Syntheses and 5-HT_2 Antagonist Activity of Bicyclic 1,2,4-Triazol-3(2*H*)-one and 1,3,5-Triazine-2,4(3*H*)-dione Derivatives

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A series of bicyclic 1,2,4-triazol-3(2*H*)-one and 1,3,5-triazine-2,4(3*H*)-dione derivatives with a 4-[bis(4-fluorophenyl)methylene]piperidine or 4-(4-fluorobenzoyl)piperidine group has been prepared and tested for 5-HT_2 and α_1 receptor antagonist activity. Among the compounds prepared, 2-[2-[4-[bis(4-fluorophenyl)methylene]-piperidin-1-yl]ethyl]-5,6,7,8-tetrahydro-1,2,4-triazolo[4,3-*a*]pyridin-3(2*H*)-one (**7b**) had the most potent 5-HT_2 antagonist activity, which was greater than ritanserin (**2**), while **7b** did not show α_1 antagonist activity in vivo. The central 5-HT_2 receptor antagonism was approximately $^1/_{30}$ that of **2** when tested for the ability to block head twitches induced by 5-hydroxytryptophan. Compound **21b**, 3-[2-[4-(4-fluorobenzoyl)piperidin-1-yl]ethyl]-6,7,8,9-tetrahydro-2*H*-pyrido[1,2-*a*]-1,3,5-triazine-2,4(3*H*)-dione also displayed potent 5-HT_2 antagonist activity. The compound had moderate α_1 receptor antagonism, and the potency inhibiting head twitches was about one-third that of ketanserin (**1**). These results indicate that 5,6,7,8-tetrahydro-1,2,4-triazolo[4,3-*a*]pyrimidin-3(2*H*)-one and 6,7,8,9-tetrahydro-2*H*-pyrido-[1,2-*a*]-1,3,5-triazine-2,4(3*H*)-dione ring systems are useful components of 5-HT_2 antagonists.

Serotonin (5-HT) has potent vasoconstriction and platelet aggregation activities and synergistically amplifies the effects of the vasoactive and/or platelet aggregative substances such as TXA_2, norepinephrine, angiotensin II, ADP, and collagen.[1-4] Since these activities have been demonstrated to be mediated through 5-HT_2 receptors,[5-7] peripheral 5-HT_2 antagonists are expected to be useful in the treatment of cardiovascular diseases. Recently, a peripheral 5-HT_2 antagonist, ketanserin (**1**), has been developed and launched as an antihypertensive agent, and it has also been reported to be beneficial in the treatment of myocardial ischemia[8] and Raynaud's phenomenon.[9] The compound is not only a potent 5-HT_2 antagonist but also has considerable α_1-adrenoceptor antagonist activity which has been confirmed to be responsible for the blood pressure reduction.[10,11] In contrast to that, ritanserin (**2**), which is a central 5-HT_2 antagonist with a related structure, exhibits anxiolytic activity[12,13] but not antihypertensive activity,[14,15] suggesting that **2** is a weak α_1 antagonist. Despite different pharmacological profiles, both compounds interrupt cyclic flow reductions (CFRs) in stenosed coronary arteries of dogs[16-18] and seem to be beneficial in the treatment of unstable angina which tends to be refractory to conventional cardiovascular drugs.

We felt that the characteristic difference in the pharmacological profile between **1** and **2** could be ascribed to the 4-substituted piperidine moieties. The 4-[bis(4-fluorophenyl)methylene]piperidine group of **2** seems favorable for conferring low antihypertensive activity, but we desired reduced CNS activity. We expected that more hydrophilic analogues of **2** might have the reduced CNS activity because in a series of synthetic compounds, permeability of the blood–brain barrier was dependent on

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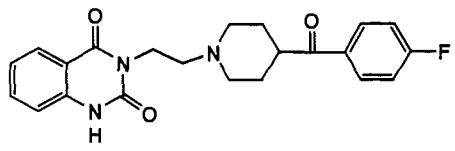
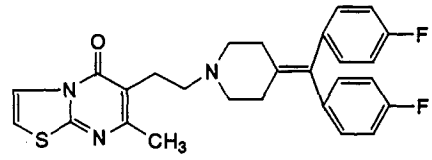


1 Ketanserin



2 Ritanserin

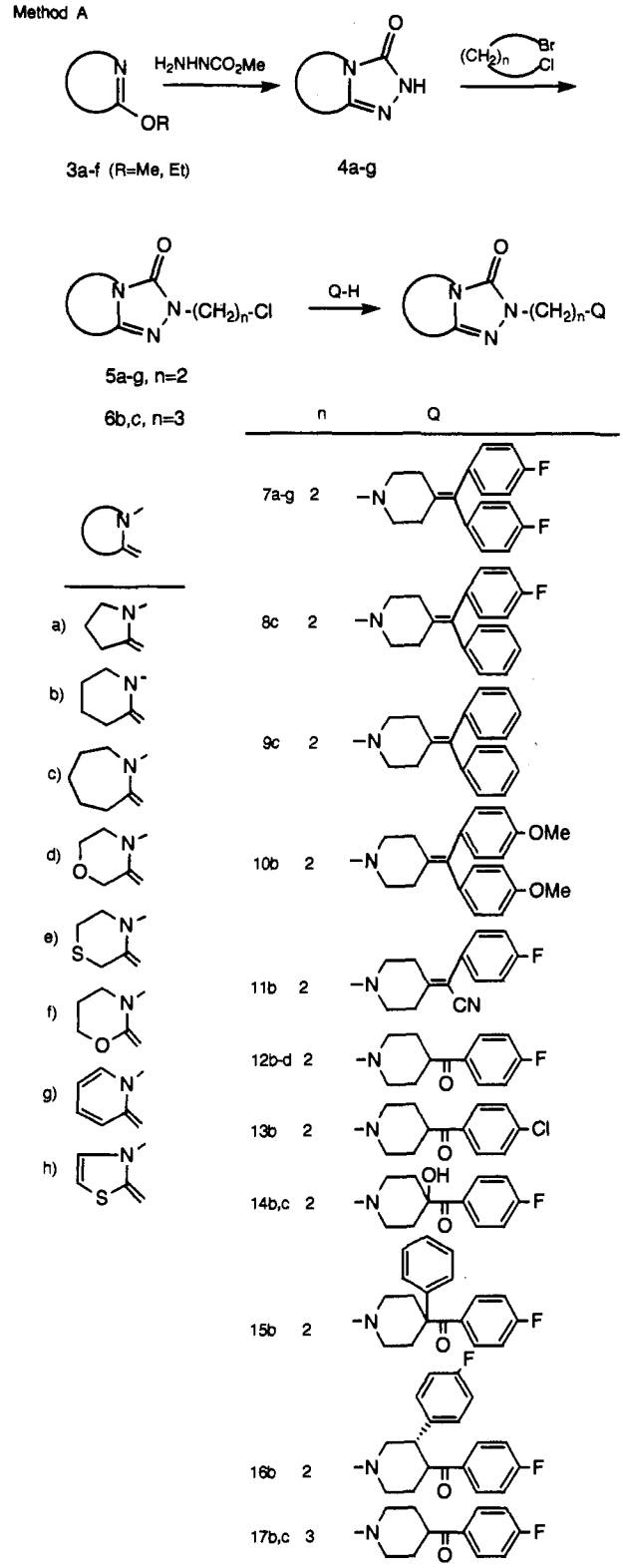
the oil/water partition coefficient.[19,20] In a search for 5-HT_2 antagonists, we found that conversion of the quinazoline-2,4(1*H*,3*H*)-dione group of **1** into the corresponding 5,6,7,8-tetrahydroquinazoline-2,4(1*H*,3*H*)-dione group retained the 5-HT_2 antagonist activity,[21] and we felt that further modification of this ring could produce peripherally selective 5-HT_2 antagonists. Thus, we designed and synthesized bicyclic 1,3,5-triazine-2,4(3*H*)-diones and 1,2,4-triazol-3(2*H*)-ones as potential 5-HT_2 antagonists. In this paper, we describe the syntheses and pharmacological evaluations of these compounds.

Chemistry

Bicyclic 1,2,4-triazol-3(2*H*)-one derivatives **7–17** were prepared as outlined in Scheme I. The 1,2,4-triazol-3-(2*H*)-ones **4** were synthesized by the procedure of Peterson et al.[22] followed by alkylation of **4** with ω-chloroalkyl bromide to give chlorides **5** or **6** in moderate yields. The reaction of the chlorides **5** or **6** with the appropriate amines, which were prepared according to the known methods, afforded **7–17** in moderate yields.

Bicyclic 1,3,5-triazine-2,4(3*H*)-dione derivatives **20–23** were prepared as outlined in Scheme II. Pyrido[1,2-*a*]-1,3,5-triazine-2,4(3*H*)-dione **19g** was prepared by the procedure of Kamal et al.[23] from 2-aminopyridine (**18g**) and chlorocarbonyl isocyanate, but its 5,6,7,8-tetrahydro derivative **19b** could not be obtained by the similar reaction of the corresponding 2-amino-3,4,5,6-tetrahydropyridine **18b** with chlorocarbonyl isocyanate. We therefore employed phenoxycarbonyl isocyanate instead of the above reagent to give the desired tetrahydro analogue **19b** in a moderate yield. Other 1,3,5-triazine-2,4(3*H*)-dione derivatives **19** were also prepared in a similar method. Compounds **19** were found to be relatively unstable under basic conditions. We therefore adopted a neutral alkylation method involving the Mitsunobu reaction[24] of **19** with

Scheme I



1-(2-hydroxyethyl)- or 1-(3-hydroxypropyl)piperidine derivatives which gave **20–23** in moderate yields.

Biological Evaluation and Discussion

Both 5-HT_2 and α_1 antagonist activities were assessed. The in vitro activities were determined and expressed as pA_2 values for rat-isolated thoracic aorta by the method

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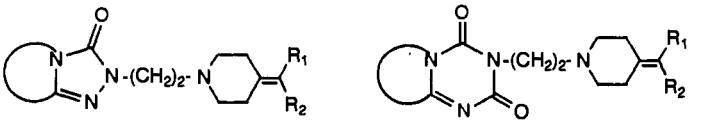
Table I. 4-[Bis(4-fluorophenyl)methylene]piperidine Derivatives and Their Congeners



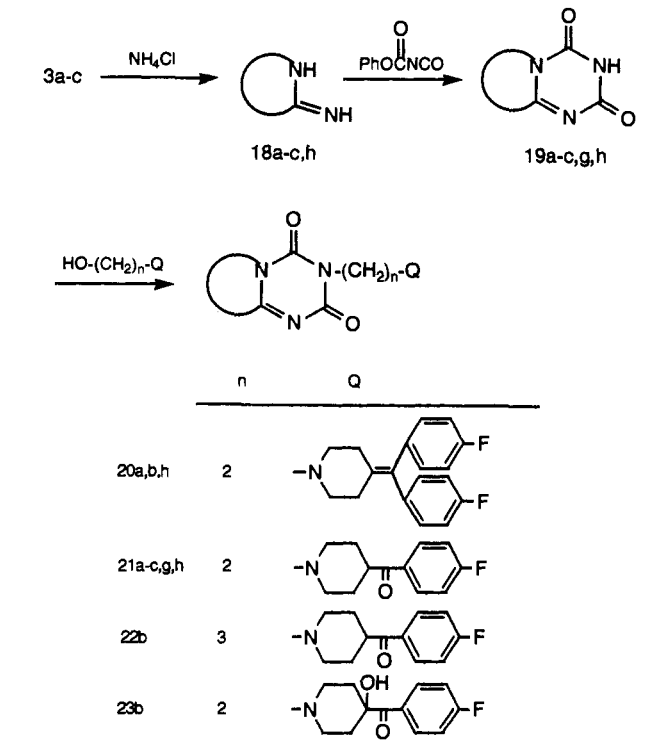
(A) (B)

compd[a]	structure type[b]	R_1	R_2	mp (°C)	formula[c]	in vitro (pA_2)[d] 5-HT$_2$[e]	α_1[f]	dose (mg/kg)	in vivo (% inhibn)[g] 5-HT$_2$	α_1
7a	A	4-F-C_6H_4	4-F-C_6H_4	123–125	$C_{25}H_{26}F_2N_4O\cdot HCl\cdot CH_4O$[i]	7.1	6.9	10	74 ± 2	6 ± 3
7b	A	4-F-C_6H_4	4-F-C_6H_4	127–130	$C_{26}H_{28}F_2N_4O\cdot HCl\cdot {}^3/_2H_2O$	8.6	7.0	10	96 ± 2	14 ± 8
								10[h]	87 ± 7	4 ± 4
								3	76 ± 11	10 ± 5
								3[h]	78 ± 6	11 ± 5
7c	A	4-F-C_6H_4	4-F-C_6H_4	108–120	$C_{27}H_{30}F_2N_4O\cdot HCl\cdot {}^1/_2H_2O$	8.5	7.0	10	90 ± 7	3 ± 3
7d	A	4-F-C_6H_4	4-F-C_6H_4	139–144	$C_{25}H_{26}F_2N_4O_2\cdot HCl$	7.2	6.6	10	60 ± 10	10 ± 2
7e	A	4-F-C_6H_4	4-F-C_6H_4	178–180	$C_{25}H_{26}F_2N_4OS\cdot HCl\cdot {}^1/_2H_2O$	7.8	7.5	10	67 ± 7	4 ± 3
7f	A	4-F-C_6H_4	4-F-C_6H_4	138–139	$C_{25}H_{26}F_2N_4O_2\cdot HCl\cdot {}^1/_2H_2O$	7.4	7.8	10	89 ± 6	16 ± 4
7g	A	4-F-C_6H_4	4-F-C_6H_4	127–129	$C_{26}H_{24}F_2N_4O\cdot HCl$	7.0	6.6	10	58 ± 9	6 ± 4
8c	A	4-F-C_6H_4	Ph	119–121	$C_{27}H_3FN_4O\cdot HCl\cdot C_2H_6O$[j]	7.3	6.4	10	84 ± 3	7 ± 7
9c	A	Ph	Ph	117–119	$C_{27}H_{32}N_4O\cdot HCl\cdot C_2H_6O$[j]	7.0	<6	10	42 ± 7	3 ± 2
10b	A	4-MeO-C_6H_4	4-MeO-C_6H_4	193–203	$C_{28}H_{30}N_4O_3\cdot HCl\cdot H_2O$	6.8	7.2	10	41 ± 6	4 ± 2
11b	A	4-F-C_6H_4	CN	114–115	$C_{21}H_{24}FN_5O$	6.7	6.3	10	32 ± 3	2 ± 2
20a	B	4-F-C_6H_4	4-F-C_6H_4	173–180	$C_{26}H_{26}F_2N_4O_2\cdot 2HCl$	8.3	6.8	10	83 ± 7	8 ± 5
20b	B	4-F-C_6H_4	4-F-C_6H_4	192–195	$C_{27}H_{28}F_2N_4O_2\cdot 2HCl$	8.3	6.8	10	93 ± 3	12 ± 4
20h	B	4-F-C_6H_4	4-F-C_6H_4	251–253	$C_{25}H_{22}F_2N_4O_2S\cdot HCl$	7.1	<6	10	93 ± 5	20 ± 4
2	(ritanserin)					8.8	7.5	10	84 ± 6	7 ± 7

[a] See Schemes I and II. [b] See top of the table. [c] All compounds analyzed for C, H, and N were within 0.4% of the calculated formula values. [d] pA_2 values were obtained by a single determination for isolated rat thoracic aortic strips. [e,f] Antagonistic activity against the contraction induced by 5-HT and phenylephrine, respectively. [g] Inhibitory activity (mean ± SEM) in rats against elevation of blood pressure caused by 5-HT (300 μg/kg, iv) or phenylephrine (30 μg/kg, iv) after 1 h of oral administration of the test compound. [h] Inhibitory activity (mean ± SEM) after 3 h of oral administration of the test compound. [i] Methanolate. [j] Ethanolate.

Scheme II

Method B



of Arunlakshana et al.[25] It has been reported that injection of 5-HT elicits a triphasic pressure response, namely a short-lasting depressor phase, a pressor phase, and a prolonged hypotension which are mediated by 5-HT$_3$, 5-HT$_2$, and 5-HT$_1$ receptors, respectively.[26] Then, the potency of the in vivo 5-HT$_2$ receptor antagonism was estimated by measurement of the inhibitory activity against the second pressor response to intraveneous administration of 5-HT. The in vivo α_1 receptor antagonism was similarly evaluated on the basis of the response to phenylephrine, a selective α_1 agonist.

The antagonistic activities against 5-HT$_2$ and α_1 receptors obtained here are shown in Tables I and II. In a series of 4-[bis(4-fluorophenyl)methylene]piperidine derivatives, compounds **7b**, **7c**, and **20b** showed strong 5-HT$_2$ antagonist activity in vitro, and the in vivo potency of **7b** was higher than that of the parent compound **2**. Interestingly, the corresponding unsaturated 1,2,4-triazolo[4,3-*a*]-pyridin-3(2*H*)-one derivative **7g** did not show potent activity. Introduction of a heteroatom into the 7-position of the 5,6,7,8-tetrahydro-1,2,4-triazolo[4,3-*a*]pyridin-3-(2*H*)-one group diminished the activity (**7d** and **7e**), suggesting that this part could interact with a lipophilic site of the 5-HT$_2$ receptor. On the other hand, introduction of a heteroatom into the 8-position appeared to be acceptable because compound **7f** with an oxygen atom had potent in vivo activity. Compound **7c** with a 2,5,6,7,8,9-hexahydro-3*H*-1,2,4-triazolo[4,3-*a*]azepin-3-one moiety also displayed comparable activity to that of **7b**; however the potency of **7a** with a 2,5,6,7-tetrahydro-3*H*-pyrrolo[2,1-*c*]-1,2,4-triazol-3-one group was decreased.

In a series of 4-(4-fluorobenzoyl)piperidine derivatives, compound **21b** with a 6,7,8,9-tetrahydro-2*H*-pyrido[1,2-*a*]-1,3,5-triazine-2,4(3*H*)-dione moiety displayed potent in vitro 5-HT$_2$ antagonist activity and in vivo potency comparable to that of ketanserin (**1**). The corresponding

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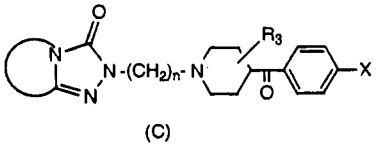
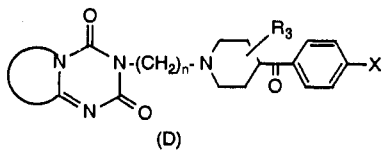
Table II. 4-(4-Fluorobenzoyl)piperidine Derivatives and Their Congeners



(C)



(D)

compd[a]	structure: type[b]	n	R_3	X	mp (°C)	formula[c]	in vitro (pA_2)[d]: 5-HT_2[e]	α_1[f]	dose (mg/kg)	in vivo (% inhibn)[g]: 5-HT_2	α_1
12a	C	2	H	F	215–217	$C_{19}H_{23}FN_4O_2 \cdot HCl$	6.8	6.4	10	65 ± 12	36 ± 7
12b	C	2	H	F	217–219	$C_{20}H_{26}FN_4O_2 \cdot HCl$	7.4	<6	10	91 ± 8	32 ± 7
12c	C	2	H	F	229–231	$C_{21}H_{27}FN_4O_2 \cdot HCl$	NC[i]	NC[i]		NT	NT
12d	C	2	H	F	236–239	$C_{19}H_{23}FN_4O_3 \cdot HCl \cdot {}^1/_2H_2O$	7.4	6.3	10	62 ± 4	36 ± 4
13b	C	2	H	Cl	247–258	$C_{20}H_{25}ClN_4O_2 \cdot HCl$	7.2	7.2	10	83 ± 7	16 ± 6
14b	C	2	4-OH	F	202–204	$C_{20}H_{25}FN_4O_3 \cdot HCl$	6.8	7.0	10	85 ± 10	15 ± 5
14c	C	3	4-OH	F	195–197	$C_{21}H_{27}FN_4O_3 \cdot HCl$	7.3	7.6	10	72 ± 11	9 ± 3
15b	C	2	4-(4-F-C_6H_4)	F	139–142	$C_{26}H_{29}FN_4O_2 \cdot HCl \cdot H_2O$	6.0	<6	10	11 ± 11	6 ± 6
16b	C	2	*trans*-3-(4-F-C_6H_4)	F	90–95	$C_{26}H_{29}F_2N_4O_2 \cdot C_4H_4O_4$[j]	6.8	6.8	10	90 ± 4	15 ± 8
17b	C	3	H	F	209–211	$C_{21}H_{27}FN_4O_2 \cdot HCl \cdot {}^1/_2H_2O$	7.7	7.8		NT	NT
17c	C	3	H	F	213–215	$C_{22}H_{29}FN_4O_2 \cdot HCl$	NC[i]	NC[i]		NT	NT
21a	D	2	H	F	253–255	$C_{20}H_{23}FN_4O_3 \cdot HCl \cdot {}^1/_2H_2O$	7.3	7.1	10	67 ± 9	23 ± 8
21b	D	2	H	F	254–258	$C_{21}H_{25}FN_4O_3 \cdot HCl \cdot {}^1/_2H_2O$	8.1	6.9	10	93 ± 4	33 ± 5
									10[h]	90 ± 4	22 ± 12
									3	93 ± 4	8 ± 2
									3[h]	83 ± 8	7 ± 3
21c	D	2	H	F	172–176	$C_{22}H_{27}FN_4O_3 \cdot 2HCl$	7.8	6.9	10	78 ± 15	15 ± 6
21g	D	2	H	F	243–244	$C_{21}H_{21}FN_4O_3 \cdot 2HCl$	8.1	7.5	10[h]	56 ± 10	14 ± 6
21h	D	2	H	F	278–280	$C_{19}H_{19}FN_4O_3S \cdot HCl \cdot {}^1/_2H_2O$	7.9	NC[i]	10	79 ± 9	50 ± 9
22b	D	3	H	F	87–89	$C_{22}H_{27}FN_4O_3 \cdot C_4H_4O_4 \cdot {}^1/_2H_2O$[j]	6.5	7.3	10	78 ± 9	53 ± 2
23b	D	2	4-OH	F	178–184	$C_{21}H_{25}FN_4O_4 \cdot 2HCl \cdot {}^1/_2H_2O$	6.7	7.2	10	83 ± 4	20 ± 10
1	(ketanserin)						8.6	7.8	10	96 ± 4	91 ± 1
									10[h]	100 ± 0	80 ± 2
									3	96 ± 4	52 ± 5
									3[h]	84 ± 9	46 ± 6

[a] See Schemes I and II. [b] See top of the table. [c] All compounds analyzed for C, H, and N were within 0.4% of the calculated formula values. [d] pA_2 values were obtained by a single determination for isolated rat thoracic aortic strips. [e,f] Antagonistic activity against the contraction induced by 5-HT and phenylephrine, respectively. [g] Inhibitory activity (mean ± SEM) in rats against elevation of blood pressure caused by 5-HT (300 μg/kg, iv) or phenylephrine (30 μg/kg, iv) after 1 h of oral administration of the test compound. [h] Inhibitory activity (mean ± SEM) after 3 h of oral administration of the test compound. [i] Noncompetitive antagonism. [j] Maleate.

5,6,7,8-tetrahydro-1,2,4-triazolo[4,3-*a*]pyridin-3(2*H*)-one derivative **12b** also had potent activity in vivo. The elongation of the alkylene chain between the triazole and piperidine rings from an ethylene to a trimethylene group resulted in a tendency toward noncompetitive 5-HT_2 antagonist activity.

Although all of the 4-[bis(4-fluorophenyl)methylene]-piperidine derivatives had moderate in vitro α_1 antagonist activity, they exhibited little activity in vivo as expected from the pharmacological profile of ritanserin (**2**).[14,15] On the other hand, 4-(4-fluorobenzoyl)piperidine derivatives had more potent α_1 antagonist activity. The discrepancy between the in vitro and in vivo α_1 antagonist activities of the 4-[bis(4-fluorophenyl)methylene]piperidine derivatives can be explained by the difference in drug–receptor dissociation time. Leysen et al. have reported that ritanserin (**2**) dissociates from α_1-adrenoceptor faster than the 5-HT_2 receptor, but ketanserin (**1**) has almost the same dissociation time with respect to the both receptors.[27] Comparing the two structures of the 4-(4-fluorobenzoyl)-piperidine and 4-[bis(4-fluorophenyl)methylene]piperidine groups, we speculated that introduction of a bulky substituent around the 4-position of the piperidine ring of **12b** could decrease α_1 antagonist activity. We then synthesized compounds substituted by a bulky group at the 4- or 3-position. Compound **16b**, with a 4-fluorophenyl group at the 3-position, appeared to have reduced α_1 antagonist activity in vivo without a serious decrease in the 5-HT_2 antagonist activity. Introduction of a phenyl group into the 4-position caused a marked decrease in the 5-HT_2 antagonist activity (**15b**), possibly indicating that the bulky 4-phenyl group forces the 4-fluorobenzoyl group into an unfavorable conformation because a smaller hydroxy group at the same position did not substantially affect the potency. In addition, we investigated the possibility of exchanging the 4-fluorophenyl groups of 4-[bis(4-fluorophenyl)methylene]piperidine moiety for other substituents. Replacement of one of the two 4-fluorophenyl groups of the 4-[bis(4-fluorophenyl)methylene]piperidine moiety with a phenyl group almost retained the in vivo 5-HT_2 antagonist activity, while similar modification of the both groups resulted in a marked decrease in potency (**7c** ≥ **8c** ≫ **9c**). Other modifications of the moiety also led to loss of activity (**10b** and **11b**).

Table III. Blockage of Head Twitches in Mice

dose, mg/kg	% inhibition in mice (n = 4)[a]: **7b**	ritanserin (**2**)	**21b**	ketanserin (**1**)
0.1			49[b]	49[b]
0.3			59[b]	88[b]
1		80	81[b]	93[b]
3	13	95	67	92
10	55	100	80	100
30	85			

[a] Inhibitory activities were measured for 5 min and expressed as percentages versus control values (n = 6) of head twitches induced by 5-HTP (100 mg/kg, ip). [b] Inhibitory activities for 8 min.

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Compounds **7b**, **21b**, **1**, and **2** were tested for inhibitory activity on head twitches induced by 5-hydroxytryptophan (5-HTP) to estimate central 5-HT_2 antagonist activity (Table III). The reference compound **1**, classified as a peripheral 5-HT_2 antagonist,[28] was almost as potent as **2** in blocking head twitches. Compound **2** caused 80% inhibition at the oral dose of 1 mg/kg; however, **7b** had no effect at 3 mg/kg and blocked it by 85% at 30 mg/kg. The potency of the central 5-HT_2 receptor antagonism of **7b** was approximately $^1/_{30}$ that of **2**. As seen in Tables I and II, compound **7b** had potent in vitro and in vivo 5-HT_2 antagonist activities comparable to those of **1** and **2**, but the central 5-HT_2 antagonist activity of **7b** was weaker than that of the reference compounds. On the other hand, compound **21b** had fairly potent activity in blocking head twitches but was approximately three times less potent than **1**; **21b** and **1** exhibited almost the same activity at 1 mg/kg and 0.3 mg/kg, respectively. However, toxicological evaluation of **7b** in rats revealed hepatotoxicity when orally administered at 50 mg/kg per day for a week.[29] Since all of the compounds with the 4-[bis(4-fluorophenyl)methylene]piperidine group showed the same toxicity, it may be due to the common amine moiety. By contrast, **21b** did not exhibit toxicity. Thus, although **7b** was more favorable than **21b** in respect to the lack of central 5-HT_2 antagonist activity and hypotensive activity, **7b** would not be safe enough to be developed as a cardiovascular drug. On the other hand, compound **21b** also displayed peripheral 5-HT_2 antagonist activity comparable to that of ketanserin (**1**), without potent α_1 antagonist activity. Considering that **1** is used as an antihypertensive agent with occasional CNS side effects such as dizziness and dry mouth,[30] the central 5-HT_2 antagonist activity of **21b** may be acceptable for development of the compound for treatment of myocardial ischemia associated with unstable angina in which 5-HT has been suggested to play an important role.[31-34] Further pharmacological evaluation of **21b** will be reported elsewhere.

Experimental Section

Melting points are uncorrected. Analyses for C, H, and N were within ±0.4% of theoretical values, and ^{1}H NMR spectra were recorded with JEOL JNM-FX90Q spectrometers (Me_4Si as an internal standard). For column chromatography, silica gel (Merck, Kieselgel 60, 0.05–0.2 mm) was used.

Method A. (a) 2-(2-Chloroethyl)-5,6,7,8-tetrahydro-1,2,4-triazolo[4,3-*a*]pyridin-3(2*H*)-one (5b). A mixture of 5,6,7,8-tetrahydro-1,2,4-triazolo[4,3-*a*]pyridin-3(2*H*)-one (**4b**) (61.0 g, 0.438 mol), 1-bromo-2-chloroethane (164.0 g, 1.14 mol), and K_2CO_3 (90.8 g, 0.657 mol) in MeCN (500 mL) was refluxed for 8 h. After removal of the insoluble material by filtration, the filtrate was concentrated in vacuo, and the residue was extracted with $CHCl_3$. After evaporation of the solvent, the residual oil was purified by column chromatography ($CHCl_3$/EtOH, 20:1) to give an oil, which was crystallized from a mixture of Et_2O and 2-PrOH to give **5b** (59.8 g, 67.6%) as colorless crystals: mp 46–49 °C; NMR ($CDCl_3$) δ 1.7–2.1 (4 H, m), 2.68 (2 H, t), 3.62 (2 H, t), 3.77 (2 H, t), 4.07 (2 H, t). Anal. ($C_8H_{12}ClN_3O$) C, H, N.

(b) 2-[2-[4-[Bis(4-fluorophenyl)methylene]piperidin-1-yl]ethyl]-5,6,7,8-tetrahydro-1,2,4-triazolo[4,3-*a*]pyridin-3(2*H*)-one Hydrochloride Sesquihydrate (7b). After a mixture of **5b** (26.8 g, 0.133 mol) and NaI (31.5 g, 0.21 mol) in MeCN (400 mL) was refluxed for 0.5 h, 4-[bis(4-fluorophenyl)methylene]-piperidine (38.2 g, 0.134 mol) and K_2CO_3 (27.6 g, 0.2 mol) were added to the mixture, and the mixture was refluxed for 8 h. After removal of the insoluble material by filtration, the solvent was evaporated in vacuo. The residue was extracted with $CHCl_3$, and the extract was concentrated in vacuo. The residue was purified by column chromatography ($CHCl_3$/EtOH, 20:1) to give the free base of **7b** (53.0 g, 88.5%) as an oil: NMR ($CDCl_3$) δ 1.7–2.2 (4 H, m), 2.2–2.9 (12 H, m), 3.6 (2 H, t), 3.92 (2 H, t), 6.9–7.2 (8 H, m).

The free base of **7b** obtained above was treated with concentrated HCl to give **7b** as colorless crystals: mp 128–130 °C; NMR (DMSO-d_6) δ 1.68–2.0 (4 H, m), 2.3–3.8 (14 H, m), 4.11 (2 H, t-like), 7.18 (4 H, s), 7.22 (4 H, s). Anal. ($C_{26}H_{28}F_2N_4O\cdot HCl\cdot^3/_2H_2O$) C, H, N.

Method B. (a) 6,7,8,9-Tetrahydro-2*H*-pyrido[1,2-*a*]-1,3,5-triazine-2,4(3*H*)-dione (19b). 2-Amino-3,4,5,6-tetrahydropyridine hydrochloride (**18b**) (4.8 g, 36 mmol) was added to a solution which was prepared from Na (0.83 g, 36 mmol) and EtOH (40 mL). After the mixture was stirred for 0.5 h, the insoluble material was filtered, and the filtrate was concentrated to dryness in vacuo. The residue was dissolved in THF (30 mL), and freshly prepared phenoxycarbonyl isocyanate (5.9 g, 36 mmol) was added dropwise to the solution with ice-cooling. After being stirred at room temperature for 16 h, the mixture was concentrated in vacuo, and the residue was purified by column chromatography ($CHCl_3$/MeOH, 20:1) to give **19b** (2.04 g, 57%) as colorless crystals: mp 185–187 °C; NMR (DMSO-d_6) δ 1.6–1.9 (4 H, m), 2.65 (2 H, t), 3.64 (2 H, t), 11.39 (1 H, br). Anal. ($C_7H_9N_3O_2$) C, H, N.

(b) 3-[2-[4-(4-Fluorobenzoyl)piperidin-1-yl]ethyl]-6,7,8,9-tetrahydro-2*H*-pyrido[1,2-*a*]-1,3,5-triazine-2,4(3*H*)-dione Hydrochloride (21b). A solution of diethyl azodicarboxylate (2.1 g, 12 mmol) in DMF (5 mL) was added dropwise to an ice-cooled solution of **19b** (1.67 g, 10 mmol), 2-[4-(4-fluorobenzoyl)piperidin-1-yl]ethanol (2.76 g, 11 mmol) and triphenylphosphine (3.15 g, 12 mmol) in DMF (50 mL). The reaction mixture was stirred at room temperature for 0.5 h. After the mixture was concentrated in vacuo, the residue was purified by column chromatography ($CHCl_3$/MeOH, 30:1) to give the free base of **21b** (1.70 g, 43%) as colorless crystals: mp 153–155 °C; NMR ($CDCl_3$) δ 1.6–2.2 (10 H, m), 2.5–2.9 (4 H, m), 3.1 (3 H, m), 3.84 (2 H, t-like), 4.05 (2 H, t), 7.13 (2 H, t), 7.95 (2 H, dd).

The free base of **21b** obtained above was treated with concentrated HCl to give **21b** as colorless crystals: mp 254–258 °C; NMR (DMSO-d_6) δ 1.6–2.1 (8 H, m), 2.2 (3 H, m), 2.69 (2 H, t), 3.7 (4 H, m), 4.16 (2 H, t-like), 7.38 (2 H, t), 8.1 (2 H, dd), 10.2 (1 H, br). Anal. ($C_{21}H_{25}FN_4O_3$) C, H, N.

5-HT_2 or α_1 Antagonistic Activity in the Rat Thoracic Aortic Strips. The thoracic aorta was removed from a male SD rat weighing 220–250 g (Japan SLC, Inc.) and cut into vascular rings of 4–5 mm in length. Preparations were suspended under a tension of 1 g in a modified Krebs–Henseleit solution at 35 °C and aerated by a gas mixture of 95% O_2 and 5% CO_2. The composition of the solution was as follows (nM): NaCl 112, KCl 5, $CaCl_2$ 1.5, $MgSO_4$ 1.2, NaH_2PO_4 1.2, $NaHCO_3$ 25, and glucose 11. Isometric tension was recorded using a TB-652T (Nihon Kohden) transducer connected to a polygraph (Nihon Kohden). After an equilibration period of 120 min, aortic strips were contracted with 60 mM KCl.

To test the anti-5-HT_2 activity, the preparations were contracted with 5-HT (0.1–100 μM), cumulatively. Then the prep-

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arations were washed, and after 1 h, the second cumulative contractions of aortic strips by 5-HT were observed in the presence of test drugs. The anti-5-HT_2 activity of the drug was calculated from the dose–response curve and expressed as a pA_2 value if its blockade was competitive.

To test the anti-α_1 activity, phenylephrine (0.01–10 μM) was given to the preparations instead of 5-HT.

5-HT_2 or α_1 Antagonistic Activity in Rats in Vivo. A male SD rat was anesthetized with urethane (1 g/kg, ip) and alpha-chloralose (80 mg/kg, ip). A catheter, connected to a pressure transducer, was inserted into the carotid artery to measure blood pressure, and a venous catheter was cannulated for drug injection. After 1 h of oral administration of a test drug or vehicle, 5-HT (300 μg/kg, iv) was injected, and the change of the hypertensive phase, immediately after a transient hypotensive phase, was measured. The anti-5-HT_2 activity was expressed as an inhibition percentage.

To test anti-α_1 activity, phenylephrine (30 μg/kg, iv) was injected into rats instead of 5-HT and the change in the blood pressure was measured.

Inhibition of Head-Twitch Response in Mice. A test drug was orally given to mice 1 h before administration of 5-hydroxytryptophan (5-HTP: 100 mg/kg, ip). Numbers of head-twitch responses were counted for 5 min (or 8 min) at 30 min after injection of 5-HTP. Inhibitory activities were expressed as percentages versus control values.

Registry No. **4a**, 116056-07-6; **4b**, 118801-67-5; **4c**, 27182-43-0; **4d**, 133365-36-3; **4e**, 133365-38-5; **4f**, 133365-37-4; **4g**, 6969-71-7; **5a**, 133365-54-5; **5b**, 133365-52-3; **5c**, 133365-53-4; **5d**, 134951-50-1; **5e**, 134951-51-2; **5f**, 133365-55-6; **5g**, 137540-94-4; **6b**, 133365-56-7; **6c**, 133365-61-4; **7a**, 133364-92-8; **7a**·HCl, 133364-93-9; **7b**, 133364-85-9; **7b**·HCl, 133364-84-8; **7c**, 133372-09-5; **7c**·HCl, 133364-87-1; **7d**, 133364-94-0; **7d**·HCl, 133364-95-1; **7e**, 133364-98-4; **7e**·HCl, 133364-99-5; **7f**, 133364-96-2; **7f**·HCl, 133364-97-3; **7g**, 137540-95-5; **7g**·HCl, 133364-91-7; **8c**, 133364-88-2; **8c**·HCl, 133364-89-3; **9c**, 137540-96-6; **9c**·HCl, 133364-90-6; **10b**, 137540-97-7; **10b**·HCl, 133365-00-1; **11b**, 137540-98-8; **12a**, 137540-99-9; **12a**·HCl, 133365-11-4; **12b**, 133365-03-4; **12b**·HCl, 133365-04-5; **12c**, 133365-07-8; **12c**·HCl, 133365-08-9; **12d**, 133365-13-6; **12d**·HCl, 133365-14-7; **13b**, 133365-27-2; **13b**·HCl, 133365-28-3; **14b**, 133365-15-8; **14b**·HCl, 133365-16-9; **14c**, 133365-17-0; **14c**·HCl, 133365-18-1; **15b**, 133365-25-0; **15b**·HCl, 133365-26-1; **16b**, 133365-19-2; **16b**·maleate (1:1), 133365-20-5; **17b**, 133365-05-6; **17b**·HCl, 133365-06-7; **17c**, 133365-09-0; **17c**·HCl, 133365-10-3; **18b**, 16011-96-4; **19b**, 133365-43-2; **20a**, 133364-77-9; **20a**·2HCl, 133364-78-0; **20b**, 133365-29-4; **20b**·2HCl, 133364-76-8; **20h**, 137541-00-5; **20h**·HCl, 133364-79-1; **21a**, 133405-29-5; **21a**·HCl, 133364-64-4; **21b**, 133364-61-1; **21b**·HCl, 133364-62-2; **21c**, 133364-65-5; **21c**·2HCl, 133364-66-6; **21g**, 137541-01-6; **21g**·2HCl, 137541-02-7; **21h**, 133364-74-6; **21h**·HCl, 133364-75-7; **22b**, 133364-67-7; **22b**·maleate (1:1), 133364-68-8; **23b**, 133364-69-9; **23b**·2HCl, 133364-70-2; 1-bromo-2-chloroethane, 107-04-0; 4-[bis(4-fluorophenyl)methylene]piperidine, 58113-36-3; phenoxycarbonyl isocyanate, 5843-43-6; 2-[4-(4-fluorobenzoyl)piperidin-1-yl]ethanol, 106088-85-1.

Communications to the Editor

Synthesis, Stability, and Biological Evaluation of Water-Soluble Prodrugs of a New Echinocandin Lipopeptide. Discovery of a Potential Clinical Agent for the Treatment of Systemic Candidiasis and *Pneumocystis carinii* Pneumonia (PCP)

The development of more efficacious agents against opportunistic infections is critical due to the growing population of immunocompromised individuals.[1] Mycoses are common among AIDS, organ transplant, and cancer chemotherapy patients.[2] It has been shown that 1,3-β-glucan synthesis inhibitors are effective antifungal agents against *Candida* species, especially *Candida albicans*.[3] These inhibitors were believed to be of very narrow spectrum; however, it appears that a broader range of organisms is susceptible.[4,8a] Of particular importance was the discovery that the cell wall of the cyst form of *Pneumocystis carinii* contained 1,3-β-glucans.[5a] *P. carinii*, whose phylogeny has been the subject of recent controversy,[5] is an opportunistic organism responsible for an often fatal pneumonitis among HIV patients and other

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