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Development of a Presynaptic 5-HT$_{1A}$ Antagonist

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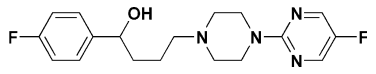
Abstract—A new 5-HT$_{1A}$ silent antagonist **14** (5-HT$_{1A}$ IC$_{50}$ = 2.2 nM) antagonizes the effects of agonists on reciprocal forepaw treading behavior, on neuronal firing in the rat dorsal raphé, and on 5-HT$_{1A}$ release in the raphé and hippocampus. While **14** alone was inactive in the social interaction paradigm, it completely reversed the social interaction activity of the serotonergic compounds (buspirone, **1**, and **2**).

Defining a 5-HT$_{1A}$ antagonist is made difficult by the complex pharmacology and neuroanatomy of these serotonergic pathways. Serotonergic 5-HT$_{1A}$ receptors are expressed both as presynaptic autoreceptors on the soma and dendrites of serotonergic neurons and as postsynaptic receptors on a variety of other neurons. Activation of either the presynaptic autoreceptors or the postsynaptic receptors causes hyperpolarization and inhibition of neuronal firing.[1] At the postsynaptic receptors, partial agonists tend to have weak agonist effects and block the effects of full antagonists. At the presynaptic autoreceptors, partial agonists tend to exhibit a full agonist effect, due to the high receptor reserve[2] at these autoreceptors. The term 'silent antagonist'[3] has been used to distinguish full antagonists, which have no intrinsic activity at either pre- or postsynaptic 5-HT$_{1A}$ receptors, from partial agonists, which have intrinsic agonist activity at the presynaptic autoreceptors.

1 

The atypical antipsychotic agent, BMS-181100[4] (**1**, BMY 14802), binds to 5-HT$_{1A}$ receptors, where it produces pharmacologic actions consistent with partial agonist activity. Compound **1** inhibits the firing of serotonergic dorsal raphé neurons[5] (a presynaptic agonist effect), and inhibits forskolin-stimulated adenylyl cyclase activity in rat hippocampal membranes similar to buspirone[6] (a postsynaptic antagonist effect). In addition, **1** induces reciprocal forepaw treading in reserpinized rats, and produces dose-dependent hypothermia in mice similar to agonists and partial agonists.[7] Like buspirone, **1** produces anxiolytic-like effects in a modified rat social interaction paradigm.[8]

In an investigation of cyclohexyl analogues[9] of **1**, two compounds, **2** and **3**, were distinguished from the rest by their affinities for 5-HT$_{1A}$ receptors. These compounds are of particular interest because they contain a benzyl piperazine, rather than the well-known heteroaryl piperazine pharmacophore. These findings sparked our interest and led to the investigation of the SAR of these benzyl piperazine 5-HT$_{1A}$ ligands, and subsequently produced **14**, a new 5-HT$_{1A}$ silent antagonist.

Compounds **2**–**29** (Tables 1 and 2) were prepared[10] by the method shown in Scheme 1. The monoketal of 1,4-cyclohexanedione was reacted with an aryl Grignard

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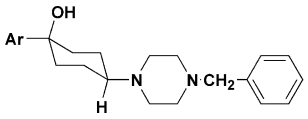
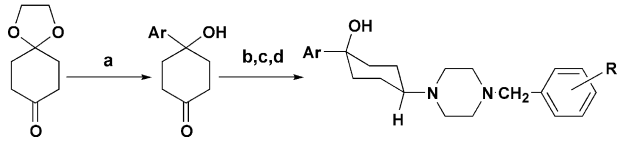
Table 1. 5-HT_{1A} receptor binding of aryl cyclohexanol derivatives[13]



Compd	Ar	5-HT_{1A} IC_{50} (nM)[12]
2	3,4-(–OCH_2O–)-Ph–	20
3	4-OMe–Ph–	50
4	3-OMe–Ph–	50
5	2-OMe–Ph–	60
6	3,4-$(OMe)_2$–Ph–	140
7	4-F–Ph–	260
8	Ph–	460



Scheme 1. Reagents and conditions: (a) ArMgBr/THF, 1 N HCl; (b) CBZ-piperazine/$Ti(OiPr)_4/NaBH_4$; (c) H_2, Pd/C; (d) $ArCH_2X/K_2CO_3$/MeCN.

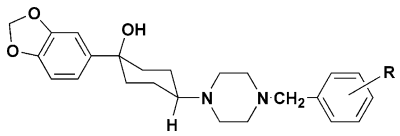
reagent to give the 4-hydroxy cyclohexanone intermediate after mild hydrolysis. This ketone intermediate was reductively coupled[11] with CBz-piperazine to preferentially give the cis alcohol intermediate, and the CBz protecting group was then cleaved by hydrogenation. The substituted benzyl groups were appended by simple alkylation.

The 5-HT_{1A} binding was measured by published methodology[12] and the data expressed as IC_{50} values with the radioligand at the K_d concentration. Compounds with electron withdrawing substituents, such as **7** (Table 1), bind poorly to the 5-HT_{1A} receptor as does the unsubstituted phenyl analogue, **8**. The 3,4-dimethoxyphenyl analogue, **6** (135 nM), binds to the 5-HT_{1A} receptor with significantly less affinity than the monomethoxy-compounds, **3**–**5** (50–60 nM), and the 3,4-methylenedioxy compound, **2** (20 nM), binds with higher affinity. This suggests that oxygen lone electron pairs might be ideally situated in the latter compound to take part in a hydrogen bonding interaction with the 5-HT_{1A} receptor that is not available to the dimethoxy analogue.

The effect of substituents on the benzyl group was also examined (Table 2). With *ortho*-substituted compounds, halogens increase 5-HT_{1A} affinity (**9**–**12**), while methoxy attenuates affinity (**13**). In the *meta*-substituted benzyl compounds (**14**–**18**) 5-HT_{1A} affinity is potentiated by halogen and alkoxy substituents. None of the *para*-substituents in **19**–**21** potentiated 5-HT_{1A} binding.

The synergism between *ortho*- and *meta*-benzyl substituents was also investigated. The six possible difluorobenzyl compounds, **22**–**27**, were prepared. Only the 2,5-difluoro benzyl derivative, **22**, possessed higher 5-HT_{1A} receptor affinity than either the *ortho*-fluoro compound, **12**, or the *meta*-fluoro compound, **18**. Similarly, when the *ortho*-fluoro and *meta*-methoxy groups are combined in a 2-fluoro, 3-methoxy-compound, **29**, and a 2-fluoro, 5-methoxy-compound, **28**, only the latter binds

Table 2. 5-HT_{1A} receptor binding of substituted benzyl piperazine derivatives[13]



Compd	R	5-HT_{1A} IC_{50} (nM)[12]
9	2-Br	1.2
10	2-I	1.2
11	2-Cl	1.9
12	2-F	10
13	2-OMe	43
14	3-OMe	2.2
15	3-Br	2.8
16	3-Cl	6
17	3-OEt	6.7
18	3-F	13
19	4-F	27
20	4-Cl	79
21	4-OMe	380
22	2,5-F_2	2.6
23	2,3-F_2	17
24	3,5-F_2	21
25	3,4-F_2	34
26	2,4-F_2	57
27	2,6-F_2	110
28	2-F, 5-OMe	0.75
29	2-F, 3-OMe	19

with greater affinity than either the ortho-fluoro compound, **12**, or the *meta*-methoxy compound, **14**. These results establish that only the 2,5-substitution pattern potentiates 5-HT_{1A} binding.

The affinity of **2** and **14** for several other receptor systems was examined to determine the selectivity of these compounds (Table 3). Compound **2** demonstrated weak affinity for α_1 and α_2 receptors, while **14** bound weakly to 5-HT_{1D} receptors. Both compounds were inactive at the other receptors examined.

Compound **2** produced partial agonist effects and **14** produced antagonist effects in blocking reciprocal forepaw treading behavior[14,15] which is mediated by postsynaptic 5-HT_{1A} receptors in rats. The presynaptic 5-HT_{1A} antagonist activity of **14** was tested both electrophysiologically by examining the firing of dorsal raphé neurons and neurochemically by measuring the extracellular concentrations of 5-HT using in vivo microdialysis.

Table 3. Receptor binding specificity[13] of **2** and **14**

Receptor binding assay[a]	**2**	**14**
5-HT_{1B} (5-HT, rat striatum)	>1000	>100,000
5-HT_{1C} (mesulergine, bovine choroid plexus)	>1000	>100,000
5-HT_{1D} (5-HT, bovine caudate)	13,000	700
5-HT_2 (spiperone, rat cortex)	11,000	28,000
5-HT_3 (GR 65630, bovine area postrema)	—	>100,000
5-HT uptake (5-HT, rat cortex)	>1000	3000
α_1 (WB 4101, rat cortex)	700	1500
α_2 (clonidine, rat cortex)	410	1100
D_2 (spiperone, rat striatum)	1700	4800

[a]Binding assay ([^{3}H] ligand, tissue; data reported as IC_{50} values, nM.

Electrophysiological recordings[16] of the effects of **14** on the firing of dorsal raphé neurons support the presynaptic 5-HT_{1A} antagonist activity of this compound (Fig. 1). The inhibition of dorsal raphé activity induced by 8-OH-DPAT was studied in combination with **14**. The dose-response curve for 8-OH-DPAT is shifted markedly to the right in the presence of **14** (ED_{50} 15.8 mg/kg versus 1.5 mg/kg, iv) in the manner of an antagonist.

In addition to the electrophysiology experiments, **14** was also tested for presynaptic 5-HT_{1A} antagonist activity using in vivo microdialysis[17] (Fig. 2). Partial agonists, such as **1** and buspirone, effectively antagonize the post-synaptically mediated forepaw treading behavior induced by agonists, but act as presynaptic agonists in decreasing 5-HT release in rat hippocampus.[18] Rats were implanted with microdialysis probes in the hippocampus and extracellular concentrations of 5-HT were measured by HPLC-ECD. While compound **14** alone (1 mg/kg, ip) did not change hippocampal extracellular 5-HT concentrations, **14** (1.0 mg/kg, ip) significantly ($p < 0.01$) blocked the decrease in hippocampal 5-HT induced by buspirone (2.5 mg/kg, ip). Thus compound **14** at 1 mg/kg failed to give an agonist effect and effectively antagonized the buspirone-induced agonist effect. These data demonstrate that **14** is an effective antagonist at 5-HT_{1A} presynaptic autoreceptors.

All known classes of clinically effective anxiolytics demonstrate activity in the social interaction paradigm,[19,20] and 5-HT_{1A} agonists are active in this model after discrete dorsal raphé injections.[21] In addition, such agonists, given systemically, do not increase social interaction in animals in which the presynaptic 5-HT_{1A} neurons in the dorsal raphé were lesioned with 5,7-dihydroxy tryptamine.[22] All of this data suggest the anxiolytic effect is mediated by a blockade of presynaptically controlled 5-HT release.

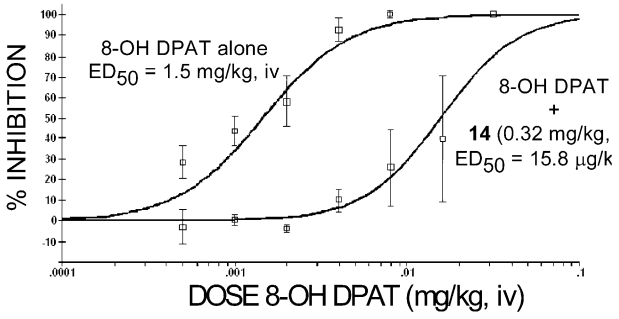


Figure 1. Dose–response curve for 8-OH-DPAT-induced inhibition of dorsal raphé firing alone and in the presence of **14**.

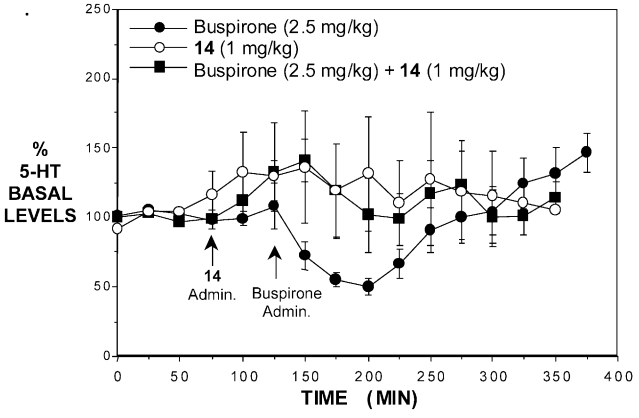


Figure 2. Effects of buspirone and **14**, alone and in combination, on in vivo hippocampal 5-HT levels as measure by microdialysis.

Table 4. Rat social interaction

Compd	Dose (mg/kg, ip)	Social interaction time (s±SEM)	Motor activity (counts±SEM)
Saline control	—	56±7	5800±700
14	1	65±8	5140±5
Diazepam	1.7	120±13[a]	5100±500
Buspirone	0.1	125±12[a]	6700±700
1	0.1	123±6[a]	6800±500
2	0.1	104±10[a]	4700±400
Diazepam	1.7	113±7[a]	4900±700
+**14**	1		
Buspirone	0.1	51±9	4600±500
+**14**	1		
1	0.1	49±9	5400±700
+**14**	1		
2	0.1	53±10	5100±500
+**14**	1		

[a]Test significantly different from saline control ($p > 0.05$).

Diazepam (1.7 mg/kg), buspirone (0.1 mg/kg), **1** (0.1 mg/kg), and **2** (0.1 mg/kg), all significantly increased the social interaction times (Table 4). However, the antagonist, **14**, by itself (1.0 mg/kg, ip), had no effect in the rat social-interaction paradigm. Of these, only **1** significantly increased total motor activity, while the others had no effect. To further define the antagonist activity of the molecule, **14** was studied in combination with diazepam, buspirone, **1**, and **2**. Compound **14** was without effect on social interaction activity of diazepam, but completely reversed the social interaction activity of the serotonergic compounds, buspirone, **1**, and **2**, without significant effects on total motor activity. These results further confirm the full antagonist effects of **14** on presynaptic 5-HT_{1A} receptors.

These data demonstrate that **14** is a 5-HT_{1A} pre- and postsynaptic antagonist that blocks the activity of 5-HT_{1A} agonists in the rat social-interaction paradigm. The data also suggest that buspirone, **1**, and **2** show activity in this paradigm through 5-HT_{1A} partial agonist effects, and support the presynaptic dorsal raphé 5-HT_{1A} receptors as a key site through which 5-HT_{1A} anxiolytics express their activity in the rat social-interaction task.

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16. **In vivo electrophysiology.** Adult male albino Sprague–Dawley rats were anesthetized with chloral hydrate and placed in a stereotaxic device (David Kopf, Model 1700). Extracellular single-unit recordings were obtained from spontaneously active serotonergic neurons in the dorsal raphé nucleus using glass micropipettes containing 1.0 M NaCl and 0.5% Pontamine Sky Blue dye. A dye spot was deposited at the tip of the electrode by iontophoresis at the conclusion of each recording session, and data was used only for recording locations confirmed histologically to be in the dorsal raphé nucleus. Changes in neuronal firing rates caused by intravenous injections of 8-OH-DPAT were determined by first recording stable baseline activity for 3–15 min from a single cell, and then injecting 8-OH-DPAT through a tail vein via a syringe pump. Percent change in firing rate was calculated by comparing the discharge rate taken over a 1-min period at peak drug effect following injection of 8-OH-DPAT to the baseline firing rate during the 1-min period preceding initiation of 8-OH-DPAT injections. Only one cell per rat was recorded, but more than one dose of 8-OH-DPAT could be given to a single rat, in which case 'dose' was cumulative dose. For antagonism experiments, **14** was injected intravenously (0.32 mg/kg) at least 10 min prior to beginning dosing with 8-OH-DPAT. Dose–response curves were calculated by an iterative computer algorithm in which the logistic equation was fitted to the data using a least-squares criterion, and constrained to pass through 0 and 100%. The ED_{50} was defined as the estimated dose required to produce a 50% reduction in the rate of firing of a serotonergic dorsal raphé neuron.
17. **In vivo microdialysis.** Method. Male Sprague–Dawley rats were anesthetized (im) with a mixture of ketamine HCl (80 mg/kg) and xylazine (6 mg/kg) and stereotaxically implanted with I-shaped fused silica microdialysis probes in the ventral hippocampus. Microdialysis experiments were carried out 24 h after surgery when the hippocampus was perfused with artificial cerebrospinal fluid (aCSF, NaCl 140.3 mM, KCl 2.7 mM, $CaCl_2$ 2.2 mM, pH 6.5) at 1.0 μL/min using a microinfusion pump. The dialysate was collected directly into a 25 μL loop of the injection valve and automatically injected (Digital Valve Sequence Programmer, Valco Instruments Inc., Houston, TX, USA) every 25 min onto the HPLC column. After stabilization of baseline dialysate levels, **14** (1–20 mg/kg, ip) was injected and the dialysates continuously collected. In the antagonism experiment, **14** (1.0 mg/kg, ip) was administered 50 min before buspirone (2.5 mg/kg, ip). **Chromatographic conditions**. Dialysate concentrations of 5-HT was measured by HPLC (ODS-Hypersil column; 3 μ, 150 × 3.0 mm; Keystone Scientific, Bellefonte, PA, USA), maintained at 30 °C with a column heater and detected coulometrically (ESA Model 5200 Coulochem II, Bedford, MA, USA) using a high sensitivity analytical cell (ESA Model 5011). The first electrode was set at +0.02 V and the second at +0.35 V; a guard cell placed before the injector was set at +0.45 V. The mobile phase consisted of 59 mM potassium phosphate (pH 4.8), 2.3 mM 1-heptanesulfonic acid sodium salt, 1 mL/L triethylamine, 17% methanol and 2% acetonitrile and was delivered at a flow rate of 0.5 mL/min by a Waters 610 pump. Retention time, peak area and peak height were measured with a data management software system (Baseline 810, Waters).
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