Journal of Medicinal Chemistry Article

J. Med. Chem. **2010,** *53,* 7219–7228 **7219**
DOI: 10.1021/jm100899z

Bioisosteric Replacement Leading to Biologically Active [2.2]Paracyclophanes with Altered Binding Profiles for Aminergic G-Protein-Coupled Receptors

Marika Skultety, Harald Hübner, Stefan Löber, and Peter Gmeiner*

Department of Chemistry and Pharmacy, Emil Fischer Center, Friedrich Alexander University, Schuhstrasse 19, 91052 Erlangen, Germany

Received July 19, 2010

Exploring the chemical diversity space of GPCR ligands, we recently discovered [2.2]paracyclophanes as valuable atypical bioisosteres for secondary affinity and selectivity generating moieties. To find out if such an exchange also works for structural moieties that simulate the endogenous neurotransmitter, π1 or π2 or both systems π1 and π2 of three representative privileged structures of types **1**, **2**, and **3** were replaced by a [2.2]paracyclophane unit. Contributions of the respective functionalities to the binding affinities of a panel of relevant monoaminergic GPCRs were systematically examined. The study led to the paracyclophanylpiperazine **3a** displaying excellent D_3 affinity (K_i = 1.6 nM) and a strongly attenuated binding to D_4, 5-HT_1 and α_1. Whereas functional experiments showed neutral D_3 antagonist properties, mutagenesis studies indicated a binding mode that is similar to its lead compounds of type **3**.

Introduction

G-Protein-coupled receptors (GPCRs[a]) represent the largest class of membrane proteins in the human genome. Within the family of aminergic GPCR ligands, 1,4-disubstituted aromatic piperidines and piperazines (1,4-DAPs) are known as privileged structural moieties simulating endogenous neurotransmitters, like dopamine, serotonin, and (nor)epinephrine. Representative examples are the CNS active drugs haloperidol, iloperidone, paliperidone, fluanisone, fluspirilene, aripiprazole, buspirone,[1−6] and the drug candidates F-13640 (bifiradol), RGH-188 (cariprazine), ABT-724, and FAUC 346.[7−10] Interestingly, 1,4-DAPs have a common pattern of ligand−receptor recognition that depends upon favorable interactions with highly conserved amino acids of the receptor binding site crevice.[11] Containing a conjugated π-system (π1) and a basic nitrogen, the phenylpiperidine/phenylpiperazine scaffold represents the primary recognition element that targets the binding site of the native biogenic amines. Additionally, in position 4 of the central piperidine/piperazine, unsaturated carbocyclic or heterocyclic appendages (π2) are attached via a spacer element. Interacting with a hydrophobic microdomain provided by specific residues of the transmembrane domains (TMs) 2, 3, and 7 as well as parts of the extracellular loop 2, this structural entity controls both binding affinity and subtype selectivity.[12]

Exploring the chemical diversity space of bioactive compounds, we recently discovered [2.2]paracyclophanes as valuable atypical bioisosteres for the secondary affinity and selectivity generating system π2. Thus, we have discovered novel paracyclophane derived dopamine D_3 receptor antagonists displaying particular binding profiles that might be a starting point for the development of highly beneficial CNS active drugs, especially for the treatment of schizophrenia and addiction.[13] Thus, we could show that the high steric demand of [2.2]paracyclophanes is well tolerated by the hydrophobic microdomain of the dopamine D_3 receptor. Moreover, we found that indoloparacyclophanes can be used as double layered aryl bioisosteres of highly selective D_4 receptor ligands.[14]

To further validate the scope and limitations of the [2.2]paracyclophane scaffold, π1, π2, or both systems π1 and π2 of the three representative 1,4-DAPs of types **1**, **2**, and **3** were replaced by a [2.2]paracyclophane unit. To be able to unambiguously allocate biological effects, 1,4-xylene derived

*To whom correspondence should be addressed. Phone: +49 9131 85-29383. Fax: +49 9131 85-22585. E-mail: peter.gmeiner@medchem.uni-erlangen.de.

[a] Abbreviations: GPCR, G-protein-coupled receptor; 1,4-DAPs, 1,4-disubstituted aromatic piperidines and piperazines; CNS, central nervous system; haloperidol, 4-[4-(4-chlorophenyl)-4-hydroxy-1-piperidyl]-1-(4-fluorophenyl)butan-1-one; iloperidone, 1-[4-[3-[4-(6-fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]propoxy]-3-methoxyphenyl]ethanone; paliperidone, 3-[2-[4-(6-fluorobenzo[*d*]isoxazol-3-yl)-1-piperidyl]ethyl]-7-hydroxy-4-methyl-1,5-diazabicyclo[4.4.0]deca-3,5-dien-2-one; fluanisone, 1-(4-fluorophenyl)-4-[4-(2-methoxyphenyl)piperazin-1-yl]butan-1-one; fluspirilene, 8-[4,4-bis(4-fluorophenyl)butyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one; aripiprazole, 7-[4-[4-(2,3-dichlorophenyl)piperazin-1-yl]butoxy]-3,4-dihydroquinolin-2(1*H*)one; buspirone, 8-[4-(4-pyrimidin-2-ylpiperazin-1-yl)butyl]-8-azaspiro[4.5]decane-7,9-dione; F-13640, 3-chloro-4-fluorophenyl-[4-fluoro-4-[[(5-methylpyridin-2-yl)methylamino]methyl]-piperidin-1-yl]methanone; RGH-188, *trans*-*N*-[4-[2-[4-(2,3-dichlorophenyl)piperazin-1-yl]ethyl]cyclohexyl]-*N*′,*N*′-dimethylurea; ABT-724, 2-(4-pyridin-2-ylpiperazin-1-ylmethyl)-1*H*-benzimidazole; FAUC 346, *N*-[4-[4-(2-methoxyphenyl)piperazin-1-yl]butyl]benzo[*b*]thiophene-2-carboxamide; TM, transmembrane helix; 5-HT, serotonin (5-hydroxytryptamine); IBX, 2-iodoxybenzoic acid; TBTU, *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate; SCH 23390, 7-chloro-3-methyl-1-phenyl-1,2,4,5-tetrahydro-3-benzazepin-8-ol; spiperone, 8-[4-(4-fluorophenyl)-4-oxobutyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one; WAY100635, *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridyl)-cyclohexanecarboxamide; ketanserin, 3-{2-[4-(4-fluorobenzoyl)piperidin-1-yl]ethyl}quinazoline-2,4(1*H*,3*H*)-dione; prazosin, 2-[4-(2-furoyl)-piperazin-1-yl]-6,7-dimethoxyquinazolin-4-amine; CHO, Chinese hamster ovary; HEK, human embryonic kidney; SAR, structure−activity relationship; NMR, nuclear magnetic resonance; MHz, megahertz; LC/MS, liquid chromatography/mass spectrometry; APC, atmospheric pressure chemical; TLC, thin layer chromatography; HRMS, high resolution mass spectrometry; HPLC, high performance liquid chromatography; EI-MS, electron ionization mass spectrometry; DMF, dimethylformamide.

© 2010 American Chemical Society

Published on Web 09/14/2010

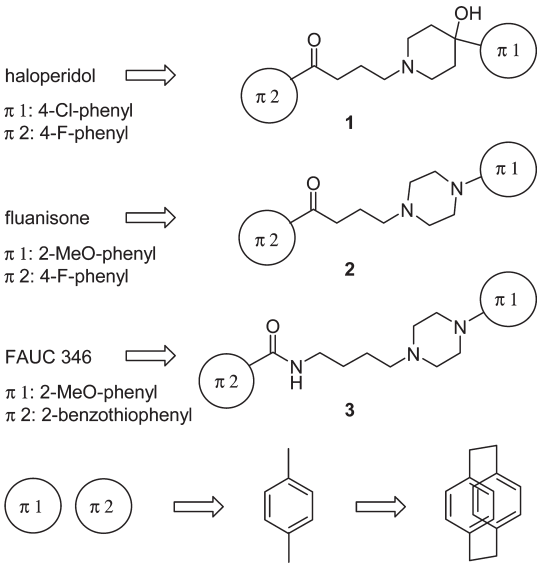


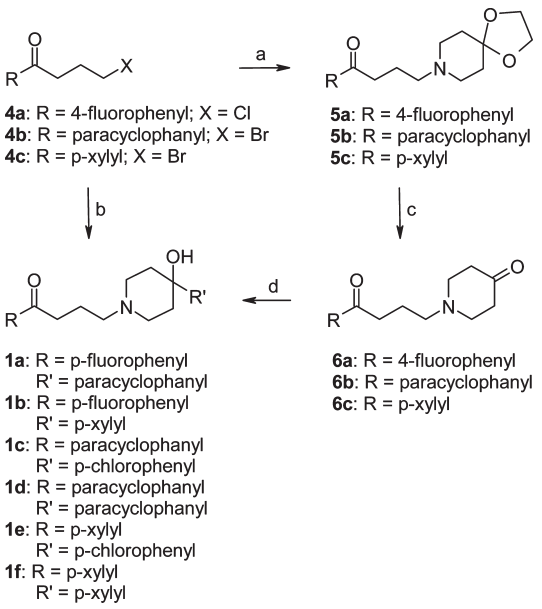
Figure 1. Bioisosteric replacement leading to the target compounds of types **1**, **2**, and **3**.

analogues should be prepared and investigated for every paracyclophane based test compound. Thus, the contributions of the respective functionalities to the binding affinities of a panel of relevant monoaminergic GPCRs were systematically detected for the classical *N*-piperidinylbutyrophenones **1**, the piperazinylbutyrophenones **2**, and the homologous (hetero)arene carboxamides **3** (Figure 1). To find out if such a bioisosteric exchange also works for the structural moieties that simulate the endogenous neurotransmitter, not only the secondary unsaturated system $\pi 2$ but also the primary recognition element $\pi 1$ was replaced. To learn if the increase of the volume by introducing the paracyclophane functionality leads to a general modification of GPCR affinity and specificity profiles, the binding properties toward eight structurally related GPCRs including D_1, D_{2long}, D_{2short}, D_3, D_4, 5-HT_{1A}, 5-HT_2, and α_1 receptors were investigated. To examine ligand efficacy and binding modes, highly promising test compounds were further characterized by functional experiments and mutagenesis studies, respectively.

Results and Discussion

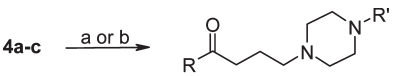
Chemistry. All paracyclophane based target compounds were synthesized in racemic form. Our initial investigations were directed to the chemical synthesis of hydroxypiperidine derivatives of type **1**. The construction of the 4-aminobutyrophenone moiety started from the commercially available haloperidol intermediate **4a** and the bromobutyrophenones **4b** and **4c**, which could be prepared by Friedel−Crafts acylation of [2.2]paracyclophane and *p*-xylene, respectively (Scheme 1). Introduction of an acetal-protected piperidone unit was done by treatment of **4a**−**c** with 1,4-dioxa-8-azaspirodecane. Ketal cleavage of the thus formed tertiary amines **5a**−**c** in the presence of aqueous hydrochloric acid gave access to the piperidones **6a**−**c**. Finally, introduction of the second π-system was performed by halogen metal exchange of bromofluorobenzene, bromoparacyclophane, and bromoxylene and subsequent treatment with the piperidones **6a**−**c**. Thus, the test compounds **1a**,**b**,**d**,**f** could be efficiently prepared. An alternative root was elaborated for the 4-(*p*-chlorophenyl)piperidines **1c** and **1e** when **4b** and **4c**, respectively, were reacted with commercially available chlorophenyl-substituted piperidine-4-ol.

Scheme 1[a]



[a] Reagents and conditions: (a) 1,4-dioxa-8-azaspirodecane, TEA or Na_2CO_3/KI, DMF or toluene (29-98%); (b) 4-(4-chlorophenyl)-4-hydroxypiperidine, Et_3N, DMF (62−65%); (c) HCl (2 N) (63−68%); (d) Br-R′, *n*-BuLi, Et_2O (24−47%).

Scheme 2[a]



2a: R = p-fluorophenyl; R' = p-chlorophenyl
2b: R = p-fluorophenyl; R' = paracyclophanyl
2c: R = p-fluorophenyl; R' = p-xylyl
2d: R = paracyclophanyl; R' = p-chlorophenyl
2e: R = p-xylyl; R' = p-chlorophenyl
2f: R = paracyclophanyl; R' = paracyclophanyl
2g: R = p-xylyl; R' = p-xylyl

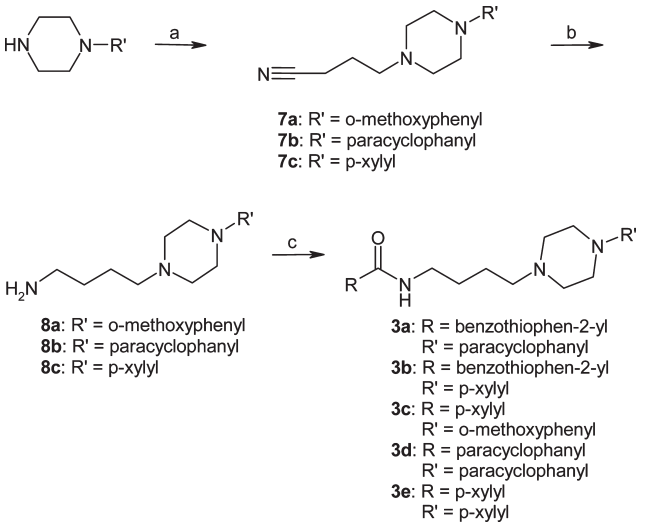
[a] Reagents and conditions. (a) For **2a**−**e** and **2g**: *N*-arylphenylpiperazine, NaI, $NaHCO_3$, CH_3CN (27−96%). (b) For **2f**: (1) $NaHCO_3$, DMSO (36%), (2) IBX, DMSO (41%), (3) 4-[2.2]paracyclophanylpiperazine, $Na(OAc_3)BH$, CH_2Cl_2 (55%).

Starting from **4a**−**c** and 4-substituted piperazines, nucleophilic displacement reaction gave the fluanisone-like test compounds **2a**−**e** and **2g** (Scheme 2). As the bis-paracyclophane **2f** could not be prepared following this method, the synthesis had to be modified for this particular target compound. The final product **2f** could be accessed by hydrolysis of the bromide **4b** and subsequent IBX-promoted oxidation of the resulting alcohol to a corresponding carbaldehyde. Reductive amination with paracyclophanylpiperazine[14b] in the presence of sodium triacetoxyborohydride afforded the test compound **2f**.

For the synthesis of the carboxamides of type **3**, the *o*-methoxyphenyl-, paracyclophanyl-, and piperazines were subjected to N-alkylation with 4-bromobutyronitrile (Scheme 3). Subsequent reduction of the intermediates **7a**−**c** by lithium aluminum hydride yielded the primary amines **8a**−**c**. Finally, TBTU-promoted coupling reaction of **8a**−**c** with benzothiophene, *p*-xylene, and paracyclophanecarboxylic acids resulted in formation of the carboxamides **3a**−**e**.

Biological Investigations. Receptor binding experiments were established to evaluate the binding properties of the

Scheme 3[a]



[a] Reagents and conditions: (a) 4-bromobutyronitrile, Na_2CO_3, CH_3CN (84−98%); (b) $LiAlH_4$, Et_2O (93−99%); (c) RCOOH, TBTU, DIPEA, CH_2Cl_2, DMF (65−83%).

paracyclophanes **1a**,**c**,**d**, **2b**,**d**,**f**, **3a**,**d**, and **3f**[13] and the *p*-xylene derived analogues **1b**,**e**,**f**, **2c**,**e**,**g**, **3b**,**c**,**e**. Haloperidol, the fluanisone analogue **2a**, and FAUC 346 were used as reference agents (Table 1). D_1 receptor affinities were determined utilizing porcine striatal membranes and the D_1 selective radioligand [^{3}H]SCH 23390.[15] $D2_{long}$, $D2_{short}$, D3, and D4 receptor affinities were investigated employing the cloned human dopamine receptor subtypes $D2_{long}$, $D2_{short}$,[16] D3,[17] and D4.4[18] stably expressed in Chinese hamster ovary cells (CHO) and the radioligand [^{3}H]spiperone.[15] The competition data were analyzed according to a sigmoid model by nonlinear regression. Because of the observation that our lead compounds are known for serotonergic and adrenergic activity as well, the test compounds were also investigated for their potency to displace [^{3}H]WAY 100635, [^{3}H]ketanserin, and [^{3}H]prazosin when employing porcine 5-HT_{1A}, 5-HT_2, and α_1 receptors, respectively. The data of the radioligand binding studies unambiguously proved that the double-layered paracyclophane derived moiety of the target compounds is specifically recognized by the binding sites of the GPCRs investigated (Table 1). Whereas only weak to moderate binding affinities were determined for D_1, 5-HT_2, and 5-HT_{1A}, respectively, D2, D4, and α_1 receptor recognition resulted in K_i values reaching even the single-digit nanomolar range.

Initially, we analyzed the impact of the replacement of the π1-system of haloperidol, the fluanisone derivative **2a**, and FAUC 346 by the [2.2]paracyclophane scaffold. Interestingly, the paracyclophane **1a** and its xylene analogue **1b** displayed a binding pattern that was very similar to that of its lead compound haloperidol with comparable or slightly lower affinities. However, the D_4 receptor binding of **1a** turned out to be significantly stronger resulting in a K_i value of 0.72 nM. Thus, the introduction of a [2.2]paracyclophane scaffold led to a significant change of the overall affinity profile. Besides significant binding affinity to adrenergic, serotonergic, cholinergic, and histaminergic receptors, the atypical antipsychotic clozapine displays D_4 preference within the dopaminergic receptor family. Thus, the above-mentioned bioisosteric replacement could exert substantial therapeutic advantages when compared to the classical antipsychotic haloperidol. It is interesting to note that the same structural modification on the piperazine analogues leading to the test compound **2b** caused a significant reduction of D_4 affinity (K_i = 120 nM). In contrast, **2b** showed strongly improved D_3 binding (K_i = 9.9 nM) when compared to the lead compound **2a**. The analogous π1-exchange within the compounds of type **3** led to an almost unchanged binding to D_1, D_2, and D_3 and 5-HT_2. Interestingly, the paracyclophane **3a** displayed excellent D_3 affinity (K_i = 1.6 nM) and a strongly attenuated binding to D_4, 5-HT_1, and α_1. As a consequence, **3a** revealed a 50- to 500-fold target selectivity. Compared to the lead compound FAUC 346, the 300-fold preference over the antitarget α_1 indicates a more than 10-fold enhancement, which is worthy of note because α_1 antagonism is known to induce cardiovascular side effects limiting the therapeutic value of many CNS-active drug candidates.[19] Comparison of the above-mentioned effects with the binding profiles of the *p*-xylene derived reference compounds **1b**, **2c**, and **3b** indicated that none of the above-mentioned improvements could be performed by the xylene moiety. Thus, the major SAR effects are obviously due to the huge steric demand of the double-layered system.

Displacement of π2 by [2.2]paracyclophane was less effective. Thus, reduced GPCR affinities were observed for **1c** and **2d**. Interestingly, however, the bioisosteric exchange that was leading to the recently described paracyclophanyl carboxamide **3f**[13] resulted in a binding pattern that was competitive to the lead compound FAUC 346. The binding properties of the xylene analogues **1e**, **2e**, and **3c** were comparable, leading to the observation that the structural modifications on π2 are in fact less crucial than those of the primary recognition element π1.

Analysis of the bis-paracyclophanes **1d**, **2f**, and **3d** in comparison to the bis-xylene analogues **1f**, **2g**, and **3e** indicated preferential D_2, D_3, and D_4 binding for all compounds when α_1 affinity was substantially attenuated for the paracyclophanes. Because α_1 is regarded to be an antitarget leading to cardiovascular problems, this observation might be highly beneficial for further efforts in the drug discovery of selective dopamine receptor agonists and antagonists.

As a measure of functional D_3 activity, ligand efficacy of the [2.2]paracyclophanylpiperazine **3a** was determined by a mitogenesis assay measuring the rate of [^{3}H]thymidine incorporation into a CHO dhfr$^-$ cell line stably expressing the human D3 receptor when the full agonist quinpirole and the partial agonist FAUC 346 were used as reference agents.[17,20,21] Interestingly, the test compound **3a** did not show any ligand efficacy at the D3 subtype, indicating neutral D_3 antagonist properties.

Site-directed mutagenesis was employed to identify crucial interactions between the binding pocket of the D_3 receptor and the paracyclophanylpiperazine **3a** compared to the xylene analogue **3b** as a representative test compound. The binding site crevice of dopamine receptors is thought to be determined by several highly conserved amino acids incuding F6.52 as a part of the aromatic microdomain binding the catechol substructure of the endogenous ligand dopamine (Figure 2).[22−24] As a crucial residue for aromatic carboxamide moiety of compounds of type **3**, V2.61 was identified.[12] To inspect the binding mode of **3a**, we mutated V2.61 and F6.52 into the phenylalanine and tryptophan derivatives D_3 V2.61F and D_3 F6.52W,[25] respectively.

In accordance with previously recorded mutagenesis data for D_3 ligands of type **3**,[12] the single mutant V2.61F induced

Table 1. Receptor Binding Data of **1a**−**f**, **2b**−**g**, and **3a**−**e** Compared to the Reference Compounds Haloperidol, **2a**, **3f**,[13] and FAUC 346, Respectively, Utilizing Human D_{2long}, D_{2short}, D_3, and $D_{4.4}$ Receptors as Well as Porcine D_1, 5-HT_{1A}, 5-HT_2, and α_1 Receptors[a]

			$K_i \pm$ SD/SEM[b] (nM)[c]							
				[^{3}H]spiperone						
compd	π1	π2	[^{3}H]SCH 23390 D_1	D_{2long}	D_{2short}	D_3	$D_{4.4}$	[^{3}H]WAY 100635 5-HT_{1A}	[^{3}H]ketan-serin 5-HT_2	[^{3}H]prazosin α_1
haloperidol	4-Cl-Ph	4-F-Ph	98 ± 15[b]	1.1 ± 0.15[b]	0.87 ± 0.071[b]	7.5 ± 2.5[b]	6.0 ± 1.8[b]	62 ± 6.3	30 ± 0.71	6.8 ± 1.8
1a	CyPhanyl	4-F-Ph	100 ± 23	4.8 ± 1.1	3.6 ± 0	53 ± 3.5	0.72 ± 0.042	410 ± 150	19 ± 6.4	2.9 ± 0.64
1b	*p*-Xylyl	4-F-Ph	19 ± 9.9	3.4 ± 1.3	6.5 ± 4.5	27 ± 5.7	20 ± 1.4	250 ± 78	110 ± 38	1.3 ± 0.70
1c	4-Cl-Ph	CyPhanyl	9800 ± 1700	21 ± 3.5	13 ± 0.71	17 ± 0.71	39 ± 5.7	6400 ± 210	2400 ± 640	160 ± 14
1d	CyPhanyl	CyPhanyl	4900 ± 1200	140 ± 35	200 ± 49	38 ± 0	30 ± 13	3400 ± 780	1200 ± 390	2800 ± 210
1e	4-Cl-Ph	*p*-xylyl	3800 ± 1900	15 ± 5.0	43 ± 2.1	73 ± 11	61 ± 7.1	400 ± 35	690 ± 110	35 ± 6.4
1f	*p*-xylyl	*p*-xylyl	590 ± 35	55 ± 2.1	46 ± 2.1	280 ± 120	30 ± 4.2	410 ± 49	1700 ± 1100	11 ± 0
2a	4-Cl-Ph	4-F-Ph	340 ± 57	63 ± 0	61 ± 12	180 ± 35	7.6 ± 1.6	1200 ± 71	440 ± 250	13 ± 7.0
2b	CyPhanyl	4-F-Ph	590 ± 240	19 ± 12	22 ± 11	9.9 ± 4.5	120 ± 36	340 ± 140	450 ± 7.1	71 ± 30
2c	*p*-xylyl	4-F-Ph	210 ± 78	13 ± 2.1	16 ± 2.1	76 ± 19[b]	17 ± 3.3[b]	550 ± 0	850 ± 180	3.2 ± 0.50
2d	4-Cl-Ph	CyPhanyl	5400 ± 140	620 ± 50[b]	440 ± 67[b]	410 ± 31[b]	120 ± 16[b]	4000 ± 640	1700 ± 640	180 ± 71
2e	4-Cl-Ph	*p*-xylyl	690 ± 150	200 ± 49	190 ± 28	290 ± 130	10 ± 2.9	2300 ± 2000	180 ± 78	26 ± 14
2f	CyPhanyl	CyPhanyl	2000 ± 420	17 ± 2.1	30 ± 9.9	4.2 ± 1.5	19 ± 11	2200 ± 570	310 ± 21	1000 ± 390
2g	*p*-xylyl	*p*-xylyl	9000 ± 4200	400 ± 110	260 ± 110	420 ± 110	45 ± 12	4900 ± 1600	5700 ± 1600	60 ± 0
FAUC 346	2-MeO-Ph	benzothiop	820 ± 100[b]	95 ± 5.5[b]	72 ± 14[b]	0.27 ± 0.019[b]	24 ± 6.3[b]	41 ± 4.2[b]	350 ± 99	14 ± 7.2[b]
3a	CyPhanyl	benzothiop	4000 ± 1200	83 ± 11	150 ± 35	1.6 ± 0.28	880 ± 63	8500 ± 2100	1000 ± 420	490 ± 160
3b	*p*-xylyl	benzothiop	2400 ± 1100	84 ± 21	65 ± 2.1	2.5 ± 0.071	300 ± 21	2000 ± 140	1500 ± 1100	20 ± 2.1
3c	2-MeO-Ph	*p*-xylyl	2100 ± 710	22 ± 6.4	17 ± 2.8	4.7 ± 1.3	23 ± 17	16 ± 2.8	1500 ± 350	14 ± 0.71
3d	CyPhanyl	CyPhanyl	7800 ± 2300	32 ± 7.8	48 ± 3.5	4.0 ± 2.9	170 ± 64	16000 ± 4200	8200 ± 2200	840 ± 220
3e	*p*-xylyl	*p*-xylyl	3200 ± 490	99 ± 0.71	86 ± 18	28 ± 2.1	220 ± 28	530 ± 42	3600 ± 2900	29 ± 6.4
3f	2-MeO-Ph	CyPhanyl	680 ± 160	24 ± 2.5[b]	18 ± 1.8[b]	0.49 ± 0.077[b]	11 ± 1.3[b]	65 ± 2.7[b]	2200 ± 71	4.2 ± 0.99

[a] Abbreviations: CyPhanyl = [2.2]paracacylophan-4-yl; benzothiop = benzo[*b*]thiophen-2-yl. [b] $K_i \pm$ SEM; all other K_i values are determined with a standard error of ±SD. [c] K_i values in nM are based on the mean of 2−15 experiments each done in triplicate.

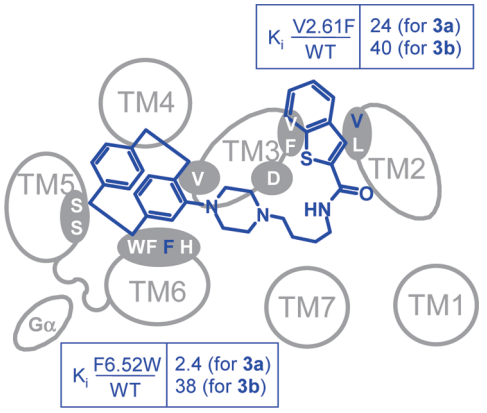


Figure 2. Conceptional binding model for D_3 and the paracyclophane **3a** as well as mutagenesis data for **3a** compared to the xylene analogue **3b**.

a significant loss of binding affinity. Comparison of wild-type D_3 and D_3 V2.61F, which were both expressed in transiently transfected HEK cells, led to a 24-fold and 40-fold reduction of affinity for the benzothiophene carboxamides **3a** and **3b**, respectively, indicating the same molecular environment of $\pi 2$. On the other hand, the D_3 F6.52W resulted in a significant reduction of binding for the xylene **3b** (38-fold) but only in a slight impairment (2.4-fold) for the paracyclophane **3a**. Thus, the structural modification of the catechol-simulating moiety $\pi 1$ leads to a differential susceptibility of the ligand−receptor contact in position 6.52 of the aromatic microdomain.

Conclusion

In conclusion, bioisosteric exchange of $\pi 1$, $\pi 2$, or both systems $\pi 1$ and $\pi 2$ of the three representative privileged structures of types **1**, **2**, and **3** by a [2.2]paracyclophane unit led to GPCR ligands with novel selectivity profiles. As an example, the paracyclophane **1a** and its xylene analogue **1b** displayed a binding pattern that was very similar to that of its lead compound haloperidol with comparable or slightly lower affinities. However, the D_4 receptor binding of **1a** (K_i = 0.72 nM) turned out to be significantly stronger and the selectivity higher (5- to 70-fold over D_{2long}, D_{2short}, and D_3). The contribution of the respective functionalities to the binding affinities of a panel of relevant monoaminergic GPCRs was systematically detected. The study led to the paracyclophanylpiperazine **3a** with excellent D_3 affinity (K_i = 1.6 nM) and a strongly attenuated binding to D_4, 5-HT_{1A}, and α_1 resulting in selectivity ratios of 550, 5300, and 300, respectively. The $\pi 1$ exchange within the compounds of type **3** led to an almost unchanged binding to D_1, D_2, and D_3 and 5-HT_2. As a consequence, **3a** revealed a 50- to 500-fold target selectivity. Compared to the lead compound FAUC 346, the 300-fold preference over the antitarget α_1 indicates a more than 10-fold enhancement, which is worthy of note because α_1 antagonism is known to induce cardiovascular side effects limiting the therapeutic value of many CNS-active drug candidates. Because of the chirality of the paracyclophane-derived final products, aymmetric synthesis or separation of the enantiomers and pharmacological investigations of a configurational impact will be investigated.

Experimental Section

Chemistry. IR spectra were registered on a JASCO model FTIR 410 instrument via KBr pellet. ^{1}H NMR (360 or 600 MHz) and ^{13}C NMR (90 or 150 MHz) spectra were determined on a Bruker AM 360 or a Bruker AVANCE 600 spectrometer in solution. LC/MS analyses were conducted in an Agilent binary gradient system (MeOH/0.1% aqueous HCO_2H, 10/90 to 90/10) in combination with ChemStation software and UV detection at 254 nm using a Zorbax SB-C8 (4.6 mm × 150 mm, 5 μm) with a flow rate of 0.5 mL/min. Mass detection was done with a Bruker Esquire 2000 ion-trap mass spectrometer using an APC ionization source. HRMS spectra were recorded on JEOL GCmatell instrument. Flash chromatography was done using silica gel (40−63 μm) as stationary phase. TLC analyses were done on Merck 60 F_{254} glass plates and analyzed by UV light (254 nm) or by iodine vapor. Purity was determined by elementary analysis or by HPLC using an Agilent 1100 HPLC systems in combination with UV detection. As column, a Zorbax Eclipse XDB-C8 (4.6 mm × 150 mm, 5 μm) was used. HPLC was run with MeOH (eluent I) and 0.1% aqueous formic acid (eluent II) and the following gradient: MeOH 10% for 3 min, ascending to 100% in 15 min, 100% for 6 min. The flow rate was 0.5 mL/min, and the wavelength λ was 254 nm. All SAR compounds were determined to be >95% pure.

4-Bromo-1-([2.2]paracyclophan-4-yl)butan-1-one (4b). To a solution of [2.2]paracyclophane (1.2 g, 5.7 mmol) in dichloromethane (6.0 mL) was added a mixture of $AlCl_3$ (1.4 g, 10.3 mmol) and 4-bromobutyric acid chloride (1.4 mL, 12 mmol) in dichloromethane (3.3 mL) at −50 °C. The mixture was stirred for 20 min when it was allowed to warm to −20 °C. Then the suspension was filtered and added to a mixture of 6 N HCl (10 mL) and ice−water (20 mL). After extraction with diethyl ether, the organic layer was subsequently extracted with saturated $NaHCO_3$ solution and saturated NaCl solution. The organic layer was dried (Na_2SO_4) and evaporated and the residue was purified by flash chromatography (hexane/EtOAc 4:1) to give pure **4b** (1.6 g, 79%) as a pale greenish solid (mp 77 °C). ^{1}H NMR ($CDCl_3$, 360 MHz) δ (ppm): 2.12−2.36 (m, 2H), 2.77−2.87 (m, 2H), 2.98−3.08 (m, 3H), 3.12−3.22 (m, 5H), 3.50−3.59 (m, 2H), 6.37 (dd, J = 7.8, 1.7 Hz, 1H), 6.45−6.56 (m, 4H), 6.66 (dd, J = 7.8, 1.7 Hz, 1H), 6.93 (d, J = 1.7 Hz, 1H). ^{13}C NMR ($CDCl_3$, 90 MHz) δ (ppm): 27.2, 33.7, 35.0, 35.2, 36.0, 38.4, 131.2, 132.1, 132.9, 133.0, 133.4, 136.4, 137.5, 139.2, 139.9, 140.2, 141.5, 201.1. IR (NaCl) ν (cm^{-1}): 3008, 2927, 1674, 1551, 1500, 1230, 721. HPLC (254 nm) t_R = 22.6 min, purity 100%. HRMS (m/z): $[M]^+$ calcd for $C_{20}H_{21}BrO$, 356.0776; found, 356.0776.

4-Bromo-1-(2,5-dimethylphenyl)butan-1-one (4c). To a mixture of 4-bromobutyric acid chloride (1.1 mL, 9.8 mmol) and *p*-xylene (2.8 mL, 22.8 mmol) was added $AlCl_3$ (1.4 g, 10.5 mmol) at 0 °C. After 30 min, stirring was continued at room temperature for another 30 min. Then the mixture was added to aqueous HCl (1.5%) at 0 °C. After extraction with hexane, the organic layer was dried ($MgSO_4$) and evaporated and the residue was purified by flash chromatography (hexane/EtOAc 10:1) to give pure **4c** (70%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm): 2.26−2.30 (m, 2H), 2.37 (s, 3H), 2.45 (s, 3H), 3.09 (t, J = 7.0 Hz, 2H), 3.54 (t, J = 6.3 Hz, 2H), 7.13 (d, J = 7.8 Hz, 1H), 7.19 (dd, J = 7.8, 1.7 Hz, 1H), 7.47 (d, J = 1.7 Hz, 1H). ^{13}C NMR ($CDCl_3$, 90 MHz) δ (ppm): 20.9, 21.0, 27.1, 33.6, 39.4, 129.1, 131.9, 132.2, 134.9, 135.3, 137.6, 203.0. IR (NaCl) ν (cm^{-1}): 3021, 2964, 1685, 1567, 1496, 1241, 818. HPLC (254 nm) t_R = 19.1 min, purity 100%. HRMS (m/z): $[M]^+$ calcd for $C_{12}H_{15}BrO$, 245.0306; found, 245.0305.

4-(1,4-Dioxa-8-azaspiro[4.5]dec-8-yl)-1-(4-fluorophenyl)butan-1-one (5a). A mixture of 8-aza-1,4-dioxaspiro[4.5]decane (1.8 mL, 14.0 mmol), 4-chloro-4′-fluorobutyrophenone (2.0 mL, 12.0 mmol), KI (30 mg, 0.2 mmol), Na_2CO_3 (25 mg, 0.24 mmol), and toluene (75 mL) was heated to reflux for 77 h. The mixture was filtered at room temperature, and the filtrate was extracted with saturated $NaHCO_3$ solution. The organic layer was dried (Na_2SO_4), evaporated and the residue was purified by flash chromatography (CH_2Cl_2/MeOH 9:1) to give pure **5a** (48%) as a pale yellow oil.

^{1}H NMR ($CDCl_3$, 360 MHz) δ (ppm): 1.68−1.71 (m, 4H), 1.90−1.98 (m, 2H), 2.45 (t, J = 7.2 Hz, 2H), 2.51−2.54 (m, 4H), 2.97 (t, J = 7.0 Hz, 2H), 3.94 (s, 4H), 7.10−7.15 (m, 2H), 7.98−8.02 (m, 2H). IR (NaCl) ν (cm^{-1}): 2955, 2812, 1685, 1598, 1506, 1228, 1096, 836. EIMS (m/z): $[M]^+$ 307.

1-([2.2]Paracyclophan-4-yl)-4-(1,4-dioxa-8-azaspiro[4.5]dec-8-yl)-butan-1-one (5b). To a solution of **4b** (520 mg, 1.5 mmol) in DMF (5.9 mL) were added 8-aza-1,4-dioxaspiro[4.5]decane (0.23 mL, 1.8 mmol) and triethylamine (0.26 mL). After the mixture was stirred at room temperature for 3 h, H_2O (40 mL) was added. After extraction with diethyl ether, the organic layer was dried (Na_2SO_4) and evaporated and the residue was purified by flash chromatography (CH_2Cl_2/MeOH 93:7) to give **5b** (99%) as a colorless solid (mp 69 °C). ^{1}H NMR ($CDCl_3$, 360 MHz) δ (ppm): 1.74−1.78 (m, 4H), 1.85−1.96 (m, 2H), 2.45 (dt, J = 7.3, 3.0 Hz, 2H), 2.55−2.58 (m, 4H), 2.70 (ddd, J = 16.8, 7.7, 6.4 Hz, 1H), 2.79−2.93 (m, 2H), 2.96−3.05 (m, 2H), 3.11−3.22 (m, 4H), 3.86 (ddd, J = 12.4, 9.5, 2.5 Hz, 1H), 3.95 (s, 4H), 6.34 (dd, J = 7.7, 1.8 Hz, 1H), 6.48 (dd, J = 7.7, 1.8 Hz, 1H), 6.50 (d, J = 7.7 Hz, 1H), 6.51−6.56 (m, 2H), 6.63 (dd, J = 7.7, 1.8 Hz, 1H), 6.91 (d, J = 1.8 Hz, 1H). ^{13}C NMR ($CDCl_3$, 90 MHz) δ (ppm): 22.0, 34.8, 35.1, 35.2, 35.9, 38.3, 51.3, 57.2, 64.2, 107.3, 131.2, 132.2, 132.8, 132.9, 133.3, 136.1, 136.3, 138.0, 139.2, 139.7, 140.2, 141.2, 202.6. IR (NaCl) ν (cm^{-1}): 2927, 2810, 1674, 1593, 1231, 1095. HPLC (254 nm) t_R = 17.3 min, purity 97%. HRMS (m/z): $[M]^+$ calcd for $C_{27}H_{33}NO_3$, 419.2460; found, 419.2460.

1-(2,5-Dimethylphenyl)-4-(1,4-dioxa-8-azaspiro[4.5]dec-8-yl)-butan-1-one (5c). Compound **4c** was reacted and worked up as described for the synthesis of **5b** to give **5c** (52%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 360 MHz) δ (ppm): 1.75−1.78 (m, 4H), 1.89−1.97 (m, 2H), 2.35 (s, 3H), 2.43 (s, 3H), 2.49 (t, J = 7.4 Hz, 2H), 2.58−2.61 (m, 4H), 2.92 (t, J = 6.9 Hz, 2H), 3.95 (s, 4H), 7.11 (d, J = 7.7 Hz, 1H), 7.16 (dd, J = 7.7, 1.7 Hz, 1H), 7.43 (d, J = 1.7 Hz, 1H). ^{13}C NMR ($CDCl_3$, 90 MHz) δ (ppm): 20.7, 20.9, 21.7, 34.6, 39.3, 51.2, 57.1, 64.3, 107.1, 129.0, 131.8, 134.6, 135.1, 138.2, 204.4. IR (NaCl) ν (cm^{-1}): 2956, 2812, 1684, 1576, 1207, 1095, 814. HPLC (254 nm) t_R = 14.5 min, purity 100%. HRMS (m/z): $[M]^+$ calcd for $C_{19}H_{27}NO_3$, 317.1991; found, 317.1991.

1-[4-(4-Fluorophenyl)-4-oxobutyl]piperidin-4-one (6a). A mixture of **5a** (1.5 g, 5.0 mmol), 2 N HCl (12.4 mL), and acetone (24.5 mL) was refluxed for 29 h. The mixture was neutralized with $NaHCO_3$ at room temperature, and acetone was removed under vacuum. The residue was extracted with ethyl acetate after addition of water. The organic layer was dried (Na_2SO_4) and evaporated and the residue was purified by flash chromatography (Ca_2Cl_2/MeOH 95:5) to give pure **6a** (72%) as a colorless solid (mp 63 °C). ^{1}H NMR ($CDCl_3$, 360 MHz) δ (ppm): 1.98−2.05 (m, 2H), 2.37−2.42 (m, 4H), 2.56 (t, J = 6.4 Hz, 2H), 2.74−2.78 (m, 4H), 3.03 (t, J = 6.7 Hz, 2H), 7.11−7.16 (m, 2H), 7.99−8.03 (m, 2H). IR (NaCl) ν (cm^{-1}): 2924, 2809, 1715, 1683, 1598, 1506, 1226, 1096, 837. EIMS (m/z): $[M]^+$ 263.

1-[4-([2.2]Paracyclophan-4-yl)-4-oxobutyl]piperidin-4-one (6b). Compound **5b** was reacted and worked up as described for the preparation of **6a** to give pure **6b** (68%) as a colorless solid (mp 127 °C). ^{1}H NMR ($CDCl_3$, 360 MHz) δ (ppm): 1.90−1.98 (m, 2H), 2.41−2.44 (m, 4H), 2.52 (t, J = 6.8 Hz, 2H), 2.73−2.76 (m, 4H), 2.80−3.06 (m, 5H), 3.12−3.20 (m, 4H), 3.88 (ddd, J = 12.2, 8.9, 3.2 Hz, 1H), 6.35 (dd, J = 7.7, 1.7 Hz, 1H), 6.48 (dd, J = 7.7, 1.7 Hz, 1H), 6.50−6.53 (m, 2H), 6.56 (dd, J = 7.7, 1.7 Hz, 1H), 6.65 (dd, J = 7.7, 1.7 Hz, 1H), 6.92 (d, J = 1.7 Hz, 1H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ (ppm): 22.2, 35.1, 35.2, 36.0, 38.1, 41.2, 51.0, 56.6, 131.2, 132.1, 132.9, 133.0, 133.3, 136.2, 136.4, 137.9, 139.2, 139.8, 140.3, 141.2, 202.4, 209.3. IR (NaCl) ν (cm^{-1}): 2925, 2810, 1718, 1674, 1592, 1227, 1093. EIMS (m/z): $[M]^+$ 375.

1-[4-(2,5-Dimethylphenyl)-4-oxobutyl]piperidin-4-one (6c). Compound **5c** was reacted and worked up as described for **6a** to give **6c** (78%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm): 1.93−1.98 (m, 2H), 2.36 (s, 3H), 2.41−2.43 (m, 4H), 2.45 (s, 3H), 2.53 (t, J = 7.1 Hz, 2H), 2.74−2.76 (m, 4H), 2.96 (t, J = 6.7 Hz, 2H), 7.13 (d, J = 7.8 Hz, 1H), 7.18 (dd, J = 7.8, 1.6 Hz, 1H), 7.46 (d, J = 1.6 Hz, 1H). ^{13}C NMR ($CDCl_3$, 90 MHz) δ (ppm): 20.8, 21.0, 22.1, 39.2, 41.2, 53.1, 56.6, 129.0, 131.8, 131.9, 134.7, 135.1, 138.3, 204.3, 209.2. IR (KBr) ν (cm^{-1}): 2960, 2808, 1718, 1682, 1568, 1221, 1093, 816. HPLC (254 nm) t_R = 16.1 min, purity 100%. HRMS (m/z): $[M]^+$ calcd for $C_{17}H_{23}NO_2$, 273.1729; found, 273.1728.

1-(4-Fluorophenyl)-4-[4-([2.2]paracyclophan-4-yl)-4-hydroxypiperidin-1-yl]butan-1-one (1a). Compound **6a** and 4-bromo-[2.2]paracyclophane were reacted and worked up as described for **1b** to give **1a** (47%) as a pale orange oil. ^{1}H NMR ($CDCl_3$, 360 MHz) δ (ppm): 1.78−1.83 (m, 2H), 1.95−1.99 (m, 2H), 2.08 (t, J = 7.1 Hz, 2H), 2.30−2.41 (m, 2H), 2.60−2.75 (m, 4H), 2.90−3.19 (m, 9H), 3.75−3.81 (m, 1H), 6.30 (dd, J = 7.8, 1.5 Hz, 1H), 6.34−6.39 (m, 2H), 6.43 (dd, J = 7.8, 1.5 Hz, 1H), 6.50 (d, J = 1.5 Hz, 1H), 6.59−6.63 (m, 2H), 7.11−7.15 (m, 2H), 7.99−8.03 (m, 2H). ^{13}C NMR ($CDCl_3$, 90 MHz) δ (ppm): 23.2, 35.2, 35.6, 36.1, 36.2, 37.1, 49.1, 49.3, 57.4, 71.3, 115.6, 115.8, 128.4, 130.7, 130.8, 132.0, 132.1, 132.3, 132.5, 132.7, 137.3, 137.7, 139.5, 139.6, 139.7, 167.2, 198.0. IR (NaCl) ν (cm^{-1}): 3364, 2928, 2852, 1685, 1598, 1230, 1157, 836, 731. HPLC (254 nm) t_R = 18.3 min, purity 100%. HRMS (m/z): $[M]^+$ calcd for $C_{31}H_{34}FNO_2$, 471.2574; found, 471.2572.

4-[4-(2,5-Dimethylphenyl)-4-hydroxypiperidin-1-yl]-1-(4-fluorophenyl)butan-1-one (1b). To a mixture of 2-bromo-*p*-xylene (0.05 mL, 0.35 mmol) in diethyl ether (20 mL) were added *n*-butyllithium (0.28 mL, 2.5 M in hexane) at −78 °C. The mixture was allowed to warm to room temperature within 90 min. After the mixture was cooled to −30 °C, a precooled solution of **6a** (210 mg, 0.80 mmol) in diethyl ether (6.4 mL) was added. After the mixture was stirred at −30 °C for 5 min, stirring was continued for 5.5 h at room temperature. Then H_2O was added, and the mixture was extracted by CH_2Cl_2. The organic layer was dried (Na_2SO_4) and evaporated and the residue was purified by flash chromatography (CH_2Cl_2/MeOH 95:5) to give pure **1b** (61 mg, 47%) as a pale yellow solid (mp 99 °C). ^{1}H NMR ($CDCl_3$, 360 MHz) δ (ppm): 1.88−1.92 (m, 2H), 1.94−2.02 (m, 2H), 2.06−2.10 (m, 2H), 2.30 (s, 3H), 2.45−2.52 (m, 4H), 2.54 (s, 3H), 2.75−2.80 (m, 2H), 2.98 (t, J = 7.1 Hz, 2H), 6.97 (dd, J = 7.7, 1.7 Hz, 1H), 7.04 (d, J = 7.7 Hz, 1H), 7.09−7.14 (m, 2H), 7.15 (d, J = 1.7 Hz, 1H), 7.99−8.03 (m, 2H). ^{13}C NMR ($CDCl_3$, 90 MHz) δ (ppm): 21.2, 21.8, 22.0, 36.3, 36.9, 49.4, 57.8, 72.4, 115.5, 115.7, 126.1, 127.7, 130.6, 130.8, 133.0, 133.4, 133.7, 135.0, 144.7, 167.0, 198.5. IR (NaCl) ν (cm^{-1}): 3466, 2921, 2850, 1686, 1598, 1230, 1121, 773. EIMS (m/z): $[M]^+$ 369.

4-[4-(4-Chlorophenyl)-4-hydroxypiperidin-1-yl]-1-([2.2]paracyclophan-4-yl)butan-1-one (1c). To a solution of **4b** (24 mg, 0.067 mmol) and 4-(4-chlorophenyl)-4-hydroxypiperidine (14 mg, 0.067 mmol) in DMF (0.27 mL) was added triethylamine (0.01 mL, 0.073 mmol) at room temperature. After the mixture was stirred for 6 h, H_2O (4 mL) and diethyl ether were added. The organic layer was dried (Na_2SO_4) and evaporated and the residue was purified by flash chromatography (CH_2Cl_2/MeOH 9:1) to give pure **1c** (20 mg, 62%) as a colorless solid (mp 75 °C). ^{1}H NMR ($CDCl_3$, 360 MHz) δ (ppm): 1.77−1.81 (m, 2H), 2.02−2.10 (m, 2H), 2.32−2.42 (m, 2H), 2.66−2.90 (m, 6H), 2.94−3.06 (m, 5H), 3.14−3.20 (m, 4H), 3.85−3.92 (m, 1H), 6.37 (dd, J = 7.7, 1.7 Hz, 1H), 6.46 (dd, J = 7.7, 1.7 Hz, 1H), 6.50−6.57 (m, 3H), 6.66 (dd, J = 7.7, 1.7 Hz, 1H), 6.93 (d, J = 1.7 Hz, 1H), 7.31−7.33 (m, 2H), 7.43−7.46 (m, 2H). ^{13}C NMR ($CDCl_3$, 90 MHz) δ (ppm): 20.9, 35.1, 35.2, 36.0, 38.0, 49.1, 49.3, 57.5, 70.6, 126.1, 128.6, 131.2, 132.2, 132.9, 133.0, 133.1, 133.4, 136.4, 137.7, 139.3, 139.9, 140.2, 141.4, 146.0, 201.9. IR (NaCl) ν (cm^{-1}): 3366, 2926, 2853, 1673, 1490, 1242, 1093, 826, 753. HRMS (m/z): $[M]^+$ calcd for $C_{31}H_{34}NO_2Cl$, 487.2278; found, 487.2279.

4-[4-([2.2]Paracyclophan-4-yl)-4-hydroxypiperidin-1-yl]-1-([2.2]-paracyclophan-4-yl)butan-1-one (1d). Compound **6b** and 4-bromo-[2.2]paracyclophane were reacted and worked up as described for **1b** to give pure **1d** (24%) as a colorless oil. ^{1}H NMR ($CDCl_3$,

360 MHz) δ (ppm): 1.88−1.99 (m, 4H), 2.22−2.29 (m, 2H), 2.45−2.54 (m, 4H), 2.67−2.79 (m, 2H), 2.82−2.93 (m, 4H), 2.96−3.06 (m, 4H), 3.08−3.19 (m, 8H), 3.78−3.91 (m, 2H), 6.29 (dd, J = 7.7, 3.0 Hz, 1H), 6.34−6.37 (m, 3H), 6.46 (d, J = 8.7 Hz, 1H), 6.49−6.56 (m, 5H), 6.61−6.65 (m, 3H), 6.92 (d, J = 1.7 Hz, 1H). ^{13}C NMR ($CDCl_3$, 90 MHz) δ (ppm): 21.8, 35.1, 35.2, 35.3, 35.7, 36.0, 36.2, 37.9, 38.4, 39.1, 49.2, 49.4, 49.6, 49.7, 57.8, 71.8, 128.4, 131.3, 132.1, 132.2, 132.3, 132.6, 132.8, 132.9, 133.3, 136.1, 136.3, 137.2, 137.8, 138.0, 139.2, 139.5, 139.6, 139.7, 140.3, 141.3, 202.6. IR (NaCl) ν (cm^{-1}): 3332, 2927, 2852, 1672, 1591, 1219, 1120, 756. HPLC (254 nm) t_R = 20.2 min, purity 95%; t_R = 17.8 min, purity 100%. HRMS (m/z): $[M]^+$ calcd for $C_{41}H_{45}NO_2$, 583.3450; found, 583.3448.

4-[4-(4-Chlorophenyl)-4-hydroxypiperidin-1-yl]-1-(2,5-dimethylphenyl)butan-1-one (1e). Compound **4c** and 4-(4-chlorophenyl)-4-hydroxypiperidine were reacted and worked up as described for **1c** to give pure **1e** (1.1 g, 65%) as a colorless solid (mp 83 °C) when CH_2Cl_2/MeOH (95:5) was used for flash chromatography. ^{1}H NMR ($CDCl_3$, 360 MHz) δ (ppm): 1.69−1.75 (m, 2H), 1.95−2.02 (m, 2H), 2.10−2.18 (m, 2H), 2.36 (s, 3H), 2.44 (s, 3H), 2.47−2.55 (m, 4H), 2.84−2.90 (m, 2H), 2.94 (t, J = 7.1 Hz, 2H), 7.13 (d, J = 7.9 Hz, 1H), 7.18 (dd, J = 7.9, 1.7 Hz, 1H), 7.29−7.31 (m, 2H), 7.40−7.43 (m, 2H), 7.46 (d, J = 1.7 Hz, 1H). ^{13}C NMR ($CDCl_3$, 90 MHz) δ (ppm): 20.8, 21.0, 21.5, 38.3, 39.3, 49.4, 57.8, 71.0, 126.1, 128.4, 129.1, 131.8, 131.9, 132.9, 134.7, 135.1, 138.2, 146.7, 204.3. IR (NaCl) ν (cm^{-1}): 3387, 2924, 2817, 1682, 1492, 1219, 1094, 825, 772. APCIMS (m/z): $[M]^+$ 386, $[M + 2]^+$ 388

1-(2,5-Dimethylphenyl)-4-[4-(2,5-dimethylphenyl)-4-hydroxypiperidin-1-yl]butan-1-one (1f). Compound **6c** and 2-bromo-*p*-xylene were reacted and worked up as described for the preparation of **1b** to give **1f** (45%) as a colorless solid (mp 108 °C). ^{1}H NMR ($CDCl_3$, 360 MHz) δ (ppm): 1.92−2.03 (m, 4H), 2.20−2.27 (m, 2H), 2.30 (s, 3H), 2.35 (s, 3H), 2.44 (s, 3H), 2.54 (s, 3H), 2.55−2.63 (m, 4H), 2.84−2.91 (m, 2H), 2.95 (t, J = 7.1 Hz, 2H), 6.97 (dd, J = 7.9, 1.7 Hz, 1H), 7.05 (d, J = 7.9 Hz, 1H), 7.11 (d, J = 7.8 Hz, 1H), 7.17 (dd, J = 7.8, 1.5 Hz, 1H), 7.20 (d, J = 1.7 Hz, 1H), 7.45 (d, J = 1.5 Hz, 1H). ^{13}C NMR ($CDCl_3$, 90 MHz) δ (ppm): 20.7, 20.9, 21.2, 21.5, 21.7, 36.7, 39.3, 49.3, 57.7, 72.2, 126.1, 127.9, 129.0, 131.8, 133.0, 133.3, 134.7, 135.1, 138.2, 144.4, 204.4. IR (NaCl) ν (cm^{-1}): 3533, 2924, 2816, 1682, 1500, 1248, 1124, 812. HRMS (m/z): $[M]^+$ calcd for $C_{25}H_{33}NO_2$, 379.2511; found, 379.2510.

4-[4-(4-Chlorophenyl)piperazin-1-yl]-1-(4-fluorophenyl)butan-1-one (2a). To a solution of 4-chlorophenylpiperazine (200 mg, 1.0 mmol), NaI (4.4 mg, 0.3 mmol), and $NaHCO_3$ (84 mg, 1.0 mmol) in acetonitrile (3.5 mL) was added **4a** (0.08 mL, 0.5 mmol). The mixture was heated to reflux for 22 h. After evaporation, saturated $NaHCO_3$ solution and CH_2Cl_2 were added. The organic layer was dried (Na_2SO_4) and evaporated and the residue was purified by flash chromatography (hexane/EtOAc 1:1) to give pure **2a** (37 mg, 46%) as a pale yellow solid (mp 92 °C). ^{1}H NMR ($CDCl_3$, 360 MHz) δ (ppm): 1.94−2.02 (m, 2H), 2.46 (t, J = 7.4 Hz, 2H), 2.56−2.59 (m, 4H), 3.00 (t, J = 7.2 Hz, 2H), 3.09−3.11 (m, 4H), 6.80−6.82 (m, 2H), 7.10−7.14 (m, 2H), 7.17−7.20 (m, 2H), 7.98−8.02 (m, 2H). ^{13}C NMR ($CDCl_3$, 90 MHz) δ (ppm): 21.6, 36.2, 49.1, 53.0, 57.6, 115.5, 115.7, 117.2, 124.4, 128.9, 130.6, 130.7, 133.7, 150.0, 167.1, 198.4. IR (NaCl) ν (cm^{-1}): 2951, 2819, 1678, 1595, 1236, 1138, 810. APCI-MS (m/z): $[M]^+$ 361, $[M + 2]^+$ 363.

4-[4-([2.2]Paracyclophan-4-yl)piperazin-1-yl]-1-(4-fluorophenyl)-butan-1-one (2b). Compound **4a** and ([2.2]paracyclophan-4-yl)-piperazine[14b] were reacted and worked up as described for **2a** to give **2b** (46%) as a pale yellow solid (mp 83 °C). ^{1}H NMR ($CDCl_3$, 360 MHz) δ (ppm): 1.97−2.04 (m, 2H), 2.49−2.54 (m, 2H), 2.59−2.72 (m, 6H), 2.83−2.90 (m, 2H), 2.93−3.05 (m, 8H), 3.24 (ddd, J = 12.7, 7.8, 7.8 Hz, 1H), 3.36 (ddd, J = 12.7, 9.5, 2.3 Hz, 1H), 5.69 (d, J = 1.7 Hz, 1H), 6.26 (dd, J = 7.7, 1.7 Hz, 1H), 6.35 (dd, J = 7.7, 1.7 Hz, 1H), 6.40 (d, J = 7.7 Hz, 1H), 6.44 (dd, J = 7.7, 1.7 Hz, 1H), 6.52 (dd, J = 7.7, 1.7 Hz, 1H), 6.68 (dd, J = 7.7, 1.7 Hz, 1H), 7.10−7.15 (m, 2H), 7.99−8.03 (m, 2H). ^{13}C NMR ($CDCl_3$, 90 MHz) δ (ppm): 21.7, 34.1, 35.1, 35.2, 35.3, 36.2, 51.6, 53.8, 57.8, 115.5, 115.8, 121.4, 127.0, 128.8, 130.7, 130.8, 131.3, 132.3, 132.8, 133.2, 133.7, 136.2, 138.8, 139.9, 140.9, 150.5, 167.1, 198.5. IR (NaCl) ν (cm^{-1}): 2924, 2812, 1686, 1597, 1232, 1155, 835, 773. APCI-MS (m/z): $[M + 1]^+$ 457.

4-[4-(2,5-Dimethylphenyl)piperazin-1-yl]-1-(4-fluorophenyl)-butan-1-one (2c).[26] Compound **4a** and 2,5-dimethylphenypiperazine were reacted and worked up as described for **2a** to give **2c** (96%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 360 MHz) δ (ppm): 1.95−2.02 (m, 2H), 2.24, 2.30, 2.48 (t, J = 7.0 Hz, 2H), 2.56−2.61 (m, 4H), 2.86−2.88 (m, 4H), 3.01 (t, J = 7.3 Hz, 2H), 6.77−6.79 (m, 2H), 7.04 (d, J = 7.6 Hz, 1H), 7.11−7.15 (m, 2H), 8.00−8.04 (m, 2H). ^{13}C NMR ($CDCl_3$, 90 MHz) δ (ppm): 17.4, 21.2, 21.8, 36.2, 51.7, 53.7, 57.8, 115.5, 115.7, 119.7, 123.7, 129.3, 130.7, 130.8, 130.9, 133.7, 136.1, 151.4, 167.1, 198.6. IR (NaCl) ν (cm^{-1}): 2943, 2812, 1686, 1597, 1234, 1136, 834, 808. APCI-MS (m/z): $[M + 1]^+$ 355.

4-[4-(4-Chlorophenyl)piperazin-1-yl]-1-([2.2]paracyclophan-4-yl)butan-1-one (2d). Compound **4b** and 4-chlorophenylpiperazine were reacted and worked up as described for **2a** to give **2d** (27%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm): 1.89−1.97 (m, 2H), 2.42−2.51 (m, 2H), 2.59−2.63 (m, 4H), 2.74 (ddd, J = 16.8, 7.4, 6.9 Hz, 1H), 2.82−2.86 (m, 1H), 2.91 (ddd, J = 16.8, 7.4, 7.4 Hz, 1H), 2.99−3.04 (m, 2H), 3.12−3.20 (m, 8H), 3.87 (ddd, J = 12.4, 10.1, 2.2 Hz, 1H), 6.34 (dd, J = 7.6, 1.6 Hz, 1H), 6.49 (dd, J = 7.6, 1.6 Hz, 1H), 6.50−6.53 (m, 2H), 6.55 (dd, J = 7.6, 1.6 Hz, 1H), 6.65 (dd, J = 7.6, 1.6 Hz, 1H), 6.82−6.84 (m, 2H), 6.92 (d, J = 1.6 Hz, 1H), 7.19−7.20 (m, 2H). ^{13}C NMR ($CDCl_3$, 90 MHz) δ (ppm): 21.7, 35.1, 35.2, 36.0, 38.2, 49.2, 53.1, 57.7, 117.2, 124.5, 129.0, 131.2, 132.1, 132.8, 132.9, 133.9, 136.1, 136.4, 138.0, 139.2, 139.7, 140.3, 141.2, 150.0, 202.6. IR (NaCl) ν (cm^{-1}): 2926, 2823, 1678, 1595, 1236, 1136, 806, 721. HPLC (254 nm) t_R = 20.5 min, purity >99%. HRMS (m/z): $[M]^+$ calcd for $C_{30}H_{33}ClN_2O$, 472.2281; found, 472.2284.

4-[4-(4-Chlorophenyl)piperazin-1-yl]-1-(2,5-dimethylphenyl)-butan-1-one (2e). Compound **4c** and 4-chlorophenylpiperazine were reacted and worked up as described for **2a** to give **2e** (31%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 360 MHz) δ (ppm): 1.90−1.98 (m, 2H), 2.35 (s, 3H), 2.44 (s, 3H), 2.46 (t, J = 7.1 Hz, 2H), 2.58−2.61 (m, 4H), 2.94 (t, J = 6.7 Hz, 2H), 3.13−3.16 (m, 4H), 6.81−6.84 (m, 2H), 7.12 (dd, J = 7.7, 1.0 Hz, 1H), 7.16−7.20 (m, 3H), 7.44 (d, J = 1.0 Hz, 1H). ^{13}C NMR ($CDCl_3$, 90 MHz) δ (ppm): 20.7, 21.0, 21.5, 39.3, 49.2, 53.1, 57.6, 117.2, 124.5, 129.0, 131.8, 134.5, 135.1, 138.3, 150.0, 204.5. IR (NaCl) ν (cm^{-1}): 2956, 2819, 1684, 1597, 1236, 1136, 816. HPLC (254 nm) t_R = 19.4 min, purity 100%. HRMS (m/z): $[M]^+$ calcd for $C_{22}H_{27}ClN_2O$, 370.1812; found, 370.1813.

4-[4-([2.2]Paracyclophan-4-yl)piperazin-1-yl]-1-([2.2]paracyclophan-4-yl)butan-1-one (2f). A mixture of **4b** (200 mg, 0.56 mmol) and $NaHCO_3$ (320 mg, 3.8 mmol) in DMSO (3.8 mL) was heated to reflux for 1 h. Then saturated $NaHCO_3$ solution and CH_2Cl_2 were added at room temperature. The organic layer was extracted with saturated NaCl solution, dried ($MgSO_4$), and evaporated and the residue was purified by flash chromatography (hexane/EtOAc 1:1) to give 4-hydroxy-1-([2.2]paracyclophan-4-yl)butan-1-one (97 mg, 59%) as a colorless solid (mp 93 °C). ^{1}H NMR ($CDCl_3$, 360 MHz) δ (ppm): 1.89−2.07 (m, 2H), 2.76−2.89 (m, 2H), 2.96−3.06 (m, 3H), 3.11−3.24 (m, 4H), 3.72−3.76 (m, 2H), 3.87 (ddd, J = 12.5, 9.0, 2.5 Hz, 1H), 6.35 (dd, J = 7.8, 1.8 Hz, 1H), 6.47 (dd, J = 7.8, 1.8 Hz, 1H), 6.49−6.57 (m, 3H), 6.65 (dd, J = 7.8, 1.8 Hz, 1H), 6.92 (d, J = 1.8 Hz, 1H). ^{13}C NMR ($CDCl_3$, 90 MHz) δ (ppm): 27.3, 35.1, 35.2, 36.0, 37.4, 62.5, 131.2, 132.2, 132.9, 133.0, 133.4, 136.3, 136.4, 137.7, 139.2, 139.8, 140.2, 141.4, 203.1. IR (NaCl) ν (cm^{-1}): 3413, 3009, 2927, 2852, 1672, 1551, 721. HPLC (254 nm) t_R = 20.9 min, purity >99%. HRMS (m/z): $[M]^+$ calcd for $C_{20}H_{22}O_2$, 294.1620; found, 294.1619.

A solution of 4-hydroxy-1-([2.2]paracyclophan-4-yl)butan-1-one (43 mg, 0.15 mmol) and IBX (73 mg, 0.30 mmol) in DMSO (2.2 mL) was stirred at room temperature for 150 min. After addition of saturated $NaHCO_3$ solution and CH_2Cl_2, the organic

layer was separated, dried ($MgSO_4$), and evaporated and the residue was purified by flash chromatography (hexane/EtOAc 4:1) to give 4-oxo-4-([2.2]paracyclophan-4-yl)butyraldehyde (33 mg, 77%) as a colorless solid (mp 85 °C). ^{1}H NMR ($CDCl_3$, 360 MHz) δ (ppm): 2.76−2.91 (m, 3H), 2.96−3.07 (m, 3H), 3.13−3.23 (m, 4H), 3.28−3.37 (m, 1H), 3.86−3.92 (m, 1H), 6.41 (dd, J = 7.7, 1.7 Hz, 1H), 6.49−6.56 (m, 4H), 6.67 (dd, J = 7.7, 1.7 Hz, 1H), 7.01 (d, J = 1.7 Hz, 1H), 9.95 (s, 1H). ^{13}C NMR ($CDCl_3$, 90 MHz) δ (ppm): 32.8, 35.0, 35.2, 36.0, 38.0, 131.4, 132.2, 132.9, 133.0, 133.6, 136.4, 136.5, 137.2, 139.2, 139.9, 140.3, 141.7, 200.0, 200.8. IR (NaCl) ν (cm^{-1}): 3012, 2927, 2852, 1720, 1672, 1551, 723. HPLC (254 nm) t_R = 21.0 min, purity 97%; t_R = 23.6 min, purity 100%. HRMS (m/z): $[M]^+$ calcd for $C_{20}H_{20}O_2$, 292.1463; found, 292.1463.

To a mixture of 4-oxo-4-([2.2]paracyclophan-4-yl)butyraldehyde (6.1 mg, 0.02 mmol) and 4-[2.2]paracyclophanylpiperazine[14] (8.6 mg, 0.03 mmol) in CH_2Cl_2 (0.27 mL) was added sodium triacetoxyborohydride (5.8 mg, 0.03 mmol). After the mixture was stirred for 16 h at room temperature, saturated $NaHCO_3$ solution and CH_2Cl_2 were added. The organic layer was dried (Na_2SO_4) and evaporated and the residue was purified by flash chromatography (hexane/EtOAc 1:1) to give **2f** (6.5 mg, 55%) as a colorless solid (mp 65 °C). ^{1}H NMR ($CDCl_3$, 360 MHz) δ (ppm): 1.89−2.02 (m, 2H), 2.52 (t, J = 7.0 Hz, 2H), 2.63−2.80 (m, 6H), 2.85−3.08 (m, 13H), 3.13−3.21 (m, 4H), 3.25 (ddd, J = 12.5, 8.2, 8.0 Hz, 1H), 3.38 (ddd, J = 12.5, 10.1, 1.8 Hz, 1H), 3.85−3.94 (m, 1H), 5.71 (d, J = 1.7 Hz, 1H), 6.27 (dd, J = 7.7, 1.7 Hz, 1H), 6.32−6.38 (m, 2H), 6.40 (d, J = 7.7 Hz, 1H), 6.44 (dd, J = 7.7, 1.7 Hz, 1H), 6.48−6.58 (m, 5H), 6.64 (dd, J = 7.6, 1.6 Hz, 1H), 6.70 (dd, J = 7.7, 1.7 Hz, 1H), 6.94 (d, J = 1.6 Hz, 1H). ^{13}C NMR ($CDCl_3$, 90 MHz) δ (ppm): 21.7, 34.1, 35.1, 35.2, 35.3, 36.0, 38.2, 51.7, 53.9, 57.8, 121.5, 127.1, 128.8, 131.2, 132.2, 132.3, 132.8, 133.0, 133.2, 133.4, 136.2, 136.3, 138.0, 138.8, 139.2, 139.8, 140.0, 140.3, 140.9, 141.3, 150.5, 202.7. IR (NaCl) ν (cm^{-1}): 3010, 2926, 2810, 1674, 1587, 1221, 771. HPLC (254 nm) t_R = 20.4 min, purity >99%. HRMS (m/z): $[M]^+$ calcd for $C_{40}H_{44}N_2O$, 568.3454; found, 568.3453.

1-(2,5-Dimethylphenyl)-4-[4-(2,5-dimethylphenyl)piperazin-1-yl]butan-1-one (2g). Compound **4c** and 2,5-dimethylphenylpiperazine were reacted and worked up as described for **2a** to give **2g** (31%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 360 MHz) δ (ppm): 1.91−1.99 (m, 2H), 2.25 (s, 3H), 2.29 (s, 3H), 2.36 (s, 3H), 2.44 (s, 3H), 2.48 (t, J = 7.2 Hz, 2H), 2.58−2.62 (m, 4H), 2.90−2.93 (m, 4H), 2.97 (t, J = 7.0 Hz, 2H), 6.79 (dd, J = 7.6, 1.3 Hz, 2H), 6.82 (d, J = 1.3 Hz, 1H), 7.05 (d, J = 7.6 Hz, 1H), 7.12 (d, J = 7.7 Hz, 1H), 7.17 (dd, J = 7.7, 1.5 Hz, 1H), 7.46 (d, J = 1.5 Hz, 1H). ^{13}C NMR ($CDCl_3$, 90 MHz) δ (ppm): 17.5, 20.7, 21.0, 21.2, 21.6, 39.4, 51.8, 53.7, 57.8, 119.7, 123.7, 129.0, 129.3, 130.9, 131.8, 134.6, 135.1, 136.1, 138.4, 151.4, 204.7. IR (NaCl) ν (cm^{-1}): 2943, 2812, 1684, 1606, 1207, 1136, 808. HPLC (254 nm) t_R = 19.6 min, purity 96%. HRMS (m/z): $[M]^+$ calcd for $C_{24}H_{32}N_2O$, 364.2515; found, 364.2512.

4-[4-(2-Methoxyphenyl)piperazin-1-yl]butyronitrile (7a).[13] To a solution of 2-methoxyphenylpiperazine (600 mg, 3.1 mmol) and $NaHCO_3$ (800 mg, 7.5 mmol) in acetonitrile (16.4 mL) was slowly added 4-bromobutyronitrile (0.25 mL, 2.5 mmol). After heating to reflux for 16 h, the mixture was evaporated and CH_2Cl_2 and water were added. The organic layer was dried ($MgSO_4$) and evaporated and the residue was purified by flash chromatography ($CHCl_3$/EtOAc 1:1) to give **150** (640 mg, 99%) as a colorless solid (mp 77 °C). ^{1}H NMR ($CDCl_3$, 360 MHz) δ (ppm): 1.82−1.90 (m, 2H), 2.45 (t, J = 7.1 Hz, 2H), 2.53 (t, J = 6.7 Hz, 2H), 2.62−2.65 (m, 4H), 3.07−3.10 (m, 4H), 3.86 (s, 3H), 6.86 (dd, J = 7.9, 1.0 Hz, 1H), 6.91−6.94 (m, 2H), 7.00 (ddd, J = 7.9, 6.2, 2.7 Hz, 1H). IR (NaCl) ν (cm^{-1}): 2941, 2816, 2245, 1500, 1450, 1240, 1140, 1024, 750. EI-MS (m/z): $[M]^+$ 259.

4-[4-([2.2]Paracyclophan-4-yl)piperazin-1-yl]butyronitrile (7b). [2.2]Paracyclophan-4-yl)piperazine was reacted and worked up as described for **7a** to give **7b** (84%) as a pale yellow solid (mp 150 °C). ^{1}H NMR ($CDCl_3$, 360 MHz) δ (ppm): 1.85−1.93 (m, 2H), 2.46 (t, J = 7.1 Hz, 2H), 2.55−2.59 (m, 2H), 2.62−2.73 (m, 5H), 2.88−3.06 (m, 9H), 3.24 (ddd, J = 12.8, 9.4, 6.4 Hz, 1H), 3.36 (ddd, J = 12.8, 9.4, 2.4 Hz, 1H), 5.71 (d, J = 1.7 Hz, 1H), 6.27 (dd, J = 7.7, 1.7 Hz, 1H), 6.36 (dd, J = 7.7, 1.7 Hz, 1H), 6.40 (d, J = 7.7 Hz, 1H), 6.44 (dd, J = 7.7, 1.7 Hz, 1H), 6.53 (dd, J = 7.7, 1.7 Hz, 1H), 6.67 (dd, J = 7.7, 1.7 Hz, 1H). ^{13}C NMR ($CDCl_3$, 90 MHz) δ (ppm): 15.0, 22.7, 34.1, 35.0, 35.2, 35.3, 51.5, 53.8, 56.4, 119.7, 121.5, 127.2, 128.7, 131.3, 132.2, 132.8, 133.2, 136.3, 138.9, 139.9, 140.9, 150.3. IR (NaCl) ν (cm^{-1}): 2924, 2815, 2245, 1490, 1449, 1233, 1138, 772. HPLC (254 nm) t_R = 17.0 min, purity 100%. HRMS (m/z): $[M]^+$ calcd for $C_{24}H_{29}N_3$, 359.2361; found, 359.2361.

4-[4-(2,5-Dimethylphenyl)piperazin-1-yl]butyronitrile (7c). 2,5-Dimethylphenylpiperazine was reacted and worked up as described for **7a** to give **7c** (99%) as a pale yellow solid (mp 45 °C). ^{1}H NMR ($CDCl_3$, 360 MHz) δ (ppm): 1.82−1.90 (m, 2H), 2.25 (s, 3H), 2.30 (s, 3H), 2.45 (t, J = 7.1 Hz, 2H), 2.53 (t, J = 6.7 Hz, 2H), 2.56−2.61 (m, 4H), 2.90−2.93 (m, 4H), 6.81 (dd, J = 7.5, 2.0 Hz, 1H), 6.83 (d, J = 2.0 Hz, 1H), 7.05 (d, J = 7.5 Hz, 1H). ^{13}C NMR ($CDCl_3$, 90 MHz) δ (ppm): 15.0, 17.5, 21.2, 22.9, 51.8, 53.7, 56.4, 119.8, 123.8, 129.3, 130.9, 136.1, 151.3. IR (NaCl) ν (cm^{-1}): 2943, 2814, 2245, 1504, 1450, 1244, 1138, 771. EI-MS (m/z): $[M]^+$ 257.

4-[4-(2-Methoxyphenyl)piperazin-1-yl]butylamine (8a).[13] To a solution of **7a** (670 mg, 2.6 mmol) in diethyl ether (33 mL) was added $LiAlH_4$ (6.4 mL, 6.4 mmol, 1 M in diethyl ether) at 0 °C. After being stirred for 1 h at room temperature, the solution was cooled to 0 °C when saturated $NaHCO_3$ solution was added. After filtration through Celite, the filtrate was evaporated to give pure **8a** (670 mg, 99%) as a colorless solid. ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm): 1.49−1.54 (m, 2H), 1.56−1.62 (m, 2H), 2.43 (t, J = 7.0 Hz, 2H), 2.63−2.69 (m, 4H), 2.75 (t, J = 6.4 Hz, 2H), 3.07−3.14 (m, 4H), 3.86 (s, 3H), 6.86 (dd, J = 7.7, 1.3 Hz, 1H), 6.90−6.96 (m, 2H), 6.99 (ddd, J = 7.7, 7.0, 1.6 Hz, 1H). EI-MS (m/z): $[M]^+$ 263.

4-[4-([2.2]Paracyclophan-4-yl)piperazin-1-yl]butylamine (8b). Compound **7b** was reacted and worked up as described for **8a** to give **8b** (99%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 360 MHz) δ (ppm): 1.52−1.60 (m, 4H), 2.46 (t, J = 7.1 Hz, 2H), 2.61−2.77 (m, 7H), 2.89−3.05 (m, 9H), 3.20−3.28 (m, 1H), 3.37 (ddd, J = 12.8, 9.4, 2.4 Hz, 1H), 5.71 (d, J = 1.7 Hz, 1H), 6.27 (dd, J = 7.7, 1.7 Hz, 1H), 6.35 (dd, J = 7.7, 1.7 Hz, 1H), 6.40 (d, J = 7.7 Hz, 1H), 6.44 (dd, J = 7.7, 1.7 Hz, 1H), 6.53 (dd, J = 7.7, 1.7 Hz, 1H), 6.68 (dd, J = 7.7, 1.7 Hz, 1H). ^{13}C NMR ($CDCl_3$, 90 MHz) δ (ppm): 24.4, 29.7, 30.9, 34.1, 35.0, 35.2, 35.3, 51.4, 53.8, 58.5, 121.5, 127.1, 128.7, 131.2, 132.2, 132.8, 133.2, 136.2, 138.9, 139.9, 140.9, 150.4. IR (NaCl) ν (cm^{-1}): 3375, 3007, 2927, 2813, 1587, 1490, 1232, 1129, 753. HPLC (254 nm) t_R = 14.4 min, purity >99%. APCI-MS (m/z): $[M + 1]^+$ 364.

4-[4-(2,5-Dimethylphenyl)piperazin-1-yl]butylamine (8c). Compound **7c** was reacted and worked up as described for **8a** to give **8c** (93%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 360 MHz) δ (ppm): 1.46−1.62 (m, 4H), 2.24 (s, 3H), 2.29 (s, 3H), 2.42 (t, J = 7.0 Hz, 2H), 2.55−2.63 (m, 4H), 2.74 (t, J = 6.4 Hz, 2H), 2.92−2.93 (m, 4H), 6.78 (dd, J = 7.6, 1.3 Hz, 1H), 6.82 (d, J = 1.3 Hz, 1H), 7.04 (d, J = 7.6 Hz, 1H). ^{13}C NMR ($CDCl_3$, 90 MHz) δ (ppm): 17.5, 21.2, 24.4, 31.6, 42.1, 51.8, 53.8, 58.6, 119.8, 123.8, 129.3, 130.9, 136.1, 151.5. IR (NaCl) ν (cm^{-1}): 3350, 2939, 2808, 1608, 1575, 1504, 1244, 1136, 995, 806. HPLC (254 nm) t_R = 11.9 min, purity >99%. HRMS (m/z): $[M]^+$ calcd for $C_{16}H_{27}N_3$, 261.2205; found, 261.2206.

***N*-[4-[4-([2.2]Paracyclophan-4-yl)piperazin-1-yl]butyl]benzo[*b*]thiophenyl-2-carboxamide (3a).** Compound **8b** and benzothiophene-2-carboxylic acid were reacted and worked up as described for **3c** to give pure **3a** (75%) as a colorless solid (mp 114 °C). ^{1}H NMR ($CDCl_3$, 360 MHz) δ (ppm): 1.62−1.68 (m, 4H), 2.45 (t, J = 7.1 Hz, 2H), 2.58−2.66 (m, 5H), 2.81−2.88 (m, 4H), 2.91−2.98 (m, 5H), 3.16 (ddd, J = 12.7, 9.4, 6.3 Hz, 1H), 3.29 (ddd, J = 12.7, 9.4, 2.2 Hz, 1H), 3.45 (dt, J = 6.2, 5.9 Hz, 2H), 5.60 (d, J = 1.6 Hz, 1H), 6.20 (dd, J = 7.5, 1.6 Hz, 1H), 6.27

(dd, J = 7.5, 1.6 Hz, 1H), 6.33 (d, J = 7.5 Hz, 1H), 6.37 (dd, J = 7.5, 1.6 Hz, 1H), 6.45 (dd, J = 7.5, 1.6 Hz, 1H), 6.52 (br t, J = 5.9 Hz, 1H), 6.60 (dd, J = 7.5, 1.6 Hz, 1H), 7.31 (ddd, J = 7.2, 7.2, 1.5 Hz, 1H), 7.35 (ddd, J = 7.2, 7.2, 1.6 Hz, 1H), 7.69 (br s, 1H), 7.72−7.79 (m, 2H). ^{13}C NMR ($CDCl_3$, 90 MHz) δ (ppm): 24.4, 27.4, 34.1, 35.0, 35.2, 35.3, 40.1, 51.5, 54.0, 58.1, 121.5, 122.7, 124.9, 125.0, 125.1, 126.3, 127.1, 128.8, 131.2, 132.3, 132.8, 133.2, 136.2, 138.7, 138.8, 139.1, 139.9, 140.7, 140.9, 150.4, 162.4. IR (NaCl) ν (cm^{-1}): 3314, 2923, 2850, 1628, 1543, 1295, 1219, 772. APCI-MS (m/z): $[M + 1]^+$ 524.

***N*-[4-[4-(2,5-Dimethylphenyl)piperazin-1-yl]butyl]benzo[*b*]thiophenyl-2-carboxamide (3b).** Compound **8c** and benzothiophene-2-carboxylic acid were reacted and worked up as described for **3c** to give pure **3b** (73%) as a pale yellow solid (mp 58 °C). ^{1}H NMR ($CDCl_3$, 360 MHz) δ (ppm): 1.72−1.76 (m, 4H), 2.23 (s, 3H), 2.27 (s, 3H), 2.66 (t, J = 7.2 Hz, 2H), 2.77−2.81 (m, 4H), 2.97−3.00 (m, 4H), 3.52 (dt, J = 6.2, 5.9 Hz, 2H), 6.80 (dd, J = 7.7, 1.4 Hz, 1H), 6.77 (d, J = 1.4 Hz, 1H), 6.81 (dd, J = 7.6, 1.4 Hz, 1H), 6.98 (br t, J = 5.9 Hz, 1H), 7.05 (d, J = 7.6 Hz, 1H), 7.37 (ddd, J = 7.2, 7.2, 1.6 Hz, 1H), 7.40 (ddd, J = 7.2, 7.2, 1.8 Hz, 1H), 7.81−7.85 (m, 3H). ^{13}C NMR ($CDCl_3$, 90 MHz) δ (ppm): 17.4, 21.2, 23.6, 27.1, 39.7, 50.9, 53.7, 57.9, 119.9, 122.7, 124.2, 124.9, 125.1, 125.2, 126.2, 129.3, 130.9, 136.3, 138.9, 139.3, 140.8, 150.6, 162.6. IR (NaCl) ν (cm^{-1}): 3305, 2941, 2812, 1630, 1545, 1294, 1246, 754. APCI-MS (m/z): $[M + 1]^+$ 422.

***N*-[4-[4-(2-Methoxyphenyl)piperazin-1-yl]butyl]-2,5-dimethylbenzamide (3c).** To a solution of 2,5-dimethylbenzoic acid (47 mg, 0.32 mmol) in CH_2Cl_2 (12 mL) was added diisopropylethylamine (0.22 mL) at 0 °C. Then TBTU (120 mg, 0.38 mmol) in DMF (0.9 mL) and subsequently **8a** (100 mg, 0.38 mmol) in CH_2Cl_2 were added. The mixture was stirred for 1 h at room temperature when saturated $NaHCO_3$ solution was added. After extraction with CH_2Cl_2 the organic layer was dried ($MgSO_4$) and evaporated and the residue was purified by flash chromatography (CH_2Cl_2/MeOH 95:5) to give pure **3c** (88 mg, 71%) as a colorless solid (mp 126 °C). ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm): 1.69−1.71 (m, 4H), 2.28 (s, 3H), 2.38 (s, 3H), 2.54 (t, J = 6.5 Hz, 2H), 2.65−2.71 (m, 4H), 2.90−2.97 (m, 4H), 3.46 (dt, J = 6.2, 5.9 Hz, 2H), 3.85 (s, 3H), 6.80 (dd, J = 7.7, 1.4 Hz, 1H), 6.85 (dd, J = 7.9, 1.0 Hz, 1H), 6.90 (ddd, J = 7.5, 7.0, 1.0 Hz, 1H), 6.93−6.95 (m, 1H), 7.00 (ddd, J = 7.9, 7.0, 1.7 Hz, 1H), 7.06−7.09 (m, 2H), 7.15 (d, J = 1.4 Hz, 1H). ^{13}C NMR ($CDCl_3$, 90 MHz) δ (ppm): 19.3, 20.8, 24.2, 27.6, 39.5, 49.9, 53.3, 55.4, 58.0, 111.3, 118.3, 121.1, 123.1, 127.4, 130.3, 130.8, 132.4, 135.3, 137.1, 141.0, 152.3, 170.5. IR (NaCl) ν (cm^{-1}): 3284, 2939, 2816, 1643, 1500, 1240, 1146, 748. EI-MS (m/z): $[M]^+$ 395.

***N*-[4-[4-([2.2]Paracyclophan-4-yl)piperazin-1-yl]butyl][2.2]paracyclophanyl-4-carboxamide (3d).** [2.2]Paracyclophane carboxylic acid and **8b** were reacted and worked up as described for **3c** to give **3d** (65%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$, 360 MHz) δ (ppm): 1.65−1.70 (m, 4H), 2.48 (t, J = 5.7 Hz, 2H), 2.56−2.70 (m, 6H), 2.80−3.16 (m, 14H), 3.21−3.27 (m, 2H), 3.34 (ddd, J = 12.6, 9.5, 2.1 Hz, 1H), 3.43−3.47 (m, 2H), 3.61−3.70 (m, 1H), 5.64 (d, J = 1.6 Hz, 1H), 6.12 (br t, J = 5.6 Hz, 1H), 6.26 (dd, J = 7.5, 1.6 Hz, 1H), 6.33 (dd, J = 7.5, 1.6 Hz, 1H), 6.39 (d, J = 7.5 Hz, 1H), 6.41−6.47 (m, 3H), 6.51−6.56 (m, 4H), 6.63−6.66 (m, 2H), 6.82 (dd, J = 7.9, 1.8 Hz, 1H). ^{13}C NMR ($CDCl_3$, 90 MHz) δ (ppm): 24.5, 27.6, 34.0, 34.8, 35.0, 35.1, 35.2, 35.3, 35.5, 39.7, 51.2, 53.9, 58.0, 121.5, 127.1, 128.7, 131.2, 131.6, 132.2, 132.0, 132.4, 132.5, 132.6, 132.8, 133.1, 134.8, 135.3, 135.9, 136.2, 138.8, 139.0, 139.1, 139.8, 139.9, 140.1, 140.8, 150.4, 169.4. IR (NaCl) ν (cm^{-1}): 3305, 3007, 2926, 2850, 1641, 1512, 1290, 771. HPLC (254 nm) t_R = 20.7 min, purity 96%; t_R = 20.3 min, purity 100%. HRMS (m/z): $[M]^+$ calcd for $C_{41}H_{47}N_3O$, 597.3719; found, 597.3720.

***N*-[4-[4-(2,5-Dimethylphenyl)piperazin-1-yl]butyl]-2,5-dimethylbenzamide (3e).** 2,5-Dimethylbenzoic acid and **8c** were reacted and worked up as described for **3c** to give **3e** (83%) as a pale yellow solid (mp 121 °C). ^{1}H NMR ($CDCl_3$, 360 MHz) δ (ppm): 1.67−1.72 (m, 4H), 2.21 (s, 3H), 2.30 (s, 3H), 2.31 (s, 3H), 2.39 (s, 3H), 2.49 (t, J = 6.7 Hz, 2H), 2.54−2.60 (m, 4H), 2.72−2.74 (m, 4H), 3.46 (dt, J = 6.2, 5.9 Hz, 2H), 6.67 (d, J = 1.3 Hz, 1H), 6.79 (dd, J = 7.7, 1.4 Hz, 1H), 7.02 (br t, J = 5.9 Hz, 1H), 7.03 (d, J = 7.5 Hz, 1H), 7.09−7.10 (m, 2H), 7.16 (d, J = 1.4 Hz, 1H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ (ppm): 17.4, 19.2, 20.8, 21.2, 24.5, 27.7, 39.6, 51.2, 53.7, 58.0, 119.8, 123.9, 127.4, 129.3, 130.3, 130.8, 132.4, 135.2, 136.0, 137.2, 151.0, 170.4. IR (NaCl) ν (cm^{-1}): 3284, 2941, 2812, 1643, 1539, 1308, 1244, 810. APCIMS (m/z): $[M + 1]^+$ 394.

Receptor Binding Studies. Receptor binding studies were carried out as previously described.[15] In brief, the dopamine D_1 receptor assay was done with porcine striatal membranes at a final protein concentration of 40−60 μg/assay tube and the radioligand [^{3}H]SCH 23390 at 0.3 nM (K_D = 0.53−0.95 nM). Competition experiments with human D_{2long},[16] D_{2short},[16] D_3,[17] and $D_{4.4}$[18] receptors were run with preparations of membranes from CHO cells stably expressing the corresponding receptor and [^{3}H]spiperone at a final concentration of 0.1−0.3 nM. The assays were carried out at a protein concentration of 1−8 μg/assay tube and K_D values of 0.05−0.11, 0.03−0.15, 0.06−0.28, and 0.11−0.28 nM for the D_{2long}, D_{2short}, D_3, and $D_{4.4}$ receptors, respectively. 5-HT and α_1 receptor binding experiments were performed with homogenates prepared from porcine cerebral cortex as described.[27] Assays were run with membranes at a protein concentration per assay tube of 80, 100, and 55 μg/mL for 5-HT_{1A}, 5-HT_2, and α_1 receptor, respectively, and radioligand concentrations of 0.1 nM ([^{3}H]WAY100635 and [^{3}H]-prazosin) and 0.5 nM ([^{3}H]ketanserin) with K_D values of 0.06 nM for 5-HT_{1A}, 1.1 nM for 5-HT_2, and 0.04−0.14 nM for the α_1 receptor. Protein concentration was established by the method of Lowry using bovine serum albumin as standard.[28]

Mitogenesis Experiments. Determination of the intrinsic activity of the representative compound was carried out by measuring the incorporation of [^{3}H]thymidine into growing cells after stimulation with the test compound as described in the literature.[10,21] For this assay D_3 expressing CHO dhfr$^-$ cells have been incubated with 0.02 μCi [^{3}H]thymidine per well (specific activity 25 μCi/mmol). Dose response curves of six experiments have been normalized and pooled to get a mean curve from which the EC_{50} value and the maximum intrinsic activity of each compound could be compared to the effects of the full agonist quinpirole.

Site Directed Mutagenesis. The pcDNA3.1(+) of hDRD3 receptor was used as described previously.[29] Oligonucleotidic primers were purchased from Biomers.net or MWG Biotech AG. Site directed mutagenesis was performed by polymerase chain reaction (PCR) using oligonucleotides bearing the desired mutation. Fidelity of PCR amplification and introduction of mutations in the receptor cDNA were confirmed by sequencing with the ABI sequencer system (ABI Systems, Weiterstadt, Germany) at the laboratory of C.-M. Becker (Department of Biochemistry, FAU Erlangen, Germany) using oligonucleotidic primers.

Mutant Receptor Preparation. HEK-293 cells, transiently transfected with the wild type and mutant receptor cDNA by the $CaHPO_4$ method or using TransIT-293 transfection reagent (Mirus Bio Corp.), were cultured in 150 mm Petri plates containing 20 mL of MEM α medium supplemented with 10% (v/v) fetal bovine serum, 100 U/mL penicillin G, 100 μg/mL streptomycin, and 2 mM L-glutamine at 37 °C and 5% CO_2.

HEK-293 cells were harvested 48 h after transfection. Cells were harvested by removal of the medium, followed by a wash with phosphate buffered saline, which was discarded, resuspension in 10 mL of harvest puffer (10 mM Tris-HCl, 0.5 mM EDTA, 5.4 mM KCl, and 140 mM NaCl, pH 7.4), scraping of the cells with a rubber spatula into a centrifuge tube, and collection of the cells by centrifugation at 220g for 8 min. The cellular pellet was resuspended in 5 mL of homogenate buffer for D_2 and D_4 receptors (50 mM Tris-HCl, 5 mM EDTA, 1,5 mM $CaCl_2$, 5 mM $MgCl_2$, 5 mM KCl, and 120 mM NaCl, pH 7,4)

and for the D_3 receptor (10 mM Tris-HCl and 5 mM $MgSO_4$, pH 7,4). Cells were used as they were or stored at −80 °C.

After thawing or being used directly, the cells were diluted in homogenate buffer, homogenized using a Polytron (20 000 rpm, 5 × 5 s each in an ice bath), and spun at 50000*g* for 18 min. The membrane pellet was always resuspended in homogenate buffer for D_2 and D_4 receptors, homogenized with a Potter-Elvehjem homogenizer, and stored in small aliquots at −80 °C. Protein concentration was estimated by the method of Lowry et al. using bovine serum albumin as a standard.[28]

Data Analysis. The resulting competition curves of the receptor binding experiments were analyzed by nonlinear regression using the algorithms in PRISM 3.0 (GraphPad software, San Diego, CA). The data were fit using a sigmoid model to provide an IC_{50} value, representing the concentration corresponding to 50% of maximal inhibition. IC_{50} values were transformed to K_i values according to the equation of Cheng and Prusoff.[30]

Binding curves resulting from the mitogenesis assay were analyzed by nonlinear regression. Each data point was normalized (basal effect = 0%; maximum effect of the full agonist quinpirole = 100%) and then combined to get a mean curve. Nonlinear regression analysis of this curve provided the EC_{50} values representing the concentration corresponding to 50% of maximal stimulation as a measure of potency.

Acknowledgment. We thank Dr. J.-C. Schwartz and Dr. P. Sokoloff (INSERM, Paris, France), Dr. H. H. M. Van Tol (Clarke Institute of Psychiatry, Toronto, Canada), and Dr. J. Shine (The Garvan Institute of Medical Research, Sydney, Australia) for providing D3, D4.4, and D2 receptor expressing cell lines. This work was supported by the Deutsche Forschungsgemeinschaft (DFG).

Supporting Information Available: Elementary analysis results for **1b**, **1c**, **1e**, **1f**, **2b**, **3a**−**c**, **6b**, and **7c**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Lopez-Munoz, F.; Alamo, C. The consolidation of neuroleptic therapy: Janssen, the discovery of haloperidol and its introduction into clinical practice. *Brain Res. Bull.* **2009**, *79*, 130–141.
(2) Scott, L. J. Iloperidone: in schizophrenia. *CNS Drugs* **2009**, *23*, 867–880.
(3) Citrome, L. Paliperidone palmitate—review of the efficacy, safety and cost of a new second-generation depot antipsychotic medication. *Int. J. Clin. Pract.* **2010**, *64*, 216–239.
(4) Janssen, P. A.; Niemegeers, C. J.; Schellekens, K. H.; Lenaerts, F. M.; Verbruggen, F. J.; van Nueten, J. M.; Marsboom, R. H.; Herin, V. V.; Schaper, W. K. The pharmacology of fluspirilene (R 6218), a potent, long-acting and injectable neuroleptic drug. *Arzneimittelforschung* **1970**, *20*, 1689–1698.
(5) Harrison, T. S.; Perry, C. M. Aripiprazole. A review of its use in schizophrenia and schizoaffective disorder. *Drugs* **2004**, *64*, 1715–1736.
(6) New, J. S. The discovery and development of buspirone: a new approach to the treatment of anxiety. *Med. Res. Rev.* **1990**, *10*, 283–326.
(7) Deseure, K.; Breand, S.; Colpaert, F. C. Curative-like analgesia in a neuropathic pain model: Parametric analysis of the dose and the duration of treatment with a high-efficacy 5-HT1A receptor agonist. *Eur. J. Pharmacol.* **2007**, *568*, 134–141.
(8) Kiss, B.; Horvath, A.; Nemethy, Z.; Schmidt, E.; Laszlovszky, I.; Bugovics, G; Fazekas, K.; Hornok, K.; Orosz, S.; Gyertyan, I.; Agai-Csongor, E.; Domany, G.; Tihanyi, K.; Adham, N.; Szombathelyi, Z. Cariprazine (RGH-188), a dopamine D3 receptor-preferring, D3/D2 dopamine receptor antagonist−partial agonist antipsychotic candidate: in vitro and neurochemical profile. *J. Pharmacol. Exp. Ther.* **2010**, *333*, 328–340.
(9) Brioni, J. D.; Moreland, R. B.; Cowart, M.; Hsieh, G. C.; Stewart, A. O.; Hedlund, P.; Donnelly-Roberts, D. L.; Nakane, M.; Lynch, J. J., III; Kolasa, T.; Polakowski, J. S.; Osinski, M. A.; Marsh, K.; Andersson, K.-E.; Sullivan, J. P. Activation of dopamine D4 receptors by ABT-724 induces penile erection in rats. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 6758–6763.
(10) Bettinetti, L.; Schlotter, K.; Hübner, H.; Gmeiner, P. Interactive SAR studies: rational discovery of super-potent and highly selective dopamine D3 receptor antagonists and partial agonists. *J. Med. Chem.* **2002**, *45*, 4594–4597.
(11) Kortagere, S.; Gmeiner, P.; Weinstein, H.; Schetz, J. A. Certain 1,4-disubstituted aromatic piperidines and piperazines with extreme selectivity for the dopamine D4 receptor interact with a common receptor microdomain. *Mol. Pharmacol.* **2004**, *66*, 1491–1499.
(12) Ehrlich, K.; Götz, A.; Bollinger, S.; Tschammer, N.; Bettinetti, L.; Härterich, S.; Hübner, H.; Lanig, H.; Gmeiner, P. Dopamine D2, D3, and D4 selective phenylpiperazines as molecular probes to explore the origins of subtype specific receptor binding. *J. Med. Chem.* **2009**, *52*, 4923–4935.
(13) Schlotter, K.; Böckler, F.; Hübner, H.; Gmeiner, P. Fancy bioisosteres: novel paracyclophane derivatives as super-affinity dopamine D3 receptor antagonists. *J. Med. Chem.* **2006**, *49*, 3628–3635.
(14) (a) Ortner, B.; Waibel, R.; Gmeiner, P. Indoloparacyclophanes: synthesis and dopamine receptor binding of a novel arylbioisostere. *Angew. Chem, Int. Ed.* **2001**, *40*, 1283–1285. (b) Löber, S.; Ortner, B.; Bettinetti, L.; Hübner, H.; Gmeiner, P. Analogs of the dopamine D4 receptor ligand FAUC 113 with planar- and central-chirality. *Tetrahedron: Asymmetry* **2002**, *13*, 2303.
(15) Hübner, H.; Haubmann, C.; Utz, W.; Gmeiner, P. Conjugated enynes as nonaromatic catechol bioisosteres: synthesis, binding experiments, and computational studies of novel dopamine receptor agonists recognizing preferentially the D3 subtype. *J. Med. Chem.* **2000**, *43*, 756–762.
(16) Hayes, G.; Biden, T. J.; Selbie, L. A.; Shine, J. Structural subtypes of the dopamine D2 receptor are functionally distinct: expression of the cloned D2A and D2B subtypes in a heterologous cell line. *Mol. Endocrinol.* **1992**, *6*, 920–926.
(17) Sokoloff, P.; Andrieux, M.; Besançon, R.; Pilon, C.; Martres, M.-P.; Giros, B.; Schwartz, J.-C. Pharmacology of human dopamine D3 receptor expressed in a mammalian cell line: comparison with D2 receptor. *Eur. J. Pharmacol.* **1992**, *225*, 331–337.
(18) Asghari, V.; Sanyal, S.; Buchwaldt, S.; Paterson, A.; Jovanovic, V.; Van Tol, H. H. M. Modulation of intracellular cyclic AMP levels by different human dopamine D4 receptor variants. *J. Neurochem.* **1995**, *65*, 1157–1165.
(19) Klabunde, T.; Evers, A. GPCR antitarget modeling: pharmacophore models for biogenic amine binding GPCRs to avoid GPCR-mediated side effects. *ChemBioChem* **2005**, *6*, 876–889.
(20) Mierau, J.; Schneider, F. J.; Ensinger, H. A.; Chio, C. L.; Lajiness, M. E.; Huff, R. M. Pramipexole binding and activation of cloned and expressed dopamine D2, D3 and D4 receptors. *Eur. J. Pharmacol.* **1995**, *290*, 29–36.
(21) Hübner, H.; Kraxner, J.; Gmeiner, P. Cyanoindole derivatives as highly selective dopamine D4 receptor partial agonists: solid-phase synthesis, binding assays, and functional experiments. *J. Med. Chem.* **2000**, *43*, 4563–4569.
(22) Boeckler, F.; Ohnmacht, U.; Lehmann, T.; Utz, W.; Hübner, H.; Gmeiner, P. CoMFA and CoMSIA investigations revealing novel insights into the binding modes of dopamine D3 receptor agonists. *J. Med. Chem.* **2005**, *48*, 2493–2508.
(23) Cho, W.; Taylor, L. P.; Mansour, A.; Akil, H. Hydrophobic residues of the D2 dopamine receptor are important for binding and signal transduction. *J. Neurochem.* **1995**, *65*, 2105–2115.
(24) Javitch, J. A.; Ballersteros, J. A.; Weinstein, H.; Chen, J. A cluster of aromatic residues in the sixth membrane-spanning segment of the dopamine D2 receptor is accessible in the binding-site crevice. *Biochemistry* **1998**, *37*, 998–1006.
(25) Tschammer, N.; Dörfler, M.; Hübner, H.; Gmeiner, P. Engineering a GPCR−ligand pair that simulates the activation of D2L by dopamine. *ACS Chem. Neurosci.* **2010**, *1*, 25–35.
(26) Janssen, P. A. J. U.S. Patent US 2997472, 1961.
(27) Schlotter, K.; Boeckler, F.; Hübner, H.; Gmeiner, P. Fancy bioisosteres: metallocene-derived G-protein-coupled receptor ligands with subnanomolar binding affinity and novel selectivity profiles. *J. Med. Chem.* **2005**, *48*, 3696–3699.
(28) Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **1951**, *193*, 265–275.
(29) Dörfler, M.; Tschammer, N.; Hamperl, K.; Hübner, H.; Gmeiner, P. Novel D3 selective dopaminergics incorporating enyne units as nonaromatic catechol bioisosteres: synthesis, bioactivity, and mutagenesis studies. *J. Med. Chem.* **2008**, *51*, 6829–6838.
(30) Cheng, Y.; Prusoff, W. H. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50% inhibition (IC_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099–3108.