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, [¹²⁵I](R,S)-trans-8-hydroxy-2-[N-n-propyl-N-(3'-iodo-2'propenyl)amino]tetralin, (R,S)-trans-8-OH-PIPAT $(K_d =$ $0.38 \text{ nM} \pm 0.03 \text{ nM}, B_{\text{max}} = 310 \pm 20 \text{ fmol/mg of protein},$ as a selective radioiodinated ligand for the 5-HT_{1A} receptor.⁸ An ¹²⁵I-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 ¹²³I-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 (IC₅₀ = 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 Homogenates⁸

compound	$K_{\rm i}$ (nM)
1 a (<i>p</i> -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
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 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 $(K_i = 10 \text{ nM})$. 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 orthoiodinated 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 [123I]or [125I]-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 (\pm) -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, (\pm) -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 (\pm) -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 (\pm) -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-

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PIPAT, reported previously,⁸ may provide excellent probes for the investigation and characterization of 5-HT_{1A} receptors.¹⁴ The corresponding ¹²³I- and ¹⁸F- ($T_{1/2} = 110$ 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.

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