Isoindol-1-one Analogues of 4-(2′-methoxyphenyl)-1-[2′-[*N*-(2″-pyridyl)-*p*-iodobenzamido]ethyl]piperazine (*p*-MPPI) as 5-HT$_{1A}$ Receptor Ligands

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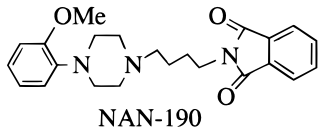
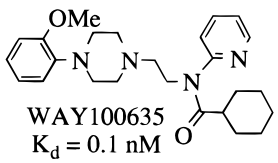
In developing radioiodinated antagonists for in vivo imaging of 5-HT$_{1A}$ receptors with SPECT, a series of new arylpiperazine benzamido derivatives, including 4-(2′-methoxyphenyl)-1-[2′-[*N*-(2″-pyridyl)-*p*-iodobenzamido]ethyl]piperazine (*p*-MPPI, **31**) (K_d = 0.36 nM), as potential ligands for 5-HT$_{1A}$ receptors were reported previously. However, rapid in vivo metabolism may have caused the breakdown of the amide bond of [^{123}I]-**31** and rendered this agent obsolete as an in vivo imaging agent in humans. To improve the in vivo stability of **31**, a series of cyclized amide analogues were designed and synthesized. In vitro binding, metabolic stability, and in vivo biodistribution of these new derivatives were investigated. Several five-membered-ring isoindol-1-ones displayed very high in vitro binding affinity, especially 2-{2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl}-6-nitro-3-phenyl-2,3-dihydroisoindol-1-one, **15**, 3-hydroxy-6-iodo-2-{2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl}-3-phenyl-2,3-dihydroisoindol-1-one, **18**, and 6-iodo-2-{2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl}-3-phenyl-2,3-dihydroisoindol-1-one, **21**, which showed K_i values of 0.05, 0.65, and 0.07 nM, respectively. The affinities for 5-HT$_{1A}$ receptors of other cyclized amide derivatives, 5-(4-bromophenyl)-1-{2-[4-(2-methoxyphenyl)-piperazin-1-yl]ethyl}pyrrolidin-2-one, **25**, 5-(4-iodophenyl)-1-{2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl}pyrrolidin-2-one, **27**, and 2-{2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl}-2,3-dihydro-isoindol-1-one, **29**, were 1.09, 2.54, and 14.9 nM, respectively. Compared to [^{125}I]-**31**, iodinated cyclized amide derivatives [^{125}I]-**21** and [^{125}I]-**27** displayed a slower metabolism in human liver microsomal and cytosolic preparations. Biodistribution of [^{125}I]-**21** and [^{125}I]-**27** in rats (after an iv injection) displayed moderate to low brain uptakes with little or no specific localization in hippocampal region, where 5-HT$_{1A}$ receptors are concentrated. These data indicate that the new iodinated ligands showed high binding affinities and better metabolic stability but displayed unexpectedly low selective binding to 5-HT$_{1A}$ receptors in vivo. Additional structural modifications may be needed to correct the unfavorable properties displayed for these iodinated cyclized amide derivatives for in vivo biodistribution in rats.

Introduction

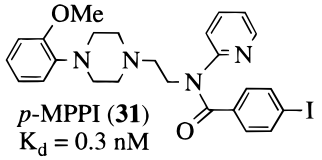
Serotonin receptors play important roles in normal brain function. One of the serotonin receptor subtypes, 5-HT$_{1A}$, plays an important function as the somatodendritic autoreceptor (presynaptic) in the dorsal raphe nucleus and as a postsynaptic receptor for 5-HT in terminal areas.[1] Buspirone and ipsapirone, which are agonists and display high affinity to 5-HT$_{1A}$ receptors (IC_{50} = 60 and 35 nM, respectively), are presently being used as antianxiety agents.[2,3] It is potentially useful to develop ligands that are selective for the 5-HT$_{1A}$ subtype to facilitate the study and characterization of this receptor.[4,5] The most commonly used ligand, 8-OH-DPAT (8-hydroxy-2-(*N*,*N*-di-*n*-propylamino)tetralin), is a potent 5-HT$_{1A}$ agonist, and the tritium-labeled compound is the ligand of choice for 5-HT$_{1A}$ receptor binding studies (K_d = 0.5 nM, rat hippocampal homogenates). It produces full 5-HT$_{1A}$ receptor agonist activity in vitro (negatively coupled to forskolin-stimulated adenylyl cyclase activity) and in vivo (hypothermia and serotonin behavior syndromes such as rapid jaw movements and forepaw treading).[6−9]



NAN-190
K_i = 0.6 nM



WAY100635
K_d = 0.1 nM



p-MPPI (**31**)
K_d = 0.3 nM

Several early front-runners of pure antagonists are arylpiperazine derivatives such as NAN-190, which displays high affinity for 5-HT$_{1A}$ receptors (K_i = 0.6 nM), but also has a high potency for the α_1 receptor.[10−12] In addition, NAN-190 displays partial agonist-like activity in a radioligand binding assay.[13−15] BMY7378 appears to exhibit the same partial agonist property.[10] Another

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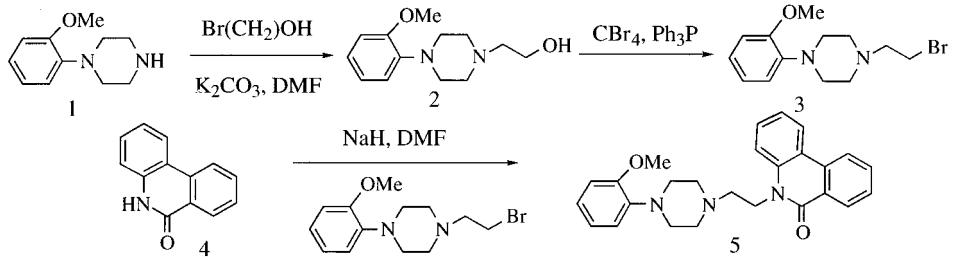
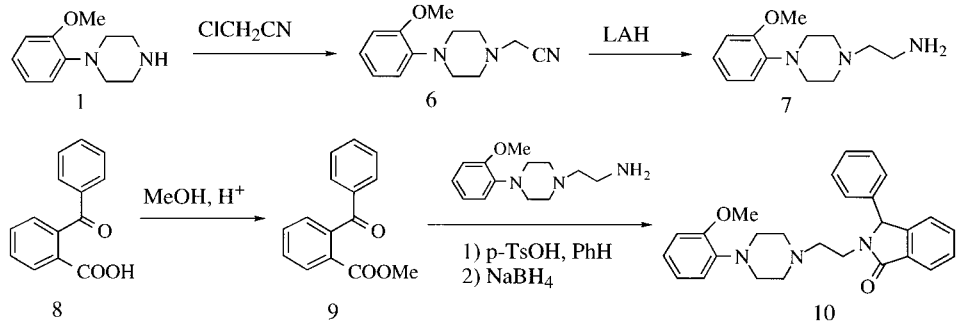
Scheme 1



Scheme 2



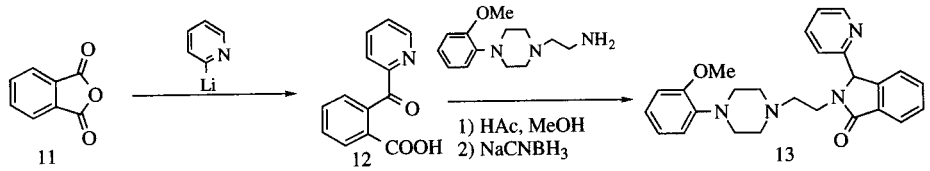
arylpiperazine, WAY100135, was recently reported.[16,17] However, it was quickly surpassed by a similar compound, WAY100635, which displayed a higher binding affinity (K_d = 0.10 nM) and better selectivity[18,19] while exhibiting no partial agonist property.[20,21] Recently, newer versions of arylpiperazines as 5-HT_{1A} receptor ligands have been reported.[22] To develop radioiodinated ligands for in vivo imaging of 5-HT_{1A} receptors, we have previously reported a series of new arylpiperazine benzamido derivatives, including the antagonist ligand, **31**,[23] and the iodinated agonist ligand, 8-OH-PIPAT.[24] Results of in vitro binding and adenylyl cyclase assays for **31** demonstrated that it is an excellent "pure" antagonist with high affinity (K_d = 0.36 nM) and selectivity for 5-HT_{1A} receptors.[25] Evaluation of the in vivo properties of **31** and the related *p*-fluoro derivative, *p*-MPPF, in rats clearly demonstrated that they act as 5-HT_{1A} receptor antagonists.[26,27] The novel iodinated compound, **31**, is the first selective 5-HT_{1A} receptor antagonist which can be labeled with iodine-125 and iodine-123. The ^{125}I-labeled ligand is a useful probe for pharmacological characterization for 5-HT_{1A} receptors, whereas the ^{123}I-labeled ligand is potentially useful for in vivo imaging of 5-HT_{1A} receptors by single photon emission computed tomography (SPECT).

Recent reports have indicated that [^{3}H]- or [^{11}C]-WAY100635, a close analogue of **31**, displayed good in vivo biodistribution properties suitable for imaging with positron emission tomography (PET).[28−34] Regional distribution of 5-HT_{1A} receptors in normal human brain has been reported.[8,30,35] As expected, [^{11}C]WAY100635 exhibited high density in the hippocampal region. A SPECT study carried out in monkeys after an iv injection of [^{123}I]-**31** clearly indicated selective brain uptake in the frontal cortex, cingulate gyrus, hippocampus, and entorhinal cortex, regions with high 5-HT_{1A} receptor density.[36] In vivo displacement experiments in monkey using SPECT demonstrated that hippocampal localization is saturable with cold **31**. The displacement experiment showed the same competitive binding using (±)-8-OH-DPAT, a 5-HT_{1A} receptor agonist. On contrast, when using ketanserin, a selective 5-HT_2 receptor antagonist, as the chasing agent, SPECT images displayed no in vivo displacement of activity localized in hippocampus of monkey brain. The data strongly suggest that in vivo hippocampal binding of [^{123}I]-**31** is selective to 5-HT_{1A} receptors. Surprisingly, preliminary studies in humans with [^{123}I]-**31** displayed low brain uptake after 20 min post iv injection.[36] Initial SPECT images (0−20 min) were close to those of brain perfusion images. It is likely that the low brain uptake observed in the human subject could be due to a rapid metabolism (amide hydrolysis), whereas in other species (i.e., rats and monkeys), the metabolic pathway (cleavage of amide bond by liver enzymes) appears to be much slower.[37] To improve the in vivo stability and bioavailability, isoindol-1-one and other cyclic amides were prepared. The rationale for choosing this ring system is that the cyclized amide bond may extend in vivo stability to allow delivery of the agent into specific brain regions for receptor-directed binding without extensive in vivo degradation. The synthesis, in vitro binding, metabolic stability, and in vivo biodistribution studies of these new ligands are reported herein.

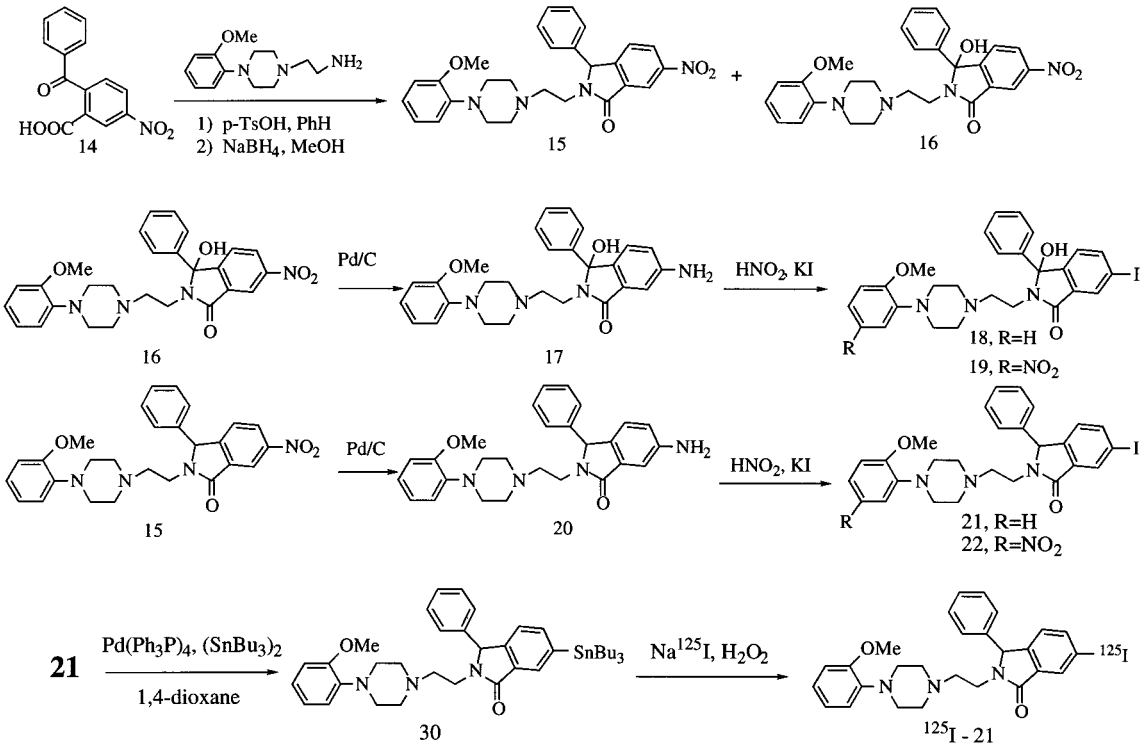
Chemistry

The synthesis of a group of isoindol-1-ones, cyclic amide analogues of 4-(2′-methoxyphenyl)-1-[2′-[*N*-(2″-pyridyl)-*p*-iodobenzamido]ethyl]piperazine (*p*-MPPI, **31**), as 5-HT_{1A} receptor ligands was achieved by reactions shown in Schemes 1−5. Commercially available phenanthridinone **4** and bromide **3** were coupled in the presence of NaH to produce compound **5** in moderate yield (45%). Alternatively, a reductive condensation reaction was carried out between γ-keto acid **12**[38] or **14**[39] or γ-keto ester **9**, **24**, or **28** and the amine **7**[40] using a HOAc−MeOH, $NaCNBH_3$ system or under a refluxing condition in benzene with a catalytic amount of *p*-TsOH followed by $NaBH_4$ as the reducing agent. When the nitro derivative of benzoylbenzoate methyl ester, **14**, was condensed with amine **7** under the same reductive amination condition, in addition to the expected fused

Scheme 3



Scheme 4



ring derivative **16** (8% yield), a second product, undehydrated compound **15**, was obtained (29%). This side product persisted even after the reaction was refluxed for 3 days. Compound **16** was reduced by a hydrogenation reaction in the presence of Pd/C as the catalyst, under which the 6-nitro group was successfully converted to a 6-amino group (compound **17**). The diazotization reaction converted the 6-amino to 6-iodo to give compound **18** (yield 16%). This reaction gave an unexpected product, **19** (yield 39%), on which an extra nitro group was added on the 2-methoxyphenyl ring. This side product probably was produced due to the nitration reaction derived by an oxidation of sodium nitrite under the reaction condition. Using the similar reaction sequence, the desired product compound **21** (yield 19%) was prepared, while the yield for the side product with an extra nitro group, compound **22**, was 41%.

The bromo compound, **25**, which was obtained by reductive amination with γ-keto ester **24** and amine **7**, was converted to the iodo compound **27** via the tin intermediate formed through normal Pd-catalyzed reaction. Similarly, 2-(methoxycarbonyl)benzaldehyde reacted with amine **7** gave another fused ring compound **29**. All of the reactions were not optimized, and in general they gave poor to moderate yields.

Biological Results

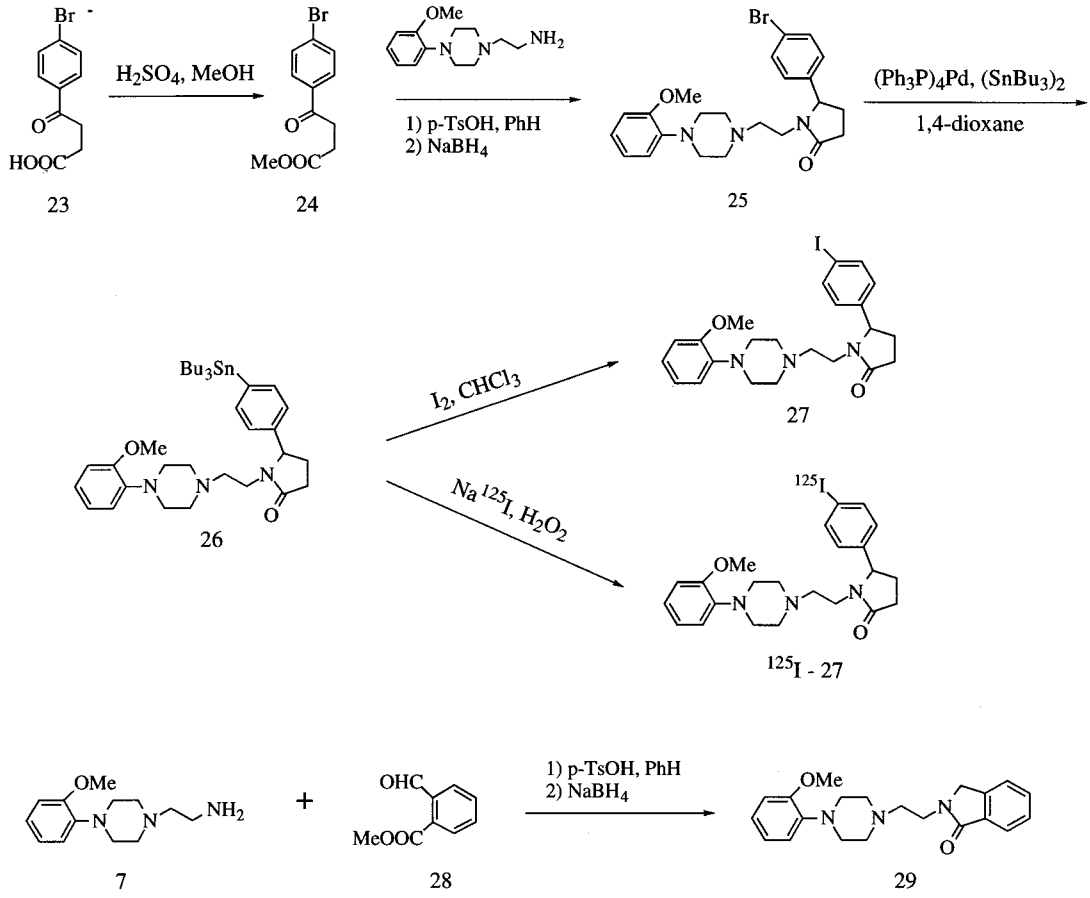
Several isoindol-1-ones containing a cyclic amide ring displayed very high in vitro binding affinity for 5-HT$_{1A}$ receptors, comparable to or exceeding that of the parent compound, **31**. 2-{2-[4-(2-Methoxyphenyl)piperazin-1-yl]ethyl}-6-nitro-3-phenyl-2,3-dihydroisoindol-1-one, **15**, 3-hydroxy-6-iodo-2-{2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl}-3-phenyl-2,3-dihydroisoindol-1-one, **18**, and 6-iodo-2-{2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl}-3-phenyl-2,3-dihydroisoindol-1-one, **21**, showed K_i values of 0.05, 0.65, and 0.07 nM, respectively (Table 1). The affinities of other cyclized amide derivatives, 5-(4-bromophenyl)-1-{2-[4-(2-methoxyphenyl)piperazin-1-yl]-ethyl}pyrrolidin-2-one, **25**, 5-(4-iodophenyl)-1-{2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl}pyrrolidin-2-one, **27**, and 2-{2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl}-2,3-dihydroisoindol-1-one, **29**, were 1.09, 2.54, and 14.9 nM, respectively. The in vitro binding data strongly suggested that the formation of a cyclic amide ring system

Table 1. Comparison of Inhibition Constants, K_i (nM), of 5-HT$_{1A}$ Receptor Ligands[a]

compd	K_i (nM)	compd	K_i (nM)
5	8.9 ± 1.20	**19**	9.3 ± 0.83
10	0.5 ± 0.80	**21**	0.069 ± 0.006
13	6.5 ± 0.94	**22**	51.7 ± 16
15	0.045 ± 0.021	**25**	1.09 ± 0.13
16	0.34 ± 0.03	**27**	2.54 ± 0.59
17	1.85 ± 1.06	**29**	14.9 ± 0.40
18	0.65 ± 0.13	**31**	0.2 ± 0.05

[a] Specific inhibition of [^{125}I]-**31** binding to rat hippocampal membrane preparations. Values are the mean ± SD of three independent experiments, each performed in duplicate.

Scheme 5



(isoindol-1-one) was acceptable in maintaining the ligand affinity to 5-HT_{1A} receptors.

Two cyclized amide ligands, **21** and **27**, which contain the iodo group on the molecules, were subjected for radiolabeling and further in vitro and in vivo evaluations. Radioiodinated compounds [^{125}I]-**21** and [^{125}I]-**27** were successfully prepared using the corresponding tributyltin intermediates, and the products were successfully purified by HPLC (yield 60−80%, radiochemical purity >95%). The identities of the ligands were confirmed with the cold standards on HPLC with similar retention times (t_R = 11.2 and 6.4 min for [^{125}I]-**21** and [^{125}I]-**27**, respectively) with an isocratic solvent system of CH_3CN:5 mM DMGA (pH 7.4), 90:10, eluting at a flow rate of 1 mL/min.

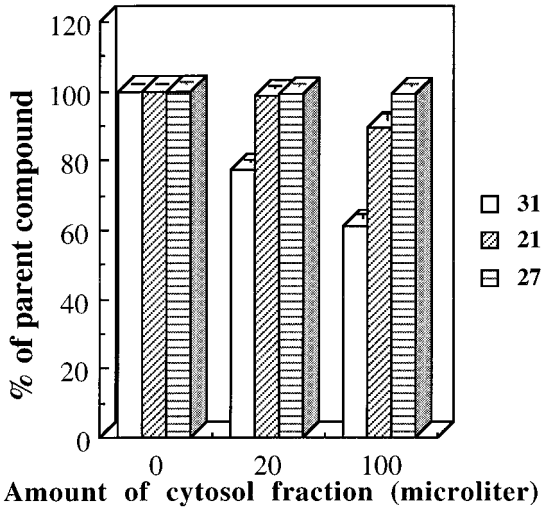
The studies carried out for [^{11}C]WAY100635, a potential PET ligand for imaging 5-HT_{1A} receptors, clearly indicated rapid metabolism of this tracer (amide hydrolysis) by human liver (but not by rat liver).[37,41] As an analogue of WAY100635, [^{125}I]-**31** may encounter a rapid degradation by human liver enzymes, which was evidenced by the successful signal and imaging for 5-HT_{1A} receptors in rats and monkeys but not in humans.[36,42] To improve in vivo metabolic stability of the ligands, we synthesized a series of cyclized amide derivatives as shown in the present study. These cyclized amide derivatives together with **31** were tested for their metabolic stability using a metabolic model system derived from human liver. [^{125}I]-**31**, as expected, was metabolized rapidly by both human liver microsomal fraction (>60% degradation in the presence of 0.75 mg of microsomal protein) and by human liver cytosolic fractions (approximately 40% degradation in the presence of 1.3 mg of cytosol protein; Figure 1). Unlike [^{125}I]-**31**, [^{125}I]-**21** and [^{125}I]-**27** were not metabolized significantly by the same human liver microsomal or cytosolic preparations. The results, demonstrating an increased metabolic stability for cyclized amide derivatives, are consistent with the observation reported for the sterically hindered derivatives of WAY100635.[37,41] In a control experiment using rat or monkey liver microsomal preparations, [^{125}I]-**31** displayed little decomposition after in vitro incubation (unpublished results, data not shown).

Despite high in vitro binding affinity and better metabolic stability, the biodistribution of [^{125}I]-**21** in rats (after an iv injection) displayed a lower brain uptake than that observed for the parent compound [^{125}I]-**31** (Table 2). The brain uptake (percentage dose/organ) was 0.26, 0.11, and 0.04 for [^{125}I]-**21** as compared to 1.22, 0.22, and 0.12 for [^{125}I]-**31** at 2, 30, and 60 min, respectively. The data on regional brain uptake indicated that the compound, [^{125}I]-**21**, did not show any specific uptake in the hippocampal region, which contains a high density of 5-HT_{1A} receptors (hippocampus/cerebellum ratios were 0.87, 1.12, and 1.06 vs 1.35, 3.28, and 3.17 for [^{125}I]-**21** and [^{125}I]-**31**, respectively). The ligand appears to be more lipophilic than [^{125}I]-**31** (PC = 1800 ± 300 and 2400 ± 400 for [^{125}I]-**31** and [^{125}I]-**21**, respectively, and the HPLC retention times were 7.6 and 11.2 min for [^{125}I]-**31** and [^{125}I]-**21**, respectively, on the reversed-phase PRP-1 column). The high lipophilicity of [^{125}I]-**21** was further evidenced by the high nonspecific binding (~30% around the K_d value) in the

Table 2. Biodistribution of [^{125}I]-**21** and [^{125}I]-**27** in Rats after iv Injection[a]

organ[b] or region[c]	[^{125}I]-**21**			[^{125}I]-**27**		
	2 min	30 min	60 min	2 min	30 min	60 min
blood	2.84 ± 0.07	1.92 ± 0.36	1.22 ± 0.04	5.60 ± 0.35	3.45 ± 0.28	2.08 ± 0.42
heart	1.42 ± 0.08	0.30 ± 0.06	0.15 ± 0.0001	0.77 ± 0.04	0.33 ± 0.03	0.18 ± 0.04
muscle	9.45 ± 0.73	14.35 ± 1.71	8.83 ± 1.94	14.07 ± 4.77	19.08 ± 9.13	9.05 ± 1.00
lung	2.05 ± 0.26	0.70 ± 0.05	0.56 ± 0.01	1.54 ± 0.09	0.75 ± 0.04	0.42 ± 0.06
kidney	4.01 ± 0.24	2.32 ± 0.01	1.42 ± 0.16	3.02 ± 0.12	2.20 ± 0.32	1.00 ± 0.24
spleen	0.26 ± 0.07	0.22 ± 0.03	0.15 ± 0.02	0.27 ± 0.01	0.22 ± 0.001	0.15 ± 0.03
liver	21.36 ± 2.4	18.65 ± 1.69	10.76 ± 0.28	19.49 ± 3.79	23.02 ± 2.23	17.95 ± 2.54
skin	3.66 ± 0.54	5.80 ± 0.21	4.96 ± 0.99	6.45 ± 1.45	7.41 ± 1.80	6.93 ± 0.06
thyroid	0.073 ± 0.003	0.05 ± 0.009	0.06 ± 0.003	0.06 ± 0.01	0.04 ± 0.01	0.03 ± 0.005
brain	0.26 ± 0.01	0.11 ± 0.001	0.04 ± 0.007	0.71 ± 0.02	0.21 ± 0.009	0.08 ± 0.003
brain	(1.22 ± 0.22)	(0.22 ± 0.01)	(0.12 ± 0.005)			
cerebellum	0.202 ± 0.006	0.080 ± 0.011	0.035 ± 0.005	0.46 ± 0.04	0.12 ± 0.003	0.051 ± 0.007
striatum	0.204 ± 0.009	0.093 ± 0.007	0.035 ± 0.002	0.55 ± 0.006	0.15 ± 0.009	0.053 ± 0.008
hippocampus	0.177 ± 0.011	0.090 ± 0.005	0.037 ± 0.005	0.47 ± 0.018	0.13 ± 0.004	0.045 ± 0.013
cortex	0.242 ± 0.012	0.081 ± 0.003	0.028 ± 0.004	0.57 ± 0.009	0.14 ± 0.002	0.048 ± 0.007
HP/CB	**0.87**	**1.12**	**1.06**	**1.02**	**1.08**	**0.88**
HP/CB	**(1.35)**	**(3.28)**	**(3.17)**			

[a] Values for previously reported [^{125}I]-**31** were listed in parentheses for comparison. [b] Percentage dose/organ, average of three rats ± SD. [c] Regional distribution (percentage dose/g).



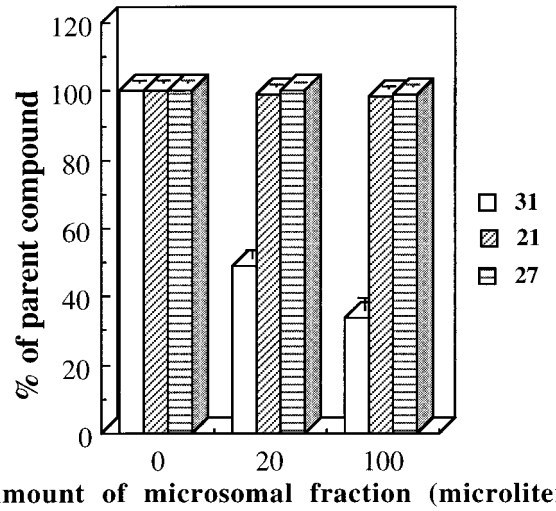


Figure 1. Metabolism of *p*-MPPI, **31,** and analogues **21** and **27** by human liver microsomal and cytosolic preparations. The incubations time was 10 min at 37 °C, and the concentrations of the radioligands used were between 5 and 6 nM.

saturation experiment in vitro (K_d = 0.2 nM, data not shown). Consequently, it is likely that the high lipophilicity and the high nonspecific binding of this ligand may lead to the low specific localization in brain regions such as the hippocampus, where 5-HT$_{1A}$ receptors are concentrated.

Selective in vitro autoradiographic localization of [^{125}I]-**21** in hippocampal and cortical regions where 5-HT$_{1A}$ receptors are concentrated was, however, clearly illustrated (Figure 2). The background level (nonspecific binding due to high lipophilicity) of section labeling of [^{125}I]-**21** was higher than that of [^{125}I]-**31** under the same conditions. In addition, it required a longer time period (18 h in ice) to wash out the nonspecific binding of the ligand from the sections compared to the short interval of 15 min that was required for [^{125}I]-**31**. This is an additional line of evidence suggesting that the high lipophilicity may lead to high nonspecific binding, even under conditions in which the blood−brain barrier is not a factor.

Another iodinated and cyclized amide analogue, [^{125}I]-**27**, was similarly evaluated for its in vitro and in vivo binding properties. The Scatchard analysis of the saturation experiment with [^{125}I]-**27** in rat hippocampal homogenates resulted in a K_d value of 1.0 nM (data not shown), which is comparable to the K_i value obtained with the unlabeled **27** (K_i = 2.54 nM; see Table 1). However, [^{125}I]-**27** also displayed comparable in vitro binding to dopamine D2 receptors (K_d = 1.7 nM, data not shown) which could confound the detection of 5-HT$_{1A}$ receptors *in vivo*. Initial brain uptake of [^{125}I]-**27** (0.71% dose/organ at 2 min postinjection) was comparable to the value obtained with [^{125}I]-**31**. However, because of the lack of selective localization in the rat hippocampal region in vivo, this cyclized amide analogue, [^{125}I]-**27**, is not useful as an in vivo imaging agent.

In conclusion, a new series of isoindol-1-ones analogues of *p*-MPPI, **31** (4-(2′-methoxyphenyl)-1-[2′-[N-(2″-pyridyl)-*p*-iodobenzamido]ethyl]piperazine), as potential ligands for 5-HT$_{1A}$ receptors was reported. Several new novel ligands, **15**, **18**, **21**, and **27**, displayed high binding affinity to 5-HT$_{1A}$ receptors as measured by in vitro binding study. Among these cyclized amide derivatives, two iodinated ligands, **21** and **27**, showed an improved metabolic stability over that of **31** using the human liver enzyme model system. However, in vivo biodistribution

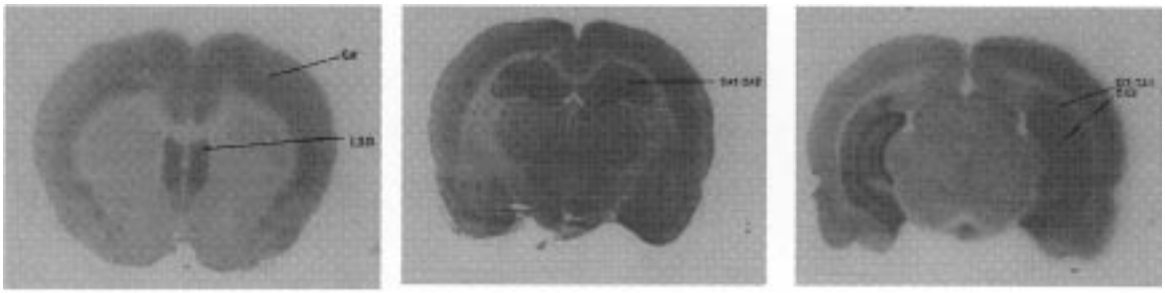


Figure 2. In vitro autoradiography showing [^{125}I]-21 localized in 5-HT$_{1A}$ concentrated areas such as cortex (CX), lateral septal nucleus (LSD), dentate gyrus (DG), and CA1, CA2 of hippocampal regions.

studies of [^{125}I]-**21** and [^{125}I]-**27** in rats after an iv injection suggested that these compounds did not localize in the target area of the brain (hippocampus) where the 5-HT$_{1A}$ receptor concentration is high. Further structural modification is warranted to improve the in vivo selectivity for this series of 5-HT$_{1A}$ receptor ligands.

Experimental Section

Proton NMR spectra were obtained on Bruker AMX300 or AMX500 spectrometers. The chemical shifts were reported in ppm downfield from the tetramethylsilane standard. Infrared spectra were recorded on Mattson Polaris FT-IR spectrometer. Low- and high-resolution mass spectra were carried out on the VG mass spectrometer (Model ZAB-E) and MS-50 with NH_3 and isobutane as gases at the Department of Chemistry, University of Pennsylvania, and the Nebraska Center for Mass Spectrometry. Elemental analyses were performed by Atlantic Microlabs, Inc., Atlanta, GA, or by the Department of Chemistry, University of Pennsylvania.

5-{2-[4-(2-Methoxyphenyl)piperazin-1-yl]ethyl}-5*H*-phenanthridin-6-one (5). To a suspension of NaH (24 mg, 0.6 mmol, 60% dispersion in mineral oil) in a mixed solvent of DMF (1 mL) and toluene (0.5 mL) was added phenanthridinone, **4** (100 mg, 0.5 mmol). The mixture was heated at 90 °C in an oil bath for 1 h. 2-[4-(2-Methoxyphenyl)piperazinyl]-ethyl bromide, **3** (150 mg, 1 equiv), in DMF (1 mL) was added dropwise, and the mixture was stirred at 90 °C for 4 h. Water was added after the reaction mixture was cooled to room temperature and extracted with methylene chloride. The organic layer was dried over Na_2SO_4, filtered, and concentrated to produce a crude product that was purified by PTLC (Hex/EtOAc, 1:1) twice to give product **5** (94 mg, 45%).

IR (film, cm^{-1}): 3058, 2938, 2817, 1646, 1606, 1589, 1502, 1237, 1141 cm^{-1}. ^{1}H NMR ($CDCl_3$, δ): 2.84 (2H, t, J = 7.9 Hz, CONCH$_2$CH_2), 2.90 (4H, s, br, NCH_2), 3.17 (4H, s, br, NCH_2), 3.87 (3H, s, OCH_3), 4.62 (2H, t, J = 7.9 Hz, CONCH_2-CH$_2$), 6.86 (1H, d, d, J = 7.8, 1.4 Hz, ArH), 6.90−7.04 (3H, m, ArH), 7.33 (1H, d, d, d, J = 8.1, 6.4, 1.8 Hz, ArH), 7.51−7.62 (3H, m, ArH), 7.77 (1H, d, d, d, J = 8.4, 7.2, 1.5 Hz, ArH), 8.30 (2H, t, d, J = 8.1, 1.1 Hz, ArH), 8.55 (1H, d, d, J = 8.0, 1.3 Hz, ArH). Anal. ($C_{26}H_{27}N_3O_2 \cdot {}^1/_2H_2O$) Calcd: C, 73.91; H, 6.69; N, 9.94. Found: C, 73.62; H, 6.47; N, 9.82.

2-{2-[4-(2-Methoxyphenyl)piperazin-1-yl]ethyl}- 3-phenyl-2,3-dihydroisoindol-1-one (10). To a solution of 2-benzoylbenzoic acid, **8** (2.8 g, 12.4 mmol), in methanol (25 mL) was added H_2SO_4 (concentrated, 1 mL) dropwise at room temperature. The mixture was refluxed for 3 h. Methanol was removed after cooling. Water was added, and the mixture was extracted with ethyl acetate. The organic layer was washed with saturated $NaHCO_3$ solution followed by saturated NaCl solution, dried, and evaporated to give 2.9 g of methyl 2-benzoylbenzoate, **9**, which was used for the next reaction without further purification.

To a mixture of 2-[4-(2-methoxyphenyl)piperazinyl]ethylamine, **7** (118 mg, 0.5 mmol), and methyl 2-benzoylbenzoate, **9** (120 mg, 0.5 mmol), in benzene (10 mL) was added *p*-TsOH (50 mg) in solid form. The mixture was refluxed for 3 days. The solvent was removed after cooling, and MeOH (5 mL) was added. To the solution was added $NaBH_4$ (100 mg) in solid form at 0 °C. The mixture was stirred at room temperature for 1 h. MeOH was removed, and water was added. The aqueous phase was extracted with CH_2Cl_2. The combined organic phases were dried over Na_2SO_4, filtered, and concentrated to give a crude product which was purified by PTLC (Hex/EtOAc, 1:1) to give 10 mg of desired product **10** (4.6%).

IR (film, ν_{max}): 3048, 2935, 2815, 1695, 1611, 1592, 1469, 1237, 1023 cm^{-1}. ^{1}H NMR ($CDCl_3$, δ): 2.53−2.72 (7H, m, NCH_2, CONCH_aH$_b$), 3.02−3.09 (4H, m, NCH_2), 3.84 (3H, s, CH_3O), 4.10 (1H, d, t, J = 14.3, 5.7 Hz, CONCH$_a$$H_b$), 5.76 (1H, s, NC$H$Ph), 6.85 (1H, d, J = 7.8 Hz, ArH), 6.90−7.00 (3H, m, ArH), 7.13−7.46 (8H, m, ArH), 7.90 (1H, d, d, J = 5.2, 2.0 Hz, ArH). MS: m/z 428 (M$^+$ + 1), 279, 236, 205, 150, 124.

2-{2-[4-(2-Methoxyphenyl)piperazin-1-yl]ethyl}-3-pyridin-2-yl-2,3-dihydroisoindol-1-one (13). To a solution of 2-[4-(2-methoxyphenyl)piperazinyl]ethylamine, **7** (118 mg, 0.5 mmol), and acetic acid (0.2 mL) in methanol (2 mL) was added 2-[2-(hydroxycarbonyl)benzoyl]pyridine, **12** (114 mg, 0.5 mmol), followed by $NaCNBH_3$ (32 mg, 0.5 mmol). The mixture was stirred at room temperature for 3 days. Water was added. The mixture was made basic with NaOH (1 M) and extracted with CH_2Cl_2. The organic layers were dried and evaporated to give a crude product that was purified by PTLC to give 48 mg of product **13** (22%).

IR (film, ν_{max}): 3048, 2944, 2832, 1690, 1611, 1590, 1498, 1463, 1237, 1023 cm^{-1}. ^{1}H NMR ($CDCl_3$, δ): 2.53−2.94 (7H, m, NCH_2, CONCH_aH$_b$), 3.04−3.16 (4H, m, NCH_2), 3.84 (3H, s, CH_3O), 4.23 (1H, d, t, J = 14.4, 6.2 Hz, CONCH$_a$$H_b$), 5.95 (1H, s, NC$H$Ph), 6.84 (1H, d, J = 7.8 Hz, ArH), 6.88−7.04 (3H, m, ArH), 7.21−7.35 (2H, m, ArH), 7.44−7.51 (2H, m, ArH), 7.63 (1H, d, t, J = 7.68, 1.8 Hz, ArH), 7.71−7.93 (2H, m, ArH), 8.65 (1H, d, d, d, J = 4.9, 1.7, 0.9 Hz, ArH). MS: m/z 429 (M$^+$ + 1), 205.

2-{2-[4-(2-Methoxyphenyl)piperazin-1-yl]ethyl}-6-nitro-3-phenyl-2,3-dihydroisoindol-1-one (15) and 3-Hydroxy-2-{2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl}-6-nitro-3-phenyl-2,3-dihydroisoindol-1-one (16). To a mixture of 2-benzoyl-5-nitrobenzoic acid, **14** (1.36 g, 5 mmol), and 2-[4-(2-methoxyphenyl)piperazinyl]ethylamine, **7** (1.18 g, 5 mmol), in benzene (20 mL) was added *p*-toluenesulfonic acid (200 mg). The mixture was stirred under reflux for 3 days. The solvent was removed on vacuum, and the residue was dissolved in MeOH (30 mL). $NaBH_4$ (500 mg) was added in portions at 0 °C in an ice bath. The mixture was stirred at room temperature for 1 h. MeOH was removed, and water was added. The separated aqueous phase was extracted with CH_2Cl_2. The combined organic phases were dried over Na_2SO_4 and concentrated to give a crude product which was purified by MPLC (Hex/EtOAc, 1:1) to give 3-hydroxy-2-{2-[4-(2-methoxyphenyl)-piperazin-1-yl]ethyl}-6-nitro-3-phenyl-2,3-dihydroisoindol-1-one, **16** (690 mg, 29%), and 2-{2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl}-6-nitro-3-phenyl-2,3-dihydroisoindol-1-one, **15** (187 mg, 8%).

3-Hydroxy-2-{2-[4-(2-methoxyphenyl)piperazin-1-yl]-ethyl}-6-nitro-3-phenyl-2,3-dihydroisoindol-1-one (16). IR (film, ν_{max}): 2950, 2828, 1706, 1592, 1530, 1498, 1345, 1243 cm^{-1}. ^{1}H NMR ($CDCl_3$, δ): 2.46 (1H, d, d, J = 12.6, 1.7 Hz, CONCH_aH$_b$), 2.72−3.17 (10H, m, NCH_2), 3.86 (3H, s, CH_3O),

4.22 (1H, d, t, J = 13.0, 2.4 Hz, CONCH$_a$*H*$_b$), 6.88 (1H, d, d, J = 7.6, 1.2 Hz, ArH), 6.93−7.08 (3H, m, ArH), 7.33−7.46 (6H, m, ArH), 8.33 (1H, d, d, J = 8.3, 2.1 Hz, ArH), 8.67 (1H, d, d, J = 2.1, 0.4 Hz, ArH). HRMS: calcd ($C_{27}H_{28}N_4O_5$) m/z 488.2060 (M^+), found m/z 488.2073 (M^+).

2-{2-[4-(2-Methoxyphenyl)piperazin-1-yl]ethyl}-6-nitro-3-phenyl-2,3-dihydroisoindol-1-one (15). IR (film, ν_{max}): 2934, 2821, 1702, 1598, 1533, 1502, 1341, 1237 cm^{-1}. ^{1}H NMR (CDCl$_3$, δ): 2.50−2.84 (7H, m, NC*H*$_2$, CONC*H*$_a$H$_b$), 3.00−3.10 (4H, m, NC*H*$_2$), 3.84 (3H, s, C*H*$_3$O), 4.13 (1H, d, t, J = 14.5, 5.4 Hz, CONCH$_a$*H*$_b$), 5.94 (1H, s, NC*H*Ph), 6.86 (1H, d, d, J = 7.4, 1.1 Hz, Ar*H*), 6.88−7.07 (4H, m, Ar*H*), 7.13−7.18 (2H, m, Ar*H*), 7.32−7.45 (4H, m, Ar*H*), 8.33 (1H, d, d, J = 8.3, 2.2 Hz, Ar*H*), 8.73 (1H, d, J = 2.1 Hz, Ar*H*); HRMS: calcd ($C_{27}H_{28}N_4O_4$) m/z 472.2110 (M^+), found m/z 472.2125 (M^+).

3-Hydroxy-6-iodo-2-{2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl}-3-phenyl-2,3-dihydroisoindol-1-one (18) and 3-Hydroxy-6-iodo-2-{2-[4-(2-methoxy-5-nitrophenyl)piperazin-1-yl]ethyl}-3-phenyl-2,3-dihydroisoindol-1-one (19). The mixture of 3-hydroxy-2-{2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl}-6-nitro-3-phenyl-2,3-dihydroisoindol-1-one, **16** (550 mg, 1.2 mmol), and Pd/C (10%, 100 mg) in a mixed solvent (60 mL, EtOAc/EtOH, 5:1) was hydrogenated at 40 psi for 1 h. The mixture was then filtered and evaporated to give a white solid (3-hydroxy-2-{2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl}-6-amino-3-phenyl-2,3-dihydroisoindol-1-one, **17**; 500 mg) that was clean enough to run in the next reaction without further purification.

To a mixture of 3-hydroxy-2-{2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl}-6-amino-3-phenyl-2,3-dihydroisoindol-1-one, **17** (200 mg, 0.45 mmol), HCl (concentrated, 0.4 mL), and ice (0.6 g) was added a solution of $NaNO_2$ (62 mg, 2 equiv) dropwise at 0 °C in an ice bath. The mixture was stirred at 0 °C for 30 min and then added to a solution of KI (700 mg) in water (3 mL) dropwise at room temperature. The mixture became a foam which was stirred at room temperature for 30 min and extracted with CH_2Cl_2. The organic phase was dried and evaporated to give a crude product which was purified by PTLC (Hex/EtOAc, 1:1) followed by HPLC (reverse-phase column, MeCN/NH_4OAc [0.1 M], 60:40) to give 3-hydroxy-6-iodo-2-{2-[4-(2-methoxy-5-nitrophenyl)piperazin-1-yl]ethyl}-3-phenyl-2,3-dihydroisoindol-1-one, **19** (106 mg, 39%), and 3-hydroxy-6-iodo-2-{2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl}-3-phenyl-2,3-dihydroisoindol-1-one, **18** (40 mg, 16%).

3-Hydroxy-6-iodo-2-{2-[4-(2-methoxy-5-nitrophenyl)-piperazin-1-yl]ethyl}-3-phenyl-2,3-dihydroisoindol-1-one (19). IR (film, ν_{max}): 2959, 2923, 2846, 1700, 1589, 1513, 1445, 1336, 1261, 1023 cm^{-1}. ^{1}H NMR (CDCl$_3$, δ): 2.45 (1H, d, d, J = 14.6, 2.6 Hz, CONC*H*$_a$H$_b$), 2.60−3.32 (10H, m, NC*H*$_2$), 3.95 (3H, s, C*H*$_3$O), 4.16 (1H, d, d, J = 13.2, 2.3 Hz, CONCH$_a$*H*$_b$), 6.93 (1H, d, J = 8.9 Hz, ArH), 7.00 (1H, d, d, J = 8.0, 0.5 Hz, Ar*H*), 7.31−7.45 (5H, m, Ar*H*), 7.74 (1H, d, J = 2.5 Hz, Ar*H*), 7.79 (1H, d, d, J = 8.0, 1.6 Hz, Ar*H*), 7.90 (1H, d, d, J = 8.8, 2.5 Hz, Ar*H*), 8.19 (1H, d, d, J = 1.6, 0.5 Hz, Ar*H*). MS: m/z 615 ($M^+ + 1$).

3-Hydroxy-6-iodo-2-{2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl}-3-phenyl-2,3-dihydroisoindol-1-one (18). IR (film, ν_{max}): 3060, 2948, 2834, 1708, 1594, 1498, 1449, 1371, 1237 cm^{-1}. ^{1}H NMR (CDCl$_3$, δ): 2.36 (1H, d, d, J = 14.6, 2.6 Hz, CONC*H*$_a$H$_b$), 2.62−3.08 (10H, m, NC*H*$_2$), 3.78 (3H, s, C*H*$_3$O), 4.10 (1H, d, d, d, J = 13.9, 3.4, 1.6 Hz, CONCH$_a$*H*$_b$), 6.80 (1H, d, J = 7.7 Hz, ArH), 6.85−7.00 (4H, m, ArH), 7.24−7.37 (5H, m, ArH), 7.70 (1H, d, d, J = 8.0, 1.6 Hz, ArH), 8.10 (1H, d, d, J = 1.5, 4.2 Hz, ArH). MS: m/z 570 ($M^+ + 1$).

6-Iodo-2-{2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl}-3-phenyl-2,3-dihydroisoindol-1-one (21) and 6-Iodo-2-{2-[4-(2-methoxy-5-nitrophenyl)piperazin-1-yl]ethyl}-3-phenyl-2,3-dihydroisoindol-1-one (22). A mixture of 2-{2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl}-6-nitro-3-phenyl-2,3-dihydroisoindol-1-one, **15** (140 mg, 0.3 mmol), and Pd/C (10%, 50 mg) in a mixed solvent (20 mL, EtOAc/EtOH, 5:1) was hydrogenated at 40 psi for 1 h. The mixture was filtered, and the filtrate was evaporated to give a white solid (136 mg), 2-{2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl}-6-amino-3-phenyl-2,3-dihydroisoindol-1-one, **20**, which was used to run the next reaction without further purification.

To a mixture of 2-{2-[4-(2-methoxyphenyl)piperazin-1-yl]-ethyl}-6-amino-3-phenyl-2,3-dihydroisoindol-1-one, **20** (125 mg, 0.28 mmol), HCl (concentrated, 0.4 mL), and ice (0.6 g) was added a solution of $NaNO_2$ (40 mg, 2 equiv) dropwise at 0 °C in an ice bath. The mixture was stirred at 0 °C for 30 min and then added to a solution of KI (700 mg) in water (3 mL) dropwise at room temperature. The mixture became a foam, which was stirred at room temperature for 30 min and extracted with CH_2Cl_2. The organic phase was dried and evaporated to give a crude product that was purified by PTLC (Hex/EtOAc, 1:1) and then HPLC (reverse-phase column, MeCN/NH_4OAc [0.1 M], 60:40) to give 6-iodo-2-{2-[4-(2-methoxy-5-nitrophenyl)piperazin-1-yl]ethyl}-3-phenyl-2,3-dihydroisoindol-1-one, **22** (70 mg, 41%), 6-iodo-2-{2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl}-3-phenyl-2,3-dihydroisoindol-1-one, **21** (30 mg, 19%).

6-Iodo-2-{2-[4-(2-methoxy-5-nitrophenyl)piperazin-1-yl]ethyl}-3-phenyl-2,3-dihydroisoindol-1-one (22). IR (film, ν_{max}): 2937, 2821, 1688, 1582, 1513, 1247, 1095, 1023 cm^{-1}. ^{1}H NMR (CDCl$_3$, δ): 2.54−2.68 (6H, m, NC*H*$_2$), 3.03 (1H, d, t, J = 14.4, 5.7 Hz, CONC*H*$_a$H$_b$), 3.21 (4H, br, NC*H*$_2$), 3.94 (3H, s, C*H*$_3$O), 4.10 (1H, d, t, J = 14.5, 5.9 Hz, CONCH$_a$*H*$_b$), 5.67 (1H, s, NC*H*Ph), 6.88 (1H, d, J = 8.9 Hz, ArH), 6.94 (1H, d, J = 8.0 Hz, ArH), 7.13−7.16 (2H, m, ArH), 7.36−7.40 (3H, m, ArH), 7 71 (1H, d, J = 2.49 Hz, ArH), 7.78 (1H, d, d, J = 8.0, 1.6 Hz, ArH), 7.86 (1H, d, d, J = 8.8, 2.5 Hz, ArH), 8.24 (1H, d, J = 1.4 Hz, ArH). HRMS: calcd ($C_{27}H_{27}N_4O_4I$) m/z 599.1105 ($M^+ + 1$), found m/z 599.1136 ($M^+ + 1$).

6-Iodo-2-{2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl}-3-phenyl-2,3-dihydroisoindol-1-one (21). IR (film, ν_{max}): 2938, 2817, 1688, 1584, 1502, 1237, 1029 cm^{-1}. ^{1}H NMR (CDCl$_3$, δ): 2.38−2.66 (6H, m, NC*H*$_2$), 2.90−2.99 (5H, m, NC*H*$_2$), 3.78 (3H, s, C*H*$_3$O), 4.00 (1H, d, t, J = 14.4, 5.6 Hz, CONCH$_a$*H*$_b$), 5.66 (1H, s, NC*H*Ph), 6.78 (1H, d, J = 7.6 Hz, ArH), 6.82−6.96 (4H, m, ArH), 7.05−7.08 (2H, m, ArH), 7.27−7.34 (3H, m, ArH), 7.69 (1H, d, d, J = 8.0, 1.5 Hz, ArH), 8.16 (1H, d, J = 1.3 Hz, ArH). HRMS: calcd ($C_{27}H_{28}N_3O_2I$) m/z 554.1305 ($M^+ + 1$), found: m/z 554.1302 ($M^+ + 1$).

5-(4-Bromophenyl)-1-{2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl}-pyrrolidin-2-one (25). To a solution of (4-bromobenzoyl)propanoic acid, **23** (1 g, 3.9 mmol), in methanol (25 mL) was added H_2SO_4 (concentrated, 1 mL) dropwise at room temperature. The mixture was refluxed for 0.5 h. Methanol was removed after cooling. Water was added, and the mixture was extracted with methylene chloride. The organic layer was washed with saturated $NaHCO_3$ solution followed by saturated NaCl solution, dried, and evaporated to give 1.07 g of methyl (4-bromobenzoyl)propionate, **24**, which was used for the next reaction without further purification.

To a mixture of 2-[4-(2-methoxyphenyl)piperazinyl]ethylamine, **7** (235 mg, 1 mmol), and methyl (4-bromobenzoyl)-propionate, **24** (271 mg, 1 mmol), in benzene (10 mL) was added *p*-TsOH (100 mg) in solid form. The mixture was refluxed with a Dean−Stark overnight. Benzene was removed, and MeOH (5 mL) was added. $NaBH_4$ (50 mg) was added at 0 °C, and the mixture was stirred at room temperature for 1 h. MeOH was removed, and water was added. The mixture was extracted with CH_2Cl_2−MeOH (9:1). The combined organic phases were dried over $NaSO_4$ and filtered. The filtrate was concentrated to give a crude product which was purified by PTLC (CH_2Cl_2/MeOH, 95:5) to give 143 mg of desired product **25** (31%).

^{1}H NMR (CDCl$_3$, δ): 1.75−1.84 (1H, m), 2.40−2.59 (9H, m), 2.73 (1H, d, t, J = 14.3, 6.3 Hz, COC*H*$_a$H$_b$), 3.04 (4H, br), 3.85 (3H, s, OC*H*$_3$), 3.85 (1H, d, t, J = 14.1, 6.5, COHCH$_a$*H*$_b$), 4.81 (1H, m, CONC*H*Ar), 6.86 (1H, d, J = 7.4 Hz, Ar*H*), 6.93 (2H, m, Ar*H*), 7.01 (1H, d, d, d, J = 7.8, 5.4, 3.8 Hz, Ar*H*), 7.10 (2H, d, t, J = 8.4, 2.2 Hz, BrAr*H*), 7.50 (2H, d, t, J = 8.4, 2.2 Hz, BrAr*H*). MS: m/z 458 ($M^+ + 1$), 205.

5-[4-(Tributylstannyl)phenyl]-1-{2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl}pyrrolidin-2-one (26). A mixture of 5-(4-bromophenyl)-1-{2-[4-(2-methoxyphenyl)piperazin-1-yl]-

ethyl}pyrrolidin-2-one, **25** (50 mg, 0.11 mmol), bis(tributyltin) (0.2 mL), and $(Ph_3P)_4Pd$ (10 mg) in 1,4-dioxane (5 mL) was stirred under reflux overnight. The solvent was removed, and the residue was purified by PTLC (EtOAc as developing solvent) to give 53 mg (73%) of the desired tin compound.

^{1}H NMR ($CDCl_3$, δ): 0.88 (4.5H, t, J = 7.1 Hz, CH_3), 0.92 (4.5H, t, J = 7.1 Hz, CH_3), 1.05 (4H, t, J = 8 Hz, CH_2), 1.36 (8H, m, CH_2), 1.56 (6H, m, CH_2), 1.80 (1H, m), 2.43−2.57 (9H, m), 2.78 (1H, d, t, J = 13.9, 6.6 Hz, COCH_aH$_b$), 3.03 (4H, m), 3.84 (3H, s, OCH_3), 3.85 (1H, d, t, J = 13.9, 6.6, CONCH_aH$_b$), 4.76 (1H, m, CONC*H*Ar), 6.90 (3H, Ar*H*), 7.15 (1H, m, Ar*H*), 7.49 (3H, m, Ar*H*), 7.70 (1H, d, t, J = 8.4, 2.2 Hz, Ar*H*).

5-(4-Iodophenyl)-1-{2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl}pyrrolidin-2-one (27). Thirty-four milligrams (0.05 mmol) of the tin compound, **26**, was dissolved in chloroform (5 mL), to which was added a solution of iodine in chloroform (0.1 M) dropwise at room temperature until the color was persistent. The mixture was stirred at room temperature for 5 min. KF (3 mL, 1 M in MeOH) and $NaHSO_3$ (3 mL, 5% solution) were added successively. The mixture was stirred at room temperature for 5 min and extracted with methylene chloride. The organic phase was dried over Na_2SO_4 and filtered. The filtrate was concentrated to give a crude product that was purified by PTLC (EtOAc as developing solvent) to give 12 mg of compound (48%).

^{1}H NMR ($CDCl_3$, δ): 1.75−1.83 (1H, m), 2.37−2.58 (9H, m), 2.73 (1H, d, t, J = 14.1, 6.3 Hz, COCH_aH$_b$), 3.03 (4H, br), 3.85 (3H, s, OCH_3), 3.85 (1H, d, t, J = 14.1, 6.4, CONCH_aH$_b$), 4.79 (1H, m, CONC*H*Ar), 6.86 (1H, d, J = 7.4 Hz, Ar*H*), 6.93 (2H, m, Ar*H*), 6.97 (1H, m, Ar*H*), 7.01 (2H, d, t, J = 8.4, 2.3 Hz, BrAr*H*), 7.70 (2H, d, t, J = 8.4, 2.2 Hz, BrAr*H*). MS: m/z 506 ($M^+ + 1$), 380, 205.

2-{2-[4-(2-Methoxyphenyl)piperazin-1-yl]ethyl}-2,3-dihydroisoindol-1-one (29). To a mixture of 2-[4-(2-methoxyphenyl)piperazinyl]ethylamine, **7** (95 mg, 0.4 mmol), and 2-(methoxycarbonyl)benzaldehyde, **28** (55 mg, 0.34 mmol), in benzene (10 mL) was added *p*-TsOH (50 mg) in solid form. The mixture was refluxed with a Dean−Stark overnight. Benzene was removed, and MeOH (5 mL) was added. $NaBH_4$ (50 mg) was added at 0 °C, and the mixture was stirred at room temperature for 1 h. MeOH was removed, and water was added. The mixture was extracted with CH_2Cl_2/MeOH (9:1). The combined organic phases were dried over $NaSO_4$, filtered, and concentrated to give a crude product that was purified by PTLC (EtOAc as developing solvent) to give 44 mg of desired product **29** (37%).

^{1}H NMR ($CDCl_3$, δ): 2.73 (6H, m), 3.08 (4H, m), 3.79 (2H, t, J = 6.5 Hz, CONCH_2), 3.85 (3H, s, OCH_3), 4.53 (2H, s, CONCH_2Ar), 6.85 (1H, d, J = 7.5 Hz, Ar*H*), 6.91 (2H, m, Ar*H*), 7.00 (1H, m, Ar*H*), 7.42−7.55 (3H, m, Ar*H*), 7.86 (1H, m, Ar*H*). HRMS: calcd ($C_{21}H_{25}N_3O_2$) m/z 352.2025 ($M^+ + 1$), found 352.2036 ($M^+ + 1$).

6-(Tributylstannyl)-2-{2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl}-3-phenyl-2,3-dihydroisoindol-1-one (30). A mixture 6-iodo-2-{2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl}-3-phenyl-2,3-dihydroisoindol-1-one, **21** (22 mg, 0.04 mmol), bis(tributyltin) (0.2 mL), and $(Ph_3P)_4Pd$ (10 mg) in 1,4-dioxane (2 mL) was stirred under reflux overnight. The solvent was removed, and the residue was purified by PTLC (EtOAc/Hex, 1:1, as developing solvent) to give 8 mg (28%) of the desired tin compound **30**.

^{1}H NMR (200 Hz, $CDCl_3$, δ): 0.87 (9H, t, J = 7.1 Hz, CH_3), 1.07 (4H, t, J = 7.9 Hz, CH_2), 1.32 (8H, m, CH_2), 1.53 (6H, m, CH_2), 2.62 (6H, m, NCH_2), 3.06 (4H, m, NCH_2), 3.18 (1H, m, CONCH_{6b}), 3.85 (3H, s, CH_3O), 4.10 (1H, m, CONCH_{6a}), 5.75 (1H, s, NC*H*Ph), 6.93 (3H, m, Ar*H*), 7.14 (3H, m, Ar*H*), 7.33 (4H, m, Ar*H*), 7.54 (1H, d, J = 7.2 H, Ar*H*), 8.00, (1H, s, Ar*H*).

Radiolabeling. No-carrier-added [^{125}I]-**21** and [^{125}I]-**27** were prepared by an iododestannylation reaction similar to the procedure reported previously for [^{125}I]-**31**.[25] Hydrogen peroxide (50 μL, 3% w/v) was used as the oxidant, and the products were purified by HPLC using a reverse-phase column (PRP-1 column, Hamilton Co., Reno, Nevada). The fractions containing the desired product were collected, condensed, and re-extracted with ethyl acetate (3 × 1 mL). The final no-carrier-added products were evaporated to dryness and redissolved in 100 μL of 50% EtOH with 100 μg of ascorbic acid added as an antioxidant. The radiochemical purity of the product was evaluated on HPLC, and the identity was confirmed with the coelution on HPLC with the unlabeled standard.

Partition Coefficient Determination. [^{125}I]Ligand (500000−800000 cpm) was mixed with 3 g each of 1-octanol and buffer (pH 7.0 or pH 7.4, 0.1 M phosphate) in a test tube. The test tube was vortexed for 3 min at room temperature and then centrifuged for 5 min to separate the 1-octanol and buffer layers. The radioactivity of approximately 1 g samples from each layer was counted in a gamma counter (Packard 5000). The partition coefficient (PC) was determined by calculating the ratio of counts per minute per gram of octanol to that of buffer. Samples from the octanol layer were repartitioned until consistent PC values were obtained. The measurements were repeated three times.

Binding Assays. The rat hippocampal membrane homogenates were prepared as described previously.[25] Binding assays were performed in glass tubes (12 × 75 mm) in a final volume of 0.25 mL. In saturation experiments, aliquots (100 μL corresponding to 20−30 μg of protein) of membrane suspensions were mixed with 50 mM Tris-HCl, pH 7.4, containing 0.1% BSA and radioligands of 0.05−2.0 nM [^{125}I]-**21** or [^{125}I]-**27**. Competition experiments were performed using 0.2 nM [^{125}I]-**31** and 8−10 concentrations (10^{-10}−10^{-5} M) of competing drugs (serially diluted in 50 mM Tris buffer containing 0.1% BSA). Nonspecific binding was defined with 1 μM **31**. Incubation was carried out for 20 min at 37 °C and then terminated by separation of bound from free radioligand by filtration through glass fiber filters (Schleicher & Schuell No. 25, Keene, NH) presoaked with 1% polyethylenimine. The filters were then washed three times with 3 mL of ice-cold 20 mM Tris buffer and counted in a gamma counter (Packard 5000) with 70% efficiency. The results of saturation and competition experiments were subjected to nonlinear regression analysis using EBDA[43] to obtain K_d and IC_{50} values.

Metabolic Stability. Human liver microsomal and cytosolic fractions purchased from Dr. Ted Inaba (University of Toronto) were used as a model of human liver metabolism for evaluating the stability of various tracer ligands. The radioiodinated ligands (2−3 μCi in 40 μL of 50 mM Na phosphate, pH 7.4) were incubated at 37 °C for 10 min with 160 μL of phosphate buffer containing various amount of cytosolic or microsomal fractions. At the end of the incubation, the amount of unmetabolized [^{125}I]-**31** or [^{125}I]-**27** was evaluated directly by thin-layer chromatography (TLC) with Whatman silica gel plates (PESIL G/UV) and a solvent system of EtOAc/MeOH/triethylamine, 85:15:5. Due to the lipophilic property of [^{125}I]-**21**, ethyl acetate (3 × 1 mL) extraction was carried out in the presence of 10 μg "cold" carrier followed by analysis on TLC. Similar procedures were applied to [^{125}I]-**31** for the purpose of comparison. Control samples were carried out by mixing small amount of ligands (1−2 μCi) with microsomal or cytosolic fractions without incubation, followed by the same procedures used for the incubation samples to determine the extraction efficiency and the total recovery yield.

Biodistribution in Rats. The animal studies were conducted according to protocols approved by the University of Pennsylvania Institutional Animal Care and Use Committee. Male Sprague−Dawley rats (225−300 g) allowed free access to food and water were used for in vivo biodistribution studies.[44,45] While under ether anesthesia, 0.2 mL of a saline solution containing [^{125}I]-**21** or [^{125}I]-**27** (10−20 μCi) was injected directly into the femoral vein of the rats. The rats were then sacrificed by cardiac excision at various time points postinjection. The organs of interest were removed and weighed, and the radioactivity was counted with an automatic gamma counter (Packard 5000). The percentage dose per organ was calculated by a comparison of the tissue counts to suitably diluted aliquots of the injected material. Total activities of blood and muscle were calculated under the

assumption that they were 7% and 40% of the total body weight, respectively. Regional brain distribution in rats was measured after an iv injection of the radioactive tracer. Samples from different brain regions (cortex, striatum, hippocampus and cerebellum) were dissected, weighed, and counted. The percentage dose per gram of each sample was calculated by comparing the sample counts with the counts of the diluted initial dose. The ratio of uptake in each region was obtained by dividing percentage dose per gram of each region with that of the cerebellum, which was used as a background region containing no 5-HT$_{1A}$ receptors.

In Vitro Autoradiography. Rats were decapitated and brains were removed immediately. Brains were placed in OTC embedding medium (Miles Laboratory, Elkhart, IN) and frozen with powdered dry ice. Coronal sections of 20 μm thickness were cut at −20 °C in a cryostat (Hacker Instruments, Fairfield, NJ) and thaw-mounted onto gelatin-coated glass slides. The sections were desiccated at 4 °C for 3 h and then kept at −70 °C until use.

The rat brain sections were thawed, brought to room temperature, and preincubated in Tris-HCl buffer, pH 7.5, at room temperature for 30 min. The sections were then incubated for 90 min at room temperature in the same buffer containing 0.1 nM [^{125}I]-**21** followed by washing with cold buffer for 18 h (sections were kept in ice) with one change of buffer. Adjacent sections were labeled similarly, but in the presence of 1 μM **31** to define nonspecific binding. The sections after washing were dipped in ice-cold distilled water to remove buffer salts before drying with a stream of cold air. Dried sections were apposed to DuPont X-ray films for 18 h, and the analysis of the autoradiograms was carried out using a computer-based image analysis system (NIH Image, version 1.61).

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