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Azaindole Derivatives with High Affinity for the Dopamine D4 Receptor: Synthesis, Ligand Binding Studies and Comparison of Molecular Electrostatic Potential Maps

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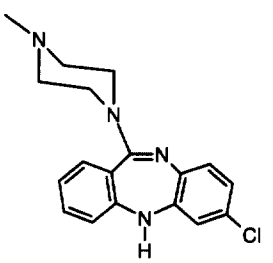
Abstract: Piperazinylmethyl substituted pyrazolo[1,5-a]pyridines and related heterocycles were synthesized and found to recognize selectively the dopamine D4 receptor. For the most potent derivative **10d** a Ki value of 2.0 nM was observed. SAR studies including the comparison of molecular isopotential surfaces were performed.

The application of molecular cloning techniques led to the characterization of five different dopamine receptors, all of which belonging to the superfamily of G protein coupled receptors.[1,2] Special interest in the D4 subtype [3] is due to the observation that the atypical antipsychotic agent clozapine (1) preferentially binds to this receptor, when compared to D1, D2 and D3.[4] Consequently, the development of selective D4 receptor antagonists has become a challenging field of research.[5,6] During the last years, the first selective D4 antagonists entered clinical evaluation.[7] Up to now, it is not clear whether subtype selectivity or the capability of exerting a specific graduation of subreceptor affinities is the essential requirement for a compound to act as a superior neuroleptic drug. Thus, structure activity relationship (SAR) studies giving insights into the molecular properties causing receptor affinity and selectivity and, thus, allowing a controlled *selectivity tuning* are of special interest.

We have recently reported stereoselective synthesis, dopamine receptor binding and computational studies of bi- and tricyclic ergoline analogs which are devoid of a heterocyclic NH function.[8,9] Employing the D4 antagonists **2a** and **2b** (L-745,870) as lead compounds,[10,11] we here describe SAR studies addressing the question whether the aromatic NH moiety as well as the position of the chloro substituent within the phenyl substructure are essential for D4 affinity and selectivity.



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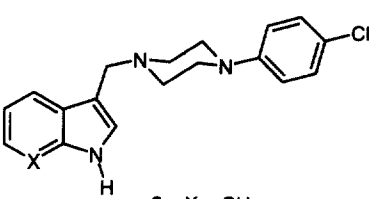

2a: X = CH
2b (L-745,870): X = N

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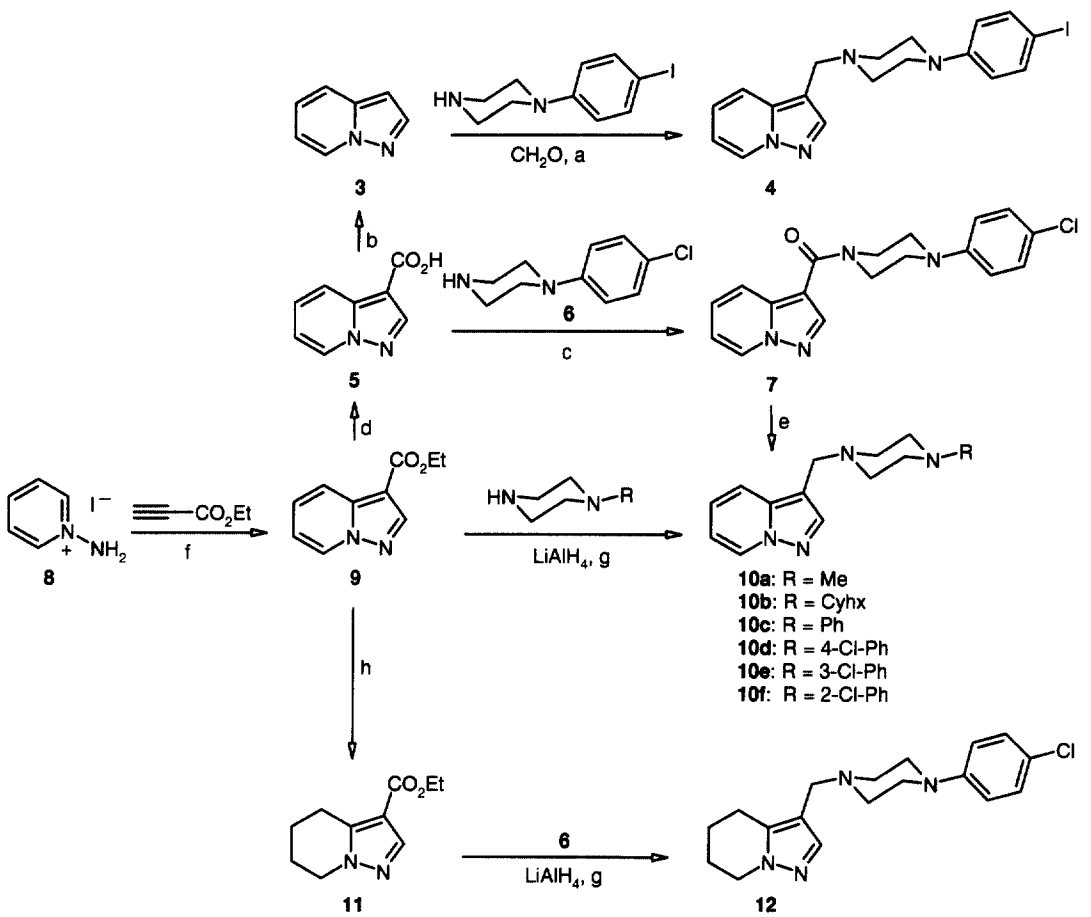
As an interesting indole congener, we chose the pyrazolo[1,5-a]pyridine framework which can be readily synthesized by 1,3-dipolar cycloaddition reaction.[12] This heterocyclic 10π system proved to be a valuable pharmacophoric moiety in recent studies and can be easily transformed into the 4,5,6,7-tetrahydro derivatives.[13] Besides the *para*-chlorophenyl group and its *ortho*- and *meta*- isomers, further aromatic and aliphatic residues were envisioned as substituents at the piperazine ring. In practice, the pyrazolo[1,5-a]pyridine carboxylate **9** was obtained by reaction of the *N*-aminopyridinium salt **8** under oxidative conditions.[14] Using standard reaction conditions saponification, activation and coupling with 4-chlorophenylpiperazine (**6**) gave the carboxamide **7** through the carboxylic acid **5**. Subsequent reduction by $LiAlH_4$ afforded the target compound **10d**. Replacement of this three-step reaction sequence by a one-step process turned out to be a very practical improvement.[15] Thus, pre-treatment of the secondary amine **6** with $LiAlH_4$ and subsequent addition of the carboxylic ester **9** resulted in formation of the reductive amination product **10d**, directly.[16] Application of this methodology for further piperazine derivatives afforded the potential D4 ligands **10a,b,c,e,f**.

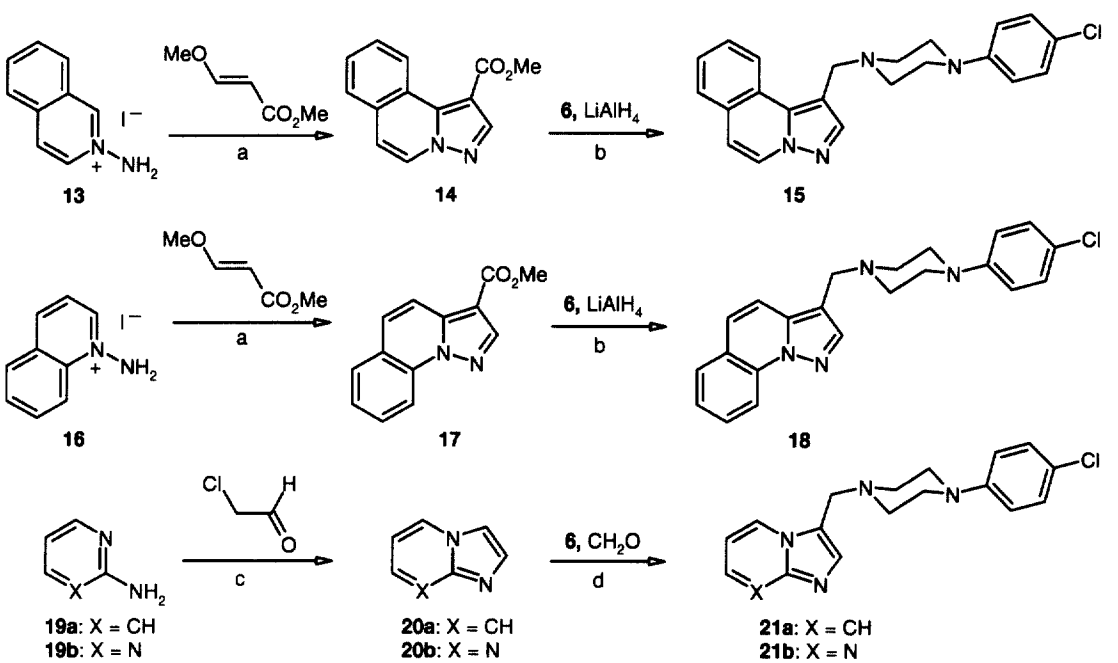


a: HOAc, 25 to 70°C, 6h (42%). b: 57% HI, reflux, 3h (78%). c: 1. $(COCl)_2$, toluene, RT to 60°C, 4.5h; 2. CH_2Cl_2, -60°C to RT, 2h (57%). d: NaOH, MeOH, RT, 48h (88%). e: $LiAlH_4$, Et_2O, reflux, 2h (71%). f: K_2CO_3, air-O_2, THF, RT, 90min, (72%). g: THF, RT to 60°C, 1h (31-43%). h: H_2, Pd/C, EtOH, 16 bar, 80°C, 5h (90%).

Reductive coupling with 4-iodophenylpiperazine [17] was not successful when reductive displacement of the iodide was observed. To avoid this, the target compound **4** was synthesized by decarboxylation of **5** followed by *Mannich* reaction of pyrazolo[1,5-a]pyridine (**3**) in position 3. Reduction of the heterocyclic 10π system into a 6π moiety was achieved by catalytic hydrogenation of the carboxylate **9** to give the tetrahydro derivative **11** which could be readily transformed into the reductive amination product **12**.

Besides the diminution of the heterocyclic aromatic region we envisioned an extension of the π system. Thus, the pyrazolo[5,1-a]isoquinoline derivative **15** and the regioisomeric pyrazolo[1,5-a]quinoline **18** were synthesized by reductive coupling of the carboxylates **14** and **17**, respectively. The intermediates were obtained efficiently from the readily available iodides **13** and **16** [18] using methodology which has been described for the respective mesitylene sulfonates.[19]

For SAR studies with respect to the distribution of charge the imidazo[1,2-a]pyridine **21a** and the imidazo[1,2-a]pyrimidine **21b** combining structural elements of **21a** with those of the lead compound **2b** (L-745,870) were expected to be of special interest. Applying *Mannich* conditions, this target compounds could be prepared from **20a,b**,[20,21] which were easily derived from the amines **19a,b**.



a: Et_3N, EtOH, reflux, 5h (72-80%). b: THF, RT then 60°C, 1h (32-34%). c: X = CH: $NaHCO_3$, H_2O, EtOH, reflux, 2h (90%); X = N: $NaHCO_3$, H_2O, RT, 16h (75%). d: HOAc, 0°C to 50°C, 16h (51-73%).

Our SAR studies were initiated by determining the binding properties of the pyrazolo[1,5-a]pyridine **10d** and its synthetic precursor **7** bearing a carboxamide function in position 3 instead of an aminomethyl group, followed by examining the effect of replacing the *para*-chlorophenyl substituent. Therefore, dopamine receptor binding assays were performed using human D3 and D4.4 receptors, expressed in CHO cells.[22,23] Striatal bovine membrane preparations were employed for evaluating D1 and D2 affinities which were shown to give suitable Ki values for describing activities at the respective human receptors, according to previous binding and sequence homology

studies. [24] The Ki values depicted in Table 1 clearly demonstrate strong and highly selective binding of the 4-chlorophenylpiperazinylmethyl substituted pyrazolo[1,5-a]pyridine **10d** with Ki values of 2.0 nM compared to 12 000 nM for D1, 4 700 nM for D2 and 5 000 nM for D3. In contrast, the carboxamide **7** was nearly uneffective. This indicates that the pyrazolo[1,5-a]pyridine framework incorporated into the lead structures **2a,b** facilitates excellent binding properties, however electronic (reduction of basicity) or stereoelectronic effects (conformational restriction of the *CN* bond) of the carboxamide function are not tolerated. Replacement of the *para*-chlorophenyl group by *meta*- or *ortho*- isomers leads only to a slight decrease in D4 affinity. However, D2 and D3 binding is significantly increased for the *meta*-chloro derivative **10e** and, more distinct for the *ortho*-isomer **10f** which reveals enhanced D1 affinity, too. Whereas the loss of the chloro substituent of the phenylpiperazine **10c** results in only an eightfold loss of D4 binding, the replacement of the aromatic moiety by a methyl or cyclohexyl group, as realized in **10a,b**, is not tolerated by the dopamine receptors. The *para*-iodophenyl derivative **4** was similar to **10d** in its dopamine receptor binding profile.

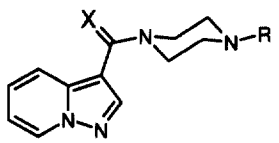


Table 1: Ki values [nM] [25] for the displacement of [^{3}H]SCH 23390 at bovine D1 and [^{3}H]spiperone at bovine D2, human D3 or human D4.4 receptors indicating binding affinities for substituted pyrazolo[1,5-a]pyridines compared to clozapine.

compound	4	7	10a	10b	10c	10d	10e	10f	1
X	H_2	O	H_2	H_2	H_2	H_2	H_2	H_2	
R	4-I-Ph	4-Cl-Ph	Me	Cyhx	Ph	4-Cl-Ph	3-Cl-Ph	2-Cl-Ph	
D1	17 000	$>10^5$	83 000	62 000	25 000	12 000	11 000	1 200	420
D2	1 400	30 000	$>10^5$	22 000	2 000	4 700	1 900	340	690
D3	6 700	61 000	34 000	5 500	1 600	5 000	560	230	960
D4	3	23 000	7 300	4 500	17	2	4	3	16

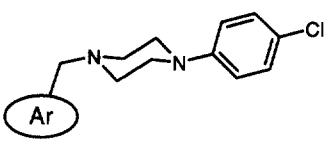
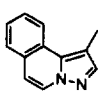
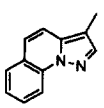


Table 2: Ki values [nM] [25] for the displacement of [^{3}H]SCH 23390 at bovine D1 and [^{3}H]spiperone at bovine D2, human D3 or human D4.4 receptors indicating binding affinities for chlorophenylpiperazinylmethyl substituted test compounds.

compound	12	15	18	21a	21b	2a	2b
Ar							
D1	22 000	7 600	45 000	15 000	21 000	330	9 900
D2	12 000	$>10^5$	4 800	5 700	30 000	270	6 100
D3	1 100	5 200	21 000	5 300	6 600	430	29 000
D4	12.5	1 800	140	13	290	1.1	0.6

Structural variations of the heterocyclic moiety are shown in Table 2. It is interesting to note, that the 4,5,6,7-tetrahydropyrazolo[1,5-a]pyridine derivative **12** exhibits D4 binding which is only reduced by the factor of 6 when compared to the 10π analog **10d** indicating that the pyrazole substructure is the part of the bicyclic ring which is important for D4 affinity. On the other hand, enlargement of the aromatic system resulted in substantial loss of affinity for the pyrazolo [5,1-a]isoquinoline **15** and the pyrazolo[1,5-a]quinoline **18**, indicating steric interactions with the receptor excluded volume. Exchange of the pyrazolo[1,5-a]pyridine framework by the regioisomeric imidazo[1,2-a]pyridine system caused 6.5 fold decrease of D4 affinity for **21a** while the high selectivity was maintained. However, introduction of a third nitrogen in position 7, as realized in the imidazo[1,2-a]pyrimidine **21b** caused strongly reduced D4 affinity. This was surprising to us, since **21b** can be regarded as a structural hybrid of the two active compounds **21a** and **2b**.

In order to understand the SARs exerted by manipulation of the heterocyclic ring, we compared the respective molecular electrostatic potential maps derived by *ab initio* molecular orbital calculations.[26] Figure 1 shows the negative electrostatic isopotential surfaces (-15 kcal/mol) for the 3-methyl substituted indole (A), pyrrolo[2,3-b] pyridine (B), pyrazolo[1,5-a]pyridine (C), tetrahydropyrazolo[1,5-a]pyridine (D), imidazo[1,2-a]pyridine (E) and imidazo[1,2-a]pyrimidine (F) representing core fragments of the lead compounds **2a** (moderate D4 selectivity) and **2b** (highly D4 selective) as well as those of the potent and selective analogs **10d**, **12**, **21a** and the modestly D4 active derivative **21b**. As a common property of A-E, representing high affinity D4 ligands, the negative potential in front and back of the plane of the 5-membered ring can be recognized. In contrast to A, however, a large negative region below the rings is typical for B-E which we expect to be responsible for the high selectivity of **2b**, **10d**, **12** and **21a**. Obviously, this molecular property is not tolerated by D1, D2 and D3 binding sites.

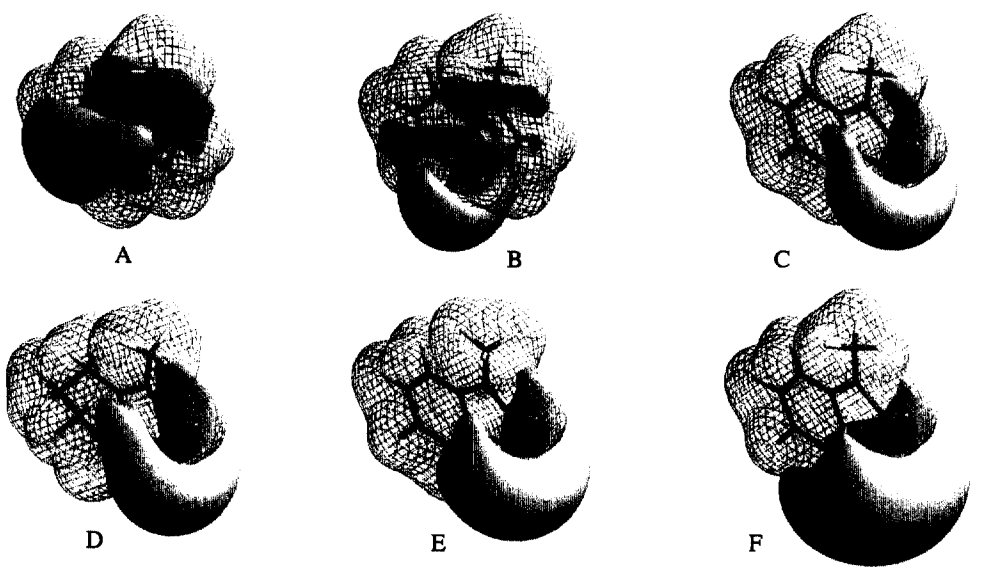


Figure 1: Molecular electrostatic isopotential maps (representing a -15 kcal/mol interaction with a point positive charge) for the core fragments A-F based on MO calculations at the RHF level of theory and the 6-31 G* basis set.

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References and Notes:

1. *The Dopamine Receptors*; Neve, K.A.; Neve, R.L., Ed.; Humana Press: Totowa, New Jersey, **1997**.
2. Sibley, D.R.; Monsma, Jr., F.J. *TiPS* **1992**, 13, 61. Sokoloff, P.; Schwartz, J.-C. *TiPS* **1995**, 16, 270.
3. Van Tol, H.H.M.; Bunzow, J.R.; Guan, H.-C., Sunahara, R.K.; Seeman, P., Niznik, H.B.; Civelli, O. *Nature* **1991**, 350, 610.
4. Strange, P.G. *TiPS* **1994**, 15, 317 and references cited therein.
5. For review: Liegeois, J.-F.; Eyrolles, L.; Bruhwyler, J.; Delarge, J. *Curr. Med. Chem.* **1998**, 5, 77.
6. For review: Steiner, G.; Bach, A.; Bialojan, S.; Greger, G.; Hege, H.-G.; Höger, T.; Jochims, K.; Munschauer, R.; Neumann, B.; Teschendorf, H.-J.; Traut, M.; Unger, L.; Gross, G. *Drugs Fut.* **1998**, 23, 191
7. For review: Kulagowski, J.J.; Patel, S. *Curr. Pharm. Design* **1997**, 3, 355 and references cited therein.
8. Gmeiner, P.; Mierau, J.; Höfner, G.; *Arch. Pharm. (Weinheim)* **1992**, 325, 57.
9. Gmeiner, P.; Sommer, J.; Mierau, J.; Höfner, G. *Bioorg. Med. Chem. Lett.* **1993**, 3, 1477.
10. Kulagowski, J.J.; Broughton, H.B.; Curtis, N.R.; Mawer, I.M.; Ridgill, M.P.; Baker, R.; Emms, F.; Freedman, S.B.; Marwood, R.; Patel, Sh.; Patel, Sm.; Ragan, C.I.; Leeson, P.D. *J. Med. Chem.* **1996**, 39, 1941.
11. Bristow, L.J.; Kramer, M.S.; Kulagowski, J.; Patel, S.; Ragan, C.I.; Seabrook, G.R. *TiPS* **1997**, 18, 186.
12. Huisgen, R.; Grashey, R.; Krischke, R. *Tetrahedron Lett.* **1962**, 387.
13. Gmeiner, P.; Schünemann, J. *Arch. Pharm. (Weinheim)* **1988**, 321, 517.
14. Gmeiner, P.; Sommer, J. *Arch. Pharm. (Weinheim)* **1988**, 321, 505.
15. Wright, Jr., W.B. *J. Org. Chem.* **1962**, 27, 1042.
16. Experimental details for the preparation of **10d**: To a solution of **6** (100mg, 0.5 mmol) in THF (10ml) was added $LiAlH_4$ (0.5ml of 1M solution in THF, 0.5mmol) at RT. After stirring for 30 min, the mixture was warmed to 60°C. Then, **9** (150mg, 0.8mmol) dissolved in THF (4ml) was added dropwise during a period of 20 min and stirring was continued for another 15 min. The mixture was cooled to RT, treated with sat. aq. $NaHCO_3$ and extracted with CH_2Cl_2. The organic layer was dried ($MgSO_4$), evaporated and the residue purified by flash chromatography (CH_2Cl_2 / MeOH 95:5) to give **10d** (59mg, 36%) as a white solid. Analytical data of **10d**: m.p.: 160°C. ^{1}H NMR ($CDCl_3$, 360 MHz): δ (ppm) = 2.60-2.63 (m, 4H, 2x NCH_2CH_2), 3.14-3.17 (m, 4H, 2x CH_2CH_2N), 3.75 (s, 2H, $ArCH_2N$), 6.75 (ddd, ^{3}J=7.0/7.0 Hz ^{4}J=1.4, 1H, 6-H), 6.79-6.84 (m, 2H, Ph), 7.10 (ddd, ^{3}J=8.5/7.0 Hz ^{4}J=0.8 Hz, 1H, 5-H), 7.16-7.20 (m, 2H, Ph), 7.63 (br d, J=8.5 Hz, 1H, 4-H), 7.91 (s, 1H, 2-H), 8.44 (br d, J=7.0 Hz, 7-H). ^{13}C NMR ($CDCl_3$, 60 MHz): δ(ppm) 49.1, 49.1, 52.1, 52.7, 52.7, 106.8, 111.6, 117.1, 117.1, 117.3, 122.8, 124.4, 128.7, 128.9, 128.9, 139.1, 142.4, 149.9. EI-MS m/z: 326 (M^+), 328 (M^+). IR (film): 2941, 2822, 1633, 1595, 1496, 1234 cm^{-1}. $C_{18}H_{19}ClN_4$ (326.8): Calc.: C 66.15 H 5.86 N 17.15; Found: C 65.97 H 5.94 N 16.92.
17. Hanson, R.N.; Hariharan, S.; Astik, R. *J. Heterocyclic Chem.* **1985**, 22, 47.
18. Huisgen, R.; Grashey, R.; Krischke, R. *Liebigs Ann. Chem.* **1977**, 506.
19. Tominaga, Y.; Ichihara, Y.; Mori, T.; Kamio, C.; Hosomi, A. *J. Heterocyclic. Chem.* **1990**, 27, 263.
20. Lombardino, J.G. *J. Org. Chem.* **1965**, 30, 2403.
21. Rival, Y.; Grassy, G.; Michel, G. *Chem. Pharm. Bull.* **1992**, 40, 1170.
22. Sokoloff, P.; Andrieux, M.; Besancon, R.; Pilon, C.; Martres, M.-P.; Giros, B.; Schwartz, J.-C. *Eur. J. Pharmacol.* **1992**, 225, 331.
23. Asghari, V.; Sanyal, S.; Buchwaldt, S.; Paterson, A.; Jovanovic, V.; Van Tol, H.H.M. *J.Neurochem.***1995**, 65, 1157.
24. Ohnmacht, U.; Tränkle, C.; Mohr, K.; Gmeiner, P. *Pharmazie*, **1998**, in press, and references cited therein.
25. Data are the means of two to three experiments performed in triplicate at eight different concentrations.
26. *Ab initio* calculations and visualizations have been done using the program package *PC SPARTAN PLUS (Wavefunction, Inc.)*.