Spiperone: Influence of Spiro Ring Substituents on 5-HT_{2A} Serotonin Receptor Binding

Kamel A. Metwally,[†] Malgorzata Dukat,[†] Christina T. Egan,[‡] Carol Smith,[‡] Ann DuPre,[‡] Colleen B. Gauthier,[‡] Katharine Herrick-Davis,[‡] Milt Teitler,[‡] and Richard A. Glennon*[,†]

Department of Medicinal Chemistry, School of Pharmacy, Virginia Commonwealth University, Richmond, Virginia 23298-0540, and Department of Pharmacology, Albany Medical College, Albany, New York 12208

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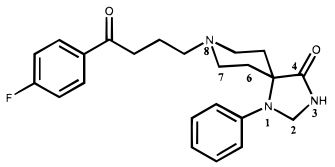
Spiperone (**1**) is a widely used pharmacological tool that acts as a potent dopamine D_2, serotonin 5-HT_{1A}, and serotonin 5-HT_{2A} antagonist. Although spiperone also binds at 5-HT_{2C} receptors, it is one of the very few agents that display some (ca. 1000-fold) binding selectivity for 5-HT_{2A} versus 5-HT_{2C} receptors and, hence, might serve as a useful template for the development of novel 5-HT_{2A} antagonists if the impact of its various substituent groups on binding was known. In the present investigation we focused on the 1,3,8-triazaspiro[4.5]decanone portion of spiperone and found that replacement of the N_1-phenyl group with a methyl group only slightly decreased affinity for cloned rat 5-HT_{2A} receptors. However, N_1-methyl derivatives displayed significantly reduced affinity for 5-HT_{1A}, 5-HT_{2C}, and dopamine D_2 receptors. Several representative examples were shown to behave as 5-HT_2 antagonists. As such, N_1-alkyl analogues of spiperone may afford entry into a novel series of 5-HT_{2A}-selective antagonists.

Serotonin (5-hydroxytryptamine, 5-HT) receptors are divided into seven major families: 5-HT_1−5-HT_7.[1−3] Prior to the identification of the latter population of 5-HT receptors (i.e., 5-HT_3−5-HT_7 receptors), the antipsychotic agent spiperone (**1**), a high-affinity D_2 dopaminergic antagonist, was one of the early agents capable of distinguishing between the original 5-HT_1 and 5-HT_2 populations of receptors. Spiperone was instrumental



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in characterizing 5-HT_1 versus 5-HT_2 pharmacology, and [^{3}H]spiperone was long used as a radioligand for labeling the latter population of receptors. With the subsequent discovery of 5-HT_{1A} receptors, it was demonstrated that spiperone binds at this 5-HT_1 subpopulation with considerable affinity (5-HT_{1A} $K_i \approx 10-100$ nM).[4] 5-HT_2 receptors are now known to be heterogeneous and the 5-HT_2 family consists of 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors;[1−3] spiperone displays significant affinity only for the 5-HT_{2A} subpopulation.[5] In fact, spiperone is one of the very few agents that bind selectively at 5-HT_{2A} receptors ($K_i \approx 1-2$ nM) versus 5-HT_{2C} receptors ($K_i \approx 1000-4000$ nM). As such, it might serve as a suitable template for the development of novel 5-HT_{2A}-selective antagonists.

We, and others, have been interested in developing 5-HT_{2A}- versus 5-HT_{2C}-selective antagonists (reviewed in ref 6) for the purpose of further investigating central 5-HT_{2A} pharmacology. Spiperone meets the necessary criteria but suffers from its high affinity for 5-HT_{1A} and dopamine D_2 receptors. The primary goal of the present study was to determine which structural features of spiperone contribute to its high affinity for 5-HT_{2A} receptors or to its lack of significant affinity for 5-HT_{2C} receptors; in other words, we asked the question: why is spiperone selective for 5-HT_{2A} versus 5-HT_{2C} receptors?

Although the structure−activity relationships of spiperone as a dopaminergic agent have been extensively investigated, little attention has been paid to the serotonergic aspects of this agent. In fact, those studies that have addressed the latter issue have focused on reducing the serotonergic character of spiperone in order to improve its selectivity for dopamine receptors. For example, introduction of substituents to the lactam (i.e., N_3) nitrogen atom of spiperone can influence selectivity; there appears to be a region of limited bulk tolerance associated with this position on both 5-HT_2 and dopamine D_2 receptors (see ref 7 and references therein for further discussion). These regions of bulk tolerance are likely different in that although small N_3-alkyl substituents seem readily accommodated by both populations of receptors, larger substituents result in enhanced dopaminergic selectivity. N_3-Benzyl and substituted-benzyl derivatives of spiperone, for example, can display >500-fold selectivity for D_2 receptors over 5-HT_2 receptors.[7]

The present investigation represents one of the first to address the serotonergic character of spiperone. We began this investigation several years ago by dissecting the spiperone molecule into two major components: the piperidine derivative **2** and the 1,3,8-triazaspirodecanone **3**.[8] Compound **3** was without affinity for 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C} receptors (i.e., K_i > 10 000 nM).

* To whom correspondence should be addressed. Tel: 804-828-8487. Fax: 804-828-7404.
[†] Virginia Commonwealth University.
[‡] Albany Medical College.

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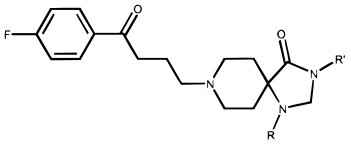
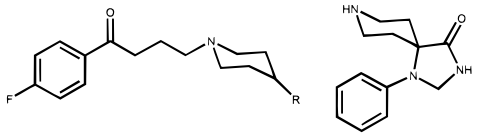
Table 1. Physicochemical Properties of Substituted 1,3,8-Triazaspiro[4.5]decanone Derivatives



compd	R	R′	mp, °C	recryst solvent[a]	% yield	empirical formula
4	−cyclohexyl	−H	245−248	acetone	27	$C_{23}H_{33}FN_3O_2 \cdot HCl$[b]
5	−*i*Pr	−H	244−247	EtOH/Et_2O	34	$C_{20}H_{28}FN_3O_2 \cdot 2HCl$[b]
6	−H	−H	227−229	EtOH/Et_2O	23	$C_{17}H_{22}FN_3O_2 \cdot 2HCl$[b]
7	−Et	−H	231−233	EtOH/Et_2O	32	$C_{19}H_{26}FN_3O_2 \cdot 2HCl$
8	−Me	−H	234−236	*i*PrOH	35	$C_{18}H_{24}FN_3O_2 \cdot 2HCl$
9	−Me	−Me	158−160	EtOH/Et_2O	28	$C_{19}H_{26}FN_3O_2 \cdot HCl$[c]
10	−Me	−Et	102−104	EtOAc/Et_2O	24	$C_{20}H_{28}FN_3O_2 \cdot 2HCl$[d]
11	−Me	−*n*Pr	188−194	EtOH	22	$C_{21}H_{30}FN_3O_2 \cdot 2HCl$
12	−Me	−*i*Pr	207−210	EtOH/Et_2O	17	$C_{21}H_{30}FN_3O_2 \cdot 2HCl$[b]
13	−Me	−*n*Bu	133−136	EtOH/Et_2O	41	$C_{22}H_{32}FN_3O_2 \cdot 2HCl$
14	−Me	−benzyl	215−217	EtOH/Et_2O	68	$C_{25}H_{30}FN_3O_2 \cdot 2HCl$

[a] EtOH, absolute ethanol; Et_2O, anhydrous ether. [b] The salt crystallized with 0.25 mol of H_2O. [c] The salt crystallized with 0.5 mol of H_2O. [d] The salt crystallized with 2 mol of H_2O.



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3

Compound **2** also lacked affinity for 5-HT_{1A} and 5-HT_{2C} receptors when R = −H but displayed modest affinity for 5-HT_{2A} receptors; however, the affinity of **2** (i.e., **2a**, R = −H, 5-HT_{2A} K_i = 140 nM) was nearly 150-fold lower than that of spiperone (**1**) itself. Incorporation of an −NH-phenyl substituent at the piperidine 4-position of **2** (i.e., **2b**, R = $-NHC_6H_5$), in an attempt to more closely approach the structure of spiperone (**1**), resulted in increased 5-HT_{2A} affinity (**2b**, 5-HT_{2A} K_i = 34 nM) relative to **2a** but in reduced selectivity for 5-HT_{2A} versus 5-HT_{2C} receptors (**2b**, 5-HT_{2C} K_i = 2300 nM) relative to spiperone.[8] From this study it was concluded that features associated with the **2** portion may represent a binding anchor point and that the imidazolinone segment of spiperone, or at least a portion thereof, may be a contributor to the modulation of 5-HT_{2A} affinity.[8] That is, the low affinity and selectivity of **2b** for 5-HT_{2A} receptors relative to spiperone (**1**) may be a function of the absence of the spiro ring (which holds the N_1-substituent in a specific location) and/or the lack of the lactam carbonyl group. Consequently, in the present investigation, we focused on the spiro portion or, more specifically, on the imidazolinone portion of spiperone.

We addressed the following: (a) what role does the N_1-phenyl group of spiperone play in binding at 5-HT_{2A} and 5-HT_{2C} receptors, (b) is the intact spiro ring required for serotonergic binding, and (c) does the lactam carbonyl group contribute to binding?

Chemistry

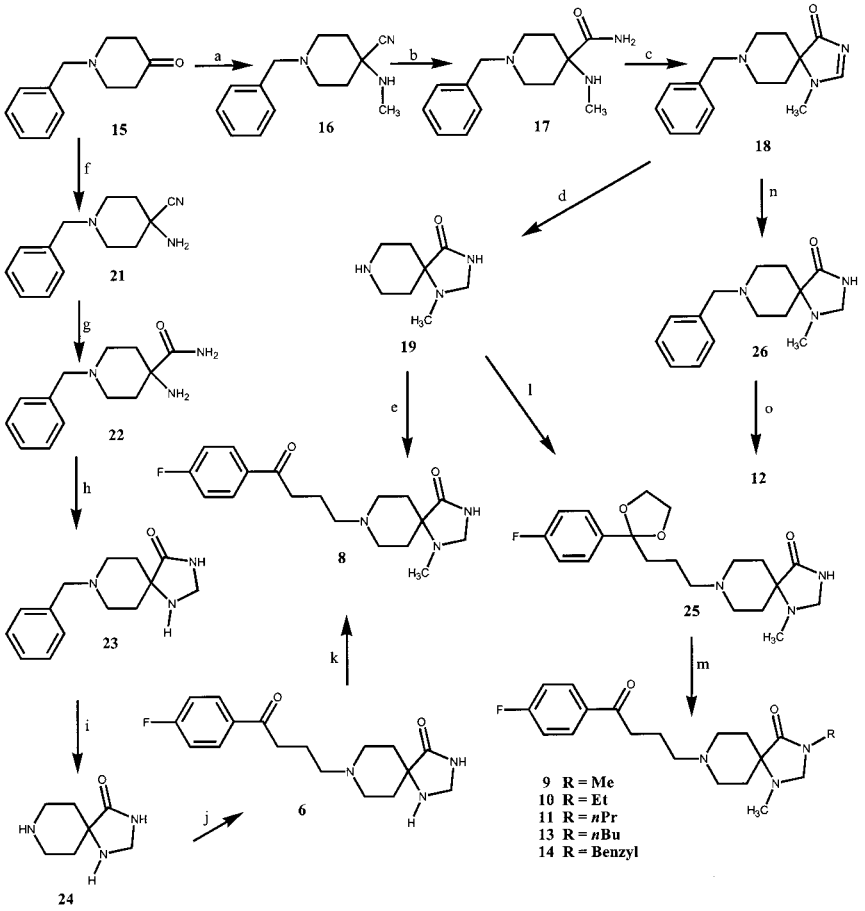
The known N_1-cyclohexyl derivative **4** (Table 1) was prepared from *N*-benzyl-4-pyridone (**15**) by a sequence of reactions previously described in the patent literature.[9] (A parallel series of reactions is shown in Scheme 1 for the preparation of the corresponding N_1-methyl analogue **8**, i.e., **15** → **16** →**17** → **18** → **19** → **8**.) Due to a disparity between the reported melting point for the dihydrochloride salt of **4** (lit.[9] mp 206−215 °C) and that obtained in the present study (mp 245−248 °C), **4** was submitted for microanalysis and was determined to be the monohydrochloride salt. The known N_1-*i*Pr derivative **5** was prepared and treated in like manner (lit.[9] mp 212.6−214 °C, present mp 244−247 °C); however, **5** was analyzed correctly as the dihydrochloride salt. The N_1-ethyl derivative **7** was obtained from the previously reported 8-benzyl-1-ethyl-1,3,8-triazaspiro[4.5]decan-4-one (**20**); deprotection of the piperidine nitrogen by hydrogenolysis, followed by reaction of the resultant amine with 4-(4-fluorophenyl)-4-oxo-*n*-butyl chloride, provided the desired product.

Synthesis of the N_1-methyl derivative **8** also followed a literature procedure (see Scheme 1), but again the melting point obtained (mp 234−236 °C) was different than that reported[9] (lit. mp 203.6−212 °C). The N_1-unsubstituted compound **6** was prepared from the known **24**[10] (Scheme 1). Although melting points were not previously reported, spectral data for compounds **21** and **22** were generally consistent with those provided in the patent.[10] Cyclization of **22** followed by deprotection afforded **24**. Compound **24** was alkylated with 4-(4-fluorophenyl)-4-oxo-*n*-butyl chloride to afford **6**. Compound **6** has been previously synthesized in a slightly different manner;[10] however, due to the lack of a reported melting point and with inconsistencies in the reported ^1H NMR spectrum, a direct comparison with the reported product could not be made. Furthermore, because alkylation of **24** might conceivably have occurred at the N_1- rather than at the N_8-position, **6** was methylated to afford **8** as confirmation of its structure.

The N_3-alkyl derivatives of **8** were prepared by alkylation of **25** (e.g., **11**) or by elaboration of **26** (i.e., **12**). A description is provided for the synthesis of **11**; compounds **9**, **10**, **13**, and **14** were prepared in the same manner (Table 1). The N_3-*i*Pr derivative **12** was prepared in a slightly different manner. Compound **18** was treated with $NaBH_4$ to afford **26**; the sodium salt of **26** was allowed to react with 2-bromopropane, and the resultant product was deprotected and allowed to react with 4-(4-fluorophenyl)-4-oxo-*n*-butyl chloride to give the desired product **12**.

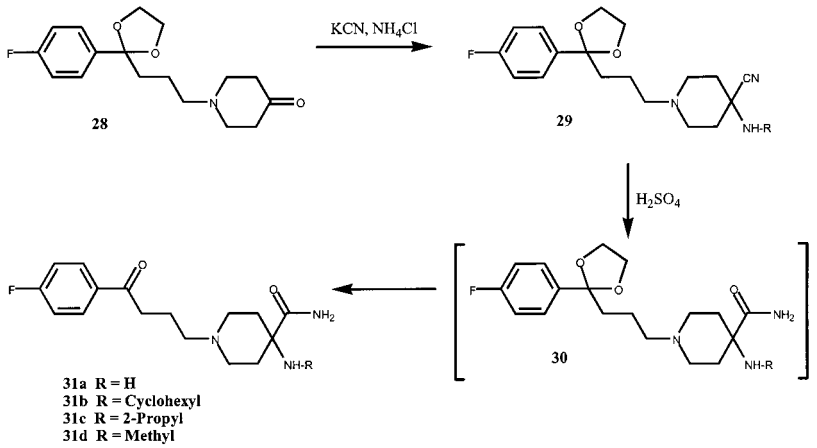
Compound **27** was prepared from compound **18**. Compound **18** was reduced by treatment with $LiAlH_4$,

Scheme 1[a]

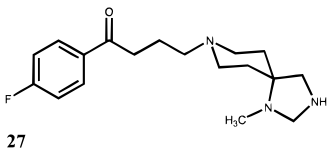


[a] (a) KCN, $MeNH_2$·HCl; (b) H_2SO_4; (c) $HC(OEt)_3$; (d) H_2/Pd; (e) 4-(4-fluorophenyl)-4-oxo-*n*-butyl chloride; (f) KCN, NH_4Cl; (g) 95% H_2SO_4; (h) i. $HCONH_2$, ii. H_2SO_4; (i) i. H_2/Pd, ii. 35% HCl; (j) 4-(4-fluorophenyl)-4-oxo-*n*-butyl chloride; (k) MeI/KOH; (l) 4-chloro-1-(4-fluorophenyl)-1,1-(ethylenedioxy)butane; (m) i. NaH/THF, ii. RX, iii. HCl; (n) $NaBH_4$; (o) i. NaH/THF, ii. 2-bromopropane, iii. H_2/Pd, iv. 4-(4-fluorophenyl)-4-oxo-*n*-butyl chloride, K_2CO_3.

Scheme 2



and the crude product was isolated as its *t*BOC derivative. Catalytic reduction, as with the compounds shown in Scheme 1, reduced the ring to an imidazoline and removed the benzyl protecting group in a single step. Subsequent alkylation with 4-(4-fluorophenyl)-4-oxo-*n*-butyl chloride followed by deprotection afforded the desired **27** at its trihydrochloride salt.



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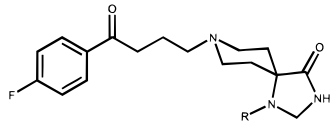
The amino carboxamides **31a**−**d** were prepared by a common method (Scheme 2). 4-Piperidone was allowed to react with 4-chloro-1-(4-fluorophenyl)-1,1-(ethylenedioxy)butane to afford the carbonyl-protected analogue **28**.[10] Compound **28** was then treated with a solution

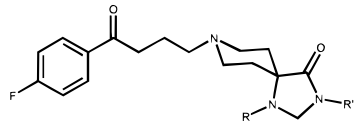
Table 2. Binding of Spiperone (**1**) and N_1-Modified Spiperone Analogues at Targeted Receptor Populations[a]



compd	R	K_i, nM (SEM) 5-HT_{2A}	5-HT_{2C}	5-HT_{1A}	D_2
1 (spiperone)	−phenyl	1.8 (±0.2)	1600 (±100)	58 (±13)	0.5 (±0.05)
4	−cyclohexyl	0.7 (±0.1)	677 (±112)		0.7 (±0.1)
5	$-CH(CH_3)_2$	38 (±7)	>10000	2340 (±16)	400 (±37)
6	−H	5600 (±800)	>10000	>10000	
7	$-C_2H_5$	170 (±40)	>10000	7960 (±30)	170 (±2)
8	$-CH_3$	23 (±3)	>10000	>10000	220 (±43)

[a] Where value is not provided, a K_i value was not determined. SEM was not determined where K_i > 10 000 nM.

Table 3. Binding of N_3-Modified Spiperone Analogues at Targeted Receptor Populations



compd	R	R′	K_i, nM (SEM) 5-HT_{2A}	5-HT_{2C}	5-HT_{1A}	D_2
8	$-CH_3$	−H	23 (±3)	>10000	>10000	220 (±43)
9[a]	$-CH_3$	$-CH_3$	45 (±15)	>10000	>10000	300 (±43)
10	$-CH_3$	$-C_2H_5$	21 (±2)	>10000	>10000	
11[a]	$-CH_3$	$-(CH_2)_2CH_3$	20 (±1)	>10000	>10000	130 (±7)
12	$-CH_3$	$-CH(CH_3)_2$	7 (±1)	>10000	6300 (±850)	46 (±17)
13	$-CH_3$	$-(CH_2)_3CH_3$	19 (±1)	>10000	>10000	13 (±3)
14	$-CH_3$	$-CH_2$-phenyl	22 (±9)	1405 (±40)	265 (±30)	

[a] Compounds **9** and **11** bind at [^{3}H]DOB-labeled 5-HT_{2A} sites with K_i = 67 ± 2 and 27 ± 2 nM, respectively.

of KCN and the appropriate amine to provide the corresponding aminonitrile **29** which was hydrolyzed to **30** and deprotected.

Ether analogue **32** was prepared by alkylation of 1-methyl-1,3,8-triazaspiro[4.5]decan-4-one (**19**) with 3-(4-fluorophenoxy)propyl chloride, and the N_3-*n*-propyl analogue **33** was prepared by alkylation of **32**.

Results and Discussion

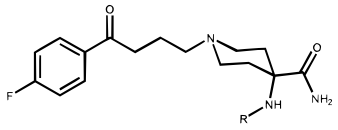
5-HT_{2A} Structure−Affinity Studies. Spiperone (**1**) binds at 5-HT_{2A} (K_i = 1.8 nM; Table 2) and 5-HT_{2C} (K_i = 1600 nM) receptors; its affinity for the latter population of receptors, however, is low. Reduction of the N_1-phenyl group of spiperone to an N_1-cyclohexyl group (i.e., **4**) doubles its affinity at both populations of receptors. Evidently, substituents at this position can influence affinity at 5-HT_{2A} and 5-HT_{2C} receptors. The cyclohexyl group was abbreviated to an isopropyl group (i.e., **5**). Compound **5** binds at 5-HT_{2A} receptors with about 20-fold lower affinity than spiperone (**1**). However, **5** displays reduced affinity at 5-HT_{2C} receptors and binds with 800-fold lower affinity than spiperone (**1**) at D_2 receptors. Results with the N_1-unsubstituted **6** (K_i = 5600 nM) further support the notion that an N_1-substituent is critical for 5-HT_{2A} binding in that **6** binds at 5-HT_{2A} receptors with >3000-fold lower affinity than **1** and lacks significant affinity for 5-HT_{2C} receptors (K_i > 10 000 nM; Table 2). Armed with the information that the presence of an N_1-substituent may be a requirement for 5-HT_{2A} binding, we incorporated the smallest possible N_1-substituent (i.e., an *N*-methyl group, **8**). Compound **8** binds at 5-HT_{2A} receptors (K_i = 23 nM; Table 2) with about 10-fold lower affinity than spiperone (**1**), but lacks affinity for 5-HT_{2C} receptors (K_i > 10 000 nM). Extension of the methyl group to an ethyl group, **7**, results in decreased 5-HT_{2A} affinity (**7**, 5-HT_{2A} K_i = 170 nM).

It has been reported that incorporation of N_3-substituents can influence the binding of spiperone (**1**) at 5-HT_2 and dopamine D_2 receptors.[7] Consequently, using the N_1-methyl counterpart of spiperone (i.e., **8**), the N_3-substituent was varied (Table 3). Variation of the N_3-substituent from −H to larger substituents such as methyl (i.e., **9**), ethyl (i.e., **10**), *n*-propyl (i.e., **11**), *n*-butyl (i.e., **13**), and even isopropyl (i.e., **12**) and benzyl (i.e., **14**) seems to have relatively little influence on 5-HT_{2A} affinity (K_i values range from 7 to 45 nM; Table 3). Most of these derivatives continue to lack affinity for 5-HT_{2C} receptors; however, the N_3-benzyl derivative **14** binds at 5-HT_{2C} receptors with an affinity comparable to that of spiperone (**1**).

Does the lactam carbonyl group contribute to binding? Compound **27** is an analogue of **8** where the lactam carbonyl oxygen atom has been eliminated. Compound **27** binds at 5-HT_{2A} receptors (**27**, 5-HT_{2A} K_i = 2400 ± 800 nM) with >1000-fold lower affinity than its parent **8**. Like **8**, **27** lacks affinity for 5-HT_{2C} receptors (**27**, 5-HT_{2C} K_i > 10 000 nM). Apparently, at least where the N_1-substituent is a methyl group, the lactam carbonyl oxygen atom contributes to 5-HT_{2A} binding.

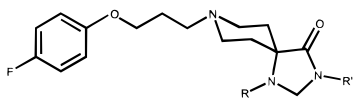
The next question to be addressed was whether an intact imidazolinone ring is necessary for 5-HT_{2A} binding. Compounds **31a**−**d** (Table 4) represent analogues of **6**, **4**, **5**, and **8**, respectively, where the imidazolinone

Table 4. Binding of Ring-Opened Analogues at Targeted Receptor Populations



		K_i, nM (SEM)			
compd	R	5-HT_{2A}	5-HT_{2C}	5-HT_{1A}	D_2
31a	−H	420 (±80)	>10000		
31b	−cyclohexyl	6 (±1)	270 (±100)	1610 (±58)	43 (±23)
31c	$-CH(CH_3)_2$	14 (±1)	426 (±18)	>10000	400 (±37)
31d	$-CH_3$	130 (±10)	4480 (±520)	>10000	

Table 5. Binding of Ether Analogues at Targeted Receptor Populations



			K_i, nM (SEM)			
compd	R	R′	5-HT_{2A}	5-HT_{2C}	5-HT_{1A}	D_2
32	$-CH_3$	−H	35 (±2)	>10000		734 (±93)
33	$-CH_3$	$-(CH_2)_2CH_3$	19 (±1)	>10000	>10000	230 (±70)

$-CH_2-$ has been excised. Results are mixed. Compound **31a** (5-HT_{2A} K_i = 420 nM; Table 4) binds at 5-HT_{2A} receptors with about 10-fold higher affinity than its parent **6**, whereas **31b** (5-HT_{2A} K_i = 6 nM) binds with 10-fold lower affinity than its parent **4**. The *i*Pr derivative **31c** binds with one-half the affinity of **5**, whereas the *N*-methyl derivative **31d** binds with about 6-fold reduced affinity at 5-HT_{2A} receptors (K_i = 130 nM) relative to its parent **8**. Interestingly, the ring-opened analogues as a group seem to display enhanced affinity for 5-HT_{2C} receptors and are, thus, less 5-HT_{2A}-selective than their parents. The spiro system, then, may be a contributor to 5-HT_{2A} selectivity.

Ether Analogues. We previously reported that the carbonyl group of spiperone (**1**) could be replaced with an ether oxygen atom with retention of 5-HT_{2A} affinity, reduction of 5-HT_{2C} affinity, and slight reduction in D_2 affinity.[8] Two such ether analogues were prepared and examined in the present study. Compounds **32** and **33** (Table 5) represent the ether analogues of **8** and **12**, respectively. Both **8** and **12** already bind at 5-HT_{2C} receptors with low affinity; thus, the effect of replacement of the carbonyl group by an ether oxygen atom was not readily apparent. Nevertheless, both **32** and **33** bind at 5-HT_{2A} receptors (K_i = 35 and 19 nM, respectively), bind at 5-HT_{2C} receptors with low affinity (K_i > 10 000 nM), and bind at D_2 receptors with somewhat lower affinity than their carbonyl counterparts. These ether analogues may represent novel templates for further exploitation.

Binding at Other Receptor Populations. The primary goal of the present study was to determine the influence of spiperone's 1,3,8-triazaspirodecanone substituents on 5-HT_{2A} receptor affinity and, to a lesser extent, on 5-HT_{2C} receptor affinity. However, because spiperone also binds at 5-HT_{1A} and dopamine D_2 receptors, it was of interest to examine the binding of the novel agents at these receptor populations as well. As shown in Table 2, the N_1-methyl group of **8** is not particularly well-accommodated by 5-HT_{2C} receptors; in fact, this is borne out by examining the compounds in Table 3. It would seem that the N_1-phenyl group of spiperone assists in 5-HT_{2C} binding, low as it might be. Replacement of the phenyl group with a cyclohexyl moiety is also tolerated; moreover, the cyclohexyl group seems to enhance affinity at both 5-HT_{2A} and 5-HT_{2C} receptors to a similar extent. Smaller alkyl substituents result in reduced 5-HT_{2C} receptor affinity and, consequently, in enhanced 5-HT_{2A} versus 5-HT_{2C} selectivity.

Spiperone (**1**) binds at 5-HT_{1A} receptors with high affinity (K_i = 58 nM; Table 2). Here, it can be concluded that the presence of the spiperone N_1-phenyl group contributes to binding in a positive fashion because its replacement with hydrogen or small alkyl groups results in compounds that lack significant 5-HT_{1A} affinity. Because the N_1-methyl derivative, compound **8**, lacks affinity for 5-HT_{1A} receptors, the role of N_3-substitution on binding is not readily apparent; nevertheless, the N_3-benzyl derivative **14** binds with about one-fourth the affinity of spiperone (**1**) suggesting that further exploration of this region might be worthwhile in attempting to understand the binding of spiperone analogues at 5-HT_{1A} receptors. That is, comparing **14** with **8**, there may exist on the receptor some auxiliary binding feature that compensates for the lack of the N_1-phenyl substituent.

Spiperone (**1**) was initially developed as a dopaminergic agent, and its affinity for dopamine D_2 receptors is higher than that which it displays for 5-HT receptors (e.g., Table 2). The N_1-phenyl group of spiperone would also seem to be a major contributor to dopamine D_2 binding. Although an N_1-cyclohexyl group as found in **4** is tolerated, replacement of the phenyl group with smaller alkyl groups results in a dramatic reduction in affinity. Consistent with what has been previously published,[7] certain bulky N_3-substituents are reasonably well-tolerated at D_2 receptors.

Functional Studies. Although it might be expected that the novel spiperone analogues would behave as 5-HT_{2A} antagonists in a manner similar to that of spiperone, we examined the functional activity of several selected compounds. 5-HT_{2A} antagonists, unlike 5-HT_{2A} agonists, bind with similar affinity regardless of whether the receptors are labeled with a labeled agonist or labeled antagonist radioligand. As a preliminary indicator of potential functional activity, compounds **9** and **11** were examined at [^{3}H]DOB-labeled 5-HT_{2A} receptors. As indicated in Table 3, the K_i values for **9** and **11** were independent of radioligand employed in the binding studies, suggesting that they might behave as antagonists. In tests of their ability to stimulate PI hydrolysis

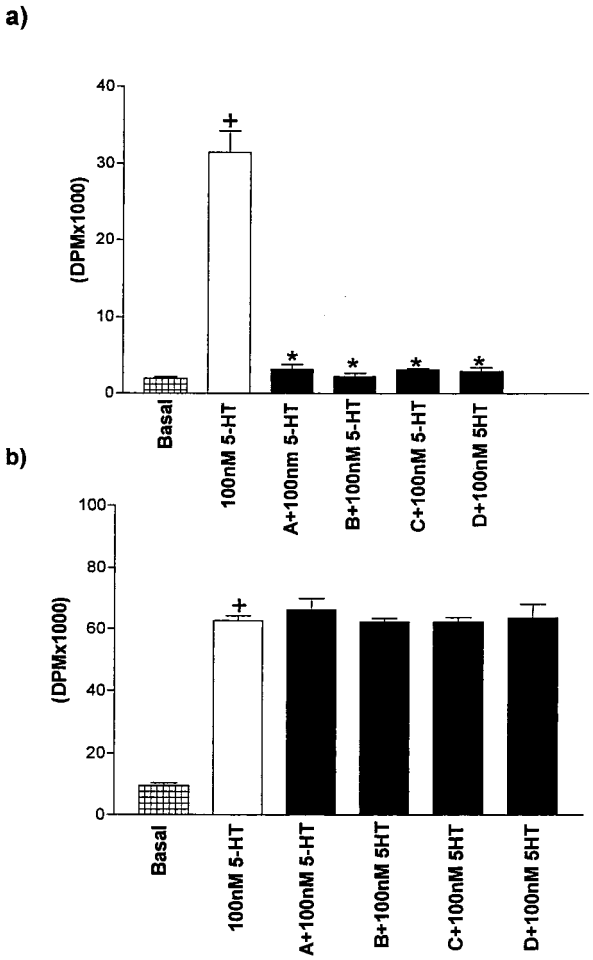


Figure 1. Inhibition of 100 nM 5-HT by **12** (A), **11** (B), **9** (C), and **31b** (D) at 5-HT_{2A} receptors (a) and 5-HT_{2C} receptors (b). 5-HT_{2A} and 5-HT_{2C} receptor stimulation by 100 nM 5-HT and inhibition by **12**, **11**, **9**, and **31b** were measured by [^{3}H]IP production using anion-exchange chromatography. Cells expressing 5-HT_{2A} or 5-HT_{2C} receptors were pretreated with 1 μM of each test compound for 10 min. Cells were then challenged with 100 nM 5-HT for 30 min. Data are expressed as mean ± SEM of three separate experiments performed in triplicate. Treatment of cells with 1 μM of each test compound was not significantly different than basal levels ($^+p < 0.001$ vs basal, $*p < 0.001$ vs 100 nM 5-HT).

in cells expressing 5-HT_{2A} receptors, compounds **9**, **11**, **12**, and **31b** were inactive as agonists at concentrations of up to 1000 nM (data not shown). In contrast, each of the compounds behaved as antagonists of 5-HT-mediated PI hydrolysis; results of representative experiments with each of the four compounds are shown in Figure 1a. Multiple experiments allowed determination of EC_{50} values; compound followed by EC_{50} (with ±SEM in parentheses): **9**, 63.2 (±1.2) nM; **11**, 23.1 (±0.4) nM; **12**, 64.0 (±18.0) nM; **31b**, 30.0 (±6.2) nM. In a similar set of experiments, all four compounds were inactive up to concentrations of 1000 nM as either agonists or antagonists of 5-HT-induced PI hydrolysis in cells expressing 5-HT_{2C} receptors; representative results for a single concentration of all four compounds are shown in Figure 1b. It is interesting to note that the ring-opened compound **31b** retains 5-HT_{2A} antagonist activity.

Summary

In summary, then, the N_1-substituent of spiperone analogues seems to be a major determinant of 5-HT_{2A} affinity and may also play a role in determining selectivity. Spiperone (**1**), with an N_1-phenyl group, displays very high affinity for 5-HT_{2A} receptors. Replacement of the N_1-phenyl group with a cyclohexyl moiety results in retention of affinity; however, replacement by a methyl group (i.e., **8**) results in a 10-fold decrease in affinity, but in a compound that lacks affinity for 5-HT_{2C} receptors. Consistent with earlier suggestions of a region of bulk tolerance,[7] N_3-substituents seem to have little impact on 5-HT_{2A} binding; for example, introduction of a variety of N_3-substituents altered the 5-HT_{2A} affinity of **8** by a maximum of about 3-fold. The lactam carbonyl oxygen atom, on the other hand, may be important for 5-HT_{2A} binding; in the single instance where this was examined (**8** → **27**), removal of the carbonyl oxygen atom reduced affinity by >1000-fold. An intact imidazolinone ring may not be required for 5-HT_{2A} binding, but it is difficult to draw specific conclusions due to the mixed results that were obtained; excision of the imidazolinone $-CH_2-$ either increased or decreased affinity by about 2−10-fold. The intact ring may determine the relative positioning of the imidazolinone substituents, and in the more conformationally flexible ring-opened analogues, the substituents may acquire a different orientation depending on the size(s) of the substituents. Replacement of the side-chain carbonyl group by an ether oxygen does not seem to significantly influence the binding profile.

The overall results of the present investigation are that replacement of the N_1-phenyl substituent of spiperone (**1**) with a methyl group results in a compound, **8**, that binds at 5-HT_{2A} receptors with about 10-fold lower affinity than spiperone itself, but in a compound with greatly reduced affinity for 5-HT_{2C}, 5-HT_{1A}, and dopamine D_2 receptors. The present compounds were not examined at 5-HT_{2B} receptors; however, 5-HT_{2B} receptors have yet to be detected in mammalian brain. Thus, **8** might serve as a useful antagonist, with reduced 5-HT_{1A} and D_2 receptor affinity, to further investigate pharmacological agents where suspected central 5-HT_{2A} versus 5-HT_{2C} mechanisms have been implicated. Additional pharmacological studies with **8** are currently in progress.

Experimental Section

Synthesis. Melting points, determined with a Thomas-Hoover melting point apparatus, are uncorrected. Proton magnetic resonance spectra were obtained with a GE QE-300 or Varian Gemini 300 spectrometer, and tetramethylsilane was used as an internal standard. Infrared spectra were recorded on a Nicolet 5ZDX FT-IR spectrometer. Elemental analysis was performed by Atlantic Microlab Inc., and determined values are within 0.4% of theory. Flash chromatography was performed on silica gel (Merck grade 60, 230−400 mesh, 60 Å).

8-[3-(4-Fluorobenzoyl)propyl]-1,3,8-triazaspiro[4.5]-decan-4-one Dihydrochloride (6). A mixture of 8-benzyl-1,3,8-triazaspiro[4.5]decan-4-one (**23**) (2.45 g, 10 mmol) and HCl (35%, 0.20 g) in MeOH (25 mL) was hydrogenated at 40 psi over Pd/C (10%, 0.40 g) at room temperature. After 8 h the catalyst was removed by filtration, and the solvent was evaporated under vacuum. The crude product was recrystallized from acetone to afford 1.80 g (79%) of **24** as a white

powder; mp 215−218 °C. IR (KBr): 3200 (NH), 1650 (C═O) cm^{-1}. ^1H NMR (D_2O) δ: 2.0−2.2 (m, 2H, CH_2), 2.2−2.4 (m, 2H, CH_2), 3.4−3.6 (m, 2H, CH_2), 3.6−3.8 (m, 2H, CH_2), 4.6−4.7 (s, 2H, CH_2).

A mixture of **24** (0.23 g, 1 mmol), 4-(4-fluorophenyl)-4-oxo-*n*-butyl chloride (0.20 g, 1 mmol), K_2CO_3 (0.28 g, 2 mmol), and a catalytic amount of KI in methyl isobutyl ketone (25 mL) was heated at 80 °C for 36 h. The reaction mixture was filtered, and the solvent was evaporated under reduced pressure. Purification by column chromatography (silica gel; $CHCl_3$ to $CHCl_3$/MeOH, 1:1) and recrystallization from acetone afforded the free base as a white solid. A solution of the free base in absolute EtOH was treated with HCl-saturated EtOH to give the hydrochloride salt. Recrystallization of the crude salt from absolute EtOH/anhydrous Et_2O gave 0.09 g (23%) of the title compound as a white solid; mp 227−229 °C. ^1H NMR (D_2O) δ: 2.3−2.5 (m, 2H, CH_2), 2.5−2.7 (m, 3H, CH_2, CH), 2.8−3.0 (m, 1H, CH), 3.4−3.7 (m, 5H, $2CH_2$, CH), 3.8−4.2 (m, 3H, CH_2, CH), 5.0 (s, 2H, CH_2), 7.5−7.7 (m, 2H, ArH), 8.3−8.5 (m, 2H, ArH). Anal. ($C_{17}H_{22}FN_3O_2$·2HCl·0.25H_2O) C, H, N.

1-Ethyl-8-[3-(4-fluorobenzoyl)propyl]-1,3,8-triazaspiro-[4.5]decan-4-one Dihydrochloride (7). A mixture of 8-benzyl-1-ethyl-1,3,8-triazaspiro[4.5]decan-4-one (**20**) (2.73 g, 10 mmol) and HCl (35%, 0.60 g) in MeOH (25 mL) was hydrogenated at 25 psi over Pd/C (10%, 0.50 g) at room temperature. After 8 h the catalyst was removed by filtration and the solvent was evaporated under reduced pressure. The crude product was recrystallized from acetone to afford 2.15 g (84%) of a white powder; mp 215−217 °C. IR (KBr): 3300 (NH), 1660 (C═O) cm^{-1}. ^1H NMR (D_2O) δ: 0.9−1.0 (t, 3H, CH_3), 1.8−1.9 (m, 4H, $2CH_2$), 2.5−2.6 (q, 2H, CH_2), 3.2−3.3 (m, 2H, CH_2), 3.4−3.5 (m, 2H, CH_2), 4.0−4.1 (s, 2H, CH_2).

A mixture of this material (2.56 g, 10 mmol), 4-(4-fluorophenyl)-4-oxo-*n*-butyl chloride (2.00 g, 10 mmol), K_2CO_3 (2.76 g, 20 mmol), and a catalytic amount of KI in methyl isobutyl ketone (50 mL) was heated at reflux for 48 h. The reaction mixture was filtered, and the filtrate was evaporated under reduced pressure. The residue was chromatographed on silica gel using CH_2Cl_2/MeOH (9:1) as an eluent. The free base was dissolved in absolute EtOH, and dry HCl gas was bubbled through the solution. Recrystallization from absolute EtOH/anhydrous Et_2O afforded 1.30 g (32%) of the hydrochloride salt as a light-beige powder; mp 231−233 °C. IR (KBr): 3300 (NH), 1690 (C═O of ketone), 1660 (C═O of amide) cm^{-1}. ^1H NMR (D_2O) δ: 1.1−1.2 (t, 3H, CH_3), 2.0−2.1 (m, 2H, CH_2), 2.2−2.3 (m, 4H, $2CH_2$), 3.0−3.2 (m, 6H, $3CH_2$), 3.5−3.6 (m, 2H, CH_2), 3.6−3.7 (m, 2H, CH_2), 4.6 (s, 2H, CH_2), 7.1−7.2 (m, 2H, ArH), 7.8−7.9 (m, 2H, ArH). Anal. ($C_{19}H_{26}FN_3O_2$·2HCl) C, H, N.

8-[3-(4-Fluorobenzoyl)propyl]-1-methyl-1,3,8-triazaspiro[4.5]decan-4-one Dihydrochloride (8). Compound **8** (KML-010) was prepared by the method of Jannsen.[9] In addition, compound **8** was prepared by the methylation of **6**, as follows. Iodomethane (0.043 g, 0.3 mmol) was added to a mixture of **6** (free base) (0.096 g, 0.3 mmol) and KOH (0.002 g, 0.03 mmol) in THF (10 mL). The reaction mixture was allowed to stir at room temperature for 30 min. The reaction mixture was diluted with H_2O (15 mL) and extracted with $CHCl_3$ (2 × 25 mL). The organic extract was washed with $NaHCO_3$ (2%, 25 mL), dried (Na_2SO_4), and evaporated under reduced pressure. The residue was subjected to column chromatography (silica gel; $CHCl_3$ to $CHCl_3$/MeOH, gradient). The free base was dissolved in 2-propanol, and HCl gas was bubbled into the solution. Recrystallization from 2-propanol afforded 0.08 g (68%) of the dihydrochloride salt as a white powder; mp 234−236 °C. Anal. ($C_{18}H_{24}FN_3O_2$·2HCl) C, H, N. Although the reported[9] mp for **8** is 203.6−212 °C, the mp and R_f (in three different solvent systems) of the present product were identical with that compound prepared following the procedure of Janssen.[9]

1-Methyl-3-*n*-propyl-8-[3-(4-fluorobenzoyl)propyl]-1,3,8-triazaspiro[4.5]decan-4-one Dihydrochloride (11). A mixture of 1-methyl-8-[3-(4-fluorophenyl)-3,3-(ethylenedioxy)-butyl]-1,3,8-triazaspiro[4.5]decan-4-one (**25**) (0.38 g, 1.0 mmol) and NaH (0.02 g, 1 mmol) in THF (15 mL) was stirred at ambient temperature for 15 min. 1-Bromopropane (0.15 g, 1.2 mmol) was added, and the mixture was heated at reflux for 15 h. The reaction mixture was evaporated under reduced pressure, and the residue was dissolved in absolute EtOH (10 mL). HCl (3 N, 2 mL) was added, and the mixture was heated at reflux for 30 min. The reaction mixture was concentrated under vacuum, treated with saturated aqueous $NaHCO_3$ (50 mL), and extracted with CH_2Cl_2 (2 × 50 mL). The combined organic extract was dried ($MgSO_4$) and evaporated under reduced pressure. The product was purified by column chromatography (silica gel, hexane to hexane/EtOAc, 1:1). The free base was dissolved in absolute EtOH and treated with an HCl-saturated solution of EtOH. The hydrochloride salt was recrystallized from absolute EtOH to afford 0.09 g (22%) of the title compound; mp 188−194 °C. ^1H NMR (D_2O) δ: 1.1−1.2 (t, 3H, CH_3), 1.8−1.9 (m, 2H, CH_2), 2.4−2.5 (m, 2H, CH_2), 2.5−2.6 (br s, 4H, $2CH_2$), 3.1 (s, 3H, NCH_3), 3.5−3.7 (complex m, 6H, $3CH_2$), 3.9−4.1 (m, 4H, $2CH_2$), 4.9 (s, 2H, CH_2), 7.5−7.6 (t, 2H, ArH), 8.4−8.5 (m, 2H, ArH). Anal. ($C_{21}H_{30}FN_3O_2$·2HCl) C, H, N.

Compounds **9**, **10**, **13**, and **14** (see Table 1) were prepared in a similar manner.

1-Methyl-3-(2-propyl)-8-[3-(4-fluorobenzoyl)propyl]-1,3,8-triazaspiro[4.5]decan-4-one Dihydrochloride (12). Solid NaH (0.24 g, 10 mmol) was added in portions to a stirred solution of 8-benzyl-1-methyl-1,3,8-triazaspiro[4.5]decan-4-one (**26**) (2.59 g, 10 mmol) in THF (25 mL) under an N_2 atmosphere, and the mixture was allowed to stir at room temperature for 5 min. 2-Bromopropane (1.23 g, 10 mmol) was added, and the mixture was heated at reflux for 12 h. The reaction mixture was concentrated under reduced pressure, quenched by the dropwise addition of H_2O (5 mL), and extracted with CH_2Cl_2 (2 × 50 mL). The organic extract was washed with H_2O (50 mL), dried (Na_2SO_4), and evaporated under reduced pressure. The residue was subjected to column chromatography (silica gel; CH_2Cl_2 to CH_2Cl_2/MeOH, 95:5) to give N_3-(2-propyl)-8-benzyl-1-methyl-1,3,8-triazaspiro[4.5]decan-4-one as a clear oil. IR ($CHCl_3$): 1650 (C═O) cm^{-1}.^1H NMR ($CDCl_3$) δ: 1.0−1.2 (d, 6H, $2CH_3$), 1.6−1.9 (m, 4H, $2CH_2$), 2.4 (s, 3H, NCH_3), 2.6−2.9 (m, 4H, $2CH_2$), 3.5−3.6 (s, 2H, CH_2), 4.2 (s, 2H, CH_2), 4.2−4.4 (m, 1H, CH), 7.2−7.4 (m, 5H, ArH).

A mixture of the above compound (0.30 g, 1 mmol) and 35% HCl solution (0.10 g) in MeOH (25 mL) was hydrogenated at 25 psi over Pd/C (10%, 0.1 g) at room temperature. After 10 h the catalyst was removed by filtration, and the solvent was evaporated under vacuum. The residue was treated with Na_2CO_3 solution (10%, 50 mL) and extracted with Et_2O (2 × 50 mL). The combined ethereal extract was dried and evaporated under reduced pressure. Purification by column chromatography (silica gel; CH_2Cl_2/MeOH, 8:2) afforded 1.60 g (76%) of the product as a clear oil. IR ($CHCl_3$): 3300 (NH), 1650 (C═O) cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.1−1.2 (d, 6H, $2CH_3$), 1.6−1.7 (m, 4H, $2CH_2$), 1.7−1.8 (br s, 1H, NH, D_2O exchangeable), 2.4 (s, 3H, NCH_3), 2.9−3.0 (m, 2H, CH_2), 3.3−3.4 (m, 2H, CH_2), 4.1 (s, 2H, CH_2), 4.3−4.4 (br m, 1H, CH).

A mixture of this oil (0.21 g, 1 mmol), K_2CO_3 (0.28 g, 2 mmol), 4-(4-fluorophenyl)-4-oxo-*n*-butyl chloride (0.20 g, 0.1 mmol), and a catalytic amount of KI in methyl isobutyl ketone (25 mL) was heated at reflux for 48 h. The reaction mixture was filtered, and the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography (silica gel; CH_2Cl_2 to CH_2Cl_2/MeOH, 9:1) to give the free base as an oil. The free base was dissolved in absolute EtOH, and HCl gas was bubbled through the solution. Recrystallization of the crude salt from absolute EtOH/anhydrous Et_2O afforded 0.07 g (17%) of the title compound as a white powder; mp 207−210 °C. IR (KBr): 1690 (C═O of ketone), 1650 (C═O of amide) cm^{-1}. ^1H NMR (D_2O) δ: 1.4−1.6 (d, 6H, $2CH_3$), 2.3−2.6 (m, 6H, $3CH_2$), 3.0 (s, 3H, NCH_3), 3.4−3.6 (m, 4H, $2CH_2$), 3.8−4.1 (m, 4H, $2CH_2$), 4.4−4.6 (br m, 1H, CH), 4.8 (s, 2H, CH_2), 7.5−7.6 (m, 2H, ArH), 8.3−8.4 (m, 2H, ArH). Anal. ($C_{21}H_{30}FN_3O_2$·2HCl·0.25 H_2O) C, H, N.

8-Benzyl-1-ethyl-1,3,8-triazaspiro[4.5]decan-4-one (20). Compound **20** was prepared by the method of Janssen[9] from

1-benzyl-4-(ethylamino)-4-piperidinecarboxamide. The crude product, however, was purified by column chromatography (silica gel; CH_2Cl_2 to CH_2Cl_2/MeOH, 9:1) and then recrystallized from EtOAc to give a 56% yield of the title compound as white crystals; mp 146−148 °C (lit.[9] mp 139−145.4 °C). IR (KBr): 3200 (NH), 1680 (C═O) cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.0−1.1 (t, 3H, CH_3), 1.6−1.7 (m, 2H, CH_2), 1.8−1.9 (m, 2H, CH_2), 2.6−2.7(q, 2H, CH_2), 2.7−2.8 (m, 4H, $2CH_2$), 3.6 (s, 2H, CH_2), 4.2 (s, 2H, CH_2), 6.5 (s, 1H, CONH, D_2O exchangeable), 7.3−7.4 (m, 5H, ArH). Anal. ($C_{16}H_{23}N_3O$) C, H, N.

8-Benzyl-1,3,8-triazaspiro[4.5]decane-4-one (23). A solution of 1-benzyl-4-piperidone (**15**) (1.89 g, 10 mmol) in absolute EtOH (5 mL) was added in a portionwise manner to a solution of KCN (0.73 g, 11 mmol) and NH_4Cl (0.61 g, 11 mmol) in H_2O (12 mL), and the mixture was allowed to stir at room temperature for 48 h. The reaction mixture was diluted with H_2O (25 mL) and extracted with CH_2Cl_2 (2 × 50 mL). The combined organic extract was washed with H_2O (2 × 50 mL), dried (Na_2SO_4), and evaporated under reduced pressure. The oily residue was subjected to column chromatography (silica gel; CH_2Cl_2 then CH_2Cl_2/MeOH, 9:1) to give a clear oil which solidified upon trituration at 0 °C with petroleum ether. Recrystallization from anhydrous Et_2O/petroleum ether afforded 2.00 g (92%) of **21** as a white solid; mp 70−72 °C. IR (KBr): 3340 (NH_2), 2220 (CN) cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.7−1.9 (m, 4H, CH_2, NH_2, D_2O exchangeable), 1.9−2.1 (m, 2H, CH_2), 2.3−2.5 (m, 2H, CH_2), 2.7−2.9 (m, 2H, CH_2), 3.6 (s, 2H, CH_2), 7.2−7.5 (m, 5H, ArH).

Compound **21** (2.15 g, 10 mmol) was added in portions to H_2SO_4 (95%, 40 mL), and the mixture was allowed to stir at room temperature for 5 h. The reaction mixture was poured into ice water (100 mL), basified with NH_4OH to pH 10−11, and extracted with CH_2Cl_2 (2 × 50 mL). The organic extract was washed with $NaHCO_3$ (5%, 100 mL), dried (Na_2SO_4), and evaporated under reduced pressure. The crude product was purified by column chromatography (silica gel; CH_2Cl_2/MeOH, 9:1). Recrystallization from absolute EtOH/anhydrous Et_2O afforded 1.70 g (75%) of **22** as a white powder; mp 158−160 °C. IR (KBr): 3400−3200 (NHs), 1660 (C═O) cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.3−1.6 (m, 4H, CH_2, NH_2, D_2O exchangeable), 2.1−2.4 (m, 4H, $2CH_2$), 2.7−2.9 (m, 2H, CH_2), 3.6 (s, 2H, CH_2), 5.6 (s, 1H, CONH, D_2O exchangeable), 7.2−7.4 (m, 5H, ArH), 7.5−7.6 (s, 1H, CONH, D_2O exchangeable).

Concentrated H_2SO_4 (97%, 2.6 g) was added in a dropwise manner to a solution of **22** (2.3 g, 10 mmol) in formamide (25 g), and the mixture was heated at reflux for 20 h. The reaction mixture was poured into ice water (100 mL), basified with NH_4OH, and extracted with CH_2Cl_2 (2 × 50 mL). The combined organic extract was washed with $NaHCO_3$ (5%, 100 mL), dried (Na_2SO_4), and evaporated under reduced pressure. The foamy residue was dissolved in MeOH (25 mL), $NaBH_4$ (0.6 g, 15 mmol) was added, and the mixture was heated at 60 °C for 1 h. The reaction mixture was diluted with H_2O (50 mL) and extracted with CH_2Cl_2 (2 × 50 mL). The organic extract was washed with H_2O (50 mL), dried (Na_2SO_4), and evaporated under reduced pressure. The crude product was purified by column chromatography (silica gel; CH_2Cl_2/MeOH, 9:1) and recrystallized from EtOAc to afford 1.3 g (54%) of the title compound; mp 142−144 °C. IR (KBr): 3400−3200 (NHs), 1670 (C═O) cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.4−1.7 (m, 4H, $2CH_2$), 2.7−3.0 (m, 4H, $2CH_2$), 3.7 (s, 2H, CH_2), 4.9 (s, 2H, CH_2), 7.1 (s, 1H, NH, D_2O exchangeable), 7.2−7.4 (m, 5H, ArH), 8.5−8.6 (s, 1H, CONH). Anal. ($C_{14}H_{19}N_3O$) C, H, N.

1-Methyl-8-[3-(4-fluorophenyl)-3,3-(ethylenedioxy)-butyl]-1,3,8-triazaspiro[4.5]decan-4-one (25). A mixture of 1-methyl-1,3,8-triazaspiro[4.5]decan-4-one dihydrochloride[9] (**19**) (0.24 g, 1 mmol), 4-chloro-1-(4-fluorophenyl)-1,1-(ethylenedioxy)butane (0.25 g, 1 mmol), K_2CO_3 (0.28 g, 2 mmol), and a catalytic amount of KI in methyl isobutyl ketone (25 mL) was heated at reflux for 48 h. The reaction mixture was filtered, and the filtrate was evaporated under reduced pressure. The residue was suspended in H_2O (50 mL) and extracted with CH_2Cl_2 (2 × 50 mL). The organic extract was dried ($MgSO_4$) and evaporated under reduced pressure. The oily residue was subjected to column chromatography (silica gel; CH_2Cl_2 then CH_2Cl_2/EtOAc, 9:1). The crude product was recrystallized from hexane/MeCN to give 0.13 g (35%) of the title compound; mp 124−126 °C. IR (KBr): 3400 (NH), 1680 (C═O) cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.5−2.0 (complex m, 8H, $4CH_2$), 2.3−2.5 (m, 5H, CH_2, NCH_3), 2.6−2.8 (m, 4H, $2CH_2$), 3.7−3.8 (t, 2H, CH_2), 3.9−4.0 (t, 2H, CH_2), 4.2 (s, 2H, CH_2), 6.2−6.3 (s, 1H, NH, D_2O exchangeable), 6.9−7.1 (m, 2H, ArH), 7.4−7.6 (m, 2H, ArH). Anal. ($C_{20}H_{28}FN_3O_3$) C, H, N.

8-Benzyl-1-methyl-1,3,8-triazaspiro[4.5]decan-4-one (26). Solid $NaBH_4$ (0.45 g, 12 mmol) was added in portions to a stirred solution of 8-benzyl-1-methyl-1,3,8-triazaspiro[4.5]dec-2-en-4-one[9] (**18**) (2.57 g, 10 mmol) in 95% EtOH (25 mL), and the mixture was heated at reflux for 1 h. The reaction mixture was evaporated under reduced pressure; the residue was suspended in H_2O (50 mL) and extracted with $CHCl_3$ (2 × 50 mL). The combined organic extract was washed with H_2O (50 mL), dried (Na_2SO_4), and evaporated under reduced pressure. The crude product was recrystallized from acetone to afford 2.00 g (78%) of the title compound as an off-white powder; mp 118−120 °C. IR (KBr): 3300 (NH), 1650 (C═O) cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.6−2.0 (m, 4H, $2CH_2$), 2.4 (s, 3H, CH_3), 2.6−3.0 (m, 4H, $2CH_2$), 3.6 (s, 2H, CH_2), 4.2 (s, 2H, CH_2), 6.4−6.6 (br s, 1H, CONH, D_2O exchangeable), 7.2−7.5 (m, 5H, ArH). Anal. ($C_{15}H_{21}N_3O$) C, H, N.

1-Methyl-8-[3-(4-fluorobenzoyl)propyl]-1,3,8-triazaspiro-[4.5]decane Trihydrochloride (27). A solution of 8-benzyl-1-methyl-1,3,8-triazaspiro[4,5]dec-2-en-4-one[9] (**18**) (1.29 g, 5 mmol) in THF (10 mL) was added, in a dropwise manner, to a stirred suspension of $LiAlH_4$ (1.50 g, 40 mmol) in anhydrous Et_2O (25 mL) at 0 °C under a nitrogen atmosphere. After addition was complete, the stirred mixture was heated at reflux for 10 h. Excess hydride was decomposed by the addition of H_2O (2 mL) followed by 2 N NaOH solution (2 mL). The inorganic precipitate was removed by filtration; the filtrate was dried (Na_2SO_4) and evaporated under reduced pressure. The oily residue was subjected to column chromatography (silica gel; CH_2Cl_2/MeOH, 9:1) to give a homogeneous product. IR ($CHCl_3$): 3300 (NH) cm^{-1}.

A solution of di-*tert*-butyl dicarbonate (0.7 g, 3.1 mmol) in CH_2Cl_2 (10 mL) was added in portions to a stirred solution of the amine (0.7 g, 3 mmol) in CH_2Cl_2 (15 mL), and the reaction mixture was allowed to stir at room temperature for 12 h. The solvent was removed under reduced pressure, and the residue was subjected to column chromatography (silica gel; petroleum ether to petroleum ether/EtOAc, 95:5) to afford 0.85 g (62%) of the BOC-protected amine as a colorless oil. IR ($CHCl_3$): 1693 (C═O) cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.4 (s, 9H, $3CH_3$), 1.5−1.7 (m, 4H, $2CH_2$), 2.2−2.4 (br s, 5H, NCH_3, CH_2), 2.5−2.6 (d, 2H, CH_2), 3.0 (s, 2H, CH_2), 3.5 (s, 2H, CH_2), 7.2−7.4 (m, 5H, ArH). The compound was used without further characterization.

A solution of the above 8-benzyl-1-methyl-3-(*tert*-butoxycarbonyl)-1,3,8-triazaspiro[4.5]decane (0.69 g, 2 mmol) in MeOH (20 mL) was hydrogenated at 40 psi over Pd/C (10%, 0.14 g) at room temperature. After 60 h, the catalyst was removed by filtration, and the solvent was evaporated under vacuum. The crude product was purified by column chromatography (silica gel; CH_2Cl_2/MeOH, gradient) to afford 0.40 g (78%) of the debenzylated amine as a colorless oil. IR ($CHCl_3$): 3380 (NH), 1690 (C═O) cm^{-1}. ^1H NMR (CD_3COCD_3) δ: 1.6 (s, 9H, $3CH_3$), 1.7−1.9 (m, 4H, $2CH_2$), 2.2 (s, 3H, NCH_3), 2.5 (s, 2H, CH_2), 2.6−2.9 (m, 4H, $2CH_2$), 3.3 (s, 2H, CH_2). A mixture of this product (0.26 g, 1 mmol), 4-(4-fluorophenyl)-4-oxo-*n*-butyl chloride (0.20 g, 1 mmol), K_2CO_3 (0.28 g, 2 mmol), and a few crystals of KI in methyl isobutyl ketone (25 mL) was heated at reflux for 24 h. The reaction mixture was filtered, and the solvent was evaporated under reduced pressure. The residue was subjected to column chromatography (silica gel; hexane/EtOAc, 9:1) to give 0.25 g (60%) of the BOC-protected amine as an oil. A solution of the protected amine in dry dioxane (5 mL) was added to 20 mL of 4 N HCl in dry dioxane, and the mixture was allowed to stir at room temperature for 1.5 h. Dioxane was removed under vacuum; the residue was dis-

solved in 10% NaOH (25 mL) and extracted with Et_2O (4 × 25 mL). The combined ethereal portion was dried (Na_2SO_4) and evaporated to dryness under reduced pressure. A solution of the free base in anhydrous Et_2O was treated with an ethereal solution of HCl. The crude salt was recrystallized from MeOH/anhydrous Et_2O to afford 0.09 g (36%) of the hydrochloride salt as a white powder; mp 241−243 °C. IR (KBr): 3418 (NH), 1685 (C═O) cm^{-1}. ^1H NMR (DMSO-d_6) δ: 2.2−2.4 (m, 2H, CH_2), 2.7−3.0 (m, 7H, $2CH_2$, NCH_3), 3.3−3.5 (m, 6H, $3CH_2$), 3.7−3.9 (m, 4H, $2CH_2$), 4.0 (s, 2H, CH_2), 7.5−7.7 (m, 2H, ArH), 8.2−8.4 (m, 2H, ArH). Anal. ($C_{18}H_{26}FN_3O$·3HCl·0.5H_2O) C, H, N.

1-[4-(4-Fluorophenyl)-4,4-(ethylenedioxy)butyl]-4-piperidone (28). A mixture of 4-piperidone hydrochloride hydrate (5.0 g, 32.5 mmol), 4-chloro-1-(4-fluorophenyl)-1,1-(ethylenedioxy)butane (8.1 g, 33.0 mmol), Na_2CO_3 (6.2 g, 73.8 mmol), and NaI (2.9 g) in DMF (125 mL) was allowed to stir at 80 °C for 24 h. The reaction mixture was filtered, and the solvent was evaporated under reduced pressure. The residue was dissolved in CH_2Cl_2 (50 mL), washed with water (50 mL), and dried ($MgSO_4$). Evaporation of the solvent under reduced pressure afforded an oil which was purified by column chromatography (silica gel; petroleum ether to petroleum ether/EtOAc 9:1) to give 4.5 g (45%) of the title compound as white crystals; mp 57−60 °C. IR (KBr): 1718 (C═O) cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.5−1.7 (m, 2H, CH_2), 1.9−2.1 (t, 2H, CH_2), 2.4−2.6 (m, 6H, $3CH_2$), 2.7−2.8 (t, 4H, $2CH_2$), 3.9−4.1 (t, 2H, CH_2), 7.0−7.1 (m, 2H, ArH), 7.4−7.5 (m, 2H, ArH). The compound was used without further characterization in the synthesis of **31a**−**d**.

1-[3-(4-Fluorobenzoyl)propyl]-4-amino-4-piperidinecarboxamide Oxalate (31a). A solution of 1-[4-(4-fluorophenyl)-4,4-(ethylenedioxy)butyl]-4-piperidone (**28**) (3.07 g, 10 mmol) in absolute EtOH (10 mL) was added portionwise to a stirred solution of KCN (6.54 g, 10 mmol), NH_4Cl (5.40 g, 10 mmol), and NH_4OH (25%, 4 mL) in H_2O (20 mL). After addition was complete, the reaction mixture was allowed to stir at room temperature for 60 h. The reaction mixture was extracted with $CHCl_3$ (2 × 50 mL), washed with H_2O (50 mL), and dried (Na_2SO_4), and the solvent was evaporated under reduced pressure. The residue was subjected to column chromatography (silica gel; $CHCl_3$/MeOH, 95:5) to afford 2.50 g (76%) of the aminonitrile as an oil. IR ($CHCl_3$): 3300 (NH_2), 2250 (CN) cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.4−1.6 (m, 2H, CH_2), 1.6−1.8 (m, 4H, CH_2, NH_2, D_2O exchangeable), 1.8−1.9 (m, 2H, CH_2), 2.0−2.1 (m, 2H, CH_2), 2.2−2.4 (m, 4H, $2CH_2$), 2.6−2.8 (m, 2H, CH_2), 3.7−3.8 (t, 2H, OCH_2), 4.0−4.1 (t, 2H, OCH_2), 7.0−7.1 (t, 2H, ArH), 7.4−7.5 (m, 2H, ArH).

This material (3.33 g, 10 mmol) was added in a portionwise manner to H_2SO_4 (95%, 36 mL), and the mixture was allowed to stir at 70 °C for 10 min and then at room temperature for an additional 60 min. Ice water (50 mL) was added, and the pH was adjusted to 10−11 with NH_4OH. Extraction with $CHCl_3$ (2 × 50 mL), washing with H_2O (50 mL), drying (Na_2SO_4), and evaporation of the solvent under reduced pressure afforded an oil. Purification by column chromatography (silica gel; $CHCl_3$/MeOH, 9:1) and recrystallization from acetone gave the free base as a white solid; mp 131−133 °C. IR (KBr): 3400, 3200 (NH), 1680 (C═O of ketone), 1655 (C═O of amide) cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.3−1.6 (m, 4H, CH_2, NH_2, D_2O exchangeable), 1.8−2.0 (m, 2H, CH_2), 2.1−2.3 (m, 4H, $2CH_2$), 2.4−2.5 (m, 2H, CH_2), 2.7−2.9 (m, 2H, CH_2), 2.9−3.1 (m, 2H, CH_2), 5.4 (s, 1H, CONH, D_2O exchangeable), 7.1−7.2 (m, 2H, ArH), 7.4−7.6 (s, 1H, CONH, D_2O exchangeable), 8.0−8.1 (m, 2H, ArH).

A solution of the free base in absolute EtOH was treated with a solution of oxalic acid in absolute EtOH, and the white precipitate was recrystallized from absolute EtOH to give 3.40 g (78%) of the title compound as a white powder; mp 181−185 °C. Anal. ($C_{16}H_{22}FN_3O_2$·1.5$C_2H_2O_4$) C, H, N.

1-[3-(4-Fluorobenzoyl)propyl]-4-(cyclohexylamino)-4-piperidinecarboxamide Dihydrochloride (31b). The compound was prepared in 25% overall yield from **28** and cyclohexylamine as described for **31a**. The crude product was recrystallized from EtOAc/anhydrous Et_2O to afford a white solid; mp 103−105 °C. A solution of the free base in absolute EtOH was treated with HCl-saturated EtOH. The white precipitate was recrystallized from absolute EtOH/anhydrous Et_2O to afford 2.5 g (54%, final reaction) of the hydrochloride salt as an off-white powder; mp 272−275 °C. ^1H NMR (D_2O) δ: 0.9−1.0 (m, 2H, CH_2), 1.1−1.2 (m, 2H, CH_2), 1.2−1.3 (m, 2H, CH_2), 1.4−1.5 (m, 1H, CH), 1.6−1.7 (m, 2H, CH_2), 1.8−1.9 (m, 2H, CH_2), 1.9−2.0 (m, 2H, CH_2), 2.1−2.2 (br s, 2H, CH_2), 2.6−2.7 (br s, 2H, CH_2), 3.0−3.1 (br s, 2H, CH_2), 3.1−3.2 (m, 4H, $2CH_2$), 3.6−3.7 (br s, 2H, CH_2), 7.0−7.1 (m, 2H, ArH), 7.9−8.0 (m, 2H, ArH). Anal. ($C_{22}H_{32}FN_3O_2$·2HCl) C, H, N.

1-[3-(4-Fluorobenzoyl)propyl]-4-(2-propylamino)-4-piperidinecarboxamide Dihydrochloride (31c). The compound was prepared in 28% overall yield from **28** and 2-propylamine as described for **31a**. The crude free base was recrystallized from anhydrous Et_2O to give a white solid; mp 96−99 °C. A solution of the free base in absolute EtOH was treated with HCl-saturated EtOH. The white precipitate was recrystallized from absolute EtOH/anhydrous Et_2O to afford 1.85 g (42%) of the hydrochloride salt as a white powder; mp 263−265 °C. IR (KBr): 3300 (NHs), 1690 (C═O of ketone), 1665 (C═O of amide) cm^{-1}. ^1H NMR (CD_3OD) δ: 1.2−1.4 (d, 6H, $2CH_3$), 1.9−2.1 (m, 2H, CH_2), 2.2−2.4 (m, 2H, CH_2), 2.6−2.7(m, 2H, CH_2), 2.9−3.2 (m, 6H, $3CH_2$), 3.3−3.5 (m, 1H, CH), 3.6−3.7 (m, 2H, CH_2), 7.0−7.1 (t, 2H, ArH), 7.8−7.9 (m, 2H, ArH). Anal. ($C_{19}H_{28}FN_3O_2$·2HCl·H_2O) C, H, N.

1-[3-(4-Fluorobenzoyl)propyl]-4-(methylamino)-4-piperidinecarboxamide Hydrogen Oxalate (31d). The compound was prepared in 55% overall yield from **28** and methylamine as described for **31a**. The crude free base was recrystallized from acetone/anhydrous Et_2O to give a white solid; mp 88−89 °C. IR (KBr): 3400−3200 (NHs), 1680 (C═O of ketone), 1660 (C═O of amide) cm^{-1}. Anal. ($C_{17}H_{24}FN_3O_2$) C, H, N. The free base was dissolved in absolute EtOH and treated with a solution of oxalic acid in absolute EtOH. The white precipitate was recrystallized from absolute EtOH/anhydrous Et_2O to afford 3.4 g (67%) of the hydrogen oxalate salt as a white powder; mp 179−182 °C. ^1H NMR (D_2O) δ: 2.2−2.4 (m, 4H, $2CH_2$), 2.4−2.6 (m, 2H, CH_2), 3.0 (s, 3H, CH_3), 3.2−3.6 (m, 6H, $3CH_2$), 3.9−4.1 (m, 2H, CH_2), 7.5−7.6 (m, 2H, ArH), 8.2−8.4 (m, 2H, ArH). Anal. ($C_{17}H_{24}FN_3O_2$·$2C_2H_2O_4$·0.5 H_2O) C, H, N.

1-Methyl-8-[3-(4-fluorophenoxy)propyl]-1,3,8-triazaspiro[4.5]decan-4-one Dihydrochloride (32). A mixture of 1-methyl-1,2,8-triazaspiro[4.5]decan-4-one hydrochloride[9] (0.21 g, 1.0 mmol), 3-(4-fluorophenoxy)propyl chloride (0.19 g, 1 mmol), K_2CO_3 (0.28 g, 2 mmol), and a catalytic amount of KI in methyl isobutyl ketone (25 mL) was heated at reflux for 48 h. Once at room temperature, the reaction mixture was filtered, and the filtrate was evaporated to dryness under reduced pressure. The solid material was recrystallized from acetone and dissolved in absolute EtOH, and dry HCl gas was bubbled through the solution. The crude product was collected by filtration and recrystallized from acetone to afford 0.17 g (42%) of **32** as a white powder; mp 235−238 °C. IR (KBr): 3300 (NH), 1675 (C═O) cm^{-1}. ^1H NMR (D_2O) δ: 2.1−2.5 (m, 6H, $3CH_2$), 2.9 (s, 3H, CH_3), 3.3−3.5 (m, 2H, CH_2), 3.6−3.9 (m, 4H, CH_2), 4.1−4.2 (m, 2H, CH_2), 4.7 (s, 2H, CH_2), 6.9−7.0 (m, 2H, ArH), 7.1−7.2 (m, 2H, ArH). Anal. ($C_{17}H_{24}FN_3O_2$·2HCl·0.25H_2O) C, H, N.

1-Methyl-3-*n*-propyl-8-[3-(4-fluorophenoxy)propyl]-1,3,8-triazaspiro[4.5]decan-4-one Dihydrochloride (33). Solid NaH (0.02 g, 1 mmol) was added in a portionwise manner under N_2 to a solution of 1-methyl-8-[3-(4-fluorophenoxy)-propyl]-1,3,8-triazaspiro[4.5]decan-4-one (**32**) (0.32 g, 1 mmol) in THF (25 mL), and the mixture was allowed to stir at room temperature for 15 min. 1-Bromopropane (0.15 g, 1.2 mmol) was added, and the mixture was heated at reflux for 15 h. The reaction mixture was quenched by the dropwise addition of H_2O (5 mL) and then 10% NaOH (5 mL) and extracted with CH_2Cl_2 (2 × 50 mL). The combined organic extract was washed with H_2O (100 mL), dried (Na_2SO_4), and evaporated

under reduced pressure. The residue was subjected to column chromatography (silica gel; CH_2Cl_2/MeOH, 9:1). The free base was dissolved in absolute EtOH, and HCl gas was bubbled into the solution. Recrystallization from acetone afforded 0.15 g (35%) of **33** as a white powder; mp 222−225 °C. IR (KBr): 3350 (NH), 1670 (C═O) cm^{-1}. ^1H NMR (D_2O) δ: 0.8−1.0 (t, 3H, CH_3), 1.5−1.7 (m, 2H, CH_2), 2.2−2.4 (m, 6H, $3CH_2$), 2.7 (s, 3H, CH_3), 3.3−3.5 (m, 4H, $2CH_2$), 3.6−3.8 (m, 4H, $2CH_2$), 4.1−4.2 (m, 2H, CH_2), 4.6 (s, 2H, CH_2), 6.9−7.0 (m, 2H, ArH), 7.1−7.2 (m, 2H, ArH). Anal. ($C_{20}H_{30}FN_3O_2$·2HCl) C, H, N.

Radioligand Binding Assays. 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 12000*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 of protein measured by bicinchoninic assay) were added to each tube containing 1 mL of assay buffer with either 0.5 nM [^{3}H]-ketanserin (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. K_i values, determined as described below, represent a minimum of three determinations. For additional details, see Egan et al.[11]

PI Hydrolysis. Inositol phosphate (IP) production has been described in detail by Herrick-Davis et al.[12] In brief, 24 h after cells expressing 5-HT_{2A} or 5-HT_{2C} receptors were plated at 1.5 × 10^5 cells/well, cells were washed with phosphate-buffered saline (PBS) and labeled with 0.25 μCi/well of [^{3}H]myoinositol (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) and centrifuged for 10 min at 10000*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.

Data Analysis. IC_{50} and EC_{50} values were generated using GraphPad Prism2. K_i values were determined from the Cheng−Prusoff equation: $^{13}K_i = IC_{50}/1 + [D]/K_D$.

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