Derivatives of 4-(2′-Methoxyphenyl)-1-[2′-(*N*-2″-pyridinyl-*p*-iodobenzamido)ethyl]piperazine (*p*-MPPI) as 5-HT_{1A} Ligands

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A series of new *p*-alkylbenzamido derivatives of 4-(2′-methoxyphenyl)-1-[2′-(*N*-2″-pyridinyl)-*p*-iodobenzamido)ethyl]piperazines (*p*-MPPI) were prepared. *In vitro* binding studies suggest that *p*-methyl and *p*-ethyl substituents on the benzamido group display the same high binding affinity to 5-HT_{1A} receptors (K_i = 2.2 and 9.3 nM, rat hippocampal homogenates). However, when the substitution groups were larger than a C_5 pentyl group, the affinity to 5-HT_{1A} receptors dropped below a useful level (K_i > 50 nM). Several irreversible binding agents (CH_2Cl, $NHCOCH_2Cl$) and a photoaffinity labeling compound (*m*-iodo *p*-azido) which showed good binding affinity to 5-HT_{1A} receptors were successfully prepared.

In the past few years, considerable progress has been made in the understanding of the central nervous system (CNS) serotonin system. It is an important neurotransmission network which regulates various physiological functions and behavior, including anxiety and affective states.[1–3] There are multiple serotonin receptor subtypes which have been reported in the literature, and the number is constantly increasing as molecular cloning techniques are being used in producing and expressing various cloned serotonin receptor subtypes. One of the serotonin receptor subtypes, the 5-HT_{1A} receptor, 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.[2,4,5] A large number of agonists and antagonists for 5-HT_{1A} receptors are reported in the literature.[6–14] Currently, 8-hydroxy-2-(*N,N*-di-*n*-propylamino)tetralin (8-OH-DPAT) is the most well-studied potent 5-HT_{1A} agonist.[15–17] The most widely studied 5-HT_{1A} putative antagonist based on arylpiperazine is 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl]piperazine (NAN-190). NAN-190 displayed high 5-HT_{1A} affinity (K_i = 0.6 nM) and had an equal potency for the α_1 receptor.[9,12] In addition, it displayed partial agonist-like activity in radioligand binding assay.[18] Replacement of the phthalimide moiety by substituted benzamides or acyl moieties produced 4-[4-(1-adamantanecarboxamido)butyl]-1-(2-methoxyphenyl)piperazine, which was found to bind to 5-HT_{1A} receptors with high affinity (K_d = 0.4 nM) and was devoid of binding affinity to other receptors.[11] Recently, a new arylpiperazine derivative, (*S*)-*N*-*tert*-butyl-3-[4-(2-methoxyphenyl)piperazin-1-yl]-2-phenylpropionamide ((S)-WAY 100135), was reported as a selective antagonist at both somatodendritic and postsynaptic receptor sites (IC_{50} = 15 nM, rat hippocampal membranes).[6,19] A similar compound, 4-(2′-methoxyphenyl)-1-[2′-(*N*-2″-pyridinylcyclohexylamido)-ethyl]piperazine (WAY 100635), displayed even higher binding affinity (IC_{50} = 2.2 nM, rat hippocampal membranes) with high selectivity.[20,21]

In order to develop antagonists for *in vitro* and *in vivo* evaluation of 5-HT_{1A} receptors, a series of new benzamido derivatives, 4-(2′-methoxyphenyl)-1-[2′-(*N*-2″-pyridinylhalobenzamido)ethyl]piperazines, was prepared.[22] The iodinated derivative (the *p*-iodobenzamido compound), [^{125}I]-*p*-MPPI, demonstrated high affinity and selectivity toward 5-HT_{1A} receptors; K_d = 0.36 nM and B_{max} = 264 fmol/mg of protein in rat hippocampal membrane homogenates. The binding was not sensitive to GTP (300 μM) or Gpp(NH)p (100 μM). In forskolin-stimulated adenyl cyclase assay using rat hippocampus, *p*-MPPI (up to 10 μM) showed no agonist activity compared to that of (±)-8-OH-DPAT. At 100 nM it completely antagonized the inhibition of forskolin-stimulated adenyl cyclase activity produced by 100 nM (±)-8-OH-DPAT.[23] This potential 5-HT_{1A} ligand is a uniquely putative antagonist which may provide a powerful tool for studies of the pharmacology of the 5-HT_{1A} receptor system. In order to further refine the structure–activity relationship of this series of compounds, a series of 4-(2′-methoxyphenyl)-1-[2′-(*N*-2″-pyridinyl-*p*-alkylbenzamido)ethyl]piperazines and several potential alkylating agents were prepared, and their binding affinity to 5-HT_{1A} receptors was evaluated using rat hippocampal homogenates. In addition, several potentially useful irreversible binding agents for 5-HT_{1A} receptors were also synthesized and evaluated.

Chemistry

Synthesis of *p*-halobenzoyl and *p*-alkylbenzoyl arylpiperazine derivatives, **2a–k**, was achieved by reactions described previously[22] (Scheme 1). Coupling of the *p*-alkylbenzoyl groups with the arylpiperazine 1 was accomplished by either using an acyl chloride in the presence of triethylamine (method A) or running the reaction with acids in the presence of oxalyl chloride in methylene chloride containing a catalytic amount of DMF (method B). Both reactions gave good yields (50–90%). The preparation of the *p*-amino and related derivatives was successfully achieved using the method shown in Scheme 2. The *p*-nitro derivatives, prepared as described previously,[22] were used as the starting

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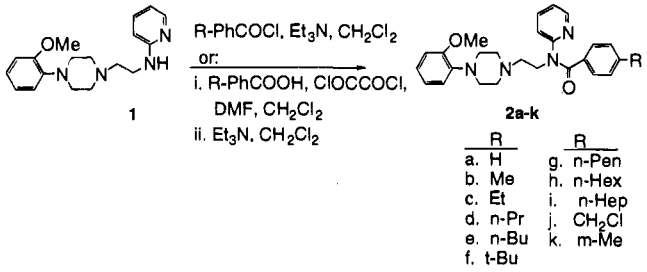
Scheme 1



Scheme 2

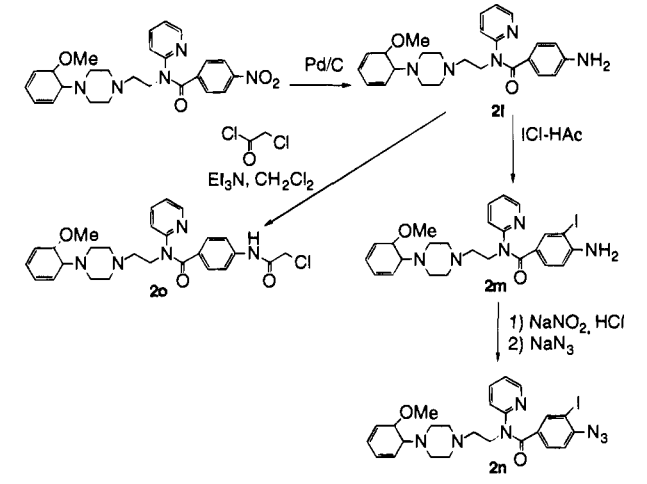


Table 1. Inhibition Constants (K_i) of Compounds **2a–q** on the Binding of [^{125}I]-(*R*)-(+)-*trans*-8-OH-PIPAT to Rat Hippocampal Homogenates

compound	K_i (nM)	compound	K_i (nM)
a, H	2.5 ± 0.7	**j**, CH_2Cl	3.5 ± 0.4
b, Me	2.2 ± 0.6	**k**, *m*-Me	16.1 ± 1.6
c, Et	9.3 ± 2.0	**l**, NH_2	10.0 ± 1.4
d, *n*-Pr	10.3 ± 2.0	**m**, NH_2, *m*-I	17.7 ± 1.1
e, *n*-Bu	13.2 ± 2.1	**n**, N_3, *m*-I	25 ± 9.0
f, *t*-Bu	27.0 ± 3.2	**o**, $NHCOCH_2Cl$	22.0 ± 0.63
g, *n*-pen	75.0 ± 4.5	**p**, I (p-MPPI)[a]	2.6 ± 0.7
h, *n*-hex	57.9 ± 5.0	**q**, F[a]	3.2 ± 0.8
i, *n*-hep	81.8 ± 10.2		

[a] Reported previously;[22] K_d = 0.3 nM for [^{125}I]MPPI to rat hippocampal homogenates.

material. The nitro group was quantitatively reduced by hydrogenation in a small volume Parr bomb. The hydrogenation method was superior to the methods using other reducing agents, which produced unknown side products and lower chemical yields during the conversion from the *p*-nitro group to the *p*-amino compound, **2k**. The *p*-amino *m*-iodo derivative, **2m**, was prepared by a simple iodination reaction of **2k**. The azido derivative, **2n**, was readily achieved by the diazo formation reaction with sodium nitrite in acidic solution followed by replacement of the diazo group with azide to give **2n**.

Results and Discussion

Results of binding studies for this series of new benzamido derivatives, 4-(2′-methoxyphenyl)-1-[2′-(*N*-2″-pyridinyl-*p*-alkylbenzamido)ethyl]piperazines, using rat brain hippocampal membrane homogenates and [^{125}I]-(*R*)-(+)-*trans*-8-OH-PIPAT[24] as the ligand, are presented in Table 1. Compounds **2a–i** are derivatives with para-substituents, from a simple hydrogen atom to a C_7 heptyl group, and the corresponding K_i values suggest that they have only limited bulk tolerance. When the para-substitution group was larger than a C_5 (pentyl) group (K_i for **2g** = 75 nM), the affinity to 5-HT_{1A} receptors dropped precipitously (**2d–h**; K_i > 50 nM). It is interesting to note that the *tert*-butyl group, which has a more compact molecular structure, actually displayed lower affinity (K_i for **2f** = 27 nM) compared to the n-butyl group (K_i for **2e** = 13.2 nM). In view of the previously reported data on para-halogenated compounds[22] (F, **2q**, and I; **2p**, K_i = 3.3 and 2.6, respectively) and that the para-halogenated compounds exhibited no steric bulk effect and showed high affinity to 5-HT_{1A} receptors, it may be possible that the halogenated compounds assume a different conformation at the 5-HT_{1A} binding site than the *p*-alkyl-substituted compounds.

In addition, several potentially irreversible binding agents, **2j** (*p*-CH_2Cl) and **2o** (*p*-$NHCOCH_2Cl$), displayed good binding affinity to 5-HT_{1A} binding sites (K_i = 3.5 and 22 nM, respectively). This type of irreversible binding agent, based on 8-OH-DPAT, has been reported in the literature.[25,26] Radioactive labeled compounds based on the Bolton–Hunter reaction have also been reported previously.[5,27] We report herein a new radioiodinated photoaffinity labeling agent, **2n** (K_i = 25 nM). It is possible that the photoaffinity labeling agents may provide another tool to specifically label the receptor site, i.e., localizing the receptor sites in autoradiography and identifying protein bands specifically related to 5-HT_{1A} receptors by gel electrophoresis.

In conclusion, a series of new *p*-benzamido derivatives, 4-(2′-methoxyphenyl)-1-[2′-(*N*-2″-pyridinylhalobenzamido)ethyl]piperazines, was prepared. *In vitro* binding studies suggest that *p*-methyl and *p*-ethyl substitutions will not change the binding affinity. However, when the substitution group was larger than the C_5 pentyl group, the affinity to 5-HT_{1A} receptors dropped below a useful level (K_i > 50 nM). Several irreversible binding agents and a photoaffinity labeling compound showing good binding affinity to 5-HT_{1A} receptors were successfully prepared, and they may be useful as pharmacological tools for studying this receptor subtype.

Experimental Section

NMR were recorded on a Varian EM 360A, a Bruker WM-250 (250 MHz), or a Bruker AM 500 (500 MHz) spectrometer. The chemical shifts are reported in ppm downfield from an internal tetramethylsilane standard. Infrared spectra were obtained with a Mattson Polaris FT-IR spectrophotometer. Mass spectra were performed with a mass spectrometer, VG70-70HS.

General Procedure for Preparation of 2a–k. Method A: With Acyl Chlorides and Triethylamine. To a solution of 4-(2′-methoxyphenyl)-1-[2′-(2″-pyridinylamino)ethyl]piperazine (amine)[22] and Et_3N in CH_2Cl_2 was added a solution of acyl chloride in CH_2Cl_2 dropwise at 0 °C in an ice bath. The mixture was stirred at room temperature for 1 h. H_2O was added, and the mixture was extracted with CH_2Cl_2. The combined organic layers were dried and then evaporated to give the crude product, which was purified by PTLC (preparative thin layer chromatography; EtOAc as the solvent) or MPLC (medium pressure liquid chromatography; EtOAc as eluent) to give the desired pure product.

Method B: With Acids and Oxalyl Chloride. To a mixture of acid and a catalytic amount of DMF in CH_2Cl_2 was added oxalyl chloride in neat at 0 °C in an ice bath.[22] The mixture was stirred at 0 °C for 30 min. Solvent and the excess oxalyl chloride were removed on vacuum, and the residue was

Table 2. NMR Data of Compounds **2a–o**

	^{1}H NMR (free base in $CDCl_3$)					
	parent moiety					
	piperazine	ethylene	$ArOCH_3$	ArH	substitute	molecular formula
a	2.64(br), 2.92(br)	2.75(t), 4.30(t)	3.84	6.76(d), 6.8–7.4, 8.43(dd)	7.19–7.40(m)	$C_{25}H_{28}N_4O_2$
b	2.64(br), 2.93(br)	2.75(t), 4.30(t)	3.84	6.74(d), 6.8–7.2, 8.43(dd)	2.24(s), 6.99(d), 7.23(d)	$C_{26}H_{30}N_4O_2$
c	2.64(br), 2.93(br)	2.75(t), 4.29(t)	3.83	6.75(d), 6.8–7.2, 8.43(dd)	1.16(t), 2.57(q), 7.00(d), 7.25(d)	$C_{27}H_{32}N_4O_2$
d	2.63(br), 2.92(br)	2.75(t), 4.29(t)	3.83	6.74(d), 6.8–7.4, 8.42(dd)	0.86(t), 1.56(hex), 2.51(t), 6.99(d), 7.24(d)	$C_{28}H_{34}N_4O_2$
e	2.64(br), 2.93(br)	2.75(t), 4.30(t)	3.84	6.74(d), 6.8–7.4, 8.43(dd)	0.88(t), 1.28(hex), 1.51(pen), 2.53(t), 7.00(d), 7.24(d)	$C_{29}H_{36}N_4O_2$
f	2.63(br), 2.93(br)	2.75(t), 4.30(t)	3.84	6.77(d), 6.8–7.4, 8.43(dd)	1.24(s), 7.21(d), 7.27(d)	$C_{29}H_{36}N_4O_2$
g	2.63(br), 2.92(br)	2.75(t), 4.29(t)	3.84	6.74(d), 6.8–7.4, 8.43(dd)	0.85(t), 1.25(m), 1.53(pen), 2.52(t), 6.99(d), 7.24(d)	$C_{30}H_{38}N_4O_2$
h	2.64(br), 2.93(br)	2.75(t), 4.29(t)	3.84	6.74(d), 6.8–7.4, 8.43(dd)	0.86(t), 1.25(m), 1.52(pen), 2.53(t), 6.99(d), 7.24(d)	$C_{31}H_{40}N_4O_2$
i	2.64(br), 2.93(br)	2.75(t), 4.30(t)	3.84	6.74(d), 6.8–7.4, 8.42(dd)	0.86(t), 1.25(m), 1.53(pen), 2.52(t), 6.99(d), 7.24(d)	$C_{32}H_{42}N_4O_2$
j	2.66(br), 2.94(br)	2.77(t), 4.30(t)	3.83	6.67–7.04(m), 8.42(dd)	4.49(s), 7.20–7.23, 7.31–7.33(d)	$C_{26}H_{29}N_4O_2Cl$
k	2.64(br), 2.93(br)	2.75(t), 4.30(t)	3.84	6.77(d), 6.8–7.4, 8.43(dd)	2.24(s), 6.98–7.05(m)	$C_{26}H_{30}N_4O_2$
l	2.64(br), 2.92(br)	2.75(t), 4.27(t)	3.83	6.73(d), 6.8–7.3, 8.42(dd)	6.42(d), 7.15(d)	$C_{25}H_{29}N_5O_2$
m	2.62(br), 2.90(br)	2.73(t), 4.23(t)	3.82	6.74(d), 6.8–7.4, 8.43(dd)	6.43(d), 7.00(d), 7.15(d)	$C_{25}H_{28}N_5O_2I$
n	2.63(br), 2.92(br)	2.73(t), 4.25(t)	3.83	6.8–7.3, 8.45(dd)	6.89(d), 7.25(d), 7.83(d)	$C_{25}H_{27}N_7O_2I$
o	2.63(br), 2.92(br)	2.75(t), 4.28(t)	3.83	6.74(d), 6.8–7.4, 8.43(dd)	4.15(s), 7.33(d), 7.42(d)	$C_{27}H_{30}N_5O_3Cl$

dissolved in CH_2Cl_2, which was added to a solution of amine and Et_3N in CH_2Cl_2 at 0 °C. The mixture was stirred at room temperature for 1 h. General work up using the same procedure described above (method A) produced the desired pure product.

Preparation of Compound 2l. To a solution of the *p*-nitro derivative (730 mg, 1.58 mmol), prepared as described previously[22] in a solvent (EtOAc:MeOH = 9:1), was added Pd/C (10%, 100 mg). The mixture was hydrogenated in a small volume Parr bomb overnight (40 psi) at RT. The mixture was filtered through Celite, and the filtrate was evaporated, producing a white solid (680 mg) in quantitative yield.

Preparation of Compound 2m. To a solution of **2l** (100 mg, 0.23 mmol) in AcOH (1 mL) was added ICl (37 mg, 1.2 equiv) in AcOH (1 mL) at room temperature. The mixture was stirred at room temperature for 4 h and then poured into water (5 mL). The mixture was neutralized with NaOH (1 M), and the resulting mixture was extracted with CH_2Cl_2. The combined organic extracts were dried over sodium sulfate and condensed to give the crude product, which was purified by PTLC (CH_2Cl_2:MeOH = 93:7) to give the desired product **2m** (51 mg) in 40% yield.

Preparation of Compound 2n. To a solution of **2m** (86 mg, 0.15 mmol), ice (0.2 g), and HCl (0.1 mL) in water (1 mL) was added $NaNO_2$ (13 mg, 1.2 equiv) in water (1 mL) at 0° C (ice bath). The mixture was stirred at 0 °C for 30 min. NaN_3 (30 mg) in water (1 mL) was added, and the mixture was stirred again at 0 °C for 2 h. The solution was made basic with NaOH (1 M) and extracted with CH_2Cl_2. The organic extracts were combined and condensed. The crude mixture was purified by PTLC (EtOAc as developing solvent) to give the product **2n** (22 mg after two purifications, 24%). Mass spectra: m/z 584 ($M^+ + 1$).

Preparation of Compound 2o. To a solution of **2l** (159 mg, 0.35 mmol) and Et_3N (0.15 mL, 3 equiv) in CH_2Cl_2 (5 mL) was added chloroacetyl chloride (47 mg, neat) at 0 °C. The mixture was stirred at room temperature for 1 h. The organic extracts were combined and condensed to give the crude product. The crude mixture was purified by MPLC (EtOAc as the eluent) to give the product **2o** (138 mg) in 78% yield.

5-HT$_{1A}$ Binding Assay. The measurements of 5-HT$_{1A}$ binding sites with [^{125}I]-(*R*)-(+)-*trans*-8-OH-PIPAT were carried out as described previously.[24] The hippocampal homogenates were prepared in 100 volumes of ice-cold Tris-HCl buffer (50 mM, pH 7.4) and centrifuged at 20000*g* for 20 min. The resulting pellets were resuspended in ice-cold water to lyse vesicles and subsequently preincubated at 37 °C and recentrifuged to remove the endogenous serotonin. The final pellets were resuspended in the Tris buffer containing 2 mM $MgCl_2$. The binding assays were carried out in a total volume of 0.2 mL containing 50 μL of tissue preparations (40–60 μg of protein), appropriate amounts of labeled ligand (0.2–0.5 nM), and different concentrations of inhibitors. The tubes were incubated at 37 °C for 15 min, and then the reactions were terminated by vacuum filtration through glass filters (Schleicher & Schuell, No. 25, Keene, NH) presoaked with 1% poly(ethylenimine). The filters were then washed three times with 3 mL of ice-cold buffer, and the radioactivity on the filters was counted in a γ counter (Packard 5000). Nonspecific binding was defined with 10 μM 5-HT. The competition experiments were analyzed using the iterative nonlinear least-squares curve-fitting program LIGAND.[28]

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References

(1) Frazer, A.; Maayani, S.; Wolfe, B. B. Subtypes of Receptors For Serotonin. *Ann. Rev. Pharm. 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. N.Y. Acad. Sci.* **1990**, *600*, 460–475.
(3) Hartig, P. R.; Adham, N.; Zgombick, J.; Macchi, M.; Kao, H. T.; Schechter, L.; Branchek, T.; Weinshank, R. Serotonin Receptor Subtypes. *Psychopharm. Ser.* **1993**, *10*, 15–25.
(4) Hamon, M.; Lanfumey, L.; El Mestikawy, S.; Boni, C.; Miquel, M.-C.; Bolanos, F.; Schechter, L.; Gozlan, H. The Main Features of Central 5-HT$_1$ Receptors. *Neuropsychopharmacology* **1990**, *3*, 349–360.
(5) Radja, F.; Daval, G.; Hamon, M.; Verge, D. Pharmacological and Physicochemical Properties of Presynaptic Versus Postsynaptic 5-Hydroxytryptamine-1A Receptor Binding Sites In the Rat Brain: A Quantitative Autoradiographic Study. *J. Neurochem.* **1992**, *58*, 1338–1346.
(6) 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.
(7) 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.
(8) 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.
(9) 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.

(10) Glennon, R. A.; Naiman, N. A.; Pierson, M. E.; Smith, J. D.; Ismaiel, A. M.; Titeler, M.; Lyon, R. A. N-(Phthalimidoalkyl) Derivatives of Serotonergic Agents: A Common Interaction At 5-HT_{1A} Serotonin Binding Sites? *J. Med. Chem.* **1989**, *32*, 1921–1926.
(11) Glennon, R. A.; Westkaemper, R. B.; Bartyzel, P. Medical Chemistry of Serotonergic Agents. In *Receptor Biochemistry and Methodology;* Peroutka, S. J., Ed.; Wiley-Liss: Stanford, CA, 1991; pp 19–64.
(12) 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.
(13) van Steen, B. J.; van Wijngaarden, I.; Tulp, M. T. M.; Soudijn, W. Structure-Affinity Relationship Studies on 5-HT_{1A} Receptor Ligands. 2. Heterobicyclic Phenylpiperazines with *N*4-Aralkyl Substituents. *J. Med. Chem.* **1994**, *37*, 2761–2773.
(14) Kuipers, W.; van Wijngaarden, I.; Ijzerman, A. P. A Model of the Serotonin 5-HT_{1A} Receptor: Agonist and Antagonist Binding Sites. *Drug Des. Disc.* **1994**, *11*, 231–249.
(15) Hacksell, U.; Liu, Y.; Yu, H.; Vallgårda, J.; Höök, B. B.; Johansson, A. M.; Lewander, T. Neuromedicinal Chemistry of 5–HT_{1A}-Receptor Agonists and Antagonists. *Drug Des. Discovery* **1993**, *9*, 287–297.
(16) Hoyer, D.; Engel, G.; Kalkman, H. O. Molecular Pharmacology of 5-HT_1 and 5-HT_2 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.
(17) Hoyer, D. The 5-HT Receptor Family: Ligands, Distribution and Receptor-Effector Coupling. In *5-HT_{1A} Agonists, 5-HT3 Antagonists and Benzodiazepines; Their Comparative Behavioural Pharmacology*, Rodgers, R. J., Cooper, S. J., eds.; John Wiley & Sons: New York, 1991; pp 31–57.
(18) Rydelek-Fitzgerald, L.; Teitler, M.; Fletcher, P. W.; Ismaiel, A. M.; Glennon, R. A. NAN-190: Agonist and Antagonist Interactions With Brain 5-HT_{1A} Receptors. *Brain Res.* **1990**, *532*, 191–196.
(19) 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.
(20) Cliffe, I. A.; Brightwell, C. I.; Fletcher, A.; Forster, E. A.; Mansell, H. L.; Reilly, Y.; Routledge, C.; White, A. C. The Design of Selective 5-HT_{1A} Receptor Antagonists. 206th ACS Meeting; American Chemical Society: Washington, DC, 1994; MEDI, Abstr. No. 30.
(21) Fletcher, A.; Cliffe, I. A.; Dourish, C. T. Silent 5-HT_{1A} Receptor Antagonists: Utility As Research Tools and Therapeutic Agents. *Trends Pharmacol. Sci.* **1993**, *14*, 441–448.
(22) Zhuang, Z.-P.; Kung, M.-P.; Kung, H. F. Synthesis and Evaluation of 4-(2′-Methoxy-)phenyl-1-[2′-(N-2″-pyridinyl-)*p*-iodobenzamido-]ethyl-piperazine (*p*-MPPI): A New Iodinated 5-HT_{1A} Ligand. *J. Med. Chem.* **1994**, *37*, 1406–1407.
(23) Kung, H. F.; Kung, M.-P.; Clarke, W.; Maayani, S.; Zhuang, Z.-P. A Potential 5-HT_{1A} Receptor Antagonist: *p*-MPPI. *Life Sci.* **1994**, *55*, 1459–1462.
(24) 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.
(25) Radja, F.; Daval, G.; Emerit, M. B.; Gallissot, M. C.; Hamon, M.; Verge, D. Selective Irreversible Blockade of 5-hydroxytryptamine1A and 5-hydroxytryptamine1C Receptor Binding Sites in the Rat Brain By 8-MeO-2′-chloro-PAT: A Quantitative Autoradiographic Study. *Neuroscience* **1989**, *31*, 723–733.
(26) Bolanos, F. J.; Schechter, L. E.; Laporte, A. M.; Hamon, M.; Gozlan, H. Recovery of 5-HT_{1A} Receptors After Irreversible Blockade By N-Ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ). *Proc. West. Pharmacol. Soc.* **1991**, *34*, 387–393.
(27) Gozlan, H.; Ponchant, M.; Daval, G.; Verge, D.; Menard, F.; Vanhove, A.; Beaucourt, J. P.; Hamon, M. ^{125}I-Bolton-Hunter-8-methoxy-2[n-propyl-n-propylamino]tetralin as a New Selective Radioligand of 5-HT_{1A} Sites in the Rat Brain - *In Vitro* Binding and Autoradiographic Studies. *J. Pharmacol. Exp. Ther.* **1988**, *244*, 751–759.
(28) Munson, P.; Rodbard, D. LIGAND: A Versatile Computerized Approach for Characterization of Ligand-Binding Systems. *Anal. Biochem.* **1980**, *107*, 220–239.