Synthesis And Evaluation of 4-(2′-Methoxyphenyl)-1-[2′-[N-(2″-pyridinyl)-p-iodobenzamido]ethyl]piperazine (p-MPPI): A New Iodinated 5-HT$_{1A}$ Ligand

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The serotonin system in the brain is an important neurotransmission network that regulates various physiological functions and behavior including anxiety and affective states.[1,2] One of the serotonin receptor subtypes, 5-HT$_{1A}$, plays an important function as the somatodendritic autoreceptor (presynaptic) in the dorsal raphe nucleus and as a postsynaptic receptor for 5-HT in terminal field areas. A large number of agonists and antagonists for 5-HT$_{1A}$ receptors are reported in the literature.[3-5] Development of radioiodinated antagonists that are selective for specific 5-HT$_{1A}$ subtypes may facilitate the study and characterization of this binding receptor subtype. On the basis of 8-hydroxy-2-(*N*,*N*-di-*n*-propylamino)tetralin (8-OH-DPAT), the most well-studied potent 5-HT$_{1A}$ agonist,[6,7] we have reported the synthesis, radiolabeling, and *in vitro* binding study of its close structural analog, [^{125}I](*R*,*S*)-*trans*-8-hydroxy-2-[*N*-*n*-propyl-*N*-(3′-iodo-2′-propenyl)amino]tetralin, (*R*,*S*)-*trans*-8-OH-PIPAT (K_d = 0.38 nM ± 0.03 nM, B_{max} = 310 ± 20 fmol/mg of protein), as a selective radioiodinated ligand for the 5-HT$_{1A}$ receptor.[8] An ^{125}I-labeled radioligand is advantageous because of high specific activity (2200 Ci/mmol) and physical characteristics of the isotope ($T_{1/2}$ = 60 d, photon energy 35–60 keV). The corresponding ^{123}I-labeled radioligand (theoretical specific specific activity 240 000 Ci/mmol, $T_{1/2}$ = 13 h, γ energy 159 keV) may be potentially useful as imaging agents for single photon emission computed tomography (SPECT). The most widely studied 5-HT$_{1A}$ antagonist, based on arylpiperazine, is NAN-190, 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl]piperazine. NAN-190 displayed high 5-HT$_{1A}$ affinity (K_i = 0.6 nM) and an equal potency for the α_1 receptor.[4,9] Replacement of phthalimide moiety by substituted benzamides or acyl moieties provides ligands with high binding affinity and selectivity. One of such agents, 4-[4-(1-adamantanecarboxamido)butyl]-1-(2-methoxyphenyl)piperazine, was found to bind to 5-HT$_{1A}$ receptor with high affinity (K_d = 0.4 nM) and was devoid of binding affinity to other receptors.[4,5] Recently, a new arylpiperazine derivative, (*S*)-WAY 100135, *N*-*tert*-butyl-3-[4-(2-methoxyphenyl)-piperazin-1-yl]-2-phenylpropionamide, was reported as a selective antagonist at both somatodendritic and postsynaptic receptor (IC_{50} = 15 nM, rat hippocampal membranes).[10,11] A similar compound, WAY 100635, 4-(2′-methoxyphenyl)-1-[2′-[*N*-2″-pyridinyl)cyclohexanecarboxamido]ethyl]piperazine, **1f**, displayed even higher binding affinity (IC_{50} = 2.2 nM, rat hippocampal membranes) with high selectivity.[12]

Scheme 1

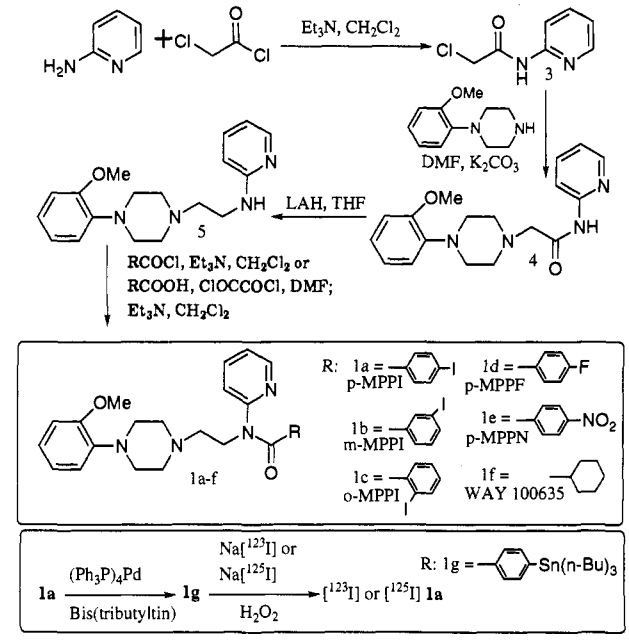

Table 1. Inhibition Constants of Compounds on the Binding of [^{125}-I]-8-OH-PIPAT to Rat Hippocampal Homogenates[8]

compound	K_i (nM)
1a (*p*-MPPI)[a]	2.6 ± 0.7
1b (*m*-MPPI)	1.7 ± 0.1
1c (*o*-MPPI)	10.4 ± 0.8
1d (*p*-MPPF)	3.3 ± 0.8
1e (*p*-MPPN)	1.6 ± 0.6
1f (WAY 100635)	0.84 ± 0.1

[a] K_d = 0.3 nM for [^{125}I]MPPI, **1a**, to rat hippocampal homogenates (Kung, unpublished data).

In order to develop radioiodinated antagonists for *in vitro* and *in vivo* evaluation of the 5-HT$_{1A}$ receptor, a series of new bezamido derivatives, 4-(2′-methoxyphenyl)-1-[2′-(*N*-2″-pyridinyl)halobenzamido]ethyl]piperazines, was prepared by Scheme 1. Synthesis of iodinated arylpiperazine derivatives was achieved by reactions described in Scheme 1. Three-step preparation of the key intermediate compound **5** was relatively straightforward and gave 81% overall yield. Coupling of the acyl groups with the arylpiperazine, **5**, was accomplished either with an acyl chloride in the presence of triethylamine or by the use of acids with oxalyl chloride in DMF. Both reactions gave good yields (50–90%). As listed in Table 1, high 5-HT$_{1A}$ binding affinity of *p*-, *m*-, *o*-MPPI, and *p*-fluoro- and *p*-nitro-MPP derivatives (**1a–f**) was observed (in rat hippocampal membrane preparations with [^{125}I](*R*,*S*)-*trans*-8-OH-PIPAT as the ligand). All of the five benzamide derivatives displayed similar potency (K_i = 1–3.3 nM) as that for the parent compound (K_i = 0.8 nM), WAY 100635, except *o*-MPPI, **1c**, which exhibited lower affinity (K_i = 10 nM). It is evident in this series of potential 5-HT$_{1A}$ antagonists that replacing the cyclohexyl group by an aromatic acyl group is feasible, except for the ortho-iodinated derivative.

To evaluate the *in vivo* biodistribution of these derivatives, radiolabeled [^{123}I]- or [^{125}I]-**1a** (*p*-MPPI), was prepared from the corresponding tri-*n*-butyltin precursor via an iododestannylation reaction with sodium [^{123}I] or [^{125}I]iodide (no-carrier-added) using an oxidative iodination method.[13] Biodistribution of the ^{123}I- or ^{125}I-labeled

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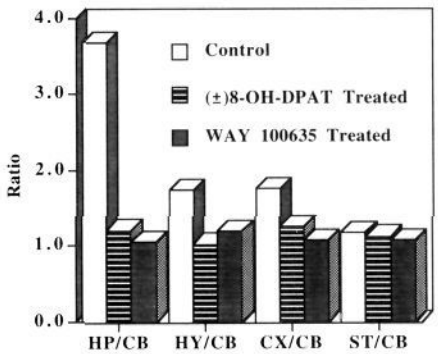

Figure 1. Ratio of regional brain uptake in rats: HP, hippocampus; CB, cerebellum; HY, hypothalamus; CX, cortex; ST, striatum. A tracer dose of [^{123}I]- or [^{125}I]-**1a** was injected in rats; at 30 min post iv injection rats were sacrificed and regional brain uptake (% injected dose/g of brain tissue) was determined. Ratios for each of the regions were calculated based on the % dose/g of each region divided by the same in CB. Blocking studies were performed in rats pretreated with (±)-8-OH-DPAT (2 mg/kg) or WAY 100635 (1 mg/kg), at 5 and 20 min, respectively, prior to the injection of the tracer.

agents (theoretical specific activity 240 000 and 2200 Ci/mmol, respectively) in rats displayed good initial brain uptake (total brain uptake was 1.22% dose/organ for **1a**, at 2 min after iv injection, n = 3–4). Uptake in hippocampal tissue, where the 5-HT$_{1A}$ receptor density is high, was 0.23 ± 0.007 and 0.127 ± 0.009% dose/g, at 30 and 60 min after the iv injection, respectively, while uptake in cerebellum, which contains no 5-HT$_{1A}$ receptor, showed 0.07 ± 0.006 and 0.04 ± 0.001% dose/g, respectively.

In order to further characterize the *in vivo* brain uptake of **1a** in rats, a blocking study was carried out to determine changes of the ratios of regional brain uptake (HP, hippocampus; CB, cerebellum; HY, hypothalamus; CX, cortex; ST, striatum) (Figure 1). A tracer dose of [^{123}I]- or [^{125}I]-**1a** was injected in rats; at 30 min post iv injection they were sacrificed and regional brain uptake (% injected dose/g of brain tissue, n = 3–4) was determined. Ratios for each of the regions were calculated based on the percent dose/g of each region divided by the same in CB. Blocking studies were performed in rats pretreated with (±)-8-OH-DPAT (2 mg/kg, iv) or WAY 100635 (1 mg/kg, iv), at 5 and 20 min prior to the injection of the tracer, respectively. With pretreatment of either an agonist, (±)-8-OH-DPAT, or an antagonist, WAY 100635, the specific uptake in hippocampus region of the brain displayed a marked decrease; the HP/CB ratio changed from 3.69 (0.203 and 0.055% dose/g for HP and CB in control rats) to 1.21 (0.074 and 0.062% dose/g for HP and CB in (±)-8-OH-DPAT treated rats) and 1.07 (0.066 and 0.061% dose/g for HP and CB in WAY 100635 treated rats). The dramatic decrease is most likely due to the competition of (±)-8-OH-DPAT or WAY 100635 binding to the same 5-HT$_{1A}$ receptor in the brain.

Additional pharmacological studies, such as *in vitro* binding with and without the presence of GTP and behavioral effects in animals, are needed to fully characterize this new series of benzamide derivatives of arylpiperazines. The potential antagonist, [^{125}I]MPPI, as well as the potential agonist, [^{125}I](*R,S*)-*trans*-8-OH-PIPAT, reported previously,[8] may provide excellent probes for the investigation and characterization of 5-HT$_{1A}$ receptors.[14] The corresponding ^{123}I- and ^{18}F- ($T_{1/2}$ = 110 min, γ energy 511 keV after positron annihilation) labeled **1a** and **1d**, respectively, may provide potentially useful ligands for *in vivo* imaging of the 5-HT$_{1A}$ receptor density in brain with single photon emission computed tomography and positron emission tomography.

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Supplementary Material Available: Experimental data for **1a–g**, **4**, and **5**; details of the preparation of [^{123}I]-**1a** and the biodistribution in rats; and tables of the biodistribution in rats after iv injection of [^{123}I]-**1a** (11 pages). Ordering information is given on any current masthead page.

References

(1) Frazer, A.; Maayani, S.; Wolfe, B. B. Subtypes of Receptors for Serotonin. *Annu. Rev. Pharmacol. Toxicol.* **1990**, *30*, 307–348.
(2) Frazer, A.; Hensler, J. G. 5-HT$_{1A}$ Receptors and 5-HT$_{1A}$-mediated Responses: Effect of Treatments that Modify Serotonergic Neurotransmission. *Ann. NY Acad. Sci.* **1990**, *600*, 460–475.
(3) Hillver, S. E.; Bjork, L.; Li, Y. L.; Svensson, B.; Ross, S.; Anden, N. E.; Hacksell, U. (S)-5-Fluoro-8-hydroxy-2-(dipropylamino)-tetralin: A Putative 5-HT$_{1A}$-receptor Antagonist [letter]. *J. Med. Chem.* **1990**, *33*, 1541–1544.
(4) Raghupathi, R. K.; Rydelek-Fitzgerald, L.; Teitler, M.; Glennon, R. A. Analogues of the 5-HT$_{1A}$ Serotonin Antagonist 1-(2-Methoxyphenyl)-4-[4-(2-phthalimido)butyl]piperazine with Reduced Alpha-adrenergic Affinity. *J. Med. Chem.* **1991**, *34*, 2633–2638.
(5) Glennon, R. A.; Naiman, N. A.; Lyon, R. A.; Titeler, M. Arylpiperazine Derivatives as High-affinity 5-HT$_{1A}$ Serotonin Ligands. *J. Med. Chem.* **1988**, *31*, 1968–1971.
(6) Hoyer, D.; Engel, G.; Kalkman, H. O. Molecular Pharmacology of 5-HT1 and 5-HT2 Recognition Sites in Rat and Pig Brain Membranes: Radioligand Binding Studies with [^{3}H]5-HT, [^{3}H]8-OH-DPAT, (-)[^{125}I]Iodocyanopindolol, [^{3}H]Mesulergine and [^{3}H]-Ketanserin. *Eur. J. Pharmacol.* **1985**, *118*, 13–23.
(7) Hjorth, S.; Carlsson, A.; Lindbery, P.; Sanchez, D.; Wikstrom, H.; Arvidsson, L. E.; Hacksell, U.; Nilsson, J. L. G. 8-Hydroxy-2-(di-n-propylamino)tetralin 8-OH-DPAT, A Potent and Selective Simplified Ergot Congener with Central 5-HT Receptor Stimulating Activity. *J. Neural Transmission* **1982**, *55*, 169–188.
(8) Zhuang, Z. P.; Kung, M. P.; Kung, H. F. Synthesis of (R,S)*trans*-8-Hydroxy-2-(N-n-propyl-N-3′-iodo-2′-propenyl)aminotetralin (*trans*-8-OH-PIPAT): A New 5-HT$_{1A}$ Receptor Ligand. *J. Med. Chem.* **1993**, *36*, 3161–3165.
(9) Greuel, J. M.; Glaser, T. The Putative 5-HT$_{1A}$ Receptor Antagonists NAN-190 and BMY 7378 are Partial Agonists in the Rat Dorsal Raphe Nucleus *In Vitro*. *Eur. J. Pharmacol.* **1992**, *211*, 211–219.
(10) Fletcher, A.; Bill, D. J.; Bill, S. J.; Cliffe, I. A.; Dover, G. M.; Forster, E. A.; Haskins, J. T.; Jones, D.; Mansell, H. L.; Reilly, Y. WAY100135: A Novel, Selective Antagonist at Presynaptic and Postsynaptic 5-HT$_{1A}$ Receptors. *Eur. J. Pharmacol.* **1993**, *237*, 283–291.
(11) Cliffe, I. A.; Brightwell, C. I.; Fletcher, A.; Forster, E. A.; Mansell, H. L.; Reilly, Y.; Routledge, C.; White, A. C. (S)-N-Tert-butyl-3-(4-(2-methoxyphenyl)-piperazin-1-yl)-2-phenylpropanamide, (S)-WAY-100135: A Selective Antagonist at Presynaptic and Postsynaptic 5-HT$_{1A}$ Receptors. *J. Med. Chem.* **1993**, *36*, 1509–1510.
(12) Cliffe, I. A. The Design of Selective 5-HT$_{1A}$ Receptor Antagonists. 206th National Meeting of the American Chemical Society, Aug 22–27, 1993, Chicago, IL, Abstr. MEDI no. 30.
(13) Chumpradit, S.; Kung, M.-P.; Panyachotipun, C.; Prapansiri, V.; Foulon, C.; Brooks, B.; Szabo, S.; Tejani-Butt, S.; Frazer, A.; Kung, H. Iodinated Tomoxetine Derivatives as Selective Ligands for Serotonin and Norepinephrine Uptake Sites. *J. Med. Chem.* **1992**, *35*, 4492–4497.
(14) Fletcher, A.; Cliffe, I. A.; Dourish C.: Silent 5HT$_{1A}$ Receptor Antagonists: Utility as Research Tools and the Therapeutic Agents. *Trends Pharm Sci.* **1993**, *14*, 441–448.