Brief Articles

Conjugated Enynes as Nonaromatic Catechol Bioisosteres: Synthesis, Binding Experiments, and Computational Studies of Novel Dopamine Receptor Agonists Recognizing Preferentially the D_3 Subtype

Harald Hübner, Christian Haubmann, Wolfgang Utz, and Peter Gmeiner*

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

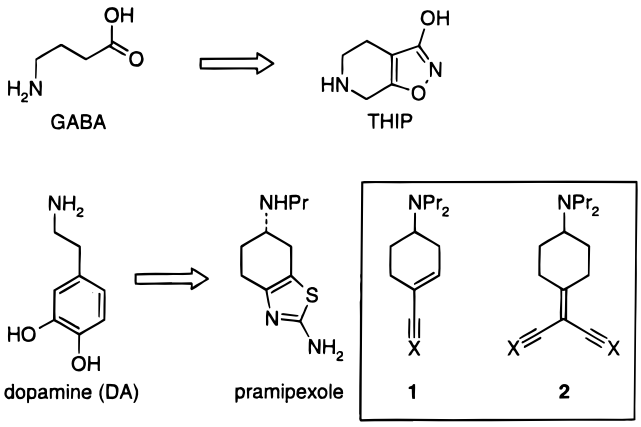
Received June 14, 1999

To evaluate nonaromatic catechol bioisosteres, the conformationally restrained enynes **1** and enediynes **2** were synthesized via palladium-catalyzed coupling as the key reaction step. Subsequent receptor binding studies at the dopamine receptor subtypes D_1, $D_{2\ long}$, $D_{2\ short}$, D_3, and D_4 showed highly interesting binding profiles for the enynes **1a** and **1b** when compared to dopamine. At the guanine nucleotide-sensitive high-affinity binding site of the D_3 receptor, the target compound **1b** (K_i = 5.2 nM) was 10-fold more potent than dopamine but less potent at the D_2 and D_4 subtypes. In contrast to dopamine the agonists **1a** and **1b** showed strong selectivity for the receptors of the D_2 family (D_2−D_4). As far as we know, this study represents the first report on nonaromatic dopamine agonists. Comparison of molecular electrostatic potentials, derived from semiempirical molecular orbital calculations, and lipophilicity maps was performed.

Introduction

Employing genuine substrates, hormones, or neurotransmitters as lead structures, the combination of conformational restrictions and bioisosteric replacements has become a valuable tool for the design of highly selective enzyme inhibitors, antimetabolites, or receptor ligands.[1,2] Starting from the respective biogenic amines, this strategy led to agonists or antagonists selectively recognizing dopamine (DA), norepinephrine (NA), serotonin (5-HT), or GABA receptors. As an example, incorporation of the inhibitory neurotransmitter GABA into a bicyclic ring system as well as bioisosteric exchange of the carboxylate function by an imidate substructure resulted in the $GABA_A$ receptor agonist THIP (Chart 1).[3] In the field of DA receptor agonists, which play an important role in the treatment of Parkinson's disease and are of potential interest as atypical antipsychotics,[4] the aminothiazole derivative pramipexole can be regarded as a result of the above-mentioned approach.[5] In this case, an aromatic heterocyclic system has been used as a bioisosteric surrogate for the catechol nucleus. The dopaminergic effects of ergoline derivatives including SAR studies with ergoline partial structures show that indole or pyrrole rings as well as aza analogues thereof can also mimic the catechol moiety of DA.[6−9] Heterocyclic bioisosteres play also an important role in the field of 5-HT receptor and adrenoreceptor agonists.[10,11] However, in all cases aromatic substructures are involved. As far as we know, nonaromatic agonists of DA receptors, 5-HT receptors, or adrenoreceptors have not been described, yet.

Chart 1

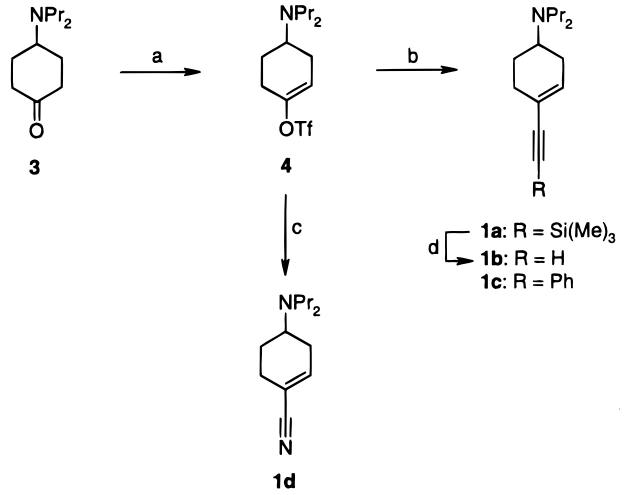


In continuation of our SAR studies on selective DA receptor ligands,[12−15] we were intrigued by the question whether nonaromatic, conjugated π-systems can mimic the catechol nucleus of DA. In general, this seemed unlikely since not only the aromatic system but also the polar hydroxy functionalities are expected to be involved in the receptor binding of DA.[16−18] However, there is more and more evidence in the literature that optimized hydrophobic effects can compensate for the attractive forces resulting from hydrogen bonds.[19] In this paper, we describe the synthesis, DA receptor binding, and calculated molecular properties of the conformationally restrained enynes of type **1** and enediynes of type **2** including aza and diaza analogues, respectively.

* To whom correspondence should be addressed. Tel: 09131/85-29383. Fax: 09131/85-22585. E-mail: gmeiner@pharmazie.uni-erlangen.de.

10.1021/jm991098z CCC: $19.00
Published on Web 01/29/2000

Scheme 1[a]



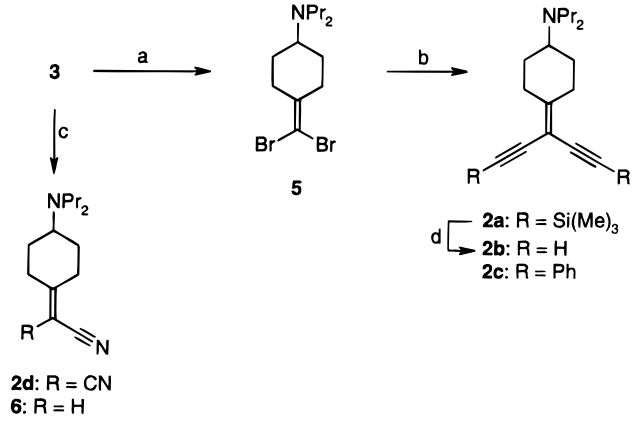
[a] Reagents and conditions: (a) Tf_2O, 2,6-di-*tert*-butyl-4-methylpyridine, 1,2-dichloroethane, reflux, 4.5 h (43%); (b) RCCH (R = $SiMe_3$, Ph), $Pd(PPh_3)_4$, CuI, $EtMe_2N$, THF, rt, 15 min (77%, 96%); (c) KCN, $Pd(PPh_3)_4$, 18-crown-6, benzene, reflux, 2.5 h (70%); (d) Bu_4NF, THF, −15 °C, 30 min (90%).

Results and Discussion

For the preparation of the target compounds we started from the aminocyclohexanone **3** that was readily prepared from 1,4-cyclohexanedione monoethylene acetal, according to the literature.[20] Transformation of the ketone **3** into an enol triflate and palladium-catalyzed coupling reactions should give access to the conjugated enynes of type **1** (Scheme 1). In practice, the central synthetic intermediate **4** was obtained by treatment of **3** with trifluoromethanesulfonic anhydride when 2,6-di-*tert*-butyl-4-methylpyridine was used as a nonnucleophilic acid scavenger.[21] Transition-metal-catalyzed reaction of the enol triflate **4** afforded the enynes **1a** and **1c**. Applying Cacchi's conditions ($Pd(PPh_3)_4$, CuI, $EtMe_2N$, THF)[22] the coupling products **1a** and **1c** were produced in 77% and 96% yield when trimethylsilylacetylene and phenylacetylene were used, respectively. Fluoride-induced desilylation of **1a** gave the terminal alkyne **1b**. Palladium-catalyzed coupling of the triflate **4** with KCN resulted in formation of the aza analogue **1d**. For this reaction, the application of 18-crown-6 in benzene was advantageous.[23]

The aminocyclohexanone **3** was also chosen as a suitable starting material for the synthesis of the geminal enediynes of type **2** (Scheme 2). Thus, synthesis of the dibromoalkene **5**, which should serve as a key intermediate, was accomplished by treatment of the ketone **3** with CBr_4 in the presence of PPh_3. Subsequent palladium-catalyzed coupling with trimethylsilylacetylene or phenylacetylene resulted in formation of the enediynes **2a** and **2c**, respectively. It is worthy to note that the reaction gave only poor product yields when $Pd(PPh_3)_4$ was employed as a catalyst. Substantial improvement could be achieved when we changed to $(Ph_3P)_2PdCl_2$.[24] Transformation of the silylalkyne **2a** into the terminal alkyne **2b** was performed in the presence of Bu_4NF. The diaza analogue **2d** was prepared from the amino ketone **3** and malononitrile under classical Knoevenagel conditions. Using KOH as a base, we also prepared the acetonitrile condensation product

Scheme 2[a]



[a] Reagents and conditions: (a) CBr_4, PPh_3, benzene, reflux, 4 h (84%); (b) RCCH (R = $SiMe_3$, Ph), $(Ph_3P)_2PdCl_2$, CuI, piperidine, THF, rt, 20 h, 24 h (78%, 75%); (c) $CH_2(CN)_2$, piperidine, MeOH, 0 °C, 2 h (47% of **2d**), CH_3CN, KOH, reflux, 3 h (48% of **6**); (d) Bu_4NF, THF, −15 °C, 30 min (70%).

6. All target compounds were synthesized in racemic form. An asymmetric synthesis is in progress.

The dopaminergic binding profiles of the test compounds **1a**−**d**, **2a**−**d**, and **6** to human DA $D_{2\ long}$, $D_{2\ short}$,[25] D_3, [26] and D_4[27] receptors heterologously expressed in Chinese hamster ovary cells (CHO) and to bovine D_1 receptors were investigated and compared with the reference agonist DA (Table 1). In our initial series of experiments, we evaluated the ability of the ligands to displace the radioligands [^{3}H]spiperone (for the receptors of the D_2 family) and [^{3}H]SCH 23390 (a selective D_1 antagonist). The binding properties of the geminal enediynes of type **2** and the aza enynes **1d** and **6** were disappointing. The data indicated K_i values in the micromolar range and only a one-site competition or the affinities were too low to be identified. Analogous results turned out for the phenylacetylene derivative **1c**. However, careful analysis of the D_2, D_3, and D_4 competition experiments for the enynes **1a** and **1b** employing a large number of test concentrations clearly showed biphasic curves. The calculated Hill coefficients (n_H) between −0.76 and −0.50 and a better fit of equations indicated a two-site competition rather a one-site model resulting only in a $K_{0.5}$ value. Whereas the K_i values for the low-affinity state were found to be guanine nucleotide-insensitive, Gpp(NH)p induced rightward shift and steepening of the curves indicating that the high-affinity binding sites are representing the G protein-coupled ternary complex. Analogous results were obtained when employing bovine striatal membranes and the selective D_2 antagonist [^{3}H]spiperone. Furthermore, high-affinity bovine D_2 receptors were exclusively labeled with [^{3}H]-pramipexole,[28,29] known as a selective autoreceptor agonist. Under this condition, monophasic curves with K_i values at 30, 15, and 1.8 nM were observed for **1a**, **1b,** and DA, respectively. In contrast to the reference agonist DA, which shows strong binding to the high-affinity binding sites of all the DA receptor subtypes investigated, the enynes **1a** and **1b** show high-affinity binding only for subtypes of the D_2 family. For the terminal enyne **1b** K_i values of 5.2, 22, 270, and 250 nM were determined for the high-affinity states of human subtypes D_3, D_4, $D_{2\ long}$, and $D_{2\ short}$, respectively. Within this group of receptors, the affinities of the more

Table 1. Binding Data of the Conjugated Enynes **1a**−**d**, **2a**−**d**, and **6** and DA to Human and Bovine DA Receptors[a]

		K_i (nM) ± SEM						
		[^{3}H]spiperone				[^{3}H]SCH 23390	[^{3}H]spiperone	[^{3}H]pramipexole
compd		human $D_{2\ long}$	human $D_{2\ short}$	human D_3	human $D_{4.4}$	bovine D_1	bovine D_2	bovine D_2
DA	$K_{i\ high}$	20 ± 2.7	17 ± 1.7	50 ± 8.1	1.2 ± 0.2	7 ± 3.6	11 ± 2.0	1.8 ± 0.3
	$K_{i\ low}$	1900 ± 140	1100 ± 76	1600 ± 180	62 ± 7.5	650 ± 200	1600 ± 550	
	R_H	42%	34%	52%	57%	36%	51%	
	$K_{i\ GppNHp}$	15000 ± 3000	15000 ± 2500	290 ± 31	94 ± 17	1600 ± 300	12000 ± 2800	
1a	$K_{i\ high}$	160 ± 20.0	970 ± 140	47 ± 9.2	160 ± 35	>20 μM	150 ± 53	30.00 ± 2.9
	$K_{i\ low}$	>20 μM	>20 μM	1600 ± 85	3800 ± 1400		7500 ± 2500	
	R_H	16%	17%	23%	54%		29%	
	$K_{i\ GppNHp}$	>20 μM	>20 μM	1900 ± 150	nd		12000 ± 2000	
	$K_{0.5}$	>20 μM	>20 μM	820 ± 90	640 ± 0		1600 ± 340	
	n_H	−0.46	−0.63	−0.74	−0.76		−0.65	
1b	$K_{i\ high}$	270 ± 33	250 ± 51	5.20 ± 1.6	22 ± 0.5	>20 μM	57 ± 9.7	15 ± 2.2
	$K_{i\ low}$	14000 ± 910	12000 ± 1600	590 ± 120	380 ± 120		4100 ± 470	
	R_H	38%	34%	23%	38%		40%	
	$K_{i\ GppNHp}$	19000 ± 3000	17000 ± 2000	720 ± 130	nd		17000 ± 1500	
	$K_{0.5}$	2800 ± 500	2900 ± 750	350 ± 100	170 ± 30		750 ± 100	
	n_H	−0.51	−0.55	−0.69	−0.69		−0.55	
1c		15000 ± 1000	15000 ± 2000	3500 ± 450	16000 ± 4400	>20 μM	nd	nd
1d		>20 μM	>20 μM	13000 ± 500	>20 μM	>20 μM	nd	nd
2a		11000 ± 2100	13000 ± 2500	2800 ± 50.0	1900 ± 150	3900 ± 150	nd	nd
2b		12000 ± 1500	>20 μM	4400 ± 1080	3900 ± 650	16000 ± 500	nd	nd
2c		5200 ± 400	7300 ± 950	1800 ± 250	1400 ± 50.0	2200 ± 500	nd	nd
2d		>20 μM	15000 ± 1000	1600 ± 50.0	>20 μM	>20 μM	nd	nd
6		>20 μM	>20 μM	13000 ± 2000	>20 μM	>20 μM	nd	nd

[a] K_i values are the means of two to five independent experiments ± SEM; $K_{i\ high}$, $K_{i\ low}$, and R_H (%) represent the inhibition constants at the high- and low-affinity sites and the relative proportion of high-affinity sites, respectively; $K_{i\ GppNHp}$ values were determined in the presence of 100 μM Gpp(NH)p for decoupling of the ternary complex; $K_{0.5}$ represents the dissociation constant using a one-site model when n_H indicates the existence of two binding sites; nd, not determined.

potent enyne **1b** were compared to those of DA. It turned out that **1b** is 5−20-fold less potent at the subtypes D_2 and D_4 but 10-fold more potent than DA at the D_3 receptor. Since the D_3 subtype is expressed predominantly in the limbic brain areas, the receptor is an important target for antipsychotic drugs.

From the physicochemical point of view, the enyne substructure of **1b** and the catechol fragment of DA look quite different. Whereas the catechol group of DA contains two acidic HO functions capable of taking part in Coulomb or H-bonding interactions, the enyne substructure of **1b** was expected less polar. Here, binding energy could result from hydrophobic forces. To gain more insight into the structural requirements that are necessary for agonist binding at the receptors of the D_2 family, we compared the molecular electrostatic potential (MEP) and lipophilicity maps of the test compounds **1b** and **1d** with those of the active analogues pramipexole and DA. To facilitate a suitable alignment, the α-rotamer of DA, representing an extended conformation with the *m*-OH group projected over the ethylamine side chain, was selected, which is in agreement with previous studies on the ligand-based design of rigid DA surrogates.[30] Figure 1 shows isopotential surfaces at −1 kcal/mol for DA, pramipexole, the active agonist **1b,** and the inactive aza analogue **1d** representing the interaction between a positive probe and the calculated charge distribution. Similar shapes and locations were observed for the MEP maps of the respective amino functions and π-systems of DA, pramipexole, and the enyne **1b**. Nevertheless, the negative electrostatic potential derived from the enyne **1b** turned out to be smaller than those generated by the aromatic moieties of DA and pramipexole. Due to the polarization of the C≡N triple bond, the negative isopotential region of the nitrile **1d** was significantly more extended away from the core of the π-system. This might be the reason for its poor DA receptor affinity. The lipophilic potentials mapped onto the Connolly surfaces, which are presented in Figure 2, indicate higher lipophilicity for **1b** and **1d** when compared to DA and pramipexole. This can be observed not only for the differently substituted amino groups but also for the π-regions.

Although the nonaromatic conjugated system of the novel DA receptor agonist **1b** revealed a more lipophilic and less polar character than the catechol ring of DA and the aminothiazole portion of pramipexole, the computational study corroborated our assumption that the conjugated enyne functionality of **1b** shows molecular properties that are similar to those of the catechol subunit of DA. Especially, at the D_3 receptor the ability of the more lipophilic enyne system to facilitate hydrophobic interactions seems to compensate for slightly reduced attractive forces resulting from electrostatic interactions.

Experimental Section

Solvents were purified and dried by standard procedures. If not otherwise stated reactions were performed under dry N_2. MS and HRMS were run on Finnigan MAT TSQ 70 and 8200 spectrometers, respectively, by EI (70 eV) with solid inlet. ^{1}H NMR spectra were obtained on a Bruker AM 360 (360 MHz) spectrometer, if not otherwise stated in $CDCl_3$ relative to TMS (*J* values in Hz); ^{13}C NMR spectra were run on a Bruker AC 250 (63 MHz) in $CDCl_3$ relative to the solvent resonance (δ = 77.0). Chromatographic purification was performed using silica gel 60 (Merck).

Dipropyl(4-trimethylsilylethynylcyclohex-3-enyl)-amine (1a). To a solution of **4** (69 mg, 0.21 mmol) in THF (6 mL) were added $EtMe_2N$ (225 μL, 2.08 mmol), trimethylsilylacetylene (60 μL, 0.42 mmol), CuI (6 mg, 0.03 mmol), and $(Ph_3P)_4Pd^0$ (12 mg, 0.01 mmol) in a nitrogen-purged flask. After being stirred at room temperature for 15 min, aqueous $NaHCO_3$ (5%) and Et_2O were added. The organic layer was dried ($MgSO_4$) and evaporated and the residue was purified by flash chromatography (petroleum ether−EtOAc−$EtMe_2N$

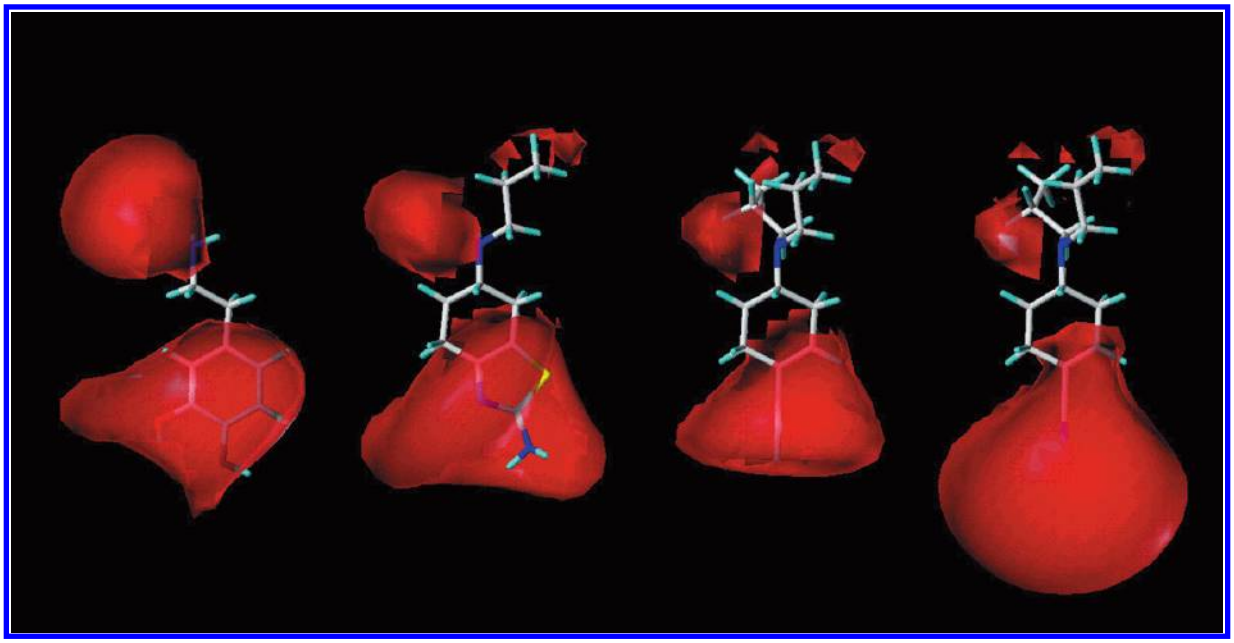


Figure 1. Molecular electrostatic isopotential maps (from left to right) contoured at −1 kcal/mol for DA, pramipexole, the nonaromatic DA receptor ligand **1b,** and the inactive aza analogue **1d**.

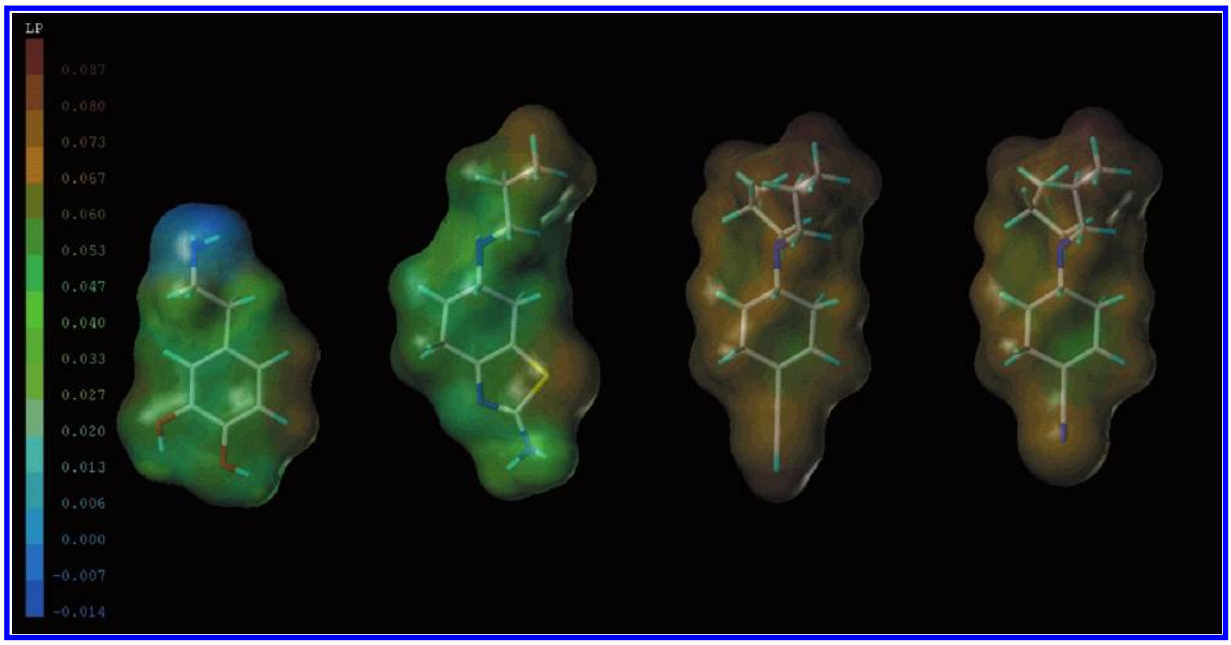


Figure 2. Lipophilic potentials (from left to right) mapped onto the calculated Connolly surfaces of DA, pramipexole, the nonaromatic DA receptor ligand **1b,** and the inactive aza analogue **1d**.

95:5:1) to give **1a** (45 mg, 77%) as a colorless oil: IR 3030, 2960, 2870, 2810, 2140, 1630, 1250 cm^{-1}; ^1H NMR δ 0.17 (s, 9H), 0.85 (t, 6H, J = 7.4), 1.35−1.49 (m, 5H), 1.79−1.88 (m, 1H), 1.99−2.11 (m, 1H), 2.14−2.28 (m, 3H), 2.34−2.41 (m, 4H), 2.74 (dddd, 1H, J = 12.0, 10.5, 5.1, 2.7), 6.11−6.16 (m, 1H); ^{13}C NMR δ 0.0, 11.8, 22.1, 25.1, 28.5, 30.3, 52.6, 55.5, 91.3, 106.6, 120.5, 135.6; EIMS 277 (M^+). Anal. ($C_{17}H_{31}NSi$) C,H,N.

(4-Ethynylcyclohex-3-enyl)dipropylamine (1b). To a solution of **1a** (24 mg, 0.086 mmol) in THF (4 mL) was added Bu_4NF (100 μL, 1 M solution in THF) at −15 °C. After being stirred at this temperature for 30 min, aqueous $NaHCO_3$ (5%) and Et_2O were added. The organic layer was dried ($MgSO_4$) and evaporated and the residue was purified by flash chromatography (petroleum ether−EtOAc−$EtMe_2N$ 95:5:1) to give **1b** (16 mg, 90%) as a colorless liquid: IR 3310, 3030, 2960, 2870, 2810, 2100, 1630 cm^{-1}; ^1H NMR δ 0.85 (t, 6H, J = 7.4), 1.36−1.52 (m, 5H), 1.81−1.89 (m, 1H), 2.00−2.12 (m, 1H), 2.15−2.30 (m, 3H), 2.32−2.45 (m, 4H), 2.76 (dddd, 1H, J = 12.1, 10.5, 5.1, 2.7), 2.79 (s, 1H), 6.13−6.18 (m, 1H); ^{13}C NMR δ 11.8, 22.1, 25.1, 28.5, 30.2, 52.6, 55.4, 74.6, 85.1, 119.5, 135.9; EIMS 205 (M^+). Anal. ($C_{14}H_{23}N$) C,H,N.

(4-Phenylethynylcyclohex-3-enyl)dipropylamine (1c). A solution of **4** (46 mg, 0.14 mmol) in THF (5 mL), $EtMe_2N$ (150 μL, 1.38 mmol), phenylacetylene (30 μL, 0.27 mmol), CuI (3 mg, 0.01 mmol), and $(Ph_3P)_4Pd^0$ (8 mg, 0.007 mmol) was reacted and worked up as described for **1a** to give **1c** (38 mg, 96%) as a colorless oil: IR 3030, 2960, 2870, 2810, 2200, 1590 cm^{-1}; ^1H NMR δ 0.87 (t, 6H, J = 7.4), 1.37−1.57 (m, 5H), 1.85−1.93 (m, 1H), 2.05−2.18 (m, 1H), 2.20−2.47 (m, 7H), 2.80 (dddd, 1H, J = 12.1, 10.6, 5.1, 2.7), 6.14−6.19 (m, 1H), 7.25−7.32 (m, 3H), 7.37−7.44 (m, 2H); EIMS 281 (M^+). Anal. ($C_{20}H_{27}N$) C,H,N.

4-Dipropylaminocyclohex-1-enecarbonitrile (1d). To a solution of **4** (66 mg, 0.20 mmol) in benzene (5 mL) were added KCN (53 mg, 0.81 mmol), 18-crown-6 (70 mg, 0.26 mmol), and $(Ph_3P)_4Pd^0$ (14 mg, 0.01 mmol). After being refluxed for 2.5 h the mixture was cooled to room temperature; aqueous $NaHCO_3$ (5%) and Et_2O were added. 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 **1d** (29 mg, 70%) as a colorless oil: IR 3030, 2960, 2870, 2810, 2215, 1640 cm^{-1}; ^1H NMR δ 0.86 (t, 6H, J = 7.4), 1.36−1.54 (m, 5H), 1.88−1.96 (m, 1H), 2.08−2.20 (m, 1H), 2.25−2.43 (m, 7H), 2.78 (dddd, 1H, J = 12.1, 10.5, 5.0, 2.7), 6.57−6.62 (m, 1H); EIMS 206 (M^+). Anal. ($C_{13}H_{22}N_2$) C,H,N.

Dipropyl[4-(3-trimethylsilyl-1-trimethylsilylethynylprop-2-ynylidene)cyclohexyl]amine (2a). To a suspension of $(Ph_3P)_2PdCl_2$ (26 mg, 0.03 mmol) and CuI (10 mg, 0.05 mmol) in THF (10 mL) were added a solution of **5** (134 mg, 0.38 mmol) in THF (5 mL), trimethylsilylacetylene (370 μL, 2.61 mmol), and piperidine (375 μL, 3.79 mmol). After being stirred at room temperature for 20 h, aqueous $NaHCO_3$ (5%) was added and the mixture was extracted with Et_2O. The organic layer was dried ($MgSO_4$) and evaporated and the residue was purified by flash chromatography (petroleum ether−EtOAc−$EtMe_2N$ 90:10:1) to give **2a** (115 mg, 78%) as a colorless solid: mp 70 °C; IR 2960, 2870, 2810, 2150, 1590, 1250 cm^{-1}; ^1H NMR δ 0.19 (s, 18H), 0.85 (t, 6H, J = 7.2), 1.29−1.47 (m, 6H), 1.83−1.92 (m, 2H), 1.98 (dt, 2H, J = 13.3, 4.4), 2.33−2.39 (m, 4H), 2.68 (tt, 1H, J = 11.4, 3.3), 3.03−3.13 (m, 2H); ^{13}C NMR δ 0.0, 11.8, 22.3, 28.8, 31.5, 52.9, 59.2, 96.1, 98.8, 101.0, 162.2; EIMS 387 (M^+). Anal. ($C_{23}H_{41}NSi_2$) C,H,N.

[4-(1-Ethynylprop-2-ynylidene)cyclohexyl]dipropylamine (2b). To a solution of **2a** (79 mg, 0.2 mmol) in THF (8 mL) was added Bu_4NF (430 μL, 1 M solution in THF) at −15 °C. After being stirred at −15 °C for 30 min, aqueous $NaHCO_3$ (5%) and Et_2O were added. The organic layer was dried ($MgSO_4$) and evaporated and the residue was purified by flash chromatography (petroleum ether−EtOAc−$EtMe_2N$ 90:10:1) to give **2b** (35 mg, 70%) as a colorless liquid: IR 3310, 2960, 2870, 2810, 2100, 1590 cm^{-1}; ^1H NMR δ 0.85 (t, 6H, J = 7.4), 1.31−1.47 (m, 6H), 1.85−1.94 (m, 2H), 2.03 (dt, 2H, J = 13.5, 4.4), 2.34−2.40 (m, 4H), 2.70 (tt, 1H, J = 11.5, 3.3), 3.07 (s, 2H), 3.08−3.15 (m, 2H); EIMS 243 (M^+). Anal. ($C_{17}H_{25}N$) C,H,N.

[4-(3-Phenyl-1-phenylethynylprop-2-ynylidene)cyclohexyl]dipropylamine (2c). A suspension of $(Ph_3P)_2PdCl_2$ (10 mg, 0.01 mmol) and CuI (4 mg, 0.02 mmol) in THF (4 mL) was reacted with a solution of **5** (50 mg, 0.14 mmol) in THF (2 mL), phenylacetylene (110 μL, 1.0 mmol), and piperidine (150 μL, 1.51 mmol). After being stirred at room temperature for 24 h the mixture was worked up as described for **2a** to give **2c** (42 mg, 75%) as a yellowish oil: IR 3050, 2950, 2870, 2810, 2210, 1590, 1490 cm^{-1}; ^1H NMR δ 0.87 (t, 6H, J = 7.3), 1.38−1.56 (m, 6H), 1.90−2.01 (m, 2H), 2.12 (dt, 2H, J = 13.3, 4.2), 2.37−2.46 (m, 4H), 2.72−2.83 (m, 1H), 3.20−3.30 (m, 2H), 7.27−7.36 (m, 6H), 7.45−7.53 (m, 4H); EIMS 395 (M^+). Anal. ($C_{29}H_{33}N$) C,H,N.

2-(4-Dipropylaminocyclohexylidene)malononitrile (2d). To a solution of **3** (50 mg, 0.25 mmol) in MeOH (3 mL) were added malononitrile (18 mg, 0.27 mmol) and piperidine (30 μL) at 0 °C. After being stirred at 0 °C for 2 h, the solvent was evaporated and the residue was purified by flash chromatography (petroleum ether−EtOAc−$EtMe_2N$ 80:20:1) to give **2d** (29 mg, 47%) as a slightly yellowish oil: IR 2960, 2870, 2810, 2230, 1640 cm^{-1}; ^1H NMR δ 0.86 (t, 6H, J = 7.4), 1.41 (sext, 4H, J = 7.4), 1.58 (dddd, 2H, J = 13.3, 12.1, 10.6, 4.0), 1.98−2.07 (m, 2H), 2.33−2.44 (m, 6H), 2.80 (tt, 1H, J = 10.6, 3.4), 3.01−3.09 (m, 2H); EIMS 245 (M^+). Anal. ($C_{15}H_{23}N_3$) C,H,N.

Trifluoromethanesulfonic Acid 4-Dipropylaminocyclohex-1-enyl Ester (4). To a solution of **3** (300 mg, 1.52 mmol), which was synthesized according to ref 20, in 1,2-dichloroethane (30 mL) were added 2,6-di-*tert*-butyl-4-methylpyridine (420 mg, 2.04 mmol) and Tf_2O (500 μL, 2.97 mmol). After being refluxed for 4.5 h, the mixture was cooled to room temperature and aqueous $NaHCO_3$ (5%) and CH_2Cl_2 were added. The organic layer was dried ($MgSO_4$) and evaporated, and the residue was purified by flash chromatography (petroleum ether−EtOAc 9:1→6:4) to give **4** (219 mg, 43%) as a yellow liquid: IR 2960, 2870, 2810, 1690, 1420, 1140 cm^{-1}; ^1H NMR δ 0.86 (t, 6H, J = 7.4), 1.42 (sext, 4H, J = 7.4), 1.64 (dddd, 1H, J = 12.5, 12.1, 11.2, 6.0), 1.90−1.98 (m, 1H), 2.08−2.28 (m, 2H), 2.32−2.54 (m, 6H), 2.81 (dddd, 1H, J = 12.1, 10.0, 5.5, 2.8), 5.69−5.74 (m, 1H); EIMS 329 (M^+), 196 ($M - 133)^+$; HREIMS (M^+) 329.1266 (329.1272 calcd for $C_{13}H_{22}O_3NF_3S$).

(4-Dibromomethylenecyclohexyl)dipropylamine (5). To a solution of PPh_3 (1.31 g, 5 mmol) in benzene (20 mL) was added CBr_4 (837 mg, 2.5 mmol). The mixture was stirred at room temperature for 30 min when a solution of **3** (200 mg, 1.01 mmol) in benzene (5 mL) was added. After being refluxed for 4 h, the mixture was cooled to room temperature and filtrated and the solvent was evaporated. The residue was dissolved in CH_2Cl_2 and washed with aqueous $NaHCO_3$ (5%). The organic layer was dried ($MgSO_4$) and evaporated and the residue was purified by flash chromatography (petroleum ether−EtOAc 3:7) to give **5** (302 mg, 84%) as a colorless oil: IR 2960, 2870, 2800, 1620, 800 cm^{-1}; ^1H NMR δ 0.85 (t, 6H, J = 7.4), 1.28−1.47 (m, 6H), 1.79−1.96 (m, 4H), 2.34−2.40 (m, 4H), 2.65 (tt, 1H, J = 11.4, 3.4), 2.93−3.01 (m, 2H); EIMS 355 (M^+), 353 (M^+). Anal. ($C_{13}H_{23}NBr_2$) C,H,N.

(4-Dipropylaminocyclohexylidene)acetonitrile (6). A suspension of KOH (28 mg, 0.5 mmol) in acetonitrile (6 mL) was refluxed for 20 min. Then a solution of **3** (90 mg, 0.45 mmol) in acetonitrile (2 mL) was added dropwise to the boiling mixture and refluxing was continued for 3 h. The mixture was poured into ice water and extracted with Et_2O. 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 **6** (48 mg, 48%) as a yellowish oil: IR 3050, 2960, 2870, 2810, 2220, 1630 cm^{-1}; ^1H NMR δ 0.86 (t, 6H, J = 7.4), 1.34−1.48 (m, 6H), 1.88−2.01 (m, 2H), 2.07−2.24 (m, 2H), 2.34−2.47 (m, 5H), 2.72 (tt, 1H, J = 11.2, 3.2), 2.96 (ddd, 1H, J = 13.9, 5.8, 3.6), 5.05 (s, 1H); ^{13}C NMR δ 11.7, 22.1, 28.8, 29.4, 31.8, 34.6, 52.7, 58.6, 92.5, 116.8, 167.5; EIMS 220 (M^+). Anal. ($C_{14}H_{24}N_2$) C,H,N.

Bovine Receptor Preparation. Fresh bovine brains were obtained from the local slaughterhouse. The striata were dissected and frozen at −80 °C. Membranes were prepared as described previously.[29] In brief, the striata were thawed, cut up, and homogenized in an aqueous solution of sucrose (0.1 M). The suspension was washed by repeated centrifugation at 1000*g*. Then, the resulting supernatant was pelleted by centrifugation at 60000*g* for 1 h. The pellet was washed twice by resuspension in Tris−EDTA buffer (50 mM TrisHCl, 1 mM EDTA; pH 7.4) and subsequent centrifugation at 60000*g* for 15 min. Finally the membranes were suspended in Tris−EDTA buffer, homogenized with a Potter-Elvehjam homogenizer, and stored at −80 °C in small aliquots.

Bovine Radioreceptor Assay. For the D_1 receptor binding assay bovine striatal membranes were diluted with binding buffer (50 mM TrisHCl, 1 mM EDTA, 5 mM $MgCl_2$, 0.1 mM dl-dithiothreitol, 100 μg/mL bacitracin, 5 μg/mL soybean trypsin inhibitor; pH 7.4) to a final concentration of 25 μg protein/assay tube. Tubes were prepared with the radioligand [^{3}H]SCH 23390 (0.3 nM) (specific activity 83.0 Ci/mmol; Amersham Pharmacia Biotech, Freiburg, Germany) and varying concentrations of test compounds (from 0.01 to 100 000 nM). Total binding of [^{3}H]SCH 23390 was measured in the absence of any competing drug; nonspecific binding was determined by coincubation with butaclamol (1 μM). Incubation was started by adding membranes to the assay tube with a final volume of 200 μL. It was carried on for 60 min at 37 °C and stopped by rapid filtration through GF/B filters precoated with 0.3% polyethylenimine, using an automated cell harvester (Inotech, Dottikon, CH). Filters were washed five times with ice-cold wash buffer (50 mM TrisHCl, 120 mM NaCl; pH 7.4) dried, and counted in a MicroBeta Trilux (Wallac ADL, Freiburg, Germany).

Protein concentration was established by the method of Lowry using bovine serum albumin as standard.[31]

Competition binding analysis with the bovine D_2 receptor was done with the radioligand [^{3}H]spiperone (0.5 nM) (specific activity 99.0 Ci/mmol; Amersham Pharmacia Biotech, Freiburg, Germany) as an antagonist and the agonist [^{3}H]pramipexole (0.5 nM) (specific activity 42.0 Ci/mmol; a generous gift from

Boehringer Ingelheim, Ingelheim, Germany) for labeling selectively the high-affinity state of the D_2 receptor. The assay was carried out in a final volume of 1500 μL by incubating the radioligand, the test compounds (8 concentrations between 0.01 and 100 000 nM, and 16 concentrations between 0.1 and 100 000 nM for two-site determinations) or butaclamol for investigating unspecific binding, and finally the membrane suspension. Membranes were diluted in binding buffer with a protein concentration of 200 μg/assay tube for [^{3}H]spiperone and 500 μg/assay tube for [^{3}H]pramipexole, respectively. Assays with [^{3}H]spiperone also included ketanserin (50 nM) to mask binding of the radioligand to serotonin sites. Incubation for 2 h at 23 °C was terminated by rapid filtration through GF/C filters, using a Brandel cell harvester (Brandel, Gaithersburg, MD). The filters were rinsed three times with ice-cold Tris−EDTA buffer, and the radioactivity trapped on the filters was counted using a Beckman LS 6500 scintillation counter (Beckman).

Cell Culture. All cell culture material was purchased from LifeTechnologies, Karlsruhe, Germany. Chinese hamster ovary cells (CHO-K1) stably expressing the human DA $D_{2\ long}$ and $D_{2\ short}$ receptors[25] were grown in DMEM-Ham's F12 medium (1:1), supplemented with 0.05% sodium bicarbonate, 10% fetal calf serum, and glutamine (2 mM). Stably expressed human D_3 receptors[26] were obtained from dihydrofolate reductase gene-deficient CHO cells. They were grown in DMEM medium containing 4500 mg/L glucose, 10% heat-inactivated dialyzed fetal calf serum, MEM amino acid supplement, and glutamine (2 mM). CHO-K1 cells expressing the human DA receptor subtype $D_{4.4}$ [27] were grown in MEM α-medium supplemented with 2.5% fetal calf serum, 2.5% horse serum, and 400 μg/mL G418. All cells were grown at 37 °C under a humidified atmosphere of 5% CO_2−95% air in the presence of 100 U/mL penicillin G and 100 μg/mL streptomycin.

Cloned Receptor Preparation. Human $D_{2\ long}$, $D_{2\ short}$, D_3, and $D_{4.4}$ receptor-expressing cell lines were grown in 145-cm^2 culture dishes to 80% confluency. Cells were rinsed twice with ice-cold PBS and scraped from the dishes using a sterile cell scraper in the presence of harvest buffer (10 mM TrisHCl, 0.5 mM EDTA, 5.5 mM KCl, 140 mM NaCl; pH 7.4) supplemented with protease inhibitors. After centrifugation (400*g*, 5 min) the cells expressing $D_{2\ long}$ $D_{2\ short}$, and $D_{4.4}$ receptors were resuspended in homogenate buffer (50 mM TrisHCl, 5 mM EDTA, 1.5 mM $CaCl_2$, 5 mM $MgCl_2$, 5 mM KCl, 120 mM NaCl; pH 7.4) and cells expressing D_3 receptors in Tris−$MgSO_4$ buffer (10 mM TrisHCl, 5 mM $MgSO_4$; pH 7.4), respectively, and stored at −80 °C. After thawing, the cells were diluted in homogenate buffer ($D_{2\ long}$, $D_{2\ short}$, and $D_{4.4}$) or Tris−$MgSO_4$ buffer (D_3), homogenized using a Polytron (20 000 rpm, 5 times for 5 s each in an ice bath), and spun at 50000*g* for 15 min. The membrane pellet was resuspended in binding buffer, homogenized with a Potter-Elvehjem homogenizer, and stored in small aliquots at −80 °C with an estimated protein concentration of ca. 500 μg/mL.

Binding Assay with Cloned Human Receptors. Binding analysis with human DA receptors was done in the same manner as described for the bovine D_1 receptors. In brief, cell membranes containing the $D_{2\ long}$, $D_{2\ short}$, D_3, and $D_{4.4}$ receptors were diluted in binding buffer to a final protein concentration of 10−20 μg/assay tube. [^{3}H]Spiperone (0.5 nM) was used as the radioligand and haloperidol (10 μM) to determine unspecific binding. Eight concentrations of the test compounds between 0.01 and 100 000 nM and 16 concentrations between 0.1 and 100 000 nM for two-site determinations were investigated.

To reveal the high-affinity sites of the receptor, competition analysis was done in the absence and presence of the nonhydrolyzable GTP analogue Gpp(NH)p (100 μM).

Data Analysis. The resulting competition curves were analyzed by nonlinear regression using the algorithms in PRISM (GraphPad Software, San Diego, CA). The data were initially fit using a sigmoid model to provide a slope coefficient (n_H) and an IC_{50} value, representing the concentration corresponding to 50% of maximal inhibition. Data were then calculated for a one-site model ($n_H - 1$) or a two-site model ($n_H < 1$) depending on the slope factor. The bases of the discrimination between monophasic and biphasic data analysis were the n_H values and the ability of the program PRISM to fit the data; −0.8 was chosen as the cutoff value. If both requirements were fulfilled (n_H below 0.8 and PRISM calculated a better fit of equations for a two-site competition) a biphasic curve was used. IC_{50} values were transformed to K_i values according to the equation of Cheng and Prusoff.[32]

Molecular Modeling. Structures were built using the SYBYL 6.5 software package.[33] Geometry optimization as well as calculation of ESP point charges of all the compounds investigated were done with the AM1 Hamiltonian[34] implemented in MOPAC 6.0.[35] All other graphical manipulations were carried out within SYBYL 6.5, including visualization of molecular electrostatic potentials (MEP; −1.0 kcal/mol) and mapping of lipophilic potentials onto the calculated Connolly surfaces (MOLCAD module). Generally, default parameters were used. To force the lipophilic potential colors of each molecule into common borders, the option *global* was selected.

Acknowledgment. The authors thank Dr. H. H. M. Van Tol (Clarke Institute of Psychiatry, Toronto), 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. Thanks are also due to Mrs. H. Käding, Mrs. B. Linke, and Mrs. P. Schmitt for skillful technical assistance. This work was supported by the DFG and the Fonds der Chemischen Industrie.

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JM991098Z