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Binding of β-carbolines and related agents at serotonin (5-HT_2 and 5-HT_{1A}), dopamine (D_2) and benzodiazepine receptors

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Abstract

A large series of β-carbolines was examined for their ability to bind at [^{3}H]agonist-labeled 5-HT_{2A} serotonin receptors. Selected β-carbolines were also examined at 5-HT_{2C} serotonin receptors, 5-HT_{1A} serotonin receptors, dopamine D_2 receptors, and benzodiazepine receptors. Indolealkylamines and phenylisopropylamines were also evaluated in some of these binding assays. The β-carbolines were found to bind with modest affinity at 5-HT_{2A} receptors, and affinity was highly dependent upon the presence of ring substituents and ring saturation. The β-carbolines displayed little to no affinity for 5-HT_{1A} serotonin receptors, dopamine D_2 receptors and, with the exception of β-CCM, for benzodiazepine receptors. Examples of β-carbolines, indolealkylamines (i.e. *N*,*N*-dimethyltryptamine analogs), and phenylisopropylamines have been previously shown to produce common stimulus effects in animals trained to discriminate the phenylisopropylamine hallucinogen DOM (i.e. 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane) from vehicle. Although the only common receptor population that might account for this action is 5-HT_{2A}, on the basis of a lack of enhanced affinity for agonist-labeled 5-HT_{2A} receptors, as well as on their lack of agonist action in the PI hydrolysis assay, it is difficult to conclude that the β-carbolines behave in a manner consistent with that of other classical hallucinogens.

Keywords: β-carbolines; Harmaline; Harmine; Hallucinogens; Dimethyltryptamine (DMT); 1-(2,5-Dimethoxy-4-methylphenyl)-2-aminopropane (DOM); Serotonin receptors; Dopamine receptors; Benzodiazepine receptors; PI hydrolysis

1. Introduction

Classical or arylalkylamine hallucinogens are divided into two broad chemical families: the indolealkylamines and the phenylalkylamines (reviewed: Glennon, 1998). The indolealkylamine category is comprised of (a) the ergolines (or lysergamides), such as derivatives of (+)-lysergic acid diethylamide (LSD), (b) the α-alkyltryptamines, such as 5-methoxy-α-methyltryptamine, and (c) the simple *N*-substituted tryptamines, such as *N*,*N*-dimethyltrptamine (DMT) (Fig. 1). The phenylalkylamine hallucinogens are divided into the (a) phenylethylamines, such as mescaline, and (b) the phenylisopropylamines, such as 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM). In tests of stimulus generalization with animals trained to discriminate DOM from vehicle, examples of agents from each category have been demonstrated to produce similar stimulus effects (reviewed: Glennon, 1996). In fact, this represents one of the criteria used for categorizing an agent as a classical hallucinogen (Glennon, 1998).

Although carefully controlled human studies are lacking, certain β-carbolines are considered to be hallucinogenic in humans (see Grella et al., 1998 for a recent

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overview). Like the above indolealkylamine hallucinogens, β-carbolines possess an indoleethylamine moiety embedded in their structures and, chemically, represent conformationally restricted analogs of the simpler *N*-substituted tryptamines. Certain β-carbolines, although not a major drug abuse problem in this country, have been used in plant-derived forms by South American natives for centuries (e.g. Deulofeu, 1967; Schultes, 1967; Rivier and Lindgren, 1972; Grob et al., 1996). Harmine, harmaline, and tetrahydroharmine are among the more common β-carbolines (Fig. 1). Harmine and harmaline have probably received the most attention and their hallucinogenic potencies are reported to be in the range of DMT (Pennes and Hoch, 1957; Naranjo, 1967, 1969, 1973). Harmaline and 6-methoxyharmalan have been shown to substitute for DOM in DOM-trained animals (Glennon et al., 1983).

Classical hallucinogens are thought to produce their behavioral effects, at least in part, via interaction with 5-HT_2 serotonin receptors in the brain (reviewed: Glennon, 1998). Although classical hallucinogens bind at multiple populations of 5-HT_2 receptors (i.e. 5-HT_{2A} and 5-HT_{2C} receptors), evidence is mounting that 5-HT_{2A} receptors are the primary targets of these agents (for further discussion see: Glennon, 1998; Nelson et al., 1999). We have investigated the binding of a limited series of β-carbolines and have shown that certain of these also bind at [^{3}H]ketanserin-labeled 5-HT_{2A} receptors (Grella et al., 1998).

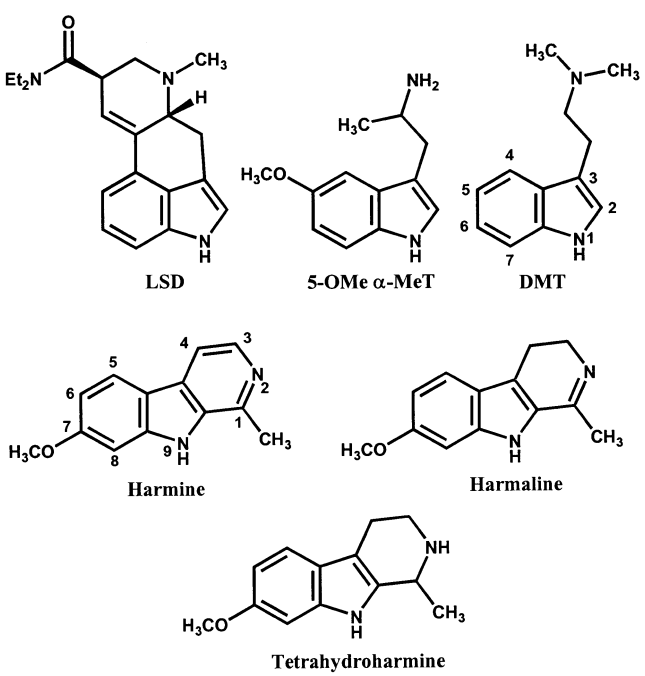


Fig. 1. Chemical structures of the ergoline hallucinogen LSD, the α-alkyltryptamine hallucinogen 5-methoxy-α-methyltryptamine (5-OMe α-MeT), the *N*,*N*-dialkyltryptamine hallucinogen *N*,*N*-dimethyltryptamine (DMT), and the β-carbolines harmine, harmaline, and tetrahydroharmine.

5-HT_{2A} receptors are thought to exist in two affinity states (i.e. a high affinity state and a low affinity state) (Battaglia et al., 1984). Radiolabeled antagonists, such as [^{3}H]ketanserin, label both states of the receptor whereas radiolabeled agonists label the high affinity state. Binding of β-carbolines at the agonist-labeled high-affinity state of 5-HT_{2A} receptors has not been previously examined. Thus, it was of interest to determine the affinities of β-carbolines at these receptors. That is, if β-carbolines are 5-HT_{2A} agonists, they should possess a higher affinity at agonist-labeled sites than at antagonist-labeled sites, and such information would serve as a preliminary indicator of functional activity.

Certain β-carbolines produce effects in humans that are similar to more established hallucinogens such as LSD; nevertheless, the effects produced by these β-carbolines allow them to be distinguished from non-β-carboline hallucinogens (Pennes and Hoch, 1957; Naranjo, 1973). It is possible that hallucinogens might, in addition to their interaction at 5-HT_2 receptors, produce some of their effects via other mechanisms. Although phenylalkylamine hallucinogens have not been demonstrated to bind with significant affinity at any population of receptors other than 5-HT_2 receptors, indolealkylamines are known to bind with high affinity at 5-HT_{1A} receptors (Glennon, 1996). In fact, it has been suggested that 5-HT_{1A} receptors may play a prominent role in the actions of hallucinogenic indolealkylamines (e.g. Deliganis et al., 1991). In addition to high affinity for 5-HT_{1A} and 5-HT_{2A} receptors, LSD binds with high affinity at dopamine D_2 receptors and displays dopamine agonist character (e.g. Giacomelli et al., 1998). The binding of various phenylalkylamines at 5-HT_{1A} serotonin receptors or D_2 dopamine receptors has not been investigated. Nevertheless, there has been long-standing speculation that these receptor populations may play a role in mediating the behavioral effects of classical hallucinogens as a class. These issues have never been satisfactorily addressed.

Finally, certain nonhallucinogenic β-carbolines (e.g. β-CCM or methyl β-carboline-3-carboxylate) bind with high affinity at benzodiazepine (BZ) receptors; some possess anxiogenic character and others display anxiolytic properties (e.g. Hollinshead et al., 1990; Allen et al., 1992; Cox et al., 1998). Certain hallucinogenic agents are also known to produce anxiety (Strassman, 1984). Given that the β-carboline hallucinogens are closely related in structure to the β-carboline anxiogenic and anxiolytic agents, it was of interest to determine if they, too, bind at BZ receptors.

Thus, the primary purpose of the present investigation was to systematically examine the binding of a series of hallucinogen-related β-carbolines at (a) agonist-labeled 5-HT_{2A} serotonin receptors (b) 5-HT_{2C} serotonin receptors (c) 5-HT_{1A} serotonin receptors (d)

D_2 dopamine receptors, and (e) benzodiazepine (BZ) receptors to determine whether or not they bind at these receptor populations and, where possible, to formulate structure–affinity relationships. A systematic binding study, such as that described herein, has not been previously reported for the β-carbolines; this is probably due to a lack of ready availability of many of the β-carbolines. Consequently, most of the necessary β-carbolines were synthesized expressly for the purpose of these investigations. A second goal of this work was to determine if 5-HT_2 serotonin receptors, 5-HT_{1A} serotonin receptors, or dopamine D_2 receptors better account for the common actions produced by indolealkylamines and phenylalkylamines. To this extent, radioligand binding data were also obtained for selected DMT analogs and phenylisopropylamines for purpose of comparison.

2. Methods

2.1. 5-HT and dopamine receptor binding studies

In general, the assay methods employed were similar to those used previously (Egan et al., 1998; Metwally et al., 1998). Cell lines expressing rat 5-HT_{1A} receptors in CHO cells (donated by Allelix Biopharmaceuticals), rat 5-HT_{2A} receptors in NIH-3T3 cells (donated by Dr. David Julius), and rat 5-HT_{2C} receptors in A-9 cells (donated by Dr Marc Caron) were subcultured and grown until confluent. Membranes were prepared by scraping and homogenizing in 50 mM Tris–HCl/5 mM $MgCl_2$/0.5 mM EDTA, pH 7.4 buffer (assay buffer), and centrifugation at $12\,000 \times g$ for 30 min. Membranes were resuspended in assay buffer, homogenized, and centrifuged again. After resuspension in assay buffer 1 ml membrane aliquots (≈ 10 μg protein measured by bicinchoninic assay) were added to each tube containing 1 ml of assay buffer with either 0.4 nM [^{3}H]DOB (5-HT_{2A}), 0.4 nM [^{3}H]8-OH DPAT (5-HT_{1A}), 1 nM [^{3}H]mesulergine (5-HT_{2C}), or 0.1 nM [^{3}H]*N*-methylspiperone (D_2) and competing test agent. Mianserin (10 μM, 5-HT_{2A}), 10 μM 8-OH DPAT (5-HT_{1A}), 10 μM mesulergine (5-HT_{2C}), or 10 μM spiperone (D_2) was used to define nonspecific binding. Samples were incubated at 37°C for 30 min, filtered on a Brandel cell harvester, and counted in Ecoscint cocktail (National Diagnostics) in a Beckman Liquid Scintillation counter at 40% efficiency. Initially, test compounds were evaluated at 10^{-5} M concentrations; those compounds displaying $< 30\%$ inhibition at this concentration were reported an possessing K_i values $> 10\,000$ nM, whereas those displaying $> 30\%$ inhibition were examined in greater detail. IC_{50} values were generated using GraphPad Prism2. K_i values were determined from the Cheng–Prusoff equation: $K_i = IC_{50}/1 + [D]/K_D$ (Cheng and Prusoff, 1973). K_i values represent a minimum of three determinations.

2.2. BZ receptor binding

Binding studies were performed in the Laboratory of Dr Arthur Jacobson (NIH) as previously described (Basile et al., 1989) with minor modifications. For measurement of RO15-1788 binding, male Sprague–Dawley rats were decapitated according to AAALAC guidelines and the brains were placed immediately in ice-cold 50 mM Tris citrate buffer. The cortex was shaved from the brains and homogenized in 50 vol. of buffer using a Polytron (Brinkmann Instruments) setting 6 for 20 s. The homogenate was washed in Tris citrate buffer; a total of $20\,000 \times g$ for 20 min. The final resuspension was in 10 vol. of Tris citrate and frozen at −20°C until use. The assay mixture contained 50 μl of 10 μM flunitrazepam for determination of nonspecific binding, buffer or test compound, 50 μL of 1 nM [^{3}H]RO15,1788 and sufficient buffer to bring the final assay volume to 0.5 ml. After a 60-min incubation at 25°C, the assay was terminated by rapid filtration over S&S glass fiber filters using a Brandel M-24R cell harvester. Filters were placed in CytoScint ES Scintillation cocktail and allowed to stand overnight before counting on a Packard Tricarb 2200CA counter. The inhibition constant (K_i) for determination of the affinity of the test compounds for BZ receptors was calculated using the Cheng–Prusoff equation (Cheng and Prusoff, 1973) with a predetermined K_D for RO17,1588 (1.0 nM) from Scatchard analysis. Experiments were performed in duplicate.

2.3. PI hydrolysis studies

Inositol phosphate (IP) production has been described in detail by Herrick-Davis et al. (1997) and Egan et al. (1998). In brief, 24 h after plating cells expressing 5-HT_{2A} or 5-HT_{2C} receptors at 1.5×10^5 cells per well, cells were washed with phosphate-buffered saline (PBS) and labeled with 0.25 μCi/well of myo-[^{3}H]inositol (New England Nuclear) in inositol-free/serum-free DMEM (GIBCO) for 18 h at 37°C. After labeling, cells were washed with PBS and preincubated in inositol-free/serum-free DMEM with 10 mM LiCl and 10 μM pargyline (assay medium) for 10 min at 37°C. Varying concentrations of antagonists were added during the 10-min preincubation period. Cells were challenged with 100 nM 5-HT (Sigma) and incubated for an additional 30 min. Assay medium was removed and cells were lysed in 250 μl of stop solution (1 M KOH/18 mM sodium borate/3.8 mM EDTA) and neutralized by adding 250 μl of 7.5% HCl. The contents of each well were extracted with three volumes of $CHCl_3$/MeOH (1:2), centrifuged 10 min at $10\,000 \times g$,

and the upper layer was loaded onto 1-ml AG1-X8 resin (100–200 mesh, Bio-Rad) columns. Columns were washed with 10 ml of 5 mM myoinositol and 10 ml of 5 mM sodium borate/60 mM sodium formate. Total IPs were eluted with 3 ml of 0.1 M formic acid/1 M ammonium formate. Radioactivity was measured by liquid scintillation counting in Ecoscint cocktail. EC_{50} values were generated using GraphPad Prism2. Assays were performed on a minimum of two separate ocassions.

2.4. Agents

The synthesis of some of the β-carbolines used in the present study was recently reported (Grella et al., 1998). Harmalol hydrochloride dihydrate (Fluka), 6-methoxytetrahydro-β-carboline (Research Biochemicals Inc.), β-carboline-3-carboxylic acid methyl ester (β-CCM; Sigma) and β-carboline-3-carboxylic acid *N*-methylamide (β-CCM amide; Sigma) were purchased from commercial sources, and tetrahydro-β-carboline hydrochloride (THBC) was a gift from Dr M. Schechter. *R*(−)3-methoxycarbonyl-3,4-dihydro-β-carboline hydrochloride (LC-032) and its *S*(+)-enantiomer LC-033 were prepared by a previously reported method (Ishida et al., 1985).

9-Methylharmalan was prepared in the following manner: N_1-methyltryptamine was acylated with acetic anhydride and the derived amide was cyclized in a Bischler–Napieralski type reaction using P_2O_5; the same product was obtained by direct methylation of harmalan in the presence of sodium hydride. The products from the two procedures were converted to their hydrochloride salts (mp: 257–259°C after recrystallization from absolute EtOH/Et_2O); results of elemental microanalysis (calculated/found) are: C, 66.56/66.28%; H, 6.44/6.36%; N, 11.93/11.78%. 1-Desmethylharmaline, prepared using the above cyclization reaction with *N*-formyl-6-methoxytryptamine as starting material, was isolated as its hydrochloride salt with 0.25 mol. of water of crystallization (mp 233–234°C); microanalysis: C, 59.76/59.74%; H, 5.64/5.46%; N, 11.61/11.37%. 5-Methoxy-1,2,3,4-tetrahydroharman was prepared by $NaBH_4$/MeOH reduction of 5-methoxyharmalan (free base) and the product was isolated as its oxalate salt (mp: 198–200°C after recrystallization from MeOH); microanalysis: C, 58.47/58.21%; H, 5.95/5.99%; N, 9.09/8.89%. The corresponding 8-methoxy-1,2,3,4-tetrahydroharman oxalate (mp: >260°C after recrystallization from MeOH/Et_2O) was prepared by similar reduction of 8-methoxyharmalan and crystallized with 0.1 mol. of water; microanalysis: C, 63.90/63.91%; 6.59/6.59%; N, 10.65/10.72%. 7-Methoxy THBC hydrochloride (mp: 267–270°C after recrystallization from MeOH) was obtained by reduction of 7-methoxytetrahydro-β-carbol-1-one with $LiAlH_4$ in a manner similar to that reported by Yamada et al. (1986) for the preparation of the corresponding 5-methoxy analog; microanalysis: C, 60.38/60.36%; H, 6.32/6.33%; N, 11.73/11.65%. The corresponding N_2-methyl derivative, N_2-methyl-7-methoxy THBC, was prepared from 7-methoxy THBC (free base) by treatment with ethyl chloroformate followed by reduction of the resulting carbamate with $LiAlH_4$. The product was isolated as its hydrochloride salt which crystallized with 0.5 moles of water (mp: 255–257°C after recrystallization from absolute EtOH); microanalysis: C, 59.65/59.87%; H, 6.93/6.58%; N, 10.70/10.51%. All proton NMR data are consistent with structure assignments. All compounds, synthesized and commercial, were homogeneous by thin-layer chromatography.

All of the other agents used in the present investigation were previously synthesized in our laboratories and include: *N*,*N*-dimethyltryptamine (DMT), 4-methoxy-*N*,*N*-dimethyltryptamine (4-OMe DMT), 5-methoxy-*N*,*N*-dimethyltryptamine (5-OMe DMT), and 6-methoxy-*N*,*N*-dimethyltryptamine (6-OMe DMT) as their hydrogen oxalate salts, and 7-methoxy-*N*,*N*-dimethyltryptamine HCl (7-OMe DMT), 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane HCl (DOM), 1-(4-bromo-2,5-dimethoxyphenyl)-2-aminopropane HCl, 1-(4-chloro-2,5-dimethoxyphenyl)-2-aminopropane HCl (DOC), 1-(2,5-dimethoxy-4-ethylphenyl)-2-aminopropane HCl (DOET), 1-(2,5-dimethoxy-4-*n*-butylphenyl)-2-aminopropane HCl (DOPR), 1-(2,5-dimethoxyphenyl)-2-aminoproane HCl (2,5-DMA), 1-(2,4,5-trimethoxyphenyl)-2-aminopropane HCl (2,4,5-TMA), 1-(2,5-dimethoxy-4-ethoxyphenyl)-2-aminopropane HCl (MEM), 1-(2,3,4-trimethoxyphenyl)-2-aminopropane HCl (2,3,4-TMA), 1-(3,4,5-trimethoxyphenyl)-2-aminopropane HCl or α-methylmescaline (3,4,5-TMA), 1-(2,4,6-trimethoxyphenyl)-2-aminopropane HCl (2,4,6-TMA), and *N*-methyl-1-(4-methoxyphenyl)-2-aminopropane HCl (PMMA).

3. Results

The β-carbolines in this study can be arbitrarily divided into three structural groups depending upon their degree of ring saturation: (a) the fully aromatic harman derivatives (i.e. those with a fully unsaturated pyridine ring), (b) the dihydro or harmalan derivatives, and (c) the tetahydro derivatives. Binding data are shown in Table 1 for each of the examined compounds. 5-HT_{2C} binding data were previously published for some of these β-carbolines (Grella et al., 1998) and are included in Table 1 for comparison with the newly examined compounds.

3.1. 5-HT_{2A} binding

In the fully aromatic series, the unsubstituted β-carboline harman ($K_i = 268$ nM) and the 7-methoxy derivative harmine ($K_i = 397$ nM) bind with nearly comparable affinity and with the highest affinities within the harman series. The 5-methoxy derivative (5-methoxyharman; $K_i = 1340$ nM) binds with several-fold lower affinity whereas the 6- and 8-methoxy derivatives display lower affinity ($K_i > 10\,000$ nM). This is unlike the trend seen with the dihydro series. 5-Methoxyharmalan ($K_i = 86$ nM) binds with 10-fold higher affinity than any of the other harmalan derivatives; harmalan itself binds with modest affinity ($K_i = 990$ nM) whereas the remaining methoxy-substituted derivatives bind with K_i values in the 1500–5000 nM range. In general, the tetrahydro derivatives bind with an affinity similar to that of their dihydro or harmalan counterparts; but, it should be kept in mind that most of these tetrahydro derivatives are racemic mixtures. In one instance, the individual optical isomers were prepared and examined; $S(-)$tetrahydroharmine ($K_i = 5000$ nM) was found to bind with higher affinity than its $R(+)$enantiomer ($K_i > 10\,000$ nM), and with an affinity comparable to that of its corresponding dihydro analog, harmaline ($K_i = 5010$ nM). In the dihydro and tetrahydro series, high affinity is associated with methoxy substitution at the 5- and 8-positions.

Several additional structural modifications in the dihydro and tetrahydro series were also examined. The 7-hydroxy analog of harmalan, harmalol ($K_i > 10\,000$), displays low affinity. Introduction of a 3-carbomethoxy group affords two optical isomers of harmalan; neither isomer binds at 5-HT_{2A} receptors (LC-032 and LC-033, $K_i > 10\,000$ nM). Introduction of an N_9-methyl substituent results in loss of affinity (9-methylharmalan; $K_i > 10\,000$ nM) as does removal of the 1-methyl substituent (desmethylharmaline; $K_i > 10\,000$ nM). Re-

Table 1
Radioligand binding data for β-carboline derivatives at 5-HT_{2A}, 5-HT_{2C}, and 5-HT_{1A} serotonin receptors, D_2 dopamine receptors, and benzodiazepine (BZ) receptors

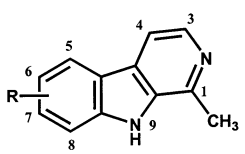
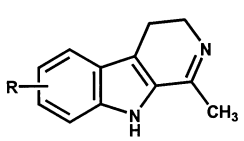
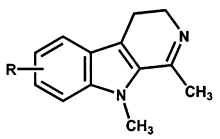
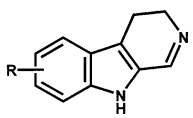
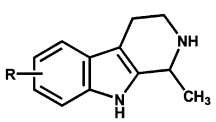
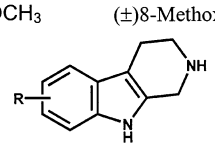
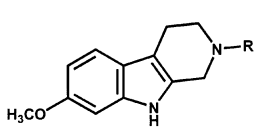
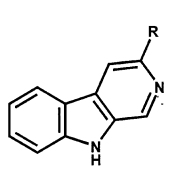
Ring system/R			K_i, nM (± SEM)				
			5-HT_{2A}	5-HT_{2C}[a]	5-HT_{1A}	D_2[b]	BZ
Fully Aromatic Derivatives							
							
-H	Harman		268 (14)	2,490†	> 10,000	-	-
5-OCH_3	5-Methoxyharman		1,340 (140)	417†	4,560 (400)	-	-
6-OCH_3	6-Methoxyharman		> 10,000	3,700†	> 10,000	-	-
7-OCH_3	Harmine		397 (13)	5,340†	> 10,000	> 10,000	> 10,000
8-OCH_3	8-Methoxyharman		> 10,000	2,530†	> 10,000	-	-
Dihydro Derivatives							
							
-H	Harmalan		990 (35)	1, 860†	3,410 (180)	> 10,000	> 10,000
5-OCH_3	5-Methoxyharmalan		86 (4)	69†	6,190 (295)	> 10,000	> 10,000
6-OCH_3	6-Methoxyharmalan		4,220 (730)	924†	> 10,000	> 10,000	-
7-OCH_3	Harmaline		5,010 (85)	9,430†	> 10,000	> 10,000	> 10,000
8-OCH_3	8-Methoxyharmalan		1,560 (990)	2,130†	3,000 (240)	> 10,000	-
7-OH	Harmalol		> 10,000	> 10,000	> 10,000	-	-
3-$COOCH_3$	R(–)-	LC-032	> 10,000	> 10,000	> 10,000	> 10,000	> 10,000
3-$COOCH_3$	S(+)-	LC-033	> 10,000	> 10,000	> 10,000	> 10,000	> 10,000

Table 1 (*Continued*)

Ring system/R		K_i, nM (±SEM)				
		5-HT_{2A}	5-HT_{2C}[a]	5-HT_{1A}	D_2[b]	BZ
						
-H	9-Methylharmalan	> 10,000	> 10,000	> 10,000	-	-
						
7-OCH_3	Desmethylharmaline	> 10,000	> 10,000	> 10,000	-	-
Tetrahydro Derivatives						
						
-H	(±)Tetrahydroharman	1,430 (290)	> 10,000†	> 10,000	> 10,000	-
5-OCH_3	(±)5-Methoxytetrahydroharman	237 (1)	1,060 (45)	2,000 (60)	> 10,000	-
6-OCH_3	(±)6-Methoxytetrahydroharman	4,360 (140)	> 10,000†	> 10,000	-	-
7-OCH_3	(±)Tetrahydroharmine	> 10,000	> 10,000†	> 10,000	> 10,000	> 10,000
7-OCH_3	R(+)-	> 10,000	> 10,000	> 10,000	-	-
7-OCH_3	S(−)-	5,000 (950)	> 10,000	> 10,000	-	-
8-OCH_3	(±)8-Methoxytetrahydroharman	770 (1)	710 (130)	450 (20)	>10,000	-
						
-H	Tetrahydro-β-carboline (THBC)	3,040 (40)	> 10,000	4,310 (380)	-	-
6-OCH_3	6-Methoxy THBC	1,100 (150)	2,700 (100)	4,175 (1330)	> 10,000	-
7-OCH_3	7-Methoxy THBC	2,210 (80)	> 10,000	> 10,000	-	-
						
-H		2,210 (80)	> 10,000	> 10,000	-	-
-CH_3	N_2-Methyl-7-methoxy THBC	2,040 (150)	3,360 (140)	> 10,000	-	-
Miscellaneous Derivatives						
						
-$COOCH_3$	β-CCM	> 10,000	> 10,000	> 10,000	> 10,000	3.0 (0.1)
-$CONHCH_3$	β-CCM amide	> 10,000	> 10,000	> 10,000	-	-

[a] Some of the 5-HT2C binding data were previously reported (Grella et al., 1998) and are included here only for comparison.
[b] Spiperone was used as control; spiperone $K_i = 0.45$ (±0.02).

moval of the 1-methyl substituent in the tetrahydro series (note: the desmethyl derivative of tetrahydroharman is commonly referred to as tetrahydro-β-carboline or THBC) also reduces affinity. For example, THBC ($K_i = 3040$ nM) binds with about half the affinity of tetrahydroharman ($K_i = 1430$ nM); a K_i value of 3900 has been previously reported for THBC in a rat cortical 5-HT_2 receptor preparation (Bojarski et al., 1993). The affinity of 7-methoxy THBC ($K_i = 2210$ nM) is about half that of $S(-)$-tetrahydroharmine. N_2-methylation of 7-methoxy THBC has no effect on affinity.

To put these binding data in perspective, and so as to investigate possible similarities in binding orientations, binding data were also obtained for a series of DMT analogs (Table 2). The hallucinogenic (Shulgin and Shulgin, 1997) DMT ($K_i = 323$ nM), 4-methoxy DMT ($K_i = 68$ nM), and 5-methoxy DMT ($K_i = 92$ nM) bind with higher affinity than 6-methoxy and 7-methoxy DMT ($K_i = 3960$ and 5440 nM, respectively). The latter two agents have not been examined in humans (Shulgin and Shulgin, 1997).

3.2. 5-HT_{2C} binding

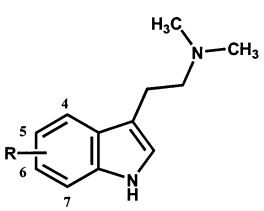
With the exception of 5-methoxyharman ($K_i = 417$ nM), the remaining harman derivatives bind with micromolar or lower affinity. In the dihydro series, harmaline binds with low affinity ($K_i = 9430$ nM) but the remaining methoxy-substituted analogs bind with 5-fold or higher affinity. Introduction of a 7-hydroxy group, a 3-carbomethoxy group, or an N_9-methyl group, all result in inactive compounds. Most of the tetrahydroharman analogs lack appreciable affinity for 5-HT_{2C} receptors; however, 5-methoxytetrahydroharman and 8-methoxytetrahydroharman are conspicuous for their somewhat higher affinity (5-HT_{2C} $K_i = 1060$ and 710 nM, respectively).

The DMT derivatives bind at 5-HT_{2C} receptors with lower affinity than they display at 5-HT_{2A} receptors (Table 2). But, as with their 5-HT_{2A} affinity, DMT ($K_i = 1450$ nM), 4-methoxy DMT ($K_i = 340$ nM), and 5-methoxy DMT ($K_i = 2450$ nM) bind with higher affinity than 6- and 7-methoxy DMT ($K_i > 10\,000$ nM).

3.3. Binding at other receptors

With few exceptions, none of the β-carbolines displayed significant affinity at 5-HT_{1A} receptors (Table 1). THBC binds at 5-HT_{1A} receptors with a $K_i = 4310$ nM (Table 1); Bojarski et al. (1993) have previously reported a K_i of 2510 nM for rat brain hippocampal 5-HT_{1A} receptors. The only β-carboline displaying a $K_i < 1000$ nM is racemic 8-methoxytetrahydroharman ($K_i = 450$ nM). None of the β-carbolines displayed affinity for D_2 dopamine receptors and of the compounds examined, only β-CCM ($K_i = 3$ nM) showed affinity for BZ receptors with a K_i value of $< 10\,000$ nM (Table 1). In contrast, all of the indolealkylamines displayed significant affinity for 5-HT_{1A} receptors (Table 2) with K_i values ranging from 11 nM for 5-methoxy DMT to 1760 nM for 7-methoxy DMT. McKenna et al. (1990) have previously reported K_i values of 75 and 14 nM for DMT and 5-methoxy DMT, respectively, at rat cortical 5-HT_{1A} receptors.

Table 2
5-HT_{2A}, 5-HT_{2C}, and 5-HT_{1A} serotonin receptor, and D_2 dopamine receptor, radioligand binding data for selected indolealkylamines

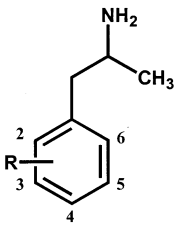


Agent[a]	R	K_i, nM (±SEM)			
		5-HT_{2A}[b]	5-HT_{2C}	5-HT_{1A}	D_2
DMT	-H	323 (60)	1450 (110)	200 (10)	–
4-Methoxy DMT	4-OCH_3	68 (5)	340 (39)	235 (6)	–
5-Methoxy DMT	5-OCH_3	92 (12)	2450 (90)	11 (1)	>10 000
6-Methoxy DMT	6-OCH_3	3960 (320)	>10 000	1210 (180)	–
7-Methoxy DMT	7-OCH_3	5440 (750)	>10 000	1760 (320)	–

[a] DMT, 4-OMe DMT, and 5-OMe DMT are hallucinogenic in humans (Shulgin and Shulgin, 1997); human data have never been reported for 6-OMe DMT or 7-OMe DMT.

[b] K_i values for ketanserin-labeled 5-HT_{2A} receptors were also determined for purpose of comparison (K_i, nM; followed by SEM): DMT, 660 (±30) nM; 4-OMe DMT, 180 (±24) nM; 5-OMe DMT, 280 (±60 nM); 6-OMe DMT, 4,190(±300) nM; 7-OMe DMT, 9200 (±830) nM.

Table 3
5-HT_{1A} serotonin receptor and D_2 dopamine receptor binding data for selected phenylisopropylamines[a]



	R_2	R_3	R_4	R_5	R_6	5-HT_{1A}, K_i nM (SEM)	D_2, K_i nM (SEM)
DOM	OCH_3	H	Me	OCH_3	H	5860 (±950)	>10 000
DOB	OCH_3	H	Br	OCH_3	H	4280 (±500)	>10 000
DOC	OCH_3	H	Cl	OCH_3	H	4520 (±210)	>10 000
DOI	OCH_3	H	I	OCH_3	H	3175 (±50)	>10 000
DOET	OCH_3	H	Et	OCH_3	H	4530 (±84)	>10 000
DOPR	OCH_3	H	nPr	OCH_3	H	>10 000	>10 000
2,5-DMA	OCH_3	H	H	OCH_3	H	1780	>10 000
2,4,5-TMA	OCH_3	H	OCH_3	OCH_3	H	>10 000	>10 000
MEM	OCH_3	H	OEt	OCH_3	H	>10 000	>10 000
2,3,4-TMA	OCH_3	OCH_3	OCH_3	H	H	>10 000	>10 000
3,4,5-TMA	H	OCH_3	OCH_3	OCH_3	H	525 (±72)	>10 000
2,4,6-TMA	OCH_3	H	OCH_3	H	OCH_3	>10 000	>10 000

[a] 5-HT_{2A} and 5-HT_{2C} binding data have been previously reported (e.g. Nelson et al., 1999). Also, with the exception of 2,3,4-TMA (which has not been extensively investigated), all compounds shown in Table 3 have been shown to be active in humans (Shulgin and Shulgin, 1991).

5-Methoxy DMT was without affinity for D_2 dopamine receptors. α-Methylmescaline (3,4,5-TMA) possessed modest affinity at 5-HT_{1A} receptors (Table 3), however, the affinities of the other phenylisopropylamines examined was, at best, in the micromolar range. All phenylisopropylamines lacked affinity for D_2 dopamine receptors.

3.4. PI hydrolysis

To obtain an indication of whether the β-carbolines can act as agonists at 5-HT_{2A} receptors, their ability to stimulate PI hydrolysis at a concentration of 10 μM was examined. The intrinsic activities for stimulation of 5-HT_{2A}-mediated PI hydrolysis by each of the phenylisopropylamines shown in Table 3 was also examined (Fig. 2). The structurally related *N*-methyl-1-(4-methoxyphenyl)-2-aminopropane or PMMA was included as a negative control. PMMA (which is the *N*-monomethyl analog of 2,4,5-TMA minus the 2- and 5-methoxy groups) does not bind at 5-HT_{2A} receptors (i.e. $K_i > 10\,000$ nM) and does not produce DOM-like effects in tests of stimulus generalization using DOM-trained animals. With the exception of PMMA, and consistent with the results of other functional assays (Glennon, 1998) all of the phenylisopropylamines behaved as agonists; most of the phenylisopropylamines possessed intrinsic efficacies of >0.8. In like manner, four of the dihydro-β-carbolines were examined: harmalan, 5-methoxyharmalan, 6-methoxyharmalan, and harmaline. At a concentration of 10 μM, none of the four dihydro-β-carbolines produced any agonist or antagonist effects (data not shown). Harmaline was also without agonist actions at a concentration of 20 μM.

4. Discussion

Depending upon the presence and location of certain substituent groups and the degree of ring saturation, the

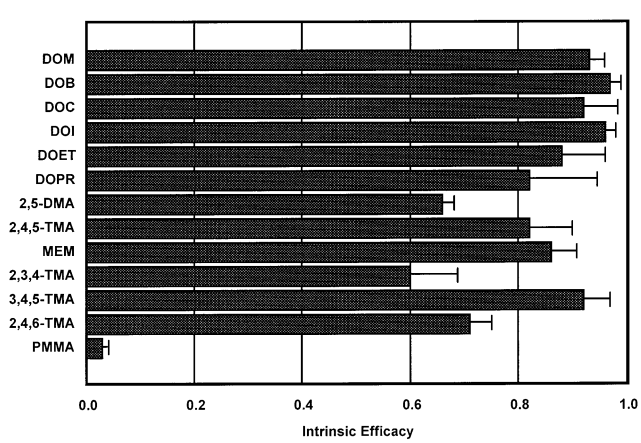


Fig. 2. Intrinsic efficacies of various phenylisopropylamines for stimulating 5-HT_{2A}-mediated PI hydrolysis.

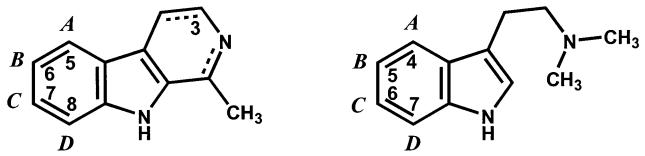


Fig. 3. General structure of a β-carboline (left) and DMT drawn in a fashion to emphasize the structural similarity associated with their indolic nuclei. The 5-, 6-, 7-, and 8-positions of the β-carboline are indicated by A–D, respectively, whereas the 4-, 5-, 6-, and 7-positions of DMT are indicated with the same letter designations, respectively.

β-carbolines bind with modest affinity at [^{3}H]agonist-labeled 5-HT_{2A} (Table 1) receptors and 5-HT_{2C} serotonin receptors (Table 1; Grella et al., 1998). In general, the β-carbolines lack significant affinity for 5-HT_{1A} serotonin receptors, dopamine D_2 receptors, and BZ receptors.

4.1. Structure-affinity relationships for serotonin receptor binding

With respect to 5-HT_{2A} affinity it is apparent that the degree of ring saturation is an important determinant for binding and that the three series do not bind in an identical manner. For example, in the fully aromatic or harman series the order of affinity is H ≈ 7-methoxy > 5-methoxy ≫ 6-methoxy or 8-methoxy, whereas in the dihydro or harmalan series, affinity decreases in the order 5-methoxy > H ≈ 8-methoxy > 6-methoxy ≈ 7-methoxy. In the tetrahydro series, the order of affinity is 5-methoxy > 8-methoxy > H > 6-methoxy ≫ 7-methoxy. The lack of parallel affinity-shifts upon substituent relocation suggests that the aromatic and dihydro series compounds bind in a different fashion. The tetrahydro series compounds seem to bind in a roughly comparable manner as the dihydro series, but even here there are some differences. But again, it should be noted that most of the tetrahydro derivatives are racemic mixtures and this might obscure valid comparisons. It will be necessary in the future to prepare and examine the individual optical isomers of more of the tetrahydro derivatives so that stricter structure-activity comparisons can be made. In the single instance where such a comparison is possible, *S*(−)-tetrahydroharmine (K_i = 5000 nM) binds with the same affinity as its dihydro counterpart, harmaline (K_i = 5010), indicating that the C_1–N_2 double bond may play only a negligible role in binding.

In general, the β-carbolines bind at 5-HT_{2C} receptors with lower affinity than they display at 5-HT_{2A} receptors. In the fully aromatic series, a 5-methoxy substituent appears optimal, the remainder of the methoxy-substituted derivatives bind with K_i values in the 2500–5000 nM range. The dihydro derivatives also bind with low affinity, but again, the 5-methoxy derivative (i.e. 5-methoxyharmalan) binds with higher affinity than most other members of the series. In the tetrahydro series the 5- and the 8-methoxy derivatives display modest affinity and the remainder of the series are essentially inactive. It would seem that a methoxy group is a greater contributor to 5-HT_{2C} binding when in the 5-position than in any of the other positions.

With respect to 5-HT_{1A} affinity, all of the compounds in the fully aromatic and dihydro series bind with low affinity; likewise, most of the tetrahydroharmalan derivatives bind with low affinity (K_i > 10 000 nM). Only 8-methoxytetrahydroharman (K_i = 450 nM) displays significant affinity for 5-HT_{1A} receptors. Two of the dihydro analogs, harmalan and 8-methoxyharmalan, display modest affinity (K_i < 5000 nM). Nevertheless, the affinity of the β-carbolines at 5-HT_{1A} receptors is too low to allow formulation of any meaningful structure–affinity relationships.

4.2. Structural relationships between the β-carbolines and the DMT analogs for 5-HT_{2A} binding

The β-carbolines are conformationally constrained indolealkylamines; that is, they are analogs of DMT where the alkyl side chain is affixed to the indole nucleus through one of the *N*-methyl groups. One consequence of this constrainment is that the 5-HT_{1A} affinity associated with the DMT analogs (Table 2) has, for all practical purposes, been eliminated.

DMT, 4-methoxy DMT, and 5-methoxy DMT bind at 5-HT_{2A} receptors with higher affinity than 6-methoxy and 7-methoxy DMT. Is there any structural relationship in the manner in which the DMT analogs and the β-carbolines bind at 5-HT_{2A} receptors? That is, because the β-carbolines are analogs of DMT, parallel substituent changes on the indole nucleus might be expected to result in parallel shifts in affinity if both types of agents bind in a common manner. Due to differences in ring numbering, it is confusing to make direct comparisons using these numbering systems. However, if the indole rings of the DMT analogs and the β-carbolines are overlayed, we can use the system shown in Fig. 3.

For example, the indole 4-position of the DMT analogs corresponds to the 5-position of the β-carbolines; these two positions can be termed the 'A' position, and so on. In the DMT series, high affinity is associated with methoxy substituents at the A or B positions (i.e. 4-methoxy DMT and 5-methoxy DMT, respectively; K_i < 100 nM) whereas methoxy substituents at the C and D positions (i.e. 6-methoxy DMT and 7-methoxy DMT; K_i ≥ 4000 nM) result in the lowest affinity compounds. In the fully aromatic β-carboline series, high affinity is associated with an unsubstituted ring or where there is a methoxy group at the C position. This suggests that the harman derivatives and the DMT

derivatives are not oriented in the 5-HT_{2A} binding pocket in a comparable manner (i.e. with overlapping indole nuclei). In the dihydro series, the highest affinity compound possesses a methoxy group at the A position, whereas the compound with a methoxy group at the C position is the lowest affinity member of the methoxy series. To this extent, there is somewhat greater similarity in the binding of members from the DMT series and the dihydro series than with the fully aromatic series. In the tetrahydro series, high affinity is associated with methoxy substitution at the B and D positions. Taken together, the evidence suggests very little correspondence between the binding orientations of the DMT analogs and the β-carbolines.

We have previously suggested that β-carbolines bind at 5-HT receptors in such a manner that the indole nuclei do not overlap in a strictly atom-by-atom fashion (Glennon, 1981). That is, although the basic amines of both series likely interact at a common amine binding site on the receptor, the benzeneoid portion of the β-carbolines likely utilize generally similar receptor features as the aromatic portion of the tryptamines, but do so in an indole-nonsuperimposable manner. Similar orientations also have been more recently considered by others (Bojarski et al., 1993). Due to the number of agents examined in this investigation, the present results offer the first evidence for our concept at central 5-HT_{2A} receptors.

4.3. BZ receptor binding

Various β-carbolines bind at BZ receptors, and previous structure–affinity studies suggest that the presence of a 3-position substituent (e.g. amide, ester, carbinol) and a fully aromatic ring system are optimal for BZ receptor binding (Hollinshead et al., 1990; Allen et al., 1992). The dihydro-β-carboline derivatives (as do most of the other analogs examined) lack affinity for BZ receptors. Even when a 3-position ester group was incorporated into the ring of harmalan (i.e. LC-032 and LC-033), the compounds did not bind at BZ receptors. β-CCM was examined for comparison and was found to bind with high affinity ($K_i = 3$ nM) at BZ receptors.

4.4. Hallucinogenic activity and binding

It has been shown that a relationship exists between the 5-HT_{2A} receptor affinity of the classical hallucinogens and their human hallucinogenic potencies (Glennon, 1996, 1998). Furthermore, examples of the different classes of agents (e.g. DMT, 5-methoxy DMT, DOB, DOPR as well as harmaline) have been demonstrated to produce common stimulus effects in rats trained to discriminate DOM from vehicle (Glennon, 1996). Most of the β-carbolines in Table 1 have not been examined in humans; however, harmine and harmaline are generally considered hallucinogenic (reviewed: Grella et al., 1998). Although their low human potencies are consistent with their modest affinities at 5-HT_{2A} receptors (Table 1; Grella et al., 1998), it cannot yet be concluded that their hallucinogenic effects are mediated via a 5-HT_{2A} mechanism. It can be concluded, however, that their hallucinogenic effects, or common stimulus effects in DOM-trained animals, likely do not involve a 5-HT_{1A}, D_2 or BZ mechanism. The β-carbolines examined, except for β-CCM, lack affinity for BZ receptors. Certain indolealkylamine hallucinogens such as DMT and 5-methoxy DMT display high affinity for 5-HT_{1A} receptors (Table 2), but harmine and harmaline lack 5-HT_{1A} receptor affinity. Furthermore, potent classical hallucinogens such as DOB and DOPR (Table 3) display little to no affinity for 5-HT_{1A} receptors. Likewise, the β-carbolines, like the phenylisopropylamine hallucinogens, lack affinity for dopamine D_2 receptors.

4.5. Functional activity

Thus far, one of the only populations of receptors that might be reasonably implicated as responsible for the common effects produced by these agents are the 5-HT_{2A} receptors. However, the affinities of the β-carbolines at agonist-labeled 5-HT_{2A} receptors (Table 1) is not very different than their affinities at [^{3}H]ketanserin-labeled sites (for those derivatives where comparisons can be made; see Grella et al., 1998). This would argue against the β-carbolines being 5-HT_{2A} agonists. Selected derivatives were examined in a PI hydrolysis assay to obtain further support for this concept. Although the phenyisopropylamines clearly possess 5-HT_{2A} agonist character in various assays (Glennon, 1996) including PI hydrolysis (Fig. 2), harmalan, 5-methoxyharmalan, 6-methoxyharmalan, and harmaline were inactive as agonists in the PI assay at concentrations of 10 μM, and harmaline was inactive as an agonist at a concentration of 20 μM. Consequently, a common 5-HT_{2A} agonist mechanism to account for the similar behavioral effects of, for example DOM, 6-methoxyharmalan, and harmaline (Glennon et al., 1983), is difficult to reconcile. Several explanations are possible: either the common stimulus effects produced by harmaline and DOM involve some other population of receptors, or 5-HT_{2A} receptors are involved but participate in an unexpected manner. For example, the β-carbolines might be metabolized in vivo to compounds that are more active than their parent compounds, the β-carbolines might behave in an indirect manner (i.e. inhibition of monoamine oxidase, release of 5-HT), or the agents might have different effects on a receptor coupling mechanism. There is also the possibility that the β-carbolines are not stable under the PI assay conditions, or that they are only very weak

partial agonists of low potency and their actions are not detected by the assay. Obviously, additional studies will be required to sort out these possibilities. Furthermore, with respect to human hallucinogenic activity, although there are only minor differences in human versus rat 5-HT_{2A} receptors (Hoyer et al., 1994), DOM and β-carbolines may behave differently in different species. Adding to this confusion, Helsley et al. (1998a,b) recently reported that harmaline and 6-methoxyharmalan produce stimulus effects similar to those produced by the hallucinogen ibogaine, another agent whose mechanism of action is unknown at this time. It was suggested that the stimulus effects might involve a nonessential 5-HT_{2A} component of action (Helsley et al., 1998a).

4.6. Conclusion

β-carbolines bind with modest affinity at 5-HT_{2A} receptors and affinity is highly dependent upon ring substituents and ring saturation. Similar comments previously have been made regarding 5-HT_{2C} binding and binding at [^{3}H]ketanserin-labeled 5-HT_2 receptors for a more limited series of β-carbolines (Grella et al., 1998). The β-carbolines bind with little to no affinity at 5-HT_{1A} serotonin receptors, dopamine D_2 receptors and, (with the exception of β-CCM) at benzodiazepine receptors. Although β-carbolines represent conformationally-constrained analogs of DMT, the orientation with which these agents bind at 5-HT_2 receptors is probably different; that is, parallel structural modifications in the DMT series and the β-carboline series did not result in parallel shifts in affinity. In fact, this was even found to be the case within the β-carboline series when β-carbolines with different degrees of saturation were compared. Binding data for the β-carbolines at agonist-labeled sites suggest that they are probably not 5-HT_{2A} agonists. In support of this concept, four β-carbolines were examined for their ability to act as agonists in a 5-HT_{2A} PI hydrolysis assay, and all were without effect at concentrations of up to 10 μM. In contrast, all the phenylisopropylamine hallucinogens, as expected, behaved as 5-HT_{2A} agonists. It is concluded that the common effects of indolealkylamine and phenylisopropylamine hallucinogens do not depend on a direct 5-HT_{1A} serotonin receptor or D_2 dopamine receptor mechanism. Prior binding data would seem to favor a common 5-HT_{2A} component of action (Grella et al., 1998); but, in light of the present results, a definitive answer has not yet been reached. In fact, at least on the basis of a lack of enhanced affinity for agonist-labeled 5-HT_{2A} receptors, as well as on their lack of agonist action in the PI hydrolysis assay, it is difficult to conclude that the β-carbolines behave in a manner consistent with that of the classical hallucinogens.

Acknowledgements

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