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

John E. Macor,* James Blake, Carol B. Fox, Celeste Johnson, B. Kenneth Koe, Lorraine A. Lebel, Jean M. Morrone, Kevin Ryan, Anne W. Schmidt, David W. Schulz, and Stevin H. Zorn

Departments of Medicinal Chemistry and Neuroscience Central Research Division Pfizer, Inc Groton, Connecticut 06340

Received July 31, 1992

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-tetrahydropyrid-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 Arndt-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}; ^1H 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 $C_{15}H_{20}N_2O$ 244.1573, found 244.1547; $[\alpha]^{25}_D$ = +100° [*c* = 1, $CHCl_3$]. Anal. Calcd for $C_{15}H_{20}N_2O$: 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: $[\alpha]^{25}_D$ = −98° [*c* = 1, $CHCl_3$]. HRMS calcd for $C_{15}H_{20}N_2O$ 244.1573, found 244.1575. Anal. Calcd for $C_{15}H_{20}N_2O$: C, 73.74; H, 8.25; N, 11.47. Found: C, 73.39; H, 8.21; N, 11.27.

(5) The ^1H 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 ^1H 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 ^1H 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 ^1H 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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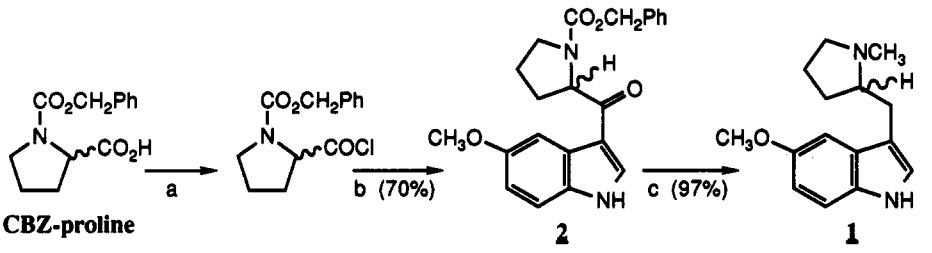
Scheme I



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 $LiAlH_4$, THF, Δ

Table I. Receptor Binding Data

	IC_{50},[a] nM					
compd	5-HT_{1A}	5-HT_{1B}	5-HT_{1C}	5-HT_{1D}	5-HT_2 [^{3}H-Ket]	5-HT_2 [^{125}I-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-Hydroxytryptamine$_2$ Receptor Subtypes Using ^{125}I-R-(−)-2,5-Dimethoxy-4-iodo-phenylisopropylamine and ^{3}H-Ketanserin. *J. Neurosci.* **1989**, *9*, 3482–3490. The aforementioned reference also describes the difference between the use of [^{125}I]DOI and [^{3}H]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

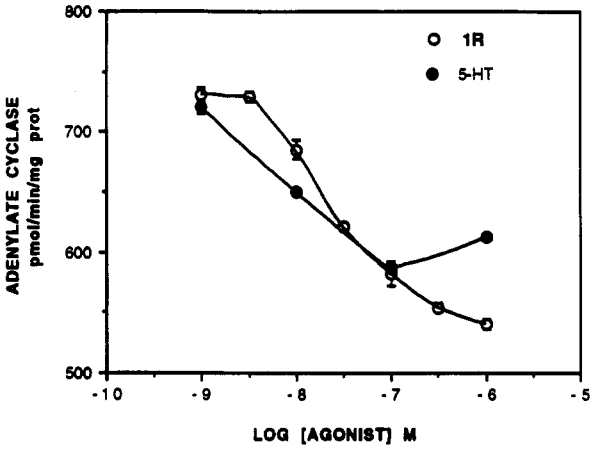


Figure 1b. 5-HT_{1D} Adenylate Cyclase Activity

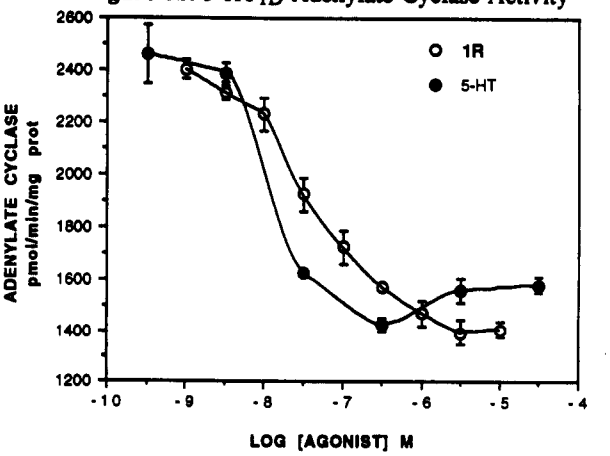


Figure 1. (a) Effects of forskolin-stimulated adenylate cyclase activity at the 5-HT_{1A} receptor (guinea pig hippocampus): 5-HT EC_{50} = 10 ± 1 nM and **1R** EC_{50} = 35 ± 11 nM. (b) Effects of forskolin-stimulated adenylate cyclase activity at the 5-HT_{1D} receptor (guinea pig substantia nigra): 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.

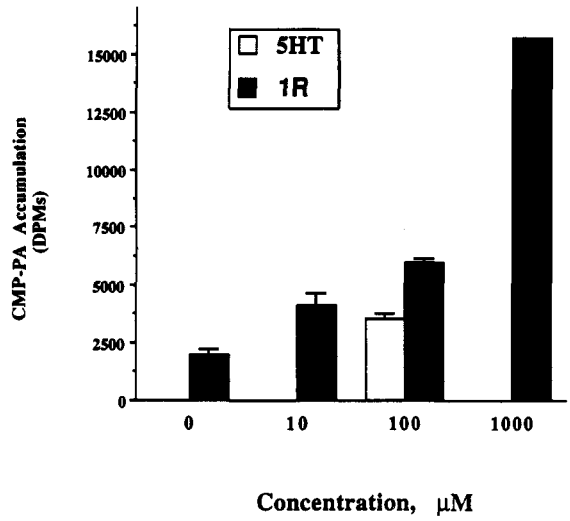


Figure 2. Hydrolysis of phosphatidylinositol by 5-HT and **1R** in rat cerebral cortical slices as measured by the accumulation of CMP-PA ([^{3}H]cytidine monophosphate-phosphatidate). Data above show mean ± SEM values for triplicate determinations within a single representative experiment.

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 [^{3}H]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-HT$_2$ 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-HT$_2$ antagonists are in progress to determine whether this response to **1R** in cortical slices is truly a 5-HT$_2$ mediated event.

Discussion. (*R*)-5-Methoxy-3-[(*N*-methylpyrrolidin-2-yl)methyl]indole (**1R**, CP-108,509) binds to 5-HT$_{1A}$, 5-HT$_{1C}$, and 5-HT$_2$ receptors as potently as the neurotransmitter itself. At 5-H$_{1D}$ receptors, **1R** is only slightly less potent than the natural substrate. Furthermore, **1R** mimics the action of serotonin as an agonist at 5-HT$_{1A}$, 5-HT$_{1D}$, and cortical 5-HT$_2$ 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-HT$_{1A}$, 5-HT$_{1D}$, 5-HT$_{1C}$, and 5-HT$_2$ receptors. The affinity of **1R** for 5-HT$_{1B}$ 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-HT$_2$ 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-HT$_{1B}$ and 5-HT$_2$ 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-HT$_{1A}$, 5-HT$_{1D}$, 5-HT$_{1C}$, and 5-HT$_2$ receptors, (2) the added bulk of the conformationally restricting pyrrolidine ring does not affect the binding of **1R** in 5-HT$_{1A}$, 5-HT$_{1C}$, and 5-HT$_2$ 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-HT$_{1D}$ 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-HT$_{1A}$, 5-HT$_{1C}$, and 5-HT$_2$ 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-HT$_{1D}$ 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-HT$_1$ and 5-HT$_2$) 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-HT$_{1A}$, 5-HT$_{1D}$, 5-HT$_{1C}$, and 5-HT$_2$ 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.

Supplementary Material Available: Details of the synthesis of **1** and **2** (including complete characterization), the NMR experiments on the chiral salts of **1R** and **1S**, and pharmacological experimental details (14 pages). Ordering information is given on any current masthead.

(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-HT$_{1B}$ 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-HT$_{1B}$ 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-HT$_{1B}$ receptor binding.

(11) Lyon, R. A.; Titeler, M.; Seggel, M. R.; Glennon, R. A. Indolealkylamine analogs share 5-HT$_2$ binding characteristics with phenylalkylamine hallucinogens. *Eur. J. Pharm.* **1988**, *145*, 291–297.

(12) Nichols, D. A.; Lloyd, D. H.; Johnson, M. P.; Hoffman, A. J. Synthesis and Serotonin Receptor Affinities of a Series of Enantiomers of α-Methyltryptamines: Evidence for the Binding Conformation of Tyrptamines of 5-HT$_{1B}$ Receptors. *J. Med. Chem.* **1988**, *31*, 1406–1412.