Synthesis and Serotonergic Pharmacology of the Enantiomers of 3-[(N-Methylpyrrolidin-2-yl)methyl]-5-methoxy-1H-indole: Discovery of Stereogenic Differentiation in the Aminoethyl Side Chain of the Neurotransmitter Serotonin

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The use of conformationally restricted analogs of biologically relevant molecules represents a potentially powerful tool which can be used to examine the molecular recognition elements of the enzymes or receptors which bind those molecules. This approach is especially relevant when a single, small molecule binds to a number of similar receptors or enzymes. The directionality of the electronic and steric characteristics of the small biomolecule can be "frozen" via synthetic organic chemistry. One can then use these "unnatural products" to probe the members of a receptor or enzyme family to ascertain the specific molecular recognition requirements of a single receptor or enzyme subtype. The knowledge gained from such a study can then be used to design novel pharmaceutical agents for treatment of diseases related to dysfunction of a specific receptor or enzyme subtype.

Since the family of serotonin [3-(2-aminoethyl)-5-hydroxyindole, 5-hydroxytryptamine, 5-HT] receptors consists of at least three major receptor families each with a number of individual subtypes,[1] there is opportunity to utilize conformationally or rotationally restricted analogs of serotonin to examine the specific binding requirements of individual 5-HT receptor subtypes. In our laboratories, we have been involved in such studies.[2] The design and synthesis of 3-(1,2,5,6-tetrahydropyrid-4-yl)pyrrolo[3,2-b]pyrid-5-one (CP-93,129) led to a selective agonist for the 5-HT$_{1B}$ receptor via rotational restriction of the C5-

hydroxy substituent. This work defined important molecular recognition requirements specific for the 5-HT$_{1B}$ receptor.[2a] Alternatively, we have synthesized a number of analogs of serotonin with the C3-(2-aminoethyl) side chain restricted via incorporation of the atoms of that side chain into variously sized rings.[2c] In this communication, we present the results involving the synthesis and initial pharmacology of the individual enantiomers of 5-methoxy-3-[(N-methylpyrrolidin-2-yl)methyl]indole (1), a conformationally restricted analog of serotonin. In 1, the C-N bond of the aminoethyl side chain of serotonin has been conformationally restricted into a pyrrolidine ring, and using 1 we can study the pharmacological consequences of this introduction of a stereogenic center α to the basic nitrogen in serotonin.

Chemistry. Due to the commercial availability of the individual enantiomers of CBZ-proline, we envisioned the enantiomers of 1 as arising from *d*- and *l*-proline. The synthesis of the enantiomers of 1 is outlined in Scheme I. Reaction of the magnesium salt of 5-methoxyindole (*two* equivalents) in benzene with either (*R*)- or (*S*)-CBZ-proline acid chloride[3] in benzene yielded the *R*- or *S*-ketones (2), respectively, in excellent yield (>70%). The use of two equivalents of the indole-magnesium salt is key to achieving satisfactory results in this reaction because one equivalent is needed to act as an equilibrating base with 2 during the course of the reaction. Complete reduction of both the ketone and the carbamate functionalities in 2 was smoothly and quantitatively accomplished with lithium aluminum hydride in refluxing tetrahydrofuran to afford the desired enantiomers 1R and 1S.[4] The use of NMR spectroscopy of chiral salts [i.e. (*S*)-(+)-1,1'-binaphthalene-2,2'-diyl hydrogen phosphate in CDCl$_3$][5] of 1R and 1S and the measurement of their individual optical rotations convinced us that no racemization had occurred in the transformation of the individual enantiomeric CBZ-prolines to 1R and 1S.

Pharmacology. Table I outlines the receptor binding data[6] of 5-HT, 1R, 1S, and 5-methoxy-3-[2-(dimethylamino)ethyl]indole (4), a serotonin analog with the basic

(1) For a review of the pharmacology of serotonin receptors, see: Schmidt, A. W.; Peroutka, S. J. 5-Hydroxytryptamine receptor "families". *FASEB J.* 1989, 3, 2242-2249.

(2) (a) Macor, J. E.; Burkhart, C. A.; Heym, J. H.; Ives, J. L.; Lebel, L. A.; Newman, M. E.; Nielsen, J. A.; Ryan, K.; Schulz, D. W.; Torgersen, L. K.; Koe, B. K. 3-(1,2,5,6-Tetrahydropyrid-4-yl)pyrrolo[3,2-b]-pyrid-5-one: A Potent and Selective Serotonin (5-HT$_{1B}$) Ligand and Rotationally Restricted Phenolic Analog of 5-Methoxy-3-(1,2,5,6-tet-rahydropyrid-4-yl)indole. *J. Med. Chem.* 1990, *33*, 2087-2093. (b) Macor, J. E.; Newman, M. E. Synthesis of a Rotationally Restricted Phenolic Analog of 5-Methoxy-3-(1,2,5,6-tetrahydropyrid-4-yl)indole (RU-24969). *Heterocycles* 1990, *31*, 805-809. (c) Macor, J. E.; Ryan, K. Synthesis of Some Conformationally Restricted Serotonin Analogs. *Heterocycles* 1990, *31*, 1497-1504. (d) Macor, J. E.; Newman, M. E. Synthesis of a Dihydropyrano[3,2-e]indole as a Rotationally Restricted Phenolic Analog of the Neurotransmitter Serotonin. *Tetrahedron Lett.* 1991, *32*, 3345-3348. (e) Macor, J. E.; Ryan, K.; Newman, M. E. The Synthesis of Pyrano-[2,3-e]indoles and Pyrano[2,3-f]indoles as Rotationally Restricted Analogs of the Neurotransmitter Serotonin. *Tetrahedron* 1992, *48*, 1039-1052. (f) Macor, J. E.; Fox, C. B.; Johnson, C.; Koe, B. K.; Lebel, L. A.; Zorn, S. H. 1-(2-Aminoethyl)-6-methyl-8,9-dihydropyrano[3,2-e]indole: A rotationally Restricted Phenolic Analog of the Neurotransmitter Serotonin and Agonist Selective for Serotonin (5-HT$_2$) Receptors. *J. Med. Chem.* 1992, *35*, 3625-3632.

(3) Aoyama, T.; Shioiri, T. New Methods and Reagents in Organic Synthesis. 17. Trimethylsilyldiazomethane (TMSCHN$_2$) as a Stable and Safe Substitute for Hazardous Diazomethane. Its application to the Arnst-Eisert Synthesis. *Chem. Pharm. Bull.* 1981, *29*, 3249-3255.

(4) (a) Physical and spectral characteristics of 1R: mp 54.0-57.0 °C; IR (CHCl$_3$) 3475, 1625, 1585, 1480, 1455 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 8.38 (br s, NH), 7.22 (d, J = 8.8 Hz, 1 H), 7.05 (d, J = 2.4 Hz, 1 H), 6.96 (d, J = 2.2 Hz, 1 H), 6.85 (dd, J = 2.4 and 8.8 Hz, 1 H), 3.86 (s, 3 H), 3.19-3.11 (m, 2 H), 2.59 (dd, J = 9.7 and 13.8 Hz, 1 H), 2.51-2.45 (m, 1 H), 2.48 (s, 3 H), 2.27-2.18 (m, 1 H), 1.90-1.72 (m, 2 H), 1.71-1.51 (m, 2 H); ^{13}C NMR (CDCl$_3$) δ 153.8, 131.5, 128.2, 122.8, 113.7, 111.8, 111.8, 101.1, 66.6, 57.5, 56.0, 40.8, 31.5, 30.0, 21.9; LRMS *m/z* (relative intensity) 244 (M$^+$, 7), 160 (20), 145 (16), 117 (21), 84 (100); HRMS calcd for C$_{15}$H$_{20}$N$_2$O 244.1573, found 244.1547; [α]^{25}$_D$ = +100° [*c* = 1, CHCl$_3$]. Anal. Calcd for C$_{15}$H$_{20}$N$_2$O: C, 73.74; H, 8.25; N, 11.47. Found: C, 73.68; H, 8.21; N, 11.33. (b) Physical and spectral characteristics for 1S are identical to those described above for 1R except: [α]^{25}$_D$ = -98° [*c* = 1, CHCl$_3$]. HRMS calcd for C$_{15}$H$_{20}$N$_2$O 244.1573, found 244.1575. Anal. Calcd for C$_{15}$H$_{20}$N$_2$O: C, 73.74; H, 8.25; N, 11.47. Found: C, 73.39; H, 8.21; N, 11.27.

(5) The ^{1}H NMR spectrum (300 MHz, CDCl$_3$) of a 1:1 salt of racemic 1 (formed by the mixture of equal parts of 1R and 1S) and (*S*)-(+)-1,1'-binaphthalene-2,2'-diyl hydrogen phosphate showed two N-CH$_3$ resonances of equal intensity at δ 2.13 and 2.19. The ^{1}H NMR spectrum (300 MHz, CDCl$_3$) of the 1:1 salt of 1S and (*S*)-(+)-1,1'-binaphthalene-2,2'-diyl hydrogen phosphate contained a single N-CH$_3$ resonance at δ 2.13, while the ^{1}H NMR spectrum (300 MHz, CDCl$_3$) of the 1:1 salt of 1R and (*S*)-(+)-1,1'-binaphthalene-2,2'-diyl hydrogen phosphate contained a single N-CH$_3$ resonance at δ 2.19. Within the limits of detection (>95% ee) only one enantiomer could be seen in the ^{1}H NMR spectra of the chiral salts of 1R and 1S. The experimental details as well as copies of the NMR spectra are available as supplementary material to this paper.

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Scheme I
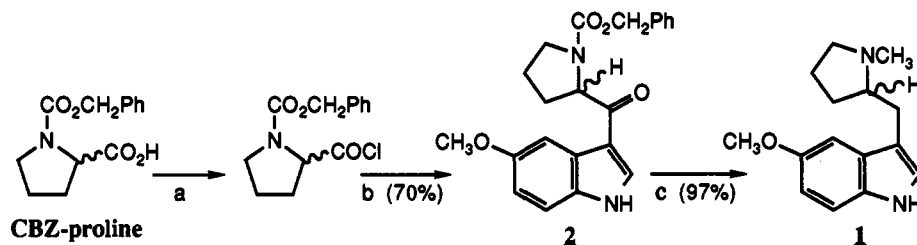
a = excess oxalyl chloride in methylene chloride at rt
b = 1) EtMgBr, 5-methoxyindole, benzene, rt; 2) CBZ-proline acid chloride in benzene
c = excess LiAlH₄, THF, Δ

Table I. Receptor Binding Data

compd	IC_{50},[a] nM					
	5-HT$_{1A}$	5-HT$_{1B}$	5-HT$_{1C}$	5-HT$_{1D}$	5-HT$_2$ [³H-Ket]	5-HT$_2$ [¹²⁵I-DOI]
5-HT	5.2 ± 1.5 [15]	5.0 ± 1.7 [12]	81 ± 30 [3]	3.0 ± 0.3 [3]	3600 ± 1800 [5]	20 ± 1 [4]
1S	140 ± 70 [4]	5800 ± 1100 [3]	1200 ± 130 [4]	420 ± 50 [5]	>8400 [3]	680 ± 70 [3]
1R	10 ± 7 [4]	830 ± 300 [4]	33 ± 5 [3]	24 ± 2 [4]	730 ± 110 [3]	17 ± 2 [3]
4	15 ± 7 [3]	550 ± 40 [3]	250 ± 70 [3]	49 ± 5 [4]	1900 ± 200 [3]	60 ± 11 [3]

[a] The number in brackets is the number of experiments; X ± SEM.

amine and C5-hydroxy groups fully methylated. The binding affinity of the enantiomer which was derived from natural (S)-proline (1S) was significantly less potent than that of 5-HT or 4 at all serotonin receptors tested (5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1C}$, 5-HT$_{1D}$, and 5-HT$_2$). These results suggest that the pyrrolidine ring of 1S was placed in a disadvantageous position in 5-HT receptors for potent binding to those receptors. However, the potency of 1R, derived from the unnatural (R)-proline, was completely comparable with that of 5-HT and 4 at 5-HT$_{1A}$, 5-HT$_{1C}$, and 5-HT$_2$ receptors. Additionally, 1R was only slightly less active than 5-HT itself at the 5-HT$_{1D}$ receptor. Only at the 5-HT$_{1B}$ receptor was 1R poor in its binding.[7] This indicates that, in 5-HT$_{1A}$, 5-HT$_{1D}$, 5-HT$_{1C}$, and 5-HT$_2$ receptors, the pyrrolidine ring in 1R must be able to occupy a position that is (at worst) not deleterious to binding, and (at best) possibly augments the energy of binding via some synergistic interaction with 5-HT receptors.

Activation of 5-HT$_1$ receptors (i.e. 5-HT$_{1A}$ and 5-HT$_{1D}$)[8] by agonists is coupled to the inhibition of adenylate cyclase activity.[1] The agonist nature of 1R can be seen in Figure 1, which depicts the ability of 1R to inhibit forskolin stimulated adenylate cyclase activity at 5-HT$_{1A}$ and 5-HT$_{1D}$ receptors. Both the potency and efficacy of 1R are comparable to serotonin in its ability to inhibit adenylate cyclase activity at 5-HT$_1$ receptors (Figure 1). These data indicate that, at 5-HT$_{1A}$ and 5-HT$_{1D}$ receptors, 1R mimics the agonist activity and efficacy of serotonin.

Activation of 5-HT$_2$ receptors by serotonin and 5-HT$_2$ receptor agonists induces hydrolysis of phosphatidyl-
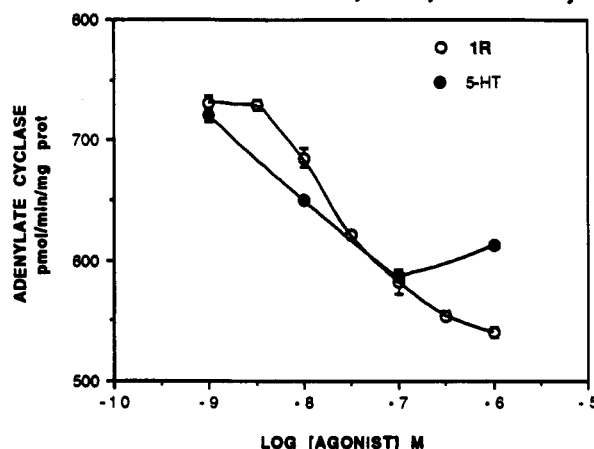
(6) Receptor binding was performed using standard methods as previously described in ref 2a, except for the 5-HT$_2$ receptor binding assay using DOI, which was done according to the methods described previously: McKenna, D. J.; Peroutka, S. J. Differentiation of 5-Hydroxytryptamine₂ Receptor Subtypes Using ¹²⁵I-R-(-)-2,5-Dimethoxy-4-iodo-phenylisopropylamine and ³H-Ketanserin. *J. Neurosci.* **1989**, *9*, 3482–3490. The aforementioned reference also describes the difference between the use of [¹²⁵I]DOI and [³H]ketanserin for binding experiments designed to study 5-HT$_2$ receptors. A more detailed description of receptor binding studies is contained in the supplementary material for this paper.
(7) The affinity of 1R at 5-HT$_3$ or 5-HT$_4$ receptors was not studied during this course of this work.
(8) The 5-HT$_{1C}$ receptor appears to be like a 5-HT$_2$-type receptor because of its functional link to phosphatidylinositol hydrolysis. For a further discussion of this, see: Hartig, P. R. Molecular biology of 5-HT receptors. *Trends Pharm. Sci.* **1989**, *10*, 64–69.
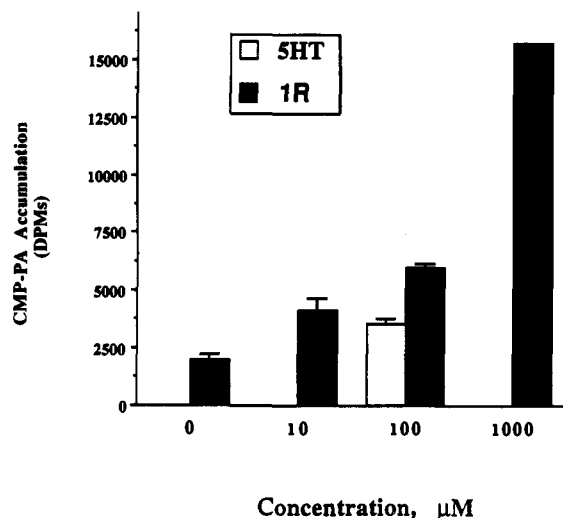
Figure 1a. 5-HT$_{1A}$ Adenylate Cyclase Activity

Figure 1b. 5-HT$_{1D}$ Adenylate Cyclase Activity
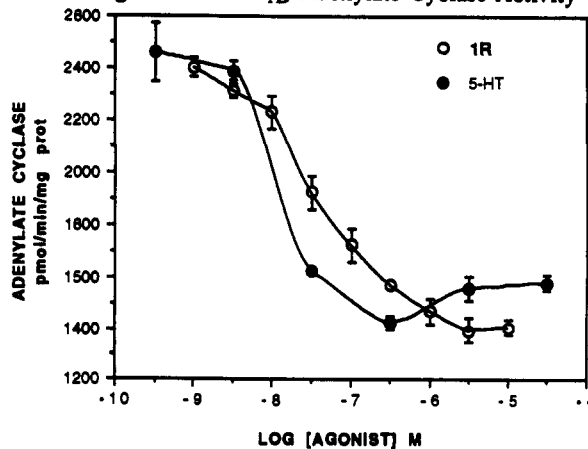
Figure 1. (a) Effects of forskolin-stimulated adenylate cyclase activity at the 5-HT$_{1A}$ receptor (guinea pig hippocampus): 5-HT EC_{50} = 10 ± 1 nM and 1R EC_{50} = 35 ± 11 nM. (b) Effects of forskolin-stimulated adenylate cyclase activity at the 5-HT$_{1D}$ receptor (guinea pig substantia nigra): 5-HT EC_{50} = 5.2 ± 0.9 nM and 1R EC_{50} = 43 ± 9 nM. Data above show mean ± SEM values for triplicate determinations within a single representative experiment. Forskolin (3 μM) elevated basal enzymatic activity 6- and 13-fold at the 5-HT$_{1A}$ and 5-HT$_{1D}$ receptors, respectively. Activity in the presence of forkolin alone was 700 and 2440 pmol/mg of protein per min, respectively.

Figure 2. Hydrolysis of phosphatidylinositol by 5-HT and 1R in rat cerebral cortical slices as measured by the accumulation of CMP-PA ([³H]cytidine monophosphate-phosphatidate). Data above show mean ± SEM values for triplicate determinations within a single representative experiment.

inositol (PI) in rat brain cerebral cortical slices.[1] Figure 2 depicts the effects of serotonin and 1R on PI turnover in rat cortical slices. The ability of 1R to induce PI turnover (as shown by the accumulation of [³H]cytidine monophosphate-phosphatidate)[9] appears to be dose dependent (Figure 2), and the response observed with 1R (10 μM) is equivalent to that of serotonin at 100 μM. These preliminary results suggests that 1R mimics the agonist action of serotonin at cortical 5-HT₂ receptors. However, in this system the maximally efficacious response of serotonin occurs at a dose of approximately 100 μM, while 1R has a much greater response than 5-HT. Studies using 5-HT₂ antagonists are in progress to determine whether this response to 1R in cortical slices is truly a 5-HT₂ mediated event.

Discussion. (R)-5-Methoxy-3-[(N-methylpyrrolidin-2-yl)methyl]indole (1R, CP-108,509) binds to 5-HT₁A, 5-HT₁C, and 5-HT₂ receptors as potently as the neurotransmitter itself. At 5-H₁D receptors, 1R is only slightly less potent than the natural substrate. Furthermore, 1R mimics the action of serotonin as an agonist at 5-HT₁A, 5-HT₁D, and cortical 5-HT₂ receptors. These results indicate that the 3-[(N-methylpyrrolidin-2-yl)methyl] group of 1R is a conformationally restricted, stereogenic surrogate for the 3-(2-aminoethyl) group of the neurotransmitter serotonin at 5-HT₁A, 5-HT₁D, 5-HT₁C, and 5-HT₂ receptors. The affinity of 1R for 5-HT₁B receptors was poor, suggesting that the 3-[(N-methylpyrrolidin-2-yl)methyl] group in 1R was not an adequate replacement for the 3-(2-aminoethyl) group in serotonin.[10] (S)-5-Methoxy-3-[(N-methylpyrrolidin-2-yl)methyl]indole (1S) has significantly less affinity for all 5-HT receptors (approximately 20-fold less) than either its R enantiomer or serotonin.

The fact that there is a stereogenic difference in binding between the enantiomers of 1 is significant because of the previous lack of such a finding in serotonin pharmacology. Titeler and co-workers found little difference in the affinities of the enantiomers of α-methyltryptamine at 5-HT₂ receptors.[11] Nichols and co-workers synthesized a series of 5-substituted-α-methyltryptamine enantiomeric pairs and found very little, if any, differences in binding to 5-HT₁B and 5-HT₂ receptors.[12] Therefore, to our knowledge, the stereogenic separation of affinity within the enantiomers of 1 is unique to serotonin pharmacology.

These observations suggest that (1) 1R can adopt (as one of its lowest energy conformers) the active conformation of the 3-(2-aminoethyl) side chain of serotonin in 5-HT₁A, 5-HT₁D, 5-HT₁C, and 5-HT₂ receptors, (2) the added bulk of the conformationally restricting pyrrolidine ring does not affect the binding of 1R in 5-HT₁A, 5-HT₁C, and 5-HT₂ receptors when compared to serotonin or 4, (3) the added bulk of the conformationally restricting pyrrolidine ring has only a small effect on the binding of 1R to 5-HT₁D receptors, and (4) the added bulk of the pyrrolidine ring in 1S is significantly deleterious to its affinity for all 5-HT receptors, especially when compared to its R enantiomer or serotonin.

The presence of the stereogenic center in 1 creates a profound effect on the binding affinity of 1R and 1S at serotonin receptors. It is remarkable that for all receptors tested, the R enantiomer (1R) was significantly more potent than the S enantiomer. This suggests that there exists within the binding site of these serotonin receptors certain common structural features which preferentially accommodate the (R)-3-[(N-methylpyrrolid-2-yl)methyl] group found in 1R. Therefore, this pair of compounds (1R and 1S) provides us with a novel tool with which we can gain insight into the ligand recognition phenomena in serotonin receptors. We are presently engaged in computational studies using these compounds and other 5-HT agonists as molecular guides to better define the specific binding requirements of the individual serotonin receptors.

In conclusion, (R)-5-methoxy-3-[(N-methylpyrrolidin-2-yl)methyl]indole (1R, CP-108,509) binds to 5-HT₁A, 5-HT₁C, and 5-HT₂ receptors with an affinity and efficacy which is comparable to that of the natural substrate, serotonin. 1R is only slightly less potent than serotonin at 5-HT₁D receptors. The S enantiomer of this compound is significantly less active than 1R at all of the 5-HT receptors tested. This pair of enantiomers demonstrates the first indication of stereogenic differentiation of ligands by serotonin (5-HT₁ and 5-HT₂) receptors. The (R)-3-[(N-methylpyrrolidin-2-yl)methyl] group in 1R is a conformationally restricted replacement for the 3-(2-aminoethyl) group in serotonin at 5-HT₁A, 5-HT₁D, 5-HT₁C, and 5-HT₂ receptors. The novel structure of 1R provides a potentially useful pharmacological tool which can be used to study the molecular recognition phenomena of individual serotonin receptors.

(9) The methodology for this study has been previously described; see: Godfrey, P. P. Potentiation by lithium of CMP-phosphatidate formation in carbachol-stimulated rat cerebral cortical slices and its reversal by myo-inositol. *Biochem. J.* **1989**, *258*, 621–624.

(10) Comparison of the affinities of 4 and 1R at 5-HT₁B receptors might suggest that the tertiary amine is responsible for the poor affinity of 1R for that receptor. However, it should be noted that the normethyl analog of 1R is equal to 1R in its poor affinity for the 5-HT₁B receptor (data not shown). This result suggests that the replacement of the 3-(2-aminoethyl) side chain of serotonin with the 3-[(N-methylpyrrolidin-2-yl)methyl] side chain contained in 1R is deleterious to 5-HT₁B receptor binding.

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