Tricyclic Pyrazoles. 3. Synthesis, Biological Evaluation, and Molecular Modeling of Analogues of the Cannabinoid Antagonist 8-Chloro-1-(2′,4′-dichlorophenyl)-*N*-piperidin-1-yl-1,4,5,6-tetrahydrobenzo[6,7]cyclohepta[1,2-*c*]pyrazole-3-carboxamide

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A series of analogues of 8-chloro-1-(2′,4′-dichlorophenyl)-*N*-piperidin-1-yl-1,4,5,6-tetrahydrobenzo-[6,7]cyclohepta[1,2-*c*]pyrazole-3-carboxamide **4a** (NESS 0327) (Ruiu, S.; Pinna, G. A.; Marchese, G.; Mussinu, J. M.; Saba, P.; Tambaro, S.; Casti, P.; Vargiu, R.; Pani, L. Synthesis and Characterization of NESS 0327: A Novel Putative Antagonist of CB_1 Cannabinoid Receptor. *J. Pharmacol. Exp. Ther.* **2003**, *306*, 363−370) was synthesized and evaluated for their affinity to cannabinoid receptors. Depending on the chemical modification of the lead structure that was chosen, compounds **4b**, **4c**, **4i**, **4l**, and **4m** still proved to be potent binders of the CB_1 receptor. Moreover, several analogues (**4c**, **4d**, **4e**, and **4m**) demonstrated superior CB_2 receptor binding affinities compared to the parent ligand. Compounds **4b**, **4c**, **4i**, and **4l** displayed the most promising pharmacological profiles, having the highest selectivity for CB_1 receptors with $K_i(CB_2)$ to $K_i(CB_1)$ ratios of 11 250, 2000, 3330 and 4625, respectively. Compound **4c** increased the intestinal propulsion in mice and antagonized the effect induced by the CB_1 receptor agonist WIN 55,212-2. Finally, molecular modeling studies were carried out on a set of tricyclic pyrazoles (**2a**−**4a**) and on rimonabant **1** (SR141716A), indicating that high CB_1 receptors affinities were consistent for the tricyclic derivatives, both with a nonplanar geometry of the tricyclic cores and with a precise orientation of the substituent (chlorine) on this ring system.

Introduction

The major psychoactive constituents of Indian hemp, *Cannabis sativa L.*, are termed cannabinoids, a group of more than 60 structurally related terpenophenolics that have been used as medicinal agents since antiquity.[2] Recently, interest in the pharmacology of cannabinoids has rapidly increased, particularly after the discovery of the endogenous cannabinoid system (ECS) in mammals at the beginning of the 1990s. This system includes a variety of cellular elements: (a) two subtypes of G-protein-coupled membrane receptors termed CB_1[3] (primarily present in the central nervous system but also expressed in peripheral tissues) and CB_2[4] (mainly present in the immune system), (b) endogenous ligands for these receptors, anandamide (*N*-arachidonoylethanolamine, AN)[5] and 2-arachidonoylglycerol (2-Ara-Gl),[6] named endocannabinoids, and (c) their multiple metabolic pathways for synthesis, degradation, and reuptake (only in the case of anandamide).[3−8]

In view of the beneficial pharmacological properties of CB_1 receptor ligands in the treatment of a number of diseases, such as neuroinflammatory disorders,[9] cognitive disorders,[10] septic shock,[10] obesity,[10,11] psychosis,[10,12] addiction,[13] and gastrointestinal disorders,[14] a major aim in medicinal chemistry is the development of novel CB_1 cannabinoid receptor ligands exhibiting more favorable pharmacological features.

Tricyclic compounds containing a 5-aryl-4-alkylpyrazole skeleton, related to rimonabant **1** (SR141716A), have been reported by us to display interesting cannabinoid binding affinity and subtype selectivity.[1,15,16] These classes of compounds have been claimed by Sanofi−Synthélabo (now Sanofi-Aventis) in WO 01 32,663.[17] Moreover, tricyclic compounds as rigid cannabinoid CB_1 receptor antagonists have been also reported by Stoit et al.,[18] with lower affinity values versus CB_1 receptors than those determined in our laboratory.[1] Medicinal chemistry strategies to cannabinoid receptor antagonists, including tricyclic compounds containing a 5-aryl-4-alkylpyrazole skeleton, have been recently reviewed by Lange and Kruse.[19]

Illustrative examples such as compounds **2**−**4**, are shown below (Figure 1). Interestingly, changes to the size and shape of the tricyclic unit in these ligands revealed intriguing effects on biological activity. Thus, a very high binding affinity for CB_2 receptors was shown by the ligands having the 1,4-dihydroindeno[1,2-*c*]-pyrazole core **2**, while 4,5-dihydro-1*H*-benzo[*g*]indazole-based compounds **3** had higher CB_1 binding affinities. 8-Chloro-1-(2′,4′-dichlorophenyl)-*N*-piperidin-1-yl-1,4,5,6-tetrahydrobenzo[6,7]cyclohepta[1,2-*c*]pyrazole-3-carboxamide **4a** (NESS 0327) showed a profile of binding

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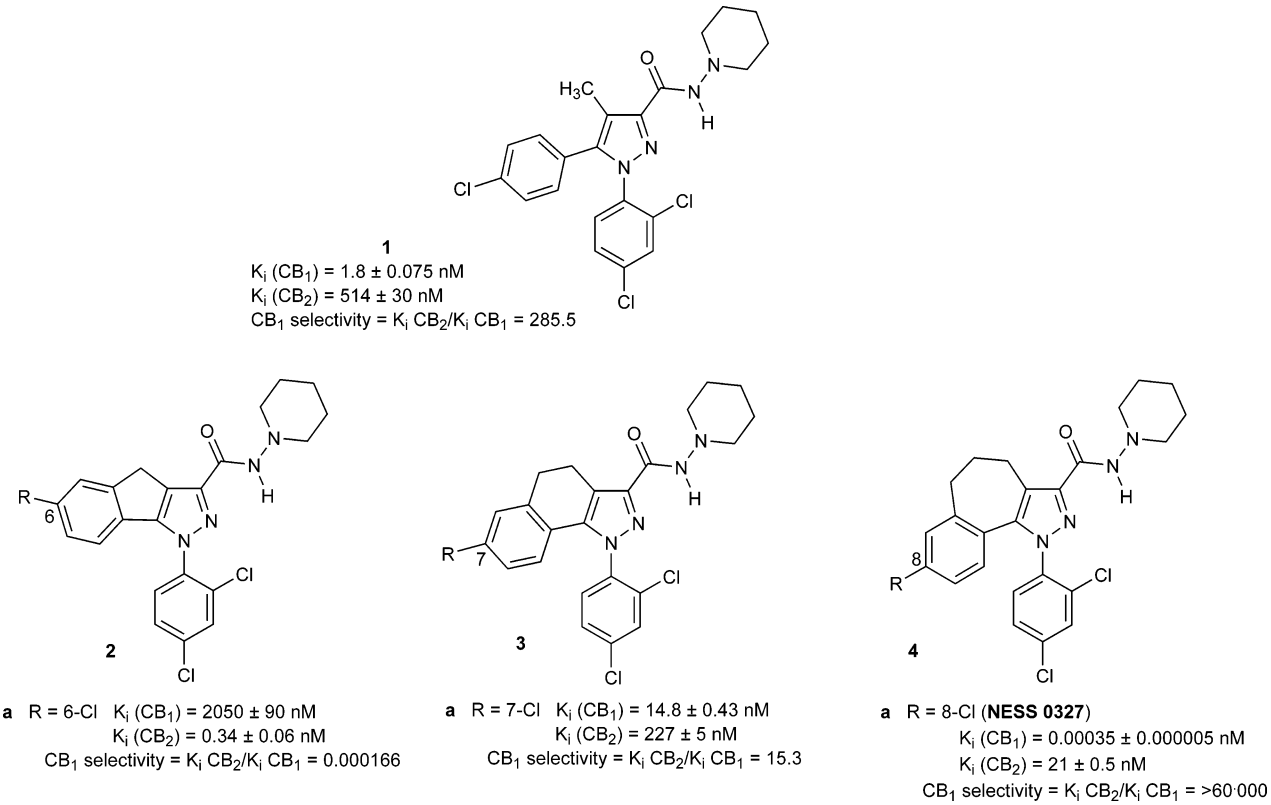


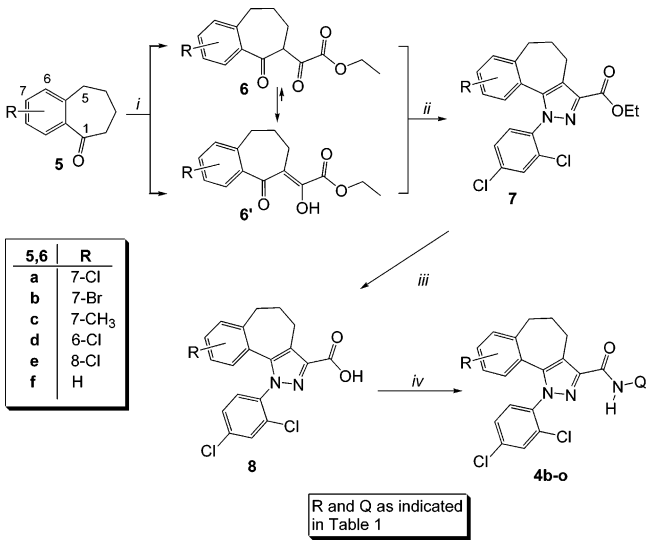
Figure 1. Cannabinoid receptor affinity and selectivity for compounds **1**−**4**.

affinities similar to those of the **3** series, yet exhibiting a far greater preference for CB_1 receptors. Moreover, affinity ratios demonstrated that **4a** was more than 60000-fold selective for the CB_1 receptor, whereas **1** was only 285-fold.[1] So increasing the length of the carbon bridge between C_4 of pyrazole and the C_5 phenyl group from one to three methylene units led to a marked increase in the CB_1 binding affinity and selectivity.

In this context, to obtain a better understanding of the structural moieties critically involved in the CB_1 receptor−ligand interaction in our tricyclic pyrazole series, novel compounds related to the **4a** have been synthesized and evaluated for their CB_1 and CB_2 binding properties.

In particular we focused our interest on two attractive modifications of **4a**. On one hand, structural variation of **4a** was brought about by modifying the piperidinyl substituent of the C_3 carboxamide group to novel synthetic variations such as **4b**−**i**, and on the other hand, we varied the C_8-chlorine atom by substitution with a bromine or a methyl group or by alteration of its position or by removing it, **4j**−**o** (Table 1).

Scheme 1[a]



[a] (i) Na, dry EtOH, $(COOEt)_2$; (ii) 2,4-$Cl_2C_6H_3NHNH_2$·HCl, EtOH; (iii) KOH, MeOH; (iv) $SOCl_2$, $C_6H_5CH_3$; then CH_2Cl_2, TEA, Q-NH_2.

Chemistry

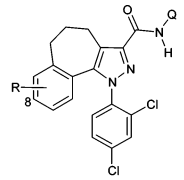
The synthesis of title compounds **4b**−**o** is outlined in Scheme 1 and was realized through the application of methodologies employed for **4a** in these laboratories.[1]

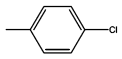
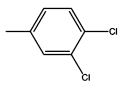
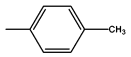
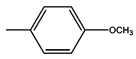
Thus, the 1,3-diketoesters **6**, as a tautomeric equilibrium shifted toward the alkenylidene structure (**6′**), were prepared from the benzosuberones **5** and diethyl oxalate in the presence of sodium ethylate. Compounds **6** and 2,4-dichlorophenylhydrazine hydrochloride were heated in ethanol to afford the benzocycloheptapyrazoles **7**. The esters **7** were hydrolyzed, and the resulting acids **8** were treated with thionyl chloride to afford the acid chlorides, which were allowed to react with the required amines to give the desired amides **4b**−**o**.

Benzosuberones that could not be purchased were synthesized as described below.

In Scheme 2, **5a**−**c** were obtained starting from aldehydes **9**. Benzaldehydes **9** were submitted to a Wittig condensation with the phosphonium bromide by means of *t*-BuOK in DMSO to yield the pentenoic acid derivatives **10**. Reduction of the double bond of **10** with H_2 over PtO_2 in ethanol at room temperature followed by cyclization with PPA afforded benzocycloheptanones **5a**−**c**.

Table 1. Structures and Binding Data of Compounds **4**

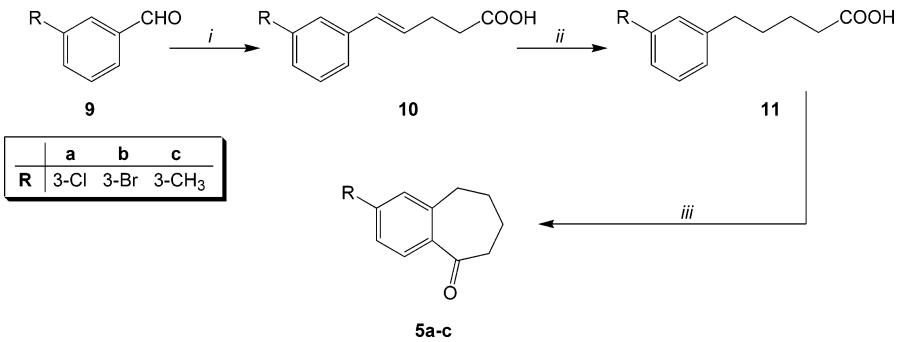


			Receptor affinity		CB_1 selectivity
Compd 4	**R**	**Q**	**K_iCB_1 (nM)[a]**	**K_iCB_2 (nM)[b]**	**K_iCB_2/ K_iCB_1**
a	8-Cl		0.00035 ± 0.000005	21 ± 0.5	60˙000:1
b	8-Cl		0.004 ± 0.0008	45 ± 5	11˙250:1
c	8-Cl		0.001 ± 0.0002	2 ± 0.2	2˙000:1
d	8-Cl		0.3 ± 0.05	0.65 ± 0.1	2.16:1
e	8-Cl		4.35 ± 0.4	1.45 ± 0.3	0.33:1
f	8-Cl		2.5 ± 0.28	79 ± 10	31.6:1
g	8-Cl		25.83 ± 3	>500	—
h	8-Cl		5.74 ± 0.36	65 ± 5	11.3:1
i	8-Cl		0.013 ± 0.0035	43.3 ± 2	3˙300:1
j	7-Cl		9.84 ± 0.6	31.6 ± 0.6	3.21:1
k	9-Cl		31.2 ± 1.2	26.8 ± 0.8	0.86:1
l	8-Br		0.008 ± 0.0015	37 ± 3	4˙625:1
m	8-CH_3		0.0052 ± 0.0002	0.460 ± 0.011	88.5:1
n	8-CH_3		0.11 ± 0.01	386 ± 23	3˙509:1
o	H		168 ± 3.75	18.1 ± 2	0.11:1
1			1.8 ± 0.075	514 ± 30	285:1

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

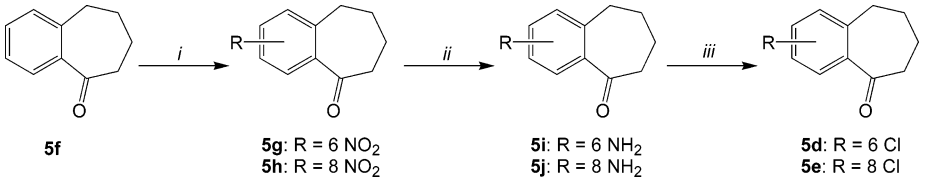
In Scheme 3, benzocycloheptanones **5d**,**e** were obtained by initial nitration of commercially available benzosuberone **5f**. Nitration occurred readily upon exposure of a solution of **5f** in H_2SO_4 at 0 °C to finely

Scheme 2[a]



[a] (i) $(Ph)_3P^+(CH_2)_3COOH{\cdot}Br^-$, *t*-BuOK, DMSO; (ii) H_2, PtO_2, EtOH; (iii) PPA.

Scheme 3[a]



[a] (i) H_2SO_4, KNO_3; (ii) Sn, HCl; (iii) $NaNO_2$, HCl, CuCl.

powdered KNO_3 to give a mixture of 6-NO_2-benzosuberone (**5g**) and 8-NO_2-benzosuberone (**5h**). Because of the preferential electrophilic attack of the nitroniun ion at the C_8 position, the isomer **5h** dominated in a ratio 13:1. Both products **5g** and **5h** could be separated by flash column chromatography.

Reduction of the nitro groups of **5g** and **5h** with Sn and HCl led to the 6-aminobenzosuberone **5i** and 8-aminobenzosuberone **5j**. Ketoamines **5i**,**j** were treated with $NaNO_2$ and HCl, and the resulting diazonium salts were quickly transformed into the desired **5d**,**e** by reaction with copper(I) chloride.

Biology

Affinities at CB_1 and CB_2 receptors for **4** were assessed by competition for [^{3}H]CP-55,940 binding in mouse brain (minus cerebellum) and spleen homogenates, respectively. Radioligand binding procedures previously reported by Ruiu et al. were adopted.[1] The results from the in vitro binding assay were compared with the K_i values of the prototypical cannabinoid ligand **1**.

Analogous to the previous studies on **1** and other tricyclic pyrazole analogues, the cannabinoid functional profile of synthesized compound **4c**, showing single digit picomolar binding for CB_1 receptors, was evaluated through the estimation of its capability to interact with cannabinoid receptors occurring in the myentheric plexus (Auerbach's plexus).[14] In fact, CB_1 cannabinoid receptors are significantly present in the enteral system of various mammalian species (i.e., human and mouse), and their function appears to be related to modulation of gastrointestinal motility.[14a] Antagonists of CB_1 have been shown not only to block the actions induced by cannabinoid agonists but also to induce an increase of the gastrointestinal motility by themselves. This effect could easily be shown using the upper gastrointestinal transit (GIT) test,[14b] where the intestinal length travelled by a nonabsorbable marker as a consequence of the active compound administration is determined.

Thus, the synthesized novel N_1-(2′,4′-dichlorophenyl)-8-chloro-1,4,5,6-tetrahydrobenzo[6,7]cyclohepta[1,2-*c*]-pyrazole-3-carboxamide derivative **4c** was evaluated in vivo by the upper gastrointestinal transit test, determining both the ability of the compound to produce a dose-dependent effect (dose response curve) and its specificity at cannabinoid receptors. The latter was experimentally determined by means of a high-affinity CB agonist administered in combination with the compound to be tested. The studies were carried out according to the previously reported procedures,[14b] using WIN 55,212-2 as the CB_1 agonist.

Results and Discussion

Radioligand Binding Assays. Examination of the CB receptor affinities of those analogues of **4a** with the C_3-carboxamide *N*-piperydinyl motif replaced by various types of moieties (**4b**−**i**) revealed a dramatic impact on CB receptor binding affinity. The marked difference in CB_1 affinity of these compounds compared to that of **1** has been further supported by new preliminary experiments carried out with **4a** on guinea pig cerebral cortex membranes, using the radioligand [^{3}H]SR141716 (data not shown).

Compounds **4b** and **4c** had a pyrrolidine or a homopiperidine ring replacement of the piperidine ring compared to **4a**. Both maintained high CB_1 receptor affinity and good CB_1 selectivity ($K_i(CB_2)/K_i(CB_1)$) even if at lower levels compared with the lead compound **4a** ($K_i(CB_1)$ = 0.000 35 nM; $K_i(CB_2)/K_i(CB_1)$ = 60 000). In particular, compounds **4b** and **4c** showed CB_1 affinities (K_i) of 0.004 and 0.001 nM with $K_i(CB_2)/K_i(CB_1)$ of 11 250 and 2000, respectively.

Replacing the piperidinyl ring of **4a** with a cyclohexyl moiety (compound **4d**) decreased the affinity for the CB_1 receptor and improved the CB_2 affinity. However, the affinity of **4d** toward CB_1 receptors ($K_i(CB_1)$ = 0.3 nM) was still higher than that of **1** ($K_i(CB_1)$ = 1.8 nM, as already determined in our laboratory).[1]

Compounds **4e**−**i** have an aryl group replacement of the piperidinyl ring. The *p*-methoxyphenyl compound,

4i, had the highest CB_1 receptor affinity among the five aryl analogues with a CB_1 receptor affinity that is 37-fold lower than **4a** (even if approximately 100-fold higher than **1**). Except for **4e** ($K_i(CB_2) = 1.45$ nM), all the compounds of this subclass had lower CB_2 receptor affinities compared to **4a**.

CB_1 receptor binding affinities are greatly influenced by the modification of the chlorine atom position on the benzene ring of the tricyclic core of **4a**. In particular, shifting the chlorine atom from C_8 to C_7 and C_9, leading to **4j** and **4k**, respectively, produced a marked decrease in affinity for CB_1 receptors, with K_i values in the order of **4j** < **4k** ($K_i(CB_1) = 31.2$ nM for **4k**).

More significant is the impact on CB_1 affinity caused by the removal of the C_8-chlorine atom leading to **4o**. In this latter case, a 480000-fold loss of CB_1 affinity was observed.

The receptor affinity trend observed for CB_2 is different from that observed for CB_1 receptors. In fact, because of the above-mentioned modification of the benzene ring of the tricyclic core of the lead compound **4a**, no substantial difference was seen in the CB_2 affinities compared with **4a**.

Substitution of the chlorine atom in the aryl ring of the tricyclic scaffold also showed some impact on CB_1 and CB_2 receptor affinity.

Compounds **4l** and **4m** contained a C_8-bromo- and C_8-methyl-substituted benzocycloheptapyrazole system, respectively. Their CB_1 receptor affinities were decreased compared to that of **4a**, while the CB_2 receptor affinity of the 8-methyl-substituted analogue was higher than that of **4a**.

The pirrolidine-substituted analogue, **4n**, showed a relatively minor decrease in both CB_1 and CB_2 receptor affinity.

In Vivo Assays. Administration of the compound **4c** (0.5−1 mg/kg ip) produced an increase in the gastrointestinal transit, up to approximately 65−70% (one-way ANOVA $F(5,104) = 11.18$, (∗) $P < 0.01$); Figure 2A). Furthermore compound **4c**, administered at a dose of 0.1 mg/kg, which did not affect propulsion in the small intestine, reversed the inhibitory effect of the CB_1 agonist compound WIN 55,212-2 (0.5 mg/kg) (Figure 2B).

Similar results have been obtained with other compounds of the studied series, i.e., **4b**, **4l**, and **4m** (data not shown).

Modeling. Understanding the preferred conformations of **1** and **4a**, as well as their analogues, would provide insight into the geometrical requirements for this class of cannabinoid antagonists. Compared with the mobility of the aryl group at pyrazole position 5 of **1**, the trimethylene bridge present in **4a** should constrain this group in a precise orientation. However, the heptacyclic ring, though inserted into a tricyclic system, maintains a certain degree of flexibility so that it might adopt more than one conformation. **1** has been the object of conformational studies at various levels of calculations.[18,20] Reggio et al. proposed[20b,c] an active conformation of **1** in which the piperidine ring is in a chair conformation with the nitrogen lone pair pointing in the same direction as the carboxamide oxygen. At the HF/6-31G*//AM1 level this conformation is 0.92 kcal/mol less stable than the global minimum.[20b] No exhaustive

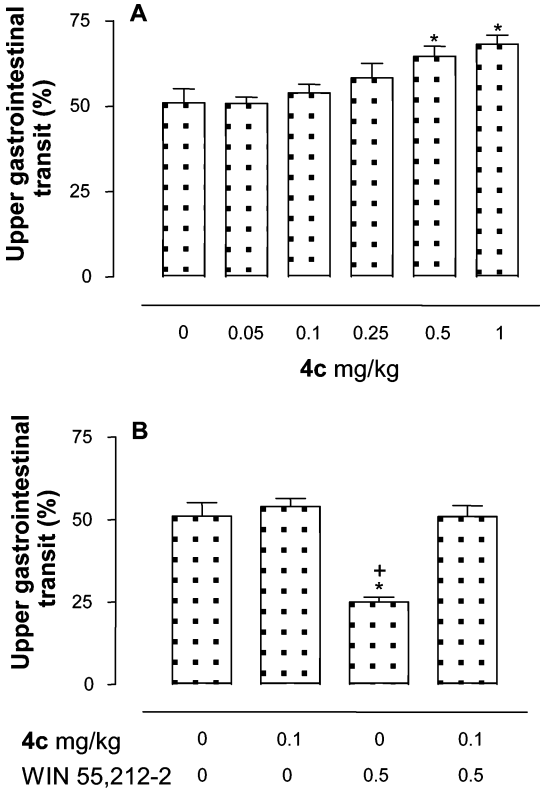


Figure 2. (A) Bar graphs showing the affect of compound **4c** on the gastrointestinal motility. Assays were performed as described in the experimental methods section. Each bar is the mean ± SEM of 15−20 mice: one-way ANOVA $F(5,104) = 11.18$; (∗) $P < 0.01$ with respect to vehicle-treated mice (Newman−Keuls test). (B) Effect of **4c** (0−0.1 mg/kg ip) alone or in combination with WIN 55,212-2 (0−0.5 mg/kg ip). Compound **4c** was given 10 min before the administration of WIN 55,212-2, and the latter was given 20 min before the marker. Gastrointestinal transit was evaluated 20 min after the administration of the marker. Each bar represents the mean ± SEM of 15−20 mice: one-way ANOVA $F(3,78) = 62.02$; (∗) $P < 0.01$ with respect to 0.1 mg/kg **4c** + 0 mg/kg WIN 55,212-2 treated mice (Newman−Keuls test); (+) $P < 0.01$ with respect to 0.1 mg/kg **4c** + 0.5 mg/kg WIN 55,212-2 treated mice (Newman−Keuls test).

modeling study of **4a** is reported in the literature; just one paper compares one conformer of **4a** and one of **1** optimized at the PM3 level with no specification about their energy ranking.[18] Because a complete conformational study of these compounds with optimizations at ab initio or DFT level has never been reported, we explored the conformational behavior of **1**, of **4a**, and of its lower homologues **3a** and **2a** using DFT methods at the B3LYP level[21] with the 6-31G* basis set. All their minimum energy conformations were located, and Figure 3 reports the three-dimensional plots of the most significant ones. Table 2 of the Supporting Information reports the geometrical features of all the conformations in a range of 3 kcal/mol above the global minimum.

In all the compounds the s-trans orientation at the bond linking the amido group to pyrazole is the only one accessible. In fact, s-cis geometries could be located through the calculations, but they are higher in energy by >7 kcal/mol with respect the corresponding s-trans geometries. The piperidine ring is in a chair conformation. In agreement with Reggio et al.,[20b,c] the orientation of its lone pair in the same direction as the carboxamide oxygen is disfavored by about 1 kcal/mol with respect

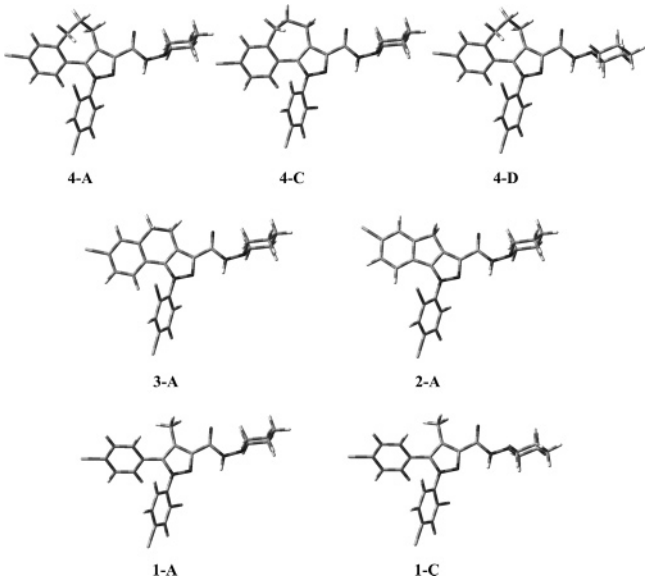


Figure 3. Three-dimensional plot of the most significant conformations of compounds **4a**, **3a**, **2a**, and **1**.

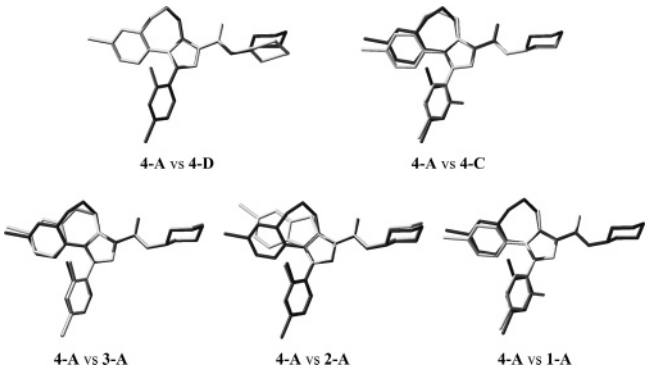


Figure 4. Superimpositions, through the best rms fit of the atoms of the pyrazole ring, of **4-A** (minimum energy conformer of compound **4a**) (dark gray) with significant conformers of the same compound and of compounds **3a**, **2a**, and **1** (light gray).

to the preferred conformation in which it is oriented in the opposite direction. The heptacyclic ring of **4a** can assume two geometries characterized by different sets of internal torsional angles, i.e., by a different fold of the trimethylene chain, and by an energy difference of about 1 kcal/mol (see, for example, **4-A** vs **4-C**). In both cases the chlorophenyl and the pyrazole rings significantly deviate from planarity (−42° in **4-A** and −35° in **4-C**). In compound **3a**, the shorter dimethylene chain forces the aryl and the pyrazole rings toward a more planar arrangement (deviation from planarity of −17°). In compound **2a**, the tricyclic system becomes completely planar. In both these cases, only one conformation of the central hexacyclic or pentacyclic ring is accessible. The 4-chlorophenyl group of **1** is connected by a single bond to the pyrazole; hence, it is prone to fluctuations at a low energy cost. At the present level of calculations the minimum energy conformation exhibits a deviation from planarity of −50°.

The similarities and the differences among the various conformations are highlighted in Figure 4 where the superimpositions of conformer **4-A** with several conformers of the same or of other compounds are reported. These superimpositions indicate a different orientation of the chlorine atom. In fact, in the superimpositions **4-A/3-A**, **4-A/2-A**, and **4-A/1-A**, the Cl−Cl distance is 0.48, 2.41, and 0.66 Å, respectively. Compounds **1** and **3a** show CB_1 affinity in the nanomolar range and compound **2a** shows CB_1 affinity in the micromolar range compared to the subpicomolar range of **4a**.

Conclusions

In this report we describe the design and synthesis of a novel series of benzocycloheptapyrazole carboxamide derivatives related to **4a**, focusing on the modification of the amide piperidine side chain and the chlorine atom of the tricyclic core. In receptor binding assays, it was revealed that alicyclic amine derivatives at the C_3 carboxamide group of **4a** showed greater affinity for the CB_1 receptor than compounds with a cyclohexyl or with an aryl moiety. It was also revealed that the presence of a halogen atom at the C_8 position of the tricyclic core is important for CB_1 affinity.

The high CB_2 binding affinities of **4d** and **4m** compared to that of the **4a** suggests that the presence of either a C_3 carboxamide cyclohexyl moiety or a C_8 methyl group in these benzocycloheptapyrazole-based derivatives can produce increased CB_2 affinity.

Among the studied compounds, a subclass of 8-chloro-1-(2′,4′-dichlorophenyl)-*N*-piperidin-1-yl-1,4,5,6-tetrahydrobenzo[6,7]cyclohepta[1,2-*c*]pyrazole-3-carboxamide **4a** derivatives was shown to possess a significant increase of CB_1 affinity and selectivity compared with the reference compound **1**. A prototipical compound, **4c**, increased the intestinal propulsion in mice and antagonized the effect induced by the CB_1 receptor agonist WIN 55,212-2.

Modeling studies provide evidence that to achieve high CB_1 binding affinity and CB_1 over CB_2 selectivity, it is important for the tricyclic architecture to be nonplanar and to bear a halogen atom (Cl, Br) or a methyl group appropriately oriented. Furthermore, the presence of a carboxamide group at tricyclic core C_3, preferably containing a cyclic amine (piperidine, pyrrolidine, or homopiperidine), is an additional favorable factor for robust CB_1 binding affinities (as in compounds **4b**,**c**,**l**,**m**). However, more studies on the in vivo pharmacology of the more active compounds in the series **2**−**4** are needed to provide the tools necessary to define the full range of their pharmacological actions and to demonstrate the therapeutic potential of these molecules.

Experimental Section

General Procedure. 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 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), qu (quintuplet), dd (doublet of doublets), m (multiplet). Atmospheric pressure ionization electrospray (API-ES) mass spectra, when reported, were obtained on an Agilent 1100 series LC/MSD spectrometer. Combustion 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 **5f**, **9a**, **9b**, **9c** 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 benzosuberones **5a**, the diketoester **6a**, the pyrazole ester **7a**, and the pyrazole acid **8a** were prepared according to the previous literature.[1]

General Procedure I: Synthesis of Carboxamides (4b−o). A mixture of the appropriate 1,4,5,6-tetrahydrobenzo-[6,7]cyclohepta[1,2-*c*]pyrazole-3-carboxylic acid **8** (1 equiv, 1.23 mmol) and $SOCl_2$ (3.0 equiv) in toluene (10 mL) was refluxed for 3 h. The solvent and the excess $SOCl_2$ were removed under reduced pressure, and the resulting dark solid in CH_2Cl_2 (6 mL) was added dropwise to a solution of requisite amine (1.5 equiv) in CH_2Cl_2 (6 mL) at 0 °C. The mixture was warmed to room temperature and stirred overnight. 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.

8-Chloro-1-(2′,4′-dichlorophenyl)-*N*-pyrrolidin-1-yl-1,4,5,6-tetrahydrobenzo[6,7]cyclohepta[1,2-*c*]pyrazole-3-carboxamide (4b). General procedure I was used to convert **8a** and *N*-aminopyrrolidine hydrochloride into the title product. Because of an excess of the hydrochloride salt, an amount of 2 equiv of TEA was used in this reaction. The mixture was purified by flash chromatography (petroleum ether/EtOAc, 4:6) to afford **4b** (0.35 g, 60%) as a white solid. $R_f = 0.33$ (petroleum ether/EtOAc 4:6); mp 127−128 °C; IR 1651, 3206; ^{1}H NMR ($CDCl_3$) δ 1.82−1.96 (m, 4H), 2.25 (qu, 2H, $J = 6.4$ Hz), 2.66 (t, 2H, $J = 6.4$ Hz), 2.90−3.10 (m, 6H), 6.57 (d, 1H, $J = 8.2$ Hz), 6.99 (dd, 1H, $J_o = 8.2$ Hz, $J_m = 2.2$ Hz), 7.28−7.31 (m, 1H), 7.37−7.49 (m, 3H), 7.66 (br s, 1H, NH exchange with D_2O); ^{13}C NMR ($CDCl_3$) δ 20.04 (CH_2), 22.20 ($CH_2 \times 2$), 31.32 (CH_2), 32.41 (CH_2), 55.31 ($CH_2 \times 2$), 122.47 (C), 126.10 (CH), 127.50 (C), 127.93 (CH), 128.11 (CH), 129.81 (CH), 130.29 (CH), 130.37 (CH), 132.36 (C), 134.06 (C), 135.84 (C), 142.13 (C), 143.34 (C), 143.67 (C), 160.72 (C), 169.98 (CO); API-ES calcd for 475.8, found 475.10. Anal. ($C_{23}H_{21}Cl_3N_4O$) C, H, Cl, N.

8-Chloro-1-(2′,4′-dichlorophenyl)-*N*-homopiperidin-1-yl-1,4,5,6-tetrahydrobenzo[6,7]cyclohepta[1,2-*c*]pyrazole-3-carboxamide (4c).[18] General procedure I was used to convert **8a** and *N*-aminohomopiperidine into the title product. The mixture was purified by flash chromatography (petroleum ether/EtOAc, 6:4) to afford **4c** (0.22 g, 35%) as a white solid. $R_f = 0.54$ (petroleum ether/EtOAc 6:4); mp 160−161 °C (162−164 °C); [18] IR 1659, 3174; API-ES calcd for 503.9, found 503.01. Anal. ($C_{25}H_{25}Cl_3N_4O$) C, H, Cl, N.

8-Chloro-1-(2′,4′-dichlorophenyl)-*N*-cyclohexyl-1,4,5,6-tetrahydrobenzo[6,7]cyclohepta[1,2-*c*]pyrazole-3-carboxamide (4d). General procedure I was used to convert **8a** and cyclohexylamine into the title product. The mixture was purified by flash chromatography (petroleum ether/EtOAc, 8:2) to afford **4d** (0.30 g, 50%) as a white solid. $R_f = 0.54$ (petroleum ether/EtOAc 8:2); mp 96−98 °C; IR 1633, 3201; API-ES calcd for 488.84, found 488.10. Anal. ($C_{23}H_{24}Cl_3N_3O$) C, H, Cl, N.

8-Chloro-1-(2′,4′-dichlorophenyl)-*N*-phenyl-1,4,5,6-tetrahydrobenzo[6,7]cyclohepta[1,2-*c*]pyrazole-3-carboxamide (4e). General procedure I was used to convert **8a** and aniline into the title product. The mixture was purified by flash chromatography (petroleum ether/EtOAc, 8:2) to afford **4e** (0.40 g, 68%) as a white solid. $R_f = 0.65$ (petroleum ether/EtOAc 8:2); mp 145 °C; IR 1673, 3383; API-ES calcd for 482.8, found 482.0. Anal. ($C_{25}H_{18}Cl_3N_3O$) C, H, Cl, N.

8-Chloro-1-(2′,4′-dichlorophenyl)-*N*-*p*-chlorophenyl-1,4,5,6-tetrahydrobenzo[6,7]cyclohepta[1,2-*c*]pyrazole-3-carboxamide (4f). General procedure I was used to convert **8a** and *p*-chloroaniline into the title product. The mixture was purified by flash chromatography (petroleum ether/EtOAc, 8:2) to afford **4f** (0.21 g, 34%) as a white solid. $R_f = 0.68$ (petroleum ether/EtOAc 8:2); mp 103 °C; IR 1682, 3377; API-ES calcd for 517.2, found 518.0. Anal. ($C_{25}H_{17}Cl_4N_3O$) C, H, Cl, N.

8-Chloro-1-(2′,4′-dichlorophenyl)-*N*-*m*,*p*-dichlorophenyl-1,4,5,6-tetrahydrobenzo[6,7]cyclohepta[1,2-*c*]pyrazole-3-carboxamide (4g). General procedure I was used to convert **8a** and 3,4-dichloroaniline into the title product. The mixture was purified by flash chromatography (petroleum ether/EtOAc, 9.5:0.5) to afford **4g** (0.25 g, 37%) as a white solid. $R_f = 0.20$ (petroleum ether/EtOAc 9.5:0.5); mp 110 °C; IR 1680, 3377; API-ES calcd for 551.7, found 552.0. Anal. ($C_{25}H_{16}Cl_5N_3O$) C, H, Cl, N.

8-Chloro-1-(2′,4′-dichlorophenyl)-*N*-*p*-methylphenyl-1,4,5,6-tetrahydrobenzo[6,7]cyclohepta[1,2-*c*]pyrazole-3-carboxamide (4h). General procedure I was used to convert **8a** and *p*-toluidine hydrochloride into the title product. Because of an excess of the hydrochloride salt, an amount of 2 equiv of TEA was used in this reaction. The mixture was purified by flash chromatography (petroleum ether/EtOAc, 9:1) to afford **4h** (0.36 g, 60%) as a white solid. $R_f = 0.38$ (petroleum ether/EtOAc 8:2); mp 120 °C; IR 1682, 3393; API-ES calcd for 496.81, found 496.0. Anal. ($C_{26}H_{20}Cl_3N_3O$) C, H, Cl, N.

8-Chloro-1-(2′,4′-dichlorophenyl)-*N*-*p*-methoxylphenyl-1,4,5,6-tetrahydrobenzo[6,7]cyclohepta[1,2-*c*]pyrazole-3-carboxamide (4i). General procedure I was used to convert **8a** and *p*-anisidine into the title product. The mixture was purified by flash chromatography (petroleum ether/EtOAc, 9:1) to afford **4i** (0.44 g, 70%) as a white solid. $R_f = 0.47$ (petroleum ether/EtOAc 8:2); mp 105 °C; IR 1667, 3314; API-ES calcd for 512.81, found 512.1. Anal. ($C_{26}H_{20}Cl_3N_3O_2$) C, H, Cl, N.

7-Chloro-1-(2′,4′-dichlorophenyl)-*N*-piperidin-1-yl-1,4,5,6-tetrahydrobenzo[6,7]cyclohepta[1,2-*c*]pyrazole-3-carboxamide (4j). General procedure I was used to convert **8d** and *N*-aminopiperidine into the title product. The mixture was purified by flash chromatography (petroleum ether/EtOAc, 6:4) to afford **4j** (0.38 g, 63%) as a white solid. $R_f = 0.59$ (petroleum ether/EtOAc 6:4); mp 199−200 °C; IR 1649, 3161; API-ES calcd for 489.8, found 489.1. Anal. ($C_{24}H_{23}Cl_3N_4O$) C, H, Cl, N.

9-Chloro-1-(2′,4′-dichlorophenyl)-*N*-piperidin-1-yl-1,4,5,6-tetrahydrobenzo[6,7]cyclohepta[1,2-*c*]pyrazole-3-carboxamide (4k).[18] General procedure I was used to convert **8e** and *N*-aminopiperidine into the title product. The mixture was purified by flash chromatography (petroleum ether/EtOAc, 6:4) to afford **4k** (0.31 g, 51%) as a white solid. $R_f = 0.63$ (petroleum ether/EtOAc 6:4); mp 271 °C (232−234 °C);[18] IR 1647, 3158; API-ES calcd for 489.8, found 491.1. Anal. ($C_{24}H_{23}Cl_3N_4O$) C, H, Cl, N.

8-Bromo-1-(2′,4′-dichlorophenyl)-*N*-piperidin-1-yl-1,4,5,6-tetrahydrobenzo[6,7]cyclohepta[1,2-*c*]pyrazole-3-carboxamide (4l). General procedure I was used to convert **8b** and *N*-aminopiperidine into the title product. The mixture was purified by flash chromatography (petroleum ether/EtOAc, 6:4) to afford **4l** (0.51 g, 78%) as a white solid. $R_f = 0.53$ (petroleum ether/EtOAc 6:4); mp 205 °C; IR 1605, 3202; API-ES calcd for 534.3, found 535.0. Anal. ($C_{24}H_{23}BrCl_2N_4O$) C, H, Cl, N.

8-Methyl-1-(2′,4′-dichlorophenyl)-*N*-piperidin-1-yl-1,4,5,6-tetrahydrobenzo[6,7]cyclohepta[1,2-*c*]pyrazole-3-carboxamide (4m). General procedure I was used to convert **8c** and *N*-aminopiperidine into the title product. The mixture was purified by flash chromatography (petroleum ether/EtOAc, 6:4) to afford **4m** (0.41 g, 72%) as a white solid. $R_f = 0.55$ (petroleum ether/EtOAc 6:4); mp 234−235 °C; IR 1645, 3163; API-ES calcd for 469.41, found 469.1. Anal. ($C_{24}H_{26}Cl_2N_4O$) C, H, Cl, N.

8-Methyl-1-(2′,4′-dichlorophenyl)-*N*-pyrrolidin-1-yl-1,4,5,6-tetrahydrobenzo[6,7]cyclohepta[1,2-*c*]pyrazole-3-carboxamide (4n). General procedure I was used to convert **8c** and *N*-aminopyrrolidine hydrochloride into the title product. Because of an excess of the hydrochloride salt, an amount of 2 equiv of TEA was used in this reaction. The mixture was purified by flash chromatography (petroleum ether/EtOAc, 4:6) to afford **4n** (0.40 g, 72%) as a white solid. $R_f = 0.42$ (petroleum

ether/EtOAc 4:6); mp 134 °C; IR 1653, 3206; API-ES calcd for 455.38, found 455.1. Anal. ($C_{24}H_{24}Cl_2N_4O$) C, H, Cl, N.

1-(2′,4′-Dichlorophenyl)-*N*-piperidin-1-yl-1,4,5,6-tetrahydrobenzo[6,7]cyclohepta[1,2-*c*]pyrazole-3-carboxamide (4o).[18] General procedure I was used to convert **8f** and *N*-aminopiperidine. The mixture was purified by flash chromatography (petroleum ether/EtOAc, 6:4) to afford **4o** (0.40 g, 73%) as a white solid. R_f = 0.63 (petroleum ether/EtOAc 6:4); mp 165−168 °C (167−169 °C);[18] IR 1650, 3165; API-ES calcd for 455.38, found 455.1. Anal. ($C_{24}H_{24}Cl_2N_4O$) C, H, Cl, N.

General Procedure II: Synthesis of Carboxylic Acids (8a−f). To a mixture of ester **7** (1.0 equiv, 5 mmol) in methanol (25 mL) was added a solution of potassium hydroxide (2.0 equiv) in methanol (18 mL). The resulting mixture was heated under reflux overnight. The mixture was allowed to cool to room temperature and then poured into water and acidified with 1 N HCl. The precipitate was filtered, washed with water, and air-dried to yield the analytically pure acid.

8-Chloro-1-(2′,4′-dichlorophenyl)-1,4,5,6-tetrahydrobenzo[6,7]cyclohepta[1,2*c*]pyrazole-3-carboxylic Acid (8a).[1] General procedure II was used to convert **7a** into the title product **8a** (1.91 g, 94.0%) as a white solid. R_f = 0.41 ($CHCl_3$/MeOH 9:1); mp 270 °C (270 °C);[1] IR 1690, 3410; ^{1}H NMR ($CDCl_3$) δ 2.25−2.30 (m, 2H), 2.68 (t, 2H, J = 6.4 Hz), 3.10−3.23 (m, 2H), 4.50 (br s, 1H, OH exchange with D_2O), 6.61 (d, 1H, J = 8.4 Hz), 7.03 (dd, 1H, J_o = 8.2 Hz, J_m = 2.2 Hz), 7.32 (d, 1H, J = 2.0 Hz), 7.39−7.44 (m, 2H), 7.52 (d, 1H, J = 8.0 Hz). Anal. ($C_{19}H_{13}Cl_3N_2O_2$) C, H, Cl, N.

8-Bromo-1-(2′,4′-dichlorophenyl)-1,4,5,6-tetrahydrobenzo[6,7]cyclohepta[1,2-*c*]pyrazole-3-carboxylic Acid (8b). General procedure II was used to convert **7b** into the title product **8b** (2.21 g, 98.0%) as a white solid. R_f = 0.76 ($CHCl_3$/MeOH 9:1); mp 144−146 °C; IR 1692, 3470; ^{1}H NMR ($CDCl_3$) δ 2.18−2.38 (m, 2H), 2.64−2.70 (m, 2H), 3.10−3.30 (m, 2H), 4.70 (br s, 1H, OH exchange with D_2O), 6.55 (d, 1H, J = 8.2 Hz), 7.18 (dd, 1H, J_o = 8.2 Hz, J_m = 1.8 Hz), 7.39−7.56 (m, 4H). Anal. ($C_{19}H_{13}BrCl_2N_2O_2$) C, H, Cl, N.

8-Methyl-1-(2′,4′-dichlorophenyl)-1,4,5,6-tetrahydrobenzo[6,7]cyclohepta[1,2-*c*]pyrazole-3-carboxylic Acid (8c). General procedure II was used to convert **7c** into the title product **8c** (1.82 g, 94.0%) as a white solid. R_f = 0.52 ($CHCl_3$/MeOH 9:1); mp 145 °C; IR 1682, 3377; ^{1}H NMR ($CDCl_3$) δ 2.18−2.35 (m, 5H), 2.66 (t, 2H, J = 6.0 Hz), 2.90−3.20 (m, 2H), 4.30 (br s, 1H, OH exchange with D_2O), 6.57 (d, 1H, J = 7.8 Hz), 6.85 (d, 1H, J = 8.0 Hz), 7.13 (s, 1H), 7.35−7.42 (m, 2H), 7.52 (d, 1H, J = 8.0 Hz). Anal. ($C_{20}H_{16}Cl_2N_2O_2$) C, H, Cl, N.

7-Chloro-1-(2′,4′-dichlorophenyl)-1,4,5,6-tetrahydrobenzo[6,7]cyclohepta[1,2-*c*]pyrazole-3-carboxylic Acid (8d). General procedure II was used to convert **7d** into the title product **8d** (1.97 g, 97.0%) as a white solid. R_f = 0.48 ($CHCl_3$/MeOH 9:1); mp 125−128 °C; IR 1720, 3419; ^{1}H NMR ($CDCl_3$) δ 2.26−2.40 (m, 2H), 2.50−3.40 (m, 4H), 4.78 (br s, 1H, OH exchange with D_2O), 6.59 (d, 1H, J = 7.6 Hz), 6.99 (t, 1H, J = 8.0 Hz), 7.30−7.45 (m, 3H), 7.54 (d, 1H, J = 8.0 Hz). Anal. ($C_{19}H_{13}Cl_3N_2O_2$) C, H, Cl, N.

9-Chloro-1-(2′,4′-dichlorophenyl)-1,4,5,6-tetrahydrobenzo[6,7]cyclohepta[1,2-*c*]pyrazole-3-carboxylic Acid (8e). General procedure II was used to convert **7e** into the title product **8e** (1.99 g, 98.0%) as a white solid. R_f = 0.60 ($CHCl_3$/MeOH 9:1); mp 250 °C; IR 1716, 3419; ^{1}H NMR ($CDCl_3$) δ 2.25−2.27 (m, 2H), 2.67 (t, 2H, J = 6.4 Hz), 3.07−3.32 (m, 2H), 4.78 (br s, 1H, OH exchange with D_2O), 6.65 (d, 1H, J = 1.8 Hz), 7.20−7.32 (m, 2H), 7.40−7.50 (m, 2H), 7.57 (d, 1H, J = 9.0 Hz). Anal. ($C_{19}H_{13}Cl_3N_2O_2$) C, H, Cl, N.

1-(2′,4′-Dichlorophenyl)-1,4,5,6-tetrahydrobenzo[6,7]-cyclohepta[1,2-*c*]pyrazole-3-carboxylic Acid (8f).[18] General procedure II was used to convert **7f** into the title product **8f** (1.71 g, 92.0%) as a yellow solid. R_f = 0.48 ($CHCl_3$/MeOH 9:1); mp 262−264 °C (262−264 °C);[18] IR 1695, 3420; ^{1}H NMR ($CDCl_3$) δ 2.25−2.31 (m, 2H), 2.71 (t, 2H, J = 6.2 Hz), 2.75−3.20 (m, 2H), 3.70 (br s, 1H, OH exchange with D_2O), 6.69 (d, 1H, J = 7.6 Hz), 7.05 (t, 1H, J = 7.0 Hz), 7.18−7.48 (m, 4H), 7.52 (d, 1H, J = 8.0 Hz). Anal. ($C_{19}H_{14}Cl_2N_2O_2$) C, H, Cl, N.

General Procedure III: Synthesis of Tricyclic Esters (7a−f). A stirred mixture of diketoester **6** (1.0 equiv, 4 mmol) and 2,4-dichlorophenylhydrazine hydrochloride (1.15 equiv) in EtOH (28 mL) was heated under reflux for 3 h. The mixture was allowed to cool to room temperature, and the solvent was removed under reduced pressure to give a red-orange solid, which was purified by flash chromatography to afford the analytically pure product.

Ethyl 8-Chloro-1-(2′,4′-dichlorophenyl)-1,4,5,6-tetrahydrobenzo[6,7]cyclohepta[1,2-*c*]pyrazole-3-carboxylate (7a).[1] General procedure III was used to convert **6a** and 2,4-dichlorophenylhydrazine hydrochloride into the title product. The residue was purified by flash chromatography (petroleum ether/EtOAc, 8.5:1.5) to afford **7a** (1.18 g, 68%) as a yellow solid. R_f = 0.39 (petroleum ether/EtOAc, 8.5:1.5); mp 158−160 °C (triturated with petroleum ether) (160−161 °C)[1]; IR 1724; ^{1}H NMR ($CDCl_3$) δ 1.43 (t, 3H, J = 7.2 Hz), 2.20−2.36 (m, 2H), 2.66 (t, 2H, J = 6.4 Hz), 3.10−3.30 (m, 2H), 4.45 (q, 2H, J = 7.2 Hz), 6.60 (d, 1H, J = 8.4 Hz), 7.02 (dd, 1H, J_o = 8.4 Hz, J_m = 2.2 Hz), 7.31 (d, 1H, J = 1.8 Hz), 7.37−7.42 (m, 2H), 7−54 (d, 1H, J = 9.2 Hz). Anal. ($C_{21}H_{17}Cl_3N_2O_2$) C, H, Cl, N.

Ethyl 8-Bromo-1-(2′,4′-dichlorophenyl)-1,4,5,6-tetrahydrobenzo[6,7]cyclohepta[1,2-*c*]pyrazole-3-carboxylate (7b). General procedure III was used to convert **7b** and 2,4-dichlorophenylhydrazine hydrochloride into the title product. The residue was purified by flash chromatography (petroleum ether/EtOAc, 8.5:1.5) to afford **7b** (1.49 g, 78%) as a white solid. R_f = 0.66 (petroleum ether/EtOAc, 8.5:1.5); mp 90 °C (triturated with petroleum ether); IR 1724; ^{1}H NMR ($CDCl_3$) δ 1.43 (t, 3H, J = 7.0 Hz), 2.20−2.40 (m, 2H), 2.66 (t, 2H, J = 6.4 Hz), 3.10−3.30 (m, 2H), 4.46 (q, 2H, J = 7.0 Hz), 6.54 (d, 1H, J = 8.2 Hz), 7.17 (dd, 1H, J_o = 8.2 Hz, J_m = 2.0 Hz), 7.35−7.42 (m, 2H), 7.46 (d, 1H, J = 2.0 Hz), 7.54 (d, 1H, J = 9.4 Hz). Anal. ($C_{21}H_{17}BrCl_2N_2O_2$) C, H, Cl, N.

Ethyl 8-Methyl-1-(2′,4′-dichlorophenyl)-1,4,5,6-tetrahydrobenzo[6,7]cyclohepta[1,2-*c*]pyrazole-3-carboxylate (7c). General procedure III was used to convert **7c** and 2,4-dichlorophenylhydrazine hydrochloride into the title product. The residue was purified by flash chromatography (petroleum ether/EtOAc, 8.5:1.5) to afford **7c** (1.05 g, 63%) as a white solid. R_f = 0.65 (petroleum ether/EtOAc, 8.5:1.5); mp 75 °C (triturated with petroleum ether); IR 1715; ^{1}H NMR ($CDCl_3$) δ 1.43 (t, 3H, J = 7.0 Hz), 2.20−2.32 (m, 5H), 2.65 (t, 2H, J = 6.2 Hz), 3.00−3.25 (m, 2H), 4.45 (q, 2H, J = 7.2 Hz), 6.56 (d, 1H, J = 7.8 Hz), 6.84 (d, 1H, J = 7.2 Hz), 7.12 (s, 1H), 7.34−7.38−7.41 (m, 2H), 7.53 (d, 1H, J = 7.8 Hz). Anal. ($C_{22}H_{20}Cl_2N_2O_2$) C, H, Cl, N.

Ethyl 7-Chloro-1-(2′,4′-dichlorophenyl)-1,4,5,6-tetrahydrobenzo[6,7]cyclohepta[1,2-*c*]pyrazole-3-carboxylate (7d). General procedure III was used to convert **6d** and 2,4-dichlorophenylhydrazine hydrochloride into the title product. The residue was purified by flash chromatography (petroleum ether/EtOAc, 8.5:1.5) to afford **7d** (0.94 g, 54%) as a white solid. R_f = 0.29 (petroleum ether/EtOAc, 8.5:1.5); mp 93 °C (triturated with petroleum ether); IR 1715; ^{1}H NMR ($CDCl_3$) δ 1.44 (t, 3H, J = 7.0 Hz), 2.20−2.40 (m, 2H), 2.60−2.83 (m, 2H), 2.90−3.24 (m, 2H), 4.46 (q, 2H, J = 7.2 Hz), 6.58 (d, 1H, J = 7.8 Hz), 6.98 (t, 1H, J = 7.8 Hz), 7.26−7.42 (m, 3H), 7−55 (d, 1H, J = 8.8 Hz). Anal. ($C_{21}H_{17}Cl_3N_2O_2$) C, H, Cl, N.

Ethyl 9-Chloro-1-(2′,4′-dichlorophenyl)-1,4,5,6-tetrahydrobenzo[6,7]cyclohepta[1,2-*c*]pyrazole-3-carboxylate (7e). General procedure III was used to convert **6e** and 2,4-dichlorophenylhydrazine hydrochloride into the title product. The residue was purified by flash chromatography (petroleum ether/EtOAc, 9:1) to afford **7e** (1.18 g, 68%) as a white solid. R_f = 0.43 (petroleum ether/EtOAc, 9:1); mp 176−177 °C (triturated with petroleum ether); IR 1709; ^{1}H NMR ($CDCl_3$) δ 1.43 (t, 3H, J = 7.0 Hz), 2.10−2.35 (m, 2H), 2.66 (t, 2H, J = 6.8 Hz), 3.10−3.40 (m, 2H), 4.46 (q, 2H, J = 7.2 Hz), 6.65 (s, 1H), 7.15−7.30 (m, 2H), 7.35−7.50 (m, 2H), 7−57 (d, 1H, J = 9.0 Hz). Anal. ($C_{21}H_{17}Cl_3N_2O_2$) C, H, Cl, N.

Ethyl 1-(2′,4′-Dichlorophenyl)-1,4,5,6-tetrahydrobenzo-[6,7]cyclohepta[1,2-*c*]pyrazole-3-carboxylate (7f).[18] General procedure III was used to convert **6f** and 2,4-dichlorophenylhydrazine hydrochloride into the title product. The residue was purified by flash chromatography (petroleum ether/EtOAc, 8.5:1.5) to afford **7f** (1.23 g, 77%) as a pink solid. R_f = 0.47 (petroleum ether/EtOAc, 8.5:1.5); mp 129−130 °C (triturated with petroleum ether); IR 1720; ^{1}H NMR ($CDCl_3$) δ 1.43 (t, 3H, J = 7.4 Hz), 2.15−2.38 (m, 2H), 2.69 (t, 2H, J = 6.4 Hz), 3.00−3.35 (m, 2H), 4.46 (q, 2H, J = 7.4 Hz), 6.68 (d, 1H, J = 7.6 Hz), 7.04 (t, 1H, J = 6.2 Hz), 7.15−7.47 (m, 4H), 7.54 (d, 1H, J = 7.6 Hz). Anal. ($C_{21}H_{18}Cl_2N_2O_2$) C, H, Cl, N.

General Procedure IV: Synthesis of α,γ-Diketoesters (6a−f). Sodium metal (2.0 equiv) was added in one small portion to dry ethanol (5 mL), and the mixture was stirred until all the sodium had reacted. Ethyl oxalate (1.0 equiv) was added, followed by dropwise addition of a solution of appropriate benzosuberone starting material (1.0 equiv, 6 mmol) in dry ethanol (30 mL). The solution was stirred at room temperature for 5−9 h. The mixture was slowly poured into ice, and 2 N HCl was added. The resulting mixture was extracted with $CHCl_3$, dried (Na_2SO_4), and concentrated to afford the analytically pure product.

Ethyl γ-(7-Chloro-1-oxo-2,3,4,5-tetrahydrobenzocyclohepten-2-yl)-α-oxoacetate (6a).[1] General procedure IV was used to convert **5a** into the title product. The mixture was stirred for 9 h at room temperature. Compound **6a** (1.45 g, 82%) was isolated as a yellowish oil. R_f = 0.71 (petroleum ether/EtOAc, 1:1); bp 93−95 °C (0.05 mmHg) (95−98 °C/0.05 mmHg);[1] IR 1680, 1730, 3440; ^{1}H NMR ($CDCl_3$) δ 1.41 (t, 3H, J = 7.0 Hz), 2.07 (qu, 2H, J = 6.8 Hz), 2.32 (t, 2H, J = 6.4 Hz), 2.72 (t, 2H, J = 6.8 Hz), 4.34 (q, 2H, J = 7.0 Hz), 7.22−7.37 (m, 2H), 7.58 (d, 1H, J = 8.2 Hz), 15.37 (br s, 1H, OH exchange with D_2O). Anal. ($C_{15}H_{15}ClO_4$) C, H, Cl.

Ethyl γ-(7-Bromo-1-oxo-2,3,4,5-tetrahydrobenzocyclohepten-2-yl)-α-oxoacetate (6b). General procedure IV was used to convert **5b** into the title product. The mixture was stirred for 9 h at room temperature. Compound **6b** (1.82 g, 90%) was isolated as a yellowish oil. R_f = 0.40 (petroleum ether/EtOAc, 9.5:0.5); bp 95−97 °C (0.05 mmHg); IR 1698, 1731, 3440; ^{1}H NMR ($CDCl_3$) δ 1.41 (t, 3H, J = 7.2 Hz), 2.07 (qu, 2H, J = 6.8 Hz), 2.31 (t, 2H, J = 6.4 Hz), 2.71 (t, 2H, J = 7.0 Hz), 4.38 (q, 2H, J = 7.0 Hz), 7.40 (s, 1H), 7.48−7.52 (m, 2H), 15.36 (br s, 1H, OH exchange with D_2O). Anal. ($C_{15}H_{15}BrO_4$) C, H.

Ethyl γ-(7-Methyl-1-oxo-2,3,4,5-tetrahydrobenzocyclohepten-2-yl)-α-oxoacetate (6c). General procedure IV was used to convert **5c** into the title product. The mixture was stirred for 9 h at room temperature. Compound **6c** (1.58 g, 96%) was isolated as a yellowish oil. R_f = 0.65 (petroleum ether/EtOAc, 9:1); bp 92−93 °C (0.05 mmHg); IR 1607, 1732, 3478; ^{1}H NMR ($CDCl_3$) δ 1.40 (t, 3H, J = 7.2 Hz), 2.05 (qu, 2H, J = 6.8 Hz), 2.32 (t, 2H, J = 6.6 Hz), 2.39 (s, 3H), 2.70 (t, 2H, J = 7.0 Hz), 4.38 (q, 2H, J = 7.0 Hz), 7.03 (s, 1H), 7.17 (d, 1H, J = 7.8 Hz), 7.54 (d, 1H, J = 7.8 Hz), 15.52 (br s, 1H, OH exchange with D_2O). Anal. ($C_{16}H_{18}O_4$) C, H.

Ethyl γ-(6-Chloro-1-oxo-2,3,4,5-tetrahydrobenzocyclohepten-2-yl)-α-oxoacetate (6d). General procedure IV was used to convert **5d** into the title product. The mixture was stirred for 5 h at room temperature. Compound **6d** (1.71 g, 97%) was isolated as a yellowish oil. R_f = 0.47 (petroleum ether/EtOAc, 9.5:0.5); bp 94−95 °C (0.05 mmHg); IR 1698, 1730, 3440; ^{1}H NMR ($CDCl_3$) δ 1.41 (t, 3H, J = 7.2 Hz), 2.06 (qu, 2H, J = 6.6 Hz), 2.28 (t, 2H, J = 6.4 Hz), 2.94 (t, 2H, J = 6.8 Hz), 4.39 (q, 2H, J = 7.0 Hz), 7.30 (d, 1H, J = 7.8 Hz), 7.49−7.56 (m, 2H), 15.37 (br s, 1H, OH exchange with D_2O). Anal. ($C_{15}H_{15}ClO_4$) C, H, Cl.

Ethyl γ-(8-Chloro-1-oxo-2,3,4,5-tetrahydro-benzocyclohepten-2-yl)-α-oxoacetate (6e). General procedure IV was used to convert **5e** into the title product. The mixture was stirred for 5 h at room temperature. Compound **6e** (1.71 g, 97%) was isolated as a yellowish oil. R_f = 0.51 (petroleum ether/EtOAc, 9.5:0.5); bp 95−97 °C (0.05 mmHg); IR 1698, 1731, 3435; ^{1}H NMR ($CDCl_3$) δ 1.41 (t, 3H, J = 7.0 Hz), 2.06 (qu, 2H, J = 7.0 Hz), 2.31 (t, 2H, J = 6.4 Hz), 2.71 (t, 2H, J = 7.0 Hz), 4.39 (q, 2H, J = 7.0 Hz), 7.16 (d, 1H, J = 7.8 Hz), 7.42 (d, 1H, J = 7.8 Hz), 7.60 (s, 1H), 15.37 (br s, 1H, OH exchange with D_2O). Anal. ($C_{15}H_{15}ClO_4$) C, H, Cl.

Ethyl γ-(1-Oxo-2,3,4,5-tetrahydrobenzocyclohepten-2-yl)-α-oxoacetate (6f).[18] General procedure IV was used to convert **5f** into the title product. The mixture was stirred for 8 h at room temperature. Compound **6f** (1.20 g, 77%) was isolated as a yellowish oil. R_f = 0.47 (petroleum ether/EtOAc, 9:1); bp 97−99 °C (0.05 mmHg); IR 1670, 1735, 3420; ^{1}H NMR ($CDCl_3$) δ 1.41 (t, 3H, J = 7.0 Hz), 2.07 (qu, 2H, J = 6.8 Hz), 2.32 (t, 2H, J = 6.6 Hz), 2.74 (t, 2H, J = 6.8 Hz), 4.39 (q, 2H, J = 7.0 Hz), 7.22 (d, 1H, J = 7.0 Hz), 7.30−7.55 (m, 2H), 7.63 (d, 1H, J = 7.6 Hz), 15.52 (br s, 1H, OH exchange with D_2O). Anal. ($C_{15}H_{16}O_4$) C, H.

General Procedure V: Synthesis of Benzosuberones (5a−c). A mixture of acid **11** (7.38 mmol) and polyphosphoric acid (8.88 g) was stirred at 130 °C for 1 h. Then it was cooled to 100 °C and a 0.2 N solution of NaOH was added. After cooling at room temperature, the solution was extracted with CH_2Cl_2, dried (Na_2SO_4), and concentrated to afford a crude product that was purified by flash chromatography.

7-Chloro-2,3,4,5-tetrahydro-benzocycloheptan-1-one (5a).[1] General procedure V was used to convert **11a** into the title product. The residue was purified by flash chromatography (petroleum ether/EtOAc, 9:1) to afford **5a** (0.92 g, 64%) as a yellowish oil. R_f = 0.65 (petroleum ether/EtOAc, 9:1); bp 91−93 °C (0.05 mmHg) (94−97 °C/0.05 mmHg);[1] IR 1677; ^{1}H NMR ($CDCl_3$) δ 1.70−1.96 (m, 4H), 2.72 (t, 2H, J = 5.0 Hz), 2.91 (t, 2H, J = 5.4 Hz), 7.19−7.30 (m, 2H), 7.67 (d, 1H, J = 8.2 Hz). Anal. ($C_{11}H_{11}ClO$) C, H, Cl.

7-Bromo-2,3,4,5-tetrahydro-benzocycloheptan-1-one (5b).[22] General procedure V was used to convert **11b** into the title product. The residue was purified by flash chromatography (petroleum ether/EtOAc, 9:1) to afford **5b** (1.12 g, 64%) as a yellowish oil. R_f = 0.50 (petroleum ether/EtOAc, 9:1); bp 103−105 °C (0.05 mmHg) (126−129 °C/0.4 mmHg);[22] IR 1673; ^{1}H NMR ($CDCl_3$) δ 1.75−1.93 (m, 4H), 2.72 (t, 2H, J = 5.2 Hz), 2.89 (t, 2H, J = 5.6 Hz), 7.35−7.46 (m, 2H), 7.59 (d, 1H, J = 8.2 Hz). Anal. ($C_{11}H_{11}BrO$) C, H.

7-Methyl-2,3,4,5-tetrahydrobenzocycloheptan-1-one (5c).[23] General procedure V was used to convert **11c** into the title product. The residue was purified by flash chromatography (petroleum ether/EtOAc, 9:1) to afford **5c** (0.79 g, 62%) as a yellowish oil. R_f = 0.65 (petroleum ether/EtOAc, 9:1); bp 100 °C (0.05 mmHg); IR 1678; ^{1}H NMR ($CDCl_3$) δ 1.73−1.93 (m, 4H), 2.37 (s, 3H), 2.71 (t, 2H, J = 5.0 Hz), 2.89 (t, 2H, J = 5.8 Hz), 7.01 (s, 1H), 7.10 (d, 1H, J = 8.0 Hz), 7.66 (d, 1H, J = 7.8 Hz). Anal. ($C_{12}H_{14}O$) C, H.

General Procedure VI: Synthesis of Chloro Derivatives (5d,e).[23,24] To a mixture of the appropriate amino derivatives **5i,j** (8.30 mmol, 1 equiv) in a 15% solution of HCl (13.5 mL) was cautiously added an aqueous solution of $NaNO_2$ (1.2 equiv, 3 mL), and the mixture was stirred at 0 °C. The resulting solution was dropwise added to a mixture of CuCl (3 equiv) in concentrated HCl (23.5 mL) at the same temperature. The resulting mixture was stirred for an additional hour 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 flash chromatography (petroleum ether/EtOAc, 8:2), furnishing the analytically pure products as yellowish oils.

6-Chloro-2,3,4,5-tetrahydrobenzocycloheptan-1-one (5d).[24] General procedure VI was used to convert **5i** into the title product. The residue was purified by flash chromatography (petroleum ether/EtOAc, 8:2) to afford **5d** (1.27 g, 79%) as a yellowish oil. R_f = 0.67 (petroleum ether/EtOAc, 8:2); bp 93−95 °C (0.05 mmHg); IR 1684; ^{1}H NMR ($CDCl_3$) δ 1.74−1.90 (m, 4H), 2.70 (t, 2H, J = 7.8 Hz), 3.09 (t, 2H, J = 6.8 Hz), 7.24 (d, 1H, J = 8.0 Hz), 7.49 (s, 1H), 7.51 (d, 1H, J = 7.8 Hz). Anal. ($C_{11}H_{11}ClO$) C, H, Cl.

8-Chloro-2,3,4,5-tetrahydrobenzocycloheptan-1-one (5e).[23] General procedure VI was used to convert **5j** into the title product. The residue was purified by flash chromatogra-

phy (petroleum ether/EtOAc, 8:2) to afford **5e** (1.27 g, 79%) as a yellowish oil. $R_f = 0.72$ (petroleum ether/EtOAc, 8:2); bp 80−82 °C (0.05 mmHg) (101 °C/0.15 mmHg);[23] IR 1678; ^{1}H NMR ($CDCl_3$) δ 1.73−1.92 (m, 4H), 2.73 (t, 2H, $J = 5.6$ Hz), 2.90 (t, 2H, $J = 6.4$ Hz), 7.15 (d, 1H, $J = 8.0$ Hz), 7.38 (dd, 1H, $J_o = 8.0$ Hz, $J_m = 2.0$ Hz), 7.68 (d, 1H, $J = 2.0$ Hz). Anal. ($C_{11}H_{11}ClO$) C, H, Cl.

General Procedure VII: Synthesis of Amino Derivatives (5i,j). To a mixture of the appropriate nitroderivatives **5g**,**h** (4.80 mmol, 1 equiv) and Sn (7 equiv) in concentrated HCl (20 mL) was added EtOH (11 mL), and the mixture was refluxed for 30 min. After cooling at room temperature, the mixture was alkalinized with a 30% aqueous solution of NaOH. The basic solution was extracted with EtOAc, dried (Na_2SO_4), and concentrated to afford the analytically pure products.

6-Amine-2,3,4,5-tetrahydrobenzocycloheptan-1-one (5i). General procedure VII was used to convert **5g** into the title product **5i** (1.66 g, 97%) as a yellowish oil. $R_f = 0.80$ ($CHCl_3$/MeOH, 9.5:0.5); bp 93−95 °C (0.05 mmHg); IR 1670, 3365−3453; ^{1}H NMR ($CDCl_3$) δ 1.80−1.89 (m, 4H), 2.66−2.78 (m, 4H), 3.73 (br s, 2H, NH_2, exchange with D_2O), 6.77−6.83 (m, 1H), 7.07−7.22 (m, 2H). Anal. ($C_{11}H_{13}NO$) C, H, N.

8-Amine-2,3,4,5-tetrahydrobenzocycloheptan-1-one (5j).[24] General procedure VII was used to convert **5h** into the title product **5j** (1.44 g, 84%) as a yellowish solid. $R_f = 0.79$ ($CHCl_3$/MeOH, 9.5:0.5); mp 103−105 °C (104−105 °C);[24] IR 1660, 3348−3430; ^{1}H NMR ($CDCl_3$) δ 1.72−1.90 (m, 4H), 2.70 (t, 2H, $J = 4.8$ Hz), 2.82 (t, 2H, $J = 6.0$ Hz), 3.70 (br s, 2H, NH_2, exchange with D_2O), 6.72−6.77 (m, 1H), 6.97−7.06 (m, 2H). Anal. ($C_{11}H_{13}NO$) C, H, N.

Synthesis of 6-Nitro-2,3,4,5-tetrahydrobenzocycloheptan-1-one (5g) and 8-Nitro-2,3,4,5-tetrahydrobenzocycloheptan-1-one (5h).[24] To a mixture of the benzosuberone **5f** (6.20 mmol, 1 equiv) in concentrated H_2SO_4 (5.4 mL) cooled at −5 °C, powdered KNO_3 (12 equiv) was portionwise added in 2 h. The mixture was stirred for 1 h at the same temperature and then poured in ice. The precipitate was filtered off, washed (H_2O), and dried. The crude product was purified by flash chromatography (petroleum ether/EtOAc, 8:2) to afford the analytically pure products, which showed two bands at $R_f = 0.54$ and 0.71. The component at $R_f = 0.54$ (0.87 g, 68%) is a white solid. IR and ^{1}H NMR spectra and elemental analysis showed it to be the 8-nitro-derivative (**5h**); mp 89−90 °C (92−92.8 °C);[24] IR 1560, 1677; ^{1}H NMR ($CDCl_3$) δ 1.82−2.02 (m, 4H), 2.80 (t, 2H, $J = 5.6$ Hz), 3.05 (t, 2H, $J = 6.6$ Hz), 7.41 (d, 1H, $J = 8.4$ Hz), 8.25 (dd, 1H, $J_o = 8.0$ Hz, $J_m = 2.0$ Hz), 8.54 (d, 1H, $J = 2.0$ Hz). Anal. ($C_{11}H_{11}NO_3$) C, H, N. The component at $R_f = 0.71$ (0.12 g, 9.40%) is a yellowish solid. IR and ^{1}H NMR spectra and elemental analysis showed it to be the 6-nitro-derivative (**5g**); mp 90−93 °C (93−95 °C); IR 1533, 1690; ^{1}H NMR ($CDCl_3$) δ 1.76−1.89 (m, 2H), 1.95−2.08 (m, 2H), 2.74 (t, 2H, $J = 6.0$ Hz), 2.98 (t, 2H, $J = 6.8$ Hz), 7.44 (t, 1H, $J = 7.8$ Hz), 7.81 (dd, 1H, $J_o = 7.6$ Hz, $J_m = 1.4$ Hz), 7.91 (dd, 1H, $J_o = 7.6$ Hz, $J_m = 1.4$ Hz). Anal. ($C_{11}H_{11}NO_3$) C, H, N.

General Procedure VIII: Synthesis of Phenylpentanoic Acids (11a−c). A mixture of pentenoic acid **10** (11.8 mmol) and PtO_2 (8.88 g) in EtOH (126 mL) was hydrogenated at room temperature for 2 h. The suspension was filtered over Celite and the solution was concentrated at reduced pressure to give an oily product analytically pure.

5-(3′-Chlorophenyl)pentanoic Acid (11a). General procedure VIII was used to convert **10a** into the title product to afford **11a** (2.30 g, 92%) as a yellow-green oil. $R_f = 0.26$ (petroleum ether/EtOAc, 9:1); bp 84 °C (27 mmHg); IR 1710, 3300; ^{1}H NMR ($CDCl_3$) δ 1.60−1.80 (m, 4H), 2.30−2.45 (m, 2H), 2.55−2.71 (m, 2H), 7.04 (d, 1H, $J = 6.0$ Hz), 7.12−7.35 (m, 3H), 9.65 (br s, 1H, OH exchange with D_2O). Anal. ($C_{11}H_{13}ClO_2$) C, H, Cl.

5-(3′-Bromophenyl)pentanoic Acid (11b). General procedure VIII was used to convert **10b** into the title product to afford **11b** (2.87 g, 95%) as a yellow-green oil. $R_f = 0.79$ (petroleum ether/EtOAc, 1:1); bp 86 °C (27 mmHg); IR 1709, 3350; ^{1}H NMR ($CDCl_3$) δ 1.62−1.69 (m, 2H), 1.80−2.02 (m, 2H), 2.33−2.63 (m, 4H), 7.05−7.17 (m, 1H), 7.28−7.32 (m, 1H), 7.45−7.53 (m, 1H), 7.69−7.78 (m, 1H), 10.00 (br s, 1H, OH exchange with D_2O). Anal. ($C_{11}H_{13}BrO_2$) C, H.

5-(*m*-Toluyl)pentanoic Acid (11c). General procedure VIII was used to convert **10c** into the title product **11c** (2.26 g, 96%) as a yellow-green oil. $R_f = 0.75$ (petroleum ether/EtOAc, 1:1); bp 83−84 °C (27 mmHg); IR 1714, 3420; ^{1}H NMR ($CDCl_3$) δ 1.60−1.72 (m, 2H), 1.82−2.05 (m,2H), 2.32 (s, 3H), 2.35−2.65 (m, 4H), 6.95−7.12 (m, 1H), 7.13−7.20 (m, 1H), 7.40−7.58 (m, 1H), 7.65−7.82 (m, 1H), 9.10 (br s, 1H, OH exchange with D_2O). Anal. ($C_{12}H_{16}O_2$) C, H.

General Procedure IX: Synthesis of (*Z*,*E*)-Phenylpentenoic Acids (10a−c). To a suspension of (3-carboxypropyl)-triphenylphosphonium bromide (32.4 mmol) in anhydrous DMSO (29.5 mL) *t*-BuOK (61.4 mmol) was added. The mixture was stirred for 20 min at room temperature, and then a solution of the appropriate meta-substitued benzaldehyde **9** (28 mmol) in DMSO was dropwise added. The resulting mixture was stirred at the same temperature for an additional 4 h and then poured in water and extracted with $CHCl_3$. The aqueous solution was acidified with concentrated HCl and extracted with $CHCl_3$. The organic layer was washed (H_2O), dried (Na_2SO_4), and concentrated to give an oily residue, which was purified by flash chromatography to afford the diastereomeric mixture as an oil.

(*Z*,*E*)-5-(3′-Chlorophenyl)pentenoic Acid (10a). General procedure IX was used to convert **9a** into the title product. The residue was purified by flash chromatography (petroleum ether/EtOAc, 1:1) to afford **10a** (7.34 g, 78%) as a yellow oil. $R_f = 0.67$ (CH_2Cl_2/$(CH_3)_2CO$, 1:1); bp 85 °C (27 mmHg); IR 1720, 3450; ^{1}H NMR ($CDCl_3$) δ 2.45−2.69 (m, 4H), 6.18−6.26 (m, 1H), 6.35−6.43 (m, 1H), 7.12−7.31 (m, 3H), 7.32 (s, 1H), 9.62 (br s, 1H, OH exchange with D_2O). Anal. ($C_{11}H_{11}ClO_2$) C, H, Cl.

(*Z*,*E*)-5-(3′-Bromophenyl)pentenoic Acid (10b). General procedure IX was used to convert **9b** into the title product. The residue was purified by flash chromatography (petroleum ether/EtOAc, 1:1) to afford **10b** (7.12 g, 78%) as a yellow oil. $R_f = 0.67$ (CH_2Cl_2/$(CH_3)_2CO$, 9:1); bp 86 °C (27 mmHg); IR 1705, 3056; ^{1}H NMR ($CDCl_3$) δ 2.49−2.68 (m, 4H), 6.17−6.26 (m, 1H), 6.34−6.43 (m, 1H), 7.11−7.39 (m, 3H), 7.48 (s, 1H), 9.56 (br s, 1H, OH exchange with D_2O). Anal. ($C_{11}H_{11}BrO_2$) C, H.

(*Z*,*E*)-5-(*m*-Toluyl)pentanoic Acid (10c). General procedure IX was used to convert **9c** into the title product. The residue was purified by flash chromatography (petroleum ether/EtOAc, 1:1) to afford **10c** (5.32 g, 69%) as a yellow oil. $R_f = 0.62$ (CH_2Cl_2/$(CH_3)_2CO$, 9:1); bp 85 °C (27 mmHg); IR 1713, 3197; ^{1}H NMR ($CDCl_3$) δ 2.32 (s, 3H), 2.33−2.64 (m, 4H), 6.17−6.24 (m, 1H), 6.36−6.44 (m, 1H), 6.99−7.25 (m, 4H), 11.00 (br s, 1H, OH exchange with D_2O). Anal. ($C_{12}H_{14}O_2$) C, H.

Animals. Male CD-1 mice (Harlan Italy S.r.l., S.Pietro al Natisone, UD, 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 of 180 Ci/mmol) was purchased from New England Nuclear (Boston, MA). CP-55,940 and WIN 55,212−2 were obtained from Tocris Cookson Ltd. (Bristol, U.K.). 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. In vivo assays were carried out dissolving drugs in a volume of 12.5 mL/kg of saline (0.1% Tween-80). Carmine (Sigma Chemical Co., St. Louis, MO) was suspended (6 wt %/v) in distilled water containing 0.5% methylcellulose and administered by gavage.

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 volumes (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 45000*g* for 30 min.

[^{3}H]CP-55,940 binding was performed by the method previously described by Ruiu et al.[1] 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 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) with 4 mL of scintillation fluid (Ultima Gold MV, Packard).

Protein determination was performed by means of the Bradford protein assay[25] 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 the Graph Pad Prism program. IC_{50} values were derived from the calculated curves and converted to K_i values as previously described.[26]

Gastrointestinal Transit (GIT). GIT in mice was measured by the upper gastrointestinal transit test, according to the previously reported procedures.[14b] Different doses of test compound were administered ip 30 min before intragastric administration of the marker (0.3 mL/mouse of red carmine) to groups of $n = 15-20$ mice. Twenty minutes later, mice were killed by cervical dislocation, the stomach and small intestine were removed, and the omentum was separated, avoiding stretching. The distance travelled by the head of the red marker was measured and expressed as a percent of the total length of the small intestine (determined from pyloric sphincter to ileocaecal junction). In the antagonism test, compound **4c** (0.1 mg/kg ip) was administered 10 min prior to the injection of WIN 55,212-2 (0.5 mg/kg ip). The marker was administered intragastrically 20 min afterward. Finally, 20 min later, mice were sacrificed and GIT was determined as described. Data were expressed as the group mean ± SEM. Data points were mean values, and vertical bars in the figures represented the SEM. In each experiment, statistical evaluation of the GIT, expressed as a percentage of the distance travelled by the head of the marker over the total length of the small intestine, was performed by a one-way analysis of variance (ANOVA), followed by the Newman−Keuls test for post hoc comparisons.

Computational Methods. All calculations were carried out using the Gaussian 03[27] program package. The conformational space of the compounds was explored through optimizations at the B3LYP level with the 6-31G* basis set.[21] All the degrees of conformational freedom were considered including the ring flexibility of the tricyclic moiety and the rotation around the single bonds of the hydrazide/piperidine moiety as well as the rotation of the aryl groups.

Supporting Information Available: Table of spectroscopic data of compounds **4c-4o** and Table with the energy and geometrical data of the populated conformations of compounds **1**, **2a**, **3a**, and **4a**, are included. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Ruiu, S.; Pinna, G. A.; Marchese, G.; Mussinu, J. M.; Saba, P.; Tambaro, S.; Casti, P.; Vargiu, R.; Pani, L. Synthesis and characterization of NESS 0327: A novel putative antagonist of CB_1 cannabinoid receptor. *J. Pharmacol. Exp. Ther.* **2003**, *306*, 363−370.

(2) Dewick, P. M. *Medicinal Natural Products. A Biosynthetic Approach*, 2nd ed.; John Wiley & Sons: New York, 2002; pp 86−89.

(3) Matsuda, L. A.; Lolait, S. J.; Brownstein, M. J.; Young, A. C.; Bonner, T. I. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature* **1990**, *346*, 561−564.

(4) Munro, S.; Thomas, K. L.; Abu-Shaar, M. Molecular characterization of a peripheral receptor for cannabinoids. *Nature* **1993**, *365*, 61−65.

(5) Devane, W. A.; Hanus, L.; Breuer, A.; Pertwee, R. G.; Stevenson, L. A.; Griffin, G.; Gibson, D.; Mandelbaum, A.; Etinger, A.; Mechoulam, R. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* **1992**, *258*, 1946−1949.

(6) Sugiura, T.; Kondo, S.; Sukagawa, A.; Nakane, S.; Shinoda, A.; Itoh, K.; Yamashita, A.; Waku, K. 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. *Biochem. Biophys. Res. Commun*. **1995**, *215*, 89−97.

(7) Wilson, R.; Nicoll, R. A. Endocannabinoid signaling in the brain. *Science* **2002**, *296*, 678−682.

(8) Recent data suggest that there may be a third cannabonoid receptor (CB_3). Breivogel, C. S.; Griffin, G.; Di Marzo, V.; Martin, B. R. Evidence for a new G protein-coupled cannabinoid receptor in mouse brain. *Mol. Pharmacol.* **2001**, *60*, 155−163.

(9) Adam, J.; Cowley, P. Recent advances in the cannabinoids. *Expert. Opin. Ther. Pat.* **2002**, *12*, 1475−1489.

(10) Pertwee, R. G. Neuropharmacology and therapeutic potential of cannabinoids. *Addict. Biol.* **2000**, *5*, 37−46.

(11) Gomez, R.; Navarro, M.; Ferrer, B.; Trigo, J. M.; Bilbao, A.; Del Arco, I.; Cippitelli, A.; Nava, F.; Pomelli, D.; de Fonseca, F. R. A peripheral mechanism for CB_1 cannabinoid receptor-dependent modulation of feeding. *J. Neurosci.* **2002**, *22*, 9612−9617.

(12) Hall, W. Cannabis and psychosis. *Drug Alcohol Rev.* **1998**, *17*, 433−444.

(13) Hungund, B. L.; Basavarajappa, B. S.; Vadasz, C.; Kunos, G.; Rodriguez de Fonseca, F.; Colombo, G.; Serra, S.; Pearson, L.; Koob, G. F. Ethanol, endocannabinoids and the cannabinoidergic signalling system. *Alcohol Clin. Exp. Res.* **2002**, *26*, 565−574.

(14) (a) Pertwee, R. G. Cannabinoids and the gastrointestinal tract. *Gut* **2001**, *48*, 859−867. (b) Casu, M. A.; Porcella, A.; Ruiu, S.; Saba, P.; Marchese, G.; Carai, M. A. M.; Reali, R.; Gessa, G. L.; Pani, L. Differential distribution of functional cannabinoid CB_1 receptors in the mouse gastroenteric tract. *Eur. J. Pharmacol.* **2003**, *459*, 97−105.

(15) Mussinu, J. M.; Ruiu, S.; Mulé, A. C.; Pau, A.; Carai, M. A. M.; Loriga, G.; Murineddu, G.; Pinna, G. A. 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. *Bioorg. Med. Chem.* **2003**, *11*, 251−263.

(16) Murineddu, G.; Ruiu, S.; Mussinu, J. M.; Loriga, G.; Grella, G. E.; Carai, M. A. M.; Lazzari, P.; Pani, L.; Pinna, G. A. Tricyclic pyrazoles. Part 2. Synthesis and biological evaluation of novel 4,5-dihydro-1*H*-benzo[*g*]indazole-based ligands for cannabinoid receptors. *Bioorg. Med. Chem.* **2005**, *13*, 3309−3320.

(17) Barth, F.; Congy, C.; Martinez, S.; Rinaldi, M. Preparation and formulation of tricyclic heterocycles containing a pyrazole-3-carboxamide subunit for pharmaceutical use as cannabinoid receptor antagonists. WO 01 32,663, , 2001; 29 pp (Sanofi-Synthelabo, France); *Chem. Abstr.* **2001**, *134*, 340504.

(18) Stoit, A. R.; Lange, J. H. M.; der Hartog, A. P.; Ronken, E.; Tipker, K.; van Stuivenberg, H. H.; Dijksman, J. A.; Wals, H. C.; Kruse C. G. Design, synthesis and biological activity of rigid cannabinoid CB1 receptor antagonists. *Chem. Pharm. Bull.* **2002**, *50*, 1109−1113.

(19) Lange, J. H. M.; Kruse, C. G. Medicinal chemistry strategies to CB_1 cannabinoid receptor antagonists. *Drug Discovery Today* **2005**, *10*, 693−702.

(20) (a) Shim, J. Y.; Welsh, W. J.; Cartier, E.; Edwards, J. L.; Howlett, A. C. Molecular interaction of the antagonist *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide with the CB_1 cannabinoid receptor. *J. Med. Chem.* **2002**, *45*, 1447−1459. (b) Hurst, D. P.; Lynch, D. L.; Barnett-Norris, J.; Hyatt, S. M.; Seltzman, H. H.; Zhong, M.; Song, Z. H.; Nie, J.; Lewis, D.; Reggio, P. H. *N*-(Piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (SR141716A) interaction with LYS 3.28(192) Is crucial for its inverse agonism at the cannabinoid CB_1 receptor. *Mol. Pharmacol.* **2002**, *62*, 1274−1287. (c) McAllister, S. D.;

Rizvi, G.; Anavi-Goffer, S.; Hurst, D. P.; Barnett-Norris, J.; Lynch, D. L.; Reggio, P. H.; Abood, M. E. An aromatic microdomain at the cannabinoid CB_1 receptor constitutes an agonist/inverse agonist binding region. *J. Med. Chem.* **2003**, *465*, 5139−5152.

(21) (a) Lee, C.; Yang, W.; Parr, R. G. Development of the Colle−Salvetti correlation-energy formula into a functional of the electron density. *Phys. Rev. B* **1988**, *37*, 785−789. (b) Becke, A. D. Density-functional thermochemistry. III. The role of exact exchange. *J. Chem. Phys.* **1993**, *98*, 5648−5652.

(22) Giardina, G.; Clarke, G. D.; Dondio, G.; Petrone, G.; Sbacchi, M.; Vecchietti, V. Selective κ-opioid agonists: Synthesis and structure−activity relationships of piperidines incorporating an oxo-containing acyl group. *J. Med. Chem.* **1994**, *37*, 3482−3491.

(23) Wong, S. C.; Sasso, S.; Howard, J.; Kaminski, J. J. Stereochemical considerations and the antiinflammatory activity of 6-amino-6,7,8,9-tetrahydro-5*H*-benzocyclohepten-5-ols and related derivatives. *J. Med. Chem.* **1984**, *27*, 20−27.

(24) Jones, D. H.; Stephenson, G. F.; Spray, G. W.; Wragg, W. R. *N*-Alkylaminoalkyl derivatives of some hexahydrobenzazocines. *J. Chem. Soc. C* **1969**, 2176−2181.

(25) Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein−dye binding. *Anal. Biochem.* **1976**, *72*, 248−254.

(26) Cheng, Y.; Prusoff, W. H. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50% inhibition (IC_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099−3108.

(27) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. *Gaussian 03*, revision B.02; Gaussian, Inc.: Pittsburgh, PA, 2003.

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