114603-35-9; (±)-**9g**, 123170-06-9; **9h**, 123170-07-0; (±)-**10a**, 123169-88-0; (±)-**10b**, 123169-89-1; (±)-(*R**,*R**)-**10c**, 123169-90-4; (±)-(*R**,*S**)-**10c**, 123169-97-1; (±)-**10d**, 123169-91-5; (±)-**10e**, 123188-08-9; **11a**, 117259-73-1; **11b**, 114603-49-5; **11c**, 123170-08-1; (±)-**11d**, 123170-09-2; **12a**, 114602-97-0; **12b**, 114602-98-1; (±)-**12c**, 123169-92-6; (±)-**12d**, 123188-09-0; PDE, 9025-82-5; succinic anhydride, 108-30-5; 4-acetyl-3,4-dihydro-1,4(2*H*)-benzoxazine, 70801-52-4; propionic anhydride, 123-62-6.

Synthesis and Serotonin Binding Site Studies of Some Conformationally Restricted Indolylethylamine Analogues Based on 2-Amino-3-(3′-indolyl)bicyclo[2.2.2]octane

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The bicycloannulation reaction between cyclohexenone and indolyl enamines yields *trans*-3-(cyclic amino)-2-(3′-indolyl)bicyclo[2.2.2]octan-5-ones, and these adducts are conformationally restricted analogues of indolylethylamine (tryptamine) which exhibit structure-dependent affinity for the serotonin $5HT_2$ and $5HT_{1a}$ receptors. The stereochemistry of the isomeric endo and exo adducts obtained is assigned from the ^1H NMR spectra of the specifically deuterated alkenes prepared from the ketones by the Bamford–Stevens reaction. Molecular mechanics calculations indicate that the conformational flexibility of the amino and indolyl groups is restricted through van der Waals interactions with the bridges of the bicyclic unit. These compounds inhibit the binding of [^3H]ketanserin to $5HT_2$ sites in mouse cerebrocortical membranes, and the binding of [^3H]-8-hydroxy-2-(di-*n*-propylamino)tetralin ([^3H]-8-OH-DPAT) to $5HT_{1a}$ sites in mouse hippocampal membranes. The endo compounds are the most potent, and molecular mechanics calculations indicate that these isomers have a less bulky bicyclo bridge proximate to the amine group and more conformational freedom about the C_α–C_β–N^+–H dihedral angle (τ^3). In the $5HT_2$ assay, *endo-trans*-3-(*N*-piperidinyl)-2-(3′-indolyl)bicyclo[2.2.2]octan-5-one (**10a**) is the most potent, and endo-*trans*-3-(*N*-pyrrolidinyl)-2-(3′-indolyl)bicyclo[2.2.2]oct-5-ene (**12a**) is the most potent in the $5HT_{1a}$ assay. A phenyl-substituted adduct shows the least affinity in these two assays. These data provide insight into the structural differences between the $5HT_{1a}$ and $5HT_2$ receptor sites.

Conformationally restricted synthetic analogues of bioactive arylethylamines can be used as effective tools for probing the structure of the binding sites for these physiologically important substances. Much effort is currently directed to the development of compounds that bind, with high specificity, to serotonin binding site subtypes in order better to evaluate structural and functional features of these numerous receptors.[1,2] The serotonin receptors are still very poorly understood, and more specific information on the requirements for binding at particular serotonin binding site subtypes should facilitate the design of more specific agonists and antagonists. Active analogues are also candidates for new selective drugs.

We are investigating the structure–activity relationships that govern the affinity of conformationally restricted analogues for serotonin binding sites, and we report here the synthesis and characterization of the indolylethylamine (tryptamine) analogues **9**, **10**, **12**, and **13** and the phenylethylamine analogue **11**, and studies on their activity at inhibiting the binding of tritiated ketanserin to $5HT_2$ sites in mouse cerebrocortical membranes and of tritiated 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) to $5HT_{1a}$ sites in mouse hippocampal membranes.[3]

We have developed a facile preparation of indolylethylamine analogues in which the indole and amine moieties are attached in a vicinal trans fashion to an ethano bridge of the bicyclo[2.2.2]octane skeleton.[4] These adducts bear a carbonyl group on one of the two remaining ethano bridges, giving rise to geometric isomers: endo when the carbonyl is syn to the amine moiety and exo when the carbonyl is syn to the indole group.[5] This structure provides a framework from which additional analogues can be made by changing the amine substituent (different sized cyclic amines), the indole substituent (substituted indole, phenyl, substituted phenyl), and the

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(5) For this series we define endo and exo on the basis of which substituent (indole or amine) is syn to the carbonyl. We assign a higher priority to the amine group since the carbon of the bicyclic skeleton is bonded to nitrogen for this substituent, versus a bond to carbon for the indole substituent. Thus the isomers **9a**, **10a**, and **11** are endo isomers, with the carbonyl syn to the higher priority substituent, and **9b** and **10b** are exo isomers. Analogous assignments are used for the corresponding alkenes.

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functional groups on the bicyclic skeleton (ketone, alkene, alkane, alcohol, etc.).

These molecules constitute conformationally restricted analogues of serotonin (or other arylethylamines) since in contrast to the relatively free rotations about the three side-chain dihedral angles in the parent neurotransmitter, the corresponding rotations in the analogues are hindered or frozen due to the rigidifying effect of the bicyclo[2.2.2]octane. This particular series of analogues is especially useful for two reasons: (1) the central dihedral angle is frozen at ≈100°, a value intermediate between the gauche angle of 60° and the anti angle of 180° found in most conformationally restricted analogues; and (2) the conformational restrictions on the two collateral dihedral angles are enforced through van der Waals repulsions between a bridge of the bicyclic portion of the molecule and the amine or aromatic group, rather than through anchoring by covalent attachment. The tryptamine-analogous portion of these compounds is thus comparatively less encumbered and more available for binding interactions.

Structure–activity correlations can be based on a number of molecular properties including polar effects, specific interactions, and conformation. The correlation between the minimum-energy conformations of a simple compound with relatively low rotational barriers and the pharmacophoric conformation is not always straightforward—there can be a large number of nonequivalent low-energy conformations, and a favorable binding energy can reciprocate for an unfavorable conformational energy. Nevertheless, when activity is found in conformationally restricted analogues, the analysis of minimum-energy conformations can provide an informative approach to structure–activity relationships. Binding site affinity is strongly dependent on the spatial relationships of the aromatic and amine groups with their putative contact points at the binding site. The bicyclo[2.2.2]octane portion of the molecule does add steric bulk to these analogues, when compared to the simple serotonin molecule, but this factor averages out in comparisons between members of the series. Correlation of the affinities of the analogues in assays for different receptor site subtypes with the molecular structure and conformations of the analogues will help to elucidate the structural differences between the serotonin binding sites.

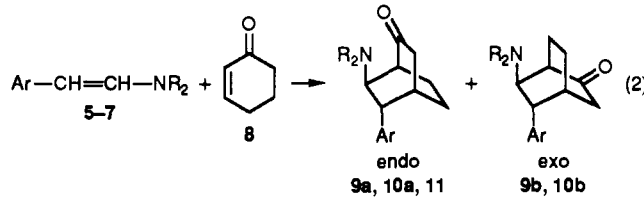
Chemistry

Following our earlier work,[4] the condensation of the arylacetaldehydes **1** or **2** with the corresponding cyclic amines **3** or **4** was effected in benzene over molecular sieves at room temperature followed by evaporation, to give quantitative yields of the enamines[6] **5–7** (eq 1). These enamines are sensitive to air oxidation and to polymerization, but can be stored below 0 °C for several days without deterioration.

$$\underset{\mathbf{1,2}}{\text{Ar–CH}_2\text{–CHO}} + \underset{\mathbf{3,4}}{\text{R}_2\text{NH}} \rightarrow \underset{\mathbf{5–7}}{\text{Ar–CH=CH–NR}_2} \qquad (1)$$

1, Ar = 3-indolyl; **2**, Ar = phenyl; **3**, $R_2 = -(CH_2)_4-$; **4**, $R_2 = -(CH_2)_5-$; **5**, Ar = 3-indolyl, $R_2 = -(CH_2)_4-$; **6**, Ar = 3-indolyl, $R_2 = -(CH_2)_5-$; **7**, Ar = phenyl, $R_2 = -(CH_2)_5-$

The bicycloannulation reaction was performed at reflux in benzene with an excess of cyclohexenone (**8**) as shown in eq 2, and was continued until the disappearance of enamine (aldehyde) by TLC. We have found that the enamine formation and bicycloannulation can be combined into a single procedure, with a 80–90% crude yield of the adducts **9–11**.



The bicycloannulation reaction of the indolyl enamines **5** and **6** with cyclohexenone yielded in each case a mixture of the two isomeric adducts **9a,b** and **10a,b** in an approximately 1:1 ratio. The phenyl enamine **7** yielded a single product, to which we assign the structure **11**. Taken together with our earlier results, it would seem that the character of both the amine and the aromatic substituents in enamines **5–7** are important determinants in whether a single one or both of the isomeric adducts are obtained.[7]

The assignment of the relative stereochemistry in adducts **9a,b**, **10a,b**, and **11** arises from our earlier work[4] and through analogy with the endo stereochemistry assigned to the bicycloannulation adduct from cyclopentanone enamine and cyclohexenone, for which an X-ray diffraction structure is available.[8] In our previous study[4] we correlated the relative stereochemistry of **9a** and **9b** through a Wolff–Kishner deoxygenation; each isomer separately yielded the same *trans*-2-(3′-indolyl)-3-*N*-pyrrolidinylbicyclo[2.2.2]octane. In a further exploration of the chemistry of this series, we have carried out the Bamford–Stevens reaction on the ketones **9a,b** and **10a,b**, to yield the corresponding alkenes **12a,b** and **13a,b** (equations 3, 4).

Interestingly, the ^{1}H NMR spectra for alkenes **12a/12b** and **13a/13b** reveal two nonequivalent vinyl proton resonances appearing as separate doublets of doublets. The observed patterns (Table I) lend additional support to the stereochemical assignments. We reason that the vinylic C–H resonances in the range δ 6.25–6.40 are relatively unperturbed as compared with the literature value of δ 6.25 for bicyclo[2.2.2]oct-2-ene.[9] In the endo alkenes, two factors could account for the upfield shift to δ 6.08–6.10: steric compression by the α-methylenes of the cyclic amine

Table I. Vinylic ^{1}H NMR Resonances for Alkenes **12a,b** and **13a,b**[a]

12a	6.10	6.40
12b	6.30	6.60
13a	6.08	6.35
13b	6.25	6.55
bicyclo[2.2.2]octene		6.25

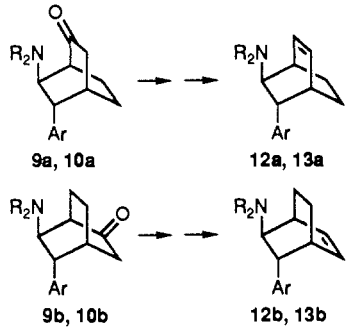
[a] Values given in δ, ppm downfield from internal tetramethylsilane. Value for bicyclo[2.2.2]octene taken from ref 9.

(6) (a) For earlier work on indolyl enamines, see: Daly, J. W.; Witkop, B. *J. Org. Chem.* **1962**, *27*, 4104. (b) For phenyl enamines, see: Mannich, C.; Davidsen, H. *Chem. Ber.* **1936**, *69*, 2106.

(7) We believe that this distinction depends on the relative rates of proton transfer vs internal rotation and Mannich closure at the stage of the enolate immonium and keto enamine intermediates. The pyrrolidine indolyl enamine **5** and the piperidine indolyl enamine **6** do give both isomers, but the morpholine indolyl enamine and the piperidine phenyl enamine give a single product. Thus both the indole aromatic ring and the more reactive pyrrolidine and piperidine enamine groups are required for the production of both isomers.

(8) Singh, Y. K.; Rao, R. B. *Chem. Lett.* **1979**, 653. Nirmala, K. A.; Gowda, D. S. S. *Acta Crystallogr.* **1982**, *B38*, 839.

(9) Pretsch, E.; Immer, H.; Pascual, C.; Schaffner, K.; Simon, W. *Helv. Chim. Acta* **1967**, *50*, 105.



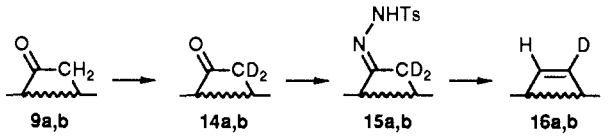
(3)

(4)

9, 12: Ar = 3-indolyl; $R_2 = -(CH_2)_4-$
10, 13: Ar = 3-indolyl; $R_2 = -(CH_2)_5-$

group will shield the vinylic C_6–H, and the same effect is predicted to result from the polarization of the olefin π-system by the nitrogen atom–lone pair dipole. For the exo isomers, in the stable conformations the indole moiety is appropriately situated for an aromatic ring current-induced deshielding effect to be exerted on the nearby vinylic C_6–H (more strongly than on the more distant C_5–H), which results in a downfield shift for this resonance, to δ 6.55–6.60.

An unambiguous assignment of the vinylic proton resonances comes from a series of deuterium-labeling experiments. Ketones **9a** and **9b** were subjected independently to the sequence shown in eq 5. Base-catalyzed



(5)

deuterium exchange on ketones **9a,b** under standard conditions ($NaOD/D_2O$) gives the deuterated ketones **14a,b**, which were converted ($TsNHNH_2/CH_3CO_2D$) to the tosylhydrazones **15a,b**. These were treated with excess methyllithium to furnish the monodeuterioalkenes **16a,b**, selectively deuterated at C_6. The ^1H NMR spectrum for alkene **16a** shows a single resonance in the vinyl region at δ 6.40, and in the spectrum for **16b** only the resonance at δ 6.30 remains. These results are consistent with the assignments given.

Pharmacology

The ability of a compound to inhibit the binding of [^{3}H]ketanserin and [^{3}H]8-OH-DPAT in the appropriate tissue preparation is taken as a measure of the affinity of the compound for serotonin subtype receptors $5HT_2$ and $5HT_{1a}$, respectively.[2] We have examined the performance in these assays of compounds **9a**, **9b**, **10a**, **10b**, **11**, **12a**, **12b**, **13a**, and **13b** as the tartrate salts, and the results are shown in Tables II and III. Also, as a point of reference, the activity of serotonin itself was evaluated (Tables II and III). The IC_{50} values were determined by computer fit[10] of the binding data, or from hand-drawn inhibition curves when the Hill numbers were less than 0.8. When the Hill numbers were sufficiently close to unity, the IC_{50} values were also converted to K_i values. In general the compounds tested were more potent in the inhibition of [^{3}H]ketanserin binding than in the inhibition of [^{3}H]8-OH-DPAT binding, except with respect to serotonin itself, which inhibited 8-OH-DPAT binding to a much greater degree (IC_{50} 0.005 μM vs 0.68 μM for ketanserin).

The pyrrolidine-substituted ketones **9a,b** demonstrated stereoselectivity in the inhibition of ketanserin binding at the $5HT_2$ site but none with respect to 8-OH-DPAT binding at the $5HT_{1a}$ site. The endo isomer (**9a**) was 3 times more active than the exo isomer (**9b**) in the inhibition of ketanserin binding (K_i values 23 vs 70, respectively, in Table II). This stereoselective effect was seen to be even more pronounced in the pair of piperidine-substituted ketones **10a,b**. As was the case with the pyrrolidine-substituted ketones, the endo isomer **10a** was more potent in the inhibition of ketanserin binding than the exo isomer **10b**, this time by a factor of 15 (K_i values 4 vs 61, respectively, in Table II). Of all the compounds tested, the ketone **10a** proved to be the most potent in the inhibition of ketanserin binding. Both isomers of the ketones **9** and **10** were equally ineffective in the inhibition of 8-OH-DPAT binding.

In contrast to the ketones, the alkenes **12a,b** and **13a,b** demonstrated a stereoselectivity in the inhibition of 8-OH-DPAT binding to the $5HT_{1a}$ receptor but none with respect to the ketanserin binding on the $5HT_2$ receptor. The endo isomers of both the piperidine- and pyrrolidine-substituted alkenes, **12a** and **13a**, demonstrated lower IC_{50} values (by a factor of 4) in the inhibition of 8-OH-DPAT binding than the exo isomers (**12b** and **13b**). Of all the compounds tested, the endo pyrrolidine-substituted alkene (**12a**) proved to be the most effective in blocking 8-OH-DPAT binding.

The Hill numbers for **9b**, **12b**, **13b**, and serotonin were below the acceptable level for simple site-selective activity in the $5HT_{1a}$ assay (0.47, 0.61, 0.69, and 0.75, respectively, Table III), and thus the K_i coefficients were not determined for these compounds.

The phenyl-substituted adduct **11** demonstrated the lowest affinity for both serotonin receptor subtypes, less

Table II. Inhibition of [^{3}H]Ketanserin Binding in Mouse Cerebral Cortex[a]

compd	IC_{50}, μM (±)	Hill no. (±)	K_i, μM (±)
9a	32 (0.8)	0.9 (0.09)	23 (0.5)
9b	96 (5.3)	1.0 (0.05)	70 (3.8)
10a	5 (0.4)	1.0 (0.04)	4 (0.3)
10b	84 (5.4)	0.9 (0.06)	61 (3.9)
11	181 (39)	1.2 (0.02)	132 (28)
12a	19 (5.5)	1.0 (0.05)	14 (3.9)
12b	24 (7.3)	0.9 (0.04)	17 (5.3)
13a	10 (0.5)	1.0 (0.07)	8 (0.3)
13b	13 (0.5)	0.9 (0.08)	9 (0.1)
5HT	0.68 (0.02)	0.85 (0.06)	0.50 (0.015)

[a] IC_{50} values are averages of two determinations ± range, calculated by computer fit[10] of inhibition curves consisting of seven points.

Table III. Inhibition of [^{3}H]-8-OH-DPAT Binding in Mouse Hippocampus[a]

compd	IC_{50}, μM (±)	Hill no. (±)	K_i, μM (±)
9a	46 (1.1)	0.95 (0.09)	32 (1.0)
9b	53 (4.0)[a,b]	0.47 (0.12)	ND
10a	86 (9)[a]	1.2 (0.13)	61 (7)
10b	160 (37)	0.88 (0.07)	115 (31)
11	358 (12)	0.89 (0.09)	274 (55)
12a	12 (2)	0.95 (0.02)	8.5 (1.5)
12b	47 (9)[a,b]	0.61 (0.05)	ND
13a	18 (7.7)	0.93 (0.12)	13.5 (5.5)
13b	89 (14)[a,b]	0.69 (0.07)	ND
5HT	0.005 (0.0017)[b]	0.75 (0.03)	ND

[a] IC_{50} values are averages of two determinations ± range, calculated by computer fit[10] of inhibition curves consisting of seven points. Values marked "a" are the averages of three to five determinations ± standard error of mean. Values marked "b" are calculated by inspection of the inhibition curves, in the cases where the low Hill numbers lead to poor analysis by the curve-fitting program. ND indicates that the K_i is not determined, in cases where the low Hill numbers exclude simple site-selective behavior.

(10) De Lean, A.; Munson, P. J.; Rodbard, D. *Am. J. Physiol.* **1978**, *235*, E97.

than half as active as the least active indole-substituted compound in each assay.

Conformational Analysis

We have used molecular mechanics calculations[11] to carry out conformational analyses[1a,1c,12] for the protonated versions of structures **9–13** in order to see what correlations can be drawn on the basis of the varying degree of conformational restriction in the members of this series. We analyze the conformational characteristics of these compounds about a six-atom axis: C_2–C_3–C_α–C_β–N^+–H (tryptamine numbering). The conformations are defined in terms of the three dihedral angles τ^1, τ^2, and τ^3, which are defined by the atom tetrads C_2–C_3–C_α–C_β, C_3–C_α–C_β–N^+, and C_α–C_β–N^+–H, respectively. Rotations about these angles significantly change the spatial relationship between the amine function and the aromatic ring. Since the interactions of these two groups with the contact points at the binding site are believed to be crucial to the molecular recognition process,[2] the degree of extension of these angles (which determines the relative placement of the groups) should be an important determinant for activity. Indeed, one hypothesis for the breadth of activity of serotonin among many receptor subtypes invokes a selective recognition of different conformations of the ligand by the various sites.[1a]

For simplicity we ignored the flexibility within the pyrrolidine ring in compounds **9** and **12**, but we explicitly considered both chairs forms of the piperidine ring in compounds **10**, **11**, and **13**. In compounds **10** and **13**, inclusion of the conformations in which the bicyclic moiety is held axial with respect to the piperidine ring does not qualitatively alter the conformation profile for the compound. In most cases, the values of τ^1, τ^2, and τ^3 for an axial conformation resemble those for an equatorial conformation about 0.2–0.3 kcal/mol lower in energy. For compound **11** these differences were somewhat higher. The lack of a qualitative impact on the profile due to conformational changes in the piperidine ring tend to validate the simplified treatment of the pyrrolidine ring in the conformational analysis of compounds **9** and **12**.

Rotation about τ^1 (i.e. the dihedral angle defined by atoms C_2–C_3–C_α–C_β) determines whether the molecule assumes an "ergoline-type" conformation ($\tau^1 \approx 180°$), or a "nonergoline-type" conformation ($\tau^1 \approx 0°$). The former arrangement mimics the structure of the highly bioactive ergoline group of compounds, but compounds of the latter conformational type can also be quite active.[1a] Rotations about τ^2 (i.e. the dihedral angle defined by atoms C_3–C_α–C_β–N^+) will also significantly affect the distance between the amine and aromatic ring. Compounds with an acute angle τ^2 such as tetrahydro-β-carboline or yohimbine generally exhibit low selectivity for serotonin receptors.[1a] Rotation about the angle τ^3 (i.e. the dihedral angle defined by atoms C_α–C_β–N^+–H) is not expected to change greatly the relative positions of the amine and the aromatic ring, since the nitrogen atom is contained in the axis of rotation. It will change the orientation of the hydrogen-bonding N^+–H, however, and this orientation should be relevant to the activity since at the binding site the fit for the amine group is believed to involve a hydrogen-bonding interaction between this N^+–H and an anionic site on the surface of the adenylate cyclase enzyme or of the phospholipase C enzyme (at the $5HT_{1a}$ and $5HT_2$ sites, respectively).[2a,12a,d] A recent report attributes the highly selective serotonergic activity found for an aporphine analogue to the hindered rotation of a phenolic hydroxyl group.[1c] Rotation at τ^3 is meaningful only in N-alkylated analogues, as the 3-fold symmetry of the $^+NH_3$ group in tryptamine or serotonin leads to a single effective conformation. For this reason N-alkylated analogues where the dihedral angle τ^3 is conformationally restricted are sensitive probes for testing the directionality requirements for the N^+–H group.

The results of the conformational analyses[11] of these compounds are shown in Tables IV–XII. The minimum-energy conformations calculated for the protonated versions of compounds **9–13** are characterized in terms of the dihedral angles τ^1, τ^2, and τ^3. For the sake of brevity, only those conformations within 3.5 kcal/mol of the ground state for a particular molecule are given.

Table IV. Compound **9a**[a]

energy, kcal/mol	dihedral angle, deg		
	τ^1	τ^2	τ^3
0.00	39	103	70
0.23	38	98	−176
0.28	33	119	66
1.35	37	84	−43
1.55	−136	97	69
1.58	107	83	45
1.60	−136	100	65
2.07	−137	94	−176
3.03	−135	83	−43
3.49	−51	87	−44

[a] The table gives the minimum-energy conformations discovered in a conformational search performed on the respective molecule as discussed in ref 10. Energy values are relative to the lowest energy structure found. Only those values within 3.5 kcal/mol of ground state are tabulated.

Table V. Compound **9b**[a]

energy, kcal/mol	dihedral angle, deg		
	τ^1	τ^2	τ^3
0.00	34	117	65
0.98	−136	103	65
1.53	34	98	−179
3.03	−135	89	179
3.14	20	86	−47
3.17	36	93	−50

[a] The table gives the minimum-energy conformations discovered in a conformational search performed on the respective molecule as discussed in ref 10. Energy values are relative to the lowest energy structure found. Only those values within 3.5 kcal/mol of ground state are tabulated.

(11) Molecular mechanics calculations are performed using the interactive graphics program PCMOD (1987, Serena Software, Bloomington, IN) to prepare input files, and the MMX force field energy minimization program (1987, Serena Software, a modification of the 1985 MM2 force field by N. L. Allinger). For the compounds **9a,b**, **10a,b**, **11**, **12a,b**, and **13a,b**, input files for the N-protonated versions are prepared with PCMOD and energy-minimized with MMX, with VESCF π calculations carried out concurrently on the aromatic system. The ROT-E option of PCMOD (rigid rotation) is used to search the angles τ^1 and τ^3 separately at 8° resolution. Files are created for each detected minimum and subjected to overall energy minimization. Each new conformation is subsequently searched over angles τ^1 and τ^3 at an 8° resolution, and for each minimum detected at this stage a new file is created and minimized. The full pool of conformations is then inspected for superimposable structures using the COMP option in PCMOD, the criterion for identity being an average atomic deviation of less than 0.200 Å.

(12) For previous work in this area, see: (a) Hearn, R. A.; Freeman, G. R.; Bugg, C. E. *J. Am. Chem. Soc.* **1973**, *95*, 7150. (b) Kopple, K. D.; Wiley, G. R.; Tauke, R. *Biopolymers* **1973**, *12*, 627. (c) Makriyannis, A.; Knittle, J. In *"QuaSAR" Research Monograph 22*; Barnett, G., Trsic, M., Willette, R., Eds.; National Institute of Drug Abuse, 1978; p 464. (d) Belleau, B. *Ann. N.Y. Acad. Sci.* **1967**, *139*, 580.

Table VI. Compound 10a[a]

energy, kcal/mol	dihedral angle, deg τ^1	τ^2	τ^3
0.00	32	114	43
0.34*	37	110	48
0.34	13	85	-32
0.35	35	90	180
0.46	35	94	-32
0.53*	34	95	-173
0.84	118	92	-34
1.62	138	102	-42
1.79	-139	100	43
1.91*	-139	99	50
1.95	-135	88	-31
2.06	-136	87	-174
2.21	-48	90	-35
2.40	-135	80	170
2.59*	30	91	165
2.66*	20	84	-28
2.70*	36	86	-29
2.72*	41	107	129
2.76*	32	92	-176
2.94*	104	81	-29
3.02*	23	127	99
3.03*	141	126	100

[a] The table gives the minimum-energy conformations discovered in a conformational search performed on the respective molecule as discussed in ref 10. Energy values are relative to the lowest energy structure found. An asterisk indicates a structure in which the bicyclic moiety is attached in an axial fashion to the piperidine nitrogen. Only those values within 3.5 kcal/mol of ground state are tabulated.

Table VII. Compound 10b[a]

energy, kcal/mol	dihedral angle, deg τ^1	τ^2	τ^3
0.00	34	107	48
0.24*	38	107	49
1.30	-137	103	48
1.34*	-137	101	52
1.98	32	84	-164
2.10	36	90	-34
2.17	21	94	137
2.52	42	103	140
2.44*	41	106	123
3.50	-133	85	-39

[a] Table gives the minimum-energy conformations discovered in a conformational search performed on the respective molecule as discussed in ref 10. Energy values are relative to the lowest energy structure found. An asterisk indicates a structure in which the bicyclic moiety is attached in an axial fashion to the piperidine nitrogen. Only those values within 3.5 kcal/mol of ground state are tabulated.

The angle τ^2 (C_3–C_α–C_β–N^+) is "fixed" in these analogues, as the atoms C_α and C_β are part of the rigid bicyclic moiety. Within a 3.5 kcal/mol envelope across all of the analogues, the angle τ^2 varies between 79° and 127°, and within the lowest 1.5 kcal/mol envelope the value is 101 ± 18°, intermediate between the gauche (60°) and anti (180°) conformations expected for simple tryptamine.

The angles τ^1 (C_2–C_3–C_α–C_β) and τ^3 (C_α–C_β–N^+–H) are restricted in their rotations by virtue of van der Waals interactions of the bridges of the bicyclic moiety with the cyclic amine group and with the aromatic group. The rotational freedom about the angles τ^1 and τ^3 is affected differently in each geometrical isomer of a pair. In the endo isomers there is more rotational flexibility in τ^3 while τ^1 is relatively rigid among the lower energy conformations (i.e. within a 1.5 kcal/mol envelope). The reverse is true for the exo isomers, where the lower energy conformations show some flexibility in the angle τ^1 but more rigidity in the angle τ^3.

Table VIII. Compound 11[a]

energy, kcal/mol	dihedral angle, deg τ^1	τ^2	τ^3
0.00	41	104	42
0.06	-48	91	-34
0.47*	43	104	50
0.57	45	92	-31
0.62	45	89	180
1.02	-16	86	-36
2.83*	46	85	-28
2.98*	-50	89	-28
3.12*	41	91	169
3.43*	3	79	-30
3.44*	-28	120	43
3.47*	40	94	-174

[a] The table gives the minimum-energy conformations discovered in a conformational search performed on the respective molecule as discussed in ref 10. Energy values are relative to the lowest energy structure found. An asterisk indicates a structure in which the bicyclic moiety is attached in an axial fashion to the piperidine nitrogen. Only those values within 3.5 kcal/mol of ground state are tabulated.

Table IX. Compound 12a[a]

energy, kcal/mol	dihedral angle, deg τ^1	τ^2	τ^3
0.00	34	109	66
0.20	37	101	-176
1.51	34	95	-46
1.58	-137	99	66
1.93	112	87	-46
2.03	-137	90	-178
2.89	137	99	-45
3.06	-135	87	-44
3.32	-45	90	-46

[a] The table gives the minimum energy conformations discovered in a conformational search performed on the respective molecule as discussed in ref 10. Energy values are relative to the lowest energy structure found. Only those values within 3.5 kcal/mol of ground state are tabulated.

Table X. Compound 12b[a]

energy, kcal/mol	dihedral angles, deg τ^1	τ^2	τ^3
0.00	57	102	66
0.21	45	104	66
1.30	-126	92	64
1.83	53	95	180
2.95	98	86	-49
3.38	-127	85	178

[a] The table gives the minimum energy conformations discovered in a conformational search performed on the respective molecule as discussed in ref 10. Energy values are relative to the lowest energy structure found. Only those values within 3.5 kcal/mol of ground state are tabulated.

Discussion

The fusion of the bicyclo[2.2.2]octane moiety to the carbons C_α and C_β of the tryptamine skeleton produces a bulkier potential ligand which may be less well tolerated at the binding sites than the parent. Moreover, the degree of tolerance may differ between binding site subtypes, and this will reflect the structural differences between the various subtypes. Comparison of the relative binding affinities for the present set of analogues with their stereochemistry and functional group arrangement gives a crude but informative picture of these binding sites.

The more active compounds share in common the endo stereochemistry. Although the differences were not significant in all cases, the overall trend is compelling. The endo isomers are characterized by having sp^2 hybridization at one (in the ketones **9a** and **10a**) or both (in the alkenes

Table XI. Compound 13a[a]

energy, kcal/mol	dihedral angle, deg		
	τ^1	τ^2	τ^3
0.00	37	93	-175
0.01	33	109	42
0.26*	38	105	47
0.38	34	97	-36
0.40*	28	107	41
0.84	124	95	-35
1.74	-136	88	-173
1.83	-139	94	39
1.88	-45	91	-36
1.91	-135	89	-33
1.98*	-139	96	40
2.50*	40	105	120
2.58*	37	92	-169
2.67*	30	93	-24
2.68*	29	93	164
3.23*	110	86	-33

[a] The table gives the minimum-energy conformational discovered in a conformational search performed on the respective molecule as discussed in ref 10. Energy values are relative to the lowest energy structure found. An asterisk indicates a structure in which the bicyclic moiety is attached in an axial fashion to the piperidine nitrogen. Only those values within 3.5 kcal/mol of ground state are tabulated.

Table XII. Compound 13b[a]

energy, kcal/mol	dihedral angle, deg		
	τ^1	τ^2	τ^3
0.00	51	102	47
0.24	38	104	48
0.31*	49	102	49
0.85	94	83	-37
1.45	-131	97	46
1.62*	-132	98	52
1.97	61	84	-39
2.05	56	98	141
2.23	49	86	-162
2.63*	43	104	123
2.69	24	81	-39
3.30	-122	82	140
3.30	-123	81	-36
3.40	-124	90	143
3.43	-125	92	140
3.47*	94	81	-32
3.54	-126	81	-163

[a] The table gives the minimum-energy conformations discovered in a conformational search performed on the respective molecule as discussed in ref 10. Energy values are relative to the lowest energy structure found. An asterisk indicates a structure in which the bicyclic moiety is attached in an axial fashion to the piperidine nitrogen. Only those values within 3.5 kcal/mol of ground state are tabulated.

12a and **13a**) of the carbons of the ethano bicyclic bridge syn to the amine. The affected carbons take on a planar geometry, which can have a 2-fold impact on binding. The van der Waals interactions between this two-carbon bridge and the α-methylene groups of the cyclic amine are diminished, and this is manifest in more flexibility of rotation for the amine substituent about the dihedral angle defined by the atoms C_α–C_β–N^+–H. Similarly, crowding interactions with the confining walls of the binding site should diminish as the carbons of the bridge become more planar.

It is not possible to differentiate cleanly between these effects at present, and both may operate simultaneously, but the first explanation can be examined semiquantitatively through conformational analysis. The idea that increased flexibility in the dihedral angle τ^3 is associated with stronger binding of these analogues at the $5HT^{1a}$ and $5HT_2$ sites is supported by the conformational data. Inspection of the Tables IV–XII reveals that the anti conformation of this angle (i.e. $\tau^3 \approx 180°$) is attainable at relatively low energies (0.00–0.35 kcal/mol) for the more active indole-substituted endo isomers **9a**, **10a**, **12a**, and **13a**. The phenyl-substituted compound **11** achieves this full extension of τ^3 at 0.62 kcal/mol. The less active indole-substituted exo isomers **9b**, **10b**, **12b**, and **13b** can attain an anti τ^3 only at relatively higher energies (1.53–2.23 kcal/mol).

The anti conformation of τ^3 cannot be the only important determinant of affinity, and there is support in the activity data for the influence of specific interactions (either positive or negative) between the ligand and the binding sites. In general, indolylethylamines display a lower affinity for $5HT_2$ sites than for $5HT_{1a}$ sites,[2b] but the compounds in the present series have a moderately higher affinity for the $5HT_2$ site. The exo ketone **10b** was more than 6 times weaker at the $5HT_2$ site than the related exo alkene **13b** even though both have a similarly restricted τ^3. There was a similar relationship between the exo ketone **9b** and the corresponding alkene **12b**, although with a 4-fold difference in this case. One explanation is that the carbonyl oxygen in the ketones **9b** and **10b** may project into a sterically encumbered zone of the binding site which is not violated when the alkenes **12b** or **13b** are bound. Alternatively the carbonyl in the exo isomers could introduce an unfavorable electrostatic interaction at the $5HT_2$ binding site. The activity at the $5HT_2$ site has been shown to improve with methyl group substitution on or adjacent to the amine nitrogen in serotonin; the ability of the $5HT_2$ site to tolerate side-chain branching is consistent with a higher relative affinity of the present analogues for that site.[2b] It has been observed that the binding of serotonin analogues to $5HT_2$ sites is somewhat insensitive to modification of the indole ring substitution pattern when [^{3}H]ketanserin is used as the radioligand,[2b] which also supports the idea of a more tolerant site. For whatever reason, the $5HT_2$ site appears to be less tolerant toward the analogues in the present series in the vicinity of their exo ethano bridge.

For the $5HT_{1a}$ site, both geometric isomers of the ketones (**9a,b** and **10a,b**) were as active as, or less so than, the weaker exo isomers of the alkenes (**12b** and **13b**). It is possible that at this binding site the carbonyl oxygen of both the endo and exo isomers is deleterious to activity for one of the reasons stated above. The fact that serotonin binds to the $5HT_{1a}$ site with approximately 10^4 greater affinity than the test compounds gives support for a very stringent steric and/or conformational demand for binding at this site. Thus the comparatively moderate affinity of the test compounds at the $5HT_{1a}$ site may be due to a poor tolerance for both the endo and exo ethano bridges of the bicyclic moiety, or to the relative conformational inflexibility of these molecules, i.e. that they are unable to achieve the conformation necessary for optimum binding.

The discrimination at the $5HT_{1a}$ site between geometric isomers was also manifest in the low Hill numbers found for three of the four exo compounds (**9b**, **12b**, and **13b**). The exo isomers do have less restriction than the endo isomers of the rotation about the angle τ^1 which governs the relative position of the aromatic ring (i.e. ergoline vs. non-ergoline). Nevertheless, no argument based only on conformation can explain the low Hill numbers for **9b**, **12b**, and **13b** and still account for the more normal behavior of **10b**. In the inhibition of 8-OH-DPAT binding, the low Hill numbers exhibited by compounds **9b**, **12b**, and **13b** as well as by serotonin indicate an interaction between these compounds and the $5HT_{1a}$ receptor that is more complex than competitive inhibition. One explanation for

the observed low Hill coefficients could be due to negative cooperativity displayed by the inhibitor in the binding to the receptor. Another possibility is the existence of distinct but interacting binding sites for the inhibitor and 8-OH-DPAT.

There was a complementary activity difference of small magnitude based on the size of the cyclic amine, where the pyrrolidine-substituted compounds were more active at the $5HT_{1a}$ site and the piperidine-substituted compounds were more active at the $5HT_2$ site. The difference was 2–3 times, except for the pair **10a**/**9a** at the $5HT_2$ site in which the difference was more than 6. The two corresponding N,N-disubstituted tryptamines [2-(3′-indolyl)ethyl]-pyrrolidine and [2-(3′-indolyl)ethyl]piperidine are both known to inhibit binding at 5HT binding sites.[13] The origin of the difference in activity observed here is not clear, whether it is due simply to the steric bulk of the cyclic amine, to the relative pK_a's or hydrogen bond donor ability of the respective $-N^+-H$ groups, or to a second-order effect on the local conformation in this portion of the molecule as discussed above.

In the lowest energy conformations of the active endo compounds the dihedral angle τ^1 (defined by the atoms $C_2-C_3-C_\alpha-C_\beta$) is approximately 35°, which corresponds to the non-ergoline-like skeleton described earlier. Ergoline-like conformations are achieved only at energies higher by about 1 kcal/mol.

Preliminary data on the ability of these compounds to inhibit the reuptake of serotonin indicate that they are active in the 0.5–10 μM range, but that there was much less stereoselectivity between the endo and exo isomers as compared to the $5HT_2$ and $5HT_{1a}$ site binding inhibition. These reuptake data also give additional support for the requirement in serotonin binding sites for an indole aromatic system, in that the phenyl-substituted adduct is significantly less active.

In summary, both the 8-OD-DPAT binding site and the ketanserin binding site are much less tolerant of a functionalized exo bridge than of a functionalized endo bridge in the bicyclic portion of these compounds. The ketanserin binding site discriminates far more between the endo and exo ketones, while the 8-OH-DPAT binding site discriminates more between the endo and exo alkenes. The phenyl-substituted adduct **11** was a relatively poor inhibitor in both assays, indicating that the presence of the indole ring is critical for significant binding. The examination of additional compounds designed as probes for binding-site structure is currently in progress, with the goal of formulating a clearer picture of the structure and binding requirements at the serotonin binding site subtypes and reuptake sites.

Experimental Section

General Procedures. Chemicals and solvents were used from commercial sources without purification unless otherwise noted. Methysergide was obtained from Sandoz Pharmaceutics, E. Hanover, NJ. Serotonin was obtained from Sigma Chemical Co., St. Louis, MO. [^{3}H]Ketanserin and [^{3}H]8-OH-DPAT were obtained from New England Nuclear, Boston, MA. Tetrahydrofuran (THF) was distilled immediately prior to use from sodium benzophenone under nitrogen. All products were purified until homogeneous in at least two TLC systems. Thin layer chromatography analyses were carried out with Analtech silica gel GF plates, 250-μM thickness; typical solvent systems include hexane/acetone (8:2), hexane/2-propanol (9:1), hexane/ethyl acetate/2-propanol (14:4:2). Infrared spectra were obtained on a Shimadzu 435 spectrometer. ^{1}H NMR spectra were determined at 90 MHz on a Varian EM390 spectrometer, and values are given in ppm downfield from tetramethylsilane standard in the following format: chemical shift (integration, multiplicity, coupling constant in hertz). ^{13}C NMR were determined at 22.5 MHz on a JEOL FX-90Q FT spectrometer, and values are given in ppm downfield from tetramethylsilane, with signals of double intensity indicated by (×2). High-resolution mass spectra were obtained through the *Mass Spectrometric Research Resource* of Rockfeller University with electron impact (EI) ionization [M^+] or hydroxyl ion negative (OH^-) ionization [$(M - 1)^-$].[14]

Indole-3-acetaldehyde (**1**) was prepared by a modification[15] of the published procedure:[16] a solution of 3.052 g (14.9 mmol) of DL-tryptophan in 100mL of water and 15 mL of 10% NaOH(aq) was prepared in a 2-L beaker. This solution was stirred mildly at room temperature with a mechanical stirrer, and 10 mL of 4 N HCl(aq) was added to bring the pH to 9–10 after the tryptophan had dissolved. The solution was diluted with 350 mL of water, 250 mL of brine, and 400 mL of benzene. The mixture was stirred and heated to 43–45 °C, and 200 mL of a 0.52% NaOCl(aq) solution (10% solution of commercial Chlorox) was added dropwise over a period of 30 min. Following the addition, another 100 mL of benzene was added, and the mixture was stirred vigorously for 35 min. Another 100 mL of benzene was added 5 min before the end of the stirring. The warm solution was transferred to a separatory funnel, and the layers were separated. The aqueous phase was extracted with 200 mL, 150 mL, and 100 mL of benzene. The combined benzene extracts were extracted with 75 mL of brine, dried (Na_2SO_4), and concentrated to give 1.765 g (74%) of crude **1** as a viscous yellow oil. This was purified by flash chromatography (44 g of silica, acetone/pentane, from 1:9 to 2:8) to yield 1.058 g (44%) of the known[16] **1** as a viscous yellow oil.

Phenylacetaldehyde (**2**) was prepared by a standard pyridinium chlorochromate oxidation of phenethanol.

The enamines (***E***)**-1-(3′-indolyl)-2-(*N*-pyrrolidinyl)ethene** (**5**), (***E***)**-1-(3′-indolyl)-2-(*N*-piperidinyl)ethene** (**6**), **and** (***E***)**-1-phenyl-2-(*N*-piperidinyl)ethene** (**7**) were prepared in quantitative yield following our published procedure.[4,6a] Spectral data for **6**: IR ($CHCl_3$) 3460 (s), 3400 (w), 3000 (m), 2930 (s), 2850 (m), 2810 (m), 1640 (s), 1450 (s), 1385 (s), 1110 (s) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.53 (6 H, br s), 2.60 (1 H, br s), 2.95 (2 H, br s), 5.54 (1 H, d, J = 16 Hz), 6.65 (1 H, d, J = 16 Hz), 6.85 (1 H, s), 7.02–7.29 (3 H, m), 7.78 (1 H, m). Spectral data for **7**: IR ($CHCl_3$) 3000 (m), 2920 (s), 2850 (m), 1730 (w), 1660 (m), 1630 (s), 1595 (m), 1450 (m), 1390 (m) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.45 (4 H, br s), 2.35 (2 H, br s), 2.82 (4 H, br s), 5.30 (1 H, d, J = 14 Hz), 5.28 (1 H, d, J = 14 Hz), 7.2 (5 H, br s).

The adducts (1(***S***,***R***),2(***R***,***S***),3(***R***,***S***),4(***S***,***R***))-***endo-trans***-**2-(3′-indolyl)-3-(*N*-pyrrolidinyl)bicyclo[2.2.2]octan-5-one** (**9a**), (1(***R***,***S***),2(***R***,***S***),3(***R***,***S***),4(***R***,***S***))-***exo-trans***-**2-(3′-indolyl)-3-(*N*-pyrrolidinyl)bicyclo[2.2.2]octan-5-one** (**9b**), (1(***S***,***R***),2(***R***,***S***),3(***R***,***S***),4(***S***,***R***))-***endo-trans***-**2-(3′-indolyl)-3-(*N*-piperidinyl)bicyclo[2.2.2]octan-5-one** (**10a**), (1(***R***,***S***),2-(***R***,***S***),3(***R***,***S***),4(***R***,***S***))-***exo-trans***-**2-(3′-indolyl)-3-(*N*-piperidinyl)bicyclo[2.2.2]octan-5-one** (**10b**), **and** (1(***S***,-***R***),2(***R***,***S***),3(***R***,***S***),4(***S***,***R***))-***endo-trans***-**2-phenyl-3-(*N*-piperidinyl)bicyclo[2.2.2]octan-5-one** (**11**) were prepared by the bicycloannulation reaction between the corresponding enamine and cyclohex-2-en-1-one (**8**) as was reported previously.[4] A new one-step procedure was also developed, detailed for the example of **9a**,**b**: a solution of 928 mg (5.83 mmol) of indole-3-acetaldehyde (**1**) in 25 mL of benzene was charged with 746 mg (10.5 mmol) of pyrrolidine (**3**) and 978 mg (9.97 mmol) of cyclohex-2-en-1-one (**8**), and this was heated to reflux under argon for 17 h. The volatiles were removed under vacuum, to produce 2.758 g of the crude product as a red oil. This was purified by flash chromatography (103 g of silica, petroleum ether/acetone, from 19:1 to

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7:3) to yield 838 mg (46%) of **9a**, 772 mg (43%) of a mixture of **9a**,**b**, and 221 mg (12%) of **9b** as red oils.[4]

Adducts **10a** and **10b** were prepared by the two-step procedure in 37% yield in a ratio of 57:43 as tan solids. Spectra data for **10a**: IR ($CHCl_3$) 3450 (s), 3400, (m br), 2920 (s), 2850 (m), 2800 (m), 1710 (s br), 1455 (s), 1420 (w), 1400 (w), 1335 (s), 1100 (s) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.42 (6 H, br s), 1.93 (1 H, br s), 2.35 (4 H, br s), 2.65 (4 H, m), 2.80 (2 H, m), 3.25 (2 H, br s), 6.83 (1 H, br s), 7.05–7.36 (3 H, m), 7.60 (1 H, m), 8.00 (1 H, m), 8.80 (1 H, br s); ^{13}C NMR ($CDCl_3$) δ 16.2, 24.5, 25.2, 25.8 (×2), 34.3, 39.8, 41.1, 46.5, 52.8 (×2), 65.0, 111.4, 118.5, 119.2 (×2), 121.0, 122.2, 126.4, 136.7, 217.8; high-resolution mass spectrum (EI) found 322.2049, calcd for $C_{21}H_{26}N_2O$ 322.204 51. Spectral data for **10b**: IR ($CHCl_3$) 3450 (s), 3350 (w), 3000 (m), 2920 (s), 2800 (m), 1710 (s br), 1450 (m), 1400 (w), 1380 (w), 1110 (m) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.38 (6 H, br s), 1.78 (1 H, br s), 2.09–2.62 (9 H, m), 2.85 (2 H, br s), 3.39 (1 H, br s), 7.00–7.29 (3 H, m), 7.31–7.55 (2 H, m), 7.7 (1 H, m), 9.02 (1 H, br s); ^{13}C NMR ($CDCl_3$) δ 19.3, 22.6, 24.6, 26.0 (×2), 34.1, 40.7, 45.9, 51.8, 53.6, 69.0, 111.5, 118.9 (×2), 119.2, 121.2, 122.2, 126.9, 136.9, 216.8; high-resolution mass spectrum (EI) found 322.2060, calcd for $C_{21}H_{26}N_2O$ 322.20451. Adduct 11 was obtained in 15% yield. Spectral data for 11: IR ($CHCl_3$) 3000 (m), 2920 (s), 2850 (m), 2800 (m), 1715 (s br), 1600 (w), 1450 (m), 1400 (w) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.40 (6 H, br s), 1.78 (3 H, br s), 2.13 (1 H, br s), 2.30 (5 H, br s), 2.76 (1 H, br s), 3.02 (4 H, br s), 7.30 (5 H, br s); ^{13}C NMR ($CDCl_3$) δ 18.6, 22.8, 24.5, 26.0 (×2), 36.1, 45.4 (×2), 49.1, 51.4 (×2), 67.7, 126.2, 127.7 (×2), 128.5 (×2), 144.2, 215.7; high-resolution mass spectrum (OH^-) found 282.1859, calcd for (M − 1) $C_{19}H_{24}NO$ 282.18579.

The alkenes **(1(*S*,*R*),2(*R*,*S*),3(*S*,*R*),4(*R*,*S*))-*endo*-*trans*-2-(3′-indolyl)-3-(*N*-pyrrolidinyl)bicyclo[2.2.2]oct-5-ene (12a)**, **(1(*R*,*S*),2(*R*,*S*),3(*S*,*R*),4(*S*,*R*))-*exo*-*trans*-2-(3′-indolyl)-3-(*N*-pyrrolidinyl)bicyclo[2.2.2]-oct-5-ene (12b)**, **(1(*S*,*R*),2-(*R*,*S*),3(*S*,*R*),4(*R*,*S*))-*trans*-2-(3′-indolyl)-3-(*N*-piperidinyl)bicyclo[2.2.2]oct-5-ene (13a)**, **and (1(*R*,*S*),2-(*R*,*S*),3(*S*,*R*),4(*S*,*R*))-*trans*-2-(3′-indolyl)-3-(*N*-piperidinyl)bicyclo[2.2.2]oct-5-ene (13b)** were obtained by the Bamford–Stevens reaction, as exemplified by the preparation of **13a**: A solution of 157 mg (0.48 mmol) of ketone **10a** in 18 mL of acetic acid was treated with 100 mg (0.53 mmol) of (*p*-tolylsulfonyl)hydrazine, and this was stirred at room temperature under argon for 49 h. The volatiles were removed under vacuum to give 311 mg of crude hydrazone, which was purified by chromatography (7.8 g of silica; petroleum ether/acetone, from 4:1 to 1:4) to yield 139 mg (61%) of the hydrazone of **10a** as a yellow foam: IR ($CHCl_3$) 3460 (m), 3400 (w), 3210 (m), 2950 (s), 2850 (m), 2480 (w), 1710 (m), 1595 (m), 1390 (m), 1335 (s), 1105 (s) cm^{-1}. The hydrazone (139 mg, 0.28 mmol) was dissolved in 15 mL of dry THF at room temperature under argon. This solution was charged with 1.66 mL (2.32 mmol) of methyllithium (1.4 M in ether), whereupon the reaction mixture changed color from orange to dark red. The solution was stirred at room temperature for 23 h, and was then diluted with 40 mL of THF and 5 mL of water. This mixture was extracted with a total of 50 mL of water and 40 mL of brine. The combined organic extracts were dried (Na_2SO_4) and concentrated to give 191 mg of the crude alkene as an orange foam. Chromatography (4.75 g of silica; petroleum ether/acetone, from 9:1 to 1:4) gave 152 mg of partially purified alkene. Preparative-layer chromatography of some mixed fractions yielded 84 mg (97% from the hydrazone, 59% overall) of alkene **13a** as a tan foam: IR ($CHCl_3$) 3460 (m), 2920 (s), 2850 (m), 2800 (w), 1455 (m), 1120 (w) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.45 (6 H, br s), 2.00 (2 H, m), 2.33 (4 H, br s), 2.70 (3 H, br s), 3.02 (3 H, br s), 6.08 (1 H, dd, J = 7.5, 7.5 Hz), 6.35 (1 H, dd, J = 7.5, 7.5 Hz), 6.9 (1 H, br s), 7.10–7.50 (3 H, m), 7.63–7.85 (1 H, m), 8.08 (1 H, br s); ^{13}C NMR ($CDCl_3$) δ 17.6, 24.6, 26.0 (×2), 26.2, 32.1, 37.8, 42.1, 53.6 (×2), 71.7, 111.1, 118.8, 119.1, 120.7, 121.8, 123.3, 126.7, 133.0, 135.3, 136.2; high-resolution mass spectrum (OH^-) calcd for (M − 1) $C_{21}H_{25}N_2$ 305.201 77, found 305.1994. Alkene **13b** was obtained in 33% overall yield as a tan solid: IR ($CHCl_3$) 3480 (m), 2930 (s), 2880 (m), 2800 (w), 1455 (m), 1100 (m) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.50 (6 H, br s), 2.16 (2 H, br s), 2.42 (4 H, br s), 2.65 (2 H, br s), 2.78–3.20 (3 H, m), 3.70 (1 H, br s), 6.25 (1 H, dd, J = 8, 8 Hz), 6.55 (1 H, dd, J = 8, 8 Hz), 7.00–7.50 (4 H, m), 7.65–7.90 (1 H, m), 8.46 (1 H, br s); ^{13}C NMR ($CDCl_3$) δ 19.3, 24.3, 25.5, 25.7 (×2), 32.6, 36.1, 39.7, 52.0 (×2), 70.5, 111.2, 118.7, 118.9 (×2), 121.1, 121.9, 127.2, 130.9, 134.5, 136.7; high-resolution mass spectrum (OH^-) calcd for (M − 1) $C_{21}H_{25}N_2$ 305.20177, found 305.2009. Alkene **12a** was obtained in 38% yield overall: IR ($CHCl_3$) 3450 (s), 3000 (m), 2950 (s), 2880 (m), 2800 (m), 1455 (m), 1330 (m), 1115 (m), 1090 (m) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.22 (4 H, m), 1.63 (4 H, br s), 2.10 (1 H, br s), 2.40 (4 H, br s), 2.80 (1 H, br s), 3.02 (2 H, br s), 6.15 (1 H, dd, J = 7.5, 7.5 Hz), 6.45 (1 H, dd, J = 7.5, 7.5 Hz), 6.85 (1 H, br s), 7.05–7.40 (3 H, m), 7.50–7.90 (1 H, m), 8.20 (1 H, br s); ^{13}C NMR ($CDCl_3$) δ 17.8, 23.4 (×2), 26.8, 35.1, 38.1, 42.0, 53.5 (×2), 72.8, 111.1, 118.9 (×2), 119.2 (×2), 120.8, 121.9, 133.3, 134.8, 136.4; high-resolution mass spectrum (OH^-) calcd for (M − 1) $C_{20}H_{23}N_2$ 291.186 12, found 291.1839. Alkene **12b** was obtained in 21% yield overall: IR ($CHCl_3$) 3450 (m), 2950 (s), 2885 (m), 2800 (w), 1455 (m), 1360 (m), 1115 (w), 1090 (w) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.20 (2 H, br s), 1.66 (4 H, br s), 2.05 (2 H, s), 2.55–3.25 (6 H, m), 3.50 (2 H, br s), 6.30 (1 H, dd, J = 7.5, 7.5 Hz), 6.6 (1 H, dd, J = 7.5, 7.5 Hz), 7.00–7.55 (4 H, m), 7.60–7.80 (1 H, m), 9.70 (1 H, br s); ^{13}C NMR ($CDCl_3$) δ 18.5, 23.0 (×2), 25.5, 34.2, 36.3, 38.3, 50.4 (×2), 66.8, 111.7, 116.4, 118.4, 121.8, 122.2, 126.8, 130.5, 135.6, 137.1.

The monodeuterated alkenes **(1(*S*,*R*),2(*R*,*S*),3(*S*,*R*),4(*R*,*S*))-*endo*-*trans*-6-deuterio-2-(3′-indolyl)-3-(*N*-pyrrolidinyl)bicyclo[2.2.2]oct-5-ene (16a) and (1(*R*,*S*),2-(*R*,*S*),3(*S*,*R*),4(*S*,*R*))-*exo*-*trans*-6-deuterio-2-(3′-indolyl)-3-(*N*-pyrrolidinyl)bicyclo[2.2.2]oct-5-ene (16b)** were prepared by Bamford–Stevens reaction on the corresponding deuterated ketones **(1(*S*,*R*),2(*R*,*S*),3(*R*,*S*),4(*S*,*R*))-*endo*-*trans*-6,6-dideuterio-2-(3′-indolyl)-3-(*N*-pyrrolidinyl)bicyclo[2.2.2]octan-5-one (14a) and (1(*R*,*S*),2(*R*,*S*),3(*R*,*S*),4(*R*,*S*))-*exo*-*trans*-6,6-dideuterio-2-(3′-indolyl)-3-(*N*-pyrrolidinyl)bicyclo[2.2.2]octan-5-one (14b)**, which in turn were synthesized by base-catalyzed deuterium exchange, as exemplified for the sequence **9a** → **14a** → **16a**: A solution of NaOD was prepared by dissolving 226 mg (9.83 mmol) of sodium in 10 mL of D_2O with stirring at room temperature for 30 min. This solution was transferred to a flask containing 608 mg (1.97 mmol) of ketone **9a**, and an additional 10 mL of D_2O was used to rinse in the solution of base. This mixture was heated gently for 30 min and then stirred at room temperature under argon for 12 h. The reaction mixture was extracted with a total of 200 mL of methylene chloride, and the combined organic extracts were dried (Na_2SO_4) and concentrated to give 411 mg (67%) of **14a** as a tan solid: ^1H NMR ($CDCl_3$) δ 1.30 (1 H, br s), 1.64 (6 H, br s), 2.03 (1 H, br d), 2.15–2.60 (5 H, m), 2.73 (2 H, br s), 3.38 (1 H, br s), 6.87 (1 H, br s), 7.09–7.50 (2 H, m), 7.52–7.78 (1 H, m), 8.07 (1 H, s), 9.00 (1 H, br s); ^{13}C NMR ($CDCl_3$) δ 16.6, 23.2 (×2), 25.7, 34.7, 39.9*, 41.5, 49.2, 52.8 (×2), 65.8, 111.4, 118.5, 119.1, 119.3, 120.9, 122.3, 126.9, 136.8, 217.3 (* = diminished intensity). A solution of 411 mg (1.32 mmol) of ketone **14a** in 12 mL of CH_3CO_2D was treated with 270 mg (1.45 mmol) of (*p*-tolylsulfonyl)hydrazine, and this was stirred at room temperature under argon for 40 h. The volatiles were removed under vacuum, to give 1.194 g of the crude hydrazone as a tan foam. Chromatography (24 g of silica, petroleum ether/ether, from 19:1 to 1:4) yielded 604 mg (95%) of pure hydrazone **15a** as a tan solid. This portion (1.26 mmol) was dissolved in 12 mL of dry THF at room temperature under argon and was treated with 4.60 mL (5.00 mmol) of methyllithium (1.4 M in ether), and the resulting mixture was stirred at room temperature for 48 h. The reaction mixture was transferred to a separatory funnel containing 50 mL of brine, and was extracted with a total of 200 mL of ether. The combined organic portions were dried (Na_2SO_4) and concentrated to yield 293 mg (79%) of the alkene **16a** was a red oil: ^1H NMR ($CDCl_3$) δ 1.23 (3 H, m), 1.49–1.79 (6 H, m), 2.44 (4 H, br s), 2.78 (1 H, s), 3.04 (1 H, s), 3.73 (1 H, m), 6.43 (1 H, m), 6.80 (1 H, s), 7.01–7.45 (2 H, m), 7.67 (1 H, m), 8.65 (1 H, br s); ^{13}C NMR ($CDCl_3$) δ 17.8, 23.2 (×2), 26.6, 34.9, 37.7, 37.9, 42.0, 53.5 (×2), 72.8, 111.1, 118.7, 118.9, 120.8, 121.7, 122.5, 127.0, 133.2, 133.4, 136.2. The deuterated ketone **14b** was obtained in 81% yield as a viscous oil: ^1H NMR ($CDCl_3$) δ 1.23 (1 H, m), 1.62 (4 H, br s), 1.84 (4 H, br s), 2.01–2.61 (5 H, m), 2.78 (2 H, m), 3.41 (1 H, br s), 7.01–7.50 (4 H, m), 7.52–7.56 (1 H, m), 9.93 (1 H, br s); ^{13}C NMR ($CDCl_3$) δ 19.1, 21.6, 23.1 (×2), 34.2, 41.8, 42.2*, 48.2, 52.1 (×2), 69.2, 111.5, 118.2, 118.5, 119.2, 121.2, 122.1, 127.1, 136.8, 216.0 (* = diminished intensity). The deuterated alkene **16b** was obtained in 85% yield as a red oil: ^1H NMR ($CDCl_3$) δ 0.81 (2 H, m), 1.20 (2 H, br s), 1.35–2.00 (4 H,

m), 2.20–2.73 (4 H, br s), 2.77–3.10 (2 H, m), 3.70 (2 H, m), 6.30 (1 H, d, J = 5.5 Hz), 6.98–7.50 (4 H, m), 7.68 (1 H, m), 8.65 (1 H, br s); ^{13}C NMR ($CDCl_3$) δ 18.8, 22.9 (×2), 24.7, 34.8, 35.9, 41.2, 52.1 (×2), 70.2, 111.1, 118.2, 118.5, 118.9, 121.1, 121.8, 127.3, 130.5, 136.5; high-resolution mass spectrum (CI) calcd for (M + 1) $C_{20}H_{24}N_2D$ 294.20795, found 294.2119.

Ketanserin Binding Assay. The binding of [^{3}H]ketanserin to $5HT_2$ receptors was conducted predominantly as described by Leysen et al.,[3a] with modification. Cerebral cortical tissue, isolated from adult BALB/cBy mice, was homogenized in 10:1 (v/w) ice-cold 0.25 M sucrose (pH 7.4) with a Brinkman polytron at a setting of 6 for 15 s and centrifuged at 1000g for 10 min. The supernatant (S_1) was removed with a pipet and diluted 1:40 (w/v) in Tris buffer (50 mM Tris-HCl, pH 7.4). This suspension was centrifuged at 35000g for 10 min. The resulting pellet was then washed once by resuspension with a polytron in an identical volume of Tris buffer and centrifuged at 35000g for 10 min. This final pellet was again resuspended, with a polytron, in Tris buffer.

One-milliliter aliquots of the membrane preparation were incubated in triplicate at 37 °C for 15 min with 0.15 nM [^{3}H]-ketanserin in the presence or absence of varying concentrations of indolylethylamine analogues in a final volume of 2 mL (Tris buffer). The incubation was terminated by the addition of 5 mL of ice-cold Tris buffer and filtration was effected with a cell harvester (Brandel) through Whatman GF/B glass-fiber filters presoaked with Tris buffer. Two 5-mL washes were used to rinse through the filters. Radioactivity was measured by scintillation spectroscopy with an efficiency of approximately 50%. Protein concentrations were determined by the method of Lowry et al.[17] Nonspecific binding of [^{3}H]ketanserin was defined with unlabeled methysergide at a final concentration of 1 μM.

8-Hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) Binding Assay. Tissue of the hippocampus, isolated from adult BALB/cBy mice, was homogenized in 40 volumes (v/w) of ice-cold 50 mM Tris-HCl (pH 7.4) using a Brinkman polytron at a setting of 4.5 for 5 s, and was centrifuged at 40000g for 20 min. The pellet was washed twice by resuspension in an identical volume of Tris with a polytron and was centrifuged at 40000g for 20 min. After the second wash and subsequent resuspension the tissue was incubated for 10 min at 37 °C to remove endogenous serotonin. After incubation the tissue was washed twice more before final resuspension in Tris buffer (method of Hall et al.).[18]

Aliquots of 100 μL of the membrane preparation were incubated in triplicate at 23 °C for 30 min (method of Cossery et al.)[19] with 1.2 nM [^{3}H]8-OH-DPAT in the presence or absence of varying concentrations of indolylethylamine analogues in a final volume of 0.5 mL of Tris buffer. The incubation was terminated by the addition of 5 mL of ice-cold Tris buffer and filtration was effected through a Whatman GF/B glass fiber filter, pretreated with 0.05% polyethylenimine. The incubation tube and the filter were each rinsed once with 5 mL of Tris buffer. Radioactivity and protein content were determined as described above. The nonspecific binding of [^{3}H]8-OH-DPAT was defined with unlabeled serotonin at a final concentration of 10 μM.

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Synthesis of Peptidyl Fluoromethyl Ketones and Peptidyl α-Keto Esters as Inhibitors of Porcine Pancreatic Elastase, Human Neutrophil Elastase, and Rat and Human Neutrophil Cathepsin G

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Comparison of MeO-Suc-Val-Pro-Phe-CO_2Me (**29**) and MeO-Suc-Ala-Ala-Pro-Phe-CO_2Me (**25**) with their corresponding trifluoromethyl ketones **9a** and **9b**, respectively, in rat and human neutrophil cathepsin G assays showed the α-keto esters to be more potent inhibitors. Likewise, Ac-Pro-Ala-Pro-Ala-CO_2Me (**21**) was more potent than its corresponding trifluoromethyl ketone (**9c**) in both porcine pancreatic elastase and human neutrophil elastase assays. Within a set of Ala-Ala-Pro-Val-CF_3 elastase inhibitors, the carbobenzyloxy (Cbz) N-protecting group conferred greater potency as a P_5 site recognition unit for elastase than did dansyl, methoxysuccinyl, or *tert*-butyloxycarbonyl. Initial inhibition of elastase was greater when trifluoromethyl ketone **9f** was added from a stock solution of dimethyl sulfoxide than when it had been buffer-equilibrated prior to assay, which suggests that the nonhydrated ketone is the more effective form of the inhibitor. The most potent elastase inhibitor we report is N^{α}-(Ad-SO_2)-N^{ϵ}-(MeO-Suc)Lys-Pro-Val-CF_3 (**16**) which has a K_i of 0.58 nM.

Replacement of the scissile amide unit of proteolytic enzyme substrate analogues by atom assemblies containing electrophilic carbonyl groups is a relatively new approach to proteinase inhibition effected through transition-state mimicry. This approach is effective for cysteine, serine, and aspartyl proteases. We[2] and others[3-13] have employed

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