Ketanserin Analogues: The Effect of Structural Modification on 5-HT2 Serotonin Receptor Binding

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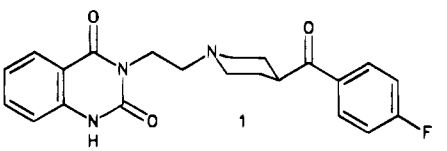
Ketanserin (**1**) is a fairly selective 5-HT2 antagonist that binds both at 5-HT2A and 5-HT2C receptors. A previous structure–affinity relationship study revealed that the structure of the piperidine-containing ketanserin molecule could be rather severely abbreviated with little effect on 5-HT2A affinity. The present investigation explores several inconsistencies identified in the earlier study and suggests that multiple modes of binding may be possible for ketanserin analogues. Perhaps the nature of the benzylic substituent is the most significant determinant of the manner in which these agents bind at 5-HT2A receptors, and it is possible that certain orientations may avail themselves of an auxiliary binding site. Depending upon the length of the piperidine *N*-alkyl chain, variation of the benzylic substituent from a carbonyl, to an alcohol, to a methylene group has a nonparallel influence on binding, and this may be further affected by the presence of a second ring nitrogen atom. The results of the present investigation provide evidence that although the structure of ketanserin can be abbreviated, and even modified by conversion of the piperidine ring to a piperazine, the resultant analogues may bind in more than one orientation at the receptors. A key structural feature that may play a prominent role in anchoring or orienting these compounds at 5-HT2A receptors is the benzylic carbonyl group.

One of the single most important discoveries in the medicinal chemistry of serotonergic agents is that ketanserin (**1**) can differentiate between multiple populations of serotonin (5-HT) receptors. Initially, **1** was used as a selective antagonist to differentiate 5-HT2-mediated effects from 5-HT1-mediated effects.[1] With the identification of more than 15 populations of 5-HT receptors,[1,2] ketanserin remains as one of the more selective agents. Although it binds with little selectivity for 5-HT2A versus 5-HT2C receptors,[3] it displays little affinity for the remaining (including 5-HT2B) populations of 5-HT receptors.[4] Because 5-HT2A receptors may be involved in various cardiovascular and mental disorders,[5] development of high-affinity 5-HT2-selective agents remains a high priority. With this in mind, we are examining possible modes of binding of serotonergic ligands at graphical models of 5-HT receptors. Binding simulations suggest that 5-HT2 agonists interact at 5-HT2A receptors in such a manner that they utilize particular aspartate (Asp-155) and phenylalanine (Phe-340) residues for binding.[6] Site-directed mutagenesis has now demonstrated the importance of Asp-155 for 5-HT2A ligand binding.[7] Interestingly, the single-point mutation Phe-340→Leu-340 greatly diminishes the affinity of 5-HT2A agonists and partial agonists but has no effect on the affinity of ketanserin.[8] These results suggest that although agonists and antagonists share a common amine binding site (i.e., Asp-155), they may be oriented somewhat differently at the receptor.

Using (+)-lysergic acid diethylamide as a template, we proposed a receptor model that seems to account for the binding of 5-HT2A agonists and certain structurally related antagonists.[6] This model, however, does not accurately describe the binding of ketanserin and ketanserin-related antagonists. This problem, although perhaps due in part to a lack of information on the binding requirements of ketanserin and related agents, may reflect different modes of binding mentioned above. Consequently, we undertook a structure–affinity study to determime exactly what structural features of ketanserin are important for binding at 5-HT2A receptors. In the course of these studies, we were struck by two, potentially related, inconsistencies that were difficult to explain.[3] (i) The structure of ketanserin (**1**; 5-HT2A K_i = 3.5 nM) can be simplified to **2** (K_i = 6.5 nM) or **3** (K_i = 5.3 nM), and the alkyl chain of **3** can shortened from four to two methylene groups (i.e., **4**; K_i = 8.5 nM), with little effect on 5-HT2A affinity; however, although reduction of the benzylic carbonyl group of ketanserin analogues to their corresponding alcohol results in a dramatic reduction in affinity, the alcohol **5** (i.e., MDL-11,939; K_i = 12.2 nM) reportedly retains high affinity for 5-HT2A receptors. (ii) Simple piperazine derivatives, such as **6** (K_i > 10 000 nM), lack affinity for 5-HT2A receptors,[3] yet **7** seems to bind with high affinity (*trans* racemate IC_{50} = 7.7 nM).[9] In light of the proposal that this (i.e., **7**) and structurally related N-substituted piperazines interact at 5-HT2A receptors in a manner that mimics the binding of the 4-benzoylpiperidine portion of ketanserin analogues,[9] the low affinity of **6** is, at least initially, difficult to rationalize. The nonfused phenyl ring of **7**-related derivatives is important for binding, and because its replacement by a hydrogen atom results in a dramatic decrease in affinity, it has been suggested that it binds at an auxiliary aromatic



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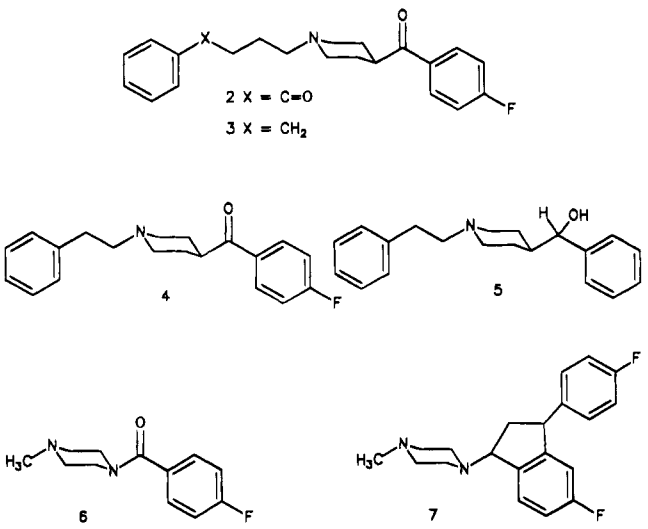
binding site (that is not necessary for the binding of ketanserin-type compounds).[9]



The purpose of the present study was 2-fold. First, we wished to compare derivatives of the 4-carbon chain analogue **3** with their 2-carbon chain counterparts, **4**, in order to conclusively establish the role of the benzylic substituent on binding and to determine the influence of the fluorine substituent on 5-HT2A affinity. Second, we wanted to examine the role of incorporating a second nitrogen atom into the piperidine ring (thereby converting it to a piperazine). In both cases, the possible existence of an auxiliary aromatic binding site would be probed by introducing benzylic substituents in place of the benzoyl carbonyl group. That is, piperazine derivatives may inherently bind at 5-HT2A receptors with lower affinity than their corresponding piperidines, but the presence of a second phenyl group (because it may take advantage of the auxiliary binding site) may account for the unexpectedly high affinity of **7**-type compounds. If this is the case, incorporation of a similar phenyl substituent into piperidine-containing ketanserin analogues may correspondingly increase their affinity. Due to the lack of selectivity of ketanserin (**1**) for 5-HT2A versus 5-HT2C receptors, 5-HT2C binding data were also obtained for all of the novel compounds in order to obtain an indication of selectivity.

Chemistry

Compounds **2-4**, **6**, and **18-20** were available from previous studies in our laboratories.[3] Compounds **8**, **9**, **21**, **23**, and **24** were prepared according to literature procedures. The 4-fluorobenzyl derivatives **10** and **15** (Table 1) were prepared from **4** and **3**, respectively, by reduction of the carbonyl group using Et_3SiH in trifluoroacetic acid (method A; Table 1). Lithium aluminum hydride reduction (method C) of **4** and **3** afforded **12** and **17**, respectively, whereas catalytic reduction of **13** provided **16** (method D). Although not characterized, both **16** and **17** have been previously mentioned in the literature.[10,11]

Compounds **11**, **13**, **14**, **26-28**, and **31-35** were obtained by direct alkylation of the corresponding piperidine or piperazine derivatives by reaction with either the appropriate alkyl or arylalkyl halide or tosylate (method B); compound **25** was prepared by Eschweiler–Clarke alkylation (method E). The starting piperidine for compounds **25**, **26**, **28**, and **33** was prepared by reduction of the corresponding 4-(α,α-diphenyl-α-hydroxymethyl)piperidine using $NaBH_4$ in trifluoroacetic acid. The 4-(diphenylmethylene)piperidine (starting material for compounds **31** and **32**) was prepared by dehydration of 4-(α,α-diphenyl-α-hydroxymethyl)piperidine using either concentrated H_2SO_4 or trifluoroacetic acid. Compounds **29** and **30** were prepared by the reaction of **13** with the appropriate (arylalkyl)magnesium bromide under Grignard conditions; the crude products were treated with trifluoroacetic acid followed by catalytic reduction.

Table 1. Physicochemical Properties of Novel Piperidine and Piperazine Derivatives

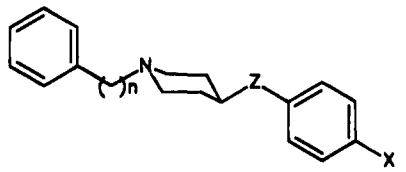
	M[a]	mp, °C	yield, %	recryst solvent[b]	formula[c]
10	A	265–267	51	EtOH	$C_{20}H_{24}FN{\cdot}HCl$[d]
11	B[k]	98–100	60	EtOH/Et_2O	$C_{20}H_{25}NO{\cdot}C_2H_2O_4$[e]
12	C	85–87	60	EtOH/Et_2O	$C_{20}H_{24}FNO{\cdot}C_2H_2O_4$[f]
13	B[l]	194–196	42	EtOH/Et_2O	$C_{22}H_{27}NO{\cdot}HCl$
14	B[k]	148–150	55	EtOH/Et_2O	$C_{22}H_{29}N{\cdot}C_2H_2O_4$
15	A	141–143	36	EtOH/Et_2O	$C_{22}H_{28}FN{\cdot}C_2H_2O_4$
16	D	55–57	86	MeOH/Et_2O	$C_{22}H_{29}NO{\cdot}C_2H_2O_4$[f]
17	C	48–51	46	MeOH/Et_2O	$C_{22}H_{28}FNO{\cdot}C_2H_2O_4$[f]
25	E	98–100	12	EtOH	$C_{19}H_{23}N{\cdot}C_2H_2O_4$[g]
26	B[l]	207–209	50	EtOH	$C_{28}H_{33}N{\cdot}C_2H_2O_4$
27	B[l]	217–219	59	EtOH/H_2O	$C_{27}H_{32}N_2{\cdot}C_2H_2O_4$[h]
28	B[i,m]	155–157	45	EtOH/Et_2O	$C_{26}H_{29}N{\cdot}C_2H_2O_4$
29	F	156–158	80	EtOH/H_2O	$C_{29}H_{35}N{\cdot}C_2H_2O_4$[m]
30	F	128–130	75	EtOH/H_2O	$C_{30}H_{37}N{\cdot}C_2H_2O_4$[f]
31	B[l]	211–213	43	EtOH	$C_{28}H_{31}N{\cdot}C_2H_2O_4$
32	B[j,m]	196–198	67	EtOH/Et_2O	$C_{26}H_{27}N{\cdot}C_2H_2O_4$
33	B[m]	225–227	37	EtOH/H_2O	$C_{28}H_{29}N_3O_2{\cdot}C_2H_2O_4$
34	B[m]	217–218	70	EtOH/H_2O	$C_{27}H_{28}N_4O_2{\cdot}C_2H_2O_4$[f]

[a] Method of preparation; see the Experimental Section. [b] Recrystallization solvent; EtOH is absolute. [c] All new compounds analyzed correctly (±0.4%) for C,H,N, except where noted. [d] Crystallized with 0.1 mol of H_2O. [e] Crystallized with 0.5 mol of EtOH (EtOH signals observed in ^{1}H-NMR). [f] Crystallized with 0.5 mol of H_2O. [g] Crystallized with 1.0 mol of EtOH. [h] Crystallized with 0.25 mol of H_2O. [i] Compound **28** was prepared from 4-(diphenylmethyl)piperidine and 2-phenylethylbromide following method G. [j] Compound **32** was prepared from 4-(diphenylmethylene)piperidine in a manner similar to **28**. [k] Refluxed for 3 h. [l] Refluxed for overnight. [m] Refluxed for 24 h.

Results

The 2-Carbon versus 4-Carbon Chain Analogues. A binding comparison of 2-carbon chain and 4-carbon chain piperidine analogues is provided in Table 2. Comparing **13** (K_i = 10 nM) with **8** (K_i = 9.6 nM), it would seem, as we have previously reported,[3] that alkyl chain length has little effect on 5-HT2A binding. However, closer inspection of the remaining members of the two series reveals that this is not the case. In the 4-carbon chain series (**3**, **13**–**17**), and consistent with our previous findings, (i) replacement of fluorine by hydrogen consistently reduces affinity by several (2–4) fold, (ii) complete reduction of the benzylic carbonyl group to a methylene group decreases affinity by about 10-fold, and (iii) reduction of the carbonyl group to an alcohol reduces affinity (by about 25-fold). In contrast, in the 2-carbon chain series, (i) the fluorine seems to play no role in binding (comparing **8** with **4**, **9** with **10**, and **11** with **12**), (ii) elimination of the carbonyl oxygen atom only halves affinity (comparing **8** with **9** or **4** with **10**), and, most importantly, (iii) reduction of the carbonyl group to an alcohol triples affinity (comparing **8** with **11** or **4** with **12**). Although it can not be excluded that

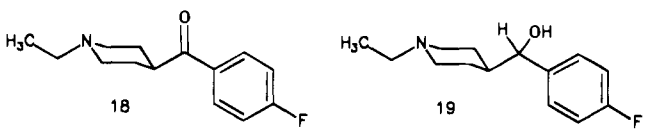
Table 2. Effect of Benzylic Modification on the Binding of 2-Carbon versus 4-Carbon Chain Analogues



	n	Z	X	K_i, nM[a] 5-HT2A	K_i, nM[a] 5-HT2C	5-HT2C/ 5-HT2A[b]
8	2	C=O	H	9.6 (±3.6)	800 (±30)	83
4	2	C=O	F	8.5	145[c]	17
9	2	CH_2	H	22 (±3)	1760 (±230)	80
10	2	CH_2	F	18 (±3)	2000 (±250)	110
11	2	CHOH	H	2.5 (±0.12)	830 (±110)	330
12	2	CHOH	F	3.0 (±1.4)	1520 (±75)	460
13	4	C=O	H	10 (±2)	2400 (±80)	240
3	4	C=O	F	5.3	620[c]	120
14	4	CH_2	H	120 (±6)	4120 (±95)	34
15	4	CH_2	F	40 (±10)	2740 (±250)	70
16	4	CH-OH	H	265 (±14)	>10 000	>35
17	4	CH-OH	F	126 (±46)	6600 (±1100)	52

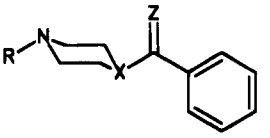
[a] K_i value followed by SEM. For pupose of comparison, ketanserin binds at 5-HT2A and 5-HT2C receptors with K_i values of 3.5 and 50 nM, respectively, under these assay conditions.[3] [b] 5-HT2A selectivity: 5-HT2C K_i ÷ 5-HT2A K_i. [c] K_i values previously reported.[3]

parallel structural modifications result in nonparallel conformational changes upon interaction at the receptor, it would appear likely that the two series of compounds are not binding in a similar manner because parallel molecular modifications do not have parallel effects on affinity. At the least, the alcohol derivatives of the shorter chain-series influence binding such that they likely bind in a manner different from that of the longer chain series. In addition, comparing the 5-HT2A affinities of **4** (K_i = 8.5 nM) and **12** (K_i = 3.0 nM) with those of their corresponding dephenyl derivatives **18** and **19** (K_i = 260 and >10 000 nM, respectively),[3] deletion of the phenyl group has nonparallel consequences (i.e., 30-fold and >3000-fold) suggesting different modes of binding even *within* the shorter chain series. Because the only structural difference between **18** and **19** is the benzylic substituent, the benzylic group must have a significant influence on the orientation of the two compounds at the binding site.



The Role of a Piperidine versus a Piperazine Ring. As previously reported,[3] piperazine **20a** does not bind at 5-HT2A receptors (K_i > 10 000 nM). Comparing **20a** with the higher affinity **21a** (K_i = 600 nM; Table 3), it is evident that the presence of the second ring nitrogen atom is detrimental to 5-HT2A binding. Likewise, we have previously shown that the 4-F analogue of **20a** is inactive whereas the 4-F analogue of piperidine **21a** binds with higher affinity (i.e. **20b** and **21b**; K_i > 10 000 and 125 nM, respectively).[3] Additional comparisons can be made by comparing **22** with **13** (K_i = 180 and 10 nM, respectively) and **23** with **14** (K_i = 240 and 120 nM, respectively). Here again, the effects are nonparallel, that is, with the carbonyl-containing pairs

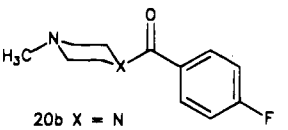
Table 3. Effect of Piperidine versus Piperazine Derivatives on 5-HT2 Binding[a]



	X	Z	R	K_i, nM 5-HT2A	K_i, nM 5-HT2C	5-HT2C/ 5-HT2A
20a	N	O	$-CH_3$	<10 000	>10 000	–
21a	CH	O	$-CH_3$	600 (±60)	>10 000	>16
22	N	O	$-(CH_2)_4Ph$	180 (±40)	8520	47
13	CH	O	$-(CH_2)_4Ph$	10 (±2)	2400 (±80)	240
23	N	H_2	$-(CH_2)_4Ph$	240 (±5)	>10 000	>40
14	CH	H_2	$-(CH_2)_4Ph$	120 (±6)	4120 (±95)	34

[a] See footnotes a and b of Table 2.

(i.e., **20/21** and **22/13**), incorporation of the second ring nitrogen atom has a greater detrimental effect than that seen with methylene pair **23/14**. Not only chain length but the presence of a second ring nitrogen atom may also influence the mode of interaction; the impact of this nitrogen atom also seems to depend on the nature of the benzylic substituent.

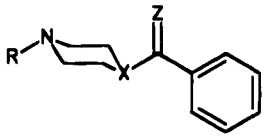


20b X = N

21b X = CH

Search for an Auxiliary Binding Site. We first examined (Table 4) two pairs of simple analogues in order to determine the influence of incorporating a second benzylic phenyl group. Comparing the affinities of **20a** (K_i > 10 000 nM) and **21a** (K_i = 600 nM) with those of their phenyl-substituted counterparts **24** (K_i = 330 nM) and **25** (K_i = 130 nM), replacement of the carbonyl oxygen atom by a phenyl group in the piperazine series results in a >30-fold enhancement in 5-HT2A affinity, whereas the corresponding modification in piperidine **21a** increases affinity only by about 5-fold. Thus, it would appear that incorporation of this second phenyl group has a greater effect on the piperazine derivative than on the piperidine derivative and that it tends to bring the affinity of the piperazine closer to that of the piperidine. In order to further explore this "equalizing" effect, we prepared and examined **26** and its piperazine counterpart **27** with the expectation that they might bind with similar affinities. Indeed piperidine **26** (K_i = 98 nM) and piperazine **27** (K_i = 140 nM) bind with comparable affinity. In this case, however, introduction of the phenyl group has relatively little impact on either the piperidine (**14** → **26**) or piperazine (**23** → **27**) derivatives. Incorporation of a phenyl group into **14** (K_i = 120 nM; Table 4) has essentially no effect (i.e., **26**; K_i = 98 nM). Incorporation of this phenyl group into the corresponding 2-carbon chain piperidine **9** (K_i = 22 nM), that is **28** (K_i = 19 nM), also has no effect. Apparently, it is likely that the absence of the carbonyl oxygen, and not the presence of the added phenyl group, accounts for the enhanced affinity of **24** over **20a**. An alternative explanation is that the presence of the second nitrogen atom in the piperazine derivatives may result in a different binding orientation relative to the

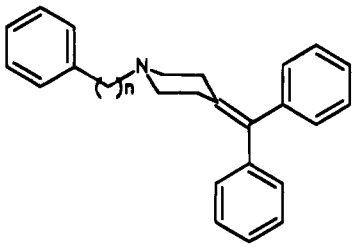
Table 4. Effect of Benzylic Modification on 5-HT2 Binding[a]



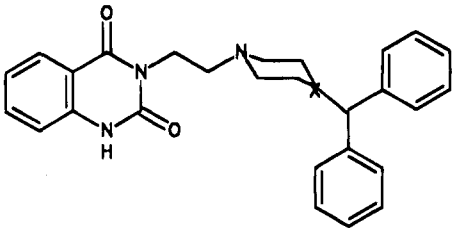
	X	Z	R	K_i, nM 5-HT2A	K_i, nM 5-HT2C	5-HT2C/5-HT2A
20a	N	O	-CH_3	>10 000	>10 000	–
24	N	H, Ph	-CH_3	330 (±50)	2865 (±500)	9
21a	CH	O	-CH_3	600 (±60)	>10 000	>16
25	CH	H, Ph	-CH_3	130 (±15)	1225 (±125)	9
14	CH	H, H	-$(CH_2)_4Ph$	120 (±6)	4120 (±95)	34
26	CH	H, Ph	-$(CH_2)_4Ph$	98 (±10)	1770 (±220)	18
27	N	H, Ph	-$(CH_2)_4Ph$	140 (±15)	2600 (±50)	19
29	CH	H, CH_2Ph	-$(CH_2)_4Ph$	100 (±40)	1390 (±10)	14
30	CH	H, CH_2CH_2Ph	-$(CH_2)_4Ph$	53 (±9)	455 (±90)	9
28	CH	H, Ph	-$(CH_2)_2Ph$	19 (±2)	–	–

[a] See footnotes to Table 2.

Table 5. Effect of Benzylic Modification on 5-HT2 Binding[a]



	n	K_i, nM 5-HT2A	K_i, nM 5-HT2C	5-HT2C/5-HT2A
31	4	20 (±5)	220 (±7)	11
32	2	6.9 (±0.2)	29 (±9)	4



	X			
33	CH	18 (±5)	52 (±1)	3
34	N	65 (±15)	635 (±125)	10

[a] See footnotes to Table 2.

binding of piperidine derivatives such that addition of the second benzylic phenyl group does not have the same impact on the two sites. Accordingly, we explored some "extended" analogues of **26** to determine if they might bind with higher affinity (Table 4). Compounds **29** and **30** (K_i = 100 and 53 nM) did not bind with significantly higher affinity than **26** (K_i = 98 nM), and none of these compared with **13** (K_i = 10 nM). Unsaturated derivative **31** (Table 5), on the other hand, displayed affinity (K_i = 20 nM) comparable to that of **13**, and unsaturated derivative **32** (K_i = 6.9 nM) had 5-HT2A affinity comparable to that of **9**. Thus, the sp^2 nature of the benzylic positions of **31** and **32** may make them appear more "carbonyl-like" to the receptor.

In a final evaluation of the influence of a second benzylic phenyl group on 5-HT2A affinity, we examined **33**, a phenyl counterpart of ketanserin (**1**), and its piperazine derivative **34**. Compound **33** (K_i = 18 nM) was found to bind at 5-HT2A receptors with an affinity comparable to that of desfluoroketanserin ($K_i = 10 \pm 4$ nM), suggesting that the second phenyl group contributes little to binding. Compound **34** (K_i = 65 nM) binds with high affinity considering it is a piperazine derivative; however, its affinity is still severalfold lower than that of **33**.

Selectivity. Ketanserin (**1**), under our assay conditions, displays about 14-fold selectivity for 5-HT2A versus 5-HT2C receptors.[3] None of the compounds in the present investigation (Tables 2–5) displayed any selectivity for 5-HT2C receptors, but most of them showed a 5-HT2A selectivity at least comparable to that of ketanserin. Table 2 reveals that the most selective derivatives in the 2-carbon chain piperidine family are the alcohol derivatives **11** and **12**; in contrast, it is the carbonyl analogues **3** and **13** that are the most selective in the 4-carbon chain series. Incorporation of a second benzylic phenyl group has relatively little effect on selectivity and, if anything, decreases selectivity some-

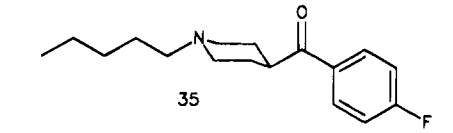
what (e.g., see Table 4 and compound **33**). The most 5-HT2A-selective agent in the present study is **12** (460-fold 5-HT2A selective). Although this makes **12** a fairly selective agent, other agents with 1000–2000-fold selectivity have been previously reported.[12] Compound **32**, although among the least selective agents, is the highest affinity 5-HT2C ligand encountered in the present investigation.

Discussion

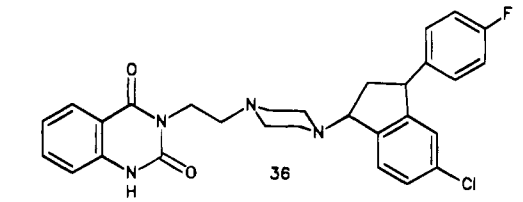
On the basis of the present investigation, it would seem that although simplification of the ketanserin structure to **3** and shortening of the butyl chain of **3** to an ethyl chain (i.e., **4**) result in retention of 5-HT2A affinity, the butyl chain analogues and the ethyl chain analogues may bind at 5-HT2A receptors in a different orientation, that is, parallel structural modifications in the two series (see Table 2) result in nonparallel changes in 5-HT2A affinity. The most dramatic difference is associated with reduction of the benzoyl carbonyl group to an alcohol. Whereas this reduction in ketanserin-related compounds, as well as with **3** and **13**, reduces 5-HT2A affinity by 25- to >100-fold, the corresponding carbonyl reduction in the ethyl chain series (i.e., **4** → **12**, **8** → **11**) enhances affinity by severalfold. There may even be a pattern to this effect, that is, carbonyl reduction in intact ketanserin and desfluoroketanserin results in >100-fold decreases in affinity,[3] carbonyl reduction in the somewhat more abbreviated derivatives **3** and **13** result in about 25-fold decreases, and carbonyl reduction in the most abbreviated derivatives (i.e., **4** and **8**) result in a reversal of this effect and in enhanced affinity.

While our work was in progress, Andersen et al.[13] developed a receptor model to account for the binding of 5-HT2A antagonists. This model, an extension of their earlier work showing a structural relationship between ketanserin and **7**-type compounds,[9] now encompasses a number of other antagonists including agents such as **5**. The high affinity of **7**-type compounds is thought to be related to interaction of the benzylic phenyl ring with an auxiliary binding site. Agents devoid of such a phenyl group (such as ketanserin) are speculated to participate in a compensating hydrogen-bond interaction with the receptor to partially account for their high affinity. In ketanserin, for example, the benzylic keto group is proposed to be responsible for this hydrogen-bond interaction; the presence of the quinazolinedione is also thought to aid in compensating for the lack of a substituent with which to interact at the auxiliary site. At first this explanation appears satisfactory. However, (i) elimination of the keto oxygen of ketanserin and desfluoroketanserin reduces affinity by only 4-fold, (ii) a reduced (i.e., alcohol) analogue of ketanserin, which should also be capable of participating in a hydrogen-bond interaction with the receptor, binds with 100-fold reduced affinity, and (iii) desoxy compounds such as **9** and **10** bind nearly as well as **8** and **4**, respectively. Furthermore, because an intact quinazolinedione nucleus is not required for 5-HT2A binding (see the introduction), it is unlikely that it, per se, is important. Perhaps some portion of the quinazolinedione nucleus, such as the fused phenyl ring, is necessary.

To test this hypothesis, we examined **35**, where the terminal phenyl group of **3** is replaced by a methyl group. Compound **35** ($K_i = 30 \pm 4$ nM) binds with only slightly reduced affinity relative to **3** ($K_i = 5.3$ nM); although it may participate, it would certainly not seem that the phenyl group is critical for binding. In addition, compound *trans*-**36**, a ketanserin/**7**-type hybrid, does not display significantly enhanced affinity ($IC_{50} = 3.8$ nM) relative to (+)-*trans*-**7** ($IC_{50} = 4.9$ nM)[9,14] which might have been expected if the quinazolinedione moiety was specifically involved in affinity enhancement. Taken together, it would seem unlikely that a hydrogen-bond interaction, coupled with participation of the quinazolinedione moiety in binding, compensates or substitutes for lack of a substituent with which to interact at the auxiliary binding site. As an alternative argument, it can be speculated that there are two different auxiliary aromatic binding sites and that interaction at the two sites does not necessarily result in additive binding or that interaction at the two sites can not be simultaneous (i.e., utilization of one aromatic binding site precludes utilization of the second site.) This latter argument, nevertheless, still invokes multiple (i.e., overlapping but nonidentical) modes of binding for different compounds.



35



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Piperidine and piperazine derivatives may also bind at 5-HT2A receptors in a different fashion, or at least, certain piperazines are not as well accommodated by the receptor as are their piperidine counterparts. Comparing the piperidine/piperazine pairs **21/20** and **13/22** (see Table 3), the piperazines bind with reduced affinity. This difference is less pronounced, however in the absence of a benzylic carbonyl group (i.e., compare **14** with **23**). Thus, once again the benzylic position seems involved. Introduction of a second benzylic phenyl group also has varying effects. Although the second phenyl has little to no effect in some cases (e.g., **14** → **26**, **9** → **28**, **21a** → **25**, **23** → **25**), it enhances the affinity of **20a** (i.e., **24**). With the exception of this last example, there is no evidence for an affinity-enhancing auxiliary binding site. Indeed, the higher affinity of **24** relative to **20a** may be related to the absence of a carbonyl oxygen atom. Introduction of a benzylic phenyl group does not enhance the affinity of **33** ($K_i = 18$ nM) relative to desflouroketeanserin ($K_i = 10$ nM). Nevertheless, the existence of such a site can not yet be discounted, that is, the benzyl substituents of **26**–**34** may not be ideally located at take advantage of the auxiliary site.

In conclusion, it would appear that there exists multiple (at least two) modes of binding for ketanserin-related analogues at 5-HT2A receptors. The particular mode of binding may be influenced by the length (or nature) of the piperidine N-substituent. Introduction of a second ring nitrogen atom reduces affinity when the piperidine N-substituent is methyl, but this effect

is not as apparent with larger substituents. Perhaps the nature of the benzylic substituent is the most significant determinant of the manner in which these agents bind at 5-HT2A receptors, and it is altogether possible that certain orientations may avail themselves of an auxiliary binding site. Variation of the benzylic substituent from a carbonyl, to an alcohol, to a methylene group has a nonparallel influence on binding, and this may be further affected by the length of the piperidine N-substituent and the presence of a second ring nitrogen atom. The results of the present investigation, then, provide evidence that although the structure of ketanserin can be abbreviated, and even modified by conversion of the piperidine ring to a piperazine, with (occasionally) relatively little influence on 5-HT2A affinity, the resultant analogues may bind in more than one orientation at these receptors. A key structural feature that may play a prominent role in anchoring or orienting these compounds at 5-HT2A receptors is the benzylic carbonyl group.

The antagonist model proposed by Andersen et al.[13] accounts for the binding of compounds that could not be accommodated by our model.[6] Their model certainly explains the binding of 7-type compounds. However, the present results (e.g., comparing the 2-carbon chain and 4-carbon chain analogues in Table 2), together with the site-directed mutagenesis studies of Choudhary et al.,[8] suggest that multiple orientations or modes of binding are possible for 5-HT2A receptor interactions. Furthermore, we have recently shown that different overlapping but nonidentical modes of binding can occur at 5-HT2A receptors within a closely related structural series of ergoline derivatives.[18] It is obvious that additional effort is necessary to continue developing and challenging 5-HT2A receptor models.

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 spectrometer, and proton magnetic resonance (NMR) spectra were obtained using a GE QE-300 spectrometer. 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. 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. Methods A–F refer to Table 1.

4-(4-Fluorobenzyl)-1-(2-phenethyl)piperidine Hydrochloride (10). Method A. Triethylsilane (0.33 g, 2.8 mmol) was added to a solution of 4-(4-fluorobenzoyl)-1-(2-phenylethyl)piperidine (100 mg, 0.29 mmol) in trifluoroacetic acid (10 mL) in a dropwise manner. The resulting reaction mixture was allowed to stir for 4 days. The solvent was removed under reduced pressure, and the organic residue was made alkaline with 10% NaOH, extracted with Et_2O (3 × 10 mL), washed with H_2O (3 × 10 mL), and dried ($MgSO_4$). The Et_2O filtrate was treated with a saturated solution of HCl gas in Et_2O (20 mL). The formed precipitate was recrystallized from absolute EtOH to give 50 mg (51%) of the title compound, mp 265–267 °C dec. ^{1}H-NMR (DMSO-d_6): δ 1.6 (m, 4 H, $2CH_2$), 2.4 (m, 2H, CH_2), 2.8 (m, 2H, CH_2), 3.05 (m, 2H, CH_2), 3.15 (m, 2H, CH_2), 3.4 (m, 1H, CH), 3.45 (d, 2H, CH_2), 7.1 (m, 2H, Ar-H), 7.15 (m, 5H, Ar-H), 10.7 (bs, 1H, NH^+). Anal. ($C_{20}H_{24}FN\cdot HCl\cdot 0.1H_2O$) C, H, N.

α-Phenyl-α-[1-(2-phenylethyl)piperidin-4-yl]methanol Oxalate (11). Method B. A mixture of α-phenyl-(piperidin-4-yl)methanol (191 mg, 1 mmol), 2-phenylethanol-*p*-toluenesulfonate (270 mg, 1 mmol), and anhydrous K_2CO_3 (140 mg, 1 mmol) in dioxane (10 mL) was heated at reflux for 3 h. The solvent was evaporated, and the residue was treated with H_2O (10 mL) and extracted with Et_2O (10, 5, and 2 mL). The combined Et_2O extract was washed with H_2O (10, 5, and 2 mL), dried, and treated with a saturated solution of oxalic acid in Et_2O (100 mg, 30 mL). The solid product was recrystallized from EtOH/Et_2O to give 219 mg (60%) of a white powder, mp 98–100 °C. ^{1}H-NMR (DMSO-d_6): δ 1.3 (m, 6H, $3CH_2$), 2.7 (m, 7H, $3CH_2$ and CH), 4.2 (d, 1H, CH), 7.1 (m, 10H, Ar-H). Anal. ($C_{20}H_{25}NO\cdot C_2H_2O_4\cdot 0.5C_2H_5OH$), C,H,N.

α-(4-Fluorophenyl)-α-[1-(2-phenethyl)-4-piperidinyl]-methanol Oxalate (12). Method C. A solution of 4-(4-fluorobenzoyl)-1-(2-phenethyl)piperidine (50 mg, 0.12 mmol) in THF (10 mL) was added, in a dropwise manner, to a suspension of $LiAlH_4$ (0.06 g, 1.6 mmol) in dry THF (15 mL) at 0 °C. The mixture was heated at reflux overnight. Excess hydride was decomposed with H_2O (2 mL) followed by 2 N NaOH solution (2 mL). The inorganic precipitate was removed by filtration; the filtrate was dried ($MgSO_4$) and evaporated. The oily residue in Et_2O (20 mL) was treated with a saturated solution of oxalic acid in Et_2O (50 mg in 20 mL). Recrystallization from EtOH/Et_2O afforded 30 mg (60%) of the title compound, mp 85–87 °C. ^{1}H-NMR (DMSO-d_6): δ 1.35 (m, 1H, CH_2), 1.5–1.8 (m, 4H, $2CH_2$), 1.85–2.0 (m, 2H, CH_2), 2.75–3.0 (m, 4H, $2CH_2$), 3.1–3.2 (m, 2H, CH_2), 3.45 (d, 1H, benzylic-CH), 4.3 (bs, 1H, OH), 7.1–7.4 (m, 9H, Ar-H). Anal. ($C_{20}H_{24}FNO\cdot C_2H_2O_4\cdot 0.5H_2O$) C,H,N.

α-Phenyl-α-[1-(4-phenylbutyl)piperidin-4-yl]methanol Oxalate (16). Method D. 4-Benzoyl-1-(4-phenylbutyl)-piperidine hydrochloride (50 mg, 0.14 mmol) in 95% EtOH (10 mL) was hydrogenated over 10% Pd/C (20 mg) for 2 h. After filtration and evaporation of solvent, the residue was treated with 10% NaOH solution (5 mL) and extracted with Et_2O (10, 5, and 5 mL). The combined Et_2O extract was washed with H_2O (10, 5, and 5 mL), dried, and treated with a saturated solution of oxalic acid in Et_2O (50 mg in 10 mL); recrystallization from MeOH/Et_2O gave 50 mg (86%) of **16**, mp 55–57 °C. ^{1}H-NMR (DMSO-d_6): δ 1.3 (m, 1H, CH), 1.45–1.75 (m, 6H, $3CH_2$), 1.8–1.95 (m, 2H, CH_2), 2.55 (t, 2H, CH_2), 2.65 (m, 2H, CH_2), 2.85 (M, 2H, CH_2), 3.25 (m, 3H, CH_2 and benzylic-CH), 4.3 (d, 1H, OH), 7.05–7.35 (m, 9H, Ar-H), Ar-H). Anal. ($C_{22}H_{29}NO\cdot C_2H_2O_4\cdot 0.5H_2O$) C,H,N.

4-(Diphenylmethyl)-1-methylpiperidine Oxalate (25). Method E. A mixture of 4-(diphenylmethyl)piperidine (126 mg, 0.5 mmol), 35% HCHO (0.5 mL), and 97% HCOOH (1 mL) was heated at reflux for 18 h. After addition of concentrated HCl (1 mL), the volatiles were removed under reduced pressure. The resulting residue was dissolved in H_2O (5 mL), and the aqueous solution was made alkaline by the addition of 30% NaOH and extracted with Et_2O (3 × 5 mL). The combined Et_2O extracts were dried ($MgSO_4$), and the solvent was removed under reduced pressure to afford the free base of **25**, mp 86–88 °C (lit.[15] mp 88–89 °C). The oxalate salt was prepared and recrystallized from absolute EtOH to yield 20 mg (12%) of **25**, mp 98–100 °C. ^{1}H-NMR ($CDCl_3$): δ 0.95 (t, EtOH), 1.2 (m, 4H, $2CH_2$), 2.4 (m, 6H, N-CH_3, CH_2 and CH), 3.2(m, 5H, CH_2, CH and CH_2 of C_2H_5-OH), 7.1 (M, 10H, Ar-H), 7.8 (bs, NH^+ and -COOH). Anal. ($C_{19}H_{23}N\cdot C_2H_2O_4\cdot$EtOH) C,H,N.

4-(1,2-Diphenylethyl)-1-(4-phenylbutyl)piperidine Oxalate (29). Method F. A solution of 1-(4-phenylbutyl)-4-benzoylpiperidine (642 mg, 2 mmol) in anhydrous Et_2O (10 mL) was added to benzylmagnesium bromide (1 M solution in Et_2O, 7 mL) in a dropwise manner. The reaction mixture was heated at reflux for 2 h and decomposed by the careful addition of a saturated solution of NH_4Cl. The Et_2O layer was separated, and the aqueous portion was extracted with Et_2O (20 mL). The combined Et_2O extract was washed with H_2O and dried ($MgSO_4$), and the solvent was evaporated. The oily residue was chromatographed on silica gel using $CHCl_3$/MeOH (9:1). After evaporation of the eluent, the residue was dissolved in Et_2O and treated with Et_2O/oxalic acid (20 mL/200 mg). Recrystallization from MeOH/Et_2O afforded 710 mg (71%) of 4-(1-hydroxy-2,3-diphenylethyl)-1-(4-phenylbutyl)piperidine oxalate, mp 78–81 °C. ^{1}H-NMR (DMSO-d_6): δ 1.2 (m,

20H, 9CH_2, CH and OH), 7 (m, 17H, Ar-H, NH^+, and -OH of carboxylic acid). Anal. ($C_{29}H_{35}NO{\cdot}C_2H_2O_4$) C,H,N.

The above intermediate (200 mg, 0.38 mmol) was treated with trifluoroacetic acid (3 mL), and the mixture was allowed to stir at room temperature for 2 h. The trifluoroacetic acid was evaporated; a solution of the residue in EtOH (20 mL) was treated with 10% Pd/C (100 mg) and hydrogenated overnight in the presence of few drops of acetic acid. The mixture was filtered, and the solvent was evaporated under reduced pressure. The oily residue was made alkaline (NaOH) and extracted with Et_2O (3 × 10 mL). The combined Et_2O extract was dried ($MgSO_4$) and treated with a saturated solution of oxalic acid in anhydrous Et_2O (20 mL). Recrystallization from EtOH (absolute) afforded 150 mg (80%) of the title compound, mp 156–158 °C. ^{1}H-NMR (DMSO-d_6): δ 1.1 (m, 8H, 4CH_2), 1.8 (m, 2H, CH_2), 2.4 (m, 10H, 4CH_2 and 2CH), 6.9 (m, 15H, Ar-H), 7.8 (bs, 2H, NH^+ and OH). Anal. ($C_{29}H_{35}N{\cdot}C_2H_2O_4{\cdot}0.25H_2O$) C,H,N.

4-(4-Fluorobenzoyl)-1-pentylpiperidine Oxalate (35). This compound was prepared as described for 11, starting with 4-(4-fluorobenzoyl)piperidine (207 mg, 1 mmol) and heating with pentanol–*p*-toluenesulfonate (242 mg, 1 mmol) and anhydrous K_2CO_3 (140 mg, 1 mmol) for 16 h in dioxane (10 mL). The free base, obtained after evaporation of dioxane, was purified via column chromatography (silica gel, $CHCl_3$/MeOH, 9:1). The free base was treated with a saturated solution of oxalic acid in anhydrous Et_2O (200 mg, 30 mL). The oxalate salt was recrystallized from EtOH to afford 200 mg (54%) of **35**, mp 165–166 °C. ^{1}H-NMR (DMSO-d_6): δ 0.7 (m, 14H, CH aliph), 2.8 (m, 6H, N-CH_2), 7.1 (d, 2H, Ar-H), 8.1 (d, 2H, Ar-H), 10.2 (bs, NH^+ and -COOH). Anal. ($C_{17}H_{24}FNO{\cdot}(COOH)_2$) C,H,N.

Radioligand Binding. Radioligand binding assays were performed as previously described in detail.[3] Briefly, frontal cortical regions of male Sprague–Dawley rats (200–250 g; Charles River) were dissected on ice, homogenized (1:10 w/v) in ice-cold buffer solution (50 mM Tris-HCl, 0.5 nM 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 recentrifuged twice more at 3000*g* for 10 min. The final pellet was resuspended in buffer that also contained 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-HT2A receptor assays and 10 mg wet weight of tissue and 1 nM [^{3}H]-mesulergine (75.8 Ci/mmol; Amersham) for 5-HT2C receptor assays. Cinanserin (1.0 μM) was used to define nonspecific binding in the 5-HT2A assay. In the 5-HT2C assay, mianserin (1.0 μM) was used to define nonspecific binding, and 100 nM spiperone (Sigma) was added to all tubes to block binding to 5-HT2A receptors. Tubes were incubated for 15 min at 37 °C, filtered on Schliecher and Schuell (Keene, NH) glass fiber filters presoaked in poly(ethylenimine), and washed with 10 mL of ice-cold buffer. Filters were counted at an efficiency of 50%.

Saturation and competition experiments were analyzed using an undated version of the program EBDA[16] 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.[17]

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