was filtered to remove mercury salts, concentrated under vacuum, dissolved in water, and washed several times with $CHCl_3$. The aqueous solution was lyophilized to give 5.5 g of crude yellow solid. Most (5.0 g) of the crude product was reacted with fumaric acid in MeOH, and the resultant salt was precipitated from solution with ether. The salt was recrystallized from water to give 1.57 g of white crystalline solid (18%): mp 149–151 °C; IR (KBr) ν_{max} 3314, 2906, 1710, 1658, 1548, 1376, 1281, 1186, 1076 cm^{-1}; FAB-MS m/e 281 (M + 1); ^1H NMR (D_2O, 90 MHz) δ 3.4–3.9 (m, 6 H), 5.4–5.6 (m, 1 H), 6.6 (s, 1.2 H, 0.6 molar equiv of fumaric acid); ^{13}C NMR (DMSO-d_6, 15.0 MHz) δ 60.9, 70.0, 72.7, 77.6, 78.0, 80.9 (ca. 0.6 C, α anomer), 83.2 (ca. 0.4 C, β anomer), 134.1 ($CHCO_2H$), 160.5 (0.4 C), 161.8 (0.6 C), 166.4 (CO_2H), 186.1 (0.6 C), 187.7 (0.4 C). Anal. ($C_8H_{16}N_4O_5S \cdot 0.6C_4H_4O_4 \cdot 2.0H_2O$) C, H, N.

1-[2-[[[(Aminoiminomethyl)amino]iminomethyl]amino]-ethyl]-1-deoxy-D-arabinose Hydrochloride Hydrate (31). To a solution of nitrile **32**[13] (18.20 g, 0.041 mol) in THF at 0 °C under nitrogen was added 41.0 mL (0.041 mol) of 1 M BH_3/THF. The ice bath was then removed and the reaction was refluxed for 5.5 h. An additional 28.0 mL of 1 M BH_3/THF (0.028 mol) was then added, and the solution was refluxed for an additional 18 h. The excess BH_3 was carefully destroyed by adding ice. The THF was removed to give a white oil suspended in water. The solution was acidified with 100 mL of 0.1 N HCl and heated for 1.5 h. The resulting HCl salt was neutralized with 3.0 N NaOH and extracted into ether. The ether extracts were dried ($MgSO_4$), filtered, and concentrated to yield 15.9 g of **33**. This free base was redissolved in ether and then acidified with ether saturated with HCl to afford 17.0 g of viscous oil (86%): CI-MS m/e 448 (M + 1), 476 (M + 29). To 21.96 g (45.3 mmol) of this amine hydochloride in DMSO (15 mL) under nitrogen was added **5** (19.07 g), and the slurry was heated at 115 °C for 7 days. The reaction mixture was dissolved in $CHCl_3$, filtered, washed with saturated aqueous NaCl, dried ($MgSO_4$), filtered, and concentrated to a brown oil. This material was triturated with ether, to facilitate removal of residual DMSO. The crude product (25.8 g) was chromatographed on dry silica gel ($CHCl_3$/MeOH/HOAc, 89:7:4) to give 9.30 g (37%) of **34**, as a brown syrup: CI-MS m/e 538 (M + 1). To 8.55 g (15.0 mmol) of **34** in methylene chloride (50 mL) at 0 °C under nitrogen was added iodotrimethylsilane (17.5 mL, 0.123 mol) via a syringe. The reaction was allowed to stir at 0 °C. After 1.5 h, the reaction was quenched with water and washed with additional methylene chloride. The aqueous phase was treated with Amberlite CG-400 anion-exchange resin (chloride anion form), filtered, treated with decolorizing carbon, filtered, and lyophilized to give 3.56 g of slightly yellow solid. Since analysis of the FAB-MS and 90-MHz ^1H NMR spectrum revealed that ca. 25% of the product still retained a benzyl group on it, the reaction was completed by catalytic hydrogenolysis of the remaining benzyl group. Thus, 1.26 g of this material was dissolved in water (50 mL), treated with 2 g of 10% Pd/C, and shaken for 2 days under 50 psig hydrogen. The catalyst was filtered and the aqueous mixture was lyophilized to yield 650 mg of tacky, white solid, pure by TLC (33%): mp 36–111 °C (softening), 111–116 °C (foaming); IR (KBr) ν_{max} 3872, 3388, 2926, 2182, 2162, 1641, 1554, 1036 cm^{-1}; FAB-MS m/e 262 (M + 1), 284 (M + Na); ^1H NMR (D_2O, 90 MHz) δ 1.7 (m, 2 H), 3.2 (t, 2 H), 3.5 (m, 2 H), 3.8 (m, 3 H). Anal. ($C_9H_{19}N_5O_4 \cdot 1.9HCl \cdot 2.0H_2O$) C, H, Cl; N: calcd, 19.10; found, 17.40.

Glucose-Tolerance Test. Male Sprague–Dawley rats (200–275 g), obtained from Charles River Breeding Laboratories (Wilmington, MA), were maintained on a regular 12 h light/dark cycle with standard rodent chow and water provided ad libitum. Rats were deprived of food for 18 h prior to glucose tolerance testing. Groups of rats were orally dosed at approximately 9 a.m. with either vehicle or test drugs 60 min prior to an oral glucose load (1.0 g/kg). Separate groups of rats received vehicle without glucose challenge. Blood samples were obtained from the tail vein (tail cut method) just before (0 min) and 30 min following glucose administration. Blood glucose was determined by the glucose oxidase method (Autoflo, Boehringer-Mannheim) on whole blood immediately deproteinized with $Ba(OH)_2$ and $ZnSO_4$. Test compounds were prepared either as a 0.5% (w/v) methylcellulose (Fisher Scientific) suspension or as an aqueous solution with control rats receiving the same vehicle as drug-treated rats. The rats were dosed based on the mean body weight per treatment group and all doses were calculated on a salt-free basis. Data are presented as the mean ± SEM for n rats per treatment group. The incremental change in glucose (mg/dL) from the t = 0 min value, in response to the glucose load, was calculated for each rat and corrected by subtracting the glucose change in vehicle-treated rats which did not receive glucose. The mean change in glucose for each drug-treated group (ΔG_D) was statistically compared to that of vehicle-treated controls (ΔG_V) with ANOVA and Dunnett's t test with a p value of 0.05 or less taken as significant. Percent inhibition of the glucose increase by each drug was calculated as $100(\Delta G_V - \Delta G_D)/(\Delta G_V)$.

Acknowledgment. We appreciate helpful discussions with Drs. Timothy Kiorpes and Richard Mohrbacher during the course of this work. We thank Dr. G. Caldwell, J. Masucci, M. Mutter, and N. Senko for obtaining most of the spectral data reported herein.

Evaluation and Synthesis of Aminohydroxyisoxazoles and Pyrazoles as Potential Glycine Agonists

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Except for structurally similar small amino acids, such as alanine, β-alanine, and serine, compounds acting as glycine-receptor agonists are an unknown class of pharmacological agents. To investigate the potential of small, substituted heterocycles to act as glycine agonists, we have evaluated the similarities between glycine and a series of hydroxy- and amino-substituted pyrazoles and isoxazoles through complementary molecular modeling techniques. Using a "scorecard approach" to determine the overall similarity of projected agonist structures to glycine, we prioritized synthesis and subsequently prepared several novel derivatives. The biological activity of these compounds was compared to that of glycine by using a [^3H]strychnine-mediated glycine receptor binding assay. Despite the close similarity in the calculated parameters when compared to glycine, no significant receptor-binding activity was observed for the targeted analogues. These results illustrate the structurally exacting nature of the glycine receptor.

Glycine, the structurally least complex of the naturally occurring amino acids, is a major inhibitory neurotransmitter located primarily in the spinal cord.[2] Activation of the glycine receptor complex by its endogenous ligand

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0022-2623/89/1832-2116$01.50/0

opens the associated chloride ion channel. The resulting inward ion flux results in cell hyperpolarization and decreases the probability of neuronal firing.[3,4] Antagonism of glycine's action is a recognized property of strychnine and related alkaloids.[5,6] More recent reports have also detailed a similar electrophysiological action for THAZ,[7] iso-THAZ,[7] THIA,[7] iso-THAO,[8] iso-THIA,[8] iso-THPO,[8] 3-PYOL,[9] and 3-PIOL.[9] Other than a weak action by small amino acids,[10] there are no reports in the literature of compounds acting as glycine agonists. The identification of compounds which bind selectively to the glycine receptor site and manifest the same electrophysiological effects would be an important step in the elucidation of the structural requirements of a glycine agonist.

With the objective of developing new pharmacological agents acting at the glycine receptor, we began to investigate the synthesis and receptor binding properties of a series of organic compounds which were projected to mimic the structural characteristics of glycine. In addition to defining novel chemical structures which bind to the glycine receptor, the need to increase lipophilicity and hence CNS bioavailability was also an objective.

Extensive structure–activity (SAR) studies have focused on the structural requirements of γ-aminobutyric acid (GABA).[11,12] The success in developing novel GABAergic agents through systematic modification of muscimol encouraged us to investigate the potential of substituted 4-amino-5-alkyl-3-hydroxyisoxazoles and pyrazoles as glycine agonists. By using a heterocycle which contains an acidic OH functionality and a proximal basic amine, it was postulated that these groups would occupy similar regions in space when compared to the glycine molecule. The work reported in this paper represents our progress in the design, synthesis, and biochemical evaluation of novel glycine agonists based on the isoxazole and pyrazole ring systems.

Molecular Modeling. Calculated spatial, distributional, and electronic properties of glycine and a number of agonists proposed for synthesis have been compared. The goals were 2-fold: first, to determine if these analogues represented reasonable approximations of glycine in terms of these properties, and second, to assess the degree of similarity between the proposed agonists and glycine as an aid in the prioritization of synthesis. The plan was to concentrate synthetic effort on those analogues calculated to be the most similar to glycine.

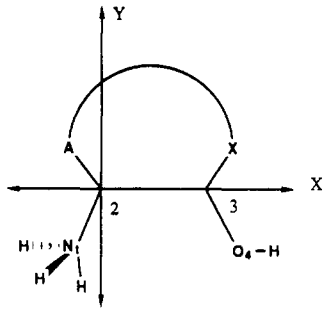

Figure 1. Orientation used for dipole moment and molecular orbital calculations. C_2 was placed at the origin, C_3 was placed along the positive X axis, and X was in the positive X–Y plane.

Within Tables I and II are given the structures and calculated properties of glycine and six agonists proposed for synthesis and/or biological testing at the outset of the study (starred structures). Note that the calculations were run on the uncharged and, where possible, the zwitterionic forms of the molecules. For the purposes of this analysis, ionization was assumed to occur on the hydroxy oxygen and protonation on the adjacent amine (or ring) nitrogen. Details regarding the programs and methods used to build and compare the structures can be found in the Experimental Section.

The reference conformation of glycine used for the comparisons corresponds to an energy minimum[13] and is stabilized by a strong hydrogen bond between the amine NH and the hydroxy oxygen. This conformer was consistent with the spatial orientation of the NH and OH groups in the less flexible synthetic targets. If this were a biologically significant conformation, then the binding affinities might be related to the properties we have calculated.

The results of the calculations are shown in Tables I and II. The "similarity scorecard" given in Table IV was an attempt to qualitatively grade the proposed agonists with regard to their similarity to glycine in the absence of more detailed information about the requirements of the glycine receptor. This was done as follows. For each calculated property listed in Tables I and II, a "similarity category" was defined (see Table III). For each category, values calculated for the various analogues were subtracted from those calculated for glycine, providing (after taking the absolute value of the result) an estimate of similarity. Thus, the smaller the difference, the more similar the analogue was to glycine for that property. For example, the difference between the calculated log P of isoxazole **2** (un-ionized form) and glycine was 3.58 (Table I, $|0.27 - (-3.31)|$), which placed **2** in similarity category 3, by using the assignments shown in Table III. Cutoffs between similarity categories were chosen to provide an even distribution of compounds across categories. Properties such as dipole moment that contained more variability among the analogues studied were spread out into more categories; fewer categories were used for properties with relatively reduced variance.

Charge distribution across the four atoms in common to glycine and the proposed agonists (Figure 1, N_1–O_4) was treated as one category; absolute values of the differences between charges on individual atoms were summed and

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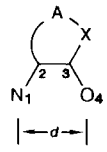
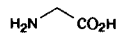
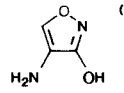
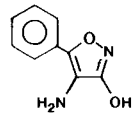
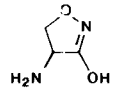
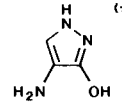
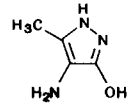
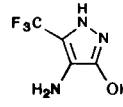
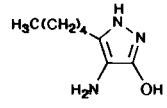
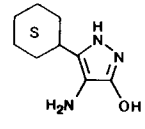
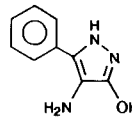
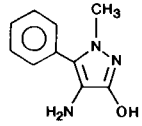
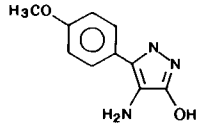
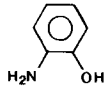
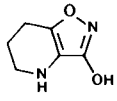
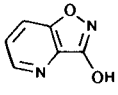
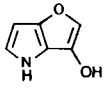
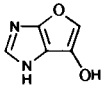
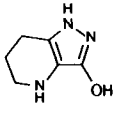
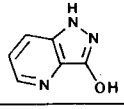
Table I. Comparison of Calculated Properties of Glycine and Proposed Agonists (Un-ionized Species)

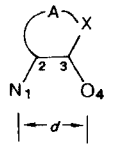


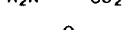
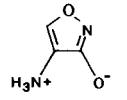
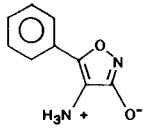
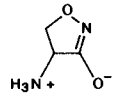
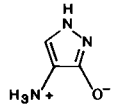
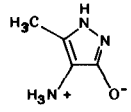
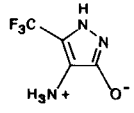
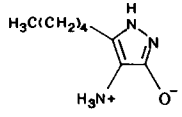
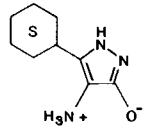
				molecular orbitals																		
												occupied					unoccupied					
				atomic charges[c]				dipole moment[d]				HOMO[e]	distribution[f]				LUMO[e]	distribution[g]				solvent (H_2O)[h]
no.[m]	structure	log p[a]	d[b]	N_1	C_2	C_3	O_4	X	Y	Z	total	energy	N_1	C_2	C_3	O_4	energy	N_1	C_2	C_3	O_4	energy
1		−3.31	2.71	−0.19	0.03	0.39	−0.26	0.54	−1.16	−1.56	2.02	−0.49	0.26[i]	−0.22[i]	0.26[i]	−0.39[i]	0.16	0.01	0.21	0.68	−0.22	−4.8
2*	 (*)	0.27	2.89	−0.17	0.01	0.29	−0.25	−0.48	−1.73	−1.50	2.34	−0.42	−0.15[i]	0.08[i]	0.39[i]	−0.48[i]	0.13	0.00	0.30	0.41	−0.14	−7.3
3		2.37	2.81	−0.16	0.00	0.28	−0.25	−0.59	−1.64	−1.38	2.22	−0.39	−0.21[i]	0.20[i]	0.35[i]	−0.44[i]	0.09	−0.02	0.30	0.23	−0.09	−4.1
4		*k*	2.98	−0.19	0.06	0.27	−0.25	−0.49	−0.81	0.78	1.23	−0.45	0.10	−0.13	0.38	−0.35	0.17	0.08	0.16	0.66	−0.20	−4.6
5*	 (*)	−1.23	2.86	−0.17	0.01	0.24	−0.25	−0.05	1.76	−1.42	2.26	−0.39	0.03[i]	−0.01[i]	0.40[i]	−0.42[i]	0.17	0.02	0.28	0.33	−0.10	−5.3
6		−0.58	2.86	−0.17	−0.01	0.24	−0.25	−0.29	1.77	−1.41	2.28	−0.38	−0.07[i]	0.06[i]	0.42[i]	−0.42[i]	0.16	0.01	0.29	0.29	−0.09	−2.6
7		0.55	2.86	−0.18	0.05	0.23	−0.25	2.28	0.59	−1.48	2.78	−0.42	0.03[i]	−0.02[i]	0.39[i]	−0.42[i]	0.12	0.00	0.34	0.26	−0.09	−5.7
8		1.53	2.86	−0.17	−0.01	0.24	−0.25	−0.30	−1.75	−1.42	2.28	−0.38	−0.08[i]	0.05[i]	0.42[i]	−0.42[i]	0.16	0.01	0.29	0.28	−0.09	−1.1
9		1.54	2.84	−0.17	−0.01	0.24	−0.25	−0.39	1.78	−1.40	2.30	−0.38	−0.09[i]	0.07[i]	0.42[i]	−0.42[i]	0.17	0.01	0.29	0.29	−0.09	−1.3

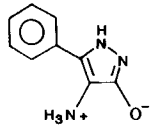
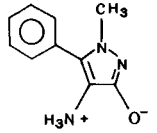
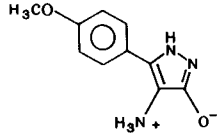
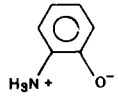
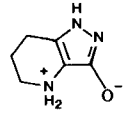
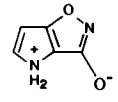
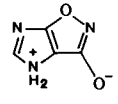
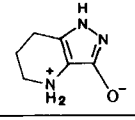
10		0.86	2.82	−0.17	0.00	0.24	−0.25	0.10	1.61	−1.32	2.08	−0.38	−0.15[i]	0.12[i]	0.41[i]	−0.39[i]	0.11	−0.01[l]	0.27[l]	0.13[l]	−0.05[l]	−3.1
11		2.60	2.82	−0.17	−0.01	0.24	−0.25	0.10	1.62	−1.27	2.06	−0.37	−0.05[i]	0.04[i]	0.38[i]	−0.35[i]	0.11	0.00[l]	0.26[l]	0.12[l]	−0.04[l]	−1.9
12		0.85	2.82	−0.17	0.00	0.24	−0.25	0.44	3.08	−1.34	3.39	−0.36	−0.22[i]	0.21[i]	0.38[i]	−0.36[i]	0.12	−0.01[l]	0.27[l]	0.13[l]	−0.05[l]	−3.9
13*		0.65	2.78	−0.20	0.09	0.13	−0.26	1.81	1.03	−1.60	2.63	−0.42	0.21[i]	−0.09[i]	0.26[i]	−0.31[i]	0.14	−0.02	0.40	0.17	−0.04	−2.8
14		1.29	2.89	−0.18	0.02	0.28	−0.25	−1.99	−2.12	−0.11	2.91	−0.37	−0.23[i]	0.00[i]	0.37[i]	−0.42[i]	0.14	−0.11	0.29	0.43	−0.14	−2.8
15*		1.00	3.14	−0.11	0.00	0.27	−0.24	−1.99	−2.12	−0.11	2.91	−0.46	−0.34	0.20	0.24	−0.26	0.09	−0.51	0.35	0.32	−0.11	−4.8
16*		1.07	3.14	−0.11	0.02	0.23	−0.24	−2.24	−3.87	0.03	4.47	−0.40	−0.54[i]	0.19[i]	0.25[i]	−0.26[i]	0.13	−0.34	0.23	0.42	−0.14	−3.5
17*		0.33	3.15	−0.05	−0.08	0.31	−0.26	−1.54	−5.50	0.04	5.71	−0.40	−0.49[i]	0.17[i]	0.29[i]	−0.32[i]	0.13	−0.35	0.22	0.42	−0.14	−6.2
18		−0.21	2.87	−0.19	0.03	0.23	−0.25	−1.36	1.21	0.08	1.82	−0.34	−0.08[i]	0.00[i]	0.41[i]	−0.40[i]	0.17	−0.10	0.28	0.34	−0.11	−1.7
19		0.60	3.14	−0.11	0.02	0.23	−0.24	−0.88	3.27	0.02	3.38	−0.40	−0.29	0.15	0.38	−0.32	0.10	−0.52	0.33	0.27	−0.10	−3.5

[a] Calculated using the CLOGP program[31] (version 3.33). [b] Distance, in angstroms, between N_1 and O_4. [c] In fractions of an electron, calculated with the CNDO/2 program within CHEMLAB-II[28] (version 9.0). [d] In debyes. Given as X, Y, and Z components and their vector sum. Calculated by using CNDO/2 charges. [e] In hartrees/mole, calculated by using CNDO/2. [f] Probability density for a comparable occupied $p_z(\pi)$ molecular orbital (the HOMO unless otherwise noted). [g] Probability density of the LUMO. In all cases, this was $p_z(\pi)$ molecular orbital. [h] In kilocalories/mole, calculated with the SOLVENT and CALCULATE options (all defaults taken) within the INITENRGY module of CHEMLAB-II. [i] The second highest HOMO (nHOMO). [j] The third highest HOMO (nnHOMO). [k] Reliable calculation not possible due to a missing fragment value. [l] Calculations show significant LUMO (and nLUMO) π molecular orbital densities reside on the carbons of the 5-phenyl ring. [m] An asterisk denotes analogues proposed for synthesis and/or biological testing at the outset of the study.

Table II. Comparison of Calculated Properties of Glycine and Proposed Agonists (Zwitterions)[a]



			HOMO									LUMO					
			atomic charges[c]					distribution					distribution[e]				solvent (H_2O)[f]
no.[j]	structure	d[b]	N_1	C_2	C_3	O_4	energy[d]	N_1	C_2	C_3	O_4	energy[d]	N_1	C_2	C_3	O_4	energy
1		2.37	0.00	−0.01	0.34	−0.56	−0.34	0.00	−0.01	0.03	−0.77	0.09					−65.9
2*		2.65	0.08	−0.12	0.29	−0.56	−0.32	−0.01	0.16	0.04	−0.77	0.09					−58.6
3		2.63	0.08	−0.14	0.34	−0.54	−0.31	−0.01	0.16	0.04	−0.70	0.08	0.16[g]	0.30[g]	0.05[g]	−0.07[g]	−38.5
4		2.57	−0.02	0.06	0.30	−0.51	−0.32	0.03	0.01	0.08	−0.62	0.09					−50.9
5*		2.68	0.08	−0.14	0.29	−0.58	−0.29	−0.01	0.26	0.10	−0.75	0.11					−55.1
6		2.68	0.07	−0.17	0.33	−0.56	−0.29	−0.01	0.27	0.11	−0.68	0.12					−41.2
7		2.68	0.05	−0.12	0.33	−0.55	−0.32	−0.01	0.31	0.10	−0.70	0.10					−46.9
8		2.69	0.07	−0.18	0.33	−0.56	−0.29	−0.01	0.28	0.11	−0.68	0.12					−38.4
9		2.66	0.07	−0.18	0.33	−0.56	−0.29	−0.01	0.27	0.11	−0.68	0.13					−28.8

10		2.65	0.09	−0.17	0.33	−0.56	−0.28	−0.01	0.26	0.10	−0.68	0.09	0.13[h]	0.25[h]	0.00[h]	−0.01[h]	−35.6
11		2.64	0.07	−0.17	0.33	−0.56	−0.29	−0.01	0.29	0.12	−0.66	0.10	0.14[i]	0.24[i]	0.01[i]	−0.02[i]	−32.1
12		2.65	0.09	−0.17	0.33	−0.56	−0.28	−0.01	0.25	0.10	−0.67	0.10	0.15[h]	0.25[h]	0.00[h]	−0.01[h]	−36.8
13*		2.62	0.04	−0.11	0.23	−0.53	−0.31	−0.02	0.39	0.07	−0.70	0.12					−45.2
14		2.74	0.11	−0.15	0.34	−0.54	−0.31	0.00	0.16	0.04	−0.69	0.13					−35.3
16*		3.10	0.09	−0.14	0.35	−0.52	−0.32	−0.01	0.15	0.03	−0.68	0.02	0.15[g]	0.32[g]	0.04[g]	−0.07[g]	−51.4
17*		2.98	0.07	−0.14	0.34	−0.52	−0.32	−0.01	0.15	0.03	−0.69	0.01	0.14[g]	0.33[g]	0.04[g]	−0.08[g]	−53.4
18		2.74	0.11	−0.14	0.28	−0.58	−0.28	0.00	0.24	0.10	−0.75	0.11					−32.7

[a] Reliable calculations on compounds 15 and 19 were not possible due to limitations in software (atom types for a protonated pyridyl nitrogen were not available). Also, reliable log P calculations were not possible due to missing fragment values for an oxy anion and a protonated amine. [b] Distance, in angstroms, between N_1 and O_4. [c] In fractions of an electron, calculated with the CNDO/2 program within CHEMLAB-II[28] (version 9.0). [d] In hartrees/mole, calculated with CNDO/2. [e] The probability density for the LUMO on those compounds with no entries lies entirely on the protonated nitrogen and attached hydrogens in σ molecular orbitals. [f] In kilocalories/mole, calculated using the SOLVENT and CALCULATE options (all defaults taken) within the INITENRGY module of CHEMLAB-II. [g] The nLUMO contains the σ molecular orbital localized on the protonated nitrogen. [h] The nLUMO contains the σ molecular orbital localized on the protonated nitrogen (the nLUMO contains a π molecular orbital localized on the phenyl ring carbons). [i] The nLUMO contains a combination of the σ molecular orbital localized phenyl ring carbons. [j] An asterisk denotes analogues proposed for synthesis and/or biological testing at the outset of the study.

Table III. Values Used in the Assignment of Similarity Categories

property	similarity category[a] 5	4	3	2	1	0
log P[b]	<2.2	2.2–3.2	3.2–4.2	4.2–5.2	>5.2	
d, N_1 to O_4	<0.35	0.35–0.40	0.40–0.50	0.50–0.60	>6.00	
charge distribution[c]	<0.35	0.35–0.45	0.45–0.50	0.50–0.60	>0.60	
dipole moment[d]	<2	2–3	3–4	4–5	5–6	>6
HOMO energy	≤0.06	0.07–0.12	0.12–0.19	0.19–0.24	>0.24	
distribution of comparable $P_z(\pi)_c$ occupied orbital[c]	<0.8	0.8–1.0	1.0–1.2	1.2–1.4	>1.4	
LUMO energy	≤0.03	0.04–0.06	>0.06			
distribution of LUMO[b,e]	<0.5	0.5–0.7	0.7–0.9	0.9–1.1	>1.1	
solvent energy, un-ionized[f]	<1	1–2	2–3	>3		
solvent energy, zwitterion[f]	<10	10–20	20–30	>30		

[a] Unless otherwise noted, the numbers given are sums of the absolute values of differences between each analogue and glycine, both in the un-ionized and zwitterionic forms. [b] For un-ionized forms only. [c] Totaled for atoms N_1, C_2, C_3, and O_4. [d] Totaled for the X, Y, and Z components and overall dipole moment. [e] For un-ionized forms only. Since the LUMO of most of the compounds (including glycine) in their zwitterionic forms was localized entirely on N_1 and the attached hydrogens in a σ molecular orbital, the differences in $P_z(\pi)$ molecular orbitals was insignificant. The similarity rankings of those compounds whose LUMOs in the zwitterionic form were not localized on N_1 were reduced by one. [f] These rankings were averaged to give the result which appears in Table IV.

Table IV. Scorecard of Qualitative Similarity[a] to Glycine

no.	log P	d	charge distrbn	dipole moment	HOMO energy	distrbn, occupied orbital	LUMO energy	LUMO distrbn	solvent energy	total
2	3	3	4	5	4	3	5	5	4	36
3	1	4	4	5	3	2	3	3	4	29
4	3	3	5	2	5	5	5	5	5	38
5	5	3	3	3	3	4	5	4	5	35
6	4	3	3	2	3	2	4	4	3	28
7	3	3	4	2	4	3	4	4	4	31
8	2	3	3	2	3	2	5	4	2	26
9	2	3	3	2	3	2	4	4	2	25
10	3	4	3	3	3	2	4	2	3	27
11	1	4	3	3	3	2	4	2	2	24
12	3	4	3	0	3	1	4	2	4	24
13	3	5	1	2	4	4	4	2	3	28
14	2	2	4	1	3	3	4	4	3	26
15	2	1	2	1	4	1	3	1	5	20
16	2	1	2	0	4	1	3	2	4	19
17	3	1	2	0	4	1	3	2	4	20
18	4	2	3	0	2	3	5	4	2	25
19	3	1	2	0	3	1	3	1	4	18

[a] Most similar = 5; least similar = 0. A breakdown and description of the similarity categories for each property are given in Table III.

the result were then used to categorize each analogue. Distribution of the LUMO and a comparable $P_z(\pi)$ occupied molecular orbital were treated similarly; differences in probability densities on individual atoms were summed before categorization.

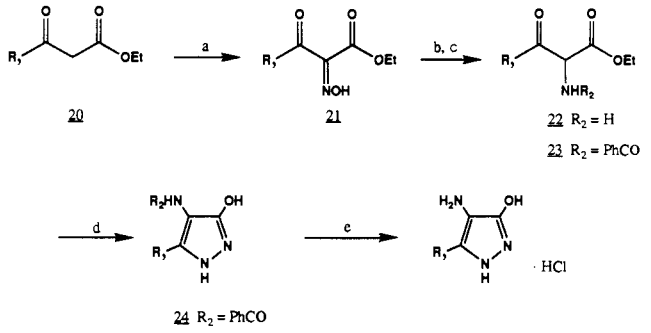
In the absence of knowledge about the ionization state of glycine, when bound to its receptor, as well as that of the various analogues, calculations were run on both the un-ionized and, where possible, the zwitterionic forms of all the structures. For the N_1 to O_4 distance, charge distribution, and HOMO and LUMO energies and distributions, the results from the calculations on the two forms were combined prior to categorization. Due to the expected large differences in calculated solvent energy between the uncharged and zwitterionic forms, separate similarity categories were maintained; the rankings were then averaged to give the result reported under "solvent energy" in Table IV.

The overall degree of similarity (Table IV, "total") was taken as the sum of the similarity rankings for each property, weighting all properties equally. These results were used in the prioritization of synthesis; vide infra.

Chemistry

The 5-substituted-4-amino-3-hydroxypyrazoles were synthesized as shown in Scheme I (the physical data for all the compounds synthesized in this study are given in Table V). For the majority of the desired compounds, the requisite 2-amino-β-keto esters were obtained from the

Scheme I[a]



[a] (a) $NaNO_2$, Ac_2O; (b) H_2, 10% Pd/C, EtOH/HCl; (c) PhCOCl, K_2CO_3/H_2O; (d) NH_2NH_2, EtOH; (e) 6 N HCl, reflux.

appropriately substituted β-keto ester[14] via oxime formation[15] and reduction.[16] Protection as benzamide derivatives, pyrazole formation, and deprotection by refluxing in 6 N HCl yielded the monohydrochloride salts of the desired pyrazoles.

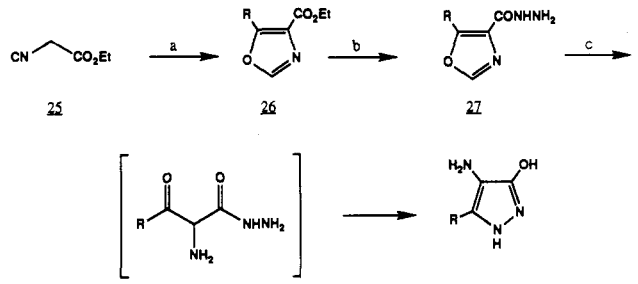
(14) For the general method used to synthesize the β-keto esters see: Wierenga, W.; Skulnick, H. I. *J. Org. Chem.* **1979**, *44*, 310.
(15) The 2-hydroxyimino β-keto esters were prepared by using the general procedure given in *J. Am. Chem. Soc.* **1938**, *60*, 1328.
(16) For the procedure used to reduce the 2-hydroxyimino β-keto esters see: Conway, T. T.; Belleau, B. R.; Doyle, T. W.; Luh, B. Y. British Patent 1 538 240, 1979.

Table V. Data for Substituted 3-Hydroxy-4-aminoisoxazoles and Pyrazoles

compd	synthetic route	mp °C	% yield	purfcn solv	formula	anal.[a]
3·HBr	C	192–194 dec	61	MeOH/ether	$C_4H_8N_2O_2$·HBr	C, H, N
5·HCl	A	185–186 dec	88	hot MeOH/ether	$C_3H_5N_3O$·HCl	C, H, N
6·HCl	A	200–203 dec	27	MeOH/ether	$C_4H_7N_3O$·HCl	C, H, N
7·HCl	B	194–195	33	hot EtOAc	$C_4H_4F_3N_3O$·HCl	C, H, N
8·HCl	A	215–216	25	hot CH_3OH	$C_8H_{15}N_3O$·HCl	C, H, N
9·HCl	A	251–253 dec	13	hot 6 N HCl	$C_9H_{15}N_3O$·HCl	C, H, N
10·HCl	A, B	222–224	67	hot EtOH/ether	$C_9H_9N_3O$·HCl	C, H, N
11·HCl	B	240–242 dec	90	hot EtOH/ether	$C_{10}H_9N_3O$·HCl	C, H, N[b]
12·HCl	B	218–219 dec	55	MeOH/ether	$C_{10}H_{11}O_2N_3$·HCl	C, H, N
14·TFA		139–140	65	TFA/ether	$C_8H_8N_2O_2$·$C_2HF_3O_2$	C, H, N
15		211–213	58	hot H_2O	$C_8H_4N_2O_2$	C, H, N
18·HCl		265–267	70	MeOH/ether	$C_6H_9N_3O$·HCl	C, H, N
19		248–251 (lit.[39] mp 247–248 °C)	20	hot H_2O	$C_6H_5N_3$	C, H, N

[a] Analytical results for C, H, and N are within ±0.4% of theoretical values except where noted. [b] C: calcd, 53.22; found, 52.50.

Scheme II[a]

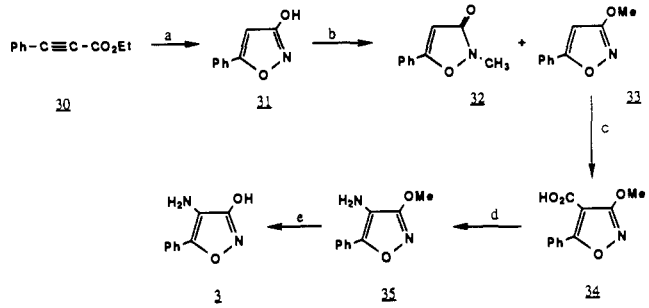


25 26 27 28

10 R = Ph
7 R = CF_3

[a] (a) RCOCl, Et_3N/THF; (b) NH_2NH_2, EtOH; (c) 3 N HCl, reflux.

Scheme III[a]

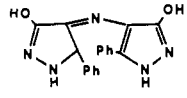


30 31 32 33 34 35 3

[a] (a) NH_2OH·HCl, NaOH/H_2O; (b) CH_2N_2, MeOH; (c) (1) *n*-BuLi/THF, −78 °C, (2) CO_2, (3) H_3O^+; (d) (1) DPPA, Et_3N, (2) $TMS(CH_2)_2OH$, (3) *n*-Bu_4NF, THF; (e) 31% HBr/HOAc.

While the above method of preparation could be used for most of the targeted compounds, the 5-phenyl and 5-(trifluoromethyl)-4-amino-3-hydroxypyrazoles presented special problems. Hydrogenation of the 2-oxime of ethyl 3-phenyl-3-oxopropionate resulted in the reduction of the β-keto ester to the corresponding benzylic alcohol. When 2-(benzoylamino)-4,4,4-trifluoro-3-oxobutanoic acid ethyl ester (**23**, R_1 = CF_3) was treated with anhydrous hydrazine, the molecule underwent a retro-Aldol reaction yielding trifluoroacetic acid hydrazide and *N*-benzoylglycine ethyl ester. To circumvent these problems, a novel method based on the procedure of Matsumoto[17] was used to synthesize the desired 5-(trifluoromethyl)-4-amino-3-hydroxypyrazole (see Scheme II). This procedure saves two steps over the method shown in Scheme I since the intermediates need not be protected as their benzamide derivatives to prevent unwanted dimerization. Although only the 5-phenyl and 5-trifluoromethyl analogues were prepared using this method, it appears to be a general method for preparation of 5-substituted-4-amino-3-hydroxypyrazoles.

Note that attempts to isolate the free bases of the 5-substituted-4-amino-3-hydroxypyrazoles by neutralization of the acid salts gave deeply colored solids. Extensive spectral analysis of the purified products established these compounds to be structurally related to **29**. The mechanism for dimerization is unknown. Similar compounds have been reported in the literature.[18]



29

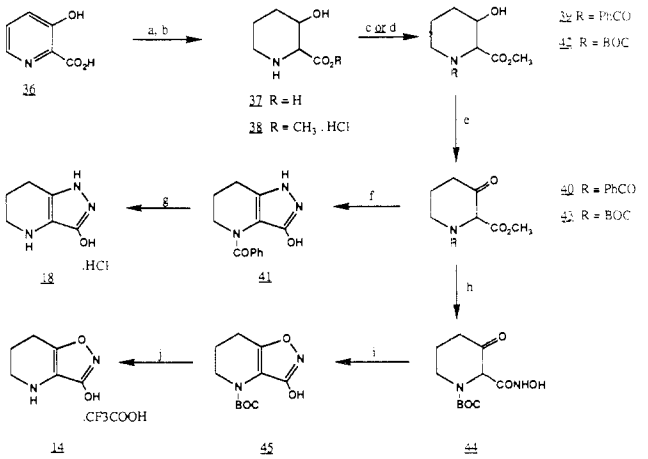
The desired unsubstituted isoxazole **2** could not be obtained by a number of synthetic routes. The 5-phenyl analogue **3** was, however, synthesized by the method shown in Scheme III. The 1:1 mixture of O- and N-methylated isomers (**32** and **33**) were separated by chromatography on silica gel. The O-methylated compound (**33**) was converted to the 4-carboxylic acid derivative by metalation at the 4-position followed by quenching with crushed dry ice.[19] The 4-amino group was introduced by a Curtius rearrangement following the method of Poutler and Capson.[20] Compound **3** proved to be more stable to treatment with base than its pyrazole analogue (**10**) as no decomposition was detected after prolonged treatment with aqueous sodium hydroxide.

The bicyclic isoxazoles and pyrazoles (**14**, **15**, **18**, and **19**) were synthesized by using different techniques appropriate for each analogue. The fused heterocyclic analogues **16** and **17** could not, however, be prepared. Scheme IV shows the synthesis of the saturated analogues **14** and **18**.

Methyl *cis*-3-hydroxypiperidine-2-carboxylate hydrochloride (**38**), common to both compounds, was obtained by hydrogenation of 3-hydroxy-2-pyridinecarboxylic acid over rhodium on carbon followed by Fisher esterification. The route to **14** and **18** then diverged. Compound **18** was prepared by protection of **38** as its benzamide (**39**). Swern oxidation of **39** gave β-keto ester **40** which when reacted with hydrazine monohydrate gave **41**. Refluxing **41** in 6 N hydrochloric acid gave **18** as its hydrochloride salt.

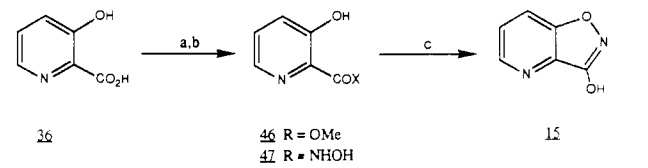
(17) Suski, M.; Iwasaki, T.; Mitoshi, M.; Okumura, K.; Matsumoto, K. *J. Org. Chem.* **1973**, *38*, 3571.
(18) Udea, T.; Oda, N.; Ito, I. *Chem. Pharm. Bull.* **1980**, *28*, 2144.
(19) Micetich, R. C.; Chin, C. G. *Can. J. Chem.* **1970**, *48*, 1371.
(20) Capson, T. L.; Poulter, C. D. *Tetrahedron Lett.* **1984**, 3515.

Scheme IV[a]



[a] (a) Rh/C, H_2, H_2O/NH_3; (b) HCl/MeOH, reflux; (c) PhCOCl, K_2CO_3/H_2O; (d) t-BOC_2O, $CHCl_3$, $NaHCO_3/NaCl/H_2O$; (e) $(COCl)_2$, CH_2Cl_2/Et_3N, DMSO, −60 °C; (f) $NH_2NH_2 \cdot H_2O$, EtOH, reflux; (g) 6 N HCl, reflux; (h) (1) EtONa/EtOH, (2) NH2OH/EtOH, (3) HCl/EtOH; (i) (1) $SOCl_2$/THF, pyridine at −70 °C, (2) Et_3N; (j) CF_3CO_2H.

Scheme V[a]

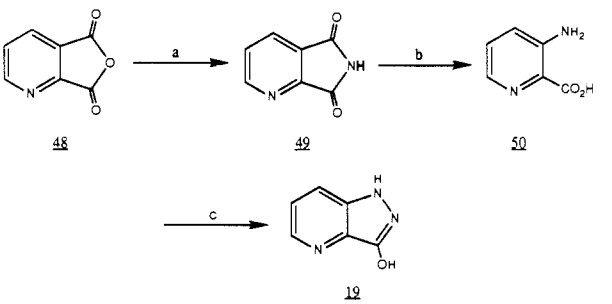


[a] (a) CH_2N_2/THF; (b) $NH_2OH \cdot HCl$, $EtOH/H_2O/NaOH$; (c) (1) $SOCl_2$/THF/Et_3N, −78 °C, (2) H_2O/HCl.

Conversion of **40** to the corresponding hydroxamic acid by treatment with hydroxylamine followed by ring closure with thionyl chloride/pyridine[21] was successful, giving the benzoyl-protected derivative of **14**. None of the deprotection strategies examined were successful, resulting in either recovered or decomposed starting material. To circumvent this problem, **38** was protected as its *tert*-butyloxycarbonyl derivative (**42**), converted to β-keto ester **43**, then reacted with hydroxylamine to give fused isoxazole **45**,[22] which when treated with trifluoroacetic acid gave **14** as its trifluoroacetate salt.

The fused aromatic analogues **15** and **19** were synthesized by different methods. The route to **15** is shown in Scheme V. β-Hydroxy acid **36** was successfully esterified with diazomethane after many other attempts gave consistently low yields. Conversion to **47** was accomplished by treatment of **46** with hydroxylamine hydrochloride in ethanol in the presence of sodium hydroxide. Reaction of **47** with thionyl chloride/triethylamine in tetrahydrofuran gave fused isoxazole **15**. As the reaction with thionyl chloride can proceed via the corresponding isocyanate, potential Lossen rearrangement would give the isomeric oxazolo[4,5-*b*]pyridin-2(3*H*)-one as the undesired product. To prove that rearrangement had not occurred, oxazolo-[4,5-*b*]pyridin-2(3*H*)-one was synthesized by an independent route.[23] Although these two compounds had the same melting points, they were shown to be different by spectral analysis and chromatographic comparison.

Scheme VI[a]



[a] (a) CH_3CONH_2, Ac_2O, heat; (b) (1) $NaOCl/H_2O$, (2) $Cu(OAc)_2$, (3) H_2S; (c) (1) $NaNO_2$, HCl/H_2O, 5 °C, (2) $NaSO_3/H_2O$, (3) HCl, heat.

Compound **19** was synthesized in an efficient manner as shown in Scheme VI. Hoffman rearrangement on phthalimide (**49**), isolation of the resulting amino acid as its copper complex, and treatment with hydrogen sulfide gave **50**.[24] Diazotization followed by reduction and acidification gave pyrazole **19**.

Results

Beside the obvious differences in overall size, calculations show that the analogues differ the most from glycine in their lipophilicity and X and Y components of the dipole moment when in the un-ionized forms, N_1 to O_4 distance (Figure 1) and energy stabilization due to solvation when in the zwitterionic forms, and HOMO energy and probability density of a comparable occupied π molecular orbital on N_1 and C_2 in both forms. The N_1 to O_4 distance and energy stabilization due to solvation in the un-ionized forms and charge distribution, occupied π molecular orbital distribution on C_3 and O_4, and LUMO energy and distribution in either form were reasonably similar between the analogues and glycine. The larger variation of N_1 to O_4 distance between the zwitterionic forms of glycine and the analogues was not surprising. The increased flexibility of glycine coupled with the strong electrostatic attraction of the protonated amine and the anionic oxygen allowed a closer approach of N_1 and O_4 than in any of the less flexible analogues. The *X* and *Y* components of the dipole moment were expected to vary, given the diversity of the structures considered, and probably best reflected the electronic variation within this set of structures. The overall dipole moment remained remarkably constant for most of the analogues, including glycine.

Ionization potential (HOMO energy) was highest for glycine and substantially lower for most of the analogues when un-ionized. These differences tended to even out when calculations were run assuming a zwitterionic form. The distribution of a comparable π molecular orbital closest to the HOMO was quite constant across X, C_3, and O_4 but varied moderately on N_1 and C_2. Once again, distributional differences of this orbital evened out for the zwitterions; the HOMO was localized primarily on the oxy anion in every case. LUMO energies and distribution were reasonably similar between glycine and the analogues, for both the un-ionized and zwitterionic forms.

Energy stabilization due to solvation was similar for glycine and the analogues when un-ionized; however, a much larger stabilization was calculated for glycine than any analogue in the zwitterionic form.

Based on the calculated properties listed in Table I, among the initial targets for synthesis/testing, the monocyclic analogues **2** and **5** were most similar to glycine,

(21) Boshagen, H. *Ber. Dtsch. Chem. Ges.* **1967**, *100*, 954.
(22) Conversion of **43** to the hydroxamic acid **44** gave two products, **44** and the corresponding oxime hydroxamic acid. Both compounds were carefully characterized by spectral methods and combustion analysis.
(23) Fraser, J.; Titensor, E. *J. Chem. Soc.* **1957**, 4625.
(24) Sucharda, E. *Ber. Dtsch. Chem. Ges.* **1925**, *58B*, 1727.

Table VI. Glycine-Receptor Affinities

compd	IC_{50}, μM[a]
1	6.0
sarcosine	350
L-alanine	78
D-alanine	17
7	418

[a] Glycine amide, glycine ethyl ester, L-phenylglycine, and compounds 3–6, 8–15, 18, and 19 all posessed IC_{50} values >10000 μM.

followed by 2-aminophenol (13). The proposed bicyclic derivatives 14, 15, 18, and 19 were clearly less similar. Of the compounds subsequently prepared and tested, calculations showed 4 to be most similar, followed by the CF_3- and CH_3-substituted derivatives 6 and 7 and the 5-phenyl analogue 3.

Although molecular calculations predicting similarity to glycine were based both on a nonionized and a zwitterionic form, experimental determination of pK_a in both water and 50% water/methanol afforded similar values for both monocyclic pyrazole 7 (3.1, 6.3 in water) and fused isoxazole 14 (3.3, 6.6 in water). These data indicate that at physiological pH a zwitterionic form for these compounds probably does not exist.

Table VI lists the binding data obtained for the potential heterocyclic glycine agonists and several additional small amino acids. Although the heterocyclic analogues synthesized were reasonable approximations of glycine from a conformational and electronic standpoint, they did not demonstrate binding at a level comparable to glycine. The best compound of the group was compound 7, which showed 50% inhibition of [^{3}H]strychnine binding at 418 nM.

Discussion

Although a number of the assumptions have been made and the methods used to do the calculations represent approximations, the aim of this study was not to calculate precisely each molecular property but rather to gain a qualitative "feel" for which of the proposed analogues might look the most similar to glycine when binding at the receptor.

Calculations revealed that the "right-hand" portion of the proposed analogues (atoms X, C_3 and O_4, Figure 1) were quite similar to glycine electronically; the major differences from glycine occur in the "left-hand part" (atoms N_1 and C_2). Although the extent to which these molecular regions may individually influence the binding of the target structures cannot be separated, the region containing the amino group may contribute more to the binding loss within the context of the electronic characteristics. In order to incorporate the functionality we desired into a heterocyclic framework, it was necessary to station the amino group directly on the aromatic ring. This resulted in a reduced basicity for the amine and altered the electronic characteristics of the "left-hand" portion of the molecule. Overall, beyond the obvious differences in size and lipophilicity, modeling has shown that the agonists proposed for synthesis were in general reasonable approximations of a low-energy conformation of glycine.

From reported strychnine binding studies and results reported herein (Table VI), which summarize the ability of simple amino acids to displace labeled strychnine from spinal cord membrane preparations, it can be concluded that the glycine receptor is highly discriminatory on the basis of steric constraints alone. It is disappointing that all of the compounds reported in this study (1–15, 18, and 19) are essentially inactive as glycine agonists. The data obtained does not permit useful conclusions to be drawn regarding receptor morphology beyond that of a strict volume constraint. We are therefore forced to conclude that the lack of affinity by these compounds for the glycine receptor may mean that the active conformation of glycine is different than that represented by any of the modeled compounds, that the target structures are incapable of ionizing in an appropriate or complete manner at physiological pH, or that all of the targets are simply too large to bind to the glycine receptor.

With the objective of further clarifying which of the structural features discussed in this report are most important in determining glycine-receptor binding, we have also synthesized several series of noncyclic, less rigid aliphatic analogues of glycine. Results from this work will be published separately.

Experimental Section

Melting points (uncorrected) were taken on a Thomas-Hoover capillary melting point apparatus. The structures of the compounds were confirmed by elemental analysis, infrared, mass, and NMR spectrometry. ^{1}H NMR spectra were obtained on a Varian Associates EM-390 (90 MHz), a Varian Associates XL-200 (200 MHz), or an IBM WP100SY (100 MHz) spectrometer. IR spectra were acquired on a Nicolet MX-1 FTIR. Mass spectra were recorded on a Finnigan 4500 (EI, CI) or a VG 7070E/HF (EI) mass spectrometer. TLC was carried out with 0.25-mm silica gel 60 F254 (E. Merck) glass plates. Unless otherwise noted, starting materials were obtained from Aldrich Chemical Co. and were used without further purification. Di-*tert*-butyl dicarbonate was purchased from Fluka. [^{3}H]Strychnine (23 Ci/mmol) was purchased from New England Nuclear. Strychnine hydrochloride, glycine, and 2-amino-2-(hydroxymethyl)-1,3-propanediol (Tris) were purchased from Sigma. THF was dried by distillation from benzophenone ketyl. Toluene was dried by distillation from sodium. Triethylamine was dried by distillation from calcium hydride. Representative procedures for the syntheses illustrated in Schemes I–VI and the procedures used to model the compounds are as follows.

Molecular Modeling. Molecules were built using the SYBYL package of programs,[25] starting from a compendium of average X-ray structures available within the program.[26] For subsequent calculations, each structure was oriented as shown in Figure 1, with C_2 at the origin, C_3 along the positive X axis, and X in the positive X–Y plane. Where possible, the amino group was rotated such that the one NH was in the plane of the ring system and pointing at O_4 to simulate a hydrogen bond. The OH group was also placed in the plane of the ring system, pointing away from N_1.

Each structure was energy minimized using MAXIMIN,[27] a molecular mechanics based minimizer within SYBYL (without considering atomic charges), and then reoriented as described above. Structures were then passed to the CHEMLAB-II program,[28] where atomic charges, dipole moments, and molecular orbital information was calculated using CNDO/2. The structures were then reminimized within SYBYL using MAXIMIN, using the CNDO/2 atomic charges. Finally, the minimized molecules were passed back to CHEMLAB-II, where the final geometries were checked and the solvent energy was calculated with the INITENRGY, MMFF, and BADCONTACT modules. A rerun of CNDO/2 on the final geometries of representative compounds revealed no appreciable change in

(25) Commercially available from Tripos Associates, Inc., 6548 Clayton Road, St. Louis, MO 63117.
(26) SYBYL, Standard Fragment Library; Tripos Associates, Inc.
(27) Labanowski, J.; Motoc, I.; Naylor, C. B.; Mayer, D.; Dammkoehler, R. A. *Quant. Struct.-Act. Relat.* **1986**, *5*, 138.
(28) (a) Pearlstein, R. A. "CHEMLAB-II Reference Manual"; Chemlab Inc. (software available from Molecular Design Limited, San Leandro, CA). (b) Childress, T., Ed. Revised MDL Edition, March, 1985; Molecular Design Limited, San Leandro, CA. (c) Potenzone, R., Jr.; Cavicchi, E.; Weintraub, H. J. R.; Hopfinger, A. J. *Comp. Chem.* **1977**, *1*, 187. (d) Weintraub, H. J. R.; Hopfinger, A. J. *Int. J. Quantum Chem. Quantum Biol. Symp.* **1975**, 203.

charge distribution or molecular orbital characteristics, thus CNDO/2 was not generally rerun on the final minimized versions.

The pyrazolopiperidine analogue (18, unionized) was built and minimized with N_1 in a trigonal planar as well as a tetrahedral geometry. The resultant energies calculated by MAXIMIN, MMFF, MNDO,[29] and AM1[30] were 1–6 kcal/mol lower for the trigonal planar version. However, the binding energy calculated via CNDO/2 was 15 kcal/mol lower for the tetrahedral version. Since the majority of methods (including the more rigorous MNDO and AM1 approaches) calculated the trigonal planar version to be more stable, compounds containing fused pyrrolidine, piperidine, and imidazole rings were modeled with N_1 in this geometry.

Due to the lack of parameterization in SYBYL for an oxyanion, the O_4 atom of the zwitterions was treated as an sp^2 oxygen attached via a single bond. Log P values were calculated using the CLOGP program.[31] All modeling and CLOGP calculations were run on a VAX 11/785.

Chemistry. Procedure A. Preparation of 3-Hydroxy-4-aminopyrazoles (Compounds 3, 5–10, 12, Table I). The starting materials for these compounds were obtained by using literature procedures.[14-16] The synthesis of 3-hydroxy-4-amino-5-methylpyrazole hydrochloride (6) is used as an example for the synthesis of these compounds.

***N*-Benzoyl-3-oxo-DL-methylalanine Ethyl Ester Hydrochloride (23) ($R_1 = CH_3$).** A mixture of DL-22 (11.38 g, 63 mmol) in methylene chloride (114 mL) was cooled to −5 °C in an ice/salt bath and triethylamine (12.47 g, 123 mmol) was added in one portion. Over 30 min, benzoyl chloride (7.94 g, 56 mmol) dissolved in methylene chloride (10 mL) was added to the cold, stirring solution. The reaction was stirred at 0 to −5 °C for 2 h and then washed with 0.5 N HCl, water, and saturated bicarbonate solution. The organic layer was dried over sodium sulfate. Filtration and concentration in vacuo gave 12.15 g (80%) of **23**: mp 112–114 °C.

***N*-(3-Hydroxy-5-methyl-1*H*-pyrazol-4-yl)benzamide (24) (R = CH_3).** A solution of the above benzamide (23, 17.68 g, 71 mmol) in absolute ethanol (50 mL) was treated with hydrazine monohydrate (4.72 g, 94 mmol). After stirring overnight at room temperature, the reaction mixture was concentrated in vacuo to give a brown solid. The crude product was taken up in hot methanol and diluted with ether until cloudy. Careful cooling, filtering, and drying gave 6.31 g (41%) of compound **24** as on off white solid: mp 255 °C dec.

4-Amino-5-methyl-1*H*-pyrazol-3-ol (6). Compound **24** (R = CH_3) (6.81 g, 29 mmol) was suspended in 6 N hydrochloric acid (100 mL). After heating at reflux overnight, the resulting solution was cooled to 0 °C, the benzoic acid was removed by vacuum filtration, and the filtrate was concentrated in vacuo. Recrystallization of the crude product from methanol/ether gave 1.16 g (27%) of **6** as an off white solid: mp 200–203 °C dec.

Procedure B. Preparation of 3-hydroxy-4-aminopyrazoles (compounds 7, 10, and 11, Table I). The precursors for these compounds were prepared by literature methods.[32,33] The synthesis of 3-hydroxy-4-amino-5-phenylpyrazole is used as an example of their preparation.

5-Phenyl-4-oxazolecarboxylic Acid Hydrazide (27) (R = Ph). A solution of 5-phenyl-4-oxazolecarboxylic acid ethyl ester (26, R = Ph, 2.78 g, 13 mmol) in absolute ethanol (25 mL) was treated with hydrazine monohydrate (0.7 g, 13.8 mmol). After stirring for 24 h, the resulting precipitate was filtered and dried to give 1.0 g (38%) of **27** (R = Ph): mp 139–140 °C.

4-Amino-5-phenyl-1*H*-pyrazol-3-ol Monohydrochloride (10). A suspension of **27** (R = Ph) in a mixture of absolute ethanol (10 mL) and 3 N hydrochloric acid (10 mL) was heated at reflux overnight. The reaction was concentrated in vacuo and the residue was dissolved in a minimal amount of hot ethanol. Ether was added until the mixture became cloudy. Standing in the refrigerator overnight gave 0.33 g (34%) of **10** after filtering and drying: mp 222–224 °C.

Procedure C. Synthesis of 3-Hydroxy-4-amino-5-phenylisoxazole Hydrobromide (Compound 3, Table I). The starting 3-hydroxy-5-phenylisoxazole (31) was synthesized by using a literature procedure.[34]

3-Methoxy-5-phenylisoxazole (33). A mixture of **31** (9.5 g, 59 mmol) in absolute methanol (25 mL) was cooled in an ice/water bath and treated with diazomethane until the yellow color persisted. The resulting yellow solution was concentrated in vacuo to give a mixture of N- and O-methylated isoxazoles. Chromatography on 200–430 mesh silica gel, eluting with 50% ether/hexane, gave 4.52 g (44%) of **33** as a white solid; R_f (50% ether/hexane) OCH_3 0.65, NCH_3 0.13; mp 69–71 °C.

3-Methoxy-5-phenyl-4-isoxazolecarboxylic Acid (34). A solution of **33** (4.47 g, 26 mmol) dissolved in dry THF (75 mL) was cooled to −78 °C and 1.3 M *n*-butyllithium in hexane (21.6 mL, 28 mmol) was added dropwise. The resulting mixture was stirred for 30 min at −78 °C and then poured onto crushed dry ice (3.12 g) and allowed to warm to room temperature. The THF was allowed to evaporate overnight. The residue was dissolved in water, extracted with ether, and acidified with concentrated hydrochloric acid. The white precipitate was filtered and air dried to give 5.22 g (92%) of compound **34**: mp 220–221 °C.

3-Methoxy-5-phenyl-4-isoxazolamine (35). To a mixture of **34** (4.22 g, 19 mmol), dry toluene (10 mL), and dry THF (10 mL) were added dry triethylamine (0.55 mL, 10 mmol) and diphenyl phosphorazidate (0.85 mL, 19 mmol). The resulting solution was heated at 80 °C for 2 h. The heat was removed, (trimethylsilyl)ethanol (4.55 g, 39 mmol) was added, and the reaction was heated at 80 °C for 6 h. After cooling to room temperature, the solvents were removed in vacuo, and the residue was diluted with ether. The ether solution was washed twice with 1 N sodium hydroxide solution, the combined aqueous layers were back-extracted with ether, and the combined organic layers were dried over anhydrous sodium sulfate. Concentration in vacuo followed by chromatography on 200–430 mesh silica gel (eluting with 20% ethyl acetate/hexane) gave 4.1 g (62%) of the silyl carbamate as a sticky, white solid. A mixture of the above silyl carbamate (4.1 g, 12 mmol) and 1 N tetrabutylammonium fluoride (47 mL, 47 mmol) was heated at 50 °C for 30 min. After cooling to room temperature, the solvents were removed in vacuo and the residue was diluted with ether and water. The two-phase mixture was rapidly stirred for 1 h, the layers were separated, the aqueous layer was extracted twice with ethyl acetate, and the combined organic layers were washed with saturated ammonium chloride solution and dried over anhydrous sodium sulfate. Concentration in vacuo followed by chromatography on 200–430 mesh silica gel (eluting with 25% ethyl acetate/hexane) gave 1.60 g (70%) of **35** as a yellow oil.

4-Amino-5-phenyl-3(2*H*)-isoxazolone Monohydrobromide (3). A suspension of **35** (1.60 g, 8.4 mmol) in 31% hydrobromic acid/acetic acid solution (27 mL) was heated at 60 °C for 30 min. A fine precipitate resulted. After cooling to room temperature, the solid was filtered and washed with ether. The crude product was recrystallized from methanol/ether to give 1.31 g (61%) of **3** as a tan solid: mp 192–193 °C dec.

***cis*-3-Hydroxy-2-piperidinecarboxylic Acid (37).** Hydrogenation of **36** (66.13 g, 0.48 mol) was accomplished at 50 psi in a mixture of water (700 mL) and concentrated ammonia (100 mL) with rhodium on carbon[35] (7.0 g) over 21 h. The catalyst was filtered, and the solvents were concentrated to give a solid, which was suspended in ethanol, filtered, and dried to give 64.5 (93%) of **37**: mp 284–285 °C dec.

***cis*-3-Hydroxy-2-piperidinecarboxylic Acid Methyl Ester Monohydrochloride (38).** Compound **37** (11.5 g, 79.3 mmol) was suspended in methanol (300 mL) and the suspension was saturated at reflux with a stream of dry hydrogen chloride gas. The solution was refluxed overnight and concentrated to an oil, which solidified after reconcentration three times from methanol.

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The solid (15.5 g, quantitative) was triturated with ether and dried at 78 °C in vacuo to give 13.9 g (90%) of compound **38**: mp 191–192 °C dec.

1-Benzoyl-3-hydroxy-2-piperidinecarboxylic Acid Methyl Ester (39). A solution of **38** (9.0 g, 46 mmol) in water (60 mL) was cooled in an ice bath (internal temperature <5 °C). Benzoyl chloride (7.2 g, 51 mmol) was added dropwise and the stirring was increased to a vigorous rate. A solution of potassium carbonate (15.6 g, 113 mmol) in water (30 mL) was added dropwise over 30 min and the reaction was stirred an additional 30 min before the ice bath was removed. After 60 min at room temperature, ethyl acetate (60 mL) was added, and the layers were separated. The water layer was washed three times with ethyl acetate (90 mL), and the organics were combined, dried over magnesium sulfate, and concentrated. The oil obtained was taken up in diethyl ether (90 mL) and allowed to stand at room temperature. An initial lot of 5.8 g of crystalline material was obtained, followed by two additional lots after concentration of the filtrate and redissolution in successively smaller amounts of ether. The combined yield was 10.2 g of compound **39** as a white, granular solid: mp 94–96 °C.

1-Benzoyl-3-oxo-2-piperidinecarboxylic Acid Methyl Ester (40). A solution of oxalyl chloride[36] (4.2 g, 33.4 mmol) in dichloromethane (75 mL) was maintained at −65 to −60 °C under nitrogen in a dry ice/acetone bath. A solution of dimethyl sulfoxide (5.7 g, 73 mmol) in dichloromethane (15 mL) was added dropwise over 5 min and the reaction was stirred 10 min before a solution of alcohol **39** (8.0 g, 30.4 mmol) in dichloromethane (32 mL) was added dropwise over 10 min. After 15 min, triethylamine (18.9 mL, 136 mmol) was added via a syringe and the cooling bath was removed. When the reaction had warmed to room temperature, water (80 mL) was added, and the layers were stirred then separated. The water was washed with dichloromethane (2 × 80 mL), and the organic phases were combined and dried over sodium sulfate. The solvent was concentrated and the product was chromatographed on 230–400 mesh silica (eluting with a gradient which started with hexane and finished with 50% ethyl acetate/hexane) to obtain an oil, which crystallized from ether (30 mL) to give 5.88 g (74%) of compound **40**: mp 108–111 °C.

4-Benzoyl-4,5,6,7-tetrahydro-1*H*-pyrazolo[4,3-*b*]pyridin-3-ol (41). A solution of hydrazine monohydrate (1.25 g, 25 mmol) in absolute ethanol (20 mL) was added to a solution of ketone **40** in ethanol (230 mL) and the reaction was refluxed overnight. The solvent was concentrated and the dark solid obtained was heated in fresh ethanol (25 mL). The suspension was refrigerated and filtered to obtain 4.2 g (78%) of compound **41**: mp 230–232 °C.

4,5,6,7-Tetrahydro-1*H*-pyrazolo[4,3-*b*]pyridin-3-ol Monohydrochloride (18). Benzamidopyrazole **41** (3.0 g, 12.3 mmol) was suspended in 6 N HCl (36 mL) and heated to reflux whereupon the solid dissolved. After 4 h the starting material was consumed as shown by TLC. The reaction was refrigerated overnight. The precipitated benzoic acid was filtered and the aqueous acid was concentrated to a syrup, which was concentrated twice from absolute ethanol to give a solid. The purified product was precipitated from warm methanol with diethyl ether to give 1.05 g (70%) of **18**: mp 265–267 °C.

***cis*-3-Hydroxy-1,2-piperidinedicarboxylic Acid 1-(1,1-Dimethylethyl) 2-Methyl Ester (42).** Amine salt **38** (20.0 g, 100 mmol) was dissolved in a mixture of water (140 mL) and chloroform (200 mL) and slowly neutralized by the addition of sodium bicarbonate (8.5 g, 100 mmol). Sodium chloride (20 g) was then added, followed by di-*tert*-butyldicarbonate (21.8 g, 100 mmol). The reaction was mechanically stirred with gentle reflux for 6 h and then stirred at room temperature overnight. The layers were separated, and the water was washed with chloroform (2 × 200 mL). The organics were combined, dried over sodium sulfate, and concentrated to an oil, which was purified by chromatography on 230–400 mesh silica gel eluting with 30% ethyl acetate/hexane. Compound **42** (17.1 g, 66%) was isolated as a viscous oil along with additional fractions (6.5 g) containing **42** in a less pure form.

3-Oxo-1,2-piperidinedicarboxylic Acid 1-(1,1-Dimethylethyl) 2-Methyl Ester (43). Alcohol **42** was oxidized by using the procedure described for **40**. The product was isolated by chromatography on 230–400 mesh silica gel (eluting with a gradient beginning with 10% ethyl acetate/heptane and finishing with 20% ethyl acetate/heptane), giving **43** as a colorless oil in 86% yield.

2-[(Hydroxyamino)carbonyl]-3-oxo-1-piperidinecarboxylic Acid 1,1-Dimethylethyl Ester (44). A solution of ester **43** (9.0 g, 35 mmol) was dissolved in absolute ethanol (135 mL) and cooled in an ice/salt bath to −5 °C under nitrogen. A sodium ethoxide solution (0.89 g, 38.5 mmol sodium in 40 mL of absolute ethanol) was added dropwise to the stirred solution. After 15 min, a solution of hydroxylamine (hydroxylamine hydrochloride, 2.7 g, 38.5 mmol) in warm ethanol (45 mL) was neutralized with a sodium ethoxide solution (0.89 g, 38.5 mmol sodium in 40 mL of absolute ethanol), which was added over 30 min, whereupon the reaction was removed from the cooling bath. After 2 h the reaction was quenched with acetic acid (4.6 g, 77 mmol), concentrated, and redissolved in ethyl acetate. The solids were filtered and washed with ethyl acetate, and the solvent was concentrated. The product was isolated by chromatography on 230–400-mesh silica gel (eluting with a gradient beginning with 50% ethyl acetate/heptane and finishing with 100% ethyl acetate) to give 3.8 g (42%) of **44** as a white solid, which gave a positive ferric chloride test: mp 124–125 °C.

4,5,6,7-Tetrahydro-3-hydroxyisoxazolo[4,5-*b*]pyridine-4-carboxylic Acid 1,1-Dimethylethyl Ester (45). The hydroxamic acid **44** (3.6 g, 13.9 mmol) was dissolved in dry THF (165 mL), and pyridine (2.7 g, 34.8 mmol) was added. The reaction was cooled to −70 °C under nitrogen in a dry ice/acetone bath and a solution of thionyl chloride (2.0 g, 16.7 mmol) in dry THF (20 mL) was added dropwise. After 1.5 h a solution of dry triethylamine (7.0 g, 69.5 mmol) was added dropwise, and the temperature was kept below −65 °C. The reaction was stirred 30 min and allowed to warm to room temperature, and the solids were filtered. The filtrate was then concentrated and the residue was taken up in ethyl acetate (400 mL) and washed with water (100 mL) and 1 N HCl (2 × 50 mL) and then dried (magnesium sulfate) and concentrated. The residue was dissolved by warming in heptane (ca. 25 mL) and adding ether (ca. 25 mL). The ether was evaporated on a steam bath and the heptane was refrigerated to precipitate a white solid, which was filtered, washed with heptane, and dried to give 0.95 g (28%) of **45**: mp 133–136 °C.

4,5,6,7-Tetrahydroisoxazolo[4,5-*b*]pyridin-3-ol Trifluoroacetate (1:1) (Salt) (14). Compound **45** (0.8 g, 3.3 mmol) was dissolved in trifluoroacetic acid (4.0 g). After 5 min the reaction was slowly diluted with ether and stirred to precipitate the product. The solvent was decanted and replaced with fresh ether, and the solid was filtered, washed with ether, and dried to give 0.55 g (65%) of **14** as a slighly yellow solid: mp 139–140 °C dec.

3-Hydroxy-2-pyridinecarboxylic Acid Methyl Ester (46). 3-Hydroxypyridine-2-carboxylic acid (31.0 g, 220 mmol) was suspended in dry THF (800 mL) and cooled in an ice bath. Diazomethane in ether [generated from Diazald (43.0 g), estimated to generate 6.0 g (140 mmol) of diazomethane] was distilled directly into the reaction as it was generated. After the addition the reaction was allowed to warm to room temperature, the solids were filtered, and the solvent was concentrated. The product was dissolved in ether and filtered and then concentrated to give 14.2 g (66% based on diazomethane) of the white solid **46**: mp 70–73 °C.[37]

***N*,3-Dihydroxy-2-pyridinecarboxamide (47).** Hydroxylamine hydrochloride (7.94 g, 114 mmol) was dissolved in absolute ethanol (125 mL) on a steam bath and neutralized by adding a sodium hydroxide solution [9.12 g, 114 mmol of a 50% wt/wt solution in water diluted with ethanol (50 mL)]. The suspension was cooled, the solids were filtered, and the filtrate was added dropwise to a solution of the ester **46** (14.0 g, 91.4 mmol) in ethanol (150 mL) cooled in an ice bath. The reaction was stirred at room temperature overnight, filtered, and concentrated. The solid was suspended in saturated sodium bicarbonate (150 mL) and dissolved by adding 2 N sodium hydroxide solution. Acidification with 2 N HCl and drying gave 4.7 g (34%) of **47** as a fluffy solid, mp 182–184 °C.

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Isoxazolo[4,5-*b*]pyridin-3-ol (15). The hydroxamic acid **47** (4.5 g, 29 mmol) was dissolved in dry THF (375 mL) and triethylamine (10.2 g, 29 mmol) was added. The solution was cooled in a dry ice/acetone bath to −78 °C and a solution of thionyl chloride (3.8 g, 32 mmol) in THF (60 mL) was added dropwise whereupon a precipitate forms. The reaction was removed from the cold bath and allowed to warm to room temperature overnight. The solid was filtered and the solvent was concentrated. The residue was dissolved in water (60 mL) and refrigerated to give a solid, which was filtered, washed with water, and dried (2.4 g). Extraction of the filtrate with chloroform (4 × 150 mL) gave an additional 1.2 g of white solid. The two lots were combined, triturated successively with ethyl acetate and then water and dried to give 2.3 g (58%) of **15** as a light brown solid: mp 211–213 °C.

1*H*-Pyrazolo[4,3-*b*]pyridin-3-ol (19). Compound **19** was prepared in 20% yield from 3-aminopyridine-2-carboxylic acid by using the general procedure of Hall[38] for pyrazolobenzene analogues: mp 248–251 °C (from water). This compound has been prepared by another method.[39]

Glycine Receptor Binding. Preparation of Membranes. Two brain stems and two spinal cords from 150–200-g Long–Evans rats (about 2-g total weight) were disrupted in 20 mL of ice-cold 50 mM Tris–citrate (pH 7.5 at 0 °C) for 30 s in a Polytron PT-10 (Brinkmann) at setting 5. The suspension was centrifuged at 50000*g* for 10 min, the supernatant was discarded, the pellet was resuspended in 20 mL of ice-cold Tris–citrate as above, recentrifuged, resuspended at 1 g/5 mL, and stored in plastic vials at −70 °C. When needed, the tissue was thawed, diluted to 1 g/20 mL in ice-cold Tris–citrate, centrifuged, resuspended, and kept on ice until used.

Incubation Conditions. All incubations were in triplicate for 60 min at 0 °C in 12 × 75 mm glass tubes containing 2 mL of Tris–citrate (pH 7.5) with 10 mg of original tissue weight of membranes and 3 nM [^{3}H]strychnine. Test compounds were dissolved at 10 mM in dimethyl sulfoxide and diluted in dimethyl sulfoxide to 100 times the final incubation concentration. Control incubations received an equal volume (20 mL) of dimethyl sulfoxide; the resulting concentration of dimethyl sulfoxide reduced specific [^{3}H]strychnine binding by about 15% but had no effect on the IC_{50} for glycine. The order of additions was test compound, membranes, and [^{3}H]strychnine. After all additions the rack of tubes was vortexed, and the tubes were incubated in an ice bath for 60 min. Incubations were terminated by filtration through 2.4 cm GF/B filters under reduced pressure followed by three rapid washes with 4 mL of ice-cold 50 mM Tris-HCl (pH 7.7 at 25 °C) with 1 M NaCl. The filtration was complete in about 12 s. Filters were counted with 8 mL of Formula 963 scintillation fluid (New England Nuclear) in a liquid-scintillation counter.

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Synthesis and Evaluation of *N*,*N*-Di-*n*-propyltetrahydrobenz[*f*]indol-7-amine and Related Congeners as Dopaminergic Agonists

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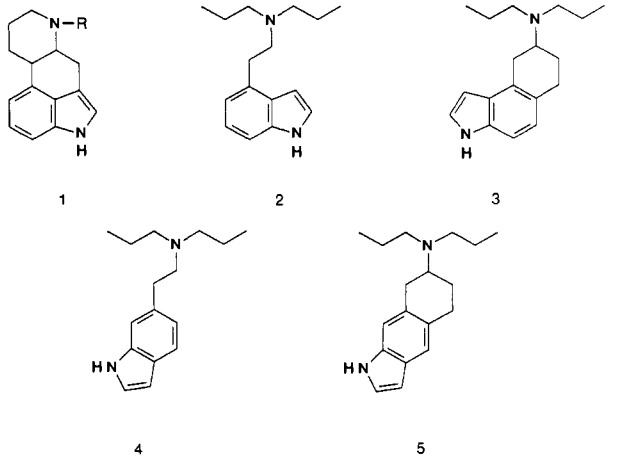
An evaluation of 6-[2-(di-n-propylamino)ethyl]indole (**4**), its rigid analogue *N*,*N*-di-*n*-propyl-5,6,7,8-tetrahydrobenz[*f*]indol-7-amine (**5**), and some related congeners, for ability to suppress serum prolactin in reserpinized rats, revealed modest biological activity in this in vivo model of dopaminergic activity. Although the indole N–H in these compounds can be considered to be oriented "meta" with respect to the ethylamine side chain, compounds with the indole N–H located in the other "meta" position (i.e. 4-[2-(di-*n*-propylamino)ethyl]indole (**2**) or its rigid benz[*e*]indole analogue **3**) were much more potent dopamine agonists. The results argue for a particular orientation of the indole N–H vector. In addition, relatively potent dopamine agonists also resulted when the pyrrole portion of the indole ring was replaced by a methanesulfonamido function, supporting the idea that the indole N–H serves as a hydrogen-bond donor.

In the past decade, considerable effort has been directed toward elucidation of the active fragment in the ergolines (**1**) that is responsible for their dopamine-like action. We have been carrying out a systematic program to elucidate the structure–activity relationships of ergoline-related dopaminergic agents. Of interest was the independent finding of Cassady et al.[1,2] and Cannon et al.[3] that 4-[2-(*N*,*N*-di-*n*-propylamino)ethyl]indole (DPAI, **2**) had substantial dopaminergic activity. In this context, it was of particular interest to examine isotryptamines with different sites of side-chain attachment and also to design conformationally restricted analogues that might be helpful in elucidating the active conformation of the side chain at its receptor.



Within intact ergolines, the pyrrole ring can serve to replace a catechol moiety, a suggestion originally made by

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