Antagonism of 1-(2,5-Dimethoxy-4-methylphenyl)-2-aminopropane Stimulus with a Newly Identified 5-HT_2- versus 5-HT_{1C}-Selective Antagonist

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DOM [i.e., 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane] is a 5-$HT_{1C/2}$ serotonin agonist that exerts stimulus control of behavior in animals. In order to determine if the discriminative stimulus effect of DOM is 5-HT_{1C}- or 5-HT_2-mediated, it would be informative to conduct tests of stimulus antagonism with a 5-HT_{1C}- or 5-HT_2-selective antagonist. To date, no such agents exist. Although the neuroleptic agent spiperone binds at D2 dopamine receptors and 5-HT_{1A} serotonin receptors, (a) it displays about a 1000-fold selectivity for 5-HT_2 versus 5-HT_{1C} sites and (b) it has been used as a "5-HT_2-selective" antagonist. Because spiperone is a behaviorally disruptive agent, it is not suitable for use in drug-discrimination studies. Using the spiperone molecule as a starting point, a limited structure–affinity investigation was conducted in order to identify a suitable antagonist with high affinity and selectivity for 5-HT_2 receptors, and yet an antagonist that might lack the disruptive actions of spiperone. Various modifications of the spiperone molecule were examined, but most resulted in decreased 5-HT_2 affinity or in loss of selectivity. One compound, 8-[3-(4-fluorophenoxy)propyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (**26**), was shown to bind at 5-HT_2 sites with high affinity (K_i = 2 nM) and >2,000-fold selectivity versus 5-HT_{1C} sites. In tests of stimulus antagonism using rats trained to discriminate 1 mg/kg of DOM from saline vehicle, **26** behaved as a potent antagonist (ED_{50} = 0.003 mg/kg) and lacked the disruptive effects associated with spiperone. As such, (a) it would appear that the DOM stimulus is primarily a 5-HT_2-mediated, and not 5-HT_{1C}-mediated, phenomenon, and (b) compound **26** may find application in other pharmacologic investigations where spiperone may not be a suitable antagonist.

Certain 4-substituted derivatives of 1-(2,5-dimethoxyphenyl)-2-aminopropane such as DOB, DOI, and DOM, where the 4-substituent is bromo, iodo, and methyl, respectively, are considered 5-HT_2 serotonin agonists.[1] We have previously used a drug-discrimination paradigm, employing rats trained to discriminate either DOB, DOI, or DOM from vehicle, to identify novel 5-HT_2 ligands and to investigate structure–activity relationships (reviewed[2]). We have demonstrated that the stimulus effects of these agents can be antagonized by 5-HT_2 antagonists and that stimulus generalization potency is significantly correlated with 5-HT_2 receptor affinity for a substantial series of agents that substitute for the DOM stimulus.[2] The discovery of 5-HT_{1C} receptors[3] and the subsequent demonstration that they possess a significant sequence homology, and share a common second messenger system, with 5-HT_2 receptors led to the suggestion that 5-HT_2 and 5-HT_{1C} receptors be termed 5-$HT_{2\alpha}$ and 5-$HT_{2\beta}$ receptors.[4] Indeed, the aminopropane derivatives DOB, DOI, and DOM display little selectivity for one of these receptor populations versus the other and are more correctly referred to now as 5-$HT_{1C/2}$ agonists.[5] All of the "standard 5-HT_2 antagonists" (e.g. ketanserin) used to characterize the stimulus properties of these agents also appear to lack selectivity and bind nearly equally well at the two populations of sites.[1] Ketanserin, for example, binds at 5-HT_2 receptors only with 14-fold selectivity.[6] In addition to the lack of agents selective for 5-HT_2 versus 5-HT_{1C} receptors has come the realization that many pharmacological and physiological effects once attributed to 5-HT_2 receptors may in fact involve a 5-HT_{1C} mechanism. Hence, there is a need to better define 5-HT_{1C} versus 5-HT_2 pharmacology.

The question now arises: is the DOM stimulus 5-HT_2- or 5-HT_{1C}-mediated? Despite the correlation between stimulus generalization and 5-HT_2 receptor affinity, a similar correlation exists between stimulus generalization and 5-HT_{1C} receptor affinity for the same series of agents.[7] One way in which to clarify a role for one receptor type versus the other would be to use a selective agent to antagonize the DOM stimulus. To date, no 5-HT_{1C}-selective nor 5-HT_2-selective antagonists exist.[8] One agent that has found some application as a 5-HT_2-selective antagonist is the neuroleptic spiperone (**1**); spiperone is generally considered to bind at 5-HT_2 versus 5-HT_{1C} receptors with about 500–1000-fold selectivity.[1] However, this agent also binds at another population of serotonin receptors (5-HT_{1A}), and binds at D2 dopamine receptors with an affinity (K_i = 0.2 nM) at least comparable to, if not higher than, its affinity for 5-HT_2 sites. Nevertheless, because the DOM stimulus does not appear to involve either a 5-HT_{1A} serotonin or D2 dopamine mechanism,[2] a demonstration of stimulus antagonism by low doses of spiperone would provide evidence that the DOM stimulus is likely 5-HT_2-mediated, whereas lack of antagonism (or antagonism only at very high doses of spiperone) would suggest involvement of a 5-HT_{1C} mechanism. Consequently, tests of DOM-stimulus antagonism with spiperone were conducted; unfortunately, these studies were inconclusive in that low doses of spiperone administered in combination with DOM resulted in severe behavioral disruption that prevented the animals from responding.[2]

Spiperone was instrumental in originally defining and differentiating the 5-HT1 and 5-HT_2 families of receptors (reviewed[1]) and may be viewed as the first known (albeit

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Table I. Physicochemical Data for Spiperone Analogues

	method[a]	% yield	RS[b]	mp, °C	empirical formula[c]
3	A	72	AE	199–201[d]	
5	A	93	A	168–171	$C_9H_{12}FNO$·HCl
6	B[e]	42	AE	119–121	$C_{11}H_{16}FNO$·HCl
7	C	61	CA	133–135	$C_{12}H_{16}FNO$·HCl
8	B	48	AE	175–177[f]	
9	B	35	HA	202–204	$C_{21}H_{25}FN_2O$·$C_2H_2O_4$[g,h]
10	B	6	A	171–173	$C_{22}H_{26}FNO$·HCl
12	B	37	AE	219–221	$C_{22}H_{25}FN_2O_2$·$C_2H_2O_4$[h,i]
13	B	60	HA	175–177	$C_{20}H_{25}FN_2O$·$C_2H_2O_4$
14	D	38	AE	145–147	$C_{21}H_{27}FN_2O$·$C_2H_2O_4$
15	E	53	AE	195–197	$C_{21}H_{25}FN_2O_2$·$C_4H_4O_4$[j]
16	B	36	A	168–170	$C_{21}H_{25}FN_2O_2$·$C_2H_2O_4$
17			AE	187–189[k]	
18	F	17	A	204–207	$C_{23}H_{28}FN_3O$·HCl
19	F[l]	50	N	188–191	$C_{23}H_{29}N_3O$·$C_4H_4O_4$[m]
20	F[n]	21	AE	194–196[o]	$C_{22}H_{27}N_3O$·$C_4H_4O_4$
21	F[n]	44	AE	225–227[p]	$C_{21}H_{25}N_3O$·$C_4H_4O_4$
22	F[r]	30	AE	228–231[q]	$C_{22}H_{27}N_3O_2$·HCl
23	F[r]	37	A	207–209[s]	$C_{23}H_{29}N_3O_2$·HCl
24	F[r]	60	A	217–219[t]	$C_{23}H_{29}N_3O_3$·HCl
25	F[r]	50	AE	273–275	$C_{22}H_{26}N_4O_4$·HCl
26	F[r]	60	MH	171–174[u]	

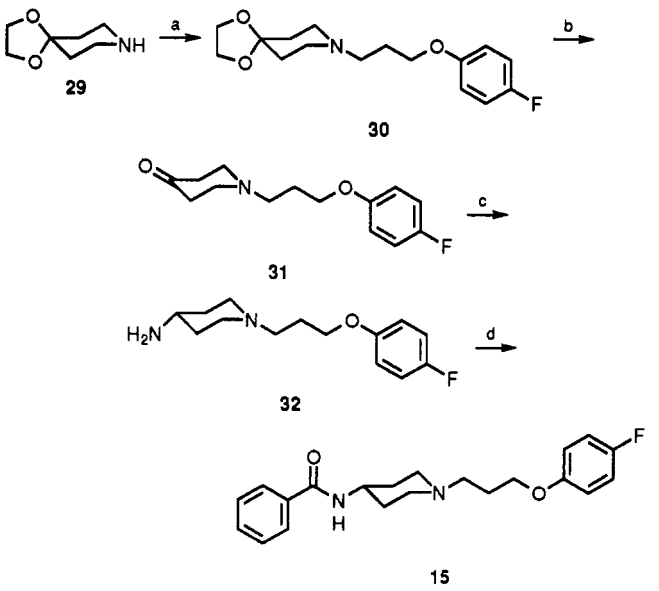
[a] See Experimental Section for representative methods. [b] Recrystallization solvents: absolute EtOH (A), anhydrous Et_2O (E), methanol (M), H_2O (H), MeCN (N), EtOAc (C). [c] Microanalytical (C, H, N) data within 0.4% of the theoretical values. [d] Literature[9] mp 202–203 °C. [e] Reaction time of 18 h using MeCN as solvent. [f] Literature[10] mp 177–178 °C. [g] Hydrogen oxalate salt. [h] Crystallized with 0.25 mol of H_2O. [i] Previously reported as free base, but no melting point reported.[11] [j] Maleate salt. [k] Prepared by literature method.[12] [l] Reaction time of 24 h using ethylene glycol dimethyl ether as solvent. [m] Crystallized with 0.5 mol of H_2O. [n] Reactants were fused together at 95–100 °C for 2 h. [o] Previously reported only as free base; free base mp 198–200 °C (lit[13] mp 199–200 °C). [p] Previously reported only as free base; free base mp 171–172 °C (lit[13] mp 170–172 °C). [q] Previously reported only as free base; free base mp 154–156 °C (lit[13] mp 154–156 °C). [r] Reaction time 3 h using dioxane as solvent and K_2CO_3. [s] Previously reported only as free base; free base mp 169–171 °C (lit[14] mp 85–112 (*sic*) °C). [t] Previously reported only as free base; free base mp 167–168 °C (lit.[14] mp 164–165.5 °C). [u] Literature[14] free base mp 173–174 °C.

nonselective) 5-HT_2 ligand. Spiperone is also the only agent that has found any significant application as a 5-HT_2- versus 5-HT_{1C}-selective antagonist. And yet, essentially nothing has been reported about its 5-HT_2 (or 5-HT_{1C}) structure–affinity relationships. In an attempt to identify a novel ligand with 5-HT_2 versus 5-HT_{1C} selectivity at least comparable to that of spiperone, and yet an agent that might not disrupt animals in drug discrimination studies, we undertook a limited structure–affinity investigation of spiperone. 5-HT_2 and 5-HT_{1C} binding data were obtained and, because spiperone binds at 5-HT_{1A} receptors, 5-HT_{1A} binding data were also acquired. Our intent was not to exhaustively investigate structure–affinity relationships nor to develop an agent specific for 5-HT_2 receptors. Rather, we were interested in obtaining a 5-HT_2- versus 5-HT_{1C}-selective agent that would be useful in our drug-discrimination studies.

Chemistry

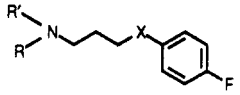
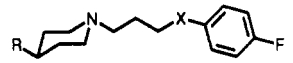
Most of the compounds (Table I) were prepared by simple alkylation procedures. For example, 4-chloro-1-(4-fluorophenyl)-1-butanone (**27**) or 1-chloro-3-(4-fluorophenoxy)propane (**28**) was aminated by reaction with potassium phthalimide followed by hydrazinolysis to afford the primary amines **3** and **5** (method A). The carbonyl group of **3** was protected as a ketal, followed by Eschweiler–Clarke alkylation and subsequent deprotection, to give

Scheme I[a]



[a] (a) **28**/K_2CO_3/KI/dioxane; (b) 30% HOAc/HCl; (c) $NaCNBH_4$/NH_4Ac/MeOH; (d) PhC(O)Cl/NEt_3/$CHCl_3$.

Table II. Radioligand Binding Data for Some Spiperone-Related Compounds

	R	R′	X	K_i,[a] nM 5-HT_{1A}	5-HT_{1C}	5-HT_2	ratio[b]
							
3	H	H	C=O	>100000	>100000	>100000	
5	H	H	O	ND[c]	>10000	1400(95)	>5
6	Me	Me	O	ND	>10000	295 (45)	>30
7	Me	Me	C=O	ND	3300 (285)	80 (10)	40
							
8	H		C=O	>10000	>10000	140 (10)	>70
9	NHPh		C=O	250 (12)	2300 (135)	34 (2)	65
10	CH_2Ph		C=O	180 (14)	1650 (300)	50 (14)	35
11	$C(O)C_6H_4$(4-F)		C=O	305 (68)	345[d]	4[d]	85
12	NHC(O)Ph		C=O	ND	>10000	70 (10)	>140
13	NHPh		O	120 (2)	3600 (270)	88 (12)	40
14	$N(CH_3)Ph$		O	110 (15)	2870 (15)	32 (5)	90
15	NHC(O)Ph		O	4000 (50)	>10000	70 (2)	>140
16	C(O)NHPh		O	ND	>10000	95 (37)	>100
17	$C(O)C_6H_4$(4-F)		O	285 (45)	335 (35)	8 (2)	40

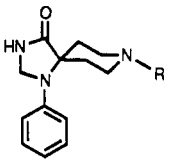
[a] K_i values are followed by SEM; SEM not determined where the $Ki > 10000$ nM. [b] 5-HT_2 selectivity ratio (i.e., 5-HT_{1c} K_i + 5-HT_2 K_i). [c] ND = not determined. [d] 5-HT_2 and 5-HT_{1C} binding data previously reported.[6]

the *N,N*-dimethyl derivative **7** (method C). Other substituted amines were prepared by reaction of **27** (*viz*: **8**, **9**, **10**, **12**) or **28** (*viz*: **6**, **13**, **16**) either with *N,N*-dimethylamine (i.e., **6**) or with the appropriately substituted piperidine derivative (method B). See Table I for physicochemical data.

Reductive alkylation of **13** with formaldehyde under catalytic conditions afforded **14** (method D). Compound **15** was prepared from the ketal-protected 4-piperidone **29** (Scheme I). Alkylation of **29** with **28**, followed by deprotection, provided **31**. Reductive amination of **31** with $NaCNBH_4$ and acylation of the resulting amine **32** with benzoyl chloride gave **15** (method E).

The triazaspirodecanone derivatives shown in Table III were prepared by alkylation of 1-phenyl-1,3,8-triazaspiro-[4.5]decan-4-one (**2**) with the appropriate alkyl bromide

Table III. Radioligand Binding Data for Triazaspirodecanone Derivatives



	R	K_i,[a] nM 5-HT$_{1A}$	5-HT$_{1C}$	5-HT$_2$	ratio[b]
2	H	>10000	>10000	>10000	
1	$(CH_2)_3C(O)C_6H_4(4\text{-}F)$	49(10)	1000(100)	1(0.5)	1000
18	$(CH_2)_3CH_2C_6H_4(4\text{-}F)$	110(20)	3200(285)	5(0.5)	650
19	$(CH_2)_4Ph$	85(2)	>10000	30(15)	>300
20	$(CH_2)_3Ph$	105(5)	>10000	230(45)	>30
21	$(CH_2)_2Ph$	1025(130)	6000(900)	570(140)	10
22	$(CH_2)_3OPh$	75(1)	>10000	5(0.1)	>2000
23	$(CH_2)_4OPh$	30(3)	>10000	325(30)	>30
24	$(CH_2)_3OC_6H_4(4\text{-}OMe)$	445(30)	>10000	1000(100)	>10
25	$(CH_2)_3OC_6H_4(4\text{-}NO_2)$	1160(20)	>10000	600(160)	>10
26	$(CH_2)_3OC_6H_4(4\text{-}F)$	50(2)	4300(770)	2(0.2)	2100

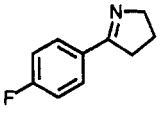
[a] K_i values are followed by SEM; SEM not determined where the K_i > 10000 nM. [b] 5-HT$_2$ selectivity (i.e., 5-HT$_{1C}$ K_i ÷ 5-HT$_2$ K_i).

(i.e, **19, 23**), chloride (i.e., **22, 26**), or tosylate (i.e., **18, 20, 21, 24, 25**) (method F).

Results and Discussion

In the present study, spiperone (1; 5-HT$_2$ K_i = 1 nM) was found to bind with a 1000-fold selectivity for 5-HT$_2$ versus 5-HT$_{1C}$ receptors (Table III). Our first goal was to identify how, and to what extent, each end of the spiperone (1) molecule contributes to 5-HT$_{1C}$ and 5-HT$_2$ affinity and selectivity.

Benzoylalkylamine-Related Compounds. The triazaspirodecanone portion of spiperone (i.e., **2**) binds with low affinity (K_i > 10 000 nM) both at 5-HT$_{1C}$ and 5-HT$_2$ sites (Table III) leading us to believe that the fluorobenzoyl portion may be the more important for binding. Surprisingly, the primary amine **3** was also without affinity for these sites (Table II). We surmised that under the conditions of the binding assay, or upon dissolution in buffer prior to the assay, **3** might undergo internal Schiff base formation to give a cyclic product. Indeed, the ^{1}H-NMR spectrum of a solution of **3** in D_2O revealed the appearance of new peaks within a matter of hours. When examined at 72 h, these peaks were clearly resolved and suggested the possibility of the formation of imine **4**. This



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was subsequently confirmed by independent synthesis of **4** and a comparison of spectral data. Consequently, we prepared ether **5**, which cannot undergo cyclization, and found that it binds, albeit with rather modest affinity, at 5-HT$_2$ sites (K_i = 1400 nM). The corresponding *N,N*-dimethylamino ether **6** (K_i = 295 nM) was found to bind with a several-fold improvement in 5-HT$_2$ affinity. Because the structure of interest was an aminopropiophenone (and not the corresponding ether) we prepared and examined the N,N-dimethyl analogue of **3** (i.e., **7**) with the expectation that it would not as readily undergo cyclization. Compound **7** was found to bind at 5-HT$_2$ sites with respectable affinity (K_i = 80 nM) but only with a modest 40-fold selectivity.

It would appear then that the fluorobenzoyl portion of spiperone (e.g. **7**) is important for binding, but that it does not bind with the selectivity of spiperone (**1**) itself. Evidently, it is a portion of the spiperone molecule that has been excised that is responsible for high affinity and selectivity. Consequently, portions of the spiperone molecule were reintroduced in a stepwise manner. The terminal amine substitutent of **7** was elaborated to a piperidine moiety (i.e., spiperone minus all but the spiro atom of the phenylimidazolinone ring); this compound, **8** (K_i = 140 nM), results in retention of 5-HT$_2$ affinity and in a slight improvement in selectivity. The anilino function of spiperone was incorporated into **8** (i.e., **9**) with further improvement in 5-HT$_2$ affinity (K_i = 34 nM) but with little effect on selectivity (65-fold selectivity). Replacement of the anilino nitrogen atom by a methylene group (i.e., **10**) had little overall effect.

Examining a series of ketanserin analogues, we previously reported that 4-fluorobenzoylpiperidines bind at 5-HT$_2$ sites with high affinity; for example, **11** binds with a K_i value of 4 nM.[6] Incorporation of this carbonyl moiety into anilino analogue **9** had little effect on affinity (**12**; K_i = 70 nM) but resulted in an agent that is essentially inactive at 5-HT$_{1C}$ sites (i.e., in a >140-fold selectivity for 5-HT$_2$ versus 5-HT$_{1C}$ receptors). This is further evidence that the amine substituents influence affinity and selectivity.

Early work on this project showed that the butyrophenone carbonyl group could be replaced by an ether oxygen atom. Compound **13** is the ether counterpart of **9**; **14** is the *N*-methyl derivative of **13**. Both compounds retain the 5-HT$_2$ affinity and selectivity of **9**. The ether counterpart of amide **12** (i.e., **15**), and its reverse amide **16**, both behaved like **12**. In each of these instances however, 5-HT$_2$ affinity is still 30–100 times less than that of spiperone. To complete the series, we prepared and examined the ether analogue of the higher affinity **11** (i.e., **17**); once again, replacement of the carbonyl group by an ether oxygen atom had a negligible effect. Although **17** binds at 5-HT$_2$ sites with high affinity (K_i = 8 nM), it displayed only 40-fold selectivity over 5-HT$_{1C}$ affinity.

Triazaspirodecanones. Because the highest affinity and most selective compound (i.e., spiperone) is associated with an intact azaspirodecanone ring, and because none of the structurally simpler analogues rivalled its affinity or selectivity, additional triazaspirodecanone derivatives were examined (Table III). Compound **18** (5-HT$_2$ K_i = 5 nM) is simply spiperone minus the alkyl-chain carbonyl oxygen; it retained high (>600-fold) selectivity for 5-HT$_2$ receptors. Removal of the fluoro group (**19**; K_i = 30 nM) somewhat decreased 5-HT$_2$ affinity but still resulted in a fairly (>300-fold) selective compound. Contraction of the 4-membered alkyl chain of **19** to a *n*-propyl (**20**; K_i = 230 nM) or ethyl group (**21**; K_i = 570 nM) decreased 5-HT$_2$ affinity; in the case of **21**, selectivity was also reduced (Table III).

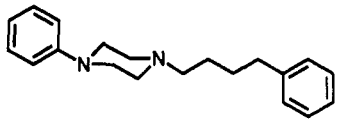
With the realization that a carbonyl group is not required for 5-HT$_2$ binding (e.g. see **19**), and as suggested by studies with the ether derivatives (e.g. compare **12** with **15**), we prepared and examined **22**. Compound **22** binds at 5-HT$_2$ sites with high affinity (K_i = 5 nM) and >2000-fold selectivity. Homologation of the alkyl chain (i.e., **23**), and incorporation of a *p*-methoxy (i.e., **24**) or nitro group (i.e., **25**), reduced 5-HT$_2$ affinity (Table III). However, incorporation of a *p*-fluoro group resulted in a slight enhance-

ment of 5-HT_2 affinity (26; 5-HT_2 K_i = 2 nM) with retention of >2000-fold 5-HT_2 versus 5-HT_{1C} selectivity.

Compound 26 (AMI-193) binds at 5-HT_2 sites with an affinity comparable to that of spiperone (1) and, by virtue of its lower affinity for 5-HT_{1C} sites (26; 5-HT_{1C} K_i = 4300 nM), displays about twice spiperone's 5-HT_2 versus 5-HT_{1C} selectivity. Compound 26 retains affinity for 5-HT_{1A} sites (K_i = 50 nM) and also binds at dopamine D2 sites (K_i = 3 ± 1 nM), but possesses low affinity for dopamine D1 sites (K_i = 2530 ± 50 nM). Overall, this compound is quite spiperone-like in its binding profile except that it binds with about 10-fold lower affinity than spiperone (K_i = 0.2 nM) at D2 sites. As such, this compound was a likely candidate for examination in drug-discrimination studies.

5-HT_{1C} Binding. Nearly all of the compounds were found to bind at 5-HT_{1C} sites with relatively low affinity. Indeed, only two compounds (11 and 17) displayed K_i values of <1000 nM. Consequently, it is difficult to comment on 5-HT_{1C} structure–affinity relationships for these compounds.

5-HT_{1A} Binding. Most of the compounds displayed modest affinity for 5-HT_{1A} serotonin receptors. General structure–affinity requirements for 5-HT_{1A} binding have been thoroughly investigated (reviewed[1,15]) and the present work adds relatively little new insight. That is, 1-arylpiperazines are known to bind with high affinity at 5-HT_{1A} sites when they bear at the piperazine 4-position an alkyl (or alkoxy) group of two to five atoms separating the amine from an aryl substituent; replacement of the 1-position nitrogen atom by a carbon atom decreases 5-HT_{1A} affinity by several fold.[15] Thus, most of the present compounds (e.g. 9–17) would be expected to bind only with modest affinity, and 8 (lacking a piperidine 4-position substituent) would be predicted to be inactive. On the other hand, essentially nothing is known about the 5-HT_{1A} affinity of triazaspirodecanone analogues. Evidently, this moiety is tolerated by 5-HT_{1A} receptors; however, to put this in proper perspective, 1-phenyl-4-[4-phenylbutyl]piperazine (35) binds with a K_i value of 1 nM[16] compared to the corresponding triazaspirodecanone 19 (5-HT_{1A} K_i = 85 nM).



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Drug-Discrimination Studies. Five rats were trained to discriminate 1.0 mg/kg of the 5-$HT_{2/1C}$ agonist DOM from saline vehicle such that they made >90% of their responses on the drug-appropriate lever 15 min after administration of the training dose of the training drug, and <20% of their responses on the same lever after administration of saline. Tests of stimulus antagonism were conducted with 26 much in the same way we have previously examined spiperone.[2] The animals were administered 26 and, 45 min later, we administered 1.0 mg/kg of DOM. Each animal served as its own control. As shown in Figure 1, administration of 26 in combination with DOM resulted in antagonism of DOM-appropriate responding in a dose-related manner. Response rates at 0.04 mg/kg of 26 (11.6 ± 2.5 responses/min) were not different from response rates after the administration of 1.0 mg/kg of DOM in the absence of antagonist (11.9 ± 2.1 responses/min). Administration of 0.04 mg/kg of 26 in the absence of DOM (60 min prior to testing) resulted in less than 20% drug-appropriate responding (results not shown).

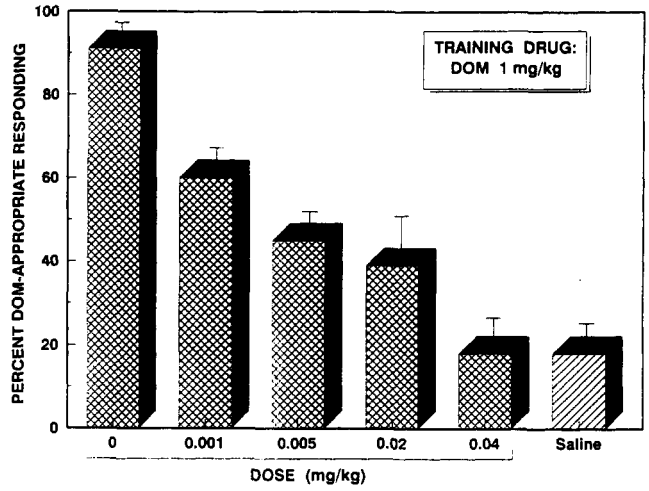

Figure 1. Antagonist effect of doses of 26 administered in combination with 1.0 mg/kg of DOM. The number of animals responding/the number treated, and the response rates (responses/min ± SEM), are given for each dose of 26: 0 (5/5; 11.9 ± 2.1), 0.001 (7/7; 18.2 ± 4.0), 0.005 (4/5; 13.2 ± 2.6), 0.02 (5/5; 10.6 ± 3.5), 0.04 (4/5; 11.6 ± 2.5). Administered alone, saline elicited 18% drug-appropriate response (5/5, 11.2 ± 2.2 responses/min). At the 0.001 mg/kg dose of 26, two of the animals were inadvertantly tested twice. The ED_{50} dose calculated for 26 is 0.003 mg/kg (95% CL, 0.002–0.06 mg/kg).

Unlike spiperone (1), which results in disruption of the animals' behavior when administered in combination with DOM,[2] 26 is capable of antagonizing the DOM stimulus. That is, at the doses necessary for stimulus antagonism, 26 appears to lack the behaviorally disruptive effects of spiperone. Not only does 26 display a >2000-fold selectivity for 5-HT_2 versus 5-HT_{1C} sites but the doses required for stimulus antagonism are also quite low (ED_{50} = 0.003 mg/kg). Thus, although a contributory role for 5-HT_{1C} receptors cannot be conclusively ruled out, the present results convincingly argue that the DOM stimulus involves a 5-HT_2 mechanism.[17]

An alternative, although less likely, explanation for the present results is that DOM produces a compound stimulus (i.e., the DOM stimulus may involve both a 5-HT_2 *and* a 5-HT_{1C} mechanism), and that interference with either one of the two components could result in an apparent antagonism. This would seem unlikely in the present situation because training animals to recognize a compound stimulus (in the few instances where this has been recognized) typically requires an extended period of time, and unusually long training periods are not required to train rats to discriminate DOM from vehicle.[2] However, the only way to provide support for this possibility would be to demonstrate that 5-HT_{1C} antagonists (of which none currently exist) are equally effective in antagonizing the DOM stimulus.

DOM, in addition to being a 5-$HT_{2/1C}$ agonist, is also a potent prototypical hallucinogen (see refs 2 and 7 and literature cited therein). In fact, although the exact mechanism of action of classical hallucinogens is unknown at this time, it has been proposed that such agents produce their hallucinogenic effects by virtue of being 5-HT_2 and/or 5-HT_{1C} agonists (reviewed[7]). Although the primary stimulus effect of DOM in animals is likely 5-HT2-

mediated (this investigation), it still remains to be seen whether a 5-HT2 or 5-HT1C mechanism is more important for human hallucinogenic activity.

Summary. Spiperone (1) binds at 5-HT_2 receptors, relative to 5-HT_{1C} receptors, with high affinity and with 1000-fold selectivity. Due to its disruptive effects on animal behavior, spiperone is unsuitable for use in drug-discrimination studies. A limited structure–affinity study was undertaken to identify a spiperone substitute with comparable 5-$HT_{2/1C}$ binding characteristics that might be useful in animal studies. Opening of the triazaspirodecanone ring (e.g. 9) resulted in a dramatic decrease in selectivity but in retention of modest 5-HT_2 affinity. Optimal 5-HT_2 affinity and selectivity seem to be associated with an intact triazaspirodecanone ring. Replacement of the carbonyl group of spiperone (1) with a methylene group (i.e., 18), and subsequent shortening of the alkyl chain (i.e., 19–21), decreased 5-HT_2 affinity and selectivity. However, replacement of the carbonyl group of spiperone (1) with an ether oxygen atom (i.e., 26) resulted in retention of spiperone's 5-HT_2 affinity and selectivity. Unlike spiperone, compound 26 (at least at the doses employed) (a) lacked the behaviorally disruptive effects of spiperone and (b) was found to be a potent antagonist of the DOM stimulus in rats. The present study suggests, due to the 5-HT_2 versus 5-HT_{1C} selectivity and behavioral potency of 26, that the stimulus effects of DOM are likely mediated primarily by a 5-HT_2, and not a 5-HT_{1C}, mechanism.

Experimental Section

Synthesis. Melting points were determined using a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared spectra were recorded on a Nicolet 5ZDX FT-IR and proton magnetic resonance (NMR) spectra on a JEOL FX90Q FT-NMR spectrometer at 89.55 MHz. ^{13}C-NMR spectra were obtained using a GE QE-300 at 300.64 MHz. All spectral data are consistent with assigned structures. Elemental analyses were performed by Atlantic Microlab Inc. (Norcross, GA) and values are within 0.4% of the theoretical values. Unless otherwise stated, salts were prepared by the following general procedure: (a) for maleates and oxalates, by the dropwise addition of a molar equivalent solution of maleic acid or oxalic acid in anhydrous Et_2O to a solution of the amine in anhydrous Et_2O or a mixture of absolute EtOH and anhydrous Et_2O, (b) for hydrochlorides, by the dropwise addition, until cessation of salt formation, of a saturated solution of HCl in anhydrous Et_2O to a solution of the amine in anhydrous Et_2O or absolute EtOH/Et_2O. Flash chromatography was performed on silica gel (Merck, grade 60, 230–400 mesh, 60 Å) using 9:1 $CHCl_3$/MeOH as eluent. MeCN was dried by distillation, THF was dried by distillation from $LiAlH_4$, and $CHCl_3$ was dried by distillation from P_2O_5; all solvents (except for Et_2O) were stored over 3- or 4-Å molecular sieves. All starting materials were purchased either from Aldrich Chemicals or from Janssen Chimica.

2-(4-Fluorophenyl)-Δ^1-pyrroline (4). A solution of 2-(4-fluorophenyl)-2-(3-aminopropyl)-1,3-dioxolane (34; 0.5 g, 2.26 mmol) in 30% aqueous HOAc was allowed to stir at room temperature for 6 h. Solvent was removed under reduced pressure. The oily residue was dissolved in Et_2O (75 mL); the solution was washed with saturated $NaHCO_3$ solution (25 mL) and then H_2O (25 mL), dried (Na_2SO_4), and evaporated to give an oil that crystallized to give 0.22 g (59%) of a yellow-orange solid upon standing: mp 37–39 °C (lit.[20] mp 38–39 °C); ^1H NMR ($CDCl_3$) δ 2.15 (m, 2H, CH_2), 2.95 (t, 2H, $CH_2C{=}N$), 4.05 (t, 2H, CH_2N), 7.1 (m, 2H, ArH), 7.85 (m, 2H, ArH); IR (KBr) 1621 (C=N) cm^{-1}.

3-(4-Fluorophenoxy)propylamine Hydrochloride (5). Method A. A mixture of potassium phthalimide (2.4 g, 1.3 mmol) and 1-(3-chloropropoxy)-4-fluorobenzene (2.0 g, 1.1 mmol) in dry DMF (10 mL) was heated on an oil bath (80 °C) for 18 h. Once cool, $CHCl_3$ (25 mL), and then H_2O (50 mL), was added to the reaction mixture, and the layers were separated. The aqueous portion was washed with $CHCl_3$ (3 × 10 mL), and the combined $CHCl_3$ portions were washed with NaOH (0.4 N, 10 mL), dried (Na_2SO_4), and evaporated to dryness to give 3.4 g of a fluffy white solid (mp 105–109 °C). A mixture of this phthalimide (1.9 g, 6.3 mmol) and 85% hydrazine hydrate (2.4 g) in absolute EtOH (100 mL) was heated at reflux for 3 h. After the reaction mixture had cooled to room temperature, the precipitate was removed by filtration and washed with $CHCl_3$ (20 mL). The combined filtrate and washing was evaporated to dryness in vacuo and the resulting crude product was taken up in $CHCl_3$ (50 mL), washed with H_2O (3 × 25 mL), dried (Na_2SO_4), and the solvent was removed under reduced pressure to afford 1.0 g (93%) of the free base as a clear oil. The hydrochloride salt was prepared and recrystallized from an absolute EtOH/anhydrous Et_2O mixture to afford the product as white crystalline flakes; mp 168–171 °C (see Table I).

Compound 3 was prepared in a similar manner from 4-chloro-1-(4-fluorophenyl)butan-1-one (27) (see Table I).

1-[3-(4-Fluorophenoxy)propyl]-*N*-phenylisonipecotamide (16). Method B. A mixture of *N*-phenylisonipecotamide (33) (102 mg, 0.5 mmol), 28 (74 mg, 0.5 mmol), anhydrous K_2CO_3 (70 mg, 0.5 mmol), and a few crystals of NaI in dioxane (10 mL) was heated at reflux overnight (16 h). The hot reaction mixture was filtered and the solvent was evaporated under reduced pressure; the residue was chromatographed on a silica gel column (column size: 1 × 18 cm; 6 g silica gel, 60 mesh) using a $CHCl_3$/MeOH (9:1) mixture to give an oily product. The oil was taken up in Et_2O (20 mL) and treated with a saturated solution of oxalic acid in anhydrous Et_2O to afford the hydrogen oxalate salt; mp 168–170 °C (see Table I).

Compounds 6, 8, 9, 10, 12, 13 were all prepared by a similar alkylation procedure (see Table I).

4h(*N*,*N*-Dimethylamino)-1-(4-fluorophenyl)-1-butanone Hydrochloride (7). Method C. 2-(4-Fluorophenyl)-2-(3-phthlimidopropyl)-1,3-dioxolane (2.8 g; mp 149–150 °C) was prepared by heating a solution of 2-(3-chloropropyl)-2-(4-fluorophenyl)-1,3-dioxolane (2.1 g, 8.7 mmol) and potassium phthalimide (2 g) in dry DMF (8 mL) at 80 °C under N_2 for 18 h. The resultant crude product was subjected to hydrazinolysis to afford 1.1 g (90%) of the ketal-protected keto amine 34 (clear oil; bp 48 °C, 0.04 mmHg) as described for compound 5. [34: ^1H NMR ($CDCl_3$) δ 1.5 (m 2H, CH_2), 1.9 (m, 2H, CH_2), 2.6 (t, 2H, CH_2), 3.6–4.1 (m, 4H, CH_2), 6.9–7.1 (m, 2H, ArH), 7.2–7.5 (m, 2H, ArH); IR (neat) 3400 (NH_2) cm^{-1}.] Formaldehyde solution (37%; 0.5 mL, 6.5 mmol) was slowly added to a cooled solution of the dioxolane (0.5 g, 2.2 mmol) in formic acid (0.25 mL) and the mixture was heated at 80 °C for 24 h. The reaction mixture was acidified by the addition of 6 N HCl (10 mL) and was subsequently washed with Et_2O (3 × 5 mL). The aqueous solution was basified by addition of 50% NaOH (10 mL) and extracted with Et_2O (3 × 10 mL). The combined Et_2O fractions were dried ($MgSO_4$), and the solvent was removed under reduced pressure to give 0.28 g (61%) of the free base as an oil. Treatment of the free base with an ethereal solution of HCl afforded 7 as off-white crystals after recrystallization from a mixture of EtOAc and absolute EtOH; mp 133–135 °C (see Table I).

1-[3-(4-Fluorophenoxy)propyl]-4-(*N*-methyl-*N*-phenylamino)piperidine Hydrogen Oxalate (14). Method D. A mixture of 13 (free base, 50 mg, 0.12 mmol) and formaldehyde solution (37%; 3 mL) in 95% EtOH (10 mL) was hydrogenated overnight (*ca.* 16 h) in the presence of 10% Pd/C (10 mg). The catalyst was removed by filtration and the solvent was evaporated under reduced pressure to afford a crude product. The oil was chromatographed on a silica gel column (column size: 1 × 10 cm; 3 g of silica gel) using a $CHCl_3$/MeOH (9:1) mixture as eluent. Treatment of the homogeneous oily product with a saturated solution of oxalic acid in anhydrous Et_2O gave 0.02 g (38%) of 14 after recrystallization from an absolute EtOH/anhydrous Et_2O mixture; mp 145–147 °C (see Table I).

4-Benzamido-1-[3-(4-fluorophenoxy)propyl]piperidine Maleate (15) Method E. A solution of 31 (260 mg, 0.5 mmol), ammonium acetate (3.8 g, 4.9 mmol), and $NaCNBH_4$ (25 mg, 0.45 mmol) in MeOH (10 mL) was allowed to stir at room temperature for 48 h. The pH was adjusted to 2 by the addition of concentrated hydrochloric acid and the MeOH was removed

by evaporation under reduced pressure. Water (10 mL) was added and the solution was extracted with Et_2O (3 × 10 mL). The aqueous portion was adjusted to pH 10 by addition of solid KOH and the solution was again extracted with Et_2O (3 × 10 mL); the Et_2O extract was washed with H_2O (3 × 10 mL) and dried ($MgSO_4$), and the solvent was removed by evaporation under reduced pressure to afford 150 mg of the crude amine **32**, which was used without further purification or characterization. Benzoyl chloride (84 mg, 0.6 mmol) in $CHCl_3$ (10 mL) was added in a dropwise manner to a stirred solution of **32** (150 mg, 0.6 mmol) and NEt_3 (60 mg, 0.6 mmol) in $CHCl_3$ (10 mL). After the addition was complete, stirring was allowed to continue for an additonal 6 h. The solvent was evaporated under reduced pressure, the solid residue was dissolved in Et_2O (20 mL), the solution was filtered, and the filtrate was washed with 5% NaOH (5, 3, and 2 mL) and then with like portions of H_2O. The Et_2O solution was dried (Na_2SO_4) and evaporated to dryness. The homogeneous solid residue (mp 145–147 °C) was treated with maleic acid in Et_2O to afford 150 mg (53%) of **15** after recrystallization from an absolute EtOH/anhydrous Et_2O mixture: mp 195–197 °C; ^{1}H NMR (free base; DMSO-d_6): δ 1.7 (m, 6H, $3CH_2$), 2.9 (m, 4H, $2CH_2$), 3.4 (m, 2H, CH_2), 3.8 (m, 3H, CH_2 and CH), 6.0 (s, 2H, —CH=CH—), 6.8–7.8 (m, 9H, Ar-H), 8.3 (d, 1H, NH) (see Table I).

8-[4-(4-Fluorophenyl)butyl]-1-phenyl-1,3,8-triazaspiro-[4.5]decan-4-one Hydrochloride (18) Method F. A mixture of 4-(4-fluorophenyl)butanol *p*-tosylate (0.5 g, 1.4 mmol) and 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (0.3 g, 1.2 mmol) in toluene (10 mL) was allowed to stir overnight (*ca.* 16 h) at 80 °C. When the reaction mixture was cool, the solvent was evaporated under reduced pressure and the residue was triturated with Et_2O (50 mL). Insoluble material was removed by filtration and the filtrate was evaporated to dryness to give **18** as the free base. An Et_2O solution of the amine was treated with HCl gas to give 0.08 g (17%) of **18** as a white solid material; mp 204–207 °C (see Table I).

Compounds **19–26** were prepared in a similar manner (see Table I).

N-Phenylisonipecotamide (33). A solution of isonipecotic acid (0.52 g, 4 mmol) in trifluoroacetic anhydride (2 mL) was heated at reflux for 2 h and then allowed to stir at room temperature overnight. Solvent was evaporated under reduced pressure and the residue was triturated with Et_2O to give 0.6 g of *N*-(trifluoroacetyl)isonipecotic acid after recrystallization from 2-PrOH/hexanes; mp 119–120 °C (lit.[21] mp 117 °C). Thionyl chloride (4 mL) was added to 0.5 g of the N-protected isonipecotic acid and the mixture was heated at reflux for 2 h. Excess $SOCl_2$ was removed by evaporation and a solution of the residue in $CHCl_3$ (10 mL) was treated with a solution of aniline (0.23 g, 2.5 mmol) in $CHCl_3$ (5 mL). After stirring of the reaction mixture at room temperature for 2 h, solvent was removed by evaporation under reduced pressure and the residue was dissolved in Et_2O (20 mL). The ethereal solution was washed with 10% HCl (10 and 5 mL), 10% NaOH (10 and 5 mL), and H_2O (10 and 5 mL), dried ($MgSO_4$), and evaporated to dryness. Recrystallization of the crude product from MeOH gave 0.7 g of the amide; mp 144–146 °C. Removal of the trifluoroacetyl protecting group was achieved by allowing the amide (0.5 g) to stir in 10% NaOH (10 mL) at room temperature for 2 h. The product was extracted from the aqueous solution with Et_2O (10, 5, 2 mL) and the combined Et_2O solution was washed with H_2O (10, 5, 2 mL), dried ($MgSO_4$), and evaporated to dryness. Recrystallization from an acetone/hexanes mixture gave 200 mg of **33**; mp 138–139 °C (lit.[22] mp 137–138 °C).

1-[3-(4-Fluorophenoxy)propyl]piperidone (31). A mixture of **28** (1.9 g, 0.1 mol) and 1,4-dioxa-8-azaspiro[4.5]decane (**29**) (1.3 g, 0.1 mol), K_2CO_3 (1.4 g), and a few crystals of KI in dioxane (40 mL) was heated at reflux for 6 h. Upon cooling, the reaction mixture was filtered and the solvent was evaporated under reduced pressure. The residue in Et_2O (30 mL) was washed with H_2O (3 × 10 mL) and dried ($MgSO_4$), and a saturated solution of oxalic acid in anhydrous Et_2O was added to afford 1.6 g (42%) of **30** as the salt; mp 166–168 °C. Treatment of an HOAc (30%; 10 mL) solution of **30** with a few drops of concentrated HCl and gentle warming overnight removed the protecting group. The reaction mixture was neutralized by the dropwise addition of 10% NaOH; the resultant oil was extracted with Et_2O (3 × 10 mL) and the Et_2O solution was washed with H_2O (3 × 10 mL) and dried ($MgSO_4$). (A sample of the oxalate salt was prepared and recrystallized from absolute EtOH/anhydrous Et_2O; mp 186–188 °C.) ^{1}H NMR (DMSO-d_6) δ 2.1 (m, 2H, CH_2), 2.7 (t, 4H, $2CH_2$), 3.2 (m, 6H, $3CH_2$), 4.2 (t, 2H, CH_2), 7.2 (m, 4H, Ar-H); IR (KBr) 1735 cm^{-1}. Compound **31** was used without further characterization for the preparation of **15**.

Radioligand Binding. Radioligand binding assays were performed as previously reported in detail.[23,24] Briefly, frontal cortical regions of male Sprague–Dawley rats (200–250 g, Charles River) were dissected on ice and homogenized (1:10 w/v) in ice-cold buffer solution (50 mM Tris HCl, 0.5 mM EDTA, and 10 mM $MgCl_2$ at pH 7.4) and centrifuged at 3000*g* for 15 min. The pellet was resuspended in buffer (1:30 w/v), incubated at 37 °C for 15 min and then centrifuged twice more at 3000*g* for 10 min (with resuspension between centrifugations). The final pellet was resuspended in buffer which also included 0.1% ascorbate and 10^{-5} M pargyline.

Assays were performed in triplicate in a 2.0-mL volume containing 5-mg, wet weight of tissue and 0.4 nM [^{3}H]ketanserin (76 Ci/mmol; New England Nuclear) for 5-HT2 receptor assays, and 10 mg wet weight of tissue and 1 nM [^{3}H]mesulergine (75.8 Ci/mmol; Amersham) for 5-HT1C receptor assays. Cinanserin (1.0 μM) was used to define nonspecific binding in the 5-HT2 assay. In the 5-HT1C assays, mianserin (1.0 μM) was used to define nonspecific binding, and 100 nM spiperone was added to all tubes to block the binding of the tritiated radioligand to 5-HT2 receptors. The 5-HT_{1A} assay[24] utilized 0.1 nM [^{3}H]-8-OH-DPAT [8-hydroxy-2-(*N*,*N*-di-*n*-propylamino)tetralin] (157 Ci/mmol; New England Nuclear) and 4 mg wet weight of rat hippocampal tissue; 8-OH-DPAT (1 μM) was used to determine nonspecific binding. Tubes were incubated for 15 min at 37 °C, filtered on Schliecher and Schuell (Keene, NH) glass-fiber filters (presoaked in polyethyleneimine), and washed with 10 mL of ice-cold buffer. The filters were counted at an efficiency of 50%.

Saturation and competition experiments were analyzed using an updated version of the program EBDA[25] to obtain equilibrium dissociation constants (K_D), B_{max}, Hill coefficients, and IC_{50} values. K_i values for competition experiments were obtained using the equation $K_i = IC_{50}/1 + (D^*/K_D^*)$ where IC_{50} is the experimentally observed concentration of competing drug that inhibits 50% of specific binding, K_{D^*} is the equilibrium dissociation constant determined in saturation studies, and D^* is the concentration of radioactive ligand used in the competition assays.[26] Serotonin hydrogen oxalate and spiperone were obtained from Sigma (St. Louis, MO).

Drug-Discrimination Studies. We have previously trained rats to discriminate DOM from saline vehicle;[27] an identical training procedure was used in the present investigation. The subjects were five male Sprague–Dawley rats weighing 250–300 g at the start of the study. The animals were first trained to lever-press for a sweetened milk reward using standard two-lever operant chambers (Coulbourn Instruments Model E10-10) housed within sound- and light-attenuating outer chambers. Once lever-pressing behavior was acquired, the animals were trained to discriminate 1.0 mg/kg of DOM from 1.0 mL/kg of 0.9% saline. Briefly, rats were trained to respond on a variable-interval 15-s (VI 15-s) schedule of reinforcement. Once rates of responding stabilized, the animals received an injection of DOM or saline 15 min prior to each session. DOM or saline was administered on a double-alternation schedule (i.e., 2 days DOM, 2 days saline) and training sessions were of 15 min duration. On every fifth day, learning was assessed during an initial 2.5-min nonreinforced (extinction) period followed by a 12.5-min training session. Data collected during the extinction period included percent drug-appropriate lever responding (i.e., the number of responses on the drug designated lever ÷ total number of responses, expressed as a percent) and total responses made during the entire 2.5-min extinction session (expressed as responses/min).

Once the rats consistently made >80% of their responses on the drug-appropriate lever after administration of DOM, and <20% of their responses on the same lever after injection of saline, stimulus antagonism studies were begun. During these investigations, test sessions were interposed among the training sessions; however, after the 2.5-min extinction period, the animals

were returned to their home cages. During antagonism studies the rats were injected with doses of 26, and then, after 45 min, with 1.0 mg/kg of DOM. Fifteen minutes later, the animals were tested under extinction conditions. Stimulus antagonism was said to have occurred when the animals made ≤20% of their responses on the drug-appropriate lever when administered drug in combination with DOM. ED_{50} (effective inhibition dose 50%) values were determined by the method of Finney.[28]

1-(2,5-Dimethoxy-4-methylphenyl)-2-aminopropane hydrochloride (DOM) was obtained from NIDA. All solutions were prepared fresh daily in sterile 0.9% saline and administered via the intraperitoneal route in a 1 mL/kg injection volume.

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