Design, Synthesis, and Binding Affinities of Potential Positron Emission Tomography (PET) Ligands for Visualization of Brain Dopamine D_3 Receptors

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We here report the synthesis of compounds structurally related to the high-affinity dopamine D_3 receptor ligand *N*-[4-[4-(2,3-dichlorophenyl)piperazin-1-yl]butyl]-7-methoxy-2-benzofurancarboxamide (**1**). All compounds were specifically designed as potential PET radioligands for brain D_3 receptors visualization, having lipophilicity within a range for high brain uptake and weak nonspecific binding ($2 < \text{ClogP} < 3.5$) and bearing a methoxy substituent for easy access to labeling with the positron emitter isotope ^{11}C. *N*-[4-[4-(5-methoxy-2-benzisoxazolyl)piperazin-1-yl]butyl]-4-(4-morpholinyl)benzamide (**22**), *N*-[4-[4-(5-methoxy-2-benzisoxazolyl)piperazin-1-yl]butyl]-4-(1*H*-imidazol-1-yl)benzamide (**23**), and *N*-[4-[4-(5-methoxy-2-benzisoxazolyl)piperazin-1-yl]butyl]-5-(2-furanyl)-1*H*-pyrazole-3-carboxamide (**24**) displayed good D_3 receptor affinities (K_i values 38.0, 22.6, and 21.3 nM, respectively) and were selective over D_2 receptor. Moreover, compounds **22**−**24** were able to permeate the Caco-2 cell monolayer, differently from compound **1**. Although the goal to identify potential PET radioligands with subnanomolar affinities for D_3 receptor was not achieved, the proposed strategy could be a starting point for future developments.

The five known mammalian dopamine receptor subtypes (D_1−D_5), which, on the basis of protein homology and function, can be divided into two receptor families, D_1-like (D_1 and D_5) and D_2-like (D_2, D_3, and D_4), are all G-protein coupled receptors. The D_3 receptor subtype was first cloned and characterized by Sokoloff et al. and reported in 1990.[1] The greatest densities of D_3 mRNA in rat brain are found primarily in limbic brain areas (islands of Calleja, ventral striatum/nucleus accumbens, dentate gyrus, and striate cortex). Expression of D_3 receptor mRNA in the human brain follows a similar pattern as in the rodent brain. The distribution pattern of D_3 receptors in both rodent and human brain is compatible with a major role in emotion, cognition, and processing of motor and sensory information.[2] Recently, the availability of selective D_3 agents has allowed new insight into the involvement of dopamine D_3 in mechanisms of drug dependence and abuse. In fact, the D_3 selective antagonist SB-277011 (Chart 1) can reduce cocaine-, nicotine-, ethanol-, and heroin-seeking behaviors. Moreover, other selective D_3 receptor antagonists such as SB-414796 (Chart 1) and NGB-2904 (Chart 2) possess similar in vivo properties as SB-277011, and this further supports the hypothesis that central D_3 receptors play an important role in the rewarding and incentive motivating effects of cocaine. Moreover, a series of in vivo studies assessed the efficacy of the D_3 partial agonist BP 897 (Chart 1) in animal models of drug addition.[3] Furthermore, it has been evidenced that D_3 receptor participated in both dyskinesia and the therapeutic action levodopa, thus the therapeutic use of D_3 agents for Parkinson's disease (PD) has been proposed.[4] Moreover, pramipexole, a D_2/D_3 agent, is effective in early stages of PD and an effective adjunct therapy to levodopa in treating late PD.[5]

Currently, positron emission tomography (PET) is one of the most advanced techniques available for noninvasive, in vivo studies of cerebral receptors for the study of normal and pathological brain function and diseases and for drug development research. PET imaging requires an appropriate radioligand labeled with a positron-emitting isotope.

To develop a tracer for a receptor in the central nervous system a number of demands have to be met.[6] First of all, the tracer needs to have high affinity for the target receptor. In particular, it is preferable that the B_{max} clearly exceeds the K_d of the ligands (ideally $B_{max}/K_d > 10$). Furthermore, the selectivity over other receptors needs to be good (about 100-fold). Although the affinity is an important factor to achieve a high signal-to-noise ratio, also the lipophilicity of the tracer is relevant. The lipophilicity should not be too high in order to avoid nonspecific binding. This is an essentially nonsaturable component of the total tissue uptake of a radioligand, usually attributed to adhesion to protein and lipids. On the other hand, it is well understood that an optimal lipophilicity (log *P* near 2) of drugs is required for good blood−brain barrier permeability.[7] Therefore, it appears that there is an optimal range of lipophilicity for brain radioligands, wherein brain uptake is high and nonspecific binding comparatively weak. Frequently, the selection of a candidate PET radioligand has been done on the basis of the affinity value and selectivity, leading to poor results when the lipophilicity of the candidate was high. From literature data a value of log $P = 3.5$ appears to be the acceptable upper limit of lipophilicity for a PET radioligand.[8] Another emerging concept for the selection of a brain penetrant drug or radioligand is affinity for P-glycoprotein that is responsible for transportation of drugs out of the brain.[9] Finally, an important consideration is that the radiolabeling of a potent ligand may lead to a new chemical entity with a different pharmacological profile as compared to that of the original compound. Therefore, it would be preferable to optimize compounds with structural features that allow labeling leading to a radioligand that is undistinguishable, from a physiological point of view, from its unlabeled counterpart.

As applied to imaging of dopamine D_3 receptors into the brain, PET is still in the early stages of development because of a lack of suitable radioligands. A limited number of

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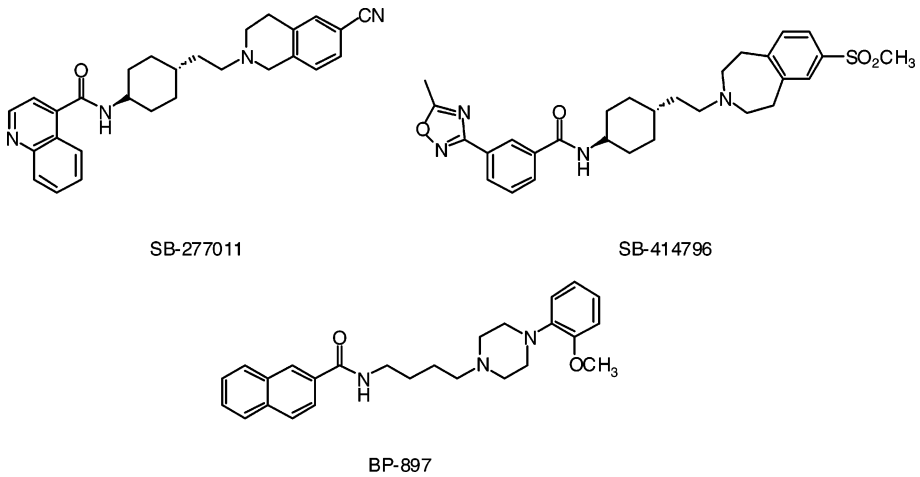
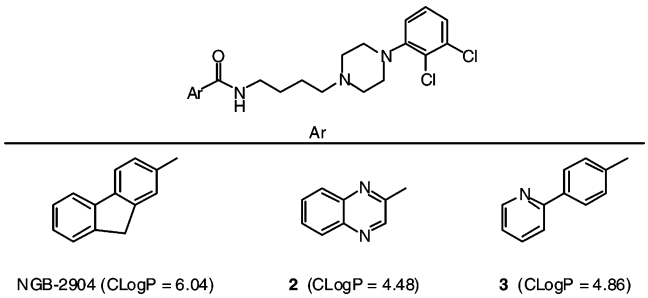
Chart 1



SB-277011 SB-414796

BP-897

Chart 2

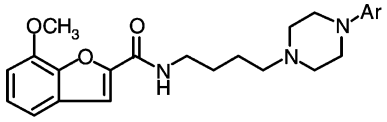


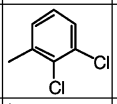
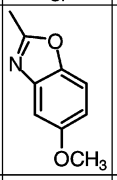
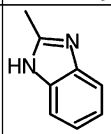
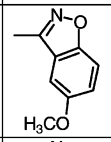
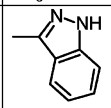
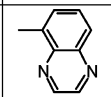
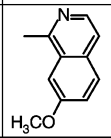
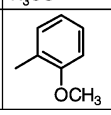
NGB-2904 (CLogP = 6.04) **2** (CLogP = 4.48) **3** (CLogP = 4.86)

radioligands have been prepared, and validation studies are in progress.[10−12] The aim of the present study is to identify a D_3 receptor ligand as a potential PET radioligand, taking into account all the above-mentioned requirements in an early stage of development. Therefore, we have designed compounds displaying (i) the structural features that are necessary for binding at D_3 receptors; (ii) a ClogP[13] (calculated log *P*) value between 2 and 3.5; (iii) a methoxy group in the structure that can give an easy access to labeling with the positron emitter isotope ^{11}C.

Previously, we have published a structure−affinity relationship study on *N*-[4-(4-arylpiperazin-1-yl)butyl]arylcarboxamides as potent and selective dopamine D_3 receptor ligands.[14] The highest D_3 receptor affinity values were obtained when the aryl substituent linked to the N-1 of the piperazine ring was the 2,3-dichlorophenyl and the arylcarboxamide moiety originated from an aromatic bicyclic carboxylic acid. Among the studied compounds, derivative **1** (Table 1) showed some features of a potential PET radioligand, i.e., high D_3 receptor affinity and high selectivity over D_2 receptor. In a subsequent study,[15] derivative [^{11}C]**1** was prepared, but it did not display an in vivo behavior suitable to image D_3 receptor expression using PET. The observed nonspecific accumulation of [^{11}C]**1** could be related to its high lipophilicity (ClogP = 4.98). In a recent paper Newman et al.[16] have studied some *N*-{4-[4-(2,3-dichlorophenyl)piperazin-1-yl]butyl}arylcarboxamides specifically designed to obtain compounds with reduced lipophilicity as compared to the prototypical antagonist NGB-2904 (ClogP = 6.04). In particular, among the reported compounds the 2-quinoxalinocarboxamide **2** and the 4-(2-pyridyl)benzamide **3** showed the lowest ClogP values (4.48 and 4.86, respectively). Although compounds **2** and **3** displayed lower lipophilicity than NGB-2904, their lipophilicity is still high. Therefore, on the basis of these findings, to obtain compounds endowed with lower lipophilicity, we replaced the 2,3-dichlorophenyl ring in compound **1** with a heteroaromatic bicyclic ring (derivatives **11**−**16**, Table 1). We have also prepared compound **17** (Table 1) because it is

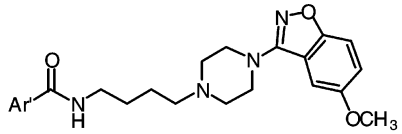
Table 1. ClogP Values and Binding Affinities of Compounds **1**, **11**−**17**

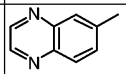
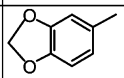
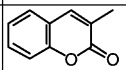
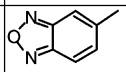
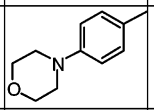
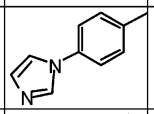
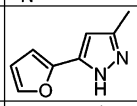
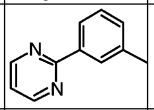
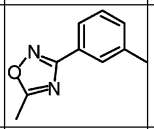
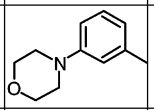
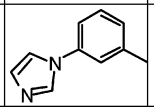


			$K_i \pm$ S.E.M., nM	
Compd	**Ar**	**CLogP**	**D_3**	**D_2**
1[a]		4.98	0.13	373
11		3.39	>8500 (40%)[b]	>750 (20%)
12		3.54	>8500 (30%)	>750 (28%)
13		3.42	98 ± 7.0	>750 (32%)
14		3.46	127 ± 50	56.6 ± 9.0
15		3.14	262 ± 12	1246 ± 150
16		3.79	102 ± 20	519 ± 35
17		3.30	25.5 ± 2.1	369 ± 20

[a] See ref 14. [b] Full K_i not obtained, percentage inhibition at the concentration shown given in parentheses.

Table 2. ClogP Values and Binding Affinities of Compounds **18**−**28**



			K_i ± S.E.M., nM	
Compd	**Ar'**	**CLogP**	**D_3**	**D_2**
18		2.53	220 ± 15	>750 (23%)[a]
19		2.51	118 ± 30	>750 (33%)
20		2.78	>2700 (40%)	>750 (28%)
21		3.14	85 ± 6.2	>750 (24%)
22		2.74	38.0 ± 7.5	>750 (26%)
23		3.31	22.6 ± 3.5	>750 (39%)
24		3.18	21.4 ± 4.1	>750 (13%)
25		2.55	1140 ± 350	>1000 (27%)
26		2.59	1285 ± 125	>1000 (27%)
27		2.74	1440 ± 210	>1000 (28%)
28		3.31	5539 ± 400	>1000 (48%)

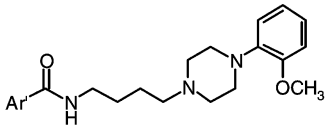
[a] Full K_i not obtained, percentage inhibition at the concentration shown given in parentheses.

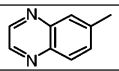
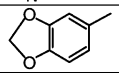
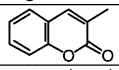
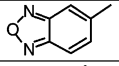
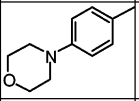
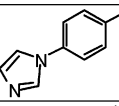
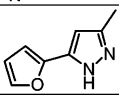
structurally related to the well-known D_3 receptor ligand BP-897 and displays a ClogP value within the target range. As shown in Table 1, this first modification fulfilled the goal of lowering lipophilicity, but it was detrimental for D_3 receptor affinity. Therefore, a second set of compounds was prepared by replacing the 7-methoxy-2-benzofurane ring of compounds **13** and **17** with a 2-naphthalene-like or a 1,4-biphenyl-like ring systems in order to increase the D_3 affinity and further lower the lipophilicity (compounds **18**−**24**, Table 2 and compounds **29**−**35**, Table 3).

Chemistry

The synthetic pathways followed to prepare the target compounds are depicted in Scheme 1. N-Substituted 7-methoxy-2-benzofurancarboxamide derivatives **11**−**17** were prepared

Table 3. ClogP Values and Binding Affinities of Compounds **29**−**35**



			K_i ± S.E.M., nM	
Compd	**Ar'**	**CLogP**	**D_3**	**D_2**
29		2.40	77.9 ± 42	179 ± 20
30		2.38	14.7 ± 3.4	86.2 ± 6.5
31		2.66	60.1 ± 4.5	146 ± 25
32		3.02	58.5 ± 9.2	>1000 (47%)[a]
33		2.62	23.9 ± 1.5	141 ± 30
34		2.55	4.79 ± 0.18	27.2 ± 7.3
35		3.06	19.3 ± 0.9	130 ± 15

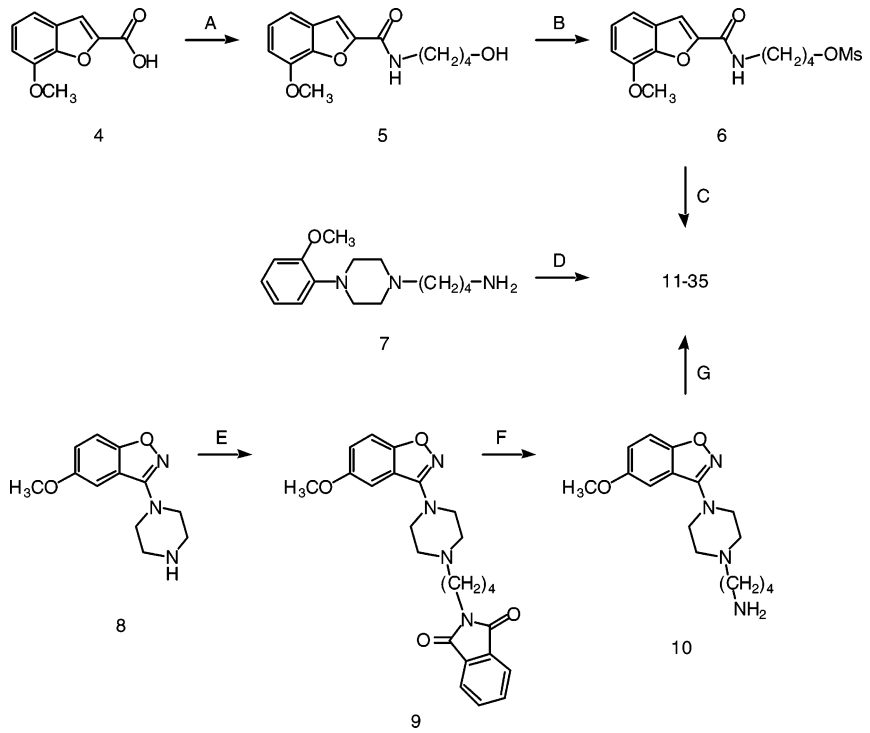
[a] Full K_i not obtained, percentage inhibition at the concentration shown given in parentheses.

starting from 7-methoxy-2-benzofurancarboxylic acid (**4**) that was condensed with 4-aminobutanol to give the corresponding amide **5**. This latter compound was transformed into its mesylate derivative **6** by means of methanesulfonyl chloride. The key intermediate **6** was reacted with the appropriate 1-arylpiperazine to give target compounds **11**−**17**. All arylpiperazines were prepared according to literature methods, as detailed in the Experimental Section. The synthesis of compounds **18**−**28** started from 5-methoxy-3-(1-piperazinyl)-1,2-benzisoxazole (**8**) that was alkylated with *N*-(4-bromobutyl)phthalimide to give the corresponding phthalimido derivative **9** which was hydrolyzed with hydrazine hydrate to afford the amine **10**. Condensation of this latter amine with the appropriate carboxylic acid in the presence of 1,1′-carbonyldiimidazole (CDI) gave the expected final compounds **18**−**28**. Similarly, the final compounds **29**−**35** were prepared from the condensation of 4-(2-methoxyphenyl)-1-piperazinebutanamine (**7**) with the appropriate carboxylic acid in the presence of CDI or methyl chloroformate. All carboxylic acids were commercially available or were prepared as described into the literature, as detailed in the Experimental Section.

Results and Discussion

The first set of compounds (Table 1) originated from **1** by replacing the 2,3-dichlorophenyl with a bycyclic ring. Previously, we have observed that this modification was tolerated for D_3 affinity binding. As shown by the affinity data reported in Table 1, this modification of **1** led to a considerable loss in D_3 receptor affinity. In fact, derivatives **13**−**16** retained only moderate D_3 affinity values, whereas compounds **11** and **12** were devoid of D_3 receptor affinity. The orientation of the bicyclic ring seems of great importance in the interaction with the D_3 receptor. In fact, **11** and **12** are devoid of D_3 receptor affinity, whereas their corresponding isomers **13** and **14** still retained D_3 receptor

Scheme 1[a]



[a] Reagents: (A) 4-Aminobutanol; (B) Methanesulfonyl Chloride, Triethylamine; (C) 1-Arylpiperazine; (D) Arylcarboxylic Acid, 1,1′-Carbonyl Diimidazole or Methyl Chloroformate; (E) *N*-(4-Clorobutyl)phthalimide; (F) Hydrazine hydrate, concd HCl; (G) Arylcarboxylic Acid, 1,1′-Carbonyl Diimidazole.

affinity. The 2-methoxyphenyl derivative **17** showed the highest D_3 affinity within this group of compounds.

At this point, other structural modifications were necessary to achieve compounds with enhanced D_3 receptor affinity values. With this aim, we replaced the 7-methoxy-2-benzofuranyl ring with a 2-naphthalene-like or a 1,4-biphenyl-like ring systems. These types of ring systems were proved to be tolerated in D_3 receptor binding. This modification was effected on compounds **13** and **17** because they showed a good compromise between D_3 receptor affinity, selectivity, and lipophilicity. To prepare the new derivatives, we extracted from the SciFinder database[17] some carboxylic acids showing the above-mentioned structural features. The search was limited to commercially available compounds that allowed a further decrease in ClogP values. Considering the compounds **18**−**24** (Table 2) that are structurally related to **13**, it can be noted that carboxamides **18**, **19**, and **21** showed D_3 receptor affinity in the same range of **13**, whereas compounds **22**−**24** were more potent than **13**. Derivative **20** was devoid of D_3 receptor affinity. These data indicated that 1,4-biphenyl-like rings in the carboxamide moiety were tolerated better than 2-naphthalene-like rings. As far as the affinity for D_2 receptor is concerned, compounds **18**−**24** were found inactive. Considering the compounds **29**−**35** (Table 3) that are structurally related to **17**, it can be noted that all compounds displayed D_3 receptor affinities in the same range as **17**. Moreover, differently from the 1,2-benzisoxazolyl derivatives, within the 2-methoxyphenyl series there was no difference in D_3 receptor affinity between the carboxamides with a 2-naphthalene-like ring or a 1,4-biphenyl-like ring. 2-Methoxyphenyl derivatives **24**−**35** showed higher D_3 affinities than their counterparts **18**−**24** but proved to be poorly selective over D_2 receptors.

Because in a recent paper[18] several 3-heteroarylbenzamides were reported as potent D_3 ligands, we included in our study the derivatives **25**−**28**. Unfortunately, these compounds were devoid of D_3 receptor affinity. These data evidenced that the 1,3-substitution pattern in the arylcarboxamide moiety was not tolerated by this class of compounds.

Table 4. Appearent Permeability, Lipophilicity Data, and Topological Polar Surface Area (TPSA) of Compounds **1**, **22**−**24**

	1	**22**	**23**	**24**
P_{app} ($\times 10^{-6}$, cm sec^{-1})	ND (<1)[a]	36.3 ± 0.11	6.56 ± 0.96	7.39 ± 0.74
ClogP	4.98	2.74	3.31	3.18
log *P*		3.16	2.63	2.98
log $D_{7.4}$		2.96	2.42	2.43
TPSA (Å^2)[b]	57.948	83.312	88.666	112.663

[a] Not detectable. [b] See ref 20.

Taken together these results indicated that a significant lowering in lipophilicity of our reference compound **1** can be achieved replacing the 2,3-dichlorophenyl ring. However, this structural feature is essential for high D_3 receptor affinity. By contrast, replacement of the 7-methoxybenzofurane ring with less lipophilic fragments might lead to an increasing of D_3 receptor affinity.

Although derivatives **22**−**24** did not show D_3 receptor affinity values in the subnanomolar range, we wanted to explore further whether these compounds met the above indicated lipophilicity requirement for a PET radioligand.

Experimental log *P* of compounds **22**−**24** were obtained by the pH metric technique using a GlpK_a apparatus, as detailed in the Experimental Section. All compounds showed log *P* values well within the range that we had chosen, although experimental values were slightly different from the calculated values (Table 4). The potential of compounds **22**−**24** to pass the blood−brain barrier was confirmed by topological polar surface area values (Table 4) that were within the limits of brain penetrant drugs.[19,20] Finally, permeation studies in vitro with human colon carcinoma cell line (Caco-2) revealed that only **22**−**24** were able to cross the cell monolayer, whereas compound **1** was not (Table 4).

In this study we have proposed a new strategy to design potential PET radioligands specifically for the visualization of

brain dopamine D_3 receptors. We have performed structural modification on the high-affinity D_3 receptor ligand *N*-[4-[4-(2,3-dichlorophenyl)piperazin-1-yl]butyl]-7-methoxy-2-benzofurancarboxamide (**1**), to lower lipophilicity within an optimal range and still retaining good D_3 receptor affinity. A significant reduction in lipophilicity was achieved mainly by substituting the 2,3-dichlorophenyl group. However, this portion of the molecule revealed to be essential for high D_3 receptor affinity. Among the studied compounds, derivatives *N*-[4-[4-(5-methoxy-2-benzisoxazolyl)piperazin-1-yl]butyl]-4-(4-morpholinyl)benzamide (**22**), *N*-[4-[4-(5-methoxy-2-benzisoxazolyl)piperazin-1-yl]butyl]-4-(1*H*-imidazol-1-yl)benzamide (**23**), and *N*-[4-[4-(5-methoxy-2-benzisoxazolyl)piperazin-1-yl]butyl]-5-(2-furanyl)-1*H*-pyrazole-3-carboxamide (**24**) displayed good D_3 receptor affinities (K_i values 38.0, 22.6, and 21.3 nM, respectively) and were selective over D_2 receptor. Moreover, on the basis of their experimental log *P* values and their ability to cross the Caco-2 monolayer, compounds **22**−**24** are likely to permeate the blood−brain barrier, differently from compound **1**. Although the goal to obtain a potent potential PET D_3 receptor was not achieved, there are elements to consider the proposed strategy a good starting point for future developments.

Experimental Section

1. Chemistry. Column chromatography was performed with 1:30 Merck silica gel 60A (63−200 μm) as the stationary phase. Melting points were determined in open capillaries on a Gallenkamp electrothermal apparatus. Elemental analyses (C, H, N) were performed on Eurovector Euro EA 3000 analyzer; the analytical results were within ±0.4% of the theoretical values for the formula given. ^{1}H NMR spectra were recorded at 300 MHz on a Varian Mercury-VX spectrometer. All spectra were recorded on free bases. All chemical shift values are reported in ppm (δ). Recording of mass spectra was done on an HP6890-5973 MSD gas chromatograph/mass spectrometer; only significant *m*/*z* peaks, with their percentage of relative intensity in parentheses, are reported. ESI$^+$/MS/MS analysis were performed with an Agilent 1100 Series LC−MSD trap System VL workstation. All spectra were in accordance with the assigned structures. The purity of new compounds that were essential to the conclusions drawn in the text were determined by HPLC on a Perkin-Elmer series 200 LC instrument using a Phenomenex Prodigy ODS-3 RP-18 column, (250 × 4.6 mm, 5 μm particle size) and equipped with a Perkin-Elmer 785A UV−vis detector setting λ = 254 nm. All compounds were eluted with $CH_3OH/H_2O/EtN_3$, 4:1:0.01, v/v at a flow rate of 1 mL/min except compounds **25**−**28**, **29**−**31**, and **33**−**35** that were eluted at a flow rate of 0.8 mL/min. When necessary, a standard procedure was used to transform final compounds into their hydrochloride or oxalate salts. Melting points and recrystallization solvents of final compounds are available in the Supporting Information.

The following compounds were synthesized according to published procedures: 5-methoxy-2-(1-piperazinyl)benzoxazole,[21] 2-(1-piperazinyl)-1*H*-benzimidazole,[22] 5-methoxy-3-(1-piperazinyl)-1,2-benzisoxazole,[23] 3-(1-piperazinyl)-1*H*-indazole,[24] 5-(1-piperazinyl)-quinoxaline,[25] 7-methoxy-1-(1-piperazinyl)isoquinoline,[26] 3-(4-morpholinyl)benzoic acid,[27] 3-(2-pyrimidinyl)benzoic acid,[28] 3-(1*H*-imidazol-1-yl)benzoic acid,[29] 3-(5-methyl-1,2,4-oxadiazol-3-yl)-benzoic acid,[30] 4-(2-methoxyphenyl)-1-piperazinebutanamine (**7**).[31]

***N*-(4-Hydroxybutyl)-7-methoxy-2-benzofurancarboxamide (5).** To a cooled mixture containing 4-aminobutanol (0.20 g, 2.2 mmol) in 1.2% aqueous NaOH (9 mL) was added dropwise under vigorous stirring a CH_2Cl_2 solution (30 mL) of 7-methoxy-2-benzofuranecarboxyl chloride, prepared from the acid **4** (0.50 g, 2.6 mmol) and $SOCl_2$ (3 mL) in the presence of few drops of triethylamine. Then, the aqueous layer was separated and extracted with CH_2Cl_2 (15 mL). The combined organic layers were washed first with a saturated aqueous solution of $NaHCO_3$, then with H_2O. The separated organic phase was dried over Na_2SO_4 and evaporated to dryness under reduced pressure. The crude residue was chromatographed ($CHCl_3$/AcOEt, 1:1 as eluent) to give pure carboxamide **5** as a colorless oil (0.45 g, 77% yield). GC−MS *m*/*z* 264 (M^+ + 1, 4), 263 (M^+, 25), 191 (32), 175 (100). ^{1}H NMR ($CDCl_3$): δ 1.65−1.77 (m, 4H), 1.93 (s, 1H, D_2O exchanged), 3.51 (q, 2H, J = 6.5 Hz), 3.72 (t, 2H, J = 5.7 Hz), 4.01 (s, 3H), 6.89 (dd, 1H, J = 1.5 Hz, 7.3 Hz), 7.00 (br t, 1H), 7.17−7.24 (m, 2H), 7.45 (s, 1H).

***N*-(4-Methanesulfonyloxybutyl)-7-methoxy-2-benzofurancarboxamide (6).** Triethylamine (0.8 mL, 5.8 mmol) and methanesulfonyl chloride (0.3 mL, 3.9 mmol) were added to a solution of **5** (1.00 g, 3.8 mmol) in CH_2Cl_2 cooled at −10 °C. The mixture was stirred at room temperature for 6 h. Then the reaction mixture was first washed with a saturated aqueous solution of $NaHCO_3$ and then with 3 N HCl. The separated organic phase was dried over Na_2SO_4 and concentrated under reduced pressure yielding a pure **6** as a white semisolid (0.96 g, 74% yield). ^{1}H NMR ($CDCl_3$): δ 1.77−1.88 (m, 4H), 3.02 (s, 3H), 3.52 (q, 2H, J = 6.5 Hz), 4.01 (s, 3H), 4.28 (t, 2H, J = 6.0 Hz), 6.83 (br t, 1H), 6.90 (dd, 1H, J = 1.5 Hz, 7.3 Hz), 7.18−7.24 (m, 2H), 7.45 (s, 1H).

4-(5-Methoxy-2-benzisoxazolyl)-1-piperazinebutanamine (10). A stirred mixture of *N*-(4-bromobutyl)phthlimide (0.51 g, 1.8 mmol), 5-methoxy-3-(1-piperazinyl)-1,2-benzisoxazole (0.51 g, 2.2 mmol), and a slight excess of K_2CO_3 in acetonitrile was refluxed overnight. After cooling, the mixture was evaporated to dryness and H_2O was added to the residue. The aqueous phase was extracted twice with AcOEt. The collected organic layers were dried over Na_2SO_4 and evaporated under reduced pressure. The intermediate phthlimide derivative **9** was isolated by chromatography ($CHCl_3$/AcOEt, 1:1 as eluent), then used for the next step. Compound **9** was mixed with an excess of hydrazine hydrate in ethanol, and it was refluxed for 3 h. Then the mixture was cooled on an ice bath and acidified with concentrated HCl. The mixture was refluxed for 10 min. Then the mixture was cooled and filtered. The filtrate was concentrated under reduced pressure, and the residue was partitioned between 10% NaOH and $CHCl_3$. The separated organic layer was dried over Na_2SO_4 and concentrated in vacuo to give the pure amine **10** as a pale yellow oil (0.37 g, 67% yield). GC−MS *m*/*z* 305 (M^+ + 1, 3), 304 (M^+, 14), 246 (25), 191 (38), 149 (41), 127 (100), 97 (97). ^{1}H NMR ($CDCl_3$): δ 1.46−1.60 (m, 6H, 2H D_2O exchanged), 2.42 (t, 2H, J = 7.3 Hz), 2.64 (app t, 4H), 2.72 (t, 2H, J = 6.7 Hz), 3.53 (app t, 4H), 3.84 (s, 3H), 7.00 (d, 1H, J = 2.5 Hz), 7.10 (dd, 1H, J = 2.5 Hz, 9.1 Hz), 7.34 (d, 1H, 9.1 Hz).

***N*-[4-[4-(5-Methoxy-2-benzoxazolyl)piperazin-1-yl]butyl]-7-methoxy-2-benzofurancarboxamide (11).** A stirred mixture of **6** (0.80 g, 2.3 mmol), 5-methoxy-2-(1-piperazinyl)benzoxazole (0.56 g, 2.4 mmol), and a slight excess of K_2CO_3 in acetonitrile was refluxed overnight. After cooling, the mixture was evaporated to dryness and H_2O was added to the residue. The aqueous phase was extracted 2 times with CH_2Cl_2. The collected organic layers were dried over Na_2SO_4 and evaporated under reduced pressure. The crude residue was chromatographed with $CHCl_3/CH_3OH$, 19:1, to afford the pure benzamide (0.11 g, 10% yield). The same synthetic procedure was followed to prepare carboxamides **12**−**17**. GC−MS *m*/*z* 479 (M^+ + 1, 9), 478 (M^+, 34), 289 (36), 246 (50), 175 (100), 123 (99). ^{1}H NMR ($CDCl_3$): δ 1.60−1.73 (m, 4H), 2.44 (t, 2H, J = 6.9 Hz), 2.56 (app t, 4H), 3.50 (q, 2H, J = 6.4 Hz), 3.71 (app t, 4H), 3.80 (s, 3H), 3.99 (s, 3H), 6.57 (dd, 1H, J = 6.2 Hz, J = 2.5 Hz), 6.88−6.92 (m, 2H), 7.00 (br t, 1H), 7.11 (d, 1H, J = 8.7 Hz), 7.18−7.24 (m, 2H), 7.46 (s, 1H). The hydrochloride salt melted at 196−198 °C (from CH_3OH/Et_2O). Anal. ($C_{26}H_{30}N_4O_5$·2HCl) C, H, N.

General Procedure for the Synthesis of Carboxamides 18−**30, 33**−**35.** A mixture of the appropriate carboxylic acid (0.48 mmol) and 1,1′-carbonyldiimidazole (0.50 mmol) in 10 mL of anhydrous THF was stirred for 8 h. A solution of amine **7** or **10** (0.48 mmol) in 10 mL of anhydrous THF was added, and the mixture was stirred until the carboxylic acid disappeared (TLC). The reaction mixture was partitioned between AcOEt and H_2O. The organic layer was washed with aqueous Na_2CO_3 solution, dried (Na_2SO_4), and concentrated in vacuo. The crude residue was

chromatographed with $CHCl_3/CH_3OH$, 19:1, unless otherwise indicated, to afford the pure benzamide.

***N*-[4-[4-(5-Methoxy-2-benzisoxazolyl)piperazin-1-yl]butyl]-4-(4-morpholinyl)benzamide (22).** 33% Yield. ESI^+/MS *m/z* 494 (MH^+). ESI^+/MS/MS *m/z* 190 (100). ^1H NMR ($CDCl_3$): δ 1.68 (br s, 4H), 2.58 (br t, 2H), 2.71 (app t, 4H), 3.21 (app t, 4H), 3.46−3.49 (m, 2H), 3.58 (m, 4H), 3.82−3.85 (s + m, 7H), 6.47 (br t, 1H, D_2O exchanged), 6.86 (d, 2H, J = 8.8 Hz), 6.99 (d, 1H, J = 2.2 Hz), 7.11 (dd, 1H, J = 2.2 Hz, 9.1 Hz), 7.35 (d, 1H, J = 9.1 Hz), 7.70 (d, 2H, J = 8.8 Hz). Mp 181−182 °C (from $CHCl_3$/*n*-hexane). Anal. ($C_{27}H_{35}N_5O_4$) C, H, N.

***N*-[4-[4-(5-Methoxy-2-benzisoxazolyl)piperazin-1-yl]butyl]-4-(1*H*-imidazol-1-yl)benzamide (23).** 73% Yield. ESI^+/MS *m/z* 475 (MH^+). ESI^+/MS/MS *m/z* 242 (74), 171 (100). ^1H NMR (DMSO-d_6): δ 1.48−1.62 (m, 4H), 2.37 (app t, 2H), 2.54 (br s, 4H), 3.25−3.31 (m, 2H), 3.41 (br s, 4), 3.80 (s, 3H), 7.11 (s, 1H), 7.16 (dd, 1H, J = 2.5, 9.0 Hz), 7.28 (d, 1H, J = 2.5 Hz), 7.46 (d, 2H, J = 9.1), 7.75 (d, 2H, J = 8.5 Hz), 7.82 (t, 1H, J = 1.1 Hz), 7.97 (d, 2H, J = 8.5 Hz), 8.34 (s, 1H), 8.53 (br t, 1H). Mp 169 °C dec (from $CHCl_3$/*n*-hexane). Anal. ($C_{26}H_{30}N_6O_3$) C, H, N.

***N*-[4-[4-(5-Methoxy-2-benzisoxazolyl)piperazin-1-yl]butyl]-5-(2-furanyl)-1*H*-pyrazole-3-carboxamide (24).** 50% Yield. ESI^+/MS *m/z* 465 (MH^+). ESI^+/MS/MS *m/z* 288 (29), 232 (100).^1H NMR ($CDCl_3$): δ 1.62−1.67 (m, 4H), 2.48 (t, 2H, J = 6.7 Hz), 2.67 (br t, 4H), 3.47 (q, 2H, J = 6.0 Hz), 3.57 (br t, 4H), 3.84 (s, 3H), 6.46 (dd, 1H, J = 1.9 Hz, 3.3 Hz), 6.65 (d, 1H, J = 3.3 Hz), 6.95 (s, 1H), 7.00 (d, 1H, J = 2.5 Hz), 7.11 (dd, 1H, J = 2.5 Hz, 9.1 Hz), 7.26−7.29 (m, 2H, D_2O exchanged), 7.33 (d, 1H, J = 8.9 Hz), 7.43 (d, 1H, J = 1.4 Hz). The hydrochloride salt melted at 125 °C dec (from CH_3OH/Et_2O). Anal. ($C_{24}H_{29}N_6O_4 \cdot 2HCl \cdot 0.5H_2O$) C, H, N.

***N*-[4-[4-(2-Methoxyphenyl)piperazin-1-yl]butyl]quinoxaline-6-carboxamide (29).** 66% Yield. GC−MS *m/z* 420 (M^+ + 1, 30), 419 (M^+, 100), 257 (22), 205 (81). ^1H NMR ($CDCl_3$): δ 1.68−1.78 (m, 4H), 2.49 (t, 2H, J = 6.4 Hz), 2.65 (br s, 4H), 3.01 (br s, 4H), 3.55 (q, 2H, J = 5.7 Hz), 3.82 (s, 3H), 6.74−6.87 (m, 3H), 6.94−6.99 (m, 1H), 7.35 (br t, 1H, D_2O exchanged), 8.13−8.22 (m, 2H), 8.45 (s, 1H), 8.86−8.88 (m, 2H). The hydrochloride salt melted at 174 °C dec (from CH_3OH/Et_2O). Anal. ($C_{24}H_{29}N_5O_2 \cdot 2HCl$) C, H, N.

***N*-[4-[4-(2-Methoxyphenyl)piperazin-1-yl]butyl]-2-oxo-2*H*-1-benzopyran-3-carboxamide (31).** A mixture of carboxylic acid (0.42 g, 2.2 mmol) in $CHCl_3$ (20 mL) and triethylamine (0.35 mL, 2.5 mmol) was stirred at room temperature for 15 min. After cooling at −10 °C methyl chloroformate (0.19 mL, 2.5 mmol) was added, and the mixture reacted at the same temperature for 1 h. Then a solution of 4-(2-methoxyphenyl)-1-piperazinebutanamine (**7**) (0.69 g, 2.6 mmol) in $CHCl_3$ was dropped into the mixture, and the resulting mixture was kept at −10 °C to − 5 °C for 1 h. After stirring overnight at room temperature, the reaction mixture was washed with 5% aqueous NaOH, with water, and dried over Na_2SO_4 Evaporation of the solvent in vacuo afforded a crude product. The final compounds were purified by column chromatography (eluent $CHCl_3$/MeOH, 19:1) to give the expected compounds (0.65 g, 70% yield). The same procedure was followed to prepare compound **32**. GC−MS *m/z* 436 (M^+ + 1, 3), 435 (M^+, 12), 420 (25), 273 (25), 205 (100). ^1H NMR ($CDCl_3$): δ 1.68 (app t, 4H), 2.49 (br t, 2H), 2.70 (br s, 4H), 3.12 (br s, 4H), 3.50 (q, 2H, J = 6.1 Hz), 3.86 (s, 3H), 6.84−7.02 (m, 4H), 7.36−7.42 (m, 2H), 7.64−7.71 (m, 2H), 8.85 (br t, 1H), 8.91 (s, 1H). The hydrochloride salt melted at 215 °C dec (from CH_3OH/Et_2O). Anal. ($C_{25}H_{29}N_3O_4 \cdot 2HCl$) C, H, N.

2. Lipophilicity Data. Lipophilicity data of compounds **22**−**24** were obtained by the pH metric technique using a GlpK_a apparatus (Sirius Analytical Instruments Ltd., Forrest Row, East Sussex, United Kingdom) as described elsewhere.[32−35] The low aqueous solubility of the investigated compounds required pK_a measurements to be performed in the presence of methanol as cosolvent. Three separate 20 mL semiaqueous solutions of approximately 5×10^{-5} M, in 30−60% w/w of MeOH, were initially acidified with 0.5 M HCl to pH 3.5. The solutions were then titrated with 0.5 M KOH to pH 11. The initial estimates of the p$_s K_a$ values, which are the apparent ionization constants in the mixed solvent, were obtained by *Bjerrum* plots. These values were then refined by a weighted nonlinear least-squares procedure (Refinement Pro 1.0 software) to create a multiset, where the refined values were extrapolated to zero cosolvent concentration using the Yasuda−Shedlovsky equation.[36] To obtained log *P* data, at least three separate titrations were performed on each compound, on approximately 5×10^{-5} M, containing various volumes of *n*-octanol, from 0.3 to 10 mL of organic solvent/10 mL of H_2O. The biphasic solutions were initially acidified to pH 3.5 with 0.5 M HCl and then titrated with 0.5 M KOH to pH 11. The obtained data were refined as described above. The log *P* values were obtained by the multiset approach, as described elsewhere.[33,34] All titrations were carried out at 25 ± 0.1 °C under an inert nitrogen gas atmosphere to exclude CO_2.

3. Permeability Experiments. 3.1. Preparation of Caco-2 Monolayers. Caco-2 cells were grown in DMEM medium with 15% heat-inactivated fetal calf serum, 100 U/mL penicillin, 100 μg/mL streptomycin, and 2 mM L-glutamine. Then, cells were harvested with trypsin−EDTA and seeded onto MultiScreen Caco-2 assay system at a density of 10 000 cells per well. The culture medium was replaced every 48 h for the first 6 days and every 24 h thereafter, and after 21 days in culture, the Caco-2 monolayer was utilized for the permeability experiments. The transepitelial electrical resistance (TEER) of the monolayers was measured daily before and after the experiment using a Millicell-ERS system (Millipore). Generally, TEER values obtained are greater than 1000 Ω for a 21 day culture.

3.2. Drug Transport Experiment. Apical to basolateral permeability of drugs were measured under various conditions of incubation time (30, 60, 120 min) and drugs concentrations (10−100 μM). Drugs were dissolved in Hanks' balanced salt solution (HBSS, pH 7.4) and sterile filtered. After 21 days of cell growth, the medium was removed from filter wells and from the receiver plate. The filter wells were filled with 75 μL of fresh HBSS buffer and the receiver plate with 250 μL per well of the same buffer. This procedure was repeated twice, and the plates were incubated at 37 °C for 30 min. After incubation time, the HBSS buffer was removed and drug solutions added to the filter well (75 μL). HBSS without the drug was added to the receiver plate (250 μL). The plates were incubated at 37 °C for the desired time. After incubation time, samples were removed from the apical (filter well) and basolateral (receiver plate) side of the monolayer and then were stored in a freezer (−20 °C) pending analysis.

3.3. Analytical Methods. Samples from in vitro permeation studies were analyzed by using a reversed-phase HPLC equipped with a Perkin-Elmer series 200 LC pump and a Perkin-Elmer 785A UV−vis detector. UV signals were monitored and obtained peaks integrated using a personal computer running Perkin-Elmer Turbochrom software. The column used was a Phenomenex Prodigy ODS-3 RP-18 (250 × 4.6 mm, 5 μm particle size). The samples were eluted with ammonium formate (5 mM; pH adjusted to 5 with formic acid) and acetonitrile (90:10 v/v for compound **1**, 80:20 v/v for compounds **22**−**24**) at a flow rate of 1 mL/min. The wavelength for UV absorbance was set as follows: 230 nm for compound **1**; 295 nm for **22**; 254 nm for derivatives **23** and **24**. The sample injection volume was 20 μL. All of the concentration determinations were well within the detection range for the analysis method used.

3.4. Permeability Calculations. The apparent permeability coefficient, (P_{app}), in units of centimeters per second, of each compound was calculated according to the following equation:[37]

$$P_{app} = \frac{V_a}{(\text{area})(t)} \frac{[\text{drug}]_{\text{acceptor}}}{[\text{drug}]_{\text{initial}}}$$

where V_a is the volume in the acceptor well, area is the surface area of the membrane, and *t* is the total transport time.

4. Biological Methods. 4.1. General. Human recombinant D_3 dopamine receptors expressed in CHO cells were obtained from RBI (Research Biochemicals International, Natick, MA). Human

recombinant D_{2L} dopamine receptors expressed in rat C6 glioma cells were gifted by Professor Roberto Maggio (University of Pisa, Italy). For receptor binding studies, the compounds were dissolved in absolute ethanol. Haloperidol was from Sigma-Aldrich (Milan, Italy); [^{3}H]spiroperidol was obtained from NEN Life Science Products (Milan, Italy); 7-OH-DPAT was purchased from Tocris Cookson Ltd. (Bristol, U.K.).

4.2. Radioligand Binding Assay at Rat Cloned D_3 Dopaminergic Receptors. Binding of [^{3}H]-spiroperidol at human cloned receptors was performed according to Haadsma-Svensson et al. with minor modifications.[38] The incubation buffer (5.0 mM $MgCl_2$, 50 mM Tris, pH 7.4) contained 4 μg of dopamine dilute membranes, 1.0 nM [^{3}H]spiroperidol (K_d = 0.54 nM) and six to nine concentrations of drug solution in a final volume of 500 μL. The samples were incubated for 30 min at 25 °C, then the incubation was stopped by rapid filtration through Whatman GF/C glass fiber filters (presoaked in 0.5% polyethylenimine for 120 min). The filters were washed with 2 × 4.0 mL of ice-cold incubation buffer. Nonspecific binding was determined in the presence 10 μM 7-OH-DPAT. The radioactivity bound to the filters was measured by liquid scintillation using LS6500 Multi-Purpose scintillation counter, Beckman.

4.3. Radioligand Binding Assay at Human Cloned D_{2L} Dopaminergic Receptors. Binding of [^{3}H]spiroperidol at human cloned receptors was performed according to Scarselli et al.[39] with minor modifications. The incubation buffer (120 mM NaCl, 5.0 mM KCl, 5.0 mM $MgCl_2$, 1 mM EDTA, 50 mM Tris, pH 7.4) contained 100 μg of dopamine dilute membranes, 0.30−0.50 nM [^{3}H]spiroperidol (K_d = 0.093 nM) and six to nine concentrations of drug solution in a final volume of 500 μL. The samples were incubated for 120 min at 25 °C, then the incubation was stopped by rapid filtration through Whatman GF/C glass fiber filters (presoaked in 0.5% polyethylenimine for 60 min). The filters were washed 3 × 1 mL of ice-cold 50 mM Tris, 0.9% NaCl, pH 7.4. Nonspecific binding was determined in the presence of 10 μM haloperidol. The radioactivity bound to the filters was measured by liquid scintillation using LS6500 Multi-Purpose scintillation counter, Beckman.

4.4. Statistical Analysis. The inhibition curves on the different binding sites of the compounds reported in Tables 1−3 were analyzed by nonlinear curve fitting utilizing the GraphPad Prism program.[40] The value for the inhibition constant, K_i, was calculated by using the Cheng−Prusoff equation.[41]

Supporting Information Available: Spectral data of compounds **12**−**21**, **25**−**28**, **30**, **32**; melting points and recrystallization solvents of compounds **11**−**35**; elemental analysis data of target compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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