Fancy Bioisosteres: Metallocene-Derived G-Protein-Coupled Receptor Ligands with Subnanomolar Binding Affinity and Novel Selectivity Profiles

Karin Schlotter, Frank Boeckler, Harald Hübner, and Peter Gmeiner*

Department of Medicinal Chemistry, Emil Fischer Center, Friedrich Alexander University, Schuhstrasse 19, D-91052 Erlangen, Germany

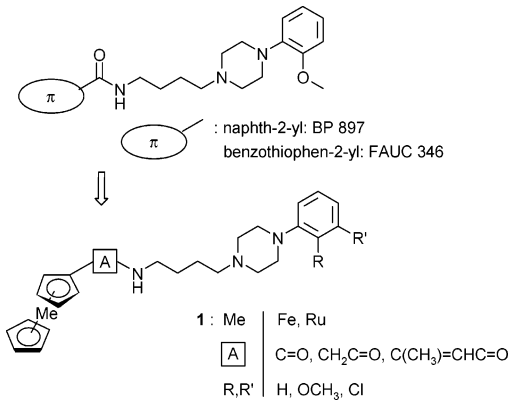
Received February 22, 2005

Abstract: Metallocene-derived bioisosteres lead to exceptionally strong binding G-protein-coupled receptor ligands, indicating substantial plasticity of the receptor excluded volume. Novel binding profiles of ferrocenylcarboxamides combining subnanomolar K_i values for the dopamine D4 receptor (**1a**, 0.52 nM; **1b**, 0.63 nM) with superpotent serotonin 5-hydroxytryptamine$_{1A}$ (**1a**, 0.50 nM) and dopamine D3 receptor binding (**1b**, 0.64 nM) and selective D4 agonist properties of the ruthenocene **1c** may be a starting point for highly beneficial central nervous system active drugs.

Bioisosteric replacement is commonly accepted as a valuable strategy in drug discovery, facilitating an improvement of pharmacodynamic and pharmacokinetic properties.[1] Taking advantage of the structural variety of heteroarene-derived scaffolds, we developed subtype-specific dopamine D3 receptor ligands[2] and selective D4 modulators.[3] An extension of our SAR studies demonstrated that the utilization of uncommon structural moieties (fancy bioisosteres) substantially expands the chemical diversity space of bioactive compounds. Besides the successful introduction of enynes and endiynes into dopaminergics,[4] we found that paracyclophane derivatives can be used as double-layered aryl bioisosteres.[5] To learn more about the obvious plasticity of G-protein-coupled receptors (GPCRs) and to approach central nervous system (CNS) active drugs with novel subtype selectivity profiles, these studies were continued. Employing the D3 partial agonists BP 897[6] and FAUC 346[2] as lead compounds, we herein report the evaluation of metallocenes of type **1** as potential ligands of GPCRs involving dopamine D1, D2$_{short}$, D1$_{long}$, D3, D4, serotonin 5-HT$_{1A}$ and 5-HT$_2$ subtypes, and adrenergic α_1-receptors (Chart 1).

Our metallocene-derived target compounds should involve ferrocenyl- and ruthenocenylcarboxamides. For the ferrocenes,[7] we additionally planned to modify the distance between the double-layered system and the phenylpiperazine moiety by inserting a methylene or a (methyl)ethenylene group. Finally, different phenyl substituents were evaluated. In detail, HATU promoted coupling of ferrocenylcarboxylic acid (**2a**) and the ruthenocene-derived analogue **2b**[8] with the 2-methoxyphenylpiperazinyl-substituted butylamine **3a** and its 2,3-dichlorophenyl analogue **3b**, being readily prepared following previously described protocols, which gave a

Chart 1



69−95% yield of the ferrocenyl- and ruthenocenylcarboxamides **1a**,**b** and **1c**,**d**, respectively (Scheme 1). For the preparation of the structural hybrid **1e**, a practical synthetic route to the building block **3c** had to be elaborated when we took advantage of a Buchwald−Hartwig cross-coupling[9,10] as a key reaction step. Thus, palladium promoted amination of 2,6-dichloroanisole (**4**) with piperazine gave the intermediate **5**. Subsequent treatment with bromobutyronitrile and reduction with $LiAlH_4$ resulted in formation of the primary amine **3c**. Coupling with ferrocenylcarboxylic acid was induced by TBTU to furnish the final product **1e**.

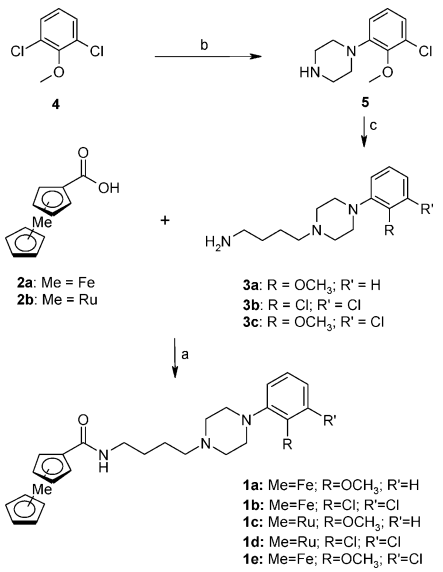
To properly adjust the relative disposition of the pharmacophoric elements, the distance between the double-layered arene bioisostere and the carboxamide moiety should be increased by insertion of small spacer elements. Thus, ferrocenylacetic acid (**6**) and the double-layered cinnamic acid analogue **7**,[11] which we prepared by treatment of ferrocene with acetoacetic acid ethyl ester under acidic conditions and subsequent saponification, should be used as additional molecular scaffolds. In fact, activation by TBTU or HATU and subsequent coupling with the primary amine **3a** gave access to the carboxamides **1f** and **1g** in 59% and 69% yield, respectively (Scheme 2).

Radioligand binding assays, mitogenesis, and [^{35}S]GTPγS binding experiments were employed to analyze affinity, selectivity profiles, and ligand efficacy of target compounds of type **1**. The K_i and EC_{50} values of the test compounds were compared to those of the D3 partial agonist BP 897, which is known for highly interesting antidyskinetic properties and for beneficial effects on cocaine-seeking behavior.[6b] The binding data were generated by measuring their ability to compete with [^{3}H]spiperone for the cloned human dopamine receptor subtypes D2$_{long}$, D2$_{short}$,[12] D3,[13] and D4.4[14] stably expressed in Chinese hamster ovary cells (CHO).[4a] D1 receptor affinities were determined utilizing porcine striatal membranes and the D1 selective radioligand [^{3}H]SCH 23390.[4a] Because of the observation that the lead compound BP 897 reveals serotoninergic and adrenergic activity,[6a] the test compounds were investigated for their potency to displace [^{3}H]-8-OH-DPAT, [^{3}H]ketanserin, and [^{3}H]prazosin when employing porcine 5-HT$_{1A}$, 5-HT$_2$, and α_1-receptors, respectively.

* To whom correspondence should be addressed. Phone: +49-(0)9131-8529383. Fax: +49-(0)9131-8522585. E-mail: gmeiner@pharmazie.uni-erlangen.de.

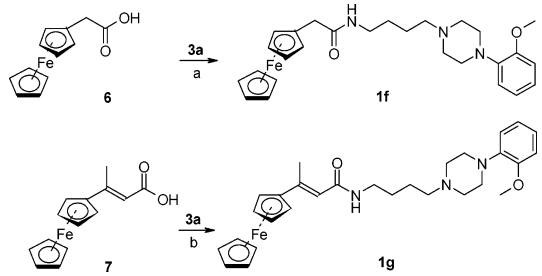
10.1021/jm050170s CCC: $30.25
Published on Web 04/29/2005

Scheme 1[a]



[a] Reagents and conditions: (a) for **1a,b**, HATU, DIPEA, DMF, **3a,b**, 0 °C to room temp, 1−2 h (69−71%); for **1c,d**, HATU, DIPEA, DMF, CH_2Cl_2, **3a,b**, CH_2Cl_2, 0 °C to room temp, 1−2h (83−95%); for **1e**, TBTU, DIPEA, DMF, CH_2Cl_2, **3c,** CH_2Cl_2, 0 °C to room temp, 30 min (79%); (b) $Pd(OAc)_2$, (*o*-biphenyl)P(*t*-Bu)$_2$, NaO*t*-Bu, toluene, piperazine, 80 °C, 21 h (37%); (c) (1) $Br(CH_2)_3CN$, Na_2CO_3, MeCN, reflux, 15 h (77%); (2) $LiAlH_4$, Et_2O, 0 °C to room temp, 1 h (95%).

Scheme 2[a]



[a] Reagents and conditions: (a) TBTU, DIPEA, DMF, **3a**, CH_2Cl_2, 0 °C to room temp, 1 h (59%); (b) HATU, DIPEA, DMF, **3a**, CH_2Cl_2, 0 °C to room temp, 21 h (69%).

The characterization of the test compounds was initiated by investigating the ferrocenylcarboxamides **1a**,**b**,**e** when the 2-methoxyphenylpiperazine **1a** showed the highest degree of similarity with the target compound BP 897. In fact, the binding data depicted in Table 1 clearly indicate that the double-layered metallocene partial structure is tolerated by the binding sites of the GPCRs investigated. Significant radioligand displacement was observed, leading to K_i values in the nanomolar or single-digit micromolar range. Compared to the D3 selective lead compound BP 897, the ferrocenylcarboxamide **1a** displayed a similar affinity pattern for D1, $D2_{long}$, $D2_{short}$, 5-HT_2, and α_1. Competition experiments at the D3 subtype led to a K_i of 6.5 nM, indicating only a 5-fold lower target recognition when compared to BP 897 (K_i = 1.3 nM). The most exciting binding data were observed for the D4 and the 5-HT_{1A} receptors resulting in K_i values of 0.52 and 0.50 nM. Thus, ferrocene **1a** (FAUC 378) proved to be a superaffinity ligand for 5-HT_{1A} and D4, displaying also substantial affinity for D3 and α_1. Exchange of the 2-methoxyphenyl group to a 2,3-dichlorophenyl moiety significantly modified the binding profile of the test compound **1b** when D3 affinity substantially increased (K_i = 0.64 nM). On the other hand, reduction of affinity for 5-HT_{1A} and an unchanged behavior toward D4 were observed when we determined a subnanomolar K_i value (0.63 nM). In fact, FAUC 382 (**1b**) turned out to be a superpotent D3/D4 modulator with more than 30-fold selectivity over D1, $D2_{long}$, $D2_{short}$, 5-HT_{1A}, 5-HT_2, and α_1. The structural similarity of the 3-chloro-2-methoxyphenyl derivative **1e** displayed a binding profile with K_i values between those of the 2-methoxyphenylpiperazine **1a** and the 2,3-dichloro analogue **1b**. Formal exchange of the Fe^{2+} central ion by Ru^{2+} led to additional highly potent GPCR ligands when the affinity patterns of the ruthenocenes **1c** and **1d** were very similar to those of the ferrocene analogues **1a** and **1b**, respectively. However, a highly significant exchange was noticed for the 5-HT_{1A} binding of the methoxyphenyl derivatives. Thus, the lower K_i value (20 nM) for the ruthenocene **1c** yielded an enhanced D4 selectivity (K_i = 0.37 nM for D4; selectivity over 5-HT_{1A} is >50). The binding affinities of the ferrocene **1g** as a structurally extended homologue of **1a** clearly indicate a moderate ability to compete with the radioligands in the binding assay. Thus, insertion of a two-carbon spacer between the double-layered π-system and the carboxamide moiety induced a reduced target recognition. On the other hand, formal insertion of a methylene unit leading to the ferrocenylacetamide **1f** significantly caused a decrease of binding affinity for only D4 (~6-fold) and 5-HT_{1A} (~25-fold).

As a measure of functional activity, ligand efficacy of the ferrocenylcarboxamides **1a**,**b**,**e** and the ruthenocyl derivatives **1c**,**d** was determined by a mitogenesis assay measuring the rate of [^{3}H]thymidine incorporation into growing CHO dhfr$^-$ cells and a CHO10001 cell line stably expressing the human D3 and D4.2 receptor, respectively.[13,15,16] The data listed in Table 2 clearly show substantial D3 ligand efficacy of the test compounds **1a**−**e** (24−50%, EC_{50} = 2.0−9.8 nM) that proved to be similar to the data we determined for the partial agonist BP 897 with respect to intrinsic activity and EC_{50} values. For the D4 subtype, the intrinsic activity of the ferrocene-derived 2-methoxyphenyl derivative **1a** (67%, EC_{50} = 0.55 nM) was superior to the respective effects of the 2,3-dichloro analogue **1b** and the 3-chloro-2-methoxy-substituted phenylpiperazine **1e**, whereas for the ruthenocenyl type compounds both derivatives (the 2-methoxyphenyl derivative **1c** (60%, EC_{50} = 1.2 nM) and the 2,3-dichloro analogue **1d** (60%, EC_{50} = 35 nM)) showed an intrinsic activity similar to that of **1a**. In contrast, the reference compound BP 897 displayed only weak D4 partial agonist properties.

To prove the functional activity of the test compounds detected in the mitogenesis experiments, a [^{35}S]GTPγS binding assay was established. When membrane preparations from human D4.4 receptor expressing CHO cells were used, the dose-dependent stimulation of [^{35}S]GTPγS binding was determined to distinguish between agonist and antagonist properties and to measure the potency and efficacy of agonists to the D4 receptor.[17,18] The intrinsic effects of **1a**−**e** listed in Table 2 indicate that the 2-methoxyphenylpiperazine moiety is a privileged structural feature to stimulate the D4

Table 1. Receptor Binding Data of **1a**−**g** Compared to the Reference Compound BP 897 Utilizing Human $D2_{long}$, $D2_{short}$, D3, and D4.4 Receptors and Porcine D1, 5-HT_{1A}, 5-HT_2, and α_1 Receptors

					K_i [a] (nM)							
					[^{3}H]SCH 23390	[^{3}H]spiperone				[^{3}H]8-OH-DPAT	[^{3}H]ketanserin	[^{3}H]prazosin
compd	Me	A	R	R′	D1	$D2_{long}$	$D2_{short}$	D3	D4.4	5-HT_{1A}	5-HT_2	α_1
1a	Fe	C=O	OMe	H	1500 ± 150	110 ± 10	78 ± 1.5	6.5 ± 0.54	0.52 ± 0.086	0.50 ± 0.080	310 ± 10	9.7 ± 0.30
1b	Fe	C=O	Cl	Cl	630 ± 25	31 ± 2.0	19 ± 0.50	0.64 ± 0.11	0.63 ± 0.027	27 ± 10	250 ± 15	73 ± 5.0
1c	Ru	C=O	OMe	H	720 ± 25	120 ± 24	60 ± 6.7	10 ± 0.93	0.37 ± 0.012	20 ± 1.9	370 ± 20	5.5 ± 0.25
1d	Ru	C=O	Cl	Cl	330 ± 25	43 ± 12	12 ± 0.58	0.84 ± 0.11	0.60 ± 0.025	77 ± 2.0	610 ± 30	26 ± 0.0
1e	Fe	C=O	OMe	Cl	2200 ± 300	99 ± 12	72 ± 9.1	3.5 ± 0.54	0.91 ± 0.19	14 ± 3.0	110 ± 0.0	35 ± 1.0
1f	Fe	CH_2C=O	OMe	H	750 ± 330	66 ± 5.0	45 ± 14	2.0 ± 0.17	3.3 ± 0.45	12 ± 3.9	700 ± 30	5.1 ± 0.80
1g	Fe		OMe	H	1700 ± 500	170 ± 5.0	120 ± 5.0	20 ± 4.5	21 ± 2.5	210 ± 0.0	2500 ± 300	26 ± 1.0
BP897					760 ± 60	220 ± 19	200 ± 5.0	1.3 ± 0.096	44 ± 7.6	81 ± 8.0	840 ± 120	5.0 ± 0.40

[a] K_i ± SEM are based on the mean of two to five experiments each done in triplicate.

Table 2. Intrinsic Activities of **1a**−**e** and of the Reference Compounds Quinpirole and BP 897 Derived from the Stimulating Effect on Mitogenesis of D3 and D4 Receptor Expressing Cells and from the D4 Receptor Mediated Binding of [^{35}S]GTPγS

	[^{3}H]thymidine uptake (mitogenesis)				[^{35}S]GTPγS binding	
	D3 receptor[a]		D4 receptor[b]		D4 receptor[c]	
compd	agonist effect[d] (%)	EC_{50} (nM)[e]	agonist effect[d] (%)	EC_{50} (nM)[e]	agonist effect[f] (%)	EC_{50} (nM)[g]
1a	38	2.0	67	0.55	74	2.5
1b	28	3.5	37	7.6	43	13
1c	24	3.9	60	1.2	94	1.9
1d	50	9.1	61	35	43	14
1e	38	9.8	45	22	33	23
BP 897	38	2.7	22	49	nd	nd
quinpirole	100	1.4	100	19	100	49

[a] Determined with CHO dhfr$^-$ mutant cells stably expressing the human D3 receptor. [b] Determined with CHO10001 cells stably expressing the human D4.2 receptor. [c] Determined with CHO K1 cells stably expressing the human D4.4 receptor. [d] Rate of incorporation of [^{3}H]thymidine as evidence for mitogenesis activity relative to the maximal effect of the full agonist quinpirole (=100%) used as a reference. [e] EC_{50} values derived from the mean of three or four independent experiments. [f] Maximum binding of [^{35}S]GTPγS induced by receptor activation at 1 μM relative to the effect of the reference agonist quinpirole (=100%) (at 10 μM) derived from six independent experiments. [g] EC_{50} values derived from the mean of two independent experiments.

receptor when the ferrocenylcarboxmide **1a** (74%, EC_{50} = 2.5 nM) and the ruthenocene analogue **1c** (94%, EC_{50} = 1.9 nM) is superior to the 2,3-dichlorophenylpiperazine derivatives **1b** and **1d** (43%, EC_{50} = 13 nM; 43%, EC_{50} = 14 nM) and to the 3-chloro-2-methoxypiperazine **1e** (33%, EC_{50} = 23 nM). Interestingly, the ruthenocene **1c** (FAUC 413) shows full agonist effects (94%, EC_{50} = 1.9 nM) but a 25-fold lower EC_{50} value when compared to the reference agent quinpirole.

To investigate the chemical stability of metallocene-derived receptor ligands, we observed the representative **1a** and **1c** under conditions of biological testing (37 °C, incubation up to 20 h) when HPLC−MS analysis did not give evidence of any degradation. Furthermore, we were interested in investigating a probable cytotoxic effect of the test compounds in a cell-based experiment utilizing nontransfected CHO K1 cells that were incubated with different concentrations of **1a**. As presented in Figure 1, analysis of the number of living cells indicated a significant cytotoxic effect only at 100 μM (76% loss of cells) and 10 μM (32% loss of cells), whereas 0.01 and 1 μM did not result in statistically significant cell toxicity. Nevertheless, since metallocene-derived bioisosteres have not been investigated clinically yet, their utility as drugs remains unclear.

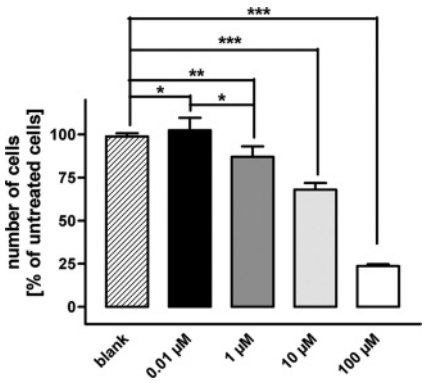


Figure 1. Investigation of the cytotoxic effect of the ferrocenyl derivative **1a** tested with nontransfected CHO K1 cells in the presence of 100 μM (not filled), 10 μM (light-gray), 1 μM (dark-gray), and 0.01 μM (black) compared to the number of living cells without any test compound (odd lines). Significance was derived from an unpaired *t*-test: (*) no significant difference; (**) $p < 0.05$; (***) $p < 0.0001$.

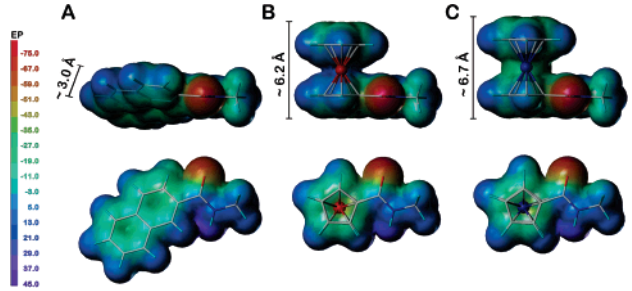


Figure 2. Electrostatic potentials mapped onto the van der Waals surfaces of the *N*-methylcarboxamide fragments **A**−**C** of the lead compound BP 897 and the ferrocene and ruthenocene derived bioisosteres of type **1.**

To understand the molecular similarity of the naphthaline-2-carboxamide moiety of BP 897 (**A**) and the respective ferrocene and ruthenocene derived bioisosteric elements **B** and **C**, we investigated the steric demand and the charge distribution by mapping the electrostatic potentials onto van der Waals surfaces.[19] In detail, the structures were derived from X-ray data[20] of suitable precursors. Electrostatic potential charges were calculated using the PM3(tm) Hamiltonian in the program package Spartan.[21] The distribution of charge on the molecular surfaces was visualized with MOLCAD implemented in SYBYL 6.9.1.[22] Figure 2 shows a high degree of similarity when the molecular electrostatic properties are compared. This might be the structural origin for the remarkable high target binding of the

double-layered bioisosteres. Nevertheless, the ability of the double-layered ligands of type **1** to specifically bind to the GPCRs investigated is surprising because the height of the metallocene-derived π-systems is more than doubled (6.2 and 6.7 Å) when compared to the naphthalene substructure of BP 897 (3.0 Å). Obviously, the plasticity of the binding region is substantially higher than expected.

In conclusion, we demonstrate that the high steric demand of ferrocene and ruthenocene derivatives of type **1** is well tolerated by the binding sites of a number of family 1 GPCRs. The novel binding profiles including subnanomolar K_i values of D4/5-HT_{1A} (for **1a**) and D3/D4 (for **1b**) and the D4 selective agonist properties of **1c** may be a starting point for highly beneficial CNS active drugs because these subtypes are extraordinarily relevant for neuropathological diseases such as schizophrenia, L-dopa-induced dyskinesia, attention deficit hyperactivity disorder, psychostimulant abuse, and sexual dysfunction. The high binding affinity of fancy bioisosteres of type **1** indicating substantial plasticity of the receptor excluded volumes of family 1 GPCRs will be a valuable tool for the investigation of GPCR−ligand interactions.

Acknowledgment. The authors thank Dr. H. H. M. Van Tol (Clarke Institute of Psychiatry, Toronto, Canada), Dr. J.-C. Schwartz and Dr. P. Sokoloff (INSERM, Paris), and Dr. J. Shine (The Garvan Institute of Medical Research, Sydney) for providing dopamine D_4, D_3, and D_2 receptor expressing cell lines, respectively, and Dr. R. Huff (Pfizer, Kalamazoo, MI) for providing dopamine D4.2 receptor expressing cells. This work was supported by the BMBF and Fonds der Chemischen Industrie.

Supporting Information Available: Experimental Section, including details on synthesis, analytical characterization, biological studies, and literature references. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Chen, X.; Wang, W. The use of bioisosteric groups in lead optimization. *Annu. Rep. Med. Chem.* **2003**, *38*, 333−346.

(2) (a) 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. (b) Boeckler, F.; Leng, A.; Mura, A. Bettinetti, L.; Feldon, J.; Gmeiner, P.; Ferger, B. Attenuation of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) neurotoxicity by the novel selective dopamine D3-receptor partial agonist FAUC 329 predominantly in the nucleus accumbens of mice. *Biochem. Pharmacol.* **2003**, *66*, 1025−1032.

(3) (a) Löber, S.; Hübner, H.; Utz, W.; Gmeiner, P. Rationally based efficacy tuning of selective dopamine D4 receptor ligands leading to the complete antagonist 2-[4-(4-chlorophenyl)piperazin-1-ylmethyl]pyrazolo[1,5-*a*]pyridine (FAUC 213). *J. Med. Chem.* **2001**, *44*, 2691−2694. (b) Boeckler, F.; Russig, H.; Zhang, W.; Löber, S.; Schetz, J.; Hübner, H.; Ferger, B.; Gmeiner, P.; Feldon, J. FAUC 213, a highly selective dopamine D4 receptor full antagonist, exhibits atypical antipsychotic properties in behavioural and neurochemical models of schizophrenia. *Psychopharmacology (Berlin)* **2004**, *175*, 7−17.

(4) (a) 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. (b) Lenz, C.; Haubmann, C.; Huebner, H.; Boeckler, F.; Gmeiner, P. Fancy bioisosteres: synthesis and dopaminergic properties of the endiyne FAUC 88 as a novel non-aromatic D3 agonist. *Bioorg. Med. Chem.* **2004**, *13*, 185−191.

(5) (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) Ortner, B.; Hübner, H.; Gmeiner, P. Planar chiral indoles: synthesis and biological effects of the enantiomers. *Tetrahedron: Asymmetry* **2001**, *12*, 3205−3208.

(6) (a) Pilla, M.; Perachon, S.; Sautel, F.; Garrido, F.; Mann, A.; Wermuth, C. G.; Schwartz, J.-C.; Everitt, B. J.; Sokoloff, P. Selective inhibition of cocaine-seeking behaviour by a partial dopamine D3 receptor agonist. *Nature* **1999**, *400*, 371−375. (b) Bezard, E.; Ferry, S.; Mach, U.; Stark, H.; Leriche, L.; Boraud, T.; Gross, C.; Sokoloff, P. Attenuation of levodopa-induced dyskinesia by normalizing dopamine D3 receptor function. *Nat. Med.* **2003**, *9*, 762−767.

(7) (a) van Staveren, D. R.; Metzler-Nolte, N. Bioorganometallic chemistry of ferrocene. *Chem. Rev.* **2004**, *104*, 5931−5985. (b) Wenzel, M.; Wu, Y. Ferrocene, ruthenocene and rhodocene analogs in haloperidol synthesis and organ distribution after labeling with 103Ru and 103mRh. *Int. J. Radiat. Appl. Instrum., Part A* **1988**, *39*, 1237−1241.

(8) (a) Rausch, M. D.; Fischer, E. O.; Grubert, H. The aromatic reactivity of ferrocene, ruthenocene, and osmocene. *J. Am. Chem. Soc.* **1960**, *82*, 76−82. (b) Reeves, P. C. Carboxylation of aromatic compounds: ferrocenecarboxylic acid. *Org. Synth.* **1977**, *56*, 28−31.

(9) (a) Wolfe, J. P.; Tomori, H.; Sadighi, J. P.; Yin, J.; Buchwald, S. L. Simple, efficient catalyst system for the palladium-catalyzed amination of aryl chlorides, bromides, and triflates. *J. Org. Chem.* **2000**, *65*, 1158−1174. (b) Hartwig, J. F. Transition metal catalyzed synthesis of arylamines and aryl ethers from aryl halides and triflates: scope and mechanism. *Angew. Chem., Int. Ed.* **1998**, *37*, 2046−2067.

(10) (a) Nishiyama, M.; Yamamoto, T.; Koie, Y. Synthesis of *N*-arylpiperazines from aryl halides and piperazine under a palladium tri-*tert*-butylphosphine catalyst. *Tetrahedron Lett.* **1998**, *39*, 617−620. (b) Torisawa, Y.; Nishi, T.; Minamikawa, J.-i. Progress in arylpiperazine synthesis by the catalytic amination reaction. *Bioorg. Med. Chem.* **2002**, *10*, 4023−4027.

(11) Plazuk, D.; Zakrzewski, J. Friedel−Crafts Type reaction of ferrocene with β-ketoesters. *J. Org. Chem.* **2002**, *67*, 8672−8674.

(12) Hayes, G.; Biden, T. J.; Selbie, L. A.; Shine, J. Structural subtypes of the dopamine D2 receptor are functionally distinct: expression of the cloned $D2_A$ and $D2_B$ subtypes in a heterologous cell line. *Mol. Endocrinol.* **1992**, *6*, 920−926.

(13) 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.

(14) 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.

(15) (a) 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. (b) 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.

(16) Chio, C.; Lajiness, M. E.; Huff, R. M. Activation of heterologously expressed D3 dopamine receptors: comparison with D2 dopamine receptors. *Mol. Pharmacol.* **1994**, *45*, 51−60.

(17) Gardner, B. R.; Hall, D. A.; Strange, P. G. Pharmacological analysis of the dopamine stimulation of [^{35}S]GTPγS binding via human $D2_{short}$ and $D2_{long}$ dopamine receptors expressed in recombinant cells. *Br. J. Pharmacol.* **1996**, *118*, 1544−1550.

(18) Newman-Tancredi, A.; Audinot, V.; Chaput, C.; Verriele, L.; Millan, M. J. [^{35}S]Guanosine-5′-*O*-(3-thio)triphosphate binding as a measure of efficacy at human recombinant dopamine D4.4 receptors: actions of antiparkinsonian and antipsychotic agents. *J. Pharmacol. Exp. Ther.* **1997**, *282*, 181−191.

(19) Meyer, E. A.; Castellano, R. K.; Diederich, F. Interactions with aromatic rings in chemical and biological recognition. *Angew. Chem., Int. Ed.* **2003**, *42*, 1210−1250.

(20) Allen, F. H. The Cambridge Structural Database: a quarter of a million crystal structures and rising. *Acta Crystallogr.* **2002**, *B58*, 380−388.

(21) *PC Spartan Plus*; Wavefunction Inc.: Irvine, CA, 1998.

(22) *SYBYL 6.9.1*, Tripos Inc. (1699 South Hanley Rd, St. Louis, MO, 63144).

JM050170S