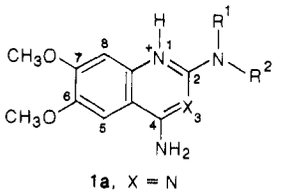
1,3-Diamino-6,7-dimethoxyisoquinoline Derivatives as Potential α_1-Adrenoceptor Antagonists

Jon Bordner,† Simon F. Campbell,* Michael J. Palmer, and Michael S. Tute

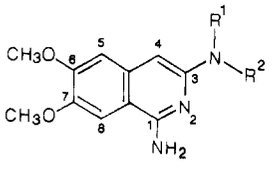
Department of Discovery Chemistry, Pfizer Central Research, Sandwich, Kent, United Kingdom, and Pfizer Central Research Laboratories, Groton, Connecticut 06340. *Received June 17, 1987*

Treatment of 2-methyl-4,5-dimethoxybenzonitrile (3) with LDA followed by reaction with an N,N-disubstituted cyanamide provided a series of 1,3-diamino-6,7-dimethoxyisoquinolines (2), which were evaluated for α-adrenoceptor binding affinity and antihypertensive activity. 1-Amino-3-(dimethylamino)-6,7-dimethoxyisoquinoline (4) showed no significant affinity ($K_i \gg 10^{-6}$ M) for α_1-adrenoceptors, while the corresponding 3-(2-furoylpiperazin-1-yl) analogue (8; $K_i = 1.6 \times 10^{-7}$ M) was some 1000-fold less potent than prazosin. pK_a data showed that N-2 protonation (34%) of 4 ($pK_a = 7.1$) would occur at physiological pH, in agreement with X-ray crystallographic analysis of 8·HCl. Comparison of positive charge distribution following protonation of 4 with the corresponding quinoline and quinazoline cations confirmed N-1 protonation is required for these heterocyclic nuclei to bind efficiently to the α_1-adrenoceptor. Computer-assisted comparison of the X-ray structures of 8 and prazosin suggested that the 4.0 kcal/mol difference in α_1-adrenoceptor binding energies was largely due to salt-bridge formation (ca. 3.0 kcal/mol) between the protonated quinazoline and the receptor protein. None of the isoquinolines (2) proved to be effective antihypertensive agents in rats even when administered at relatively high doses (10 mg/kg). These results support the hypothesis that the antihypertensive activity of prazosin, doxazosin, and related derivatives derives solely from α_1-adrenoceptor blockade.

In an accompanying paper,[1] the synthesis and biological activities of a series of 2,4-diamino-6,7-dimethoxyquinoline derivatives are presented. Like their quinazoline counterparts,[2] most of these compounds are orally active antihypertensive agents in rats, and they also display high affinity and selectivity for α_1-adrenoceptors. These latter results are consistent with the fact that both 2,4-diaminoquinoline and -quinazoline derivatives undergo N-1 protonation at physiological pH to provide **1a,b**, key pharmacophores for α_1-adrenoceptor recognition.[3] This paper deals with a representative group of 1,3-diamino-6,7-dimethoxyisoquinoline derivatives (**2**), which are closely related, structurally, to both the quinolines and quinazolines reported previously. However, since N-1 protonation to provide a pharmacophore corresponding to **1a,b** is not feasible, series **2** provides a critical test for previous α_1-adrenoceptor modeling studies.[4]



1a, X = N
b, X = CH



2

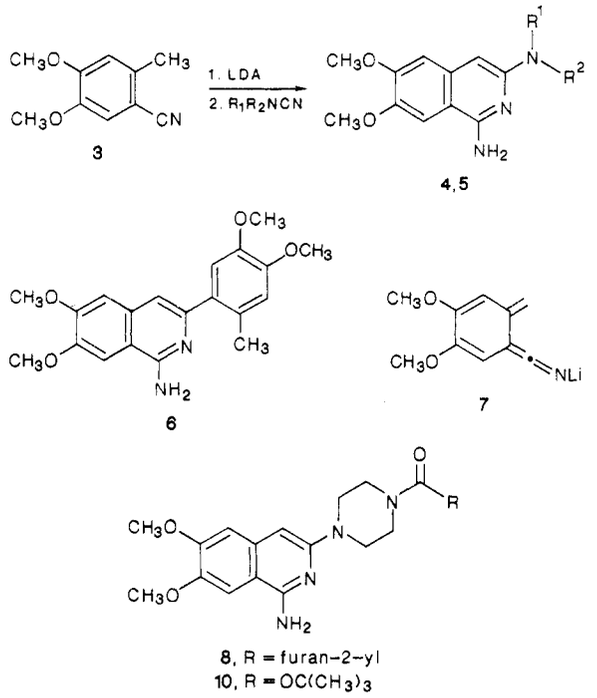
Chemistry

Treatment of 2-methyl-4,5-dimethoxybenzonitrile (**3**) with LDA at −70 °C followed by reaction of the blood-red solution with an appropriate cyanamide derivative allowed rapid acess to **4**, **5**, albeit in only a 20% yield[5] (Scheme I). Poor conversion to the 1,3-diaminoisoquinoline system appeared to result from competing formation of dimer **6**, and side reactions possibly derived from **7**.[6] Direct synthesis of **8** from **3** and 1-cyano-4-(furan-2-ylcarbonyl)-piperazine (**9**) was not successful due to competing deprotonation in the furan moiety. However, preparation of the protected derivative **10** proceeded satisfactorily, and acidic hydrolysis followed by acylation with furoyl chloride provided **8**. NMR analysis of **4**, **5**, and **8** indicated a characteristic singlet around 6.3 ppm (C_4-H) consistent with formation of an isoquinoline ring system.[7]

Results and Discussion

Structure–Activity Relationships (SAR) for in Vitro α_1-Adrenoceptor Affinity. The results in Table I show that the 1,3-diaminoisoquinoline derivatives **4**, **5** display no significant affinity ($K_i \gg 10^{-6}$ M), for α_1-adrenoceptors. Comparison of **4** with the corresponding quinoline[1] and quinazoline[2,4] analogues **11** and **12** clearly confirms the marked superiority of the latter heterocyclic systems as α_1-adrenoceptor ligands. In addition, **8** is approximately 1000-fold less potent than prazosin, which

Scheme I



3 → **4,5** (1. LDA; 2. R_1R_2NCN)

6

7

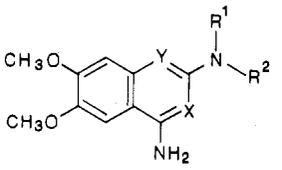
8, R = furan-2-yl
10, R = $OC(CH_3)_3$

† Pfizer Central Research Laboratories.

(1) Campbell, S. F.; Hardstone, J. D.; Palmer, M. J. *J. Med. Chem.* preceding paper in this issue.
(2) Campbell, S. F. *X-Ray Crystallography and Drug Action*; Horn, A. S., De Ranter, C. J., Eds.; Clarendon: Oxford, 1984; p 347.
(3) For example, see: Campbell, S. F.; Plews, R. M. *J. Med. Chem.* **1987**, *30*, 1794.
(4) Campbell, S. F. *Second SCI-RSC Medicinal Chemistry Symposium*; Emmett, J. C., Ed.; Royal Society of Chemistry; Letchworth, 1984; p 18.
(5) Van der Goot, H.; Nauta, W. Th. *Chim. Ther.* **1972**, *7*, 185.
(6) Evidence for the intermediacy of 7 will be presented separately.
(7) Liepa, A. J. *Aust. J. Chem.* **1982**, *35*, 1391.

0022-2623/88/1831-1036$01.50/0

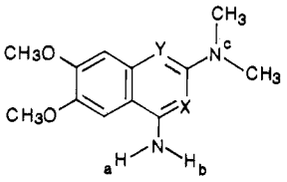
Table I. Binding, pK_a, and Antihypertensive Results for Isoquinoline, Quinoline, and Quinazoline Derivatives



no.	Y	X	R^1,R^2	α-receptor binding[a] affinity, K_i, nM		pK_a	% reduction in SHR (n = 5) blood pressure		dose, mg/kg, po
				α_1[b]	α_2[b]		1 h	4.5 h	
4	CH	N	$(CH_3)_2$	NA	NA	7.1 ± 0.09	12	10	10
5	CH	N	$(CH_2)_5$	NA	NA	NT[c]	15	11	10
8	CH	N	$(CH_2CH_2)_2$NCO-2-furyl	160 ± 29[d]	NT[c]	NT[c]	4	6	10
11[1]	N	CH	$(CH_3)_2$	11.37 ± 2.00	NA	9.3 ± 0.09	12	11	3
12[2]	N	N	$(CH_3)_2$	4.1 ± 0.62	NA	8.1 ± 0.08	NT	NT	
prazosin	N	N	$(CH_2CH_2)_2$NCO-2-furyl	0.19 ± 0.02	4830 ± 1280	6.8 ± 0.04	24	15	3

[a] Rat brain homogenate preparation, all results are the means of at least three experiments performed in triplicate. [b] NA indicates a $K_i \gg 10^{-6}$ M for displacement of [^{3}H]prazosin or [^{3}H]clonidine. [c] NT, not tested. [d] Approximate K_i due to nonsigmoidal binding curve.

Table II. Calculated Positive Charge Distribution in the Protonated Forms of **4**, **11**, and **12**[a]



no.	Y	X	O_6	O_7	(Y)-H	(X)-H	H_a, H_b	N_c
4	CH	$^+$NH	−0.23	−0.24	0.03	0.15	0.17, 0.17	−0.15
11	$^+$NH	CH	−0.24	−0.23	0.14	0.04	0.14, 0.14	−0.13
12	$^+$NH	N	−0.24	−0.23	0.14		0.15, 0.16	−0.13

[a] See Table I, CNDO/2 Mulliken population analysis, only selected centers shown. (Note different numbering systems for **4** and **11**, **12**).

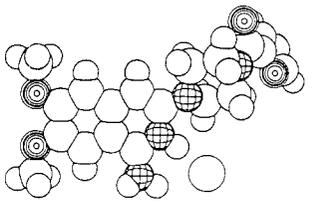


Figure 1. Spacefill representation of X-ray structure for **8**, hydrochloride salt.

corresponds to a reduction in binding energy of 4.0 kcal/mol. Except for prazosin, none of the other compounds tested in Table I showed any affinity for α_2-adrenoceptors.

The pK_a data in Table I show that while **11** and **12** are more basic than **4**,[8] the isoquinoline derivative should still undergo substantial protonation at physiological pH (99%, 81%, and 34%, respectively), and only a small spread in α_1-adrenoceptor affinities would be expected. However, protonation of **11** and **12** will occur on N-1 whereas for **4** only N-2 is available, as confirmed by X-ray analysis of the hydrochloride salt of **8** (Figure 1). Comparison of the calculated positive charge distribution on protonation of **4**, **11**, and **12** (Table II) shows marked similarities in overall electron densities, for example, on the dimethoxy, amino, and dimethylamino functions. However, the poor α_1-adrenoceptor activity of **4** suggests that these groups play only a secondary role in receptor interactions. In particular, an alternative receptor binding mode in which the primary amino moiety in **4**, **11**, and **12** could function as a bioisostere for the benzylic hydroxyl function of norepinephrine does not appear to be feasible. The major difference between **4**, **11**, and **12** is clearly the ring protonation site (Table II), and these SAR studies support previous proposals that N-1 protonation is a fundamental requirement for effective interaction of these heterocyclic nuclei with the α_1-adrenoceptor. Indeed, the K_i value for **8** (Table I) is only approximate, since nonsigmoidal binding curves were observed, suggesting a noncompetitive displacement of [^{3}H]prazosin. Thus, it appears that pharmacophores **1a**,**b** play a key role in specifically locking both the quinoline and quinazoline systems on to the α_1-adrenoceptor active site.

Extrapolation of the pK_a data in Table I suggests that the prazosin analogue **8** would not be efficiently protonated (<5%) at physiological pH, and the moderate α_1-adrenoceptor affinity observed probably derives from general hydrophobic interactions largely involving the 3-substituent (cf. **4** and **5**). The difference in potency between **8** and prazosin (binding energies 13.3 and 9.3 kcal/mol) suggests that charge-reinforced hydrogen bonding between the protonated species **1** and a carboxylic counterion[2-4] on the α_1-adrenoceptor contributes approximately 4.0 kcal/mol in binding energy. Interestingly, recent results from site-directed mutagenic modification of dihydrofolate reductase suggest a surprisingly low value of 1.8 kcal/mol for the contribution to binding free energy of salt bridge formation between a protonated pteridine system and an aspartate anion in the protein.[10] However, assignment of

(8) A reported[9] pK_a of 5.70 for 1,3-diaminoisoquinoline, determined by potentiometric titration, is lower than expected when compared to that of **4**, which was measured by spectrometry.

(9) Cox, J. M.; Elvidge, J. A.; Jones, D. E. H. *J. Chem. Soc.* **1964**, 1423.

(10) Howell, E. E.; Villafranca, J. E.; Warren, M. S.; Oatley, S. J.; Kraut, J. *Science (Washington, D.C.)* **1986**, *231*, 1123.

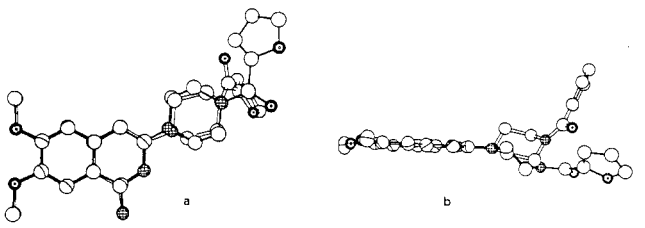


Figure 2. Computer-assisted superimposition of X-ray structures of **8** (hollow bonds) and prazosin[11] (solid bonds), face-on (a) and side-view (b) illustrated (hydrogen atoms omitted).

4.0 kcal/mol as the ionic contribution to binding between prazosin and the α_1-adrenoceptor assumes that the only important difference from **8** is the site of protonation and that other structural features are essentially identical. Computer-assisted superimposition of the X-ray structures of prazosin[11] and **8** confirms an exact spatial correspondence of the parent heterocyclic rings, but the piperazino moieties are displaced from one another (Figure 2). For prazosin, the exocyclic N-2 atom is sp^2 hybridized[12] (sum of bond angles 359°) whereas the corresponding center in **8** tends more toward sp^3 (sum of bond angles 343°) with the piperazine moiety twisted out of the isoquinoline plane (ca. 30°), presumably to minimize steric interactions with the C_4 and N-2 hydrogen atoms. In addition, different orientations of the furan-2-ylcarbonyl systems are also apparent (Figure 2), but molecular mechanics and PCILO molecular orbital calculations indicate only minor energy differences between the two conformations, and interconversion would be expected at room temperature.[13]

Rotation of the piperazine system in **8** through approximately 30° allows coplanar orientation with the isoquinoline ring and almost exact superimposition with prazosin (not shown), although with an energy penalty of approximately 1.0–1.6 kcal/mol.[14] Thus, if a coplanar arrangement of **8**, similar to prazosin, is required for interaction with the α_1-adrenoceptor, then salt-bridge formation between the N-1 protonated quinazoline and a carboxylate counterion on the protein can be estimated to contribute between 2.4 and 3.0 kcal/mol in binding energy.[17]

In conclusion, these results provide substantial support for previous α_1-adrenoceptor modeling studies and confirm the importance of pharmacophores **1a,b** for effective interaction of diaminoquinazoline and -quinoline systems at the receptor active site.[19]

SAR for in Vivo Antihypertensive Activity. As expected from the poor in vitro α_1-adrenoceptor activity, none of the isoquinolines in Table I proved to be effective antihypertensive agents in rats even at relatively high doses (10 mg/kg). Indeed, **8** was very poorly active and the weak effects observed with **4**, **5** are probably due to a nonspecific mechanism of action. These results provide further support for the hypothesis that the antihypertensive activity of prazosin, doxazosin, and related derivatives derives solely from α_1-adrenoceptor blockade.

Experimental Section

Chemistry. Melting points were determined in a Büchi apparatus in glass capillary tubes and are uncorrected. Spectroscopic data for all compounds were recorded on Perkin-Elmer 257 (IR), AEI MS12 or VG 7070F (MS), Perkin-Elmer R12B, Varian XL 100, and Nicolet (QE300 (NMR) instruments and were consistent with assigned structures. Where analyses are indicated only by symbols of the elements, results obtained were within ±0.4% of the theoretical values.

1-Amino-6,7-dimethoxy-3-piperidin-1-ylisoquinoline Hydrochloride (5). A solution of 2-methyl-4,5-dimethoxybenzonitrile[20] (0.50 g, 2.8 mmol) in THF (5 mL) was added dropwise to a stirred solution of LDA (3.1 mmol) and 1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone (0.40 g, 3.1 mmol) in dry THF (10 mL) at −70 °C under a nitrogen atmosphere. A blood-red color developed immediately, and after 0.1 h a solution of *N*-cyanopiperidine (0.46 g, 4.2 mmol) in THF (5 mL) was added dropwise. The mixture was allowed to attain room temperature and then stirred overnight. Water (5 mL) was then added to the cooled (10 °C) mixture followed by dichloromethane (20 mL), the organic layer was separated, and the aqueous phase was further extracted with dichloromethane. The combined extracts were washed with water, dried (Na_2SO_4), and then evaporated, and the residue was separated into two products by chromatography on silica gel (30 g), with dichloromethane/methanol (100:0 → 96:4) as eluant. The first product was dissolved in chloroform and treated with ethereal HCl, and the precipitate was collected and crystallized from 2-propanol/ether to give 1-amino-6,7-dimethoxy-3-piperidin-1-ylisoquinoline hydrochloride (0.12 g, 18%): mp 258–260 °C; MS, (M^+) 287; NMR (DMSO-d_6) δ 1.60 (6 H, br s), 3.38 (4 H, br s), 3.86 (3 H, s), 3.90 (3 H, s), 6.30 (1 H, s, exchanges with TFA-*d*), 7.13 (1 H, s), 7.67 (1 H, s), 8.46 (2 H, br s, exchanges with TFA-*d*). Anal. ($C_{16}H_{21}N_3O_2$·HCl) C, H, N.

The second product was dissolved in ethyl acetate/methanol and treated with ethereal hydrogen chloride, and then the precipitate was collected and recrystallized from ethanol/methanol to give 1-amino-6,7-dimethoxy-3-(2-methyl-4,5-dimethoxyphenyl)isoquinoline hydrochloride (**6**) (0.07 g, 16%): mp 292 °C dec; MS, (M^+) 354; NMR (DMSO-d_6) δ 2.30 (3 H, s), 3.79 (3 H, s), 3.82 (3 H, s), 3.95 (6 H, br s), 7.00 (1 H, s), 7.08 (1 H, s), 7.13 (1 H, s), 7.45 (1 H, s), 7.98 (1 H, s) 8.65 (2 H, br s, exchanges with D_2O). Anal. ($C_{20}H_{22}N_2O_4$·HCl) C, H, N.

1-Amino-6,7-dimethoxy-3-(dimethylamino)isoquinoline hydrochloride (4), mp 268–269 °C, was prepared similarly (19% yield): MS, (M^+) 247; NMR (DMSO-d_6) δ 3.03 (6 H, s) 3.83 (3 H, s), 3.89 (3 H, s), 6.13 (1 H, s, exchanges with TFA-*d*), 7.07 (1 H, s), 7.61 (1 H, s), 8.60 (2 H, br s, exchanges with TFA-*d*). Anal. ($C_{13}H_{17}N_3O_2$·HCl) C, H, N.

1-Amino-6,7-dimethoxy-3-[4-(furan-2-ylcarbonyl)-piperazin-1-yl]isoquinoline Hydrochloride $^1/_3$-Ethanolate (8). (a) A solution of 2-methyl-4,5-dimethoxybenzonitrile (0.76 g, 4.3 mmol) in dry THF (10 mL) was added dropwise to a stirred solution of LDA (4.7 mmol) and 1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone (0.60 g, 4.7 mmol) in THF (10 mL) at −65 °C under an atmosphere of nitrogen. After 0.10 h, a solution of 1-cyano-4-(*tert*-butoxycarbonyl)piperazine (1.35 g, 6.4 mmol)

(11) Bordner, J., unpublished results.
(12) Theoretical bond angle sums for nitrogen: sp^2, 360°; sp^3, 328.5°.
(13) Rotational barrier around the amide bond in **8** is calculated to be ca. 20 kcal/mol with no difference between the two minimum-energy conformers. The barrier to rotation for the furan moiety is 5.6 kcal/mol with the antiperiplanar arrangement for the carbonyl and furan oxygen atoms slightly preferred (1.25 kcal/mol) over the syn form.
(14) Rotational barrier (ΔG) around the isoquinoline–piperazine bond in **8** (free base) is calculated to be 6.75 kcal/mol (method of ref 16, but using HMO π bond orders) or 9.11 kcal/mol (empirical method[16]). The energy penalty for a rotation of θ° from the minimum is approximated by $\Delta G(1 - \cos \theta)$.
(15) Barbieri, G.; Benassi, R.; Grandi, R.; Pagnoni, U. M.; Taddei, F. *J. Chem. Soc., Perkin Trans. II* **1979**, 330.
(16) Allinger, N. L.; Sprague, J. T. *J. Am. Chem. Soc.* **1973**, *95*, 3893.
(17) Formation of a hydrogen bond between an uncharged side chain on an enzyme and a charged group on the substrate can contribute approximately 3.5–4.5 kcal/mol in binding energy.[18]
(18) Fersht, A. R.; Shi, J.-P.; Knill-Jones, J.; Lowe, D. M.; Wilkinson, A. J.; Blow, D. M.; Brick, P.; Carter, P.; Waye, M. M. Y.; Winter, G. *Nature (London)* **1985**, *314*, 235.
(19) Quinoline and quinazoline derivatives based on general structure **1** have been shown to be competitive antagonists of the α_1-mediated vasoconstrictor effects of norephinephrine.[1,3]
(20) Luff, B. D. W.; Perkin, W. H.; Robinson, R. *J. Chem. Soc.* **1910**, *97*, 1130.
(21) Baker, J. A. *Chem. Abstr.* **1971**, *75*, 140 886*s*.

in THF (10 mL) was added dropwise, and then the mixture was stirred at −65 °C for a further 0.75 h. The reaction mixture was allowed to attain room temperature, stirred for 1.75 h, and cooled (10 °C). Water (10 mL) and dichloromethane (20 mL) were then added. The organic layer was separated, the aqueous phase was further extracted with methylene chloride (2 × 20 mL), and the combined organic extracts were washed with water, dried (Na_2SO_4), and evaporated. The residue was purified by chromatography on silica gel (50 g) to give 1-amino-6,7-dimethoxy-3-[4-(*tert*-butoxycarbonyl)piperazin-1-yl]isoquinoline (**10**) (0.59 g, 21%), NMR.

(b) A solution of the product (0.58 g, 0.9 mmol) from (a) in ethyl acetate (20 mL) was stirred with HCl (3 mL, 3 N) at room temperature for 2.75 h. Ether (50 mL) was then added, the supernatant liquid was decanted, and the solid product was further triturated with ether. The crude residue was cooled (10 °C) and treated with chloroform (40 mL) and sodium hydroxide (20 mL, 5 N), and the organic layer was separated. The aqueous phase was further extracted with chloroform (3 × 20 mL), and the combined extracts were washed with brine, dried (Na_2SO_4), and evaporated to leave 1-amino-6,7-dimethoxy-3-piperazin-1-ylisoquinoline (0.38 g, 88%), NMR.

(c) A solution of furan-2-carbonyl chloride (0.10 g, 0.8 mmol) in chloroform (5 mL) was added dropwise to a stirred solution of the crude product (0.34 g, 0.8 mmol) from (b) in chloroform (15 mL) and triethylamine (0.11 g, 1.1 mmol) at 7 °C. After 0.75 h, sodium carbonate solution (5 mL, 10%) was added, the organic layer was separated, and the aqueous phase was further extracted with chloroform (2 × 20 mL). The combined organic extracts were washed with water, dried (Na_2SO_4), and evaporated, and then the residue was purified by chromatography on silica gel (25 g) with dichloromethane/methanol (100:0 → 96:4) as eluant. The product was dissolved in chloroform, treated with ethereal HCl, and then evaporated, and the residue was recrystallized from ethanol/methanol to give 1-amino-6,7-dimethoxy-3-[4-(furan-2-ylcarbonyl)piperazin-1-yl]isoquinoline hydrochloride $^1/_3$-ethanolate (0.19 g, 56%): mp 258–261 °C; MS, (M^+) 382; NMR (DMSO-d_6) δ 3.35 (4 H, br, s), 3.87 (10 H, br m), 6.38 (1 H, s, exchanges with TFA-*d*), 6.67 (1 H, m), 7.08 (1 H, m), 7.17 (1 H, s), 7.75 (1 H, s), 7.88 (1 H, s), 8.54 (2 H, br s, exchanges with TFA-*d*). Anal. ($C_{20}H_{22}N_4O_4$·HCl·0.33EtOH) H, N; C: calcd, 57.2; found, 56.6.

1-Cyano-4-(*tert*-butoxycarbonyl)piperazine. A solution of di-*tert*-butyl dicarbonate (3.24 g, 15 mmol) in THF (25 mL) was added dropwise to a stirred solution of 1-cyanopiperazine[21] (1.5 g, 13.5 mmol) in THF (25 mL) at 5–10 °C under an atmosphere of nitrogen. The reaction mixture was then allowed to attain room temperature and was stirred for a further 2 h. The mixture was evaporated, and the residue was dissolved in ethyl acetate, washed with citric acid solution and brine, dried (Na_2SO_4), and evaporated. The residue was purified by chromatography on silica gel (25 g), and the product was recrystalllized from ethyl acetate/hexane to give 1-cyano-4-(*tert*-butoxycarbonyl)piperazine (1.48 g, 52%): mp 101 °C; MS, (M^+) 211. Anal. ($C_{10}H_{17}N_3O_2$) C, H, N.

Biology. Experimental details for evaluation of α-adrenoceptor binding and antihypertensive activities have been provided previously.[1]

Acknowledgment. We gratefully thank Drs. V. A. Alabaster and P. M. Greengrass for biological results, D. J. Greenan for pK_a data, and V. A. Horne for valuable help with modeling studies.

Registry No. **3**, 58814-69-0; **4**, 113533-94-1; **4** (free base), 113533-95-2; **5**, 113533-96-3; **5** (free base), 113533-97-4; **6**, 113533-98-5; **6** (free base), 23023-37-2; **8**, 113533-99-6; **8** (free base), 113534-00-2; **9**, 40172-93-8; **10**, 113534-01-3; Me_2NCN, 1467-79-4; 2-furoyl chloride, 527-69-5; *N*-cyanopiperidine, 1530-87-6; 1-cyano-4-(*tert*-butoxycarbonyl)piperazine, 113534-02-4; 1-amino-6,7-dimethoxy-3-piperazin-1-ylisoquinoline, 113534-03-5; 1-cyanopiperazine, 34065-01-5.

Supplementary Material Available: X-ray data are available for 1-amino-6,7-dimethoxy-3-[4-(furan-2-ylcarbonyl)piperazin-1-yl]isoquinoline hydrochloride (**8**) (9 pages). Ordering information is given on any current masthead page.

Dopamine D-2 Receptor Imaging Radiopharmaceuticals: Synthesis, Radiolabeling, and in Vitro Binding of (*R*)-(+)- and (*S*)-(−)-3-Iodo-2-hydroxy-6-methoxy-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]benzamide

Hank F. Kung,* Ravindra Kasliwal, Sangren Pan, Mei-Ping Kung, Robert H. Mach, and Yu-Zhi Guo

Division of Nuclear Medicine, Department of Radiology, University of Pennsylvania, Philadelphia, Pennsylvania 19104.
Received November 5, 1987

In developing central nervous system (CNS) dopamine D-2 receptor imaging agents, enantiomers, *R*-(+) and *S*-(−) isomers, of 3-[^{125}I]iodo-2-hydroxy-6-methoxy-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]benzamide, [^{125}I]IBZM, were synthesized, and their in vitro binding characteristics were evaluated in rat striatum tissue preparation. The (*S*)-(−)-[^{125}I]IBZM showed high specific dopamine D-2 receptor binding (K_d = 0.43 nM, B_{max} = 0.48 pmol/mg of protein). Competition data of various ligands for IBZM binding displayed the following rank order of potency: spiperone > (*S*)-(−)-IBZM > (+)-butaclamol ≫ (*R*)-(+)-IBZM > (*S*)-(−)-BZM > dopamine > ketanserin > SCH23390 ≫ propanolol. The results indicate that [^{125}I]IBZM binds specifically to the dopamine D-2 receptor with stereospecificity. The [^{123}I]IBZM is potentially useful as an imaging agent for the investigation of dopamine D-2 receptors in humans.

A variety of substituted benzamide derivatives possessing antidopaminergic properties has been reported.[1-6] Of these sulpiride,[7,8] raclopride,[9] eticlopride,[10,11] iodoclebopride, and iodoazidoclebopride[12] show specific antagonistic activity, high affinity constants (K_d) in rat striatum tissue preparations (Table I), and relatively low nonspecific binding.

(1) Jenner, P.; Marsen, C. D. *Neuropharmacol.* **1981**, *20*, 1285.
(2) Florvall, L.; Ogren, S.-O. *J. Med. Chem.* **1982**, *25*, 1280.
(3) DePaulis, T.; Hall, H.; Ogren, S. O. *Eur. J. Med. Chem.* **1985**, *20*, 273.
(4) DePaulis, T.; Kumar, Y.; Johansson, L.; Ramsby, S.; et al. *J. Med. Chem.* **1986**, *29*, 61.
(5) DePaulis, T.; Kumar, Y.; Johansson, L.; et al. *J. Med. Chem.* **1985**, *28*, 1263.
(6) Seeman, P. *Pharmacol. Rev.* **1980**, *32*, 229.
(7) Martres, M. P.; Sales, N.; Bouthenet, M. L.; et al. *Eur. J. Pharmacol.* **1985**, *118*, 211.
(8) Holden-Dye, L.; Poat, J. A.; Senior, K.; et al. *Biochem. Pharmacol.* **1985**, *34*, 2905.
(9) Kohler, C.; Ogren, O.-S.; Gawell, L. *Biochem. Pharmacol.* **1985**, *34*, 2251.
(10) Kohler, C.; Hall, H.; Gawell, L. *Eur. J. Pharmacol.* **1986**, *120*, 217.
(11) Hakan, H.; Kohler, C.; Gawell, L. *Eur. J. Pharmacol.* **1985**, *111*, 191.
(12) Neumeyer, J. L.; Guan, J. M.; Niznik, M. B. *J. Med. Chem.* **1985**, *28*, 405.

0022-2623/88/1831-1039$01.50/0