Syntheses and 5-HT₂ Antagonist Activity of Bicyclic 1,2,4-Triazol-3(2H)-one and 1,3,5-Triazine-2,4(3H)-dione Derivatives

Yoshifumi Watanabe,* Hiroyuki Usui, Shozo Kobayashi, Hirotaka Yoshiwara, Toshiro Shibano, Tsuyoshi Tanaka, Yoshiyuki Morishima, Megumi Yasuoka, and Munefumi Kanao

Research Institute, Daiichi Pharmaceutical Co., Ltd., 16-13, Kitakasai 1-Chome Edogawa-ku, Tokyo 134, Japan. Received February 25, 1991

A series of bicyclic 1,2,4-triazol-3(2H)-one and 1,3,5-triazine-2,4(3H)-dione derivatives with a 4-[bis(4-fluorophenyl)methylene]piperidine or 4-(4-fluorobenzoyl)piperidine group has been prepared and tested for 5-HT₂ 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(2H)-one (7b) had the most potent 5-HT₂ antagonist activity, which was greater than ritanserin (2), while 7b did not show α_1 antagonist activity in vivo. The central 5-HT₂ 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-2H-pyrido[1,2-a]-1,3,5-triazine-2,4(3H)-dione also displayed potent 5-HT₂ 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 [1,2-a]-1,3,5-triazine-2,4(3H)-dione ring systems are useful components of 5-HT₂ 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 TXA₂, norepinephrine, angiotensin II, ADP, and collagen.¹⁻⁴ Since these activities have been demonstrated to be mediated through 5-HT₂ receptors,⁵⁻⁷ peripheral 5-HT₂ antagonists are expected to be useful in the treatment of cardiovascular diseases. Recently, a peripheral 5-HT₂ 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 ischemia⁸ and Raynaud's phenomenon.⁹ The compound is not only a potent 5-HT₂ 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),

- De Clerck, F.; David, J.-L.; Janssen, P. A. J. Inhibition of 5-Hydroxytryptamine-Induced and -Amplified Human Platelet Aggregation by Ketanserin (R41,468), a Selective 5-HT₂-receptor Antagonist. Agents Actions 1982, 12, 388-397.
- (2) Van Nueten, J. M. 5-Hydroxytryptamine and Precapillary Vessels. Fed. Proc. 1983, 42, 223-227.
- (3) De Clerck, F.; Herman, A. G. 5-Hydroxytryptamine and Platelet Aggregation. Fed. Proc. 1983, 42, 228-232.
- (4) De Clerck, F.; Van Nueten, J. M.; Reneman, R. S. Platelet-Vessel Wall Interactions: Implication of 5-Hydroxytryptamine. A Review. Agents Actions 1984, 15, 612-626.
- (5) Cohen, M. L.; Fuller, R. W.; Eiley, K. S. Evidence for 5-HT₂ Receptors Mediating Contraction in Vascular Smooth Muscle. J. Pharmacol. Exp. Ther. 1981, 218, 421-425.
- (6) Vanhoutte, P. M. 5-Hydroxytryptamine and Vascular Disease. Fed. Proc. 1983, 42, 233-237.
- (7) De Cree, J.; Leempoels, J.; Demoen, B.; Roels, V.; Verhaegen, H. The Effect of Ketanserin, a 5-HT₂-receptor Antagonist, on 5-Hydroxytryptamine-Induced Irreversible Platelet Aggregation in Patients with Cardiovascular Diseases. Agents Actions 1985, 16, 313-317.
- (8) Prevention of Atherosclerotic Complications with Ketanserin Trial Group. Prevention of Atherosclerotic Complications: Controlled Trial of Ketanserin. Br. Med. J. 1989, 298, 424-430.
- (9) van de Wal, H. J.; Wijn, P. F.; van Lier, H. J.; Skotnicki, S. H. Quantitative Study of the Effects of Ketanserin in Patients with Primary Raynaud's Phenomenon. A Randomized, Double Blind, Placebo Controlled Investigation and an Additional Long Term Open Trial. *Microcirc. Endothelium Lymphatics* 1985, 2, 657-685.
- (10) Fozard, J. R. Mechanism of the Hypotensive Effect of Ketanserin. J. Cardiovasc. Pharmacol. 1982, 4, 829-838.

which is a central 5-HT₂ 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 dogs¹⁶⁻¹⁸ 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(4fluorophenyl)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

- (11) Cohen, M. L.; Fuller, R. W.; Kurz, K. D. Evidence that Blood Pressure Reduction by Serotonin Antagonists Is Related to Alpha Receptor Blockade in Spontaneously Hypertensive Rats. Hypertension (Dallas) 1983, 5, 676-681.
- (12) Reyntjens, A.; Gelders, Y. G.; Hoppenbrouwers, M.-L. J. A.; Bussche, G. V. Thymosthenic Effects of Ritanserin (R55667), a Centrally Acting Serotonin-S₂ Receptor Blocker. Drug Dev. Res. 1986, 8, 205-211.
- (13) Hosie, J.; Stott, D. J.; Robertson, J. I. S.; Ball, S. G. Does Acute Serotonergic Type-2 Antagonism Reduce Blood Pressure? Comparative Effects of Single Doses of Ritanserin and Ketanserin in Essential Hypertension. J. Cardiovasc. Pharmacol. 1987, 10 (Suppl. 3), S86-S88.
- (14) Persson, B.; Gradin, K.; Pettersson, A.; Hedner, T. Antihypertensive Effects of Ketanserin and Ritanserin in the Spontaneously Hypertensive Rat. J. Cardiovasc. Pharmacol. 1988, 11 (Suppl. 1), S22-S24.
- (15) Hedner, T.; Persson, B. Experience with Ketanserin and Ritanserin in Hypertensive Patients. J. Cardiovasc. Pharmacol. 1988, 11 (Suppl. 1), S44-S48.
- (16) Ashton, J. H.; Benedict, C. R.; Fitzgerald, C.; Raheja, S.; Taylor, A.; Campbell, W. B.; Buja, L. M.; Willerson, J. T. Serotonin as a Mediator of Cyclic Flow Variations in Stenosed Canine Coronary Arteries. *Circulation* 1986, 73, 572-578.
- (17) Bush, L. R. Effects of the Serotonin Antagonists, Cyproheptadine, Ketanserin and Mianserin, on Cyclic Flow Reductions in Stenosed Canine Coronary Arteries. J. Pharmacol. Exp. Ther. 1987, 240, 674-682.
- (18) Torr, S.; Noble, M. I. M.; Folts, J. D. Inhibition of Acute Platelet Thrombosis Formation in Stenosed Canine Coronary Arteries by Specific Serotonin 5-HT₂ Receptor Antagonist Ritanserin. Cardiovas. Res. 1990, 24, 465-470.



1 Ketanserin



2 Ritanserin

the oil/water partition coefficient.^{19,20} In a search for 5-HT₂ antagonists, we found that conversion of the quinazoline-2,4(1H,3H)-dione group of 1 into the corresponding 5,6,7,8-tetrahydroquinazoline-2,4(1H,3H)-dione group retained the 5-HT₂ antagonist activity,²¹ and we felt that further modification of this ring could produce peripherally selective 5-HT₂ antagonists. Thus, we designed and synthesized bicyclic 1,3,5-triazine-2,4(3H)-diones and 1,2,4triazol-3(2H)-ones as potential 5-HT₂ antagonists. In this paper, we describe the syntheses and pharmacological evaluations of these compounds.

Chemistry

Bicyclic 1,2,4-triazol-3(2H)-one derivatives 7-17 were prepared as outlined in Scheme I. The 1,2,4-triazol-3-(2H)-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(3H)-dione derivatives 20–23 were prepared as outlined in Scheme II. Pyrido[1,2-a]-1,3,5-triazine-2,4(3H)-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(3H)-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 reaction²⁴ of 19 with

- (19) Hansch, C.; Steward, A. R.; Anderson, S. M.; Bentley, D. The Parabolic Dependence of Drug Action upon Lipophilic Character as Revealed by a Study of Hypnotics. J. Med. Chem. 1967, 11, 1-11.
- (20) Levin, V. A. Relationship of Octanol/Water Partition Coefficient and Molecular Weight to Rat Brain Capillary Permeability. J. Med. Chem. 1980, 23, 682–684.
- (21) Watanabe, Y.; Usui, H.; Shibano, T.; Tanaka, T.; Kanao, M. Syntheses of Monocyclic and Bicyclic 2,4(1H,3H)-Pyrimidinediones and their Serotonin 2 Antagonist Activities. Chem. Pharm. Bull. 1990, 38, 2726-2732.
- (22) Petersen, S.; Tietze, E. Reactions of Cyclic Lactim Ethers with Acylhydrazine Derivatives. *Chem. Ber.* 1957, 90, 909-921.
- (23) Kamal, A.; Sattur, P. B. A One-Pot Synthesis of Ring-Fused 1,3,5-Triazine-1,4(3H)-diones: Reactions with Chlorocarbonyl Isocyanate. Synthesis 1985, 892-3.





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

Biological Evaluation and Discussion

Both 5-HT₂ 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

⁽²⁴⁾ Mitsunobu, O. The Use of Diethyl Azodicarboxylate and Triphenylphosphine in Synthesis and Transformation of Natural Products. Synthesis 1981, 1-28.

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



	structure				in vitro $(pA_2)^d$		dose	in vivo (% inhibn) ^g		
$compd^a$	type ^b	R ₁	R_2	mp (°C)	formula ^c	5-HT2 ^e	α_1^f	(mg/kg)	$5-HT_2$	α ₁
	A	4-F-C ₆ H ₄	4-F-C ₆ H ₄	123-125	C ₂₅ H ₂₆ F ₂ N ₄ O·HCl·CH ₄ O ⁱ	7.1	6.9	10	74 ± 2	6 ± 3
7b	Α	4-F-C ₆ H₄	4-F-C ₆ H₄	127 - 130	$C_{26}H_{28}F_2N_4O \cdot HCl^{-3}/_2H_2O$	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	Α	4-F-C ₆ H₄	4-F-C ₆ H₄	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	Α	4-F-C ₆ H₄	4-F-C ₆ H₄	13 9– 144	C ₂₅ H ₂₆ F ₂ N ₄ O ₂ ·HCl	7.2	6.6	10	60 ± 10	10 ± 2
7e	Α	4-F-C _s H₄	4-F-C ₆ H₄	178-180	$C_{25}H_{26}F_2N_4OS \cdot HCl \cdot 1/_2H_2O$	7.8	7.5	10	67 ± 7	4 ± 3
7 f	A	4-F-C _e H	4-F-C ₆ H₄	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
78	Ā	4-F-C _€ H₄	4-F-C _e H₄	127-129	C ₂₆ H ₂₄ F ₂ N ₄ O·HCl	7.0	6.6	10	58 ± 9	6 ± 4
8c	Α	4-F-C ₆ H₄	Ph	11 9–12 1	C ₂₇ H ₃ FN ₄ O·HCl·C ₂ H ₆ O ⁱ	7.3	6.4	10	84 ± 3	7 ± 7
9c	Α	Ph	Ph	117-119	C ₂₇ H ₃₂ N ₄ O·HCl·C ₂ H ₆ O ^j	7.0	<6	10	42 ± 7	3 ± 2
10b	A	4-MeO-C ₆ H ₄	4-MeO-C ₆ H ₄	193-203	C ₂₈ H ₃₀ N ₄ O ₃ ·HCl·H ₂ O	6.8	7.2	10	41 ± 6	4 ± 2
11b	Α	4-F-C ₆ H₄	CN	114-115	C ₂₁ H ₂₄ FN ₅ O	6.7	6.3	10	32 ± 3	2 ± 2
20a	В	4-F-C ₆ H ₄	4-F-C ₆ H₄	173-180	$C_{26}H_{26}F_2N_4O_2\cdot 2HCl$	8.3	6.8	10	83 ± 7	8±5
20b	В	4-F-C ₆ H₄	4-F-C ₆ H ₄	192-195	$C_{27}H_{28}F_2N_4O_2 \cdot 2HCl$	8.3	6.8	10	93 ± 3	12 ± 4
20h	В	4-F-C ₆ H₄	$4 - F - C_6 H_4$	251-253	C ₂₅ H ₂₂ F ₂ N ₄ O ₂ S·HCl	7.1	<6	10	93 ± 5	20 ± 4
2	(ritanserin)					8.8	7.5	10	84 ± 6	7±7

^aSee Schemes I and II. ^bSee top of the table. ^cAll 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. ^gInhibitory activity (mean ± SEM) in rats against elevation of blood pressure caused by 5-HT (300 $\mu g/kg$, iv) or phenylephrine (30 $\mu g/kg$, iv) after 1 h of oral administration of the test compound. ^hInhibitory activity (mean ± SEM) after 3 h of oral administration of the test compound. ⁱMethanolate. ^jEthanolate.

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 5HT₃, 5-HT₂, and 5-HT₁ receptors, respectively.²⁶ Then, the potency of the in vivo 5-HT₂ 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-HT₂ 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-HT₂ 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(2H)-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-(2H)-one group diminished the activity (7d and 7e), suggesting that this part could interact with a lipophilic site of the 5-HT₂ 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 2,5,6,7,8,9hexahydro-3H-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-3H-pyrrolo[2,1c]-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[1,2a]-1,3,5-triazine-2,4(3*H*)-dione moiety displayed potent in vitro 5-HT₂ antagonist activity and in vivo potency comparable to that of ketanserin (1). The corresponding

⁽²⁵⁾ Arunlakshana, O.; Schild, H. O. Some Quantitative Uses of Drug Antagonists. Br. J. Pharmacol. 1959, 14, 48-58.

⁽²⁶⁾ Kalkman, H. O.; Engel, G.; Hoyer, D. Three Distinct Subtypes of Serotonergic Receptors Mediate the Triphasic Blood Pressure Response to Serotonin in Rats. J. Hypertension 1984, 2 (Suppl. 3), 143-145.

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





4. <u></u> 1.1	structure					in vitro $(pA_2)^d$		dose	in vivo (% inhibn)#		
compd ^a	type ^b	n	R ₃	Х	mp (°C)	formula ^c	5-HT ₂ ^e	α_1^f	(mg/kg)	$5-HT_2$	α1
12a	С	2	Н	F	215-217	C ₁₉ H ₂₃ FN ₄ O ₂ ·HCl	6.8	6.4	10	65 ± 12	36 ± 7
1 2b	С	2	Н	F	217-21 9	C ₂₀ H ₂₅ FN ₄ O ₂ ·HCl	7.4	<6	10	91 ± 8	32 ± 7
1 2c	С	2	Н	F	22 9– 231	$C_{21}H_{27}FN_4O_2 \cdot HCl$	NC^i	NC^i		NT	NT
12d	С	2	Н	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
1 3b	С	2	Н	Cl	247-258	C ₂₀ H ₂₅ ClN ₄ O ₂ ·HCl	7.2	7.2	10	83 ± 7	16 ± 6
14b	С	2	4-0H	F	202-204	C ₂₀ H ₂₅ FN ₄ O ₃ ·HCl	6.8	7.0	10	85 ± 10	15 ± 5
1 4c	С	3	4-0H	F	195–197	C ₂₁ H ₂₇ FN ₄ O ₃ ·HCl	7.3	7.6	10	72 ± 11	9±3
15 b	С	2	4-(4-F-C ₆ H ₄)	\mathbf{F}	13 9– 142	C ₂₆ H ₂₉ FN ₄ O ₂ ·HCl·H ₂ O	6.0	<6	10	11 ± 11	6±6
16b	С	2	$trans-3-(4-F-C_6H_4)$	F	90-95	C ₂₆ H ₂₉ F ₂ N ₄ O ₂ ·C ₄ H ₄ O ₄ ^j	6.8	6.8	10	90 ± 4	15 ± 8
17b	С	3	Н	F	20 9- 211	$C_{21}H_{27}FN_4O_2 \cdot HCl \cdot 1/_2H_2O$	7.7	7.8		NT	NT
17c	С	3	Н	F	213-215	C ₂₂ H ₂₉ FN ₄ O ₂ ·HCl	NC^i	NC^i		NT	NT
21a	D	2	Н	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	Н	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	Н	F	172-176	C ₂₂ H ₂₇ FN ₄ O ₃ •2HCl	7.8	6.9	10	78 ± 15	15 ± 6
21g	D	2	Н	F	243–244	$C_{21}H_{21}FN_4O_3$ ·2HCl	8.1	7.5	10^{h}	56 单 10	14 ± 6
21h	D	2	Н	F	278-280	C ₁₉ H ₁₉ FN ₄ O ₃ S·HCl ¹ / ₂ H ₂ O	7.9	NC^i	10	79 ± 9	50 ± 9
22Ь	D	3	Н	F	87-89	$C_{22}H_{27}FN_4O_3C_4H_4O_4^{-1}/_2H_2O^{-1}$	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	(keta	nse	rin)				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

^aSee Schemes I and II. ^bSee top of the table. ^cAll 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. ^{ef}Antagonistic activity against the contraction induced by 5-HT and phenylephrine, respectively. ^gInhibitory activity (mean ± SEM) in rats against elevation of blood pressure caused by 5-HT (300 μ g/kg, iv) or phenylephrine (30 μ g/kg, iv) after 1 h of oral administration of the test compound. ^hInhibitory activity (mean ± SEM) after 3 h of oral administration of the test compound. ⁱNaleate.

5,6,7,8-tetrahydro-1,2,4-triazolo[4,3-a]pyridin-3(2H)-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-HT₂ 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-HT₂ 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 3Table III. Blockage of Head Twitches in Mice

dose.	% inhibition in mice $(n = 4)^a$							
mg/kg	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.

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-HT₂ antagonist activity. Introduction of a phenyl group into the 4-position caused a marked decrease in the 5-HT₂ 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-HT₂ antagonist activity, while similar modification of the both groups resulted in a marked decrease in potency ($7c \ge 8c$ \gg 9c). Other modifications of the moiety also led to loss of activity (10b and 11b).

⁽²⁷⁾ Leysen, J. E.; Gommeren, W. Drug-Receptor Dissociation Time, New Tool for Drug Research: Receptor Binding Affinity and Drug-Receptor Dissociation Profiles of Serotonin-S₂, Dopamine-D₂, Histamine-H₁ Antagonists, and Opiates. Drug Dev. Res. **1986**, 8, 119-131.

$5-HT_2$ Antagonists

Compounds 7b, 21b, 1, and 2 were tested for inhibitory activity on head twitches induced by 5-hydroxytryptophan (5-HTP) to estimate central 5-HT₂ antagonist activity (Table III). The reference compound 1, classified as a peripheral 5-HT₂ 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-HT₂ 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-HT₂ antagonist activities comparable to those of 1 and 2, but the central 5-HT₂ 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-HT₂ 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-HT₂ 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-HT₂ 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 $\pm 0.4\%$ of theoretical values, and ¹H NMR spectra were recorded with JEOL JNM-FX90Q spectrometers (Me₄Si 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-1,2,4triazolo[4,3-a]pyridin-3(2H)-one (5b). A mixture of 5,6,7,8-

- (28) Awouters, F. The Pharmacology of Ketanserin, the First Selective Serotonin S₂-Antagonist. Drug Dev. Res. 1985, 6, 263-300.
- (29) Daiichi Pharmaceutical Co., Ltd., unpublished results.
- (30) Woittiez, A. J. J.; Wenting, C. J.; van den Meiracker, A. H.; van Eck Ritsema, H. J.; in't Veld Man, A. J.; Zantvoort, F. T. Chronic Effect of Ketanserin in Mild to Moderate Essential Hypertension. Hypertension (Dallas) 1986, 8, 167-173.
- (31) Rubanyi, G. M.; Frye, R. L.; Holmes, D. R., Jr.; Vanhoutte, P. M. Vasoconstrictor Activity of Coronary Sinus Plasma from Patients with Coronary Artery Disease. J. Am. Coll. Cardiol. 1987, 9, 1243-1249.
- (32) van den Berg, E. K.; Schmitz, J. M.; Benedict, C. R.; Malloy, C. R.; Willerson, J. T.; Dehmer, G. J. Transcardiac Serotonin Concentration Is Increased in Selected Patients with Limiting Angina and Complex Coronary Lesion Morphology. *Circulation* 1989, 79, 116-124.
- (33) Golino, P.; Piscione, F.; Willerson, J. T.; Cappelli-Bigazzi, M.; Focaccio, A.; Villari, B.; Indolfi, C.; Russolillo, E.; Condorelli, M.; Chiariello, M. Divergent Effects of Serotonin on Coronary-Artery Dimensions and Blood Flow in Patients with Coronary Atherosclerosis and Control Patients. N. Engl. J. Med. 1991, 324, 641-648.
- (34) Hillis, L. D.; Lange, R. A. Serotonin and Acute Ischemic Heart Disease. N. Engl. J. Med. 1991, 324, 688–690.

tetrahydro-1,2,4-triazolo[4,3-a]pyridin-3(2H)-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 CHCl₃. After evaporation of the solvent, the residual oil was purified by column chromatography (CHCl₃/EtOH, 20:1) to give an oil, which was crystallized from a mixture of Et₂O and 2-PrOH to give 59.8 g, 67.6%) as colorless crystals: mp 46–49 °C; NMR (CDCl₃) 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. (C₈H₁₂ClN₃O) C, H, N.

(b) 2-[2-[4-[Bis(4-fluorophenyl)methylene]piperidin-1yl]ethyl]-5,6,7,8-tetrahydro-1,2,4-triazolo[4,3-a]pyridin-3-(2H)-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 K₂CO₃ (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 CHCl₃, and the extract was concentrated in vacuo. The residue was purified by column chromatography (CHCl₃/EtOH, 20:1) to give the free base of 7b (53.0 g, 88.5%) as an oil: NMR (CDCl₃) 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_{θ}) 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. (C₂₆H₂₈F₂N₄O·HCl·³/₂H₂O) C, H, N.

Method B. (a) 6,7,8,9-Tetrahydro-2*H*-pyrido[1,2-*a*]-1,3,5triazine-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 (CHCl₈/MeOH, 20:1) to give 19b (2.04 g, 57%) as colorless crystals: mp 185–187 °C; NMR (DMSO-d₆) δ 1.6–1.9 (4 H, m), 2.65 (2 H, t), 3.64 (2 H, t), 11.39 (1 H, br). Anal. (C₇H₉N₃O₂) C, H, N.

(b) 3-[2-[4-(4-Fluorobenzoyl)piperidin-i-yl]ethyl]-6,7,8,9tetrahydro-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 (CHCl₃/MeOH, 30:1) to give the free base of 21b (1.70 g, 43%) as colorless crystals: mp 153-155 °C; NMR (CDCl₃) 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. (C₂₁H₂₅FN₄O₃) C, H, N.

5-HT₂ 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% O₂ and 5% CO₂. The composition of the solution was as follows (nM): NaCl 112, KCl 5, CaCl₂ 1.5, MgSO₄ 1.2, NaH₂PO₄ 1.2, NaHCO₃ 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-HT₂ activity, the preparations were contracted with 5-HT (0.1-100 μ M), cumulatively. Then the preparations 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-HT₂ 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 μ M) was given to the preparations instead of 5-HT.

5-HT₂ or α_1 Antagonistic Activity in Rats in Vivo. A male SD rat was anesthetized with urethane (1 g/kg, ip) and alphachloralose (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 ($300 \ \mu g/kg$, iv) was injected, and the change of the hypertensive phase, immediately after a transient hypotensive phase, was measured. The anti-5-HT₂ activity was expressed as an inhibition percentage.

To test anti- α_1 activity, phenylephrine (30 $\mu g/kg$, 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 5hydroxytryptophan (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 $1,3-\beta$ 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 $1,3-\beta$ -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

 ⁽a) Hector, R. F. Recent Developments in Antifungal Agents. Current Opin. Ther. Patents 1991, 1, 457-473. (b) Walzer, P. D. Pneumocystis carinii - New Clinical Spectrum? N. Engl. J. Med. 1991, 324, 263-5. (c) Vanvoorhis, W. C. Therapy and Prophylaxis of Systemic Protozoan Infections. Drugs 1990, 40, 176-202. (d) Walsh, T. J.; Jarosinski, P. F.; Fromtling, R. A. Increasing Usage of Systemic Antifungal Agents. Diagn. Microbiol. Infect. Dis. 1990, 13, 37-40. (e) Saksena, A. K.; Girijavallabhan, V. M.; Cooper, A. B.; Loebenberg, D. Recent Advances in Antifungal Agents. Annu. Rep. Med. Chem. 1989, 24, 111-120.

^{(2) (}a) Saral, R. Candida and Aspergillus Infections in Immunocompromised Patients - An Overview. Rev. Infect. Dis. 1991, 13, 487-492. (b) Deepe, G. S.; Bullock, W. E. Immunological Aspects of Fungal Pathogenesis. Eur. J. Clin. Microbiol. Infect. Dis. 1990, 9, 377-80. (c) Mills, J.; Masur, H. AIDS-related Infections. Scientific American 1990, August, 50-57. (d) Hughes, W. T.; Kuhn, S.; Chaudhary, S.; Feldman, S.; Verzosa, M.; Aur, R. J. A.; Pratt, C.; George, S. L. Successful Chemoprophylaxis for Pneumocystis carinii Pneumonitis. N. Engl. J. Med. 1977, 297, 1419.

^{(3) (}a) Tkacz, J. S. In Emerging Targets In Antibacterial And Antifungal Chemotherapy; Sutcliffe, J., Georgopapadakou, N. H., Eds.; Chapman and Hall: New York, 1991, in press. (b) Bartizal, K.; Abruzzo, G.; Trainor, C.; Krupa, D.; Nollstadt, K.; Schmatz, D.; Schwartz, R.; Hammond, M.; Balkovec, J.; Van-Middlesworth, F. β-1,3 Glucan Synthesis Inhibitors L-671,329, L-646,991, Tetrahydroechinocandin B and Papulacandin in a New Target Organ Kidney Assay (TOKA) of Systemic Candidiasis. Proc. 30th ICAAC, Atlanta, GA, Oct. 20-23, 1990, Abstr. No. 584.

^{(4) (}a) Denning, D. W.; Stevens, D. A. Cilofungin Treatment of Disseminated Aspergillosis in a Murine Model. Proc. 30th ICAAC, Atlanta, GA, Oct. 20-23, 1990, Abstr. No. 286. (b) Galgiani, J. N.; Sun, S. H.; Clemons, K. V.; Stevens, D. A. Activity of Cilofungin Against Coccidioides Immitis: Differential In Vitro Effects on Mycelia and Spherules Correlated with In Vivo Studies. J. Infect. Dis. 1990, 162, 944-948.

^{(5) (}a) Matsumoto, Y.; Matsuda, S.; Tegoshi, T. Yeast Glucan in the Cyst Wall of *Pneumocystis carinii*. J. Protozool. 1989, 36, 21S. (b) Edman, J. C.; Kovacs, J. A.; Masur, H.; Santi, D. V.; Elwood, H. J.; Sogin, M. L. Ribosomal RNA Sequence Shows *Pneumocystis carinii* to be a Member of the Fungi. Nature (London) 1988, 334, 519-522. (c) Stringer, S.; Stringer, J.; Blase, M.; Walzer, P.; Cushion, M. Pneumocystis carinii: Sequence from Ribosomal RNA Implies a Close Relationship with Fungi. Exp. Parasitol. 1989, 68, 450-461. (d) Edman, U.; Edman, J. C.; Lundgren, B.; Santi, D. V. Isolation and Expression of the Pneumocystis carinii Thymidylate Synthase Gene. Proc. Natl. Acad. Sci. U.S.A. 1989, 86, 6503-6507.