Synthesis and Pharmacological Evaluation of Potent and Highly Selective D_3 Receptor Ligands: Inhibition of Cocaine-Seeking Behavior and the Role of Dopamine D_3/D_2 Receptors†

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The synthesis, pharmacological evaluation, and structure−activity relationships (SARs) of a series of novel arylalkylpiperazines structurally related to BP897 (**3**) are described. In binding studies, the new derivatives were tested against a panel of dopamine, serotonin, and noradrenaline receptor subtypes. Focusing mainly on dopamine D_3 receptors, SAR studies brought to light a number of structural features required for high receptor affinity and selectivity. Several heteroaromatic systems were explored for their dopamine receptor affinities, and combinations of synthesis, biology, and molecular modeling, were used to identify novel structural leads for the development of potent and selective D_3 receptor ligands. Introduction of an indole ring linked to a dichlorophenylpiperazine system provided two of the most potent and selective ligands known to date (D_3 receptor affinity in the picomolar range). The intrinsic pharmacological properties of a subset of potent D_3 receptor ligands were also assessed in [^{35}S]-GTPγS binding assays. Evidence from animal studies, in particular, has highlighted the dopaminergic system's role in how environmental stimuli induce drug-seeking behavior. We therefore tested two novel D_3 receptor partial agonists and a potent D_3-selective antagonist in vivo for their effect in the cocaine-seeking behavior induced by reintroduction of cocaine-associated stimuli after a long period of abstinence, and without any further cocaine. Compound **5g**, a nonselective partial D_3 receptor agonist with a pharmacological profile similar to **3**, and **5p**, a potent and selective D_3 antagonist, reduced the number of active lever presses induced by reintroduction of cocaine-associated stimuli. However, **5q**, a highly potent and selective D_3 partial agonist, did not have any effect on cocaine-seeking behavior. Although brain uptake studies are needed to establish whether the compounds achieve brain concentrations comparable to those active in vitro on the D_3 receptor, our experiments suggest that antagonism at D_2 receptors might significantly contribute to the reduction of cocaine craving by partial D_3 agonists.

Introduction

Drug abuse, and the consequent drug addiction and dependence, are serious threats to public health. Illness, crime, domestic violence, reduced productivity, and lost opportunities are direct consequences. The economic cost of drug abuse to society is enormous and rising. There are several reasons, frequently of social origin, for drug abuse and various causes of relapse. In view of its potent psychostimulating effects, cocaine (**1**) is one of the most abused drugs, and the number of users among people 12−40 years old who have used cocaine at least once is extremely high in developed countries.[1−3] Despite huge advances in our understanding of the biological basis of drug abuse and dependence, there is still no effective long-term pharmacological therapy for cocaine craving and for preventing relapses to abuse,[1] although very recently a candidate drug has been proposed.[4] Treatments are anyway needed for all phases of drug abuse, not only for the related problems of relapse and toxicity.

Craving is one of the most important factors underlying relapse.[5−7] In humans, environmental stimuli that are reliably associated with the effects of many drugs of abuse can produce craving and relapse.[8−10] In animals, such cues can induce and maintain drug-seeking behavior and reinstate drug-seeking after extinction.[11−13] There is evidence that the dopaminergic system is involved in how environmental stimuli induce drug-seeking behavior in rats.[13,14−16] Thus D_1[13] and D_2[17a−c] receptors antagonists (raclopride, **2**) reduced the reinstatement of drug-seeking behavior induced by cocaine-associated stimuli. BP897 (**3**), a D_3 partial agonist lacking reinforcing properties, reduced cocaine-seeking

† In honor of Vito Nacci's 70th birthday.

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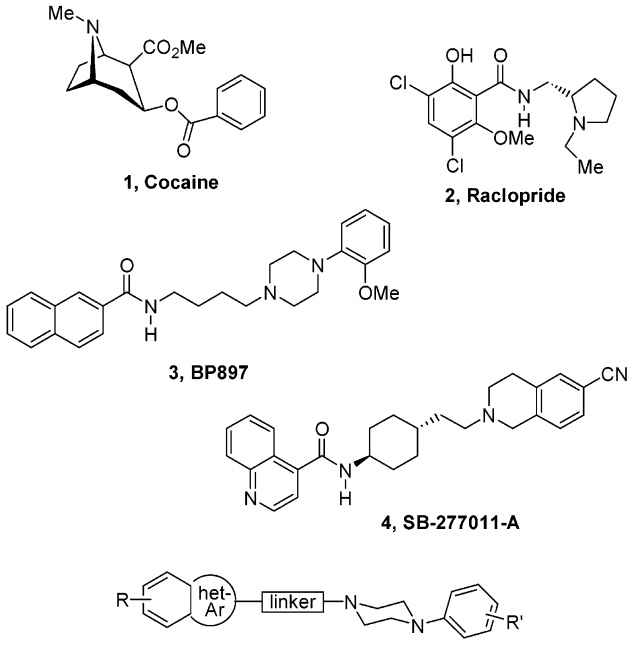
Chart 1



behavior maintained by cocaine in a second-order schedule in rats.[18] Together with the recent finding that **3** reduced cocaine-seeking behavior induced by reintroduction of environmental stimuli associated with and predictive of cocaine availability after a period of abstinence,[17c] this strongly suggests that compounds such as **3** could be used for reducing drug craving and the vulnerability to relapse that are elicited by drug-associated environmental stimuli. The mechanism underlying this effect is unknown. However, the selective D_3 receptor antagonist SB-277011-A (**4**)[19] also reduced seeking behavior maintained by cocaine in a second-order schedule,[20] suggesting that antagonists at D_3 receptors may be useful in controlling drug-seeking behavior induced by cocaine-associated cues.

Although **3** was claimed to reduce the response to cocaine cues through its D_3 receptor interaction,[18] this compound is characterized by a multireceptor affinity profile. In fact, it shows a significant D_2 receptor affinity, together with a potent antagonism at hα_{1A} and, to a lesser extent, hα_{2A} adrenoceptors and partial agonist properties at h5-HT_{1A} receptors.[21] Consequently, this compound may not prove the hypothesis that only D_3 receptors are implicated in mechanisms by which cocaine produced craving.

These considerations prompted us to develop a project aimed at identifying novel, selective, and nonselective D_3/D_2/5-HT_{1A} ligands (**5**, Chart 1) to produce compounds inhibiting cocaine-seeking behavior and to investigate the role of D_3 receptors in cocaine craving. We presented preliminary partial pharmacological results on one of the newly developed compounds (the benzofuran analogue **5g**) at the XIII Meeting of the Italian Society of Neuropsychopharmacology (Milan, 9−12 July 2002),[22] but while we were preparing this manuscript Gmeiner and co-workers reported the synthesis, binding studies, and intrinsic activity of this same analogue,[23] and Tortorella and co-workers reported the identification of other potent D_3 receptor ligands,[24] structurally related to our series **5a**−**r**.

Herein we report the synthesis and the pharmacological and biochemical characterization of a new series of selective and nonselective D_3 receptor ligands. From this series, we initially selected **5g** (a nonselective D_3 receptor partial agonist), **5p** (a highly selective and potent D_3 receptor partial agonist), and **5q** (an extremely potent and selective D_3 receptor antagonist) and tested their effects in the modulation of cocaine-seeking behavior induced by presentation of environmental stimuli associated with and predictive of cocaine availability following a period of extinction and in the absence of any further cocaine. Additionally, the rationalization of the structure−activity relationships by a molecular modeling study is discussed.

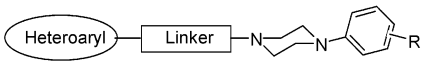
Chemistry

The synthesis of compounds **5a**−**r** (Table 1) is described in Schemes 2−5. *N*-Arylpiperazines **7a**,**b** were obtained as described in Scheme 1A starting from piperazine **6** that was N-arylated with a substituted aryl bromide through a palladium-catalyzed reaction.[25] The amine **10** was obtained, according to Scheme 1B, starting from the methoxyphenylpiperazine **8** that was alkylated with 2-bromoethanol to give the alcohol **9** that was then transformed into the corresponding amine **10** in a three-step sequence using mesyl and azido intermediates. The synthesis strategy followed to obtain compounds **5a**−**h**,**l**−**q** is reported in Scheme 2; acids **11a**,**c**−**i** or the acid chloride **11b** were transformed into the corresponding hydroxy amides **12a**,**c**−**i** and **12b**, respectively, by a standard procedure.[26−28] These latter, after substitution of a halogen for the terminal hydroxy group (**13a**−**i**),[29,30] were treated with the arylpiperazine in the presence of a base to give the desired products **5a**−**d**,**g**,**h**,**l**−**q**, and **14a**,**b**.[31] Compounds **5e**,**f** were then obtained after deprotection of the amine of **14a**,**b** by catalytic hydrogenation. The synthesis of **5r** is shown in Scheme 3. N-alkylation of the indole nitrogen (**15**) by means of 2-bromoacetonitrile (**16**) followed by treatment of **16** with cobalt boride and sodium borohydride gave the cyclic lactam **17**, which was N-alkylated with 1,4-dibromobutane in the presence of sodium hydride to yield the bromo-derivative **18**.[32−34] This was treated with 1-(2-methoxyphenyl)piperazine in the presence of a base to give the desired product **5r**. Scheme 4 describes the synthesis of **5i**. Starting from acid **11a**, the amide **19** was obtained by reaction with 4-methoxycarbonylpiperidine in the presence of 1,3-dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBT).[26] Reduction of the ester function (**20**) and bromination provided the intermediate **21** that was used in the next step to obtain the desired product **5i**. The heterocyclic compounds **5j**,**k** were obtained according to Scheme 5. Starting from the acids **11a** and **22**, after transformation to the corresponding amides by means of thionyl chloride and liquid ammonia, and subsequent formation of the oxazole ring system (**23a**,**b**), using 1,3-dichloroacetone,[35] which were used as alkylating agents, compounds **5j**,**k** were prepared.

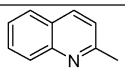
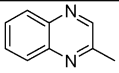
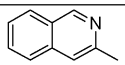
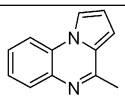
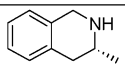
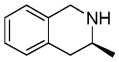
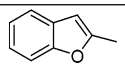
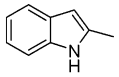
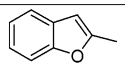
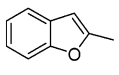
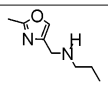
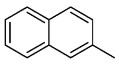
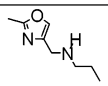
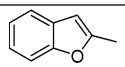
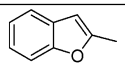
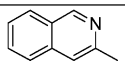
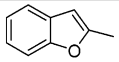
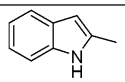
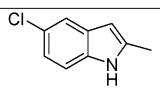
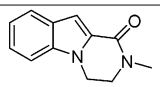
Pharmacological Studies. Results and Discussion

1. Binding Assays. The binding affinities for D_1, D_2, D_3, 5-HT_{1A}, α_1 and α_2 receptors of compounds **5a**−**r** (as

Table 1. Physical and Chemical Data for Compounds **5a**−**r**

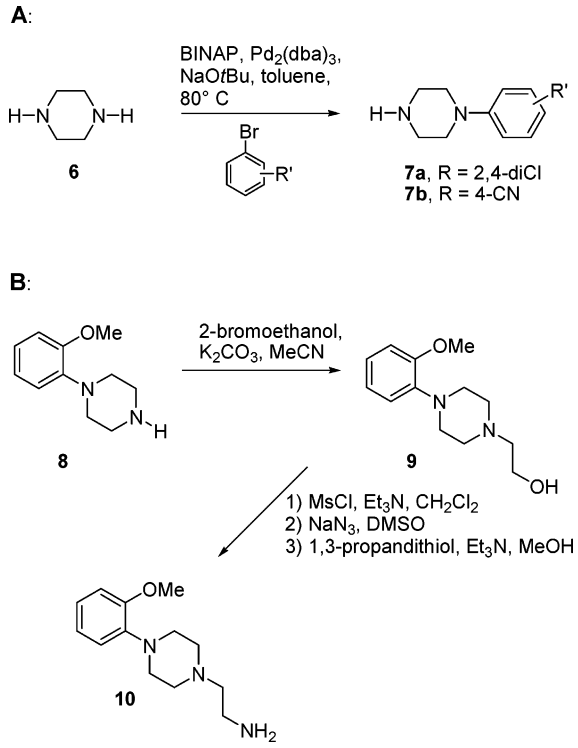


5a-r

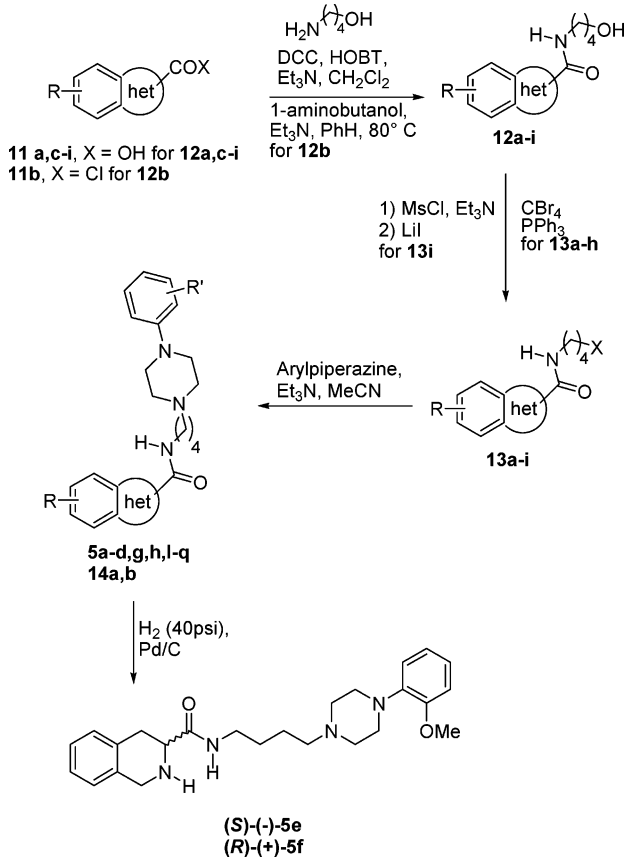
Compd	Heteroaryl	Linker	R	yield %[a]	mp (°C)	formula	anal.
5a		-CONH(CH_2)$_4$-	2-methoxy	94	oil	$C_{25}H_{30}N_4O_2$	C,H,N
5b		-CONH(CH_2)$_4$-	2-methoxy	91	oil	$C_{24}H_{29}N_5O_2$	C,H,N
5c		-CONH(CH_2)$_4$-	2-methoxy	97.5	132-33	$C_{26}H_{34}N_4O_2$	C,H,N
5d		-CONH(CH_2)$_4$-	2-methoxy	95	oil	$C_{27}H_{31}N_5O_2$	C,H,N
5e		-CONH(CH_2)$_4$-	2-methoxy	84	150-55 (dec.)	$C_{25}H_{34}N_4O_2$	C,H,N
5f		-CONH(CH_2)$_4$-	2-methoxy	81	150-55 (dec.)	$C_{25}H_{34}N_4O_2$	C,H,N
5g		-CONH(CH_2)$_4$-	2-methoxy	60	120-21	$C_{24}H_{29}N_3O_3$	C,H,N
5h		-CONH(CH_2)$_4$-	2-methoxy	48	oil	$C_{24}H_{30}N_4O_2$	C,H,N
5i		-CO[N(CH_2CH_2)$_2$CH]CH_2-	2-methoxy	60	oil	$C_{26}H_{31}N_3O_3$	C,H,N
5j			2-methoxy	18	oil	$C_{25}H_{28}N_4O_3$	C,H,N
5k			2-methoxy	16	oil	$C_{27}H_{30}N_4O_2$	C,H,N
5l		-CONH(CH_2)$_4$-	4-cyano	65	146-48	$C_{24}H_{26}N_4O_2$	C,H,N
5m		-CONH(CH_2)$_4$-	3,4-dichloro	56	121-22	$C_{23}H_{25}Cl_2N_3O_2$	C,H,N
5n		-CONH(CH_2)$_4$-	2,4-dichloro	72	127-28	$C_{24}H_{26}Cl_2N_4O$	C,H,N
5o		-CONH(CH_2)$_4$-	2,4-dichloro	68	112-13	$C_{23}H_{25}Cl_2N_3O_2$	C,H,N
5p		-CONH(CH_2)$_4$-	2,4-dichloro	66	oil	$C_{23}H_{26}Cl_2N_4O$	C,H,N
5q		-CONH(CH_2)$_4$-	2,4-dichloro	73	oil	$C_{23}H_{25}Cl_3N_4O$	C,H,N
5r		-(CH_2)$_4$-	2-methoxy	93.5	oil	$C_{26}H_{32}N_4O_2$	C,H,N

[a] Yields refer to isolated and purified materials. [b] All the compounds were analyzed within ±0.4% of the theoretical values.

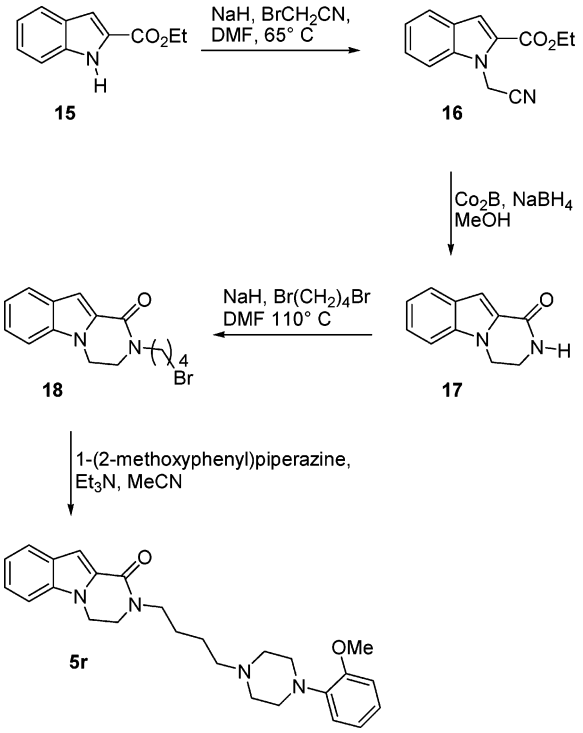
Scheme 1



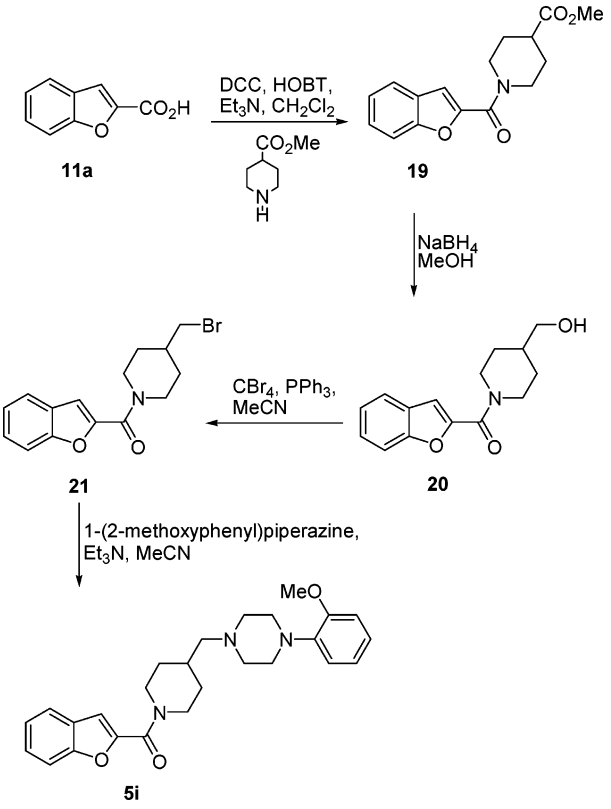
Scheme 2



Scheme 3



Scheme 4



di- or trihydrochloride salts) are given in Table 2. The intrinsic activity of a selected subset of compounds at D_3 and 5-HT_{1A} receptors was determined in [^{35}S]-GTPγS binding assays (Figures 1 and 2). The results reported in Table 2 are summarized as follows.

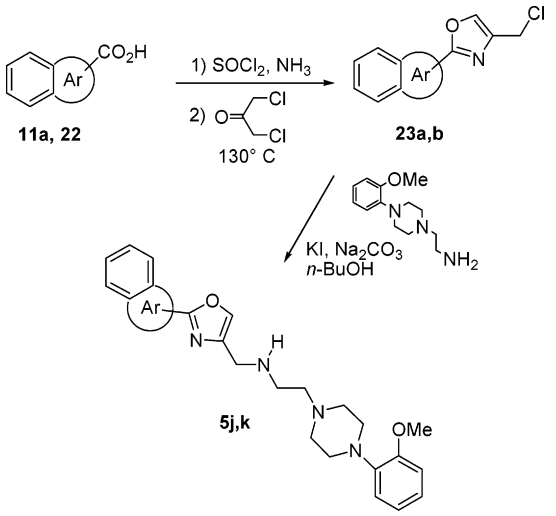
(a) The Key Role of Different Heteroaromatic Systems on D_3 Receptor Affinity and Selectivity. The naphthalene system of **3** was replaced with different heteroaromatic ring scaffolds, leading to extremely potent D_3 receptor ligands. Initially, the result of the introduction of one or two heterocyclic nitrogen atoms on the naphthalene ring was evaluated, obtaining the quinoline analogue **5a** and the quinoxaline **5b**. When

Table 2. Binding Profile of Compounds **5a**−**r** on D_{1-3}, 5-HT_{1A}, and Adrenergic Receptors (K_i nM ± SD)[a]

compd	D_1[b]	D_2[c]	D_{3r}[d]	5-HT_{1A}[e]	alpha$_{1NA}$[f]	alpha$_{2NA}$[g]
5a	1350 ± 114	83 ± 5	4.8 ± 0.05	NT[h]	NT	NT
5b	1440 ± 41	84 ± 2	11 ± 0.1	NT	NT	NT
5c	1480 ± 146	75 ± 12	0.49 ± 0.01	3.12 ± 0.31	5.80 ± 0.61	76 ± 12
5d	778 ± 128	59 ± 9	28 ± 1	NT	NT	NT
5e	NT	NT	9.2 ± 0.2	NT	NT	NT
5f	NT	NT	4.3 ± 1.0	NT	NT	NT
5g	2565 ± 342	87 ± 19	1.6 ± 0.5	2.14 ± 0.16	36 ± 9	54 ± 3
5h	970 ± 71	160 ± 19	2.9 ± 0.05	11.8 ± 0.75	666 ± 106	76 ± 15
5i	2676 ± 282	244 ± 40	74 ± 6	NT	NT	NT
5j	≫10 000	518 ± 193	759 ± 59	NT	NT	NT
5k	≫10 000	86 ± 1	295 ± 24	NT	NT	NT
5l	NT	NT	41.3 ± 8	NT	NT	NT
5m	1655 ± 461	333 ± 57	7.8 ±1.4	NT	NT	NT
5n	7750 ± 808	342 ± 53	0.66 ± 0.03	1050 ± 241	629 ± 190	1047 ± 148
5o	7664 ± 743	409 ± 65	0.41 ± 0.05	908 ± 236	779 ± 181	1887 ± 65
5p	>10 000	>10 000	0.18 ± 0.004	>10 000	547 ± 146	>10 000
5q	>10 000	>10 000	0.38± 0.005	>10 000	8100 ± 1590	>10 000
5r	1567 ± 289	45 ± 7	0.87 ± 0.16	4.9 ± 1.0	33 ± 10	89 ± 4
BP 897[i]	3000	61	0.92	84	60	83

[a] Each value is the mean ± SD of three determinations and represents the concentration giving half-maximal inhibition of [^{3}H]-ligand binding. [b] [^{3}H]-SCH 2339, rat striatum. [c] [^{3}H]-spiperone, rat striatum. [d] [^{3}H]-7-OH-DPAT, Sf9 cells. [e] [^{3}H]-8-OH-DPAT, rat hippocampus. [f] [^{3}H]-prazosin, rat cortex. [g] [^{3}H]-RX 81002, rat cortex. [h] NT = not tested. [i] Data from ref 18.

Scheme 5



tested against dopamine receptors, both compounds showed a similar low affinity for D_1 receptors, an affinity for D_2 receptors in the high nanomolar range, and nanomolar affinity for the D_3 receptor, with the quinoline analogue **5a** being twice as potent (**5a** vs **5b**). Replacement of the quinoline of **5a** by an isoquinoline system led to a 10-fold increase in D_3 receptor affinity (**5c** vs **5a**) with a D_1/D_3 affinity ratio of 3020 and a D_2/D_3 affinity ratio of 153. This selective D_3 receptor ligand was further tested on 5-HT_{1A}, α_1 and α_2 receptors. While the α_2 receptor affinity was similar to that for D_2 receptors, **5c** proved to be a potent 5-HT_{1A} and α_1 ligand. Introduction of a fused pyrrole ring (**5d**) into the structure of **5b**, leading to a pyrroloquinoxaline system, slightly decreased the D_3 receptor affinity, but doubled the D_1 affinity (K_{iD1} = 778 nM, K_{iD3} = 28 nM). The partially saturated **5c** counterparts **5e** and **5f** were less potent than **5c** at D_3 receptors (**5e** and **5f** vs **5c**), with twice the stereoselectivity at D_3 receptors, indicating a scarce stereoselective interaction at D_3 receptor for both enantiomers. The naphthalene ring of **3** was also replaced by bioisosteric ring systems, namely, the benzofuran and indole. The benzofuran provided a compound (**5g**) that was as potent as **3** at D_1, D_2, and

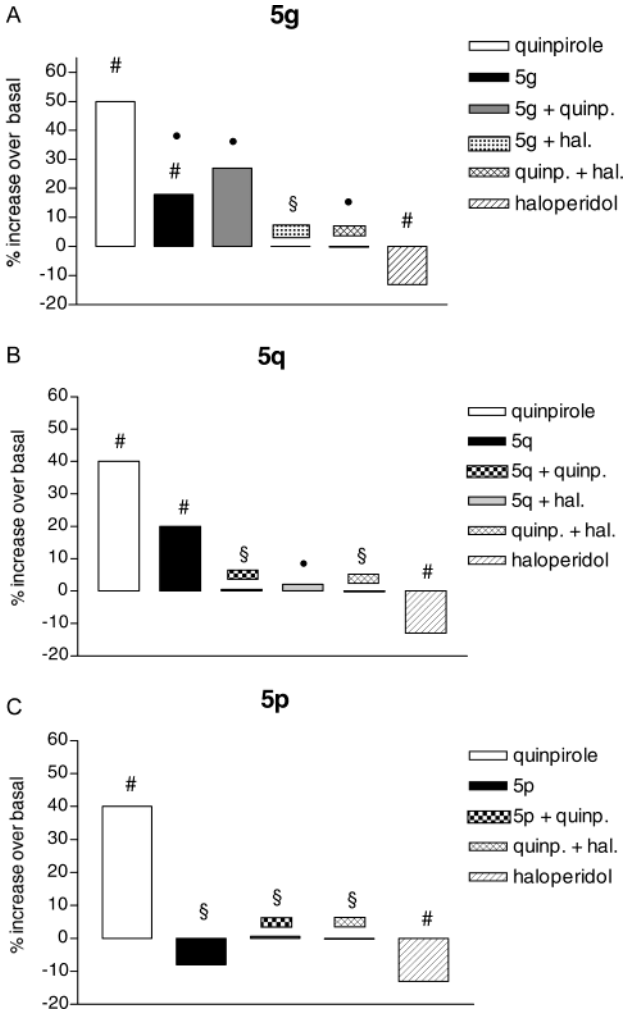


Figure 1. [^{35}S]-GTPγS binding to D_{3r} receptors expressed in Sf9 cells was carried out as described in Experimental Section. Quinpirole, **5g**, **5p**, **5q**, and haloperidol were used at 10 μM and the values are the mean of three experiments (variability less than 10%). For statistical analysis Anova and Tukey test were used. #, $P < 0.01$ different from basal. §, $P < 0.01$ different from quinpirole. •, $P < 0.01$ different from **5g** or **5q** alone.

D_3 receptors, but this structural modification improved the affinity for 5-HT_{1A} (K_i = 2.14 nM), α_1 (K_i = 36 nM),

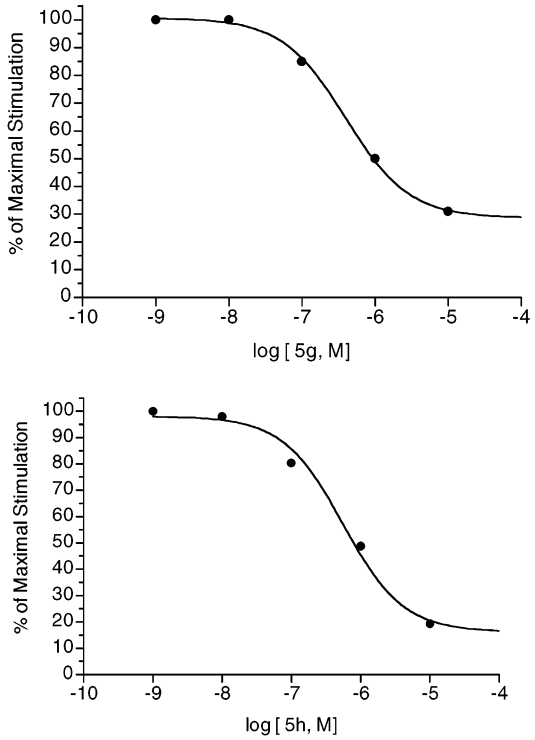


Figure 2. Representative inhibition curves of (A) **5g** and (B) **5h** on agonist-stimulated [^{35}S]-GTPγS binding in rat hippocampus. Hippocampal homogenates were incubated in the presence of 10 μM 5-HT and graded concentrations of **5g** and **5h**. Each point is the mean of triplicate values, varying by less than 10%.

and α_2 (K_i = 53.6 nM) receptors. The D_3 receptor affinity of the indole analogue **5h** (K_i = 2.9 nM) and its D_2/D_3 affinity ratio was similar to that of **5g**. However, **5h** was found less potent than 5g on the panel of receptors, with the exception of D_1 receptors.

(b) Modification of the Linker. Effect on D_3 Receptor Affinity. The selected modifications of the linker were, in general, detrimental to receptor affinity. The amide 4-methylene linker of **5g** and **3** was modified by constraining the alkyl chain in a six-membered ring (**5i**) or by replacing the amide system with a 4-aminomethyloxazole group (**5j**,**k**). In the case of **5i**, the introduction of the piperidine ring determined a 46-fold drop in D_3 receptor affinity with respect to **5g**, while the oxazole derivative **5j** proved to be poorly active at dopamine receptors. On the contrary, **5k** demonstrated a D_2 receptor affinity similar to that of the parent compound **3**, showing a dramatic drop in D_3 receptor affinity (K_{iD2} = 86 nM and K_{iD3} = 295 nM). It is noteworthy that compound **5r**, designed on the skeleton of **5h**, is the only conformationally constrained analogue showing a D_3 receptor subnanomolar affinity (**5r**, K_{iD3} = 0.87 nM) (see Molecular Modeling section).

(c) The Influence of the Substituents on the Arylpiperazine Ring System. In the next step of our SAR study, we evaluated the effect on receptor affinity and selectivity of halogen atoms or a cyano group on the phenyl at N-4 of the piperazine ring, in relation to the 2-methoxyphenylpiperazine scaffold of the previous analogues.

A cyano group at C-4 of the phenyl ring caused D_3 receptor affinity to drop 26-fold (**5l** vs **5g**), and chlorine atoms at C-3 and C-4 provided an analogue (**5m**) about 4 times less potent than **5g** itself at D_2 and D_3 receptors; introduction of chlorine atoms at positions 2 and 4 of the aromatic ring led to the identification of extremely potent and selective D_3 receptor ligands. With respect to the methoxy derivatives **5c**, **5g**, and **5h** (K_{iD3} of 0.49, 1.6, and 2.9 nM, with a D_2/D_3 affinity ratio of 153, 54, and 55, respectively), the corresponding 2,4-dichlorophenyl analogues **5n**−**p** (K_{iD3} 0.66, 0.41, 0.18 nM, with D_2/D_3 affinity ratio of 518, 998, and >55 000, respectively) showed a better selectivity profile for the D_3 receptor, and **5p** could be considered one of the most potent and selective D_3 receptor ligand known to date.

These three compounds were also tested on 5-HT_{1A}, α_1 and α_2 receptors. While **5n** and **5o** showed low affinity for the panel of receptors, **5p** showed micromolar affinity for α_1 receptors and negligible affinity for 5-HT_{1A} and α_2 receptors. Receptor selectivity was improved by introducing a chlorine (**5q**) atom at position 5 of the indole system of **5p**. The pattern of receptor affinities of **5q** is similar to that reported for **5p** (for D_3 receptors, $K_{i\mathbf{5q}}$ = 0.38 nM vs $K_{i\mathbf{5p}}$ = 0.18 nM), although it has a lower α_1 receptor affinity. In summary, **5p** and **5q** are extremely potent and selective D_3 receptor ligands.

(d) Intrinsic Pharmacological Activities. In [^{35}S]-GTP-γS binding assays, the reference agonist quinpirole increased basal binding with EC_{50} 80 ± 15 nM, reaching maximal stimulation at 1−10 μM (80% over basal value). Compound **5g** behaved as a partial agonist at the cloned D_3 receptor expressed on Sf9 cells (Figure 1A). Because of the low efficacy of **5g** in enhancing [^{35}S]-GTP-γS binding a complete dose−response curve could not be obtained. However, at equimolar saturating concentrations, it was significantly less active than quinpirole in increasing [^{35}S]-GTP-γS binding, and its effect was completely antagonized by haloperidol. In addition, like haloperidol, it significantly reduced the enhancing effect of quinpirole.

Similar results were obtained with **5q**, which also behaves as a partial agonist at D_3 receptors (Figure 1B), while **5p** was a pure antagonist lacking intrinsic activity (Figure 1C). Differently from **3**, which behaves as a 5-HT_{1A} receptor agonist, **5g** and **5h** behaved as antagonists at 5-HT_{1A} receptors in the rat hippocampus membrane, with K_i of 17.5 ± 1.8 and 23.2 ± 2.8 nM, respectively (Figure 2).

The EC_{50} of serotonin for enhancing [^{35}S]-GTP-γS binding was 523 ± 160 nM, with maximum stimulation (65% over basal value) at 10−30 μM. The effect of 5-HT was completely abolished by 10 μM WAY 100635 (data not shown).

2. Behavioral Effects: Results on 5g, 5p, and 5q. (a) Cocaine Self-Administration, Conditioning, Extinction, and Reinstatement Phases. In all the experiments, the separate groups of rats developed stable cocaine self-administration between 7 and 10 training days with no differences in responding during the first and second hour of cocaine availability; the number of lever-pressings during saline dropped gradually to less than five per session in 10 training days. The number of days required to meet the training criterion was the same for the different experimental groups (mean ± SEM, 17.2 ± 0.6 to 19.3 ± 1.1). No differences were observed during the final 3 days of the training phase (P > 0.05, Newman-Keuls test) or

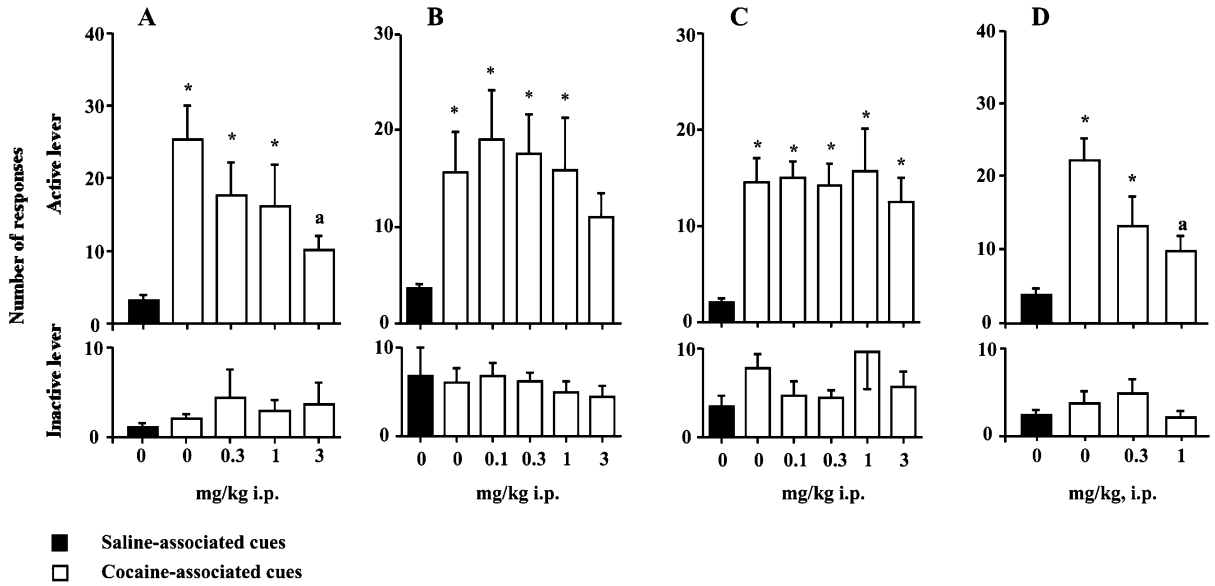


Figure 3. Effects of (A) **5g**, (B) **5p**, (C) **5q**, and (D) **3** on the number of pressings on the active and inactive levers after reintroduction of cocaine-associated stimuli. For comparison, the figure also shows the average number of lever pressings after reintroduction of stimuli associated with no reward. Histograms indicate the mean ± SEM pressings on active and inactive levers of at least seven rats. **5g**, dissolved in sterile saline, **5p** and **5q**, dissolved in 25% DMSO in sterile 0.9% saline, or vehicles were given i.p. 30 min before testing. Data for **3** are from ref 17, modified. $F_{5g}(11,83) = 15.0$, $P < 0.01$; $F_{5g}(11,83) = 1.5$, $P > 0.05$, for active and inactive levers respectively, one-way ANOVA for repeated measurements. $F_{5p}(13,78) = 7.8$, $P < 0.01$ and $F_{5p}(13,78) = 1.6$, $P > 0.05$ for active and inactive levers respectively, one-way ANOVA for repeated measures. $F_{5q}(13,91) = 7.8$, $P < 0.01$ and $F_{5q}(13,91) = 2.1$, $P < 0.05$ for active and inactive levers respectively, one-way ANOVA for repeated measures. *$P < 0.01$, different from saline-associated cue presentation and the three preceding extinction sessions (data not shown, see Results for details), Neuman-Keuls test. [a]$P < 0.01$, different from the cocaine-associated cue presentation, Newman-Keuls test.

between the first and second daily hour of self-administration ($P > 0.05$, mixed factorial or one-way ANOVA for repeated measurement). The data for the two daily cocaine sessions were pooled for all subsequent analyses. During this phase responding on the inactive lever was minimal in all experimental groups.

In our experiments, rats met the extinction criterion after an average of 16.0 ± 1.6 sessions. No differences were found between groups in the mean ± SEM number of days to extinction, which ranged from 12.0 ± 1.8 to 18.6 ± 1.9 days. Responding on the inactive lever was again negligible throughout the whole phase.

In all the experiments, the cocaine-associated stimuli produced immediate recovery of responding that was significantly higher than after the saline-associated cues ($P < 0.01$ Newman-Keuls test) and compared to the three preceding extinction sessions (data not shown, $P < 0.01$ Newman-Keuls test). In all the experiments, the overall behavioral output after presentation of the cocaine-associated cues was similar to that during cocaine self-administration ($P > 0.05$, Newman-Keuls test) and significantly different from saline self-administration ($P < 0.01$, Newman-Keuls test). The number of lever pressings during S presentation did not differ from the three preceding extinction sessions ($P > 0.05$, Newman-Keuls test). Since they had to meet the extinction criterion, lever pressing during the extinction sessions, preceding the reintroduction of cocaine- and saline-associated stimuli, did not differ between groups in all the experiments.

(b) Effects of 5g, 5p, and 5q on Cue-Induced Reinstatement of Drug-Seeking Behavior. Figure 3 shows the effects of **5g** (A), **5p** (B), and **5q** (C), all used as dihydrochloride salts, on cocaine-seeking behavior induced by reintroduction of the drug-associated stimuli. Data are compared to **3** (D). Reintroduction of the cues always increased the number of pressings on the active lever [$F_{5g}(11,83) = 15.0$, $P < 0.01$, $F_{5p}(13,78) = 7.8$, $P < 0.01$ and $F_{5q}(13,91) = 8.9$, $P < 0.01$, one-way ANOVA, $P < 0.01$ vs saline-associated cues presentation and vs the three preceding extinction days, data not shown, Newman-Keuls test] but not on the inactive lever ($P > 0.05$ vs saline-associated cues presentation and vs the three preceding extinction days, data not shown, Newman-Keuls test). Pretreatment with **5g** 3 mg/kg, but not 0.3 and 1 mg/kg, significantly reduced the number of lever pressing induced by presentation of cocaine-associated cues ($P < 0.01$ vs vehicle treated group); moreover, the number of lever responses after 3 mg/kg **5g** pretreatment with reintroduction of the drug-associated stimuli was no longer different from the saline-associated cues presentation ($P > 0.05$, Newman-Keuls test). **5g** pretreatment did not affect the number of inactive lever pressings.

Pretreatment with 0.1−1 mg/kg **5p** did not modify rats' behavior after presentation of cocaine-associated stimuli ($P < 0.01$ vs saline-associated cues presentation and vs the three preceding extinction days, data not shown, Newman-Keuls test). However, at 3 mg/kg **5p** partially reduced the number of active lever pressings induced by reintroduction of the drug-associated stimuli ($P > 0.05$ vs saline- and cocaine-associated cues, Newman-Keuls test). The numbers of responses were no longer different from those elicited by the saline-associated stimulus, and not different from those induced by cocaine-associated stimuli. There were no effects on the number of pressings of the inactive lever.

5q did not modify the number of lever pressings on the active lever ($P < 0.01$ vs saline-associated stimuli presentation and vs the three preceding extinction days,

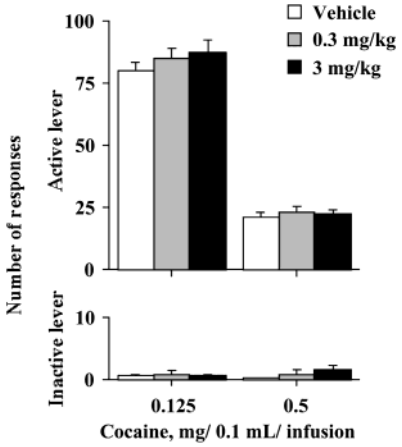


Figure 4. Effects of **5g** or vehicle on self-administration of two doses of cocaine. Rats were trained to respond for cocaine (0.125 and 0.5 mg/0.1 mL/infusion) under a fixed ratio 1 (FR1) schedule of reinforcement. Once baseline was stable, rats received i.p. **5g**, dissolved in 2 mL of sterile saline, or vehicle, 30 min before the 2 h test session. Histograms present the mean ± SEM number of self-administered infusions during a 2 h test session. The data were analyzed by one-way ANOVA for repeated measures followed by Dunnett's test. $F_{0.125\ \text{Active lever}}(2,23) = 1.8$, $P > 0.05$; $F_{0.125\ \text{Inactive lever}}(2,23) = 1.8$, $P > 0.05$; $F_{0.5\ \text{Active lever}}(2,23) = 0.6$, $P > 0.05$; $F_{0.5\ \text{Inactive lever}}(2,23) = 0.4$, $P > 0.05$, one-way ANOVA for repeated measures.

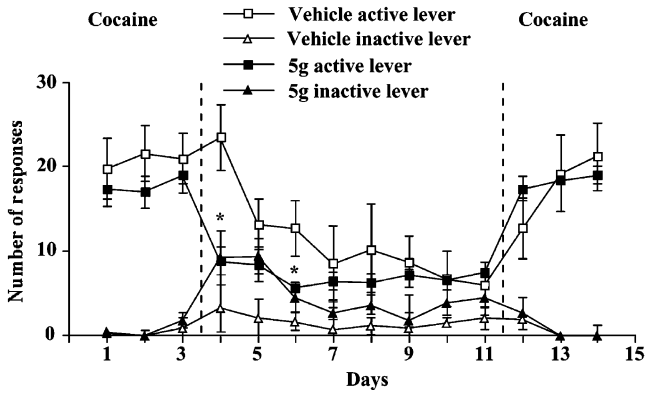


Figure 5. Effects of replacing cocaine (0.25 mg/0.1 mL/infusion) with **5g** (3 mg/mL, 0.1 mL per infusion) or saline (0.1 mL per infusion) on i.v. cocaine self-administration under a FR1 schedule of reinforcement. Data are the mean ± SEM of the number of active and inactive lever pressings by six rats during daily 2 h sessions. The data were analyzed by the one-way ANOVA for repeated measurements. $F_{\text{active lever}}(1,10) = 6.3$, $P < 0.05$, mixed one-way ANOVA for repeated measurements. $F_{\text{inactive lever}}(1,10) = 4.4$, $P > 0.05$, mixed one-way ANOVA for repeated measurements. *****$P < 0.05$, vs saline group, Newman-Keuls test.

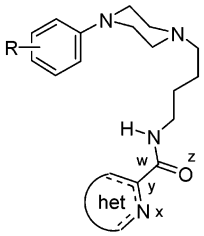
data not shown, Newman-Keuls test). But treatment did have some effect on the number of inactive lever pressings [$F(13,91) = 2.1$, $P < 0.05$, one-way ANOVA]. Post-hoc comparison by the Newman-Keuls test, however, did not reveal any significant effect of **5q** at any dose on reintroduction of cocaine-associated stimuli ($P > 0.05$).

(c) Effect of 5g on Cocaine Self-Administration. Figure 4 shows the effects of acute pretreatment with 0.3 and 3 mg/kg **5g** or vehicle on cocaine self-administration under a continuous reinforcement schedule. One-way ANOVA for repeated measurements found **5g** had no significant effect on either 0.125 or 0.5 mg/0.1 mL/infusion cocaine [$F_{0.125\ \text{Active lever}}(2,23) = 1.8$, $P > 0.05$; $F_{0.125\ \text{Inactive lever}}(2,23) = 3.3$, $P > 0.05$; $F_{0.5\ \text{Active lever}}(2,23) = 0.6$, $P > 0.05$; $F_{0.5\ \text{Inactive lever}}(2,23) = 0.4$, $P > 0.05$].

(d) Effects of Replacing Cocaine with 5g or Vehicle on Cocaine Self-Administration. Figure 5 reports the effects of replacing cocaine with **5g** (0.3 mg/0.1 mL/infusion) or saline (0.1 mL/infusion) on i.v. cocaine self-administration. The number of infusions earned by the two groups of rats did not differ during the 3 days before the cocaine substitution ($P > 0.05$, Newman-Keuls test). When cocaine was replaced with **5g** or saline treatment had an effect on the number of active lever pressings [$F(1,10) = 6.3$, $P < 0.05$, mixed one-way ANOVA for repeated measurements] but not on the inactive ones [$F(1,10) = 4.4$, $P > 0.05$, mixed one-way ANOVA for repeated measurements]. Post-hoc comparisons by the Newman-Keuls test found that **5g** induced a steeper decrease in the number of active lever pressing on the first and third substitution days ($P < 0.05$). Resubstituting cocaine for **5g** or saline resulted in prompt reinstatement of drug self-administration in both groups.

3. Behavioral Studies: Discussion. Compound **5g**, a new dopamine D_3 partial agonist with 50 times the selectivity toward D_2 receptors, significantly attenuated drug-seeking behavior induced by cocaine predictive cues even after prolonged abstinence. This effect could not be attributed to drug-induced disruption of behavior since in no instance were any signs of sedation or motor disturbance observed that could have interfered with the behavioral measurements. Also, responses on the inactive levers were not affected by **5g**. It cannot be excluded that the low rate of responding on the inactive lever prevented us from detecting some subtle motor impairment. The fact that **5g** reduced rats' behavior elicited by cues associated with and predictive of cocaine availability, with no effect on lever pressing for cocaine, makes it unlikely that **5g** disrupted the rats' behavior.

This rather supports the idea that nonselective D_3 partial agonists specifically modulate drug-seeking behavior induced by cocaine-associated cues,[18] as confirmed by our recent findings,[17c] by which, using the same experimental procedure as in the present study, that **3**, a nonselective D_3 partial agonist, reduced cocaine-seeking behavior induced by reintroduction of cocaine-associated stimuli (Figure 3D). Moreover, the effects of **5g** seem to be specific on rats' behavior elicited by cues-associated with and predictive of cocaine availability but not on cocaine primary reinforcing properties. It also seems unlikely that **5g** substituted for cocaine's effects, reducing the motivating action of cocaine-predictive stimuli since **5g**, at a dose reducing drug-seeking behavior (3 mg/kg), did not substitute for cocaine in the self-administration paradigm. This selective effect on the behavioral consequences of exposure to cocaine-associated stimuli may imply dissociable neural mechanisms underlying responding with conditioned reinforcement and responding for cocaine itself.[12,14,36] The possibility of antagonism at the 5-HT_{1A} receptors being involved in the effect of **5g** seems to be excluded by recent findings that WAY 100625, a selective 5-HT_{1A} antagonist,[37] was completely inactive on rats' behavior elicited by reintroduction of cocaine-associated stimuli.[17] The role of α_1- and α_2-adrenocep-



5a,c,h

Figure 6. x, y, w, and z indicate the atoms defining the torsion angle τ in the general structure of compounds **5a**,**c**,**h**.

tors in drug-seeking behavior elicited by presentation of cocaine-associated stimuli is not known.

A partial reduction of cocaine-seeking behavior was also observed with the selective D_3 antagonist **5p**. In no instance were any effects observed that could have interfered with the measurement of rats' behavior. This result is in agreement with a recent report that **4**, a potent and selective D_3 antagonist[19] that crosses the blood-brain barrier, reduced seeking behavior maintained by cocaine in a second-order schedule.[20]

However, differently from **5g**, the highly potent and selective partial agonist **5q** did not have any effect against cocaine-seeking behavior. Although brain uptake studies are needed to establish whether the compounds achieve brain concentrations comparable to those active in vitro on D_3 receptors, these results, together with previous findings,[17] suggest that antagonism at D_2 receptors might significantly contribute to the activity of partial D_3 agonists, like **5g** and **3**, in reducing cocaine craving.

4. Molecular Modeling Studies. The Design of 5r. The aim of this investigation was to identify the conformational features of the structures of our leads able to improve D_3 receptor affinity maintaining a significant D_2 receptor interaction, to design potential therapeutic agents against cocaine craving. We analyzed compounds **5a** (D_3, K_i = 4.8 nM, D_2, K_i = 83 nM, K_{iD2}/K_{iD3} = 17), **5c** (D_3, K_i = 0.49 nM, D_2, K_i = 75 nM, K_{iD2}/K_{iD3} = 153), and **5h** (D_3, K_i = 2.9 nM, D_2, K_i = 160 nM, K_{iD2}/K_{iD3} = 55). Since the structures of these compounds differ solely in the nature of the nitrogen-containing heteroaromatic system, this study focused on the conformational analysis of the heteroaromatic carboxamide moiety by using molecular mechanics (MM) and semiempirical (MOPAC, AM1) calculations.

As expected, MM energy minimizations indicated the trans form of the amide bond as the energetically favored, and AM1 semiempirical calculations confirmed these results (data not shown). To investigate the orientation of the heteroaromatic rings, with respect to the trans amide bond, we analyzed the rotation around the torsion angle τ (**5a**,**c**,**h**, Figure 6). It has to be underlined that the conjugation effect between the amide bond and the heteroaromatic moiety limits the rotation about the considered angle. Accordingly, MM energy minimizations identified two different minima corresponding to $\tau \sim 0°$ and $\tau \sim 180°$, herein called the syn and anti conformers, respectively. In the case of **5a** and **5c** the anti conformers resulted to be energetically favored, with an increased value of ΔE ($E_{syn} - E_{anti}$) when the calculations were carried out in a vacuum (ϵ = 1) rather than in an aqueous environment (ϵ = 80,

Table 3. Calculated Energy Differences, Expressed in kcal/mol, between the Syn and Anti Conformers of the Nitrogen-Containing Aromatic System of **5a**, **5c**, and **5h**

	$\Delta E_{syn-anti}$ (kcal/mol)		
compd	MM ϵ = 1	MM ϵ = 80*r	AM1
5a	8.88	0.87	*a*
5c	8.85	0.87	*a*
5h	−4.28	0.03	−2.06

[a] $\Delta E_{syn-anti}$ cannot be measured for the absence of the syn conformer (see text).

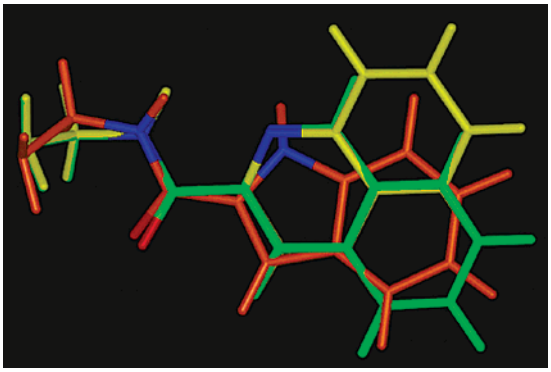


Figure 7. Comparison of the heteroarylcarboxamide moieties of **5a** (yellow), **5c** (green), and **5h** (orange) in their anti conformation. Superimposition was obtained by fitting: (i) the amide nitrogen, (ii) the carbonyl carbon, (iii) the aromatic C-1 carbon, (iv) the aromatic nitrogen. Heteroatoms are colored by atom type (N blue, O red).

Table 3). On the contrary, **5h** showed a conformational preference for the syn orientation of τ when MM calculations were performed in a vacuum (Table 3).

MM conformers of **5a**, **5c**, and **5h** have been used as starting structures for a full AM1 semiempirical geometry optimization. The results obtained confirmed the lower potential energy of the syn conformer of **5h** (Table 3), and evidenced a conformational instability of the syn conformers of **5a** and **5c**. In particular, during AM1 calculation, the anti conformers maintained their starting geometry, while the syn conformers rotated to τ = −161° and τ = −115° (**5a** and **5c**, respectively), indicating a propensity to turn into the anti conformers. This conformational behavior can be explained by the electrostatic repulsion occurring between the aromatic nitrogen and the carbonyl group of **5a** and **5c**, and, to a lesser extent, by the steric overlap between the amide hydrogen and the hydrogen at C3 (**5a**) and C4 (**5c**) of the heteroaromatic ring. On the contrary, the indole NH group of compound **5h** tends to assume a syn orientation with respect to the carbonyl group since, in this case, the syn conformer presents less dipole repulsion and, therefore, it is energetically favored. The strong influence of electrostatic interactions in the conformational behavior of **5a**, **5c**, and **5h** is confirmed by the different results obtained varying the dielectric constant in MM calculations, and using AM1 semiempirical method (Table 3). Since a low dielectric medium more closely resembles the interior of a protein, it is likely that **5a** and **5c** bind to D_3 receptor assuming an anti conformation of τ, and **5h** assuming a syn conformation.

In Figure 7 is reported the superimposition of the heteroaromatic carboxamide moieties of **5a**, **5c**, and **5h**, considered in their anti conformations. It is noteworthy the extra volume occupied by **5a** (D_3, K_i = 4.8 nM) with

respect to **5c** (D_3, $K_i = 0.49$ nM); considering their close structural similarity, we supposed that the unfavorable orientation of the bicyclic system of **5a** is responsible for its lower D_3 receptor affinity. On the other hand, the anti conformer of **5h** (D_3, $K_i = 2.9$ nM) showed a good overlap with the heteroaromatic carboxamide moiety of **5c** (Figure 7), but resulted to be energetically disfavored with respect to the syn conformer (Table 3), in which the nitrogen of the indole ring did not fit the nitrogen of **5c**. On this basis, to improve D_3 receptor affinity, we designed an ethylene bridge between the indole and the amide nitrogen of **5h** to constrain τ in the anti orientation (**5r**). Accordingly, compound **5r** showed an increased D_3 receptor affinity with respect to **5h** ($K_{i\mathbf{5r}} = 0.87$ nM vs $K_{i\mathbf{5h}} = 2.9$ nM), very similar to that of **5c** ($K_i = 0.49$ nM) while showing a better D_2/D_3 receptor affinity ratio (**5c** $K_{iD2}/K_{iD3} = 153$ vs **5r** $K_{iD2}/K_{iD3} = 52$). With respect to **5h**, the constrained analogue **5r** showed a 3-fold higher affinity at D_3 receptors, with D_1/D_3 and D_2/D_3 affinity ratios of 1801 and 52, respectively (for **5h**: D_1/D_3 and D_2/D_3 affinity ratios of 334 and 55, respectively). While the affinity for 5-HT_{1A} and α_2 receptors was similar to **5h**, **5r** was found to be 3- and 20-fold more potent than **5h** at D_2 and α_1 receptors, respectively. With respect to **5g** (a compound active against cocaine craving), **5r** showed the same D_1/D_3 and D_2/D_3 affinity ratios and a similar pattern of α_1, α_2, and 5-HT_{1A} receptor affinity. Consequently, **5r** represents an interesting lead for the generation of a new series of D_2/D_3 receptor ligands potentially active against cocaine craving, and it is a candidate for future in vivo studies.

Conclusions

A series of novel arylalkylpiperazines was synthesized and evaluated in D_1, D_2, D_3, 5-HT_{1A}, α_1 and α_2 receptor binding assays. SAR studies delineated a number of structural features required for high D_3 receptor affinity and selectivity. Several heteroaromatic systems were explored for their dopamine, serotonin, and noradrenaline receptor affinities, and novel structural leads were identified for the development of potent and selective D_3 receptor ligands. An indole ring coupled to a 2,4-dichlorophenylpiperazine ring system led to two of the most potent and selective D_3 receptor ligands known to date (**5p** and **5q**).

We also assessed intrinsic pharmacological properties of a subset of potent D_3 receptor ligands were also assessed in [^{35}S]-GTPγS binding assays. Since it was recently suggested that in vivo activity of **3** could not be attributed just to a mediated response, we investigated the effects of **5g**, a partial D_3 agonist and 5-HT_{1A} antagonist with low receptor selectivity, **5q**, a partial D_3 receptor agonist with extremely high D_3 receptor affinity and selectivity, and **5p**, a highly selective and very potent D_3 receptor antagonist, on cocaine-seeking behavior induced by reintroduction of cocaine-associated stimuli after a long period of abstinence and in the absence of any further cocaine. While **5g** had a pharmacological profile similar to **3**, **5q** had no effect on cocaine-seeking behavior. Although brain uptake studies are needed to establish whether the compounds achieve brain concentrations comparable to those active in vitro, these results, though only preliminary, do suggest that the D_2 component of the receptor binding profile of **5g** may be essential for its activity. This seems to apply only for partial D_3 agonists, since compound **4**, a selective brain penetrating D_3 receptor antagonist,[19] reduced drug-seeking behavior in rats,[20] and similar though partial, results were obtained in the present study with **5p**, a potent and selective D_3 receptor antagonist. On these basis, through a molecular modeling study, we designed the conformationally constrained analogue **5r**, characterized by a similar binding profile to **5g**, with a 2-fold higher affinity at D_2 and D_3 receptors, thus being a lead structure for the generation of a new series of D_2/D_3 receptor ligands.

In conclusion, the results suggest that **5g**, a partial agonist at D_3 receptors with significant affinity for D_2 receptors, and **5p**, a potent and selective D_3 antagonist, could be useful in the pursuit of medications for drug-seeking behavior induced by environmental stimuli associated with cocaine.

Experimental Section

Melting points were determined using an Electrothermal 8103 apparatus. IR spectra were taken with Perkin-Elmer 398 and FT 1600 spectrophotometers. ^{1}H NMR spectra were recorded on a Bruker 200 MHz spectrometer with TMS as internal standard; the value of chemical shifts (δ) are given in ppm and coupling constants (J) in Hertz (Hz). All reactions were carried out in an argon atmosphere. GC-MS were performed on a Saturn 3 (Varian) or Saturn 2000 (Varian) GC-MS System using a Chrompack DB5 capillary column (30 m $\times$ 0.25 mm i.d.; 0.25 μm film thickness). Mass spectra were recorded using a VG 70−250S spectrometer. ESI-MS and APCI-MS spectra were taken by a LCQDeca-Thermofinnigan spectrometer. Optical rotations were recorded on a Perkin-Elmer model 343 polarimeter at the sodium D line at 20 °C. Elemental analyses were done on a Perkin-Elmer 240C elemental analyzer and the results were within 0.4% of the theoretical values, unless otherwise noted. Yields refer to purified products and are not optimized. For testing, compounds **5a**−**r** were transformed into the corresponding hydrochloride salts by a standard procedure.

1-(2,4-Dichlorophenyl)piperazine (7a). A mixture of 1-bromo-2,4-dichlorobenzene (1.0 g, 4.4 mmol), piperazine (1.14 g, 13.3 mmol), sodium *tert*-butoxide (0.59 g, 6.2 mmol), tris-(dibenzylideneacetone)dipalladium-(0) (10.13 mg, 0.01 mmol), and BINAP (20.7 mg, 0.03 mmol) in anhydrous toluene (20 mL) was heated to 80 °C under argon. After the sample was stirred for 2 h, the mixture was allowed to cool to room temperature, taken up in ethyl ether (30 mL), filtered, and concentrated. The crude product was then purified by flash chromatography (20% methanol in chloroform) to give 0.65 g (65% yield) of **7a** as a yellow oil; ^{1}H NMR ($CDCl_3$) δ 2.60 (br s, 1H), 3.14 (m, 8H), 6.87 (d, 1H, $J = 8.7$ Hz), 7.09 (dd, 1H, $J = 8.5$, 2.3 Hz), 7.26 (d, 1H, $J = 2.5$ Hz). GC-MS m/z 230 $[M]^+$, 188, 172, 149 (100). Anal. ($C_{10}H_{12}Cl_2N_2$) C, H, N.

1-(4-Cyanophenyl)piperazine (7b). Starting from 4-bromobenzonitrile (1.2 g, 6.6 mmol), the title compound was obtained following the above-described procedure as a yellow oil (90% yield): IR ($CHCl_3$) 2218 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.69 (br s, 1H), 2.99 (m, 4H), 3.26 (m, 4H), 6.83 (m, 2H), 7.47 (m, 2H). Anal. ($C_{11}H_{13}N_3$) C, H, N.

1-(2-Hydroxyethyl)-4-(2-methoxyphenyl)piperazine (9). 2-Bromoethanol (0.32 mL, 4.6 mmol) was added to a vigorous stirred mixture of 1-(2-methoxyphenyl)piperazine hydrochloride (1.0 g, 4.4 mmol) and anhydrous potassium carbonate (1.50 g, 10.9 mmol) in dry acetonitrile (10 mL), and then the suspension was refluxed for 10 h under argon. After that time, the suspension was filtered and concentrated. The crude product was chromatographed (20% methanol in chloroform) to give **9** (0.34 g, 34% yield) as a yellow oil; ^{1}H NMR ($CDCl_3$) δ 2.58 (t, 2H, $J = 5.4$ Hz), 2.70 (m, 4H), 2.95 (br s, 1H), 3.09 (m, 4H), 3.65 (t, 2H, $J = 5.4$ Hz), 5.83 (s, 3H), 6.94 (m, 4H). Anal. ($C_{13}H_{20}N_2O_2$) C, H, N.

1-(2-Aminoethyl)-4-(2-methoxyphenyl)piperazine (10). 1-(2-Methanesulfonylethyl)-4-(2-methoxyphenyl)piperazine. A solution of methanesulfonyl chloride (0.41 mL, 0.54 mmol) in dry dichloromethane (2 mL) was added dropwise to a cooled solution (0 °C) of **9** (127.0 mg, 0.54 mmol) and triethylamine (0.11 mL, 0.80 mmol) in dry dichloromethane (5 mL) under argon. The resulting solution was warmed to room temperature and stirred for 12 h. After quenching of the sample with water (7 mL), the mixture was extracted with ethyl acetate. The organic layers were dried and evaporated. Chromatography of the crude product (10% methanol in chloroform) gave 118.0 mg of the methanesulfonyl derivative (70% yield) as an amorphous solid; ^{1}H NMR ($CDCl_3$) δ 2.78 (m, 6H), 3.02 (s, 3H), 3.10 (m, 4H), 3.64 (t, 2H, J = 6.9 Hz), 3.85 (s, 3H), 6.93 (m, 4H). Anal. ($C_{14}H_{22}N_2O_4S$) C, H, N.

1-(2-Azidoethyl)-4-(2-methoxyphenyl)piperazine. To a solution of the above-described methanesulfonyl-intermediate (1.0 g, 3.2 mmol) in dimethyl sulfoxide (15 mL), sodium azide (248.0 mg, 3.81 mmol) was added and the mixture was heated to 45 °C with stirring for 15 h. After cooling of the sample to room temperature, the solution was quenched with water (20 mL) and the mixture was extracted with diethyl ether. The organic layers were dried and evaporated. The residue was chromatographed (ethyl acetate) to give the azido-derivative (94% yield) as a yellow oil; ^{1}H NMR ($CDCl_3$) δ 2.72 (m, 6H), 3.12 (m, 4H), 3.40 (t, 2H, J = 5.8), 3.85 (s, 3H), 6.93 (m, 4H); GC-MS m/z 262 $[M + H]^+$, 219, 205 (100), 190, 175, 162, 150, 134, 121. Anal. ($C_{13}H_{19}N_5O$) C, H, N.

The azido-derivative (0.7 g, 2.7 mmol) was dissolved in dry methanol (10 mL) in the presence of dry triethylamine (1.12 mL, 8.0 mmol) and then 1,3-propanedithiol (0.81 mL, 8.0 mmol) was added. The solution was stirred at room temperature for 3 days and the white precipitate formed was filtered off and the solution was concentrated. The oily residue was recrystallized to give 620.0 mg of **10** (98% yield) as an amorphous solid that was used in the next step without further purification. For an analytical sample: mp (methanol) 76−77 °C; ^{1}H NMR (DMSO-d_6) δ 2.17 (t, 2H, J = 6.3 Hz), 2.35 (t, 2H, J = 2.3 Hz), 2.48 (m, 4H), 2.92 (m, 4H), 3.73 (s, 3H), 6.88 (m, 4H). Anal. ($C_{13}H_{21}N_3O$) C, H, N.

***N*-[1-(4-Hydroxy)butyl]benzo[*b*]furan-2-carboxamide (12a).** To a solution of 2-benzofurancarboxylic acid **11a** (0.50 g, 3.08 mmol) in dry dichloromethane, 1-hydroxybenzotriazole hydrate (0.46 g, 3.4 mmol) and 1,3-dicyclohexylcarbodiimide (0.7 g, 3.4 mmol) were added at 0 °C under argon; the suspension was warmed to room temperature and stirred for 1 h. Then 4-amino-1-butanol (0.28 mL, 3.08 mmol) was added and the mixture was stirred overnight at room temperature. The resulting suspension was filtered through Celite, washed with chloroform, and the filtrate evaporated. The crude product was purified by flash chromatography (10% methanol in chloroform) to give 0.7 g (97% yield) of **12a** as colorless prisms: mp (methanol) 95−96 °C; ^{1}H NMR ($CDCl_3$) δ 1.67 (m, 4H), 2.14 (br s, 1H), 3.53 (m, 2H), 3.73 (m, 2H), 6.89 (br s, 1H), 7.25−7.48 (m, 4H), 7.63 (d, 1H, J = 7.7 Hz). Anal. ($C_{13}H_{15}NO_3$) C, H, N.

***N*-[1-(4-Hydroxy)butyl]quinoxaline-2-carboxamide (12b).** Starting from quinoxaline-2-carboxylic acid chloride **11b** (166.0 mg, 0.86 mmol), the title compound was prepared following the above-described procedure, and it was obtained as colorless prisms (40% yield): mp (methanol) 138−139 °C; ^{1}H NMR ($CDCl_3$) δ 1.55 (m, 4H), 3.53 (m, 2H), 3.63 (t, 2H, J = 6.5 Hz), 7.24 (s, 1H), 7.84 (m, 2H), 8.14 (m, 3H), 9.64 (br s, 1H). Anal. ($C_{13}H_{15}N_3O_2$) C, H, N.

***N*-[1-(4-Hydroxy)butyl]pyrrolo[1,2-*a*]quinoxaline-4-carboxamide (12c).** The title compound was prepared starting from pyrrolo[1,2-*a*]quinoxaline-4-carboxylic acid **11c** (100.0 mg, 0.47 mmol) and following the procedure as described for **12a**. **12c** was obtained as a yellow oil (47% yield); ^{1}H NMR ($CDCl_3$) δ 1.68 (m, 4H), 2.45 (br s, 1H), 3.62 (m, 2H), 3,93 (m, 3H), 6.91 (m, 1H), 7.45 (m, 2H), 7.79 (m, 4H) 8.21 (br s, 1H). Anal. ($C_{16}H_{17}N_3O_2$) C, H, N.

***N*-[1-(4-Hydroxy)butyl]indole-2-carboxamide (12d).** Starting from 2-indolecarboxylic acid **11d** (150.0 mg, 0.93 mmol), the title compound was prepared following the procedure described for **12a**. The product **12d** was obtained as colorless prisms (93% yield): mp (methanol) 108−109 °C; ^{1}H NMR ($CDCl_3$) δ 1.67 (m, 4H), 3.52 (q, 2H, J = 11.5, 5.6) 3.72 (t, 2H, J = 5.8 Hz), 6.65 (br s, 1H), 6.82 (s, 1H), 7.11 (d, 1H, J = 8.0 Hz), 7.29 (m, 1H), 7.39 (d, 1H, J = 7.7 Hz), 7.59 (d, 1H, J = 7.8 Hz), 9.25 (br s, 1H). Anal. ($C_{13}H_{16}N_2O_2$) C, H, N.

***N*-[1-(4-Hydroxy)butyl]quinoline-2-carboxamide (12e).** Starting from 2-quinolinecarboxylic acid **11e** (0.5 g, 2.88 mmol), the title compound was prepared following the procedure described for **12a**. The product **12e** was obtained as colorless prisms (99% yield): mp (methanol) 131−132 °C; ^{1}H NMR ($CDCl_3$) δ 1.59 (m, 4H), 3.45 (m, 2H), 3.58 (m, 2H), 7.50 (m, 3H), 7.91 (d, 1H, J = 8.3 Hz), 8.13 (m, 2H), 8.34 (br s, 1H). Anal. ($C_{14}H_{16}N_2O_2$) C, H, N.

***N*-[1-(4-Hydroxy)butyl]isoquinoline-3-carboxamide (12f).** The title compound was prepared starting from 3-isoquinolinecarboxylic acid **11f** (100.0 mg, 0.57 mmol) and following the procedure as described to obtain **12a**. **12f** was obtained as colorless prisms (96% yield): mp (methanol) 126−127 °C; ^{1}H NMR ($CDCl_3$) δ 1.74 (m, 4H), 3.57 (q, 2H, J = 12.7, 6.3 Hz), 3.73 (t, 2H, J = 5.9 Hz), 7.40 (m, 2H), 7.75 (m, 2H), 8.39 (br s, 1H), 8.57 (s, 1H), 9.14 (s, 1H). Anal. ($C_{14}H_{16}N_2O_2$) C, H, N.

(*S*)-(−)-*N*-[4-(1-Hydroxy)butyl]-2-(benzyloxycarbonyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (12g). The title compound was prepared starting from (*S*)-(−)-2-(benzyloxycarbonyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid **11g** (190.0 mg, 0.61 mmol) and following the procedure as described to obtain **12a**. **12g** was obtained as a yellow oil (97% yield); ^{1}H NMR ($CDCl_3$) δ 1.23 (m, 4H), 1.83 (br s, 1H), 3.02−3.44 (m, 6H), 4.61−4.78 (m, 3H), 5.20 (s, 2H), 6.20 (br s, 1H), 7.18 (m, 4H), 7.34 (m, 5H); ESI-MS m/z 405 $[M + Na]^+$; ESI-MS/MS of $[M + Na]^+$ 361 (100), 269, 253, 230, 175. Anal. ($C_{22}H_{26}N_2O_4$) C, H, N. $[\alpha]_D^{20}$ −3.43 (*c* 2.04, $CHCl_3$).

(*R*)-(+)-*N*-[4-(1-Hydroxy)butyl]-2-(benzyloxycarbonyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (12h). The title compound was prepared starting from (*R*)-(+)-2-(benzyloxycarbonyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid **11h** (250.0 mg, 0.80 mmol) and following the procedure as described to obtain **12a**. **12h** was obtained as a yellow oil (98% yield); ^{1}H NMR ($CDCl_3$) δ 1.23 (m, 4H), 1.83 (br s, 1H), 3.02−3.44 (m, 6H), 4.61−4.78 (m, 3H), 5.20 (s, 2H), 6.20 (br s, 1H), 7.18 (m, 4H), 7.34 (m, 5H). Anal. ($C_{22}H_{26}N_2O_4$) C, H, N. $[\alpha]_D^{20}$ +3.43 (*c* 3.26, $CHCl_3$).

***N*-[1-(4-Hydroxy)butyl]-5-chloroindole-2-carboxamide (12i).** Starting from 5-chloroindole-2-carboxylic acid **11i** (300.0 mg, 1.53 mmol), the title compound was prepared following the procedure described for **12a**. **12i** was obtained as colorless prisms (90% yield): mp (methanol) 102−103 °C; ^{1}H NMR (CD_3OD) δ 1.61 (m, 4H), 3.40 (t, 2H, J = 6.6 Hz), 3.59 (t, 2H, J = 5.8 Hz), 6.95 (s, 1H), 7.14 (dd, 1H, J = 8.8, 1.9 Hz), 7.34 (d, 1H, J = 8.7 Hz), 7.53 (d, 1H, J = 1.7 Hz); GC-MS m/z 266 $[M]^+$, 194, 178 (100), 150, 123, 114, 88, 70. Anal. ($C_{13}H_{15}ClN_2O_2$) C, H, N.

***N*-[1-(4-Bromo)butyl]benzo[*b*]furane-2-carboxamide (13a).** To a stirred solution of **12a** (0.50 g, 2.14 mmol) in acetonitrile (25 mL), triphenylphosphine (0.86 g, 3.22 mmol) and carbon tetrabromide (1.06 g, 3.22 mmol) were added at room temperature. After 2 h, the mixture was quenched with 15% NaOH and extracted with ethyl acetate. The organic layers were dried and evaporated. The residue was chromatographed (20% *n*-hexane in ethyl acetate) to afford 0.58 g (91% yield) of **13a** as colorless prisms: mp (ethyl acetate) 65−66 °C; ^{1}H NMR ($CDCl_3$) δ 1.67 (m, 4H), 3.37 (m, 4H), 7.36 (m, 4H), 7.63 (d, 1H, J = 7.7 Hz); GC-MS m/z 297 $[M + H]^+$, 216 (100), 202, 188, 174, 161, 145, 118, 89. Anal. ($C_{13}H_{14}BrNO_2$) C, H, N.

***N*-[1-(4-Bromo)butyl]quinoxaline-2-carboxamide (13b).** The title compound was prepared starting from **12b** (84.0 mg, 0.34 mmol) and following the above-described procedure. **13b** was obtained as a yellow oil (38% yield); ^{1}H NMR ($CDCl_3$) δ 1.93 (m, 4H), 3.48 (t, 2H, J = 5.9 Hz), 3.61 (m, 2H, J = 6.3 Hz), 7.83 (m, 2H), 8.09 (m, 3H), 9.52 (br s, 1H). Anal. ($C_{13}H_{14}BrN_3O$) C, H, N.

***N*-[1-(4-Bromo)butyl]pyrrolo[1,2-*a*]quinoxaline-4-carboxamide (13c).** To a solution of **12c** (60.0 mg, 0.22 mmol) in acetonitrile (3 mL), triphenylphosphine (86.0 mg, 0.32 mmol), and carbon tetrabromide (106 mg, 0.32 mmol) were added under vigorous stirring at room temperature. After 5 h, the mixture was quenched with 15% NaOH and extracted with ethyl acetate. The organic layers were dried and evaporated. The residue was chromatographed (50% *n*-hexane in ethyl acetate) to afford 29.0 mg (38% yield) of **13c** as a yellow oil; ^{1}H NMR ($CDCl_3$) δ 1.94 (m, 4H), 3.51 (m, 4H), 6.91 (m, 1H), 7.52 (m, 2H), 7.83 (m, 4H), 8.21 (br s, 1H). Anal. ($C_{16}H_{16}BrN_3O$) C, H, N.

***N*-[1-(4-Bromo)butyl]indole-2-carboxamide (13d).** The title compound was prepared starting from **12d** (170.0 mg, 0.73 mmol) and following the procedure described to obtain **13a**. **13d** was obtained as colorless prisms (84% yield): mp (ethyl acetate) 133−134 °C; ^{1}H NMR ($CDCl_3$) δ 1.96 (m, 4H), 3.56 (m, 4H), 7.28 (m, 5H), 7.60 (d, 1H, J = 7.6 Hz), 9.80 (br s, 1H). Anal. ($C_{13}H_{15}BrN_2O$) C, H, N.

***N*-[1-(4-Bromo)butyl]quinoline-2-carboxamide (13e).** The title compound was prepared starting from **12e** (0.7 g, 2.86 mmol) and following the procedure described to obtain **13a**. **13e** was obtained as a yellow oil (57% yield); ^{1}H NMR ($CDCl_3$) δ 1.60 (m, 4H), 3.16 (t, 2H, J = 6.7 Hz), 3.29 (m, 2H), 7.29 (d, 1H, J = 7.5 Hz), 7.50 (m, 2H), 7.83 (d, 1H, J = 8.4 Hz), 8.06 (m, 2H), 8.27 (m, 1H). Anal. ($C_{14}H_{15}BrN_2O$) C, H, N.

***N*-[1-(4-Bromo)butyl]isoquinoline-3-carboxamide (13f).** To a solution of **12f** (140.0 mg, 0.57 mmol) in acetonitrile (10 mL), triphenylphosphine (225.0 mg, 0.86 mmol) and carbon tetrabromide (285.0 mg, 0.86 mmol) were added under vigorous stirring at room temperature. After 2 h, the mixture was quenched with 15% NaOH and extracted with ethyl acetate. The organic layers were dried and evaporated. The residue was chromatographed (30% *n*-hexane in ethyl acetate) to give 130.0 mg of **13f** (75% yield) as a yellow solid: mp (ethyl acetate) 72−73 °C; ^{1}H NMR ($CDCl_3$) δ 2.06 (m, 4H), 3.48 (m, 4H), 7.66 (m, 2H), 7.93 (m, 2H), 8.36 (br s, 1H), 8.55 (s, 1H), 9.08 (s, 1H). Anal. ($C_{14}H_{15}BrN_2O$) C, H, N.

(*S*)-(−)-*N*-[1-(4-Bromo)butyl]-2-(benzyloxycarbonyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (13g). To a solution of **12g** (230.0 mg, 0.60 mmol) in acetonitrile (3 mL), triphenylphosphine (237.0 mg, 0.90 mmol) and carbon tetrabromide (300.0 mg, 0.90 mmol) were added under vigorous stirring at room temperature. After 5 h, the mixture was quenched with 15% NaOH and extracted with ethyl acetate. The organic layers were dried and evaporated. The residue was chromatographed (20% *n*-hexane in ethyl acetate) to afford 180.0 mg (67% yield) of **13g** as a colorless oil; ^{1}H NMR ($CDCl_3$) δ 1.27 (m, 4H), 3.15 (m, 6H), 4.35 (br s, 3H), 5.21 (s, 2H), 5.87 (br s, 1H), 7.19 (s, 4H), 7.35 (s, 5H). Anal. ($C_{22}H_{25}BrN_2O_3$) C, H, N. $[\alpha]_D$ −0.96 (*c* 4.14, $CHCl_3$).

(*R*)-(+)-*N*-[1-(4-Bromo)butyl]-2-(benzyloxycarbonyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (13h). Starting from **12h** (250.0 mg, 0.65 mmol), the title compound was prepared following the above-described procedure and obtained as a colorless oil (65% yield); ^{1}H NMR ($CDCl_3$) δ 1.27 (m, 4H), 3.15 (m, 6H), 4.35 (br s, 3H), 5.21 (s, 2H), 5.87 (br s, 1H), 7.19 (s, 4H), 7.35 (s, 5H). Anal. ($C_{22}H_{25}BrN_2O_3$) C, H, N. $[\alpha]_D$ +0.96 (*c* 1.43, $CHCl_3$).

***N*-[1-(4-Iodo)butyl]-5-chloroindole-2-carboxamide (13i).** *N*-[1-(4-Methanesulfonyl)butyl]-5-chloroindole-2-carboxamide. To a stirred and cooled (0 °C) solution of **12i** (270.0 mg, 1.01 mmol) and triethylamine (0.18 mL, 1.31 mmol) in dry *N*,*N*-dimethylformamide (4 mL), methanesulfonyl chloride (0.85 mL, 1.10 mmol) was added dropwise. The solution was left at 0 °C under argon overnight. Water was added to the formed suspension and was extracted with chloroform. The organic layers were dried and concentrated and the residue was purified by chromatography (10% methanol in chloroform) to afford the methanesulfonyl-derivative (97% yield) as a yellow amorphous solid; ^{1}H NMR (acetone-d_6) δ 1.77 (m, 4H), 3.05 (s, 3H), 3.51 (m, 2H), 4.27 (m, 2H), 7.02 (s, 1H), 7.19 (m, 1H), 7.55 (m, 2H), 7.90 (br s, 1H), 10.88 (br s, 1H). Anal. ($C_{14}H_{17}ClN_2O_4S$) C, H, N.

The methanesulfonyl intermediate (340.0 mg, 0.99 mmol) was suspended in dry tetrahydrofuran (5 mL) and lithium iodide (200.0 mg, 1.48 mmol) was added in portions. The suspension was stirred under argon in an ultrasound bath at 40 °C. After stirring of the sample for 5 h, the solvent was removed under reduced pressure, water was added, and the mixture was extracted with ethyl acetate. The organic layers were collected, dried, concentrated and the crude product was chromatographed (10% methanol in chloroform) to give 0.37 g of pure **13i** (99% yield) as colorless prisms: mp (methanol) 124−125 °C; ^{1}H NMR (acetone-d_6) δ 1.60 (m, 2H), 1.79 (m, 2H), 3.19 (m, 2H), 3.31 (m, 2H), 6.86 (s, 1H), 7.19 (m, 1H), 7.40 (m, 2H), 7.75 (m, 1H). Anal. ($C_{13}H_{14}ClIN_2O$) C, H, N.

(*S*)-(−)-*N*-[4-[4-(2-Methoxyphenyl)piperazin-1-yl]butyl]-2-(benzyloxycarbonyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (14a). Starting from **13g** (180.0 mg, 0.40 mmol) the title compound was prepared following the procedure described for **5a** and was obtained as a colorless oil (80% yield); ^{1}H NMR (acetone-d_6) δ 1.30 (m, 4H), 2.22 (m, 2H), 2.45 (m, 4H), 2.95 (m, 4H), 3.13 (m, 4H), 3.79 (s, 3H), 4.64 (br s, 3H), 5.16 (s, 2H), 6.84 (m, 4H), 7.16 (s, 5H), 7.34 (m, 4H); ESI-MS *m*/*z* 579 $[M + Na]^+$ 557 $[M + H]^+$; ESI-MS/MS of $[M + Na]^+$ 535 (100), 443. Anal. ($C_{33}H_{40}N_4O_4$) C, H, N. $[\alpha]_D^{20}$ −5.15 (*c* 0.97, $CHCl_3$).

(*R*)-(+)-*N*-[4-[4-(2-Methoxyphenyl)piperazin-1-yl]butyl]-2-(benzyloxycarbonyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (14b). Starting from **13h** (150.0 mg, 0.34 mmol) the title compound was obtained following the procedure described for **14a** and was obtained as a colorless oil (91% yield); ^{1}H NMR (acetone-d_6) δ 1.30 (m, 4H), 2.22 (m, 2H), 2.45 (m, 4H), 2.95 (m, 4H), 3.13 (m, 4H), 3.79 (s, 3H), 4.64 (br s, 3H), 5.16 (s, 2H), 6.84 (m, 4H), 7.16 (s, 5H), 7.34 (m, 4H). Anal. ($C_{33}H_{40}N_4O_4$) C, H, N. $[\alpha]_D^{20}$ +5.15 (*c* 0.97, $CHCl_3$).

1-(Cyanomethyl)indole-2-carboxylic Acid Ethyl Ester (16). A mixture of sodium hydride (60% in mineral oil) (190.0 mg, 7.94 mmol) and ethyl indole-2-carboxylate (**15**, 1.0 g, 5.29 mmol) in *N*,*N*-dimethylformamide (4.6 mL), was stirred at room temperature for 0.5 h and to this bromoacetonitrile (0.74 mL, 10.60 mmol) in *N*,*N*-dimethylformamide (1 mL) was added. The reaction mixture was then maintained at 60−65 °C for 0.5 h, and stirred for 5−6 h further at room temperature, left overnight and decomposed with ice. The separated solid was recrystallized from ethanol to give **16** (90% yield) as colorless prisms: mp (ethanol) 83−84 °C; ^{1}H NMR ($CDCl_3$) δ 1.42 (t, 3H, J = 7.3 Hz), 4.41 (q, 2H, J = 14.2, 7.2 Hz), 5,60 (s, 2H), 7.37 (m, 4H), 7.71 (d, 1H, J = 7.9 Hz); GC-MS *m*/*z* 228 $[M]^+$ (100), 199, 182, 154, 128, 115, 101, 89, 77. Anal. ($C_{13}H_{12}N_2O_2$) C, H, N.

1,2,3,4-Tetrahydropyrazino[1,2-*a*]indole-1(2*H*)-one (17). To a warm solution (60 °C) of **16** (200.0 mg, 0.87 mmol) in dry methanol (8 mL) under argon, freshly prepared cobalt boride (450.0 mg, 3.50 mmol), was added under stirring. Sodium borohydride (166.0 mg, 4.38 mmol) was cautiously added portionwise and the mixture was refluxed for 3 h. The mixture was cooled, and the solvent removed under reduced pressure then water was added and the mixture was extracted with ethyl acetate. The organic layers were dried, evaporated and the crude product was purified by chromatography (10% methanol in chloroform) to give **17** (68% yield) as colorless prisms: mp (methanol) 261−265 °C (dec); ^{1}H NMR ($CDCl_3$) δ 3.82 (m, 2H), 4.27 (m, 2H), 6.65 (br s, 1H), 7.23 (m, 4H), 7.72 (d, 1H, J = 8.0 Hz); APCI-MS *m*/*z* 187 $[M + H]^+$; APCI-MS/MS of $[M + H]^+$ 159 (100), 144. Anal. ($C_{11}H_{10}N_2O$) C, H, N.

***N*-[1-(4-Bromo)butyl]-1,2,3,4-tetrahydropyrazino[1,2-*a*]indole-1(2*H*)-one (18).** To a suspension of **17** (130.0 mg, 0.69) in anhydrous *N*,*N*-dimethylformamide (1 mL) sodium hydride (60% in mineral oil) (20.0 mg, 0.83 mmol) was added. After stirring of the sample for 1 h at 60 °C under argon, a solution of 1,4-dibromobutane (0.41 mL, 3.47 mmol) in anhydrous *N*,*N*-dimethylformamide (0.5 mL) was added dropwise. The mixture was refluxed under argon at 110 °C for 3 h. Then the solvent was evaporated under reduced pressure, and the residue was resuspended in water and extracted with dichloromethane. The combined organic layers were dried evaporated and the

residue was chromatographed (30% ethyl acetate in *n*-hexane) to give **18** as a yellow solid (41% yield): mp (ethyl acetate) 101−102 °C; ^{1}H NMR ($CDCl_3$) δ 1.85 (m, 4H), 3.67 (m, 4H), 3.81 (m, 2H), 4.29 (m, 2H), 7.20 (m, 4H), 7.70 (d, 1H, J = 8.0 Hz); APCI-MS m/z 321 $[M + H]^+$, 241, 227, 199 (100), 187, 159, 144, 117. Anal. ($C_{15}H_{17}BrN_2O$) C, H, N.

(Benzo[*b*]furan-2-yl)-[(4-methoxycarbonyl)piperidin-1-yl]methanone (19). Starting from 2-benzofurancarboxylic acid **11a** (0.5 g, 3.1 mmol) and using methyl 4-piperidinecarboxylate (0.44 g, 3.1 mmol), the title compound was prepared following the procedure described for **12a**. Compound **19** was obtained as colorless prisms (70% yield): mp (methanol) 88−89 °C; ^{1}H NMR ($CDCl_3$) δ 1.67 (m, 4H), 2.53 (m, 1H), 3.17 (m, 2H), 3.66 (s, 3H), 4.34 (m, 2H), 7.40 (m, 5H). Anal. ($C_{16}H_{17}NO_4$) C, H, N.

(Benzo[*b*]furan-2-yl)-[4-(hydroxymethyl)piperidin-1-yl]methanone (20). To a solution of **19** (180.0 mg, 0.63 mmol) in methanol (5 mL), sodium borohydride (238.0 mg, 6.3 mmol) was added in portions and the mixture was refluxed for 2 h. Then 0.5 N HCl (2 mL) was added to decompose the excess of hydride and the solvent was removed under reduced pressure. The residue was extracted with ethyl acetate, dried, concentrated and chromatographed (50% ethyl acetate in *n*-hexane) to give 100.0 mg of **20** (61% yield) as colorless prisms: mp (ethyl acetate) 91−92 °C; ^{1}H NMR ($CDCl_3$) δ 1.19 (m, 4H), 1.78 (m, 2H), 2.99 (m, 2H), 3.66 (m, 2H), 4.58 (m, 2H), 7.41 (m, 5H). Anal. ($C_{15}H_{17}NO_3$) C, H, N.

(Benzo[*b*]furan-2-yl)-[4-(bromomethyl)piperidin-1-yl]-methanone (21). Starting from **20** (100.0 mg, 0.39 mmol), the title compound was prepared following the procedure described for **13a**. Compound **21** was obtained as colorless prisms (49% yield): mp (ethyl acetate) 63−64 °C; ^{1}H NMR ($CDCl_3$) δ 1.33 (m, 4H), 1.93 (m, 3H), 3.03 (m, 2H), 3.31 (d, 2H, J = 5.9 Hz), 4.58 (m, 2H), 7.26 (m, 3H), 7.49 (d, 1H, J = 8.3 Hz), 7.61 (d, 1H, J = 7.6 Hz). Anal. ($C_{15}H_{16}BrNO_2$) C, H, N.

2-(Benzo[*b*]furan-2-yl)-4-(chloromethyl)-1,3-oxazole (23a). To a solution of acid **11a** (301.6 mg, 1.86 mmol) in anhydrous benzene (3 mL), thionyl chloride (0.67 mL, 9.1 mmol) dissolved in anhydrous benzene (1 mL) was added dropwise. The reaction mixture was heated at reflux for 2 h under argon. After cooling the solvent was removed under vacuum and the residue was dissolved in anhydrous tetrahydrofuran (2 mL) and added to liquid ammonia at −50 °C. After 1 h of stirring, the reaction mixture was warmed to room temperature the solvent was removed, the corresponding amide was obtained in quantitative yield as a white amorphous solid and was used in the next step without purification. A sample was recrystallized from ethyl acetate: mp 158−159 °C; ^{1}H NMR (DMSO-d_6) δ 7.33 (m, 2H), 7.53 (s, 1H), 7.59 (d, 1H, J = 8.1 Hz), 7.72 (d, 1H, J = 7.6 Hz), 8.09 (br s, 2 H). The amide (300.0 mg, 1.86 mmol) and 1,3-dichloroacetone (709.0 mg, 5.6 mmol) were fused at 130 °C under argon. After fusion the mixture was stirred for 1 h. Then water was added and the mixture was extracted with dichloromethane. The organic layers were dried and evaporated and the residue was chromatographed (dichloromethane) to afford **25a** (54% yield) as an amorphous white solid; ^{1}H NMR ($CDCl_3$) δ 4.66 (s, 2H), 7.34 (m, 3H), 7.73 (m, 3H); GC-MS m/z 233 $[M]^+$ (100), 198, 170, 143, 130, 115. Anal. ($C_{12}H_8ClNO_2$) C, H, N.

4-(Chloromethyl)-2-(2-naphthyl)-1,3-oxazole (23b). The title compound was obtained following the procedure described for **23a**. The compound **23b** was obtained as an amorphous white solid (37% yield); ^{1}H NMR ($CDCl_3$) δ 4.60 (s, 2H), 7.54 (m, 2H), 7.74 (s, 1H), 7.91 (m, 4H), 8.10 (d, 1H, J = 8.8 Hz), 8.53 (s, 1H); GC-MS m/z 243 $[M]^+$ (100), 208, 180, 153, 139, 127. Anal. ($C_{14}H_{10}ClNO$) C, H, N.

***N*-[4-[4-(2-Methoxyphenyl)piperazin-1-yl]butyl]quinoline-2-carboxamide (5a).** To a solution of **13e** (482.0 mg, 1.57 mmol) in dry acetonitrile (5 mL) under argon, 1-(2-methoxyphenyl)piperazine (207.0 mg, 1.57 mmol) and triethylamine (0.35 mL, 2.54 mmol) were added; the solution was refluxed overnight with stirring. The solvent was removed under reduced pressure, water was added, and the mixture was extracted with dichloromethane. The organic layers were dried and concentrated and the crude product was chromatographed (10% methanol in chloroform) to give 0.6 g of **5a** (94% yield) as a yellow oil; ^{1}H NMR ($CDCl_3$) δ 1.29 (m, 4H), 2.04 (m, 2H), 2.22 (m, 4H), 2.67 (m, 4H), 3.18 (m, 2H), 3.42 (s, 3H), 6.50 (m, 4H), 7.18 (m, 1H), 7.34 (m, 1H), 7.44 (d, 1H, J = 7.8 Hz), 7.58 (s, 1H), 7.72 (d, 1H, J = 8.2 Hz), 7.90 (s, 1H), 8.12 (br s, 1H). Anal. ($C_{25}H_{30}N_4O_2$) C, H, N.

***N*-[4-[4-(2-Methoxyphenyl)piperazin-1-yl]butyl]quinoxaline-2-carboxamide (5b).** Starting from **13b** (40.0 mg, 0.13 mmol), the title compound was prepared following the above-described procedure and was obtained as a yellow oil (91% yield); ^{1}H NMR ($CDCl_3$) δ 1.63 (m, 4H), 2.40 (m, 2H), 2.58 (m, 4H), 3.00 (m, 4H), 3.50 (m, 2 H), 3.75 (s, 3H), 6.81 (m, 4H), 7.75 (m, 2H), 8.08 (m, 3H), 9.52 (br s, 1H). Anal. ($C_{24}H_{29}N_5O_2$) C, H, N.

***N*-[4-[4-(2-Methoxyphenyl)piperazin-1-yl]butyl]isoquinoline-3-carboxamide (5c).** Starting from **13f** (60.0 mg, 0.20 mmol), the title compound was prepared following the above-described procedure and was obtained as colorless prisms (97% yield): mp (methanol) 132−133 °C; ^{1}H NMR ($CDCl_3$) δ 1.69 (m, 4H), 2.48 (t, 2H, J = 6.7 Hz), 2.67 (m, 4H), 3.11 (m, 4H), 3.57 (q, 2H, J = 12.3, 6.2 Hz), 3.84 (s, 3H), 6.96 (m, 4H), 7.72 (m, 2H), 8.00 (m, 2H), 8.34 (br s, 1H), 8.60 (s, 1H), 9.14 (s, 1H); ^{13}C NMR (CD_3OD) δ 22.3, 27.2, 40.3, 50.4, 51.5, 56.7, 57.6, 114.3, 122.0, 122.6, 125.2, 129.5, 130.1, 130.3, 132.2, 133.7, 135.5, 138.7, 139.6, 149.7, 150.2, 153.6, 161.4. Anal. ($C_{25}H_{30}N_4O_2$) C, H, N.

***N*-[4-[4-(2-Methoxyphenyl)piperazin-1-yl]butyl]pyrrolo-[1,2-*a*]quinoxaline-4-carboxamide (5d).** Starting from **13c** (38.0 mg, 0.11 mmol), the title compound was prepared following the procedure described for **5a** and was obtained as a yellow oil (95% yield); ^{1}H NMR ($CDCl_3$) δ 1.73 (m, 4H), 2.49 (t, 2H, J = 6.7 Hz), 2.68 (m, 4H), 3.09 (m, 4H), 3.55 (m, 2H), 3.85 (s, 3H), 6.89 (m, 4H), 7.54 (m, 2H), 7.93 (m, 4H), 8.22 (m, 1H). Anal. ($C_{27}H_{31}N_5O_2$) C, H, N.

(*S*)-(−)-*N*-[4-[4-(2-Methoxyphenyl)piperazin-1-yl]butyl]-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (5e). To a methanol/ethyl acetate solution (1:1, 2 mL) under N_2 in a thick-walled Parr test tube 10% Pd/C (62.0 mg) was added. To the resulting suspension a solution of **14a** (170.0 mg, 0.30 mmol) in methanol (0.5 mL) was added. The suspension was shaken for 3 h in a Parr hydrogenator under 40 psi of H_2. The mixture was filtered through Celite, and the Celite was washed with methanol. Then the filtrate was concentrated, and the resulting crude product was chromatographed (10% methanol in chloroform) to afford 107.0 mg of **5e** (84% yield) as colorless prisms mp (methanol) 150−155 °C (dec); ^{1}H NMR (acetone-d_6) δ 1.53 (m, 4H), 2.35 (m, 2H), 2.53 (m, 4H), 2.77 (dd, 2H, J = 16.4, 10.1 Hz), 3.04 (m, 4H), 3.26 (m, 2H), 3.44 (dd, 1H, J = 9.9, 4.9 Hz), 3.79 (s, 3H), 3.95 (s, 2H), 6.86 (m, 5H), 7.02 (br s, 1H), 7.08 (m, 4H), 7.45 (br s, 1H); ^{13}C NMR (CD_3OD) δ 22.0, 27.3, 31.0, 39.2, 45.5, 50.0, 52.0, 56.2, 56.5, 57.5, 114.0, 121.5, 122.5, 127.7, 128.6, 128.8, 129.2, 129.3, 130.1, 131.8, 135.4, 153.7, 169.8. ESI-MS m/z 867 $[2M + Na]^+$ (100), 445 $[M + Na]^+$, 423 $[M + H]^+$. Anal. ($C_{25}H_{34}N_4O_2$) C, H, N. $[\alpha]^{20}_D$ −57.14 (c 1.68, $CHCl_3$).

(*R*)-(+)-*N*-[4-[4-(2-Methoxyphenyl)piperazin-1-yl]butyl]-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (5f). Starting from **14b** (160.0 mg, 0.28 mmol), the title compound was obtained following the procedure described for **5e**. **5f** was obtained as a white solid (81% yield): mp (methanol) 150−155 °C (dec); ^{1}H NMR (acetone-d_6) δ 1.53 (m, 4H), 2.35 (m, 2H), 2.53 (m, 4H), 2.77 (dd, 2H, J = 16.4, 10.1 Hz), 3.04 (m, 4H), 3.26 (m, 2H), 3.44 (dd, 1H, J = 9.9, 4.9 Hz), 3.79 (s, 3H), 3.95 (s, 2H), 6.86 (m, 5H), 7.02 (br s, 1H), 7.08 (m, 4H), 7.45 (br s, 1H).^{13}C NMR (CD_3OD) δ 15.4, 22.0, 27.3, 30.9, 39.3, 45.6, 50.2, 52.1, 56.5, 56.5, 57.5, 66.9, 114.1, 121.5, 122.5, 127.6, 128.6, 128.9, 129.2, 129.3, 130.1, 131.9, 135.4, 153.7, 169.7. Anal. ($C_{25}H_{34}N_4O_2$) C, H, N. $[\alpha]^{20}_D$ +57.14 (c 0.39, $CHCl_3$).

***N*-[4-[4-(2-Methoxyphenyl)piperazin-1-yl]butyl]benzo-[*b*]furan-2-carboxamide (5g).** Starting from **13a** (0.5 g, 1.69 mmol) the title compound was prepared following the procedure described for **5a** and was obtained as colorless prisms (60% yield): mp (methanol) 120−121 °C; ^{1}H NMR ($CDCl_3$) δ

1.67 (m, 4H), 2.48 (m, 2H), 2.65 (m, 4H), 3.12 (m, 4H), 3.56 (m, 2H), 3.85 (s, 3H), 6.90 (m, 4H), 7.36 (m, 4H), 7.63 (d, 1H, J = 7.7 Hz). Anal. ($C_{24}H_{29}N_3O_3$) C, H, N.

***N*-[4-[4-(2-Methoxyphenyl)piperazin-1-yl]butyl]indole-2-carboxamide (5h).** Starting from **13d** (35.0 mg, 0.12 mmol) the title compound was prepared following the procedure described for **5a** and was obtained as a yellow oil (48% yield); ^{1}H NMR ($CDCl_3$) δ 1.74 (m, 4H), 2.52 (m, 2H), 2.68 (m, 4H), 3.09 (m, 4H), 3.62 (m, 2H), 3.90 (s, 3H), 6.97 (m, 4H), 7.20 (m, 3H), 7.40 (d, 1H, J = 8.0 Hz), 7.56 (d, 1H, J = 8.0 Hz), 10.43 (br s, 1H). Anal. ($C_{24}H_{30}N_4O_2$) C, H, N.

(Benzo[*b*]furan-2-yl)-[4-[[4-(2-methoxyphenyl)piperazinomethyl]piperidin-1-yl]methanone (5i). Starting from **21** (63.0 mg, 0.19 mmol) the title compound was prepared following the procedure described for **5a.** The product **5i** was obtained as a yellow oil (60% yield); ^{1}H NMR ($CDCl_3$) δ 1.25 (m, 2H), 1.89 (m, 3H), 2.27 (d, 2H, J = 6.4 Hz), 2.62 (m, 4H), 3.08 (m, 6H), 3.85 (s, 3H), 4.57 (m, 2H), 6.83 (m, 4H), 7.32 (m, 3H), 7.49 (d, 1H, J = 8.1 Hz), 7.61 (d, 1H, J = 7.6 Hz). Anal. ($C_{26}H_{31}N_3O_3$) C, H, N.

***N*-[[2-(Benzo[*b*]furan-2-yl)-1,3-oxazol-4-yl]methyl]-2-[4-(2-methoxyphenyl)piperazin-1-yl]-1-ethanamine (5j).** The amine **10** (82.0 mg, 0.35 mmol), Na_2CO_3 (37.0 mg. 0.35 mmol) and KI (57.7 mg, 0.35 mmol) were suspended in 1-butanol (2 mL). To the suspension under vigorous stirring under argon, a solution of **23a** (57.0 mg, 0.23 mmol) in 1-butanol (1 mL) was added and the resulting mixture was refluxed for 20 h. Then the mixture was filtered, the filtrate was evaporated and the residue was purified by chromatography (ethyl acetate) to afford **5j** (18% yield) as a yellow oil; ^{1}H NMR ($CDCl_3$) δ 2.22 (m, 2H), 2.63 (m, 2H), 2.70 (m, 4H), 3.15 (m, 4H), 3.70 (s, 2H), 3.85 (s, 3H), 6.92 (m, 4H), 7.33 (m, 3H), 7.65 (m, 3H). Anal. ($C_{25}H_{28}N_4O_3$) C, H, N.

***N*-[[2-(2-Naphthyl)-1,3-oxazol-4-yl]methyl]-2-[4-(2-methoxyphenyl)piperazin-1-yl]-1-ethanamine (5k).** Following a procedure as described for **5j**, the title compound was obtained as a yellow oil (16% yield); ^{1}H NMR ($CDCl_3$) δ 2.21 (m, 2H), 2.64 (m, 2H), 2.81 (m, 4H), 3.15 (m, 4H), 3.66 (s, 2H), 3.85 (s, 3H), 6.90 (m, 4H), 7.57 (m, 2H), 7.67 (s, 1H), 7.88 (m, 3H), 8.15 (d, 1H, J = 8.0 Hz), 8.56 (s, 1H). Anal. ($C_{27}H_{30}N_4O_2$) C, H, N.

***N*-[4-[4-(4-Cyanophenyl)piperazin-1-yl]butyl]benzo[*b*]-furan-2-carboxamide (5l).** To a solution of **13a** (120.0 mg, 0.40 mmol) in dry acetonitrile 6 mL under argon, **7b** (76.0 mg, 0.40 mmol) and triethylamine (92 μL, 0.65 mmol) were added and the solution was refluxed overnight with stirring. The solvent was removed under reduced pressure, water was added and the mixture was extracted with dichloromethane. The organic layers were dried and concentrated and the crude product was chromatographed (10% methanol in chloroform) to give 130.0 mg of **5l** (65% yield) as colorless prisms: mp (methanol) 146−148 °C; ^{1}H NMR ($CDCl_3$) δ 1.64 (m, 4H), 2.44 (t, 2H, J = 6.6 Hz), 2.57 (m, 4H), 3.32 (m, 4H), 3.51 (q, 2H, J = 12.3, 6.2 Hz), 6.82 (m, 2H), 6.90 (br s, 1H), 7.34 (m, 6H), 7.65 (d, 1H, J = 7.6 Hz); ^{13}C NMR (CD_3OD) δ 22.4, 27.5, 39.3, 45.9, 52.7, 57.7, 103.5, 111.4, 111.5, 112.9, 113.0, 116.6, 120.5, 123.9, 125.1, 128.4, 128.9, 134.8, 153.8. ESI-MS m/z 425 $[M + Na]^+$, 403 $[M + H]^+$; ESI-MS/MS of $[M + H]^+$ 242, 216 (100), 173. Anal. ($C_{24}H_{26}N_4O_2$) C, H, N.

***N*-[4-[4-(3,4-Dichlorophenyl)piperazin-1-yl]butyl]benzo[*b*]furan-2-carboxamide (5m).** To a solution of **13a** (50.0 mg, 0.17 mmol) in dry acetonitrile (3 mL) under argon, 1-(3,4-dichlorophenyl)piperazine (39.0 mg, 0.17 mmol) and triethylamine (38 μL, 0.27 mmol) were added; the solution was refluxed overnight under stirring. The workup was same as that for **5a**. Pure **5m** was obtained as colorless prisms (56% yield): mp (methanol) 121−122 °C; ^{1}H NMR ($CDCl_3$) δ 1.69 (m, 4H), 2.47 (m, 2H), 2.60 (m, 4H), 3.20 (m, 4H), 3.52 (m, 2H), 6.82 (m, 3H), 7.39 (m, 4H), 7.66 (d, 1H, J = 7.5 Hz). Anal. ($C_{23}H_{25}Cl_2N_3O_2$) C, H, N.

***N*-[4-[4-(2,4-Dichlorophenyl)piperazin-1-yl]butyl]isoquinoline-3-carboxamide (5n).** To a solution of **13f** (60.0 mg, 0.19 mmol) in dry acetonitrile (5 mL) under argon, 1-(2,4-dichlorophenyl)piperazine (45.3 mg, 0.19 mmol) and triethylamine (44 μL, 0.31 mmol) were added, and the solution was refluxed overnight under stirring. The workup was the same followed for **5a**. The product **5n** was obtained as colorless prisms (72% yield): mp (methanol) 127−128 °C; ^{1}H NMR ($CDCl_3$) δ 1.71 (m, 4H), 2.50 (m, 2H), 2.64 (m, 4H), 3.05 (m, 4H), 3.55 (m, 2H), 6.94 (d, 1H, J = 8.8 Hz), 7.16 (dd, 1H, J = 8.5, 2.3 Hz), 7.34 (d, 1H, J = 2.1 Hz), 7.73 (m, 2H), 8.00 (m, 2H), 8.33 (br s, 1H), 8.60 (s, 1H), 9.13 (s, 1H). Anal. ($C_{24}H_{26}Cl_2N_4O$) C, H, N.

***N*-[4-[4-(2,4-Dichlorophenyl)piperazin-1-yl]butyl]benzo[*b*]furan-2-carboxamide (5o).** Starting from **13a** (300.0 mg, 1.01 mmol) the title compound was prepared following the above-described procedure. Compound **5o** was obtained as colorless prisms (68% yield): mp (methanol) 112−113 °C; ^{1}H NMR ($CDCl_3$) δ 1.59 (m, 4H), 2.44 (t, 2H, J = 6.6 Hz), 2.61 (m, 4H), 3.02 (m, 4H), 3.50 (q, 2H, J = 12.2, 6.2 Hz), 6.88 (d, 1H, J = 8.7 Hz), 7.08 (m, 2H), 7.32 (m, 4H), 7.62 (d, 1H, J = 7.5 Hz); ^{13}C NMR ($CDCl_3$) δ 24.3, 27.5, 39.2, 51.1, 53.2, 57.8, 76.6, 77.8, 110.1, 111.6, 121.0, 122.6, 123.6, 126.7, 127.5, 127.6, 128.0, 129.3, 130.2, 148.0, 149.0, 154.6, 158.8. Anal. ($C_{23}H_{25}Cl_2N_3O_2$) C, H, N.

***N*-[4-[4-(2,4-Dichlorophenyl)piperazin-1-yl]butyl]indole-2-carboxamide (5p).** Starting from **13d** (141.0 mg, 0.48 mmol), the title compound was prepared following the above-described procedure as a yellow oil (65% yield); ^{1}H NMR ($CDCl_3$) δ 1.68 (m, 4H), 2.49 (t, 2H, J = 6.8 Hz), 2.63 (m, 4H), 3.04 (m, 4H), 3.55 (m, 2H), 6.67 (m, 1H), 6.86 (m, 2H), 6.90 (s, 1H), 7.16 (m, 2H), 7.27 (m, 1H), 7.33 (m, 1H), 7.45 (d, 1H, J = 8.2 Hz), 7.64 (d, 1H, J = 8.0 Hz), 9.97 (br s, 1H). Anal. ($C_{23}H_{26}Cl_2N_4O$) C, H, N.

***N*-[4-[4-(2,4-Dichlorophenyl)piperazin-1-yl]butyl]-5-chloroindole-2-carboxamide (5q).** Starting from **13i** (27.0 mg, 0.11 mmol), the title compound was prepared following the above-described procedure as a yellow oil (73% yield); ^{1}H NMR ($CDCl_3$) δ 1.69 (m, 4H), 2.47 (t, 2H, J = 6.5 Hz), 2.63 (m, 4H), 3.03 (m, 4H), 3.53 (m, 2H) 6.56 (br s, 1H), 6.75 (d, 1H, J = 1.4 Hz) 6.90 (d, 1H, J = 8.3 Hz), 7.19 (m, 2H), 7.37 (d, 1H, J = 8.2 Hz), 7.59 (d, 1H, J = 1.3 Hz), 9.66 (br s, 1H). Anal. ($C_{23}H_{25}Cl_3N_4O$) C, H, N.

***N*-[4-[4-(2-Methoxyphenyl)piperazin-1-yl]butyl]-1,2,3,4-tetrahydropyrazino[1,2-*a*]indole-1(2*H*)-one (5r).** To a solution of **18** (80.0 mg, 0.25 mmol) in 6 mL dry acetonitrile under argon, 1-(2-methoxyphenyl)piperazine (48.0 mg, 0.25 mmol) and triethylamine (56 μL, 0.40 mmol) were added; the solution was refluxed overnight under stirring. The solvent was removed under reduced pressure, water was added and the mixture was extracted with dichloromethane. The organic layers were dried and concentrated and the crude product was chromatographed (10% methanol in chloroform) to give 100.0 mg of **5r** (93% yield) as yellow oil; ^{1}H NMR (acetone-d_6) δ 1.68 (m, 4H), 2.49 (m, 2H), 2.63 (m, 4H), 3.10 (m, 4H), 3.66 (m, 2H), 3.79 (m, 2H), 3.85 (s, 3H), 4.27 (m, 2H), 6.91 (m, 4H), 7.14 (m, 1H), 7.31 (m, 3H), 7.70 (d, 1H, J = 8.1 Hz); ^{13}C NMR (CD_3OD) δ 22.2, 25.6, 41.2, 46.5, 47.4, 50.4, 51.3, 56.8, 57.6, 111.1, 114.3, 121.8, 122.1, 122.6, 123.3, 125.8, 127.4, 128.6, 129.8, 130.7, 133.1, 138.1, 153.6, 162.3. Anal. ($C_{26}H_{32}N_4O_2$) C, H, N.

Biological Studies

1. Animals. Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national (D. L. n. 116, G. U., suppl. 40, 18 Febbraio 1992, Circolare No. 8, G. U., 14 Luglio 1994) and international laws and policies (EEC Council Directive 86/609, OJL 358, 1, Dec. 12, 1987; Guide for the Care and Use of Laboratory Animals, U.S. National Research Council, 1996).

Male Sprague−Dawley CD-COBS rats (Charles River, Italy) were used, weighing 150 g (for binding assays) or 250−275 g (for in vivo studies) at the beginning of the experiments. They were housed individually at constant room temperature (21 ± 1 °C) and relative humidity (60%) under an inverted light/dark schedule (light 8:00 p.m. − 8:00 a.m.) with food and water ad libitum. Animals were allowed to adapt to laboratory

conditions for at least two weeks and were handled for 5 min per day during this period.

2. Binding Assays. Rats were killed by decapitation and their brains were quickly removed. The various brain regions were dissected (striatum for D_1 and D_2, cortex for α_1- and α_2-adrenoceptors, hippocampus for 5-HT_{1A}) and stored at −80 °C until use.

Tissues were homogenized in about 50 vol of ice-cold Tris HCl 50 mM, pH 7.4 using an Ultra-Turrax TP-1810 (2 × 20 s) and centrifuged (Beckman Avanti J-25) at 50000*g* for 10 min at 4 °C. The pellets were resuspended in the same volume of fresh buffer, incubated at 37 °C for 10 min, and centrifuged again as before. The pellets were then washed once by resuspension in fresh buffer, centrifuged again at 50000*g* for 10 min, resuspended just before the binding assay in the appropriate incubation buffer (for D_1 and D_2 receptors: Tris HCl, 50 mM, pH 7.4 containing 10 μM pargyline, 0.1% ascorbic acid, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$. For 5-HT_{1A} receptors: Tris HCl, 50 mM, pH 7.7 containing 10 μM pargyline, 4 mM $CaCl_2$ for [^{3}H]-8-OH-DPAT binding or Tris HCl, 50 mM, pH 7.4 containing 4 mM $MgCl_2$, 160 mM NaCl, 0.3 mM EGTA, 300 μM GDP for [^{35}S]-GTPγS binding. For α_1-adrenoceptors: Tris HCl, 50 mM, pH 7.4 containing 10 μM pargyline, 0.1% ascorbic acid. For α_2-adrenoceptors: Tris HCl, 50 mM, pH 7.5.

[^{3}H]-SCH 23390 (sa 75.5 Ci/mmol, NEN) binding[38] to D_1 receptors was assayed in a final incubation volume of 0.5 mL, consisting of 0.25 mL of membrane suspension (2 mg of tissue/sample), 0.25 mL of [^{3}H]-ligand (0.4 nM) and 10 μL of displacing agent or solvent. Nonspecific binding was obtained in the presence of 10 μM (−)-*cis*-flupentixol; samples were incubated for 15 min at 37 °C. [^{3}H]-Spiperone (sa 16.5 Ci/mmol, NEN) binding[38] to D_2 receptors was assayed in a final incubation volume of 1 mL, consisting of 0.5 mL of membrane suspension (1 mg tissue/sample) 0.5 mL of [^{3}H]-ligand (0.2 nM) and 20 μL of displacing agent or solvent. Nonspecific binding was obtained with of 100 μM (−)-sulpiride; samples were incubated for 15 min at 37 °C. [^{3}H]-7-OH-DPAT (sa 139 Ci/mmol, Amersham) binding[39] to D_3 receptors was assayed in a final incubation volume of 1 mL, consisting of 0.5 mL of membrane suspension (about 12 μg prot./sample of rat cloned dopamine receptor subtype 3 in Sf9 cells (Signal Screen), resuspended in Tris HCl, 50 mM, pH 7.4 containing 5 mM EDTA, 5 mM $MgCl_2$, 5 mM KCl, 1.5 mM $CaCl_2$, 120 mM NaCl, 0.5 mL of [^{3}H]-ligand (0.7 nM) and 20 μL of displacing agent or solvent. Nonspecific binding was obtained in the presence of 1 μM dopamine; samples were incubated for 60 min at 25 °C. [^{3}H]-Prazosin (sa 83.8 Ci/mmol, NEN) binding[40] to α_1-adrenoceptors was assayed in a final incubation volume of 1 mL, consisting of 0.5 mL of membrane suspension (10 mg of tissue/sample) 0.5 mL of [^{3}H]-ligand (0.2 nM) and 20 μL of displacing agent or solvent. Nonspecific binding was obtained with 1 μM prazosin; samples were incubated for 30 min at 25 °C. [^{3}H]-RX 821002 (sa 49 Ci/mmol, Amersham) binding[41] to α_2-adrenoceptors was assayed in a final incubation volume of 1 mL, consisting of 0.5 mL of membrane suspension (16 mg of tissue/sample) 0.5 of [^{3}H]-ligand (1 nM) and 20 μL of displacing agent or solvent. Nonspecific binding was obtained with 10 μM clonidine; samples were incubated for 30 min at 25 °C. [^{3}H]-8-OH-DPAT (sa 120 Ci/mmol, NEN) binding[40] to 5-HT_{1A} receptors was assayed in a final incubation volume of 0.5 mL, consisting of 0.25 mL of membrane suspension (10 mg tissue/sample), 0.25 mL of [^{3}H]-ligand (1 nM) and 10 μL of displacing agent or solvent. Nonspecific binding was obtained in the presence of 1 μM serotonin; samples were incubated for 30 min at 25 °C.

[^{35}S]-GTPγS (sa 1064 Ci/mmol, Amersham) binding to 5-HT_{1A} serotonin receptors[42] was assayed in a final incubation volume of 1.04 mL, consisting of 1 mL of membrane suspension from rat hippocampus (200 μg of protein/sample), 20 μL of [^{35}S]-GTPγS (final concentration 0.1 nM), and 20 μL of displacing agent or solvent. Nonspecific binding was obtained in the presence of 10 μM GTPγS, samples were preincubated for 20 min at 37 °C without [^{35}S]-GTPγS and then for 45 min at 37 °C with [^{35}S]-GTPγS.

For evaluation of [^{35}S]-GTPγS binding to D_{3r} receptors[43] the Sf9 cells were resuspended in Hepes 25 mM, pH 8, containing 100 mM NaCl, 1 mM EDTA, 3 mM $MgCl_2$, 0.5 mM DTT, 0.003% digitonine, 10 μM GDP, with a glass/Teflon potter. The binding assay was carried out in a final incubation volume of 120 μL, consisting of 100 μL of membrane suspension (about 15 μg of protein/sample), 10 μL of [^{35}S]-GTPγS (final concentration 1 nM) and 10 μL of displacing agent or solvent. Nonspecific binding was obtained in the presence of 10 μM GTPγS; samples were preincubated for 15 min at 30 °C without [^{35}S]-GTPγS and then for 30 min at 30 °C with [^{35}S]-GTPγS.

After incubation, samples were filtered through Whatman GF/B glass fiber filters using a Brandel apparatus (mod. M-48R) and washed three times with 4 mL of ice-cold buffer. The radioactivity trapped on the filters was counted in 4 mL of "Ultima Gold MV" (Packard) in an DSA 1409 (Wallac) liquid scintillation counter, with a counting efficiency of 50% for [^{3}H] or 90% for [^{35}S]. For inhibition experiments, the drugs were added to the binding mixture at 7−9 different concentrations. Inhibition curves were analyzed using the "Allfit" program[44] running on an IBM AT-PC. The K_i values were derived from the IC_{50} values using the Cheng and Prusoff equation.[45]

3. Behavioral Studies. The apparatus, surgical procedure, behavioral training, and testing were identical to those previously described in detail.[17] Animals were trained and tested using standard rodent operant test chambers (MED Associates Inc., St. Albans, VT) containing two stainless steel levers. In half the chambers the right-hand lever was designated as the active lever, and in the others, the left-hand one.

All training and testing was conducted during the dark phase of the light/dark cycle at approximately the same time each day. To facilitate acquisition of cocaine self-administration, before surgery the rats were trained to press a lever for food pellets on a fixed ratio 1 (FR-1) schedule. As soon as this behavior was mastered, rats returned to an unrestricted diet and a chronic jugular catheter was implanted.

Catheters were made in-house using guide cannulae (C313G 5UP, Plastic One), silicon tubing (0.30 × 0.60 and 0.64 × 1.19 mm, i.d. × o.d. respectively, Degania Silicone Ltd., Israel), dental cement (Paladur, Heraus Kulzer GmbH, Wehrheim/Ts., Germany) and silicon rubber (Elastosil E43, Wacker-Chemie GmbH, München, Germany) according to Caine and co-workers[46] with few modifications. They were implanted in rats deeply anaesthetised with equithesin [3.0 mL/kg intraperitoneally (i.p.)] with the proximal end reaching the heart through the right jugular vein and continuing subcutaneously (s.c.) over the right shoulder, exiting dorsally between the scapulae. Catheters were kept patent by daily intravenous (i.v.) infusions of 0.1 mL heparinised (30 units/mL) sterile 0.9% saline before and after each self-administration session.

4. Cocaine Self-Administration and Conditioning. Rats were trained in the operant cages to self-administer cocaine i.v. (MacFarlan-Smith, Edinburg, UK), 0.25 mg/0.1 mL/infusion, under a FR-1 for 2 h per day. As soon as they developed stable levels of daily cocaine intake, rats were subjected to a discriminative learning regimen that comprised three daily 1-h sessions, separated by 1 h home cage rest, when either cocaine or saline was available as the only infusion solution, in an unpredictable sequence. Each training day included one saline and two cocaine sessions presented in random order.

Sessions were started by extension of the levers and concurrent presentation of the respective discriminative stimuli that lasted throughout the session. The discriminative stimulus associated with cocaine (S^{D+}) consisted of a white noise (20 dB above background), and the S^{D-}, i.e., the stimulus predictive of vehicle solution, consisted of illumination of the house light. To prevent accidental overdosing, after each infusion of cocaine the lever remained inactive for 20-s time-out (TO), signaled by the cue light above the active lever coming on. The TO after each infusion of saline was instead signaled by an intermittent tone (7 kHz, 70 dB). The S^Ds were

not turned off during the TO periods. Responses on the inactive lever (whenever it was presented) were recorded but had no programmed consequences.

This training was conducted daily until cocaine-reinforced responding stabilized (±10% over three consecutive training days) and the rats almost stopped responding during saline sessions (≤5 responses during each of three successive sessions).

5. Extinction. Beginning 1 day after meeting the self-administration training criterion each rat was placed on extinction conditions until the end of the experiment. Sessions began by extension of both the active and inactive levers without either the cocaine- or the saline-associated stimuli. Responses on the previously active lever resulted in activation of the syringe pump motor but had no other programmed consequences; responses on the inactive lever were also recorded but had no programmed consequences. One daily 1 h extinction session was conducted, until a criterion of ≤5 responses per session over three consecutive days was met.

6. Reinstatement. Reinstatement tests began 1 day after individual animals met the extinction criterion. Tests lasted 1 h and involved exposing the rats to the cocaine- or saline-associated stimuli under conditions identical to the self-administration phase, except that cocaine or saline were not available. After the initial reinstatement test session rats were further placed on extinction conditions until retested. Test sessions for each animal were separated by at least 3 days in which rates of responding under extinction conditions remained at the criterion. To control for order effects, different drug doses and the vehicles were administered in a random sequence across the reinstatement test sessions.

7. Effects of 5g, 5p, and 5q on Cue-Induced Reinstatement of Drug-Seeking Behavior. To determine whether **5g**, **5p**, and **5q** affected rats' behavior induced by presentation of cocaine-associated stimuli after extinction and in the absence of any further cocaine, three independent groups of rats were prepared for testing. In the first experiment a group of eight rats was evaluated to see whether 0.3, 1, and 3 mg/kg **5g** affected the behavior induced by cocaine-associated cues. The compound, dissolved in 2 mL of sterile saline, or vehicle, was given i.p. 30 min before testing. In two other separate groups of seven rats **5p** and **5q**, dissolved in 25% DMSO in a final volume of 2 mL of sterile saline, or vehicle, were given i.p. 30 min before testing, at doses from 0.1 to 3 mg/kg. The test sessions in the presence of the stimuli associated with cocaine or saline were arranged in a random sequence.

8. Effect of 5g on Cocaine Self-Administration. To evaluate the effect of **5g** on the reinforcing properties of cocaine two groups of six rats were initially trained to press a lever of an operant box for food reinforcement, as described above. As soon as this behavior was mastered rats underwent chronic jugular catheter implantation.

After a five-day recovery, animals were allowed to self-administer cocaine hydrochloride, dissolved in sterile saline, for 2 h daily sessions on a FR1 schedule with 20 s TO. These sessions began by extension of both the active and inactive levers and terminated with their retraction at the end of the sessions. An active lever press resulted in a 6 s infusion of cocaine HCl (0.25 mg/0.1 mL) and illumination of the stimulus light above the active lever, which remained on for 20 s, signaling the TO period during which pressing a lever would not result in cocaine infusion. Inactive lever pressings were recorded but had no programmed consequences. Rats received a 2 h self-administration session 7 days a week. Once the animals had established a stable pattern of cocaine intake (the total number of drug infusions per session stabilized to within 10% for three consecutive days), they were randomly assigned to two experimental groups to self-administer two doses of cocaine (0.125 and 0.5 mg/0.1 mL/infusion). After 3 days of stable baseline, the effects of 30 min pretreatment with 0.3 and 3 mg/kg **5g** or vehicle on cocaine self-administration was assessed. The different dosages of **5g** and vehicle were given to rats in a counterbalanced order. The test sessions for each animal were separated by at least 3 days of stable baseline.

9. Effects of Replacing Cocaine with 5g or Vehicle on Cocaine i.v. Self-Administration. To study whether **5g** has intrinsic rewarding properties sufficient to maintain drug-taking behavior we evaluated in animals with a daily history of cocaine i.v. self-administration whether **5g** (given i.v. at the dose of 3 mg/mL) could substitute for i.v. cocaine. In this experiment rats first acquired i.v. 0.25 mg/0.1 mL cocaine self-administration under continuous reinforcement. When baseline rates of cocaine self-administration were stable (three consecutive days with the same number of responses ± 10%), **5g** 3 mg/mL or saline were substituted for cocaine and self-administration was allowed to continue for a further 7 days, after which, cocaine at the training dose was substituted for **5g** or saline.

10. Statistical Analysis. Data are expressed as mean ± SEM of the active and inactive lever-pressings during the self-administration, extinction, and reinstatement phases. First of all in each experiment the number of cocaine infusions earned in the two separate 1 h sessions were analyzed by mixed factorial ANOVA or one-way ANOVA for repeated measurements. Since no differences were observed in the first and second hour of cocaine self-administration, these data were pooled for statistical analysis. The number of lever-pressings during the last 3 days of extinction, before any or between the different reinstatement test sessions also did not differ because they had to meet the extinction criterion, so they too were pooled for statistical analysis.

Differences between the mean numbers of the last three cocaine and saline self-administration sessions, the preceding three extinction sessions, and the reinstatement sessions were analyzed by mixed factorial ANOVA or one-way ANOVA for repeated measurements. When a significant effect was found, post-hoc comparison was done by the Newman-Keuls test.

To evaluate the effects of **5g** on cocaine self-administered under fixed ratio schedule the number of reinforcers earned during the 2 h session was analyzed by one way ANOVA for repeated measurements followed by the Newman-Keuls post-hoc comparison. The ability of **5g** and vehicle to substitute for cocaine in the i.v. self-administration procedure was analyzed by mixed one-way ANOVA for repeated measurements.

11. Molecular Modeling. Molecular modeling was run on a Silicon Graphics Indigo2 R10000 workstation. Structures of compounds **5a**, **5c**, **5h**, and **5r** were built using the Builder module in Insight2000 (Accelrys, San Diego). Atomic potentials and charges were assigned using the cff91 force field.[47] All the compounds were considered protonated on the alkyl-piperazine nitrogen. The piperazine ring of **5a**, **5c**, and **5h** was assumed in a chair conformation, since, as already reported,[48] it is present in all arylpiperazines found in the Cambridge Crystallographic Structural Database, suggesting it is energetically preferred, as compared to the boat conformation. On the other hand, the butyl chain was assumed in an extended conformation to minimize intramolecular interactions which would affect the evaluation of the energy contribution of the carboxamide moiety.

The starting conformations were geometrically optimized (Discover module, Accelrys, San Diego) using the cff91 force field in a vacuum and in an aqueous environment ($\epsilon = 1$ and $\epsilon = 80$*r, respectively). Energy minimizations were done with a conjugate gradient[49] as minimization algorithm until the maximum RMS derivative was less than 0.001 kcal/mol. This protocol was applied to all the molecular mechanic (MM) calculations.

12. Analysis of Orientation of the Heteroarylcarboxamide Group of Compounds 5a, 5c, and 5h. For each compound, conformations were generated by rotating the torsion angle τ (as defined in Results and Discussion) by 30° within a 0−359° range, while the amide bond was rotated 180° within the same range. All the conformations were subjected to the MM energy minimization protocols, leading to four conformers for each molecule, both in a vacuum and in an aqueous environment. MM conformers of **5a**, **5c**, and **5h** were subjected to a full geometry optimization using the quantum mechanical method AM1 in the Mopac 6.0 package[50] in Ampac/

Mopac module of Insight2000. The AM1 method was preferred to the PM3 method because of its lower degree of pyramidalization of the conjugated nitrogens (see ref 50). The MMOK keyword was used to constrain the amide nitrogen to be planar. The NOTHIEL option was used to disable the Thiel's FSTMIN technique in the BFGS routine, so gradient minimization could be done. GNORM value was set to 0.5. To reach full geometry optimization we increased the criteria for terminating all optimizations by a factor of 100, using the keyword PRECISE.

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