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A *de Novo* Design Probe of a Dopamine Receptor Ligand Based on a Theoretical Approach

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A trial to design *de novo* a dopamine (DA) receptor ligand was made, taking as the base four structural and electrostatic requirements: (1) a group simulating the interaction of the DA amino group with the TM3 aspartic acid of the receptor, (2) a group that can simulate the interaction of the DA *m*-hydroxyl group with the TM5 serine of the receptor, (3) a distance between these groups similar to that of the DA *anti*-coplanar conformer, and (4) a rigid structure keeping the distance between the groups right. After the design "on paper" of the models of four structures, quantum chemistry calculations were performed to check the properties of the molecules, and then the most encouraging ones were synthesized. None of the compounds synthesized was able to bind D_1- and D_2-dopamine receptor subtypes; this shows that the structural and electrostatic requirements considered in this work are insufficient. In particular, the presence of an arylethylamine moiety seems to be essential for the interaction of a ligand with the DA receptor.

INTRODUCTION

The neurotransmitter dopamine (DA) is involved in various central nervous system (CNS) disorders such as schizophrenia and Parkinson's disease. Structure–activity relationship studies on DA agonists and antagonists have attracted considerable interest during the last years. The results obtained have been used to discuss optimal features for DA receptor activation, and various hypothetical DA receptor models have been suggested. The same studies have given many lead compounds as well as apomorphine, aminodihydroxytetraline (ADTN), ergoline, and benzazepine derivatives (*1*).

Recently, in addition to the structure–activity studies, some attempts have been made to approach the problem in a more rational way, based on understanding the molecular mechanism of neurotransmitter–receptor interaction (*2*). This approach has been possible since the three-dimensional structure of DA receptors has been postulated by means of molecular modeling simulations.

Part of those studies concerned conformational analysis and molecular electro-

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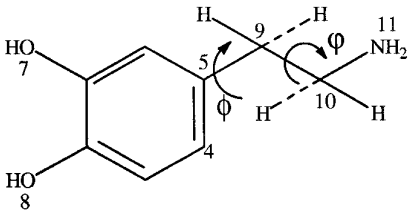


ϕ ≡ C4-C5-C9-C10 = 0.0°

φ ≡ C5-C9-C10-N11 = 180.0°

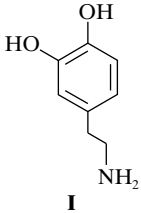
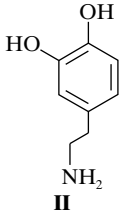
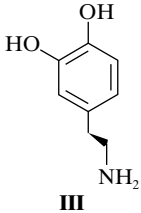
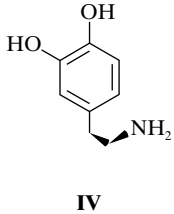
FIGURE 1

static potential distribution of DA (*3–6*). Theoretical conformational analysis performed for isolated and solvated DA molecule has revealed that its side chain may attain various conformations. The energy differences between them are small, but the *anti*-conformer with the catechol ring coplanar with the ethylamine (C–C–N) side chain (Fig. 1) is not energetically favored. This is not in agreement with experimental studies on conformationally restricted analogs which suggest that the *anti*-coplanar conformer is biologically active (*7*). Therefore, Edvardsen and Dahl (*5*) have proposed a "zipper" mechanism for the binding of flexible DA to its receptor, a suggestion based on molecular dynamics simulations. Molecular electrostatic potential calculations have revealed that the potential and charge distribution is very dependent on a conformation that can be important for DA-receptor recognition and DA agonist/antagonist selectivity.

Theoretical molecular modeling calculations have been performed for isolated DA receptor as well as for receptor–DA interacting structure (*8–12*). These studies have been possible since (1) the genes coding for different DA receptors, belonging to the large G-protein-linked family of receptors, have recently been isolated, cloned, and decoded (*13*); (2) the structure of bacteriorhodopsin—a structural analog of the G-protein-coupled receptors (GPCR)—has been resolved by cryomicroscopy study (*14*). The structural molecular modeling simulations, by homology to bacteriorhodopsin, together with hydropathicity analysis have revealed that the DA D_2 receptor (similar to other G-protein-coupled receptors) has seven α-helical transmembrane segments that form the central core with a putative ligand-binding site. It has also been found that defined amino acids can be responsible for DA–receptor interaction. The point mutations of DA receptors also support these data (*15*). Generally, it has been postulated that (1) the aspartic acidic residue on the third transmembrane domain (TM3) interacts with the protonated amino group of DA, (2) two serine residues on the fifth transmembrane domain (TM5) interact with the hydroxyl groups of DA, and (3) the aromatic nuclei of a few amino acids on TM3 and TM6 form the hydrophobic surrounding of DA, which is probably rearranged during the binding process.

Taking into acocunt the essential aspects of the molecular model of receptor–DA interaction and the results of DA structural studies, we attempted to design *de novo* a DA-receptor ligand. The design was based on four structural and electrostatic requirements: (1) a group simulating the interaction of the DA amino group with the TM3 aspartic acid of the receptor, (2) a group which can simulate the interaction

TABLE 1
Results of AM1 Calculations for DA Molecule[a]

Conformers	 **I**	 **II**	 **III**	 **IV**
	Torsional angle (degrees)			
N11–C10–C9–C5	178	−176	−177	73
C10–C9–C5–C4	−2	167	−100	−95
	Distance of atoms (Å)			
O7–N11	7.311	6.474	6.700	6.249
O8–N11	7.903	7.868	7.789	6.610
	Net atomic charge (a.u.)			
O7	−0.249	−0.250	−0.249	−0.252
O8	−0.271	−0.272	−0.271	−0.272
N11	−0.350	−0.342	−0.352	−0.348
	Energy (heat of formation) and relative energy (kcal/mol)			
E	−74.7	−74.9	−75.7	−74.6
ΔE	1.0	0.8	0.0	1.1
	Proton affinity of nitrogen atom N11 (kcal/mol)			
PA	216.2	216.2	216.2	216.2

[a] The numbering of atoms is presented in Fig. 1.

of the DA *m*-hydroxyl group with the TM5 serine of the receptor, (3) the distance between these groups should be similar, as for the *anti*-coplanar conformer **I** of DA (Table 1), and (4) a rigid structure keeping the distance between the groups right.

In accordance with those requirements the models of four structures were designed ''on paper'' (Fig. 2). Later quantum chemistry calculations were performed to check the properties of the molecules, and then the most encouraging ones were synthesized.

All well-known DA receptor ligands include in their structure an aromatic moiety. The aromatic ring is considered very important for ligand–receptor binding because it seems to interact with the aromatic nuclei of two phenylalanine residues on the DA receptor (*8*). However, the DA aromatic ring may also be seen as a flat structure able to penetrate into the receptor pocket surrounded by the aromatic moieties of the phenylalanine residues. On the other hand, studies developed in the field of β-adrenergic receptor (G-protein-coupled receptor) show that completely aliphatic molecules maintain β-blocking properties as well as the relative aromatic compounds (*16*). Moreover, we have not found any reference to negative biological test results being obtained by studying nonaromatic compounds as DA receptor ligands. Therefore, we decided to replace the aromatic ring of DA with a system of conjugated double bonds to obtain a planar structure. In addition, since a group

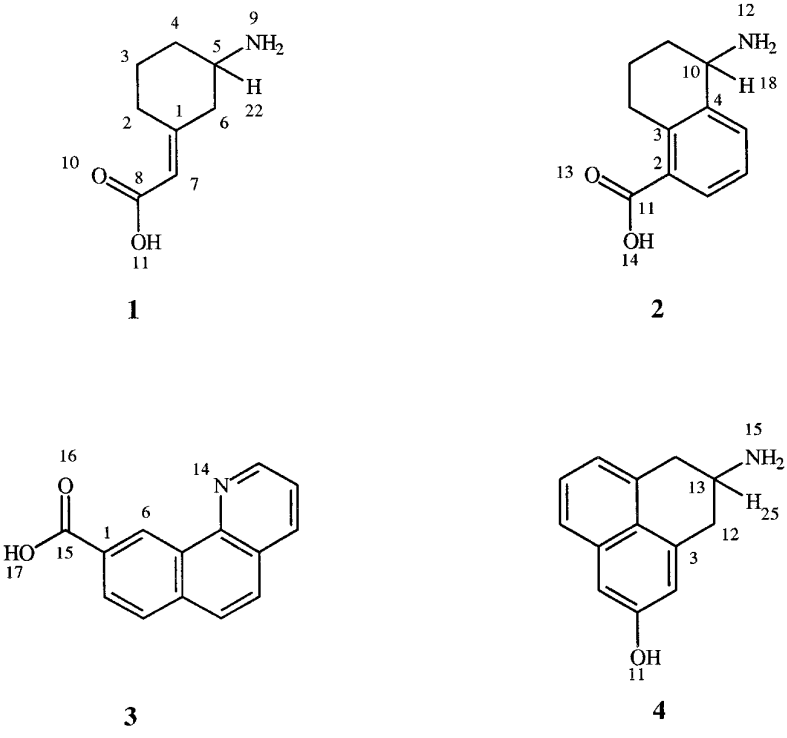


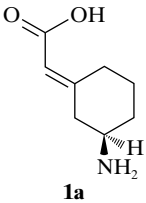
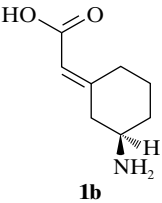
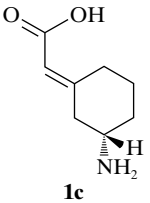
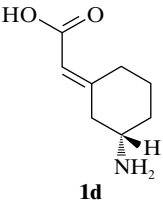
FIG. 2. The atoms were automatically numbered by the Chem3D program.

simulating the phenolic hydroxyl connected to the planar aromatic ring and able to interact with the serine residue on TM5 is necessary, we have chosen the α–β unsaturated carboxylic acid moiety. On the basis of this, we designed structure **1.** Compound **2** was seen as a more complex α–β unsaturated carboxylic acid which contains molecule **1** embedded in a more rigid framework. Moreover, the carboxyl group on compounds **1, 2,** and **3** can interact with the serine residue on TM5 as hydrogen bond donor or acceptor. Structure **4,** like DA, bears a phenolic group. Models **1, 2,** and **4** have an amino group very similar to that of DA, and compound **3** contains a nitrogen atom which can simulate the DA amino group.

RESULTS AND DISCUSSION

The quantum chemistry calculations for the four DA conformers shown in Table 1 were performed. Two of them, **I** and **II,** are *anti*-coplanar conformers. The *anti*-perpendicular conformer (**III**) is found in the crystal state and the *gauche*-perpendicular one (**IV**) is the most stable conformer found by Edvardsen and Dahl (*5*) in molecular dynamics simulations for protonated DA.

TABLE 2
Results of AM1 Calculations for Compound **1** (*E* Isomer)[a]

Conformers	 **1a**	 **1b**	 **1c**	 **1d**
	Torsional angle (degrees)			
N9–C5–C4–C3	−73	−73	179	−178
H22–C5–C4–C3	171	171	−59	−56
O10–C8–C7–C1	−148	0	−149	5
O11–C8–C7–C1	−33	180	33	−176
	Distance of atoms (Å)			
O10–N9	5.718	5.636	7.054	6.796
O11–N9	5.800	5.899	6.748	7.068
	Net atomic charge (a.u.)			
O10	−0.366	−0.375	−0.367	−0.377
O11	−0.319	−0.319	−0.320	−0.319
N9	−0.336	−0.336	−0.347	−0.347
	Energy (heat of formation) and relative energy (kcal/mol)			
E	−101.4	−102.7	−99.5	−100.9
Δ*E*	1.3	0.0	3.2	1.8
	Proteon affinity of nitrogen atom N9 (kcal/mol)			
PA	215.1	215.1	215.1	215.1

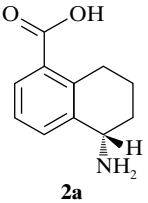
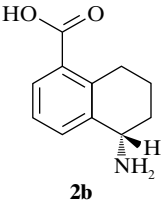
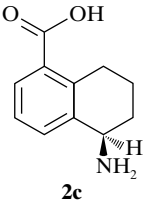
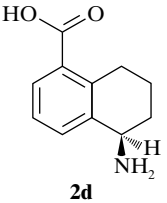
[a] The numbering of atoms is presented in Fig. 2.

In our study, conformer **III** was found to have the lowest energy (heat of formation). This result differs from the studies of Urban *et al.* (*3*), who found that the *gauche*-perpendicular conformer (our **IV**) is the most stable one for neutral DA, but at the same time they did not give energy values for the *anti*-perpendicular conformer (our **III**), so it is difficult to discuss the discrepancy.

It was also found, within our calculations, that the *anti*-coplanar conformers **I** and **II** lie only approximately 1.0 kcal/mol above conformer **III.** Generally, since the differences of energy between conformers are not very big, and conformer **I** is postulated as the active one (*7*), we chose it as the reference for other studied molecules.

The results of calculations performed for compounds **1–4** are presented in Tables 2–5. The carbon atoms C5 of **1** and C10 of **2** (Fig. 2) can have different configurations with regard to the position of the amino group. These possibilities were accounted for in the calculations (Tables 2 and 3). The energy difference between the conformers of the same compound sometimes assumes a value of several kilocalories per mole (especially for compounds **2** and **4**). However, it is difficult to analyze this, since the conformer populations at the receptor site can be different from those in

TABLE 3
Results of AM1 Calculations for Compound **2**[a]

Conformers	 **2a**	 **2b**	 **2c**	 **2d**
		Torsional angle (degrees)		
N12–C10–C4–C3	−110	−110	143	146
H18–C10–C4–C3	135	135	−97	−93
O13–C11–C2–C3	160	−10	159	−12
O14–C11–C2–C3	−21	171	−22	168
		Distance of atoms (Å)		
O13–N12	6.939	6.332	7.255	6.623
O14–N12	6.272	6.952	6.447	7.227
		Net atomic charge (a.u.)		
O13	−0.370	−0.373	−0.371	−0.373
O14	−0.316	−0.320	−0.315	−0.320
N12	−0.334	−0.334	−0.345	−0.349
		Energy (heat of formation) and relative energy (kcal/mol)		
E	−81.1	−82.0	−77.4	−78.6
ΔE	0.9	0.9	4.6	3.4
		Proteon affinity of nitrogen atom N12 (kcal/mol)		
PA	218.4	218.4	218.4	218.4

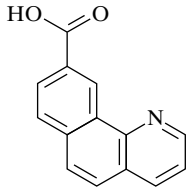
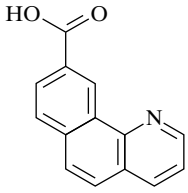
[a] The numbering of atoms is presented in Fig. 2.

the gas phase. It also means that the low energetic conformer in the gas phase need not be the active one. The same problem regards the DA molecule.

When the distances between functional groups (or rather atoms) are considered, compound **1** (conformers **1c** and **1d**), compound **2** (conformers **2c** and **2d** and, to some extent, **2a** and **2b**), and compound **4** (conformer **4a**) are the best simulators of the DA molecule. The values of net atomic charge for oxygen atoms in the compounds with carboxyl groups (**1–3**) are obviously more negative than for those in DA, so stronger electrostatic interaction of these groups with the serine residue on the DA receptor can be expected. The values of net atomic charge for the nitrogen atom are very similar for all compounds (except for compound **3**), and comparable with those for the DA molecule. The value for proton affinity of the nitrogen atom, which can be used to estimate to some extent the possibility of forming a hydrogen bond, is better for compounds **1–3** than for the DA molecule. The amino group (i.e., the nitrogen atom) in these compounds can form a similar or stronger ionic bond with aspartic acid residue on the DA receptor.

Taking into account the distance requirement, which may be very important for a possible interaction of the functional groups with the DA receptor, compound **3** (Fig. 2, Table 4) was excluded from further studies. The results of molecular modeling calculations for the other structures **1, 2,** and **4** were encouraging, so it was

TABLE 4
Results of AM1 Calculations for Compound **3**[a]

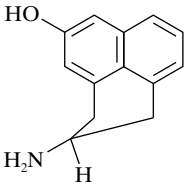
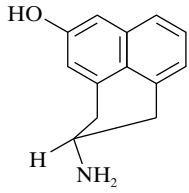
Conformers	 **3a**	 **3b**
	Torsional angle (degrees)	
O16–C15–C1–C6	0	180
O17–C15–C1–C6	180	0
	Distance of atoms (Å)	
O16–N14	5.157	6.432
O17–N14	6.468	4.954
	Net atomic charge (a.u.)	
O16	−0.361	−0.368
O17	−0.319	−0.313
N14	−0.137	−0.138
	Energy (heat of formation) and relative energy (kcal/mol)	
E	−21.2	−21.3
ΔE	0.1	0.0
	Proton affinity of nitrogen atom N14 (kcal/mol)	
PA	218.2	218.2

[a] The numbering of atoms is presented in Fig. 2.

decided to synthesize these compounds. Meanwhile, however, we had found some reports (*17*) about dopaminergic activity of 2-amino-5-hydroxyphenalene **4** derivatives, and thus only compounds **1** and **2** were synthesized. Since the hydroxyl of the carboxylic acid group is more acidic than phenolic hydroxyl, we also synthesized the ester and amide derivatives to evaluate the influence of acidity on the ligand–receptor interaction.

The synthetic approach to 3-aminocyclohexylidene acetic acid **1** (Scheme 1) started from commercially available 2-cyclohexen-1-one, which was reacted with azidotrimethylsilane and ethylene glycol to give 3-azidocyclohexanone ethylene ketal **5.** This compound was reduced by lithium aluminum hydride to the 3-aminocyclohexanone ethylene ketal **6,** which was treated with acetic anhydride to give acetamide **7.** After ketal cleavage, 3-acetamidocyclohexanone **8** was reacted with triethylphosphonacetate to obtain the 3-*N*-acetylaminocyclohexylidenacetic acid ethyl ester **9** as *E*–*Z* isomers mixture. Single isomers were separated by recrystallization; the exact structures were identified by NMR analysis observing the NOE (nuclear Overhouser effect) between the hydrogen on C7 and those on C6 (*E* isomer) (numbering in Fig. 2) or on C2 (*Z* isomer). *E* isomer **9** was hydrolyzed to target compound **1.** Then the amino group was protected with a buthyloxycarbonyl (Boc) group. The *N*-Boc-amino acid **10** was treated with ethyl chloroformate, triethylamine and ammonia to give the corresponding amide **11,** or with potassium

TABLE 5
Results of AM1 Calculations for Compound **4**[a]

Conformers	 **4a**	 **4b**
	Torsional angle (degrees)	
O15–C13–C12–C3	176	70
H25–C13–C12–C3	60	−169
	Distance of atoms (Å)	
O11–N15	7.314	6.287
	Net atomic charge (a.u.)	
O11	−0.252	−0.254
N15	−0.335	−0.343
	Energy (heat of formation) and relative energy (kcal/mol)	
E	−13.6	−10.0
	Proton affinity of nitrogen atom N15 (kcal/mol)	
PA	212.9	212.9

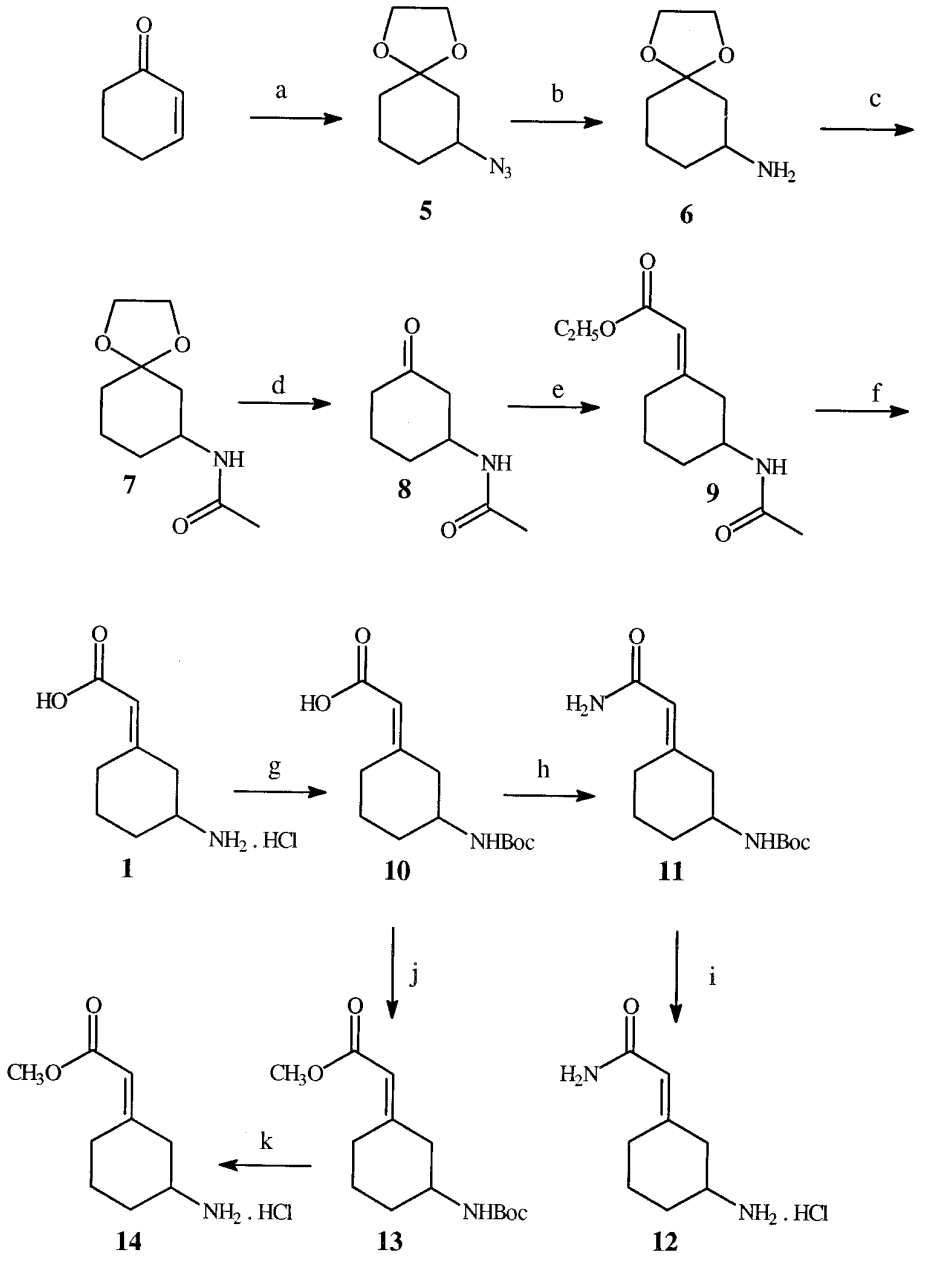
[a] The numbering of atoms is presented in Fig. 2.

carbonate and iodomethane to give ester **13.** The Boc group cleavage gave, respectively, the 3-aminocyclohexylidenacetamide **12** and the 3-aminocyclohexylidenacetic acid methyl ester **14.**

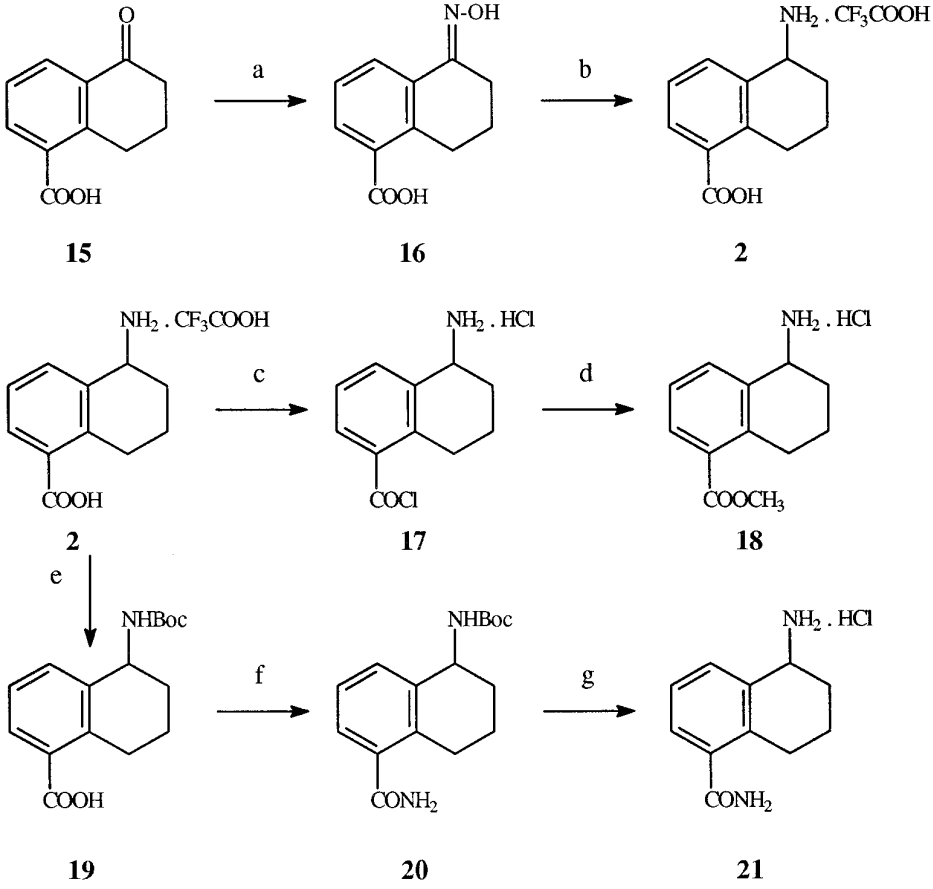
Scheme 2 shows the synthetic approach to 5-amino-5,6,7,8-tetrahydro-1-naphthalenecarboxylic acid **2.** 5-Oxo-5,6,7,8-tetrahydro-1-naphthalenecarboxylic acid **15** (*18–20*) was reacted with hydroxylamine to give the corresponding oxime **16.** This was hydrogenated over Pd/C in trifluoroacetic acid to amine **2.** The amino group was protected with a Boc group, and the *N*-Boc-amino acid **19** was treated with *N*-hydroxysuccinimide (NHS), dicyclohexylcarbodiimide (DCC), and ammonia to give the corresponding amide **20.** The Boc group cleavage gave the 5-amino-5,6,7,8-tetrahydro-1-naphthalenecarboxamide **21.** Treatment of amino acid **2** with thionyl chloride and methanol gave the 5-amino-5,6,7,8-tetrahydro-1-naphthalenecarboxylic acid ethyl ester **18.**

To evaluate the affinities for D_1 and D_2 DA receptors, compounds **1, 2, 12, 14, 18,** and **21** and the reference compound were tested in *in vitro* radioligand competition assays on rat striatal membrane using [^{3}H]SCH23390 and [^{3}H]spiperone as radioligands Table 6.

Although some molecular parameters calculated by theoretical methods for compounds **1** and **2** agree with the reference DA conformer **I** (claimed to be active), no compounds show any significant affinity to the DA receptor. These results suggest that an aromatic ring seems to be necessary for the ligand–receptor interaction. Moreover, the α–β unsaturated carboxylic moiety cannot be considered a bioisost-



SCHEME. 1. (a) $(CH_3)_3SiN_3$, $SiCl_4$, $HOCH_2CH_2OH$; (b) $LiAlH_4$; (c) $(CH_3CO)_2O$; (d) CH_3COOH; (e) $(C_2H_5O)_3POCH_2COOC_2H_5$, NaH; (f) 2 N NaOH, 6 N HCl; (g) (t-BuOCO)$_2$O, $(C_2H_5)_3N$; (h) $ClCOOC_2H_5$, $(C_2H_5)_3N$, NH_3; (i) HCl; (j) CH_3I, K_2CO_3; (k) HCl.



SCHEME. 2. (a) $NH_2OH \cdot HCl$, pyridine; (b) H_2, Pd/C, CF_3COOH; (c) $SOCl_2$; (d) CH_3OH; (e) $(t\text{-}BuOCO)_2O$, $N(C_2H_5)_3$; (f) NHS, DCC, NH_3; (g) HCl.

ere of the hydroxyphenyl group. Although compound **2** contains an aromatic group, it lacks affinity to the DA receptors. This shows that the position of the aromatic ring is also very important, and is in agreement with the suggestion that a distance equivalent to the spacing of two methylene groups in an extended conformation between the phenyl ring and amino group of DA is determinant for dopaminergic activity (*21*). However, in view of our results and the previous considerations, it is difficult to say whether the presence of the aromatic ring is needed because of sterical requirements or, rather because of electrostatic interactions. The latter can be described as aromatic–aromatic or aromatic–polar group interactions and may be important for ligand DA receptor binding. Aromatic interactions are postulated as an ordinary type of interaction inside proteins as well as between receptor and ligand (*22, 23*).

Since the main compounds do not interact with DA receptor, the influence of

TABLE 6
pK_i Values and 95% Confidence Intervals for the New and Reference Drugs Assayed

	D_1	D_2
Dopamine	6.38 (6.04–6.72)	6.14 (5.95–6.33)
Apomorphine	6.74 (6.63–6.85)	7.26 (6.97–7.56)
SCH23390	9.13 (9.09–9.17)	NT[a]
SKF38393	7.28 (7.26–7.31)	NT
(+)-Butaclamol	8.10 (7.94–8.26)	NT
cis-(*Z*)-flupentixol	7.66(7.53–7.80)	NT
Chlorpromazine	6.94 (6.78–7.11)	NT
Spiperone	NT	8.96 (8.87–9.05)
Sulpiride	NT	6.55 (6.30–6.79)
Compounds: **1, 2, 12, 14, 18,** and **21**	Inactive	Inactive

[a] Not tested.

hydroxyl acidity cannot be discussed here. In conclusion, the results show that (1) the structural and electrostatic requirements considered in this work are insufficient to design a dopaminergic ligand; (2) the presence of an aromatic ring separated by two methylenes from the amino group in an extended conformation seems to be an essential prerequisite for the interaction between a ligand and the DA receptors.

Our study has also revealed that more structural data about DA receptor are needed to understand the requirements for binding of DA to its receptor.

EXPERIMENTAL

Molecular Modeling

Molecular modeling calculations were performed by semiempirical quantum chemistry program AM1 (*24*) within the MOPAC 6.0 packet (**25**). The AM1 input structure for the DA molecule was taken from X-ray data (*26*). The structures of molecules **1–4** were built into program Chem3D[2] using standard values of bond lengths, bond angles, and torsional angles. For all four molecules, the calculations were performed for the different isomers with regard to the positions of the amino and carboxyl groups. The geometry within calculations was fully optimized. The studies for all molecules were performed in vacuo.

Proton affinity (PA) was determined by employing the heat of formation according to

$$PA = \Delta H_f^0(B) + \Delta H_f^0(H^+) - \Delta H_f^0(BH^+),$$

where $\Delta H_f^0(B)$ and $\Delta H_f^0(BH^+)$ are calculated by AM1 heats of formation for the

[2] Chem3D Molecular Modelling and Analysis is a trademark of Cambridge Scientific Computing Inc., 875 Massachusetts Avenue, Sixth Floor, Cambridge, MA 02139. It contains Allinger's MM2 program (QCPE 395).

free and protonated molecules, respectively, and $\Delta H_f^0(H^+)$ is the experimental value (367.2 kcal/mol) (*27*) of the heat of formation for H^+. Proton affinity was calculated only for the most stable conformer of each compound.

Methods

Melting points were determined on a Buchi 510 apparatus and are uncorrected. Microanalyses were performed on a 1106 Carlo Erba CHN analyzer, and the results were within ±0.4% of the calculated values. ^{1}H NMR spectra were recorded on a Varian VXR 200-MHz spectrometer. Chemical shifts are reported in parts per million (δ) downfield from the internal standard tetramethylsilane (Me_4Si). The IR spectra were run on a Perkin-Elmer Model 297 spectrometer as nujol mulls or liquid films. The identity of all new compounds was confirmed by both elemental analysis and NMR data; homogeneity was confirmed by TLC on silica gel Machery–Nagel Alugram SilG UV/254. Solutions were routinely dried over anhydrous Na_2SO_4 prior to evaporation. Chromatographic purifications were accomplished on Merck-60 silica gel columns 70–230 mesh ASTM from Merck with a reported solvent.

Syntheses

3-Aminocyclohexylidenacetic acid hydrochloride ***1.*** A suspension of 3-*N*-acetylaminocyclohexylidenacetic acid ethyl ester **9** (0.5 g, 2.2 mmol) in 2 N NaOH (5 ml) was warmed to reflux overnight. The solution was cooled in an ice bath and acidified with 6 N HCl. Water was evaporated at 30°C in vacuo and the solid residue was dried on P_2O_5 and triturated in $CHCl_3$. The suspension was filtered and the solid washed with cold anhydrous ethanol. The filtrate was evaporated to dryness and the white solid residue (0.21 g) was recrystallized from anhydrous ethanol/anhydrous Et_2O. MP 223°C. Yield 50%. IR (nujol) 1670 (C=O), 1625 (C=O) cm^{-1}. NMR (DMSO-d_6) δ: 12.20 (s, 1H, COOH); 8,20 (s, 3H, NH_3^+); 5.65 (s, 1H, CH=C); 3.46 (m, 2H, H-cyclohex); 3.10 (m, 1H, N-CH); 2.27 (t, 1H, H-cyclohex); 1.90 (m, 3H, H-cyclohex); 1.53 (m, 1H, H-cyclohex); 1.35 (m, 1H, H-cyclohex). Anal. ($C_8H_{13}NO_2 \cdot HCl$) C, H, N.

5-Amino-5,6,7,8-tetrahydro-1-naphthalenecarboxylic acid trifluoroacetate ***2.*** A suspension of 5-hydroxymino-5,6,7,8-tetrahydro-1-naphthalenecarboxylic acid **16** (2.8 g, 13.6 mmol) and 10% Pd/C (1.4 g) in trifluoroacetic acid (100 ml) was hydrogenated at 30 psi of hydrogen in a Parr shaker. Hydrogenation was stopped when the theoretical amount of hydrogen had been taken up. The reaction mixture was filtered on celite and the solvent was removed in vacuo. The residue was triturated in anhydrous Et_2O. The solid was filtered and recrystallized from ethyl acetate. MP 193–195°C. Yield 63%. IR (nujol) 1685 (C=O) cm^{-1}. NMR (DMSO-d_6) δ: 13.10 (s, 1H, COOH); 8.35 (s, 3H, NH_3^+); 7.78 (d, 1H, ArH); 7.68 (d, 1H, ArH); 7.38 (t, 1H, ArH); 4.30 (m, 1H, CH–N); 3.00 (m, 2H, CH_2); 1.92 (m, 4H, $2CH_2$). Anal. ($C_{11}H_{13}NO_2 \cdot CF_3COOH$) C, H, N.

3-Azidocyclohexanone ethylene ketal ***5.*** To a solution containing 2-cyclohexen-1-one (0.96 ml, 10 mmol) and anhydrous ethylene glycol (1.11 ml, 20 mmole) in anhydrous CH_2Cl_2 (15 ml), azidotrimethylsilane (3 ml, 22 mmol) and tetrachlorosi-

lane (0.1 ml of a solution 1 M in dichloromethane) were added. The reaction mixture was warmed to reflux for 3 h and, after cooling, was filtered on silica gel. After solvent evaporation an oily residue was obtained (1.8 g) that was not further purified. Yield 98%. IR 2080 (N_3) cm^{-1}. NMR ($CDCl_3$) δ: 3.90 (m, 4H, $2CH_2O$); 3.45 (m, 1H, CH–N); 1.95 (m, 2H, H-cyclohex); 1.46 (m, 6H, H-cyclohex). Anal. ($C_8H_{13}N_3O_2$) C, H, N.

3-Aminocyclohexanone ethylene ketal ***6.*** In a well-dried three-neck flask, lithium aluminum hydride (2.28 g, 60 mmol) was suspended in anhydrous Et_2O (100 ml), and then a solution of 3-azidocyclohexanone ethylene ketal **5** (5.4 g, 30 mmol) in anhydrous Et_2O (100 ml) was added dropwise. The reaction mixture was warmed to 65°C for 5 h. After cooling in an ice bath, the excess $LiAlH_4$ was quenched by successive dropwise additions of 2.28 ml of water, 2.28 ml 15% NaOH, and 6.84 ml of water. The solution was filtered. The solvent evaporation under reduced pressure gave an oily residue (2.98 g). Yield 63%. NMR ($CDCl_3$) δ: 3.86 (m, 4H, $2CH_2O$); 2.86 (m, 1H, CH–N); 1.35 (m, 10H, H-cyclohex and NH_2). Part of the residue was dissolved in anhydrous Et_2O and added to a saturated solution of oxalic acid in anhydrous ethanol. The solution was evaporated to dryness, and the residue recrystallized from anhydrous ethanol/anhydrous Et_2O: mp 182–184°C. Anal. ($C_8H_{15}NO_2 \cdot HCl$) C, H, N.

3-N-Acetylaminocyclohexanone ethylene ketal ***7.*** A solution of 3-aminocyclohexanone ethylene ketal **6** (1 g, 6.3 mmol) in acetic anhydride (10 ml) was warmed at 140°C for 30 min. The solvent was distilled under reduced pressure and the yellow oily residue (0.95 g) triturated in Et_2O. The white solid obtained was filtered and recrystallized from ethyl acetate. MP 112–115°C. Yield 76%; IR (nujol) 1620 (C=O) cm^{-1}; NMR ($CDCl_3$) δ: 6.36 (d, 1H, NH); 4.25 (m, 1H, NCH); 3.97 (s, 4H, $2CH_2O$); 1.97 (1, 3H,CH_3); 1.88 (dd, 1H, H-cyclohex); 1.63 (m, 7H, H-cyclohex). Anal. ($C_{10}H_{17}NO_3$) C, H, N.

3-N-Acetylaminocyclohexanone ***8.*** A solution of 3-*N*-acetylaminocyclohexanone ethylene ketal **7** (4 g, 20 mmol) in acetic acid (50 ml of 80% solution in water) was warmed at 65°C for 1.5 h. The reaction mixture was concentrated under reduced pressure and basified with a saturated solution of Na_2CO_3. The basic solution was extracted with $CHCl_3$. The collected organic layers were evaporated to dryness. The residue (2.55 g) was recrystallized from benzene. MP 86–87°C. Yield 82%. IR (nujol) 1690 (C=O), 1625 (C=O) cm^{-1}. NMR ($CDCl_3$) δ: 5,45 (d, 1H, NH); 4,28 (m, 1H, N-CH) 2,70 (dd, 1H, H-cyclohex); 2,35 (m, 3H, H-cyclohex); 1,97 (s, 3H, CH_3); 1,85 (m, 4H, H-cyclohex). Anal. ($C_8H_{13}NO_2$) C, H, N.

3-N-Acetylaminocyclohexylidenacetic acid ethyl ester ***9.*** To a suspension of NaH (0.29 g, 7.3 mmole 60% dispersion in mineral oil) in anhydrous benzene (20 ml) under nitrogen atmosphere, triethylphosphonacetate (1.46 ml, 7.3 mmol) was added dropwise over a 1-h period. During this period, the temperature was maintained at 30–35°C. The reaction mixture was stirred for 1 h at room temperature; then 3-*N*-acetylaminocyclohexanone **8** (1 g, 6.4 mmol) was added dropwise over a 1-h period, maintaining the reaction temperature at 20–30°C. The slurry was stirred for 1 h at room temperature and then warmed at 65°C for 2 h. After cooling, water (25 ml) was added and the organic layer was separated. The water solution was extracted three times with $CHCl_3$. The collected organic phases were dried. The

solvent evaporation gave the product (1.34 g, yield 82%) as a mixture of *E–Z* isomers. Pure *E* isomer was obtained by recrystallizing the crude product three times from benzene. MP 174–76°C. IR (nujol) 1705 (C=O), 1625 (C=O) cm^{-1}. NMR ($CDCl_3$) δ: 5.65 (s, 1H, CH=C); 5,42 (d, 1H, NH); 4,15 (q, 2H, CH_2); 4.05 (m, 1H, N-CH) 3.10 (m, 1H, H-cyclohex); 2.53 (m, 2H, H-cyclohex); 2.05 (m, 1H, H-cyclohex); 1.97 (s, 3H, CH_3); 1.90 (m, 1H, H-cyclohex); 1.85 (m, 1H, H-cyclohex); 1.57 (m, 2H, H-cyclohex); 1.27 (t, 3H, CH_3). Anal. ($C_{12}H_{19}NO_3$) C, H, N.

To the mother liquor Et_2O was added and the *Z* isomer precipitated was filtered and recrystallized from benzene. MP 139–143°C. IR (nujol) 1705 (C=O), 1625 (C=O) cm^{-1}. NMR ($CDCl_3$) δ: 5.75 (s, 1H, CH=C); 5.58 (d, 1H, NH); 4.15 (q, 2H, CH_2); 4.05 (m, 1H, N-CH) 3.12 (dd, 1H, H-cyclohex); 2.61 (m, 1H, H-cyclohex); 2.20 (m, 2H, H-cyclohex); 1.97 (s, 3H, CH_3); 1.85 (m, 1H, H-cyclohex); 1.65 (m, 1H, H-cyclohex); 1.57 (m, 2H, H-cyclohex); 1.27 (t, 3H, CH_3). Anal. ($C_{12}H_{19}NO_3$) C, H, N.

3-N-(ter-Butoxycarbonyl)aminocyclohexylidenacetic acid ***10.*** To a stirred solution containing 3-*N*-acetylaminocyclohexylidenacetic acid **9** (0.75 g, 3.9 mmol) and triethylamine (2.36 ml, 16.9 mmol) in methanol (25 ml), di-t-butyldicarbonate (3.4 g, 15.6 mmol) was added over a 1-h period. The reaction mixture was warmed at 50°C for 3 h. The solvent was evaporated and water (20 ml) added to the residue. The solution was acidified at pH 2 with $KHSO_4$ and immediately extracted three times with ethyl acetate. The collected organic layers were dried and evaporated to dryness. The oily residue (0.95 g) was purified by flash chromatography using a mixture of cyclohexane–ethyl acetate (8:2) as eluant. After solvent evaporation of pure fractions the residue was recrystallized from $CHCl_3$–petroleum ether. MP 135–36°C. Yield 60%. IR (nujol) 1675 (C=O), 1625 (C=O) cm^{-1}. NMR ($CDCl_3$) δ: 9.50 (bs, 1H, COOH); 5.65 (s, 1H, CH=C); 4.60 (d, 1H, NH); 3.72 (m, 1H, H-cyclohex); 3.15 (m, 1H, H-cyclohex); 2.50 (m, 2H, H-cyclohex); 2.09 (m, 1H, H-cyclohex); 1.87 (m, 2H, H-cyclohex); 1.48 (m, 11H, H-cyclohex, $3CH_3$). Anal. ($C_{13}H_{21}NO_4$) C, H, N.

3-N-(ter-Butoxycarbonyl)aminocyclohexylidenacetamide ***11.*** To a solution of 3-*N*-ter-butoxycarbonylaminocyclohexylidenacetic acid **10** (0.12 g, 0.47 mmol) and triethylamine (0.186 ml, 1.41 mmol) in anhydrous ethyl acetate (15 ml), a solution of ethyl chloroformate (0.045 ml, 0.47 mmol) in anhydrous ethyl acetate (5 ml) was added dropwise under nitrogen atmosphere. The reaction mixture was stirred at room temperature for 1 h and then filtered. Ammonia was bubbled for 10 min into the clear solution. The flask was corked and stirring was maintained at room temperature for 1 h. The solvent was removed in vacuo, and the solid residue (0.11 g) was triturated in petroleum ether, filtered, and recrystallized from ethyl acetate/cyclohexane. MP 170–171°C. Yield 75%. IR (nujol) 1665 (C=O), 1640 (C=O) cm^{-1}. NMR ($CDCl_3$) δ: 5.65 (s, 1H, CH=C); 5.42 (bs, 2H, NH_2); 4.55 (d, 1H, NH); 3.70 (m, 1H, H-cyclohex); 3.20 (m, 1H, H-cyclohex); 2.50 (m, 2H, H-cyclohex); 2.02 (m, 1H, H-cyclohex); 1.83 (m, 2H, H-cyclohex); 1.68 (m, 1H, H-cyclohex); 1.48 (m, 10H, H-cyclohex and $3CH_3$). Anal. ($C_{13}H_{22}N_2O_3$) C, H, N.

3-Aminocyclohexylidenacetamide hydrochloride ***12.*** Anhydrous HCl was bubbled for 10 min into a solution of 3-(*N*-ter-butoxycarbonyl)aminocyclohexylidenacetamide **11** (0.09 g, 0.35 mmol) in $CHCl_3$ (10 ml). The flask was corked and the reaction

mixture stirred at room temperature for 1 h. The solvent was removed in vacuo, and the solid residue (0.05 g) recrystallized from anhydrous ethanol/anhydrous Et_2O. MP 211–213°C. Yield 75%. IR (nujol) 1660 (C=O) cm^{-1}. NMR (D_2O) δ: 5.68 (s, 1H, CH=C); 3.27 (m, 1H, H-cyclohex); 2.98 (m, 1H, H-cyclohex); 2.57 (m, 1H, H-cyclohex); 2.22 (m, 1H, H-cyclohex); 2.02 (m, 2H, H-cyclohex); 1.83 (m, 1H, H-cyclohex); 1.48 (m, 2H, H-cyclohex). Anal. ($C_8H_{15}N_2O.HCl$) C, H, N.

3-N-(ter-butoxycarbonyl)aminocyclohexylidenacetic acid methyl ester ***13.*** To a solution of 3-*N*-(ter-butoxycarbonyl)aminocyclohexylidenacetic acid **10** (0.5 g, 1.95 mmol) in acetone, K_2CO_3 (0.55 g, 4 mmol) and iodomethane (0.5 ml, 8.3 mmol) were added. The reaction mixture was warmed to reflux for 8 h. The solvent was removed in vacuo and the residue purified by column chromatography using ethyl acetate–*n*-hexane (2:8) as eluant. The collected pure fractions were evaporated to dryness and the oily residue (0.5 g) recrystallized from *n*-hexane. MP 83–85°C. Yield 91%. Ir (nujol) 1690 (C=O); 1635 (C=O) cm^{-1}. NMR ($CDCl_3$) δ: 5.67 (s, 1H, CH=C); 4.50 (bs, 1H, NH); 3.70 (m, 4H, H-cyclohex and CH_3); 3.15 (m, 1H, H-cyclohex); 2.50 (m, 2H, H-cyclohex); 1.93 (m, 4H, H-cyclohex); 1.48 (m, 10H, H-cyclohex, $3CH_3$). Anal. ($C_{14}H_{23}NO_4$) C, H, N.

3-Aminocyclohexylidenacetic acid methyl ester hydrochloride ***14.*** Anhydrous HCl was bubbled for 10 min into a solution of 3-*N*-(ter-butoxycarbonyl)aminocyclohexylidenacetic acid methyl ester **13** (0.22 g, 0.81 mmol) in $CHCl_3$ (10 ml). The flask was corked and the reaction mixture stirred at room temperature for 1 h. The solvent was removed in vacuo and the solid residue (0.15 g) recrystallized from anhydrous ethanol/anhydrous Et_2O. MP 150–152°C. Yield 90%. IR (nujol) 1700 (C=O) cm^{-1}. NMR ($CDCl_3$) δ: 8.53 (bs, 3H, NH_3^+); 5.75 (s, 1H, CH=C); 3.67 (s, 3H, CH_3); 3.58 (m, 1H, H-cyclohex); 3.30 (m, 1H, H-cyclohex); 2.70 (m, 1H, H-cyclohex); 2.48 (m, 1H, H-cyclohex); 2.10 (m, 3H, H-cyclohex); 1.60 (m, 2H, H-cyclohex). Anal. ($C_9H_{15}NO_2 \cdot HCl$) C, H, N.

5-Hydroxyamino-5,6,7,8-tetrahydro-1-naphthalenecarboxylic acid ***16.*** 5-Oxo-5,6,7,8-tetrahydro-1-naphthalenecarboxylic acid **15** (1 g, 5.2 mmol) was added to a solution of hydroxylamine hydrochloride (1 g, 1.44 mmol) and anhydrous pyridine (2 ml) in anhydrous ethanol. The mixture was refluxed for 2 h. The solution was evaporated in vacuo and the residue dissolved in water and acidified with 6 N HCl. The solid precipitate was filtered and recrystallized from ethyl acetate. MP 241–242°C. Yield 94%. IR (nujol) 1690 (C=O) cm^{-1}. NMR (DMSO-d_6) δ: 13.00 (s, 1H, COOH); 11.20 (s, 1H, OH); 8.1 (d, 1H, ArH); 7.75 (d, 1H, ArH); 7.25 (t, 1H, ArH); 3.00 (t, 2H, CH_2); 2.70 (t, 2H, CH_2); 1.70 (m, 2H, CH_2). Anal. ($C_{11}H_{11}NO_3$) C, H, N.

5-Amino-5,6,7,8-tetrahydro-1-naphthalenecarboxylic acid methyl ester hydrochloride ***18.*** A solution of 5-amino-5,6,7,8-tetrahydro-1-naphthalenecarboxylic acid trifluoroacetate **2** (2 g, 6 mmol) in freshly distilled thionyl chloride (15 ml) was warmed at 75°C for 2 h. The reaction mixture was evaporated to dryness and the solid residue triturated in anhydrous Et_2O. The filtration gave the desired acyl chloride **17:** MP 189–193°C. IR (nujol) 1755 (C=O) cm^{-1}. NMR (DMSO-d_6) δ: 8.60 (bs, 3H, NH_3^+); 7.80 (t, 2H, ArH); 7.39 (t, 1H, ArH); 4.48 (m, 1H, CHN); 3.00 (m, 2H, CH_2); 1.92 (m, 4H, $2CH_2$).

The acyl chloride **17** was dissolved in methanol (20 ml) and the solution refluxed

for 1.5 h. The solvent was removed in vacuo and the oily residue was triturated in anhydrous Et_2O. The solid was filtered and recrystallized from anhydrous methanol/anhydrous Et_2O. MP 215–217°C. Yield 76%. IR (nujol) 1700 (C=O) cm^{-1}. NMR (DMSO-d_6) δ: 8.58 (bs, 3H, NH_3^+); 7.82 (d, 1H, ArH); 7.75 (d, 1H, ArH); 7.40 (t, 1H, ArH); 4.50 (m, 1H, CHN); 3.83 (s, 3H, CH_3); 2.97 (m, 2H, CH_2); 1.90 (m, 4H, $2CH_2$). Anal. ($C_{12}H_{15}NO_2 \cdot HCl$): C, H, N.

5-[N-(t-Butoxycarbonyl)amino]-5,6,7,8-tetrahydro-1-naphthalenecarboxylic acid ***19.*** To a solution of 5-amino-5,6,7,8-tetrahydro-1-naphthalenecarboxylic acid trifluoracetate **2** (2 g, 6.6 mmol) and triethylamine (4 ml, 28 mmol) in methanol (10 ml), di-t-butyldicarbonate (5.76 g, 26.4 mmol) was added portionwise over a 1-h period. The reaction mixture was warmed at 50°C for 3 h. After solvent evaporation, the residue was dissolved in water and acidified with solid $KHSO_4$ up to pH 2. The solution was immediately extracted with ethyl acetate. The organic layer was dried, filtered, and evaporated to dryness. The oily residue was recrystallized from ethyl acetate. MP 207°C. Yield 83%. IR (nujol) 1680 (C=O), 1650 (C=O) cm^{-1}. NMR (DMSO-d_6) δ: 12.80 (s, 1H, COOH); 7.60 (d, 1H, ArH); 7.30 (m, 3H, NH, 2ArH); 4.68 (m, 1H, CH−N); 2.90 (m, 2H, CH_2); 1.82 (m, 2H, CH_2); 1.62 (m, 2H, CH_2); 1.40 (s, 9H, $3CH_3$). Anal. ($C_{16}H_{21}NO_4$) C, H, N.

5-[N-(t-Butoxycarbonyl)amino]-5,6,7,8-tetrahydro-1-naphthalenecarboxamide ***20.*** To a solution of 5-[*N*-(t-butoxycarbonyl)amino]-5,6,7,8-tetrahydro-1-naphthalenecarboxylic acid **19** (0.4 g, 1.37 mmol) and *N*-hydroxysuccinimide (NHS) (0.19 g, 1.64 mmol) in $CHCl_3$ (20 ml), dicyclohexylcarbodiimide (DCC) (0.33 g, 0.0016 mmol) was added. The reaction mixture was stirred at room temperature for 2.5 h. After filtration, ammonia was bubbled into the clear solution for 10 min. The flask was corked and the reaction mixture stirred at room temperature for 1 h. After filtration, the clear solution was evaporated to dryness and the oily residue recrystallized from ethyl acetate. MP 207°C. Yield 99%. IR (nujol) 1665 (C=O), 1650 (C=O) cm^{-1}. NMR (DMSO-d_6) δ: 7.68 (d, 1H, ArH); 7.25 (m, 5H, NHCO, $CONH_2$ and 2ArH); 4.65 (m, 1H, CH–N); 2.77 (m, 2H, CH_2); 1.88 (m, 2H, CH_2); 1.68 (m, 2H, CH_2); 1.45 (s, 9H, $3CH_3$). Anal. ($C_{16}H_{22}N_2O_3$) C, H, N.

5-Amino-5,6,7,8-tetrahydro-1-naphthalene carboxamide hydrochloride ***21.*** Anhydrous HCl was bubbled for 10 min into a solution of 5-[*N*-(t-butoxycarbonyl)amino]-5,6,7,8-tetrahydro-1-naphthalene carboxaminde **20** (0.3 g, 1 mmol) in $CHCl_3$ (25 ml). The flask was corked and the reaction mixture stirred at room temperature for 1 h. The solid was filtered and recrystallized from anhydrous methanol/anhydrous Et_2O. MP 288–290°C. Yield 88%. IR (nujol) 1665 (C=O), 1650 (C=O) cm^{-1}. NMR (DMSO-d_6) δ: 8.60 (s, 3H, NH_3^+); 7.77 (s, 1H, NHCO); 7.65 (dd, 1H, ArH); 7.45 (s, 1H, NHCO); 7.30 (m, 2H, ArH); 4.45 (m, 1H, CH−N); 2.99–2.68 (m, 2H, CH_2); 2.15–1.65 (m, 4H, $2CH_2$). Anal. ($C_{11}H_{14}N_2O \cdot HCl$) C, H, N.

Pharmacology

Male Sprague–Dawley rats (300–350 g body weight) were obtained from the breeding facilities at the University of Santiago. Rats were killed by decapitation, and brains were rapidly removed and dissected on an ice-cold plate.

[^{3}H]Spiperone (95 Ci/mmol) and [^{3}H]SCH23390 (81 Ci/mmol) were purchased

from Amersham International (England), unlabeled spiperone · HCl, *R*(+)-SCH23390 · HCl, SKF38393 · HCl, (+)butaclamol · HCl, *cis*(*z*)-flupentixol · 2HCl, and *R*(−)-apomorphine · HCl from Research Biochemicals Inc. and dopamine · HCl, chlorpromazine · HCl, and sulpiride · HCl from Sigma. Reference drugs or new compounds were stored in 1 mM solutions at −20°C, and diluted to the required concentration on ice immediately before binding assays.

Striatal membrane preparations were obtained by homogenization (Polytron homogenizer, setting 6 for 10 s) of tissue in 50 mM Tris–HCl (pH 8.7 at 25°C, about 100 μl/mg of tissue) containing 5 mM EDTA. Homogenates were centrifuged (31,000*g* for 15 min at 4°C, Beckman J2-MI), then resuspended in 50 mM Tris–HCl buffer (pH 7.4 at 25°C), and then centrifuged again (same conditions). Final pellets were stored at −80°C until assay.

Just before the binding assay, pellets were resuspended (1.25 mg original wet weight per 750 μl for D_2 binding assays, 1.00 mg per 750 μl for D_1 binding assays) in 50 mM Tris–HCl buffer (pH 7.4 at 25°C) containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, and 1 mM $MgCl_2$. For D_2 binding assays, aliquots of striatal membrane preparations were added to ice-cold tubes containing (a) 100 μl of [^{3}H]spiperone plus 50 μl of ketanserin (final concentration 50 nM to block 5-HT_{2A} receptors), and either (b) 100 μl of buffer (total binding) or (c) 100 μl of sulpiride (final concentration 10 μM to allow quantification of specific binding by [^{3}H]spiperone), or (d) 100 μl of new or reference drug (concentrations between 1 nM and 3 μM). For D_1 binding assays, the same procedure was followed except that (a) was 100 μl of [^{3}H]SCH23390 plus 50 μl of buffer, and (c) was 100 μl of nonradiolabeled SCH23390 (final concentration 1 μM to allow quantification of specific binding by [^{3}H]SCH23390). The final assay volume was, thus, in all cases 1 ml. All assays were performed in duplicate.

Incubations (15 min at 37°C) were stopped by rapid filtration under vacuum through GF-52 glass-fibers filters (Schlelcher and Schuell) in a Brandel (M-30) cell harvester. Filters were rinsed three times with 3 ml of ice-cold 50 mM Tris–HCl buffer (pH 7.4). Radioactivity was determined by liquid scintillation counting in a Beckman LS-6000LL apparatus (counting efficiency approximately 50%).

Competition analyses were carried out with the aid of the Prism program (GraphPad), and K_i values were calculated as $K_i = IC_{50}/(1 + L/K_D)$, where L is the concentration and K_D the apparent dissociation constant of the ligand.

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