6.55.

Ethyl 1-(2^{I},4^{I}-dichlorophenyl)-6-fluoro-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxylate 4b. General procedure III was used to convert **3b** 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, 8/2) afforded **4b** (1.15 g, 85%) as a colourless solid. R_f = 0.59 (petroleum ether/EtOAc, 8/2); mp 197 °C (triturated with petroleum ether); IR 1725; ^{1}H NMR ($CDCl_3$) δ 1.45 (t, 3H), 3.86 (s, 2H), 4.40–4.55 (q, 2H), 6.95 (d, 1H), 7.28 (d, 1H), 7.45 (dd, 1H), 7.57 (s, 1H), 7.61 (s, 1H), 7.64 (dd, 1H). Anal. calcd for $C_{19}H_{13}FCl_2N_2O_2$: C, 58.33; H, 3.35; Cl, 18.12; F, 4.86; N, 7.16. Found: C, 58.61; H, 3.25; Cl, 18.15; F, 4.77; N, 7.38.

Ethyl 6-bromo-1-(2^{I},4^{I}-dichlorophenyl)-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxylate 4c. General procedure III was used to convert **3c** and 2,4-dichlorophenylhydrazine hydrochloride into the title product. The mixture was heated at reflux for 5 h. Purification by flash chromatography (petroleum ether/EtOAc, 8.5/1.5) afforded **4c** (1.27 g, 80%) as a yellow solid. R_f = 0.54 (petroleum ether/EtOAc, 8/2); mp 208–209 °C (triturated with petroleum ether); IR 1715; ^{1}H NMR ($CDCl_3$) δ 1.45 (t, 3H), 3.85 (s, 2H), 4.41–4.55 (q, 2H), 6.86 (d, 1H), 7.39 (dd, 1H), 7.49 (dd, 1H), 7.56 (s, 1H), 7.61 (s, 1H), 7.67 (dd, 1H). Anal. calcd for $C_{19}H_{13}BrCl_2N_2O_2$ C, 50.47; H, 2.90; Br, 17.67; Cl, 15.68; N, 6.20. Found C, 50.57; H, 3.03; Br, 17.55; Cl, 15.49; N,6.55.

Ethyl 1-(2^{I},4^{I}-dichlorophenyl)-6-iodo-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxylate 4d. General procedure III was used to convert **3d** and 2,4-dichlorophenylhydrazine hydrochloride into the title product. The mixture was heated at reflux for 4 h. Purification by flash chromatography (petroleum ether/EtOAc, 8/2) afforded **4d** (1.44 g, 81%) as a yellow solid. R_f = 0.42 (petroleum ether/EtOAc, 8/2); mp 236–238 °C (triturated with ethanol); IR 1720; ^{1}H NMR ($CDCl_3$) δ 1.45 (t, 3H), 3.83 (s, 2H), 4.38–4.53 (q, 2H), 6.75 (d, 1H), 7.25 (s, 1H), 7.45 (dd, 1H), 7.56 (s, 1H), 7,62 (dd, 1H), 7.92 (s, 1H). Anal. calcd for $C_{19}H_{13}Cl_2IN_2O_2$: C, 45.72; H, 2.63; Cl, 14.21; I, 25.43; N, 5.61. Found: C, 45.59; H, 2.50; Cl, 14.28; I, 25.31; N, 5.79.

Ethyl 5-chloro-1-(2^{I},4^{I}-dichlorophenyl)-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxylate 4e. General procedure III was used to convert **3e** 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, 9/1) afforded **4e** (0.93 g, 63%) as a off-white solid. R_f = 0.63 (petroleum ether/EtOAc, 8:2); mp 191–193 °C (triturated with petroleum ether); IR 1715; ^{1}H NMR ($CDCl_3$) δ 1.46 (t, 3H), 3.88 (s, 2H), 4.43–4.56 (q, 2H), 6.92 (d, 1H), 7.17–7.34 (m, 2H), 7.46 (d, 1H), 7.59 (d, 1H), 7.65 (d, 1H). Anal. calcd for $C_{19}H_{13}Cl_3N_2O_2$: C, 55.98; H, 3.21; Cl, 26.09; N, 6.87. Found: C, 55.76; H, 3.42; Cl, 26.31; N, 6.85.

Ethyl 7-chloro-1-(2^{I},4^{I}-dichlorophenyl)-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxylate 4f. General procedure III was used to convert **3f** 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 **4f** (0.80 g, 58%) as a yellowish solid. R_f = 0.40 (petroleum ether/EtOAc, 8/2); mp 193–195 °C (triturated with petroleum ether); IR 1730; ^{1}H NMR ($CDCl_3$) δ 1.45 (t, 3H), 3.84 (s, 2H), 4.40–4.55 (q, 2H), 6.95 (d, 1H), 7.28 (dd, 1H), 7.43–7.54 (m, 2H), 7.58 (d, 1H), 7.67 (d, 1H). Anal. calcd for $C_{19}H_{13}Cl_3N_2O_2$: C, 55.98; H, 3.21; Cl, 26.09; N, 6.87. Found: C, 55.71; H, 3.22; Cl, 26.22; N, 6.74.

Ethyl 1-(2^{I},4^{I}-dichlorophenyl)-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxylate 4g. General procedure III was used to convert **3g** 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 **4g** (0.90 g, 70%) as a off-white solid. R_f = 0.63 (petroleum ether/EtOAc, 8/2); mp 165 °C (triturated with petroleum ether); IR 1710; ^{1}H NMR ($CDCl_3$) δ 1.45 (t, 3H), 3.88 (s, 2H), 4.41–4.55 (q, 2H), 7.01 (d, 1H), 7.31 (m, 3H), 7.44 (dd, 1H), 7.56 (d, 1H), 7.62 (d, 1H). Anal. calcd for $C_{19}H_{14}Cl_2N_2O_2$: C, 61.14; H, 3.78; Cl, 19.00; N, 7.51. Found: C, 61.22; H, 3.68; Cl, 19.21; N, 7.45.

Ethyl 1-(2^{I},4^{I}-dichlorophenyl)-6-methyl-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxylate 4h. General procedure III was used to convert **3h** 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, 9.5/0.5) afforded **4h** (1.25 g, 93%) as a colourless solid. R_f = 0.55 (petroleum ether/EtOAc, 9/1); mp 199 °C (triturated with petroleum ether); IR 1715; ^{1}H NMR ($CDCl_3$) δ 1.45 (t, 3H), 2.41 (s, 3H), 3.82 (s, 2H), 4.40–4.54 (q, 2H), 6.90 (d, 1H), 7.06 (d, 1H), 7.39 (s, 1H), 7.44 (dd, 1H), 7,61 (s, 1H), 7.63 (d, 1H). 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, 62.00; H, 4.01; Cl, 18.20; N, 7.44.

Ethyl 1-(2^{I},4^{I}-dichlorophenyl)-6-methoxy-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxylate 4i. General procedure III was used to convert **3i** and 2,4-dichlorophenylhydrazine hydrochloride into the title product. The mixture was heated at reflux for 4 h. Purification by flash chromatography (petroleum ether/EtOAc, 9/1) afforded **4i** (1.20 g, 85%) as a colourless solid. R_f = 0.62 (petroleum ether/EtOAc, 8/2); mp 168–169 °C (triturated with petroleum ether); IR 1720; ^{1}H NMR ($CDCl_3$) δ 1.44 (t, 3H), 3.83 (s, 2H), 3.84 (s, 3H), 4.40–4.54 (q, 2H), 6.78 (dd, 1H), 6.91 (d, 1H), 7.12 (d, 1H), 7.44 (dd, 1H), 7,59 (d, 1H), 7.63 (d, 1H). Anal. calcd for $C_{20}H_{16}Cl_2N_2O_3$: C, 59.57; H, 4.00; Cl, 17.58; N, 6.95. Found: C, 59.38; H, 4.13; Cl, 17.29; N, 7.10.

Ethyl 6-chloro-1-(4^{I}-chlorophenyl)-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxylate 4j. General procedure III was used to convert **3a** and 4-chlorophenylhydrazine hydrochloride into the title product. The mixture was heated at reflux for 2.5 h. Purification by flash chromatography (petroleum ether/EtOAc, 9.5/0.5) afforded **4j** (1.20 g, 91%) as a yellow solid. R_f = 0.78 (petroleum ether/EtOAc, 8.5/1.5); mp 197–198 °C (triturated with ethanol); IR

1590, 1730; ^{1}H NMR ($CDCl_3$) δ 1.45 (t, 3H), 3.82 (s, 2H), 4.40–4.55 (q, 2H), 7.22–7.38 (m, 3H), 7.50–7.63 (m, 3H), 7.68 (d, 1H). Anal. calcd for $C_{19}H_{14}Cl_2N_2O_2$: C, 61.14; H, 3.78; Cl, 19.00; N, 7.51. Found: C, 61.02; H, 3.58; Cl, 19.21; N, 7.44.

Ethyl 6-chloro-1-phenyl-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxylate 4k. General procedure III was used to convert **3a** and phenylhydrazine hydrochloride into the title product. The mixture was heated at reflux for 2.5 h. Purification by flash chromatography (petroleum ether/EtOAc, 9.5/0.5) afforded **4k** (1.14 g, 97%) as a off-white solid. R_f = 0.63 (petroleum ether/EtOAc, 8.5/1.5); mp 155–157 °C (triturated with petroleum ether); IR 1600, 1710; ^{1}H NMR ($CDCl_3$) δ 1.46 (t, 3H), 3.84 (s, 2H), 4.42–4.56 (q, 2H), 7.24 (s, 1H), 7.37 (d, 1H), 7.44–7.66 (m, 5H), 7.74 (d, 1H). Anal. calcd for $C_{19}H_{15}ClN_2O_2$: C, 67.36; H, 4.46; Cl, 10.46; N, 8.27. Found: C, 67.22; H, 4.55; Cl, 10.25; N, 8.32.

Ethyl 6-chloro-1-(4^1-methoxyphenyl)-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxylate 4l. General procedure III was used to convert **3a** and 4-methoxyphenylhydrazine hydrochloride into the title product. The mixture was heated at reflux for 3 h. Purification by flash chromatography (petroleum ether/EtOAc, 8/2) afforded **4l** (1.08 g, 84%) as a yellow product. R_f = 0.52 (petroleum ether/EtOAc, 8/2); mp 199–200 °C (triturated with petroleum ether); IR 1700; ^{1}H NMR ($CDCl_3$) δ 1.45 (t, 3H), 3.82 (s, 2H), 3.90 (s, 3H), 4.40–4.55 (q, 2H), 7.06 (d, 2H), 7.22–7.36 (m, 2H), 7.54 (s, 1H), 7.62 (d, 2H,). Anal. calcd for $C_{20}H_{17}ClN_2O_3$: C, 65.13; H, 4.65; Cl, 9.61; N, 7.60. Found: C, 65.19; H, 4.52; Cl, 9.55; N, 7.41.

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 indanone 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.

Ethyl β-(5-chloro-1-oxo-2,3-dihydro-1*H*-inden-2-yl)-α-oxo-acetate 3a. General procedure IV was used to convert **2a** into the title product. The mixture was stirred for 5 h at room temperature. Compound **3a** (1.36 g, 85%) was isolated as a yellowish solid. R_f = 0.61 (petroleum ether/EtOAc, 8/2); mp 122–124 °C (triturated with petroleum ether); IR 1600, 1670, 1710, 3400; ^{1}H NMR ($CDCl_3$) δ 1.43 (t, 3H), 3.99 (s, 2H), 4.35–4.49 (q, 2H), 7.43 (d, 1H), 7.55 (s, 1H), 7.80 (s, 1H), 13.28 (br s, 1H, OH exch. with D_2O). Anal. calcd for $C_{13}H_{11}ClO_4$: C, 58.55; H, 4.16; Cl, 13.29. Found: C, 58.43; H, 4.23; Cl, 13.22.

Ethyl β-(5-Fluoro-1-oxo-2,3-dihydro-1*H*-inden-2-yl)-α-oxo-acetate 3b. General procedure IV was used to convert **2b** into the title product. The mixture was stirred for 2 h at room temperature. Compound **3b** (1.26g, 84%) was isolated as a off-white solid. R_f = 0.51 (petroleum ether/EtOAc, 8/2); mp 107 °C (triturated with petroleum ether); IR 1590, 1680, 1720, 3430; ^{1}H NMR ($CDCl_3$) δ 1.44 (t, 3H), 3.99 (s, 2H), 4.35–4.52 (q, 2H), 7.08 (m, 2H), 7.87 (t, 1H), 13.18 (br s, 1H, OH exch. with D_2O). Anal. calcd for $C_{13}H_{11}FO_4$: C, 62.40; H, 4.43; F, 7.59. Found: C, 62.33; H, 4.41; F, 7.69.

Ethyl β-(5-bromo-1-oxo-2,3-dihydro-1*H*-inden-2-yl)-α-oxo-acetate 3c. General procedure IV was used to convert **2c** into the title product. The mixture was stirred for 8 h at room temperature. Compound **3c** (1.69 g, 91%) was isolated as a yellowish solid. R_f = 0.29 (petroleum ether/EtOAc, 8/2); mp 107 °C (triturated with petroleum ether); IR 1600, 1670, 1720, 3400; ^{1}H NMR ($CDCl_3$) δ 1.43 (t, 3H), 3.98 (s, 2H), 4.35–4.52 (q, 2H), 7.57 (d, 1H), 7.70–7.82 (m, 2H), 12.95 (br s, 1H, OH exch. with D_2O). Anal. calcd for $C_{13}H_{11}BrO_4$: C, 50.18; H, 3.56; Br, 25.68. Found: C, 50.43; H, 3.61; Br, 25.44.

Ethyl β-(5-iodo-1-oxo-2,3-dihydro-1*H*-inden-2-yl)-α-oxo-acetate 3d. General procedure IV was used to convert **2d** into the title product. The mixture was stirred for 6 h at room temperature. Compound **3d** (2.0 g, 66%) was isolated as a yellow solid. R_f = 0.40 (petroleum ether/EtOAc, 8/2); mp 117–118 °C (triturated with petroleum ether); IR 1595, 1670, 1720, 3400; ^{1}H NMR ($CDCl_3$) δ 1.43 (t, 3H), 3.97 (s, 2H), 4.36–4.51 (q, 2H), 7.58 (d, 1H), 7.81 (d, 1H), 7.97 (s, 1H), 14.22 (br s, 1H, OH exch. with D_2O). Anal. calcd for $C_{13}H_{11}IO_4$: C, 43.60; H, 3.10; I, 35.44. Found: C, 43.65; H, 3.22; I, 35.32.

Ethyl β-(4-Chloro-1-oxo-2,3-dihydro-1*H*-inden-2-yl)-α-oxo-acetate 3e. General procedure IV was used to convert **2e** into the title product. The mixture was stirred for 2 h at room temperature. Compound **3e** (1.1 g, 69%) was isolated as a green solid. R_f = 0.21 (petroleum ether/EtOAc, 8/2); mp 86 °C (triturated with petroleum ether); IR 1590, 1610, 1670, 1720, 2540; ^{1}H NMR ($CDCl_3$) δ 1.45 (t, 3H), 4.00 (s, 2H), 4.39–4.56 (q, 2H), 7.43 (t, 1H), 7.64 (d, 1H), 7.78 (d, 1H), 9.19 (br s, 1H, OH exch. with D_2O). Anal. calcd for $C_{13}H_{11}ClO_4$: C, 58.55; H, 4.16; Cl, 13.29. Found: C, 58.30; H, 4.19; Cl, 13.25.

Ethyl β-(6-chloro-1-oxo-2,3-dihydro-1*H*-inden-2-yl)-α-oxo-acetate 3f. General procedure IV was used to convert **2f** into the title product. The mixture was stirred for 8 h at room temperature. Compound **3e** (1.2 g, 75%) was isolated as a brownish solid. R_f = 0.37 (petroleum ether/EtOAc, 8/2); mp 128 °C (triturated with petroleum ether); IR 1600, 1730, 3400; ^{1}H NMR ($CDCl_3$) δ 1.45 (t, 3H), 2.70 (br s, 1H, OH exch. with D_2O), 3.97 (s, 2H), 4.36–4.50 (q, 2H), 7.49 (d, 1H), 7.60 (dd, 1H), 7.83 (s, 1H). Anal. calcd for $C_{13}H_{11}ClO_4$: C, 58.55; H, 4.16; Cl, 13.29. Found: C, 58.50; H, 4.09; Cl, 13.33.

Ethyl β-(1-Oxo-2,3-dihydro-1*H*-inden-2-yl)-α-oxo-acetate 3g.[24] General procedure IV was used to convert **2g** into the title product. The mixture was stirred for 5 h at room temperature. Compound **3f** (1.5 g, 98%) was isolated as a yellow solid. R_f = 0.30 (petroleum ether/

EtOAc, 9/1); mp 68–70 °C (triturated with petroleum ether) (lit.[24] 69–70 °C); IR 1610, 1670, 1730, 3420; ^{1}H NMR ($CDCl_3$) δ 1.44 (t, 3H), 4.00 (s, 2H), 4.36–4.52 (q, 2H), 7.41 (t, 1H), 7.56 (d, 1H), 7.66 (t, 1H), 7.88 (d, 1H), 13.68 (br s, 1H, OH exch. with D_2O). Anal. calcd for $C_{13}H_{12}O_4$: C, 67.23; H, 5.21. Found C, 67.13; H, 5.35.

Ethyl β-(5-methyl-1-oxo-2,3-dihydro-1*H*-inden-2-yl)-α-oxo-acetate 3h. General procedure IV was used to convert **2h** into the title product. The mixture was stirred for 5 h at room temperature. Compound **3h** (1.16 g, 78%) was isolated as a yellow solid. R_f = 0.57 (petroleum ether/EtOAc, 9:1); mp 110–112 °C (triturated with petroleum ether); IR 1620, 1670, 1720, 3420; ^{1}H NMR ($CDCl_3$) δ 1.43 (t, 3H), 2.48 (s, 3H), 3.94 (s, 2H), 4.43 (q, 2H), 7.25 (d, 1H), 7.34 (s, 1H), 7.75 (d, 1H),13.70 (br s, 1H, OH exch. with D_2O). Anal. calcd for $C_{14}H_{14}O_4$: C, 68.28; H, 5.73. Found: C, 68.18; H, 5.52.

Ethyl β-(5-methoxy-1-oxo-2,3-dihydro-1*H*-inden-2-yl)-α-oxo-acetate 3i. General procedure IV was used to convert **2i** into the title product. The mixture was stirred for 5 h at room temperature. Compound **3i** (1.37 g, 87%) was isolated as a yellow solid. R_f = 0.47 (petroleum ether/EtOAc, 8/2); mp 110 °C (triturated with petroleum ether); IR 1610, 1660, 1720, 3420; ^{1}H NMR ($CDCl_3$) δ 1.43 (t, 3H), 3.92 (s, 3H), 3.94 (s, 2H), 4.34–4.49 (q, 2H), 6.92–7.04 (m, 2H), 7.79 (d, 1H), 13.19 (br s, 1H, OH exch. with D_2O). Anal. calcd for $C_{14}H_{14}O_5$ C, 64.12; H, 5.38. Found: C, 64.02; H, 5.45.

General procedure V: synthesis of chloroindanones

A solution of $NaNO_2$ (1.2 equiv) in water (2.6 mL) was added dropwise to a stirred solution of the amino-indanone starting material (1 equiv, 7.27 mmol) in 15% HCl (12 mL) at 0 °C. The resulting solution was added to a stirred solution of CuCl (3.5 equiv) in concd HCl (20 mL) at 0 °C. When the addition was complete, the mixture was stirred at room temperature for 2 h, poured into water and extracted with EtOAc (15 × 3 mL). The combined layers were dried over Na_2SO_4 and concentrated under reduced pressure. Purification by flash chromatography afforded the analytically pure product.

4-Chloroindan-1-one 2e. General procedure V was used to convert 4-amino-indanone[25] into the title product. Purification by flash chromatography (petroleum ether/EtOAc, 8.5/1.5) afforded **2e** (0.76 g, 63%) as a brown solid. R_f = 0.72 (petroleum ether/EtOAc, 8:2); mp 94–96 °C (triturated with petroleum ether) (lit[26] mp 93–94 °C); IR, ^{1}H NMR, and ^{13}C NMR were in agreement with previously reported spectra.[26] Anal. calcd for C_9H_7ClO: C, 64.88; H, 4.23; Cl, 21.28. Found: C, 64.59; H, 4.41; Cl, 21.19.

6-Chloroindan-1-one 2f. General procedure V was used to convert 6-amino-indanone[25] into the title product. Purification by flash chromatography (petroleum ether/EtOAc, 8.5/1.5) afforded **2f** (0.98 g, 98%) as a brown solid. R_f = 0.57 (petroleum ether/EtOAc, 8:2); mp 76–78 °C (triturated with petroleum ether) (lit[26] mp 77–80 °C); IR, ^{1}H NMR, and ^{13}C NMR were in agreement with previously reported spectra.[26] Anal. calcd for C_9H_7ClO: C, 64.88; H, 4.23; Cl, 21.28. Found: C, 64.65; H, 4.11; Cl, 21.12.

5-Iodoindan-1-one 2d. A solution of $NaNO_2$ (1.2 equiv) in water (1.1 mL) was added dropwise to a stirred solution of the 5-amino-indanone[27] 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 tiosulfate, dried over Na_2SO_4 and concentrated under reduced pressure to afforded a crude oil. Purification by flash chromatography (petroleum ether/EtOAc, 9/1) afforded **2d** (0.75g, 55.5%) as a red oil. R_f = 0. 78 (petroleum ether/EtOAc, 8/2); IR 1590, 1700; ^{1}H NMR ($CDCl_3$) δ 2.67 (t, 2H), 3.13 (t, 2H), 7.47 (d, 1H), 7.73 (d, 1H), 7.91 (s, 1H). Anal. calcd for C_9H_7IO: C, 41.89; H, 2.73; I, 49.18. Found: C, 41.77; H, 2.57; I, 49.31.

Biology

General information. Male CD1 mice weighting 20–25 g, (Charles River, Calco, LC Italy) were housed in the animal care quarters maintained at 22 ± 2 °C on a 12 h light/dark cycle, and food and water were available ad libitum. All experimental protocols were accepted by the Ethical Committee at the University of Cagliari and performed in strict accordance with the E.C. regulation for care and use of experimental animals (EEC N°86/609).

[^{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 biochemical 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.

Tissue preparation. Mice were killed by cervical dislocation and the brain (minus cerebellum) for CB_1 receptor and spleen for CB_2 receptor were rapidly removed and placed on an ice-cold plate. After thawing, tissues were homogenated in 20 vol (w/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 in a Beckman SW41 swing-out rotor, at 4 °C.

Binding study at CB_1 and CB_2 receptor. [^{3}H]-CP-55,940 binding was performed by a modification of a method previously described.[28] Briefly, the membranes (30–80 μg of protein) were incubated with 0.5–1 nM of [^{3}H]-CP55940 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 10 μM of CP55940. 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 96-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 2100, Packard, Meridien, USA) with 4 mL of scintillation fluid (Ultima Gold MV, Packard). Protein determination was performed by means of Bradford[29] protein assay using BSA as a standard according to the protocol of the supplier (Bio-Rad, Milan, Italy).

Data Analysis

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 described previously.[30]

References and Notes

1. Matsuda, L. A.; Lolait, S. J.; Young, A. C.; Bonner, T. I. *Nature* **1990**, *346*, 561.
2. Munro, S.; Thomas, K. L.; Abu-Shaar, M. *Nature* **1993**, *365*, 61.
3. Dewane, W. A.; Hanus, L.; Breuer, A.; Pertwee, R. G.; Stevenson, L. A.; Griffin, G.; Gibson, D.; Mandelbaum, A.; Etinger, A.; Mechoulam, R. *Science* **1992**, *268*, 1946.
4. Sugiura, T.; Kondo, S.; Sukagawa, A.; Natane, S.; Shinoda, A.; Itoh, K.; Yamashita, A.; Waku, K. *Biochem. Biophys. Res. Commun.* **1995**, *215*, 89.
5. Di Marzo, V. *Life Sci.* **1999**, *65*, 645.
6. Bisogno, T.; Maurelli, S.; Melk, D.; De Petrocellis, S.; Di Marzo, V. *J. Biol. Chem.* **1997**, *272*, 3315.
7. Beltramo, M.; Stella, M.; Calignano, A.; Lin, S. Y.; Makriyannis, A.; Piomelli, D. *Science* **1997**, *277*, 1094.
8. Hillard, C. J.; Edgemond, W. S.; Jarranhian, A.; Campbell, W. B. *J. Neurochem.* **1997**, *69*, 631.
9. Maccarone, M.; Van der Stelt, M.; Rossi, A.; Veldink, G. A.; Vliegenthart, J. F.; Finazzi-Agrò, A. *J. Biol. Chem.* **1998**, *273*, 32332.
10. (a) Calignano, A.; Giuffrida, A.; Piomelli, D. *Nature* **1998**, *394*, 277. (b) Wolker, J. M.; Hohmann, A. G.; Martin, W. J.; Strangman, M. N.; Huang, S. M.; Tsou, K. *Life Sci.* **1999**, *65*, 665. (c) Fernandez-Rui, J.; Berrendero, F.; Hernandez, M. L.; Ramos, J. A. *Nature* **2001**, *410*, 558. (d) Hampson, R. E.; Deadwyler, S. A. *Life Sci.* **1999**, *65*, 715. (e) Di Marzo, V.; Goparaju, S. K.; Wang, L.; Liu, J.; BatKai, S.; Jarai, Z.; Fezza, F.; Miura, G. I.; Palmiter, R. D.; Sugiura, T.; Kunos, G. *Nature* **2001**, *410*, 822. (f) Giuffrida, A.; Piomelli, D. *Chem. Phys. Lipids* **2000**, *108*, 151. (g) De Petrocellis, S.; Melk, D.; Bisogno, T.; Di Marzo, V. *Chem. Phys. Lipids* **2000**, *108*, 191.
11. (a) Calignano, A.; Kàtona, I.; Désarnaud, F.; Giuffrida, A.; La Rana, G.; Mackie, K.; Freund, T. F.; Piomelli, D. *Nature* **2000**, *408*, 96. (b) Baker, D.; Pryce, G.; Croxford, J. L.; Brown, P.; Pertwee, R. G.; Huffmann, J. W.; Laywards, L. *Nature* **2000**, *404*, 84. (c) Galve-Roperh, I.; Sanchez, C.; Cortés, M. L.; Gòmes Del Pulgar, T.; Izquierdo, M.; Guzmàn, H. *Nat. Med.* **2000**, *6*, 313. (d) Pertwee, R. G. *Curr. Med. Chem.* **1999**, *6*, 635. (e) Salzet, M.; Breton, C.; Bisogno, T.; Di Marzo, V. *Eur. J. Biochem.* **2000**, *267*, 4917.
12. Seltzman, H. H. *Curr. Med. Chem.* **1999**, *6*, 685.
13. Devane, W. A.; Dyzarzlll, F. A.; Johnson, M. R.; Melvin, L. S.; Howlett, A. C. *Mol. Pharmacol.* **1988**, *34*, 605.
14. Melvin, L. S.; Milne, G. M.; Johnson, M. R.; Wilken, G. H.; Howlett, A. C. *Drug Des. Disc.* **1995**, *13*, 155.
15. Huffman, J. W. *Curr. Med. Chem.* **1999**, *6*, 705.
16. Goutopoulos, A.; Fan, P.; Khanolkar, D. A.; Xie, X.-Q.; Lin, S.; Makriyannis, A. *Bioorg. Med. Chem.* **2001**, *9*, 1673.
17. Barth, F.; Rinaldi-Carmona, M. *Curr. Med. Chem.* **1999**, *6*, 745.
18. (a) Pertwee, R. G.; Fernando, S. R. *Br. J. Pharmacol.* **1996**, *118*, 2199. (b) Piomelli, D.; Giuffrida, A.; Calignano, A.; Rodriguez de Fonseca, F. *TiPS* **2000**, *21*, 218.
19. Fichera, M.; Cruciani, G.; Bianchi, A.; Musumarra, G. *J. Med. Chem.* **2000**, *43*, 2300.
20. Cannon, G. J. In *Analogue design, chapter nineteen*; Wolff, M. E., Ed.; *Burger's Medicinal Chemistry and drug discovery,volume1: principles and practice, 5th ed*; A Wiley-Interscience publication: New York, 1995; pp 788–791.
21. (a) Silverman, R. R. *The Organic Chemistry of Drug Design and Drug Action;* Academic Press: San Diego, 1992. (b) Wermuth, C. G. *The Practice of Medicinal Chemistry;* Academic Press: San Diego 1996.
22. Rinaldi-Carmona, M.; Barth, F.; Millian, J.; Derocq, J. M.; Casellas, P.; Congy, C.; Oustruc, D.; Sarran, M.; Bouaboula, M.; Calandra, B.; Portier, M.; Shire, D.; Brelière, J. C.; Le Fur, G. *J. Pharmacol. Exp. Ther.* **1998**, *284*, 644.
23. Boykin, D. W.; Hertzler, R. L.; Delphone, J. K.; Eisenbraun, E. J. *J. Org. Chem.* **1989**, *54*, 1418.
24. Hamilton, R. W. *J. Heterocyclic Chem.* **1976**, *13*, 545.
25. Exner, O.; Friedl, Z. *Collect. Czech. Chem. Commun.* **1978**, *43*, 3227.
26. Takeuchi, R.; Yasue, H. *J. Org. Chem.* **1993**, *58*, 5386.
27. Allinger, L. A.; Jones, E. S. *J. Org. Chem.* **1962**, *27*, 70.
28. Rinaldi-Carmona, M.; Barth, F.; Heaulme, M.; Shire, D.; Calandra, B.; Congy, C.; Martinez, S.; Maruani, J.; Neliat, G.; Caput, D.; Ferrara, P.; Soubriè, P.; Brelière, J. C.; Le Fur, G. *FEBS Lett.* **1994**, *350*, 240.
29. Bradford, M. M. *Anal. Biochem.* **1976**, *72*, 248.
30. Cheng, X.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.