N-(4-(4-(2,3-Dichloro- or 2-methoxyphenyl)piperazin-1-yl)butyl)heterobiarylcarboxamides with Functionalized Linking Chains as High Affinity and Enantioselective D3 Receptor Antagonists[||,⊥]

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In the present report, the D3 receptor pharmacophore is modified in the 2,3-diCl- and 2-OCH_3-phenylpiperazine class of compounds with the goal to improve D3 receptor affinity and selectivity. This extension of structure−activity relationships (SAR) has resulted in the identification of the first enantioselective D3 antagonists (*R*- and *S*-**22**) to be reported, wherein enantioselectivity is more pronounced at D3 than at D2, and that a binding region on the second extracellular loop (E2) may play a role in both enantioselectivity and D3 receptor selectivity. Moreover, we have discovered some of the most D3-selective compounds reported to date that show high affinity ($K_i = 1$ nM) for D3 and ∼400-fold selectivity over the D2 receptor subtype. Several of these analogues showed exquisite selectivity for D3 receptors over >60 other receptors, further underscoring their value as in vivo research tools. These lead compounds also have appropriate physical characteristics for in vivo exploration and therefore will be useful in determining how intrinsic activity at D3 receptors tested in vitro is related to behaviors in animal models of addiction and other neuropsychiatric disorders.

The dopamine D3 receptor, a member of the dopamine D2-like receptor family, has become a target of intensive research over the past decade because of several features that have revealed its potential for development of medications toward neuropsychiatric disorders, dyskinesias associated with L-DOPA treatment of Parkinson's disease, and drug addiction.[1−3] Numerous studies have been published that implicate the dopamine D3 receptors in animal models of these disorders. However, many of the pharmacological tools that have been available for in vivo investigation cannot rule out other possible underlying mechanisms of action, due to lack of D3 receptor selectivity, poor bioavailability, or predicted toxicity that precludes human testing. Indeed, another complicating factor is that although functional coupling of D3 receptors to $G_{\alpha i/o}$-proteins has been established,[4,5] the question of which G-protein signaling pathways are recruited by D3 receptor activation in vivo remains unanswered.

Nevertheless, the fact that several D3 antagonists have demonstrated efficacy in animal models of drug abuse without the concomitant motor side effects associated with nonselective D2 antagonists supports further pursuit of the D3 receptor as a potential target for medication development. One of the single most important drivers of this research is the medicinal chemistry that has ultimately broken the barriers of nonselective D2/D3 ligands and enabled the discovery of high affinity and selective D3 antagonists and partial agonists. Highly selective and fully efficacious D3 agonists have thus far remained elusive, likely due to their competition for the orthosteric binding site and the protein homology that is present within the dopamine D2-like family of receptors to bind the endogenous substrate dopamine. Nevertheless, the evolution of structure−activity relationships (SAR) that have been derived and utilized to result in D3-preferring and sometimes highly D3-selective ligands has recently been described in detail[6] and the patented compounds from the decade of 1997−2007 have been summarized.[7] Interestingly, despite significant "molecular tinkering", the compounds with highest D3 affinity and selectivity typically are extended molecules with aryl termini and functionalized linking chains resulting in relatively high molecular weights (450−600 g/mol) and concomitant lipophilicities as measured by cLogP values.[2,6,7] Significant effort has thus been focused on attaining the appropriate balance of physical properties that would allow blood−brain barrier (BBB[a]) penetration while limiting nonspecific binding. Cell-based binding and functional assays have been developed for quick screening of novel templates, and lead optimization has ensued. An excellent example of this effort has recently been published in which significant departure from the D3-selective SB 277011-A (*N*-((1*s*,4*s*)-4-(2-(6-cyano-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)cyclohexyl)quinoline-4-carboxamide[8] and second generation SB-414796 (3-(2-((1*r*,4*r*)-4-(3-(5-methyl-1,2,4-oxadiazol-3-yl)benzamido)cyclohexyl)ethyl)-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepin-7-yl methanesulfonate[9] was taken to achieve an improved pharmacological profile for in vivo testing.[10] The resulting 1,2,4-triazol-3-ylthipropyltetrahydrobenzazepines were reported to retain the desired D3-selective pharmacological profile (∼100-fold) but also showed excellent BBB penetrability and acceptable pharmacokinetics.[10] Intensive and biologically based drug design is undoubtedly key to further characterizing D3-related

[||] This manuscript is dedicated to the memory of a dear friend and colleague, Dr. Andrew Thurkauf, who first introduced A.H.N. to the dopamine D3 receptor antagonists more than a decade ago.

[⊥] Atomic coordinates for *S*-**22** have been deposited with the Cambridge Crystallographic Data Centre.

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[a] Abbreviations: E2, second extracellular loop; BBB, blood−brain barrier; PSA, polar surface area; TMS, transmembrane spanning; D2/D3E2, a human D2 receptor with the E2 loop of the D3 receptor; D3/D2E2, a human D3 receptor with the E2 loop of the D2 receptor.

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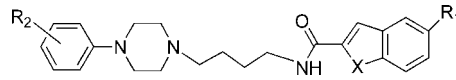
Table 1. Human D2-Family Receptor Subtype Binding Data on *N*-(4-(4-(2,3-Dichloro- or 2-methoxyphenyl)piperazin-1-yl)butyl)heterobiarylcarboxamides[a]



				$K_i \pm$ SEM (nM)				
	R_2	X	R_1	D_2	D_3	D_4	D_2/D_3	D_4/D_3
9[b]	2,3-diCl	NH	F	24.8 ± 8.61	0.52 ± 0.21	NT[e]	50	
10[c]	2-MeO	NH	H	37.4 ± 6.22	0.32 ± 0.12	476 ± 153	117	1488
11[b]	2,3-diCl	O	H	44.8 ± 10.6	0.81 ± 0.30	1220 ± 353	56	1506
12[c]	2-MeO	O	H	36.5 ± 3.91	0.92 ± 0.12	NT[e]	40	
13[d]	2,3-diCl	O	I	154 ± 13.3	1.59 ± 0.31	1720 ± 416	97	1082
14[d]	2-MeO	O	I	77.0 ± 17.2	0.72 ± 0.21	335 ± 63.2	107	465
15[b]	2,3-diCl	S	H	64.7 ± 8.91	0.81 ± 0.20	1370 ± 430	80	1691
16[c]	2-MeO	S	H	21.5 ± 1.60	0.19 ± 0.04	305 ± 108	113	1605

[a] All compounds in this table have been previously reported as noted. They were prepared as described and evaluated under the same assay conditions for more accurate and direct comparison. The methods for the determination of the binding data (cloned human dopamine D2-like receptors transfected into HEK293 cells, radioligand ^{125}I-IABN) were described earlier.[26] Inhibition of binding constants (K_i) is the mean ± SEM of at least three independent determinations. [b] Reference 26. [c] Reference 58. [d] Reference 33. [e] NT = not tested.

behaviors in vivo and potentially developing these agents as medications.

Numerous reports using some of the prototypic D3 antagonists and partial agonists have described attenuation of drug seeking behaviors and effectiveness in animal models of drug reinstatement (relapse) that support D3 receptor blockade as a plausible target for drug discovery.[11−18] Further, these studies suggest that D3 selective antagonists and/or partial agonists will likely have therapeutic utility in the treatment of drug addiction in humans.[3,7] In addition, in vivo models in rodents and nonhuman primates have been designed to more accurately assess D3 receptor-mediated behaviors.[19−21] Nevertheless, a correlation between intrinsic efficacy determined in vitro has yet to be linked to in vivo behaviors, and hence, additional biological assays are needed to clarify this apparent disconnect. Moreover, numerous ligands that show "D3-mediated" behaviors as determined by their high affinity binding to D3 receptors may have off-target receptor interactions, including (albeit low affinity) D2 receptor subtype related effects,[22] reduced bioavailability, poor pharmacokinetics, or functional selectivities[23,24] that are typically not defined. Thus, additional discovery and assessment of novel and D3 receptor selective ligands must continue to be pursued to validate this target and ultimately discover efficacious and safe compounds for human clinical trials.

Structure−activity relationships (SAR) for at least the 4-phenylpiperazine class of D3 antagonists/partial agonists have been well established. However, continued and, sometimes, incremental modification is required to effectively retain the desired D3 receptor-selective binding and functional profile while improving physical properties. This task has presented a considerable challenge, and thus far only a few D3-preferring antagonists or partial agonists have been evaluated behaviorally. Although we have also attempted to diverge from this template[25] in the present report, we continue to modify the D3 pharmacophore in the 2,3-diCl- and 2-OCH_3-phenylpiperazine class of compounds. Our goal is thus to improve both D3 receptor affinity and selectivity over the other D2-like receptor subtypes, as well as additional, related 5-HT receptors, and reduce lipophilicity so that BBB penetration and D3 receptor-rich brain localization may be achieved at concentrations that are relevant to binding affinities.

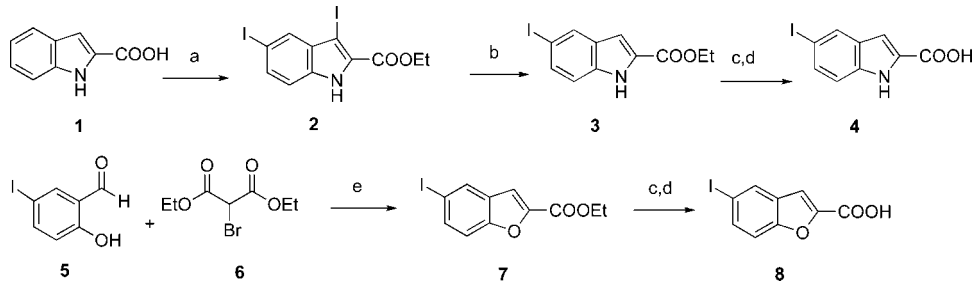
Drug Design and Synthesis

All of the saturated butyl-linked analogs (**9**−**16**) have been described in the literature, as indicated in Table 1. Compounds **13** and **14** were prepared as described in the Experimental Methods. This series of saturated analogues was evaluated for hD2, hD3, and hD4 receptor binding, under the same assay conditions, to make direct SAR comparisons. As noted previously,[2] the wide range of cell lines, radioligands, and binding assays performed across laboratories has made it impossible to directly compare K_i values for each other's novel compounds, and hence, direct comparison under the same assay conditions is required to get a true sense of SAR at human D2-like receptor subtypes.

The trans-olefin, 3-hydroxybutyl- or 2-hydroxybutyl-linked analogues (**17**−**37**) were designed based on SAR previously described[26,27] wherein the D3 pharmacophore 2-OCH_3- or 2,3-diCl-phenylpiperazine was retained. However, incorporation of variously substituted heterobiarylamides were explored to identify combinations of these D3 pharmacophores that provided the best balance between high D3 receptor affinity and selectivity, as well as to reduce the cLogP value to the druglike range of 2−5.[28] In addition, since one goal of this program has been to identify a potential D3-receptor selective radioligand, incorporation of F, I, and OCH_3 moieties was explored, as these could readily be replaced with ^{18}F, ^{125}I, O$^{11}CH_3$, or OCT_3, using a modification of the synthetic strategies devised. Finally, as we had previously discovered that the racemic 3- and 2-hydroxylated linking chain afforded several high affinity and D3 receptor selective ligands,[27] we chose to investigate enantioselectivity within this class of compounds. We previously showed that the 2-OH analogues were very similar in binding profile to the unsubstituted butyl-linked analogues,[27] suggesting that this position may not be pivotal for binding; hence, the 3-OH analogue **22** was chosen for enantiomeric separation.

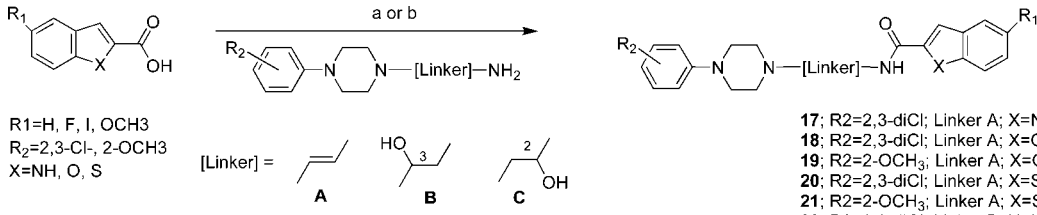
Of the indole, benzofuran, and benzothiophene carboxylates required, only the 5-iodoindole and 5-iodobenzothiophene were not commercially available. In Scheme 1, following a modification of a published procedure, 5-iodoindole-2-carboxylate (**4**) was readily obtained by converting indole-2-carboxylic acid (**1**) to its ethyl ester and iodinating to give the 3,5-diodo intermediate **2**. After workup, the crude 3,5-diodoindole ethyl ester was suspended in concentrated HCl to which Zn dust was added portionwise at room temperature. Extractive workup and ester hydrolysis in ethanol and aqueous KOH gave the desired 5-iodoindole-2-carboxylic acid (**4**). 5-Iodobenzofuran-2-carboxylic acid (**8**) was also obtained via basic hydrolysis of the ethyl ester **7**, which was prepared from 5-iodosalicylaldehyde (**5**) and diethyl bromomalonate (**6**, Scheme 1).

Scheme 1. Synthesis of 5-Iodoheterobiaryl Synthons[a]



[a] Reagents and conditions: (a) I_2, EtOH, $NaIO_3$ H_2SO_4; (b) HCl, Zn; (c) EtOH, KOH; (d) 10 M HCl; (e) $N(t\text{-}Bu)_4I$, K_2CO_3.

Scheme 2. Synthesis of Compounds **17**−**37**[a]



17; R2=2,3-diCl; Linker A; X=NH; R1=H
18; R2=2,3-diCl; Linker A; X=O; R1=H
19; R2=2-OCH_3; Linker A; X=O; R1=H
20; R2=2,3-diCl; Linker A; X=S; R1=H
21; R2=2-OCH_3; Linker A; X=S; R1=H
22; R2=2,3-diCl; Linker B; X=NH; R1=H
***R*-22**; R2=2,3-diCl; Linker B; X=NH; R1=H
***S*-22**; R2=2,3-diCl; Linker B; X=NH; R1=H
23; R2=2-OCH_3; Linker B; X=NH; R1=H
24; R2=2-O-n-Propyl; Linker B; X=NH; R1=H
25; R2=2,3-diCl; Linker B; X=NH; R1=F
26; R2=2-OCH_3; Linker B; X=NH; R1=F
27; R2=2,3-diCl; Linker B; X=NH; R1=I
28; R2=2-OCH_3; Linker B; X=NH; R1=I
29; R2=2,3-diCl; Linker B; X=NH; R1=OCH_3
30; R2=2-OCH_3; Linker B; X=NH; R1=OCH_3
31; R2=2,3-diCl; Linker B; X=O; R1=H
32; R2=2-OCH_3; Linker B; X=O; R1=H
33; R2=2,3-diCl; Linker B; X=O; R1=I
34; R2=2-OCH_3; Linker B; X=O; R1=I
35; R2=2-OCH_3; Linker B; X=S; R1=H
36; R2=2,3-diCl; Linker C; X=NH; R1=H
37; 2-OCH_3; Linker C; X=NH; R1=H

[a] Reagents and conditions: (a) CDI, THF (method A); (b) $SOCl_2$ (method B).

The syntheses of the amino synthons with linkers **A** (e.g., 4-(4-(2,3-chlorophenyl)piperazin-1-yl)-*trans*-but-2-enylamine or 4-(4-(2-methoxyphenyl)piperazin-1-yl)-*trans*-but-2-enylamine), **B** (e.g., 4-amino-1-(4-(2,3-dichlorophenyl)piperazin-1-yl)butan-2-ol or 4-amino-1-(4-(2-methoxyphenyl)piperazin-1-yl)butan-2-ol), and **C** (e.g., 1-amino-4-(4-(2,3-dichlorophenyl)-piperazin-1-yl)butan-2-ol or 1-amino-4-(4-(2-methoxyphenyl)piperazin-1-yl)butan-2-ol) have all been previously described.[26,27] The convergent reaction sequence used to prepare the compounds **17**−**37**, incorporating a butenyl (**A**), 3-hydroxybutyl (**B**), or 2-hydroxybutyl (**C**) linking chain, is depicted in Scheme 2, using classic amidation reactions. The *R*-**22** and *S*-**22**, incorporating an enantiomerically pure 3-hydroxybutyl linker (**B**), were prepared from the corresponding enantiomerically pure amines. An enantiomeric resolution of **22** using tartaric acid derivatives failed.

X-ray Results

S-**22** was crystallized in the triclinic space group *P*1 with four independent molecules in the asymmetric unit. Three of these four molecules contained a disorder dichlorophenyl ring. The absolute configuration was determined from the anomalous dispersion data collected as part of the X-ray diffraction data set, and on the basis of this, the hydroxylated C14 was found to have an *S*-configuration (Figure 1). All bond lengths and bond angles were within the expected range. Although all four molecules have unique conformations, the four can be split into two groups with molecules A and C and molecules B and D, having similar configurations at C12; the chlorine atoms all fall in the same hemisphere despite free rotation about the N19−C22 bond (see Supporting Information Figures 1 and 2).

Pharmacological Results and Discussion

All ligands were evaluated in competition binding assays in HEK 293 cells transfected with human $D2_L$, D3, or D4 dopamine receptors, as described previously.[26] The displaced radioligand was the high-affinity, selective D2-like receptor antagonist 2,3-dimethoxy-5-(^{125}I)-iodo-*N*-(9-benzyl-9-azabicyclo(3.3.1)nonan-3-yl)benzamide ([^{125}I]IABN).[30] Data for the saturated butyl analogues are compared in Table 1. In addition, cLogP values and polar surface areas (PSA) were calculated to provide a measure of lipophilicity and predicted brain penetration, respectively,[31,32] for the novel compounds described in Table 2. Most of these compounds were also evaluated in a quinpirole-stimulated mitogenesis assay for functional activity at dopamine D2 and D3 receptors and for binding affinities at the dopamine D1 and serotonin $5HT_{1A}$ receptors (Table 5).

Our initial objective was to evaluate the biarylamides including indole, benzofuran, and benzothiophene and the 5-F- and 5-I substitution on these ring system, as available, on the prototypic 2,3-diCl-or 2-OCH_3-phenylpiperazinyl butyl-linked compounds. All of these compounds had been previously reported, as indicated in Table 1, but they had not all been tested in our binding assays. Thus, to more accurately compare, we

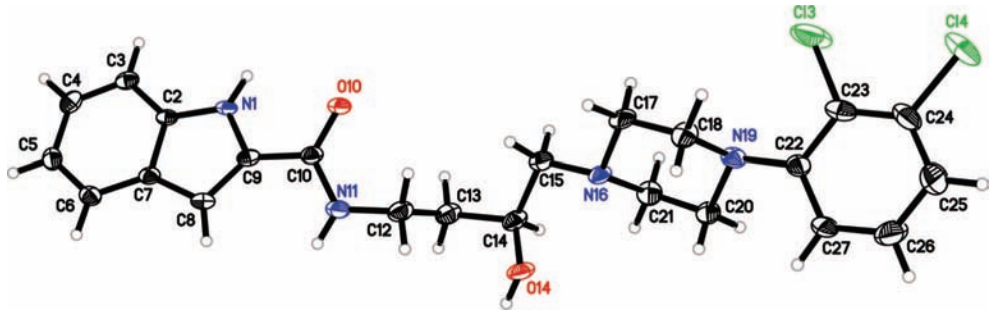
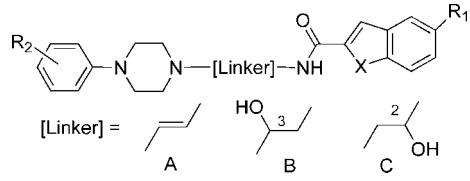


Figure 1. X-ray crystal structure of *S*-**22**. Displacement ellipsoids are at the 50% level. For clarity only one of the four independent molecules in the asymmetric unit is shown.

Table 2. Human D2-Family Receptor Subtype Binding Data on Functionalized Linking Chain Analogues[a]



							$K_i \pm$ SEM (nM)				
	R_2	[linker]	X	R_1	PSA[b] (Å)	cLogP[c]	D_2	D_3	D_4	D_2/D_3	D_4/D_3
17	2,3-diCl	A	NH	H	51	4.6	76.4 ± 6.61	0.44 ± 0.05	640 ± 43.3	174	1455
18[d]	2,3-diCl	A	O	H	49	4.6	76.5 ± 14.0	2.11 ± 0.53	1070 ± 238	36	507
19	2-MeO	A	O	H	58	3.1	78.8 ± 2.20	11.3 ± 1.40	NT[e]	7	
20[d]	2,3-diCl	A	S	H	37	5.3	149 ± 5.60	1.11 ± 0.03	1770 ± 508	135	1610
21	2-MeO	A	S	H	45	4.0	60.0 ± 7.01	2.61 ± 0.29	NT[e]	23	
22	2,3-diCl	B	NH	H	72	4.2	502 ± 51.5	1.39 ± 0.19	4900 ± 1034	358	5300
R-**22**	2,3-diCl	B	NH	H	72	4.2	433 ± 29.5	1.12 ± 0.21	3430 ± 1300	394	3116
S-**22**	2,3-diCl	B	NH	H	72	4.2	715 ± 6.23	16.6 ± 2.31	>5000	43	>300
23	2-MeO	B	NH	H	81	2.5	249 ± 44.4	1.40 ± 0.11	1920 ± 263	178	1370
24	2-n-PropO	B	NH	H	81	3.7	47.1 ± 6.01	62.1 ± 2.21	NT[e]	0.8	
25	2,3-diCl	B	NH	F	72	4.5	293 ± 34.0	1.61 ± 0.11	>5000	183	3125
26	2-MeO	B	NH	F	81	2.8	244 ± 35.4	2.41 ± 0.40	967 ± 178	102	
27	2,3-diCl	B	NH	I	72	5.5	1060 ± 155	5.21 ± 0.64	>10000	204	>1900
28	2-MeO	B	NH	I	81	3.8	520 ± 7.30	3.60 ± 0.55	455 ± 144	144	126
29	2,3-diCl	B	NH	OMe	81	4.3	489 ± 8.40	1.20 ± 0.10	>10000	408	>8000
30	2-MeO	B	NH	OMe	90	2.8	390 ± 20.3	2.32 ± 0.31	1530 ± 238	170	1173
31	2,3-diCl	B	O	H	69	4.2	622 ± 30.1	6.11 ± 1.01	>10000	102	>1600
32	2-MeO	B	O	H	78	2.6	507 ± 99.5	7.51 ± 1.10	2140 ± 623	68	286
33	2,3-diCl	B	O	I	69	5.5	581 ± 87.5	7.71 ± 0.81	>10000	75	>1300
34	2-MeO	B	O	I	78	3.8	430 ± 59.0	7.50 ± 0.70	1290 ± 412	57	172
35	2-MeO	B	S	H	65	4.9	337 ± 15.4	4.60 ± 0.20	NT[e]	73	
36	2,3-diCl	C	NH	H	72	4.2	28.4 ± 4.60	0.26 ± 0.03	1040 ± 733	108	4015
37	2-MeO	C	NH	H	81	2.5	52.5 ± 3.10	0.51 ± 0.03	176 ± 18.7	105	352

[a] The methods for the determination of the binding data (cloned human dopamine D2-like receptors transfected into HEK293 cell, radioligand ^{125}I-IABN) were described earlier.[26] Inhibition of binding constants (K_i) are the mean ± SEM of at least three independent determinations. [b] Polar surface area (PSA) was calculated using an algorithm developed by Ertl et al.[31] [c] Partition coefficients (cLogP) were calculated using ChemDraw Ultra, version 11.0, CambridgeSoft 2007.[31] [d] Reference 26. [e] NT = not tested.

Table 3. Amino Acid Sequence of the E2 Loops of the Human D2 and D3 Dopamine Receptors[45,a]

E2 loop	amino acid sequence
hD2	NNADQNE*CIIAN
hD3	-TTG-PTV-S-S-

[a] Amino acid sequence of the human D2 and D3 dopamine receptor second extracellular (E2) loop is shown using the single letter code. The asterisk (*) denotes a shift in the sequence alignment to maximize homology, and a dashed line (-) denotes sequence homology. The cysteine residue is conserved and forms a disulfide bond.

prepared and evaluated these compounds for binding at D2, D3, and D4 receptors. As expected, all of the compounds showed high affinity binding at D3 and relatively low affinities for D4. In this set, the 2-OCH_3-phenylpiperazines **10**, **14**, and **16** showed >100-fold D3-selectivity over D2. Further, the 5-F and 5-I substituents were generally well tolerated, supporting the 5-position of the biarylamide as a potential place for radioligand development, as previously suggested.[33]

Previous studies from our laboratory had demonstrated that replacing the saturated butyl linker with either a trans-olefin or hydroxylated butyl chain often improved D3 selectivity while retaining high affinity.[26,27] Thus, the compounds described in Scheme 2 were designed. These analogues are arranged in Table 2 according to linking chain template with the trans olefins having linker A, the 3-OH-subsituted analogues having linker B, and the 2-OH-butyl analogues with linker C. We have previously reported that the combinations of extended arylamides and these linking chains yielded high affinity and selective D3 antagonists and partial agonists. However, many of the previous compounds had high cLogP values, which limited H_2O solubility and potentially bioavailability, as discussed.[27] Hence, by limiting the size of the arylamides, we were typically able

Table 4. Comparison of the Binding for the Enantiomers of **22** to Wild Type and Chimeric D2-like Receptors[a]

	$K_i \pm$ SEM (nM)			
compd	D2 receptor	D2/D3E2 loop	D3 receptor	D3/D2E2 loop
R-**22**	433 ± 30 (1.0)	211 ± 40 (2.1)	1.1 ± 0.2 (394)	8.3 ± 1.8 (52)
S-**22**	715 ± 6.0 (1.0)	389 ± 66 (1.8)	16.6 ± 2.3 (43)	63.7 ± 3.5 (11)

[a] All of the dissociation constants were obtained from competitive radioligand binding studies. K_i values using transfected HEK 293 cells, and ^{125}I-IABN are expressed in nanomolar and are the mean ± the SEM for $n \geq 3$. The number shown in parentheses is the ratio of the K_i values for D2 receptor/receptor.

to keep the cLogP values less than 5 and PSA values in the range of 50−81, predicting drug-like properties.[28,32]

None of the analogues showed high affinity for D4 receptors. In the trans-olefin group (A), all except compound **19** showed high binding affinities for D3 (0.44−2.5 nM) and selectivity over D2 receptors. In the 3-OH-butyl linked group (B) high affinities for D3 were retained, but in several cases, D2 binding affinities were further reduced compared to the trans olefins, and thus, several compounds were 145- to 400-fold selective for D3 over D2 (e.g., **22**, **23**, **25**, **27**−**30**). Most of the cLogP values in this group were within the 2−5 range and, hence, were deemed as good candidates for in vivo exploration. Moreover, several contained 5-F, -I, or -OCH_3 substitutions that might be candidates for future radioligand development (e.g., **27**−**29**). In addition to the in vitro data reported in Tables 2 and 5, all three of these analogues were tested in 63 radioligand/enzyme assays at concentrations of 100 and 10 000 nM, in duplicate. Neither **27** nor **29** inhibited binding activity >50% at either concentration, and **28** showed only modest affinity at α_1 adrenergic receptors (K_i = 115 nM). Thus, these compounds are among the most selective D3 receptor ligands reported to date.

It is noted that significant effort has been directed toward the development of both D3-selective radioligands and potential PET imaging agents.[33−38] However, despite a promising in vitro profile, most of these ligands have proven unsuccessful and there is yet to be a commercially available radiolabeled D3 receptor antagonist for development of non-cell-based binding and functional assays.

Both the 2-hydroxylated analogues showed high D3 affinity and D3 selectivity over the D2-like receptor subtypes of ∼100-fold. However, because of their relatively high D2 affinity, this template will not be pursued toward radioligand development.

All of the hydroxyl-linked compounds are racemic, and thus, it was of interest to attempt to separate an enantiomeric pair and evaluate *R*- and *S*-enantiomers for enantioselectivity in the D2-like family of receptors. The *R*-enantiomer of **22** showed the highest D3 affinity and a remarkable 394-fold D3 receptor selectivity over the D2 receptor subtype. In contrast, the *S*-enantiomer, although D3-selective, was significantly less active at D3 (15-fold) than its *R*-enantiomer and enantioselectivity was less pronounced at D2 (<2-fold). Similar D3 enantioselectivities were recently reported for a series of 4-phenylpiperazine hybrid molecules, although the absolute configuration of these compounds was not described.[39]

Although the precise determination of the molecular basis for the enantioselective binding of the *R*- and *S*-**22** is beyond the scope of the present study, we investigated whether or not these enantiomers could be interacting differentially with the second extracellular loop (E2) of the D2 and D3 receptors. Initial studies on the three-dimensional structure of the bovine rhodopsin protein,[40] and more recently on members of the adrenergic receptors,[41,42] have indicated that the conserved disulfide bond, which joins the conserved cysteine residue located within the E2 loop with the top of the third transmembrane spanning (TMS) region, brings the E2 loop in proximity to the extracellular portion of the helical receptor TMS regions. In addition, several studies have suggested that amino acid residues within the E2 loop of the dopamine receptors can interact with ligands positioned in the neurotransmitter binding site, thereby influencing binding affinity.[43,44]

The primary structure of the D2 and D3 receptor E2 loops exhibits less than 50% homology (Table 3).[45] To test the possibility that the enantioselective binding of the *R*- and *S*-**22** was influenced by the composition of the D2-like receptor E2 loops, we prepared two chimeric receptor proteins: (1) a human D2 receptor with the E2 loop of the D3 receptor (D2/D3E2) and (2) a human D3 receptor with the E2 loop of the D2 receptor subtype (D3/D2E2). A comparison of the affinities of the *R*- and *S*- **22** for the wild type (D2 or D3) receptors and the two chimeric D2-like receptors (D2/D3E2 and D3/D2E2) is shown in Table 4. The binding of both enantiomers of **22** to the D2/D3E2 receptor modestly increased the affinity approximately 2-fold compared to the binding affinity at the wild type D2 receptor. In addition, the substitution of the D2E2 loop onto the D3 receptor scaffold decreased the affinity of the *R*-**22** by 8-fold and *S*-**22** by 4-fold compared to the wild type human D3 receptor. These results suggest that (1) the hydroxylated linking chain of these compounds may be in direct contact with the E2 extracellular loop of D3 and (2) this interaction may play a role in, although it does not fully account for, the enantioselective binding observed. Recently the E2 extracellular loop has been identified as contributing to the allosteric binding of 4-(3-chlorophenyl)carbamoyloxy)-2-butynyltrimethylammonium chloride (McN-A-343), a muscarinic M2 receptor partial agonist that has been described as binding to both allosteric and orthosteric binding sites on M2 muscarinic receptors.[46] Thus, we speculate that direct interaction of our novel analogues at E2 might be through an allosteric binding site that may play a pivotal role in their D3-selectivity.

In Table 5, functional activity data at both D2 and D3 receptors, using the quinpirole-stimulated mitogenesis assay in HEK 293 cells, are shown. In addition, data at the serotonin 1A (5-HT1A) receptor subtype that often shows cross-reactivity with the D3 antagonists for a few of these analogues are shown. None of the compounds showed high affinity for D1 receptors, and all were selective for D3 over the 5-HT1A receptor subtype. It is worth noting that other arylpiperazinylalkyl analogues have been developed as 5-HT1A serotonin ligands recently.[47] Although in that report D3 receptor binding affinities were not assessed, based on data from our studies and others, it appears that SAR between D3 and 5HT1A receptors is separable. Moreover, in another class of D3-preferring antagonists, the serotonergic interactions were determined to have no adverse effects on the desired in vivo profile.[48]

In addition, all of the compounds were D3-selective antagonists or partial agonists and in some cases (e.g., **16**, **25**, **26**) selectivity over D2 receptor mediated mitogenesis was ∼100-fold. In others, D2 activity was not determined because of very low D2 binding affinity (K_i > 400 nM, e.g., **29** and **30**), and they are likely >100-fold D3-selective. Finally, several of these analogues showed potent partial agonist activity in this assay (e.g., *S*-**22**, **27**, **29**). Although partial agonists, determined in vitro, have yet to be differentiated from antagonists in vivo, compounds with both high D3 affinity and selectivity will provide excellent tools with which to further pursue these

Table 5. Additional In Vitro Functional and Binding Data for Selected Compounds[a]

	$IC_{50} \pm$ SEM, nM		$K_i \pm$ SEM, nM	
compd	D_2 mitogenesis	D_3 mitogenesis	D_1 [^{3}H]SCH23390	5-HT_{1A} [^{3}H]8-OH-DPAT
11	118 ± 13.9	9.01 ± 1.36	2100 ± 359	27.9 ± 1.07
12	8.20 ± 1.70	2.39 ± 0.79	2000 ± 350	
13	1030 ± 270	520 ± 180	2920 ± 940	
14	170 ± 57.0	22.9 ± 7.60	900 ± 190	
15	94.7 ± 18.2	4.94 ± 0.26	1195 ± 47.4	198 ± 2.71
16	21.4 ± 7.10	0.22 ± 0.05	800 ± 130	
17	175 ± 2.55	7.00 ± 0.76	931 ± 181	193 ± 42.2
18	111 ± 18.5	18.7 ± 2.75	1460 ± 95.6	58.8 ± 8.91
19	23.0 ± 9.7	14.9 ± 5.4	990 ± 150	
20	254 ± 34.8	9.62 ± 0.63	1730 ± 184	388 ± 46.4
21	58.0 ± 17.0	1.09 ± 0.23	678 ± 56.0	
22	4370 ± 59.4,[c] 171.2 ± 60.5 (22%)[b]	29.9 ± 5.76	4630 ± 1300	104 ± 23.5
R-**22**	ND[d]	18.2 ± 3.50	>10000	
S-**22**	ND[d]	6.60 ± 2.02 (42.2%)[b]	>10000	
23	427 ± 97.1	12.1 ± 2.80	>10000	132 ± 30.0
24	162 ± 57.0	6.51 ± 2.82	4960 ± 140	
25	2160 ± 760	23.9 ± 7.91	>10000	
26	270 ± 100	1.76 ± 0.76	5060 ± 290	
27	ND[d]	38.0 ± 18.0 (26%)[b]	>10000	
28	ND[d]	173 ± 77.1	>10000	
29	ND[d]	1.51 ± 0.35 (34.9%)[b]	>10000	
30	ND[d]	1.58 ± 0.53	>10000	
31	ND[d]	13.7 ± 4.41,[c] 22.8 ± 9.11 (22.6%)[b]	>10000	
32	ND[d]	11.2 ± 3.31	>10000	
33	ND[d]	217 ± 56.0,[c] 84.0 ± 28.0 (23.6%)[b]	>10000	
34	405 ± 21.0	14.5 ± 3.71	3690 ± 700	
35	146.1 ± 6.20	39.1 ± 12.3	6650 ± 630	
36	340 ± 100	25.7 ± 8.12	>10000	58.3 ± 2.81

[a] Unless otherwise noted, data were obtained through the NIDA Addiction Treatment Discovery Program contract with Southern Research Institute (N01DA-1-8816) or Oregon Health & Science University (Y1 DA 5007-05). [b] Percent stimulation compared to standard agonist quinpirole. [c] The first value is the antagonist IC_{50}; the second value is the agonist EC_{50} with % stimulation in parentheses. [d] ND = not determined because of K_i value in the D2 binding assay was >400 nM (by the contractor).

comparisons and to determine whether intrinsic activity at D3 (or lack thereof) is important for therapeutic efficacy.

Selective inhibition of cocaine seeking in rodents was first described with the D3 partial agonist *N*-(4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)-2-naphthamide (BP 897)[11,12] and then followed with several reports using the now prototypic D3 antagonist **38** (SB 277011-A).[8b,13−15] Additional studies showed that not only could the D3 antagonists reduce cocaine-induced and brain stimulation reward but these agents were also able to attenuate cocaine-, cue-, and stress-induced reinstatement of cocaine taking in rodent models of relapse.[2,3] Additional reports using *N*-(4-(4-(2,3-dichlorophenyl)piperazin-1-yl)butyl)-9*H*-fluorene-2-carboxamide (NGB 2904),[15,16,49] as well as studies that have been extended to other drugs of abuse,[3,17] further demonstrate the efficacy of selective D3 antagonists in these animal models of drug reward and addiction. The similar in vivo profiles of the D3 partial agonists to the antagonists have brought into question the correlation of intrinsic activity, as measured in cell-based models, to behavior.[50,51] Nevertheless, other reports of D3 partial agonists suggest that they may indeed have different profiles in vivo than the antagonists[21,52] and that these agents might provide a therapeutic advantage. Recently, D3-preferring full agonists and highly selective partial agonists were reported and evaluated in models of contralateral rotation[39] yawning and body temperature,[53] the latter being models predictive of D3 receptor stimulation.[19,20] It will be very interesting to see how these D3 agonists affect cocaine-induced behaviors in future studies.

Summary

An extension of SAR at D3 receptors has resulted in the discovery of some of the most D3-selective compounds reported to date. Compounds such as **22** and **29** show high affinity (K_i = 1 nM) for D3 and ~400-fold selectivity over the D2 receptor subtype. Importantly, in this study we have identified the first enantioselective D3 antagonists (*R*- and *S*-**22**) to be reported wherein enantioselectivity is more pronounced at D3 than at D2 and that a binding region on the extracellular loop E2 may play a role in both enantioselectivity and D3 vs D2 binding selectivity. These lead compounds also have appropriate physical properties for in vivo exploration and therefore will be useful in determining how intrinsic activity at D3 receptors tested in vitro is related to behavior in animals. Furthermore, these novel and selective D3 antagonists and partial agonists will undoubtedly aid in further determining the role of D3 receptors in addiction and other neuropsychiatric disorders.

Experimental Methods

The ^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury Plus 400 spectrometer. Proton chemical shifts are reported as parts per million (δ ppm) relative to tetramethylsilane (0.00 ppm) as an internal standard. Coupling constants are measured in hertz (Hz). Chemical shifts for ^{13}C NMR spectra are reported as δ relative to the deuterium signal of the solvent ($CDCl_3$, 77.5 ppm; CD_3OD, 49.3 ppm). Combustion analysis was performed by Atlantic Microlab, Inc. (Norcross, GA) and agrees within 0.4% of calculated values. Melting point determination was conducted using a Thomas-Hoover melting point apparatus, and the melting points are uncorrected. Anhydrous solvents were purchased from Aldrich (pyridine, acetonitrile, dichloromethane, chloroform, hydrazine) or JT Baker (diethyl ether) and were used without further purification, except for tetrahydrofuran, which was freshly distilled from sodium benzophenone ketyl. If not stated otherwise, final compounds were purified by column chromatography (silica gel, Merck, 230−400 mesh, 60 Å) or preparative thin layer chromatography (silica gel, Analtech, 1000 μm) using EtOAc/$CHCl_3$ (5:5:1), 1% triethylamine or $CHCl_3$/MeOH (10:1), 1% triethylamine as an eluent. Yields and

reaction conditions are not optimized. Generally, yields and spectroscopic data refer to the free base. With the exception of *R*-**22** and *S*-**22** all hydroxy compounds described in this paper are racemates. Oxalate or HCl salts were prepared and recrystallized as indicated. On the basis of NMR, GC−MS (where obtainable), and combustion analysis data, all final compounds are >95% pure. The procedures to determine the binding affinities at the human dopamine D2-like receptors have been described.[26]

Synthesis. 5-Iodoindole-2-carboxylic Acid Ethyl Ester (3). Adapted from Beshore and Dinsmore,[29] to a stirring solution of indole-2-carboxylic acid (1, 2.5 g, 13 mmol) in EtOH (25 mL) was added I_2 (3.35 g, 13 mmol), $NaIO_3$ (1.42 g, 6.6 mmol), and concentrated H_2SO_4 (1.5 mL). The resulting solution was stirred at reflux for 1.5 h. After the reaction mixture was cooled to room temperature, a solution of saturated aqueous Na_2CO_3 (40 mL) was added. The product was extracted with EtOAc (3 × 40 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and the solvent was removed by distillation to yield the intermediate 3,5-diiodoindole-2-carboxylic acid ethyl ester (**2**) as a yellow solid, which was used without further purification. To a vigorously stirred suspension of this 3,5-diiodo intermediate (**2**) in EtOH (125 mL) and concentrated HCl (11 mL) was added Zn dust (13.5 g, 20.5 mmol) at room temperature over 90 min in 4 equal portions. The mixture was diluted in H_2O (60 mL), and the product was extracted with EtOAc (3 × 60 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and the solvent was removed by distillation. The crude product **3** was recrystallized with EtOAc/hexanes to yield pure product as a white solid (1.2 g). Yield: 28%. ^{1}H NMR (DMSO-d_6) δ 1.34 (t, J = 7.2, 3H), 4.34 (q, J = 7.2, 2H), 7.10 (d, J = 1.2, 1H), 7.30 (d, J = 8.8, 1H), 7.50 (dd, J = 1.6, 8.8, 1H), 8.06 (d, J = 1.6, 1H), 12.07 (s, 1H). ^{13}C NMR (DMSO-d_6) δ 14.9, 61.3, 84.6, 107.4, 115.7, 128.8, 130.1, 131.2, 133.2, 136.9, 161.7.

5-Iodoindole-2-carboxylic Acid (4). 4 was prepared from **3** in a similar manner as described for **8**. Yield: 83%. ^{1}H NMR (DMSO-d_6) δ 7.06 (d, J = 1.2, 1H), 7.29 (d, J = 8.8, 1H), 7.50 (dd, J = 1.2, 8.8, 1H), 8.06 (s, 1H), 11.97 (s, 1H), 13.14 (br s, 1H). ^{13}C NMR (DMSO-d_6) δ 84.5, 107.0, 115.6, 129.9, 130.2, 131.1, 132.9, 136.8, 163.2.

5-Iodobenzofuran-2-carboxylic Acid Ethyl Ester (7). 5-Iodosalicylaldehyde (**5**, 2.18 g, 8.53 mmol) was added to a suspension containing toluene (40 mL), *tert*-butylammonium iodide (320 mg, 0.866 mmol), diethyl bromomalonate (**6**, 2.44 g, 9.39 mmol), and K_2CO_3 (1.75 g, 12.3 mmol). The reaction flask was fitted with a Dean−Stark adapter, and the mixture was stirred at reflux for 36 h. The suspension was filtered, washed with deionized H_2O (20 mL), dried over Na_2SO_4, and concentrated. Flash chromatography ($CHCl_3$/hexanes 3:1) afforded **7** as a yellow solid (1.73 g). Yield: 65%. ^{1}H NMR ($CDCl_3$) δ 1.43 (t, J = 7.2, 3H), 4.45 (q, J = 6.8, 2H), 7.35 (d, J = 8.4, 1H), 7.43 (s, 1H), 7.69 (dd, J = 6.8, 2.0, 1H), 8.02 (d, J = 2.0, 1H). ^{13}C NMR ($CDCl_3$) δ 14.5, 61.9, 87.5, 112.7, 114.6, 129.8, 131.8, 136.4, 146.7, 155.1, 159.4.

5-Iodobenzofuran-2-carboxylic Acid (8). A suspension of **7** (2.2 g, 7.0 mmol) in ethanol (20 mL) and 2 M aqueous KOH (14 mL, 28 mmol) was stirred at reflux for 1 h. The free acid product was obtained by acidifying a hot aqueous solution of the potassium salt of **8** with 10 M aqueous HCl (pH 2−3) and collecting and drying the white precipitated product (1.33 g). Yield: 66%. ^{1}H NMR (DMSO-d_6) δ 7.53 (d, J = 9.2, 1H), 7.55 (s, 1H), 7.75 (dd, J = 1.6, 8.8, 1H), 8.15 (d, J = 1.6, 1H). ^{13}C NMR (DMSO-d_6) δ 88.6, 112.9, 115.2, 130.4, 132.2, 136.2, 136.3, 148.0, 154.9, 160.5.

General Amidation Procedures. Procedure A. CDI (1 equiv) was added to a solution of the carboxylic acid (1 equiv) in THF (10 mL/mmol). The reaction mixture was stirred at room temperature for 2 h. The solution was cooled to 0 °C, and the appropriate amine (1 equiv) was added dropwise. The reaction mixture was allowed to warm to room temperature and stirred for 2−3 h. The solvent was removed in vacuo. The residue was diluted in $CHCl_3$ (30 mL) and washed with saturated aqueous $NaHCO_3$ solution (2 × 10 mL). The organic layer was dried with Na_2SO_4 and concentrated in vacuo. The crude product was purified by crystallization from Et_2O or preparative thin layer chromatography, as indicated.

Procedure B. Thionyl chloride (2 mL/mol) was added to the carboxylic acid (1 equiv). The solution was stirred at reflux for 3 h and concentrated in vacuo. Residual thionyl chloride was removed by azeotropic distillation in dry toluene. The resulting solid was dissolved in amylene stabilized $CHCl_3$ (5 mL). To a stirring solution of amine (1 equiv) in stabilized $CHCl_3$ (20 mL) and 0.5 M aqueous NaOH (8 mL) cooled to 0 °C was added the acid chloride solution dropwise. The solution was stirred vigorously for 3 h at room temperature. The organic layer was separated, dried with Na_2SO_4, and concentrated in vacuo. The crude product was purified by crystallization from Et_2O or preparative thin layer chromatography, as indicated.

***N*-(4-(4-(2,3-Dichlorophenyl)piperazin-1-yl)butyl)-5-iodobenzofuran-2-carboxamide (13). 13** was prepared from 5-iodobenzofuran-2-carboxylic acid (**8**) and 4-(4-(2,3-dichlorophenyl)piperazin-1-yl)butan-1-amine[54,55] according to general procedure B. Yield: 85%. Mp (HCl salt, recrystallized from $CHCl_3$) 265−266 °C. ^{1}H NMR ($CDCl_3$) δ 1.68−1.73 (m, 4H), 2.48 (t, J = 7.2, 2 H), 2.66 (s, br, 4H), 3.08 (s, br, 4H), 3.53 (q, J = 5.6, 2H), 6.91 (dd, J = 2.0, 7.6, 1H), 7.00 (m, 1H), 7.11−7.16 (m, 2H), 7.25 (d, J = 8.4, 1H), 7.38 (s, 1H), 7.66 (dd, J = 1.6, 8.4, 1H), 8.01 (d, J = 1.6, 1H). ^{13}C NMR ($CDCl_3$) δ 24.6, 27.8, 39.5, 51.5, 53.6, 58.2, 87.4, 109.4, 113.8, 118.8, 124.8, 127.7, 130.5, 131.7, 134.3, 135.6, 149.9, 151.4, 154.2, 158.6. Anal. ($C_{23}H_{24}Cl_2IN_3O_2 \cdot HCl \cdot 0.5H_2O$) C, H, N.

5-Iodo-*N*-(4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)benzofuran-2-carboxamide (14). 14 was prepared from 5-iodobenzofuran-2-carboxylic acid (8) and 4-(4-(2-methoxyphenyl)piperazin-1-yl)butan-1-amine[57] according to general procedure A. Yield: 49%. Mp (oxalate, recrystallized from EtOAc) 151−154 °C. ^{1}H NMR ($CDCl_3$) δ 1.65−1.73 (m, 4H), 2.48 (t, J = 7.2, 2H), 2.68 (s, br, 4H), 3.12 (s, br, 4H), 3.53 (q, J = 5.6, 2H), 3.86 (s, 3H), 6.85 (d, J = 7.6, 1H), 6.91−6.93 (m, 2H), 6.98−7.01 (m, 1H), 7.07 (m, 1H), 7.22 (d, J = 8.8, 1H), 7.37 (s, 1H), 7.64 (dd, J = 2.0, 8.4), 8.00 (d, J = 1.6, 1H). ^{13}C NMR ($CDCl_3$) δ 24.6, 27.7, 39.5, 50.84, 53.7, 55.6, 58.2, 87.4, 109.4, 111.4, 113.9, 118.4, 121.2, 123.2, 130.5, 131.7, 135.6, 141.5, 149.9, 152.5, 154.2, 158.6. Anal. ($C_{24}H_{28}IN_3O_3 \cdot 2(COOH)_2$) C, H, N.

1*H*-Indole-2-carboxylic Acid (4-[4-(2,3-Dichlorophenyl)piperazin-1-yl]-*trans*-but-2-enyl)amide (17). 17 was prepared from 1*H*-indole-2-carboxylic acid and 4-(4-(2,3-chlorophenyl)-piperazin-1-yl)-*trans*-but-2-enylamine[26] according to general procedure A. Yield: 47%. Mp (HCl salt, recrystallized from methanol/diethyl ether) 266−268 °C (dec). ^{1}H NMR ($CDCl_3$) δ 2.65 (s, 4H), 3.06−3.10 (m, 6H), 4.16 (m, 2H), 5.80−5.82 (m, 2H), 6.39 (t, J = 5.6, 1H), 6.86 (m, 1H), 6.93 ("dd", J'' = 7.2, 2.5, 1H), 7.11−7.17 (m, 3H), 7.28 (m, 1H), 7.45 (dd, J = 8.2, 0.8, 1H), 7.64 (dd, J = 8.2, 0.8, 1H), 9.87 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 41.6, 51.7, 53.7, 60.6, 102.6, 112.5, 119.1, 121.1, 122.4, 125.0, 125.1, 127.9, 128.0, 129.7, 130.0, 131.0, 134.5, 136.9, 151.6, 162.0. Anal. ($C_{23}H_{24}Cl_2N_4O \cdot HCl \cdot 1.5H_2O$) C, H, N.

Benzo[*b*]furan-2-carboxylic Acid (4-[4-(2-Methoxyphenyl)piperazin-1-yl]-*trans*-but-2-enyl)amide (19). 19 was prepared from benzo[*b*]furan-2-carboxylic acid and 4-(4-(2-methoxyphenyl)piperazin-1-yl)-*trans*-but-2-enylamine[26] according to general procedure A. Yield: 67%. Mp (HCl salt, recrystallized from methanol/diethyl ether) 210−212 °C (dec). ^{1}H NMR ($CDCl_3$) δ 2.66 (s, 4H), 3.06−3.15 (m, 6H), 3.85 (s, 3H), 4.12 (t, J = 5.3, 2H), 5.75−5.86 (m, 2H), 6.78 (t, J = 5.0, 1H), 6.86 (d, 7.5, 1H), 6.91−7.20 (m, 4H), 7.28 (m, 1H), 7.42 (t, J = 7.5, 1H), 7.47−7.53 (m, 2H), 7.66 (d, J = 7.0, 1H). ^{13}C NMR ($CDCl_3$) δ 41.0, 50.8, 53.6, 55.5, 60.5, 110.8, 112.0, 112.3, 118.4, 121.2, 123.0, 123.2, 123.9, 127.1, 127.8, 129.4, 129.5, 141.5, 148.9, 152.5, 154.9, 158.9. Anal. ($C_{24}H_{27}N_3O_3 \cdot 2HCl \cdot H_2O$) C, H, N.

Benzo[*b*]thiophene-2-carboxylic Acid (4-[4-(2-Methoxyphenyl)piperazin-1-yl]-*trans*-butenyl)amide (21). 21 was prepared from benzo[*b*]thiophene-2-carboxylic acid and 4-(4-(2-methoxyphenyl)piperazin-1-yl)-*trans*-but-2-enylamine according to general

procedure A. Yield: 51%. Mp (HCl salt, recrystallized from methanol/diethyl ether) 225−226 °C. ^{1}H NMR ($CDCl_3$) δ 2.62 (m, 4H), 3.04−3.14 (m, 6H), 3.85 (s, 3H), 4.10 (t, J = 3.9, 2H), 5.74−5.82 (m, 2H), 6.52 (t, J = 5.4, 1H), 6.86 (d, J = 7.8, 1H), 6.89−6.95 (m, 2H), 6.99 (m, 1H), 7.35−7.44 (m, 2H), 7.76−7.86 (m, 3H). ^{13}C NMR ($CDCl_3$) δ 41.9, 50.8, 53.6, 55.6, 60.5, 111.6, 118.4, 121.2, 122.9, 123.2, 125.1, 125.3, 125.5, 126.6, 129.5, 129.6, 138.6, 139.3, 141.0, 141.4, 152.4, 162.4. Anal. ($C_{24}H_{27}N_3O_2S\cdot 2HCl\cdot 0.5H_2O$) C, H, N.

***N*-(4-(4-(2,3-Dichlorophenyl)piperazin-1-yl)-3-hydroxybutyl)-1*H*-indole-2-carboxamide (22). 22** was prepared from 1*H*-indole-2-carboxylic acid and 4-amino-1-(4-(2,3-dichlorophenyl)piperazin-1-yl)butan-2-ol[27] according to general procedure A. Yield: 72%. Mp (oxalate salt, recrystallized from EtOH) 212−214 °C. ^{1}H NMR ($CDCl_3$) δ 1.64 (m, 1H), 1.83 (m, 1H), 2.46 (m, 2H), 2.62 (s, 2H), 2.88 (dd, J = 10.0, 4.7, 2H), 3.08 (s, 4H), 3.51 (ddd, J = 12.7, 8.1, 3.9, 1H), 3.92 (m, 2H), 6.85 (dd, J = 2.1, 0.8, 1H), 6.95 (dd, J = 7.0, 2.6, 1H), 7.11−7.18 (m, 3H), 7.27 (ddd, J = 8.2, 7.0, 1.2, 1H), 7.41 (m, 1H), 7.45 (dd, J = 8.3, 0.9, 1H), 7.64 (dd, J = 8.0, 0.8), 9.51 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 33.4, 38.2, 51.5, 53.3, 63.8, 66.5, 101.9, 112.0, 118.7, 120.6, 121.9, 124.3, 124.8, 127.6, 127.6, 127.8, 131.2, 134.2, 136.3, 151.1, 161.7. Anal. ($C_{23}H_{26}Cl_2N_4O_2\cdot(COOH)_2\cdot 0.25H_2O$) C, H, N.

(*R*)-*N*-(4-(4-(2,3-Dichlorophenyl)piperazin-1-yl)-3-hydroxybutyl)-1*H*-indole-2-carboxamide (*R*-22). A solution of 27 mg (44 μmol) of (*R*,*R*)-*N*,*N*′-bis(3,5-di-*tert*-buytlsalicylidene)-1,2-cyclohexanediaminocobalt(II) (Aldrich) in 5 mL of anhydrous THF was treated with 2-(2-bromoethyl)oxirane[56] (3.32 g, 22.0 mmol) and HOAc (15 μL, 270 μmol), followed by H_2O (220 μL, 12.2 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature overnight. After this time 10 mL of DMF and 3.70 g (20.0 mmol) of phthalimide potassium salt were added and stirring at room temperature was continued for another 72 h. The reaction mixture was diluted with 50 mL of ethyl acetate and washed 3× with 10 mL of H_2O. The organics were dried with Na_2SO_4 and concentrated in vacuo. Flash chromatography ($CHCl_3$/EtOAc 3:1) yielded 2.07 g (21%) of 2-(3-hydroxy-4-(4-(2-propoxyphenyl)piperazin-1-yl)butyl)isoindoline-1,3-dione which was converted into the title compound in a similar manner as described for racemic **22**. The optical purity of *R*-**22** was determined by HPLC analysis (Daicel Chiralcel OD, hexane/*i*-PrOH = 7:3, flow rate = 1 mL/min, t_R = 2.1 min) to be >90% ee (Supporting Information). The product appeared to be somewhat acid sensitive. Attempts to increase the optical purity by crystallization with tartaric acid or camphorsulfonic acid failed because of partial racemization. Mp (free base) 204−206 °C. Anal. ($C_{23}H_{26}Cl_2N_4O_2$) C, H, N.

(*S*)-*N*-(4-(4-(2,3-Dichlorophenyl)piperazin-1-yl)-3-hydroxybutyl)-1*H*-indole-2-carboxamide (*S*-22). *S*-**22** was prepared from commercially available (*S*)-2-(2-bromoethyl)oxirane (Aldrich), using the procedure described for racemic **22**. The optical purity of *S*-22 was determined by HPLC analysis (Daicel Chiralcel OD, hexane/*i*-PrOH = 7:3, flow rate = 1 mL/min, t_R = 6.8 min) > 99% ee. Mp (free base) 206−208 °C. Its absolute configuration was confirmed by X-ray crystallography (see below). Anal. ($C_{23}H_{26}Cl_2N_4O_2$) C, H, N.

***N*-(3-Hydroxy-4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)-1*H*-indole-2-carboxamide (23). 23** was prepared from 1*H*-indole-2-carboxylic acid and 4-amino-1-(4-(2-methoxyphenyl)piperazin-1-yl)butan-2-ol according to general procedure A. Yield: 17%. Mp (oxalate salt recrystallized from absolute EtOH) 140−142 °C. ^{1}H NMR ($CDCl_3$) δ 1.64 (m, 1H), 1.86 (m, 1H), 2.43 (d, J = 6.7, 2H), 2.87 (m, 2H), 3.09 (s, 4H), 3.52 (m, 1H), 3.85 (s, 3H), 3.93 (m, 2H), 4.07 (s, br1H), 6.86 (d, J = 7.8, 1H), 6.88 (d, J = 0.8, 1H), 6.92 (m, 2H), 7.00 (m, 1H), 7.13 (m, 1H), 7.26 (m, 1H), 7.45 (dd, J = 8.2, 0.8, 1H), 7.56 (dd, J = 6.3, 3.9, 1H), 7.62 (d, 8.2, 1H). ^{13}C NMR ($CDCl_3$) δ 38.1, 50.8, 53.4, 55.4, 63.9, 66.4, 102.2, 111.3, 112.2, 118.3, 120.5, 121.1, 121.9, 123.2, 124.2, 127.8, 131.3, 136.5, 141.1, 152.3, 161.9. Anal. ($C_{24}H_{30}N_4O_3\cdot(COOH)_2\cdot 0.5H_2O$) C, H, N.

***N*-(3-Hydroxy-4-(4-(2-propoxyphenyl)piperazin-1-yl)butyl)-1*H*-indole-2-carboxamide (24).** (a) To obtain 1-amino-4-(4-(2-propoxyphenyl)piperazin-1-yl)butan-3-ol, 1.0 g (4.6 mmol) of 1-(2-propoxyphenyl)piperazine in 25 mL 2-propanol was reacted with 0.75 g (5.0 mmol) of 2-(2-(oxiran-2-yl)ethyl)isoindoline-1,3-dione in the microwave cavity (pressure vessel, 300 W, cooling, 110 °C, 15 min). The solvent was removed in vacuo, and the foamy residue was washed twice with 5 mL of 2-propanol to yield 1.2 g (77%) of 2-(3-hydroxy-4-(4-(2-propoxyphenyl)piperazin-1-yl)butyl)isoindoline-1,3-dione, which was used without further characterization. This intermediate (1.0 g, 2.3 mmol) was fully dissolved in 20 mL of warm ethanol and treated with 0.3 g (6.0 mmol) of hydrazine hydrate in the microwave (pressure vessel, 300 W, cooling, 100 °C, 15 min). The cooled reaction mixture was filtered, and the filtrate was evaporated in vacuo. Both the distillation residue and the initial precipitate were partitioned between $CHCl_3$ and 20% aqueous K_2CO_3 solution. The layers were separated and the aqueous layer was dried with Na_2SO_4 to give the amine as an oil. Yield: 0.48 g (69%). ^{1}H NMR ($CDCl_3$) δ 1.05 (t, J = 7.0, 3H), 1.71 (m, 1H), 1.76−1.91 (m, 3H), 2.46 (m, 2H), 2.61 (s, br, 2H), 2.79−2.95 (m, 3H), 3.11 (m, br, 6H), 5.62 (s, br, 2H), 6.82−6.98 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 11.1, 22.9, 34.1, 38.8, 50.8, 53.8, 64.2, 66.2, 69.7, 112.4, 118.3, 121.1, 123.0, 141.3, 151.8.)

(b) The above amine was reacted with 1*H*-indole-2-carboxylic acid and according to general procedure A. Yield: 48%. Mp (oxalate salt, EtOH) 186−188 °C. ^{1}H NMR ($CDCl_3$) δ 1.08 (t, J = 7.4, 3H), 1.64 (m, 1H), 1.79−1.90 (m, 3H), 2.44 (m, 2H), 2.61 (m, 2H), 2.88 (m, 4H), 3.14 (s, 4H), 3.51 (m, 1H), 3.84−3.94 (m, 2H), 3.95 (t, J = 7.6, 2H), 6.84 (m, 2H), 6.92 (m, 2H), 6.97 (m, 1H), 7.12 (t, J = 7.4, 1H), 7.26 (t, J = 7.4, 1H), 7.46 (d, J = 8.6, 1H), 7.50 (s, br, 1H), 7.63 (d, J = 7.8, 1H), 9.72 (s, 1H). Anal. ($C_{26}H_{34}N_4O_3\cdot 1.5(COOH)_2\cdot 0.25H_2O$) C, H, N.

***N*-(4-(4-(2,3-Dichlorophenyl)piperazin-1-yl)-3-hydroxybutyl)-5-fluoro-1*H*-indole-2-carboxamide (25). 25** was prepared from 5-fluoro-1*H*-indole-2-carboxylic acid and 4-amino-1-(4-(2,3-dichlorophenyl)piperazin-1-yl)butan-2-ol according to general procedure A. Yield: 64%. Mp (HCl salt recrystallized from absolute EtOH) 244−246 °C. ^{1}H NMR (DMSO-d_6) δ 1.49−1.54 (m, 1H), 1.74−1.81 (m, 1H), 2.39−2.40 (m, 2H), 2.58 (s, br, 4H), 2.94 (s, br, 4H), 3.30−3.47 (m 2H), 3.72 (s, br, 1H), 4.50 (s, br, 1H), 7.01 (dt, J = 2.4, 9.6, 1H), 7.08−7.10 (m, 2H), 7.25−7.27 (m, 2H), 7.35−7.41 (m, 2H), 8.50 (t, J = 5.6, 1H), 11.66 (s, 1H). ^{13}C NMR (DMSO-d_6) δ 36.0, 36.8, 51.7, 54.1, 65.1, 66.1, 102.9, 106.3 (d, J = 23), 112.5 (d, J = 25), 114.1 (d, J = 11), 120.2, 125.0, 126.7, 128.9 (d, J = 11), 129.1, 133.3, 133.8, 134.3, 151.9, 157.8 (d, J = 230), 161.44. Anal. ($C_{23}H_{25}Cl_2FN_4O_2\cdot HCl\cdot 0.25H_2O$) C, H, N.

5-Fluoro-*N*-(3-hydroxy-4-(4-(2-methoxy-phenyl)piperazin-1-yl)butyl)-1*H*-indole-2-carboxamide (26). 26 was prepared from 5-methoxy-1*H*-indole-2-carboxylic acid and 4-amino-1-(4-(2-methoxyphenyl)piperazin-1-yl)butan-2-ol according to general procedure A. Yield: 47%. Mp (free base) 158−160 °C. ^{1}H NMR (DMSO-d_6) δ 1.51−1.55 (m, 1H), 1.79−1.81 (m, 1H), 2.32 (m, 2H), 2.54 (s, br, 4H), 2.91 (s, br, 4H), 3.34−3.47 (m, 3H), 3.73 (s, 3H), 4.48 (s, 1H), 6.82−6.89 (m, 3H), 6.99−7.10 (m, 3 H), 7.36−7.43 (m, 2H), 8,53 (s, br, 1H), 11.69 (s, br, 1H). ^{13}C NMR (DMSO-d_6) δ 36.0, 36.9, 50.8, 54.3, 55.9, 65.3, 66.1, 102.9 (d, J = 5), 106.3 (d, J = 23, 1C), 112.5 (d, J = 26), 112.5, 114.2 (d, J = 10), 118.5, 121.5, 123.0, 127.9 (d, J = 11), 133.8, 134.4, 142.0, 152.6, 157.8 (d, J = 230) 161.5. Anal. ($C_{24}H_{29}FN_4O_3$) C, H, N.

***N*-(4-(4-(2,3-Dichlorophenyl)piperazin-1-yl)-3-hydroxybutyl)-5-iodo-1*H*-indole-2-carboxamide (27). 27** was prepared from 5-iodo-1*H*-indole-2-carboxylic acid (**4**) and 4-amino-1-(4-(2,3-dichlorophenyl)piperazin-1-yl)butan-2-ol according to general procedure A. Yield: 70%. Mp (free base) 224−226 °C. ^{1}H NMR (DMSO-d_6) δ 1.51−1.55 (m, 1H), 1.79−1.81 (m, 1H), 2.30−2.35 (m, 2H), 2.58 (s, br, 4H), 2.94 (s, br, 4H), 3.34−3.46 (m, 2H), 3.74 (s, 1H), 4.52 (d, J = 2.4, 1H), 7.06−7.09 (m, 2H), 7.27 (d, J = 4.8, 3H), 7.40 (d, J = 8.0, 1H), 8.00 (s, 1H), 8.58 (s, J = 4.8, 1H), 11.76 (s, br, 1H). ^{13}C NMR (DMSO-d_6) δ 36.0, 36.9, 51.7, 54.1, 65.1, 66.1, 84.1, 102.1, 115.4, 120.2, 125.0, 126.7, 129.1,

130.5, 130.6, 131.8, 133.3, 133.5, 136.0, 151.9, 161.4. Anal. ($C_{23}H_{25}Cl_2IN_4O_2 \cdot 0.5H_2O$) C, H, N.

***N*-(3-Hydroxy-4-(4-(2-methoxy-phenyl)piperazin-1-yl)butyl)-5-iodo-1*H*-indole-2-carboxamide (28). 28** was prepared from 5-iodo-1*H*-indole-2-carboxylic acid (**4**) and 4-amino-1-(4-(2-methoxyphenyl)piperazin-1-yl)butan-2-ol according to general procedure A. Yield: 65%. Mp (free base) 205−209 °C. ^{1}H NMR (DMSO-d_6) δ 1.51−1.55 (m, 1H), 1.79−1.81 (m, 1H), 2.27−2.36 (m, 2H), 2.54 (s, br, 4H), 2.92 (s, br, 4H), 3.36 (s, 2H), 3.43−3.46 (m, 1H), 3.74 (s, 3H), 4.47 (d, J = 4.0, 1H), 6.81−6.87 (m, 4H), 6.91 (s, 1H), 7.28 (d, J = 8.8, 1H), 7.40 (dd, J = 1.6, 8.8, 1H), 8.00 (s, 1H), 8.55 (t, J = 1.6, 1H), 11.77 (s, 1H). ^{13}C NMR (DMSO-d_6) δ 36.0, 36.9, 50.8, 54.4, 56.0, 65.3, 66.1, 84.1, 102.0, 112.5, 115.4, 118.5, 121.5, 123.0, 130.5, 130.6, 131.8, 133.5, 136.0, 142.0, 152.6, 161.3. Anal. ($C_{24}H_{29}IN_4O_3$) C, H, N.

***N*-(4-(4-(2,3-Dichlorophenyl)piperazin-1-yl)-3-hydroxybutyl)-5-methoxy-1*H*-indole-2-carboxamide (29). 29** was prepared from 5-methoxy-1*H*-indole-2-carboxylic acid and 4-amino-1-(4-(2,3-dichlorophenyl)piperazin-1-yl)butan-2-ol according to general procedure A. Yield: 63%. Mp (free base) 211−215 °C. ^{1}H NMR (DMSO-d_6) δ 1.51−1.54 (m, 1H), 1.77−1.79 (m, 1H), 2.34 (m, 2H), 2.58 (s, br, 4H), 2.94 (s, br, 4H), 3.34 (m, 2H), 3.43 (m, 1H), 3.73 (s, 3H), 4.51 (d, J = 3.6, 1H), 6.80 (dd, J = 8.6, 1.6, 1H), 7.00−7.09 (m, 3H), 7.25−7.31 (m, 3H), 8.40 (t, J = 5.2, 1H), 11.39 (s, 1H). ^{13}C NMR (DMSO-d_6) δ 36.1, 36.8, 51.7, 54.1, 55.9, 65.1, 66.2, 102.6, 113.8, 115.0, 120.2, 125.0, 126.7, 128.1, 129.1, 132.3, 132.9, 133.3, 151.9, 154.4, 161.8. Anal. ($C_{24}H_{28}Cl_2N_4O_3$) C, H, N.

***N*-(3-Hydroxy-4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)-5-methoxy-1*H*-indole-2-carboxamide (30). 30**was prepared from 5-methoxyindole-2-carboxylic acid and 4-amino-1-(4-(2-methoxyphenyl)piperazin-1-yl)butan-2-ol according to general procedure A. Yield: 70%. Mp (free base) 207−209 °C. ^{1}H NMR (DMSO-d_6) δ 1.48−1.53 (m, 1H), 1.72−1.79 (m, 1H), 2.34 (s, br, 2H), 2.56 (s, br, 4H), 3.35−3.43 (m, 2H), 2.93 (s, br, 4H), 3.72 (s, br, 1H), 3.73 (s, 3H), 3.74 (s, 3H), 4.50 (s, br, 1H), 6.79−6.92 (m, 5H), 7.05 (m, 2H), 7.30 (d, J = 9.2, 1H), 8.40 (s, br, 1H), 11.38 (s, br, 1H). ^{13}C NMR (DMSO-d_6) δ 36.1, 36.8, 50.7, 54.3, 55.9, 56.0, 65.2, 66.0, 102.6, 112.6, 113.8, 115.0, 118.5, 121.5, 123.0, 128.1, 132.3, 132.9, 141.9, 152.64, 154.4, 161.8. Anal. ($C_{25}H_{32}N_4O_4 \cdot 0.5H_2O$) C, H, N.

***N*-(4-(4-(2,3-Dichlorophenyl)piperazin-1-yl)-3-hydroxybutyl)-benzofuran-2-carboxamide (31). 31** was prepared from benzofuran-2-carboxylic acid and 4-amino-1-(4-(2,3-dichlorophenyl)piperazin-1-yl)butan-2-ol according to general procedure A. Yield: 45%. Mp (HCl salt recrystallized from abs. EtOH) 209−212 °C. ^{1}H NMR (DMSO-d_6) δ 1.51−1.55 (m, 1H), 1.76−1.79 (m, 1H), 2.30−2.34 (m, 2H), 2.58 (s, br, 4H), 2.95 (s, br, 4H), 3.33−3,44 (m, 2H), 3.72 (s, br, 1H), 4.53 (s, br, 1H), 7.07−7.10 (m, 1H), 7.26−7.27 (m, 2H), 7.31 (t, J = 8.0, 1H), 7.43 (t, J = 7.8, 1H), 7.51 (s, 1H), 7.61 (d, J = 8.0, 1H), 7.34 (d, J = 8.0, 1H), 8.69 (t, J = 2.6, 1H). ^{13}C NMR (DMSO-d_6) δ 35.7, 36.8, 51.6, 54.1, 65.0, 66.3, 109.8, 112.4, 120.2, 123.4, 124.3, 125.0, 126.7, 127.4, 127.9, 129.1, 133.3, 150.0, 151.9, 154.8, 158.7. Anal. ($C_{23}H_{25}Cl_2N_3O_3 \cdot HCl \cdot 0.5H_2O$) C, H, N.

***N*-(3-Hydroxy-4-(4-(2-methoxy-phenyl)piperazin-1-yl)butyl)-benzofuran-2-carboxamide (32). 32** was prepared from benzofuran-2-carboxylic acid and 4-amino-1-(4-(2-methoxyphenyl)piperazin-1-yl)butan-2-ol according to general procedure A. Yield: 77%. Mp (HCl salt recrystallized from EtOH/2-PrOH) 218−222 °C. ^{1}H NMR ($CDCl_3$) δ 1.61−1.67 (m, 1H), 1.82−1.86 (m, 1H), 2.44−2.46 (m, 2H), 2.62 (s, br, 4 H), 2.88−2.91 (m, 2H), 3.11 (s, br, 4H), 3.49−3.55 (m, 1H), 3.89 (s, 3H), 3.84−3.95 (m, 1H), 6.86 (d, J = 7.6, 1H), 6.93−6.95 (m, 2H), 6.99−7.03 (m, 1H), 7.28 (t, J = 7.8, 1H), 7.40 (t, J = 7.8, 1H), 7.45 (s, 1H), 7.50 (d, J = 8.2, 1H), 7.54 (s, br, 1H), 7.65 (d, J = 7.8, 1H). ^{13}C NMR ($CDCl_3$) δ 33.8, 37.7, 51.0, 53.6, 55.6, 64.0, 66.2, 110.3, 111.4, 112.0, 118.4, 121.2, 122.9, 123.3, 123.8, 126.9, 127.9, 141.3, 149.3, 152.5, 155.0, 159.2. Anal. ($C_{24}H_{29}N_3O_4 \cdot 2HCl$) C, H, N.

***N*-(4-(4-(2,3-Dichlorophenyl)piperazin-1-yl)-3-hydroxybutyl)-5-iodobenzofuran-2-carboxamide (33). 33** was prepared from 5-iodobenzofuran-2-carboxylic acid (**8**) and 4-amino-1-(4-(2,3-dichlorophenyl)piperazin-1-yl)butan-2-ol according to general procedure B. Yield: 78%. Mp (HCl salt recrystallized from EtOH) 251−254 °C. ^{1}H NMR (DMSO-d_6) δ 1.50−1.54 (m, 1H), 1.76−1.77 (s, br, 1H), 2.30−2.37 (m 2H), 2.58 (s, br, 4H), 2.94 (s, br, 4 H), 3.35−3.42 (m, 2H), 3.72 (s, br, 1H), 4.52 (s, br, 1H), 7.07−7.10 (m, 1H), 7.26−7.27 (m, 2H), 7.46−7.49 (m, 2H), 7.70 (dd, J = 1.6, 8.8, 1H), 8.15 (d, J = 1.6, 1H), 8.74 (t, J = 5.2, 1H). ^{13}C NMR (DMSO-d_6) δ 35.7, 36.9, 51.6, 54.1, 65.0, 66.2, 88.5, 108.9, 114.9, 120.2, 125.0, 126.7, 129.1, 130.7, 131.9, 133.3, 135.5, 150.7, 151.9, 154.2, 158.3. Anal. ($C_{23}H_{24}Cl_2IN_3O_3 \cdot HCl \cdot 0.5H_2O$) C, H, N.

***N*-(3-Hydroxy-4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)-5-iodobenzofuran-2-carboxamide (34). 34** was prepared from 5-iodobenzofuran-2-carboxylic acid (**8**) and 4-amino-1-(4-(2-methoxyphenyl)piperazin-1-yl)butan-2-ol according to general procedure B. Yield: 63%. Mp (HCl salt recrystallized from MeOH) 239−240 °C. ^{1}H NMR (DMSO-d_6) δ 1.49−1.53 (m, 1H), 1.76−1.78 (m, 1H), 2.27−2.36 (m, 2H), 2.30 (dq, J = 7.2, 12.8, 2H), 2.54 (s, br, 4H), 2.91 (s, br, 4H), 3.37−3.42 (m, 2H), 3.72 (s, 1H), 3.74 (s, 3H), 3.48 (d, J = 4.4, 1H), 6.81−6.86 (m, 2H), 6.88−6.91 (m, 2H), 7.46−7.49 (m, 2H), 7.70 (dd, J = 1.6, 8.8, 1H), 8.14 (d, J = 1.6,1H), 8.74 (t, 5.2, 1H). ^{13}C NMR (DMSO-d_6) δ 35.7, 36.9, 50.8, 54.3, 56.0, 65.2, 6 2,3Cl-2-hydroxy 6.2, 88.5, 108.9, 112.6, 114.9, 118.5, 121.5, 123.0, 130.8, 131.9, 135.5, 142.0, 150.7, 152.6, 154.2, 158.3. Anal. ($C_{24}H_{28}IN_3O_4 \cdot 2HCl$) C, H, N.

***N*-(3-Hydroxy-4-(4-(2-methoxy-phenyl)piperazin-1-yl)butyl)-benzo[*b*]thiophene-2-carboxamide (35). 35** was prepared from benzo[*b*]thiophene-2-carboxylic acid and 4-amino-1-(4-(2-methoxyphenyl)piperazin-1-yl)butan-2-ol according to general procedure A. Yield: 62%. Mp (oxalate salt, EtOH) 214−216 °C. ^{1}H NMR ($CDCl_3$) δ 1.63 (m, 1H), 1.87 (m, 1H), 3.05 (m, 2H), 3.11 (d, J = 3.9, 2H), 3.38 (s, br, 8H), 3.83 (s, 3H), 4.46 (m, 1H), 6.83−6.89 (m, 3H), 7.05 (td, J = 7.4, 1.2, 1H), 7.29 (t, J = 7.8, 1H), 7.34 (t, J = 7.0, 1H), 7.77 (t, J = 7.6, 2H), 8.16 (s, 1H), 8.37 (s, br, 1H). ^{13}C NMR ($CDCl_3$) δ 34.6, 36.3, 47.6, 53.8, 55.7, 63.2, 63.4, 111.5, 118.9, 121.4, 122.8, 124.6, 125.0, 125.5, 125.9, 126.3, 139.0, 139.3, 139.6, 141.2, 152.2, 163.4. Anal. ($C_{24}H_{29}N_3O_3S \cdot (COOH)_2 \cdot 0.25H_2O$) C, H, N.

1*H*-Indole-2-carboxylic Acid (4-[4-(2,3-Dichlorophenyl)piperazin-1-yl]-2-hydroxybutyl)amide (36). 36 was prepared from 1*H*-Indole-2-carboxylic acid and 1-amino-4-(4-(2,3-dichlorophenyl)piperazin-1-yl)butan-2-ol according to general procedure A. Yield: 40%. Mp (HCl salt recrystallized from EtOAc/2-PrOH) 226−228 °C. ^{1}H NMR ($CDCl_3$) δ 1.72 (m, 2H), 2.50−2.86 (m, 6H), 3.06 (s, 4H), 3.36 (m, 1H), 3.59 (m, 1H), 3.83 (s, 3H), 3.95 (m, 1H), 5.92 (s, br, 1H), 6.83−6.90 (m, 3H), 6.94 (m, 1H), 6.96 (m, 1H), 7.11 (s, 1H), 7.17 (t, J = 7.8, 1H), 7.46 (d, J = 7.8, 1H), 7.59 (d, J = 7.8, 1H), 8.07 (t, J, 5.4, 1H). ^{13}C NMR ($CDCl_3$) δ 10.8, 22.7, 26.2, 33.4, 37.9, 50.4, 53.5, 63.7, 66.1, 69.5, 102.2, 111.9, 112.2, 118.1, 120.5, 120.9, 121.9, 123.0, 124.2, 127.7, 131.1, 136.2, 140.8, 151.6, 161.7. Anal. ($C_{23}H_{26}Cl_2N_4O_2 \cdot HCl \cdot 0.5H_2O$) C, H, N.

1*H*-Indole-2-carboxylic Acid (2-Hydroxy-4-[4-(2-methoxyphenyl)piperazin-1-yl]butyl)amide (37). 37 was prepared from 1*H*-Indole-2-carboxylic acid and 1-amino-4-(4-(2-methoxyphenyl)piperazin-1-yl)butan-2-ol according to general procedure A. Yield: 51%. Mp (HCl salt, recrystallized from methanol/diethyl ether) 210−212 °C (dec). ^{1}H NMR ($CDCl_3$) δ 1.58 (d, J = 14.0, 1H), 1.79 (m, 1H), 2.60 (s, 2H), 2.71 (m, 2H), 2.83 (s, 2H), 3.07 (s, 4H), 3.41 (m, 1H), 3.73 (m, 1H), 3.84 (s, 3H), 4.08 (s, 1H), 6.85 (d, J = 8.2, 1H), 7.42 (d, J = 8.2, 1H), 7.61−7.69 (m, 2H), 8.62 (d, J = 4.0, 1H), 10.19 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 28.8, 45.4, 50.7, 53.4, 55.4, 57.4, 72.6, 102.7, 111.2, 112.1, 118.3, 120.5, 121.1, 122, 123.3, 123.9, 124.3, 127.7, 131, 135.3, 136.2, 136.6, 140.9, 149.8, 152.3, 162.2. Anal. ($C_{24}H_{30}N_4O_3 \cdot HCl \cdot H_2O$) C, H, N.

X-ray Crystal Structure of *S*-22. Single-crystal X-ray diffraction data on compound *S*-**22** were collected at 123 K using Mo Kα radiation and a Bruker APEX II CCD area detector. A 0.53 × 0.18 × 0.07 mm^3 crystal was prepared for data collection coating with high viscosity microscope oil (Paratone-N, Hampton Research). The oil-coated crystal was mounted on a glass rod and transferred

immediately to the cold stream on the diffractometer. The crystal was triclinic in space group $P1$ with unit cell dimensions a = 5.6352(3) Å, b = 18.4468(9) Å, c = 22.5318(11) Å, α = 71.273(1)°, β = 86.514(1)°, and γ = 88.997(1)°. Corrections were applied for Lorentz, polarization, and absorption effects. Data were 98.4% complete to 25.0° θ (approximately 0.75 Å) with an average redundancy of 2.0. The structure was solved by direct methods and refined by full-matrix least-squares on F^2 values using the programs found in the SHELXTL suite[59] (Bruker, SHELXTL, version 6.10, 2000, Bruker AXS Inc., Madison, WI). Parameters refined included atomic coordinates and anisotropic thermal parameters for all non-hydrogen atoms. Hydrogen atoms on carbons were included using a riding model [coordinate shifts of C applied to H atoms] with C−H distance set at 0.96 Å. The absolute configuration was determined from anomalous scattering (Flack parameter = 0.01(5)).[60] Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, U.K. [fax, +44(0)-1223-336033; e-mail, deposit@ccdc.cam.ac.uk].

Radioligand Dopamine Receptor Binding Assays. A filtration binding assay was used to characterize the binding properties of membrane-associated receptors. For human D2L, D3, and D4 dopamine receptors expressed in HEK 293 cells, tissue homogenates (50 mL) were suspended in 50 mM Tris-HCl/150 mM NaCl/10 mM EDTA buffer, pH 7.5, and incubated with 50 μL of ^{125}I-IABN at 37 °C for 60 min. Nonspecific binding was determined using 25 μM (+)-butaclamol. For competition experiments, the radioligand concentration was generally equal to 0.5 times the K_d value, and the concentration of the competitive inhibitor ranged over 5 orders of magnitude. Binding was terminated by the addition of cold wash buffer (10 mM Tris-HCl/150 mM NaCl, pH 7.5) and filtration over glass-fiber filters (Schleicher and Schuell no. 32). Filters were washed with 10 mL of cold buffer, and the radioactivity was measured using a Packard Cobra γ counter. Estimates of the equilibrium dissociation constant and maximum number of binding sites were obtained using unweighted nonlinear regression analysis of data modeled according to the equation describing mass action binding. Data from competitive inhibition experiments were modeled using nonlinear regression analysis to determine the concentration of inhibitor that displaced 50% of the specific binding of the radioligand. Competition curves were modeled for a single site, and the IC_{50} values were converted to equilibrium dissociation constants (K_i values) using the Cheng−Prusoff correction. Mean K_i values SEM are reported for at least three independent experiments.

Preparation of Chimeric Receptors. The D2/D3E2 and D3/D2E2 receptor chimeras were prepared using a PCR based site-directed mutagenesis (Quick-Change site-directed mutagenesis kit, Stratagene) strategy with synthetic oligonucleotides encoding the E2 loop with the appropriate 5′ and 3′ flanking regions. Both wild type receptor genes were in the pIRES expression vector (Clontech). The size of the oligonucleotide for preparation of the D2/D3E2 chimera was 69 bases and for the D3/D2E2 loop was 66 bases. The chimeric receptors were transfected into HEK-293 cells. The authenticity of the chimeric receptor was verified by DNA sequencing, and the expression of the receptor construct in HEK 293 was verified by radioligand binding using [^{125}I]IABN.

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Supporting Information Available: Elemental analysis results, HPLC spectra for *R*- and *S*-**22**, and X-ray crystallographic figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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