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Thiophene systems. 12. Analogues of ketanserin and ritanserin as selective 5-HT_2 antagonists

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Summary — A series of thieno[3,2-*d*]-, [3,4-*d*]- and [2,3-*d*]pyrimidinedione derivatives was prepared with *N*-3 substitution containing the side chains of ketanserin and ritanserin. The best of these thiophene analogues were the isosteres of ketanserin which were up to 20-fold more potent than the standards in 5-HT_2 binding assays. More importantly, in addition to their increased potency, these derivatives were more selective than the standards in that they had less affinity for 5-HT_{1A} and α_1 binding sites. This selectivity is especially noted as the ratio of α_1/5-HT_2 wherein the most interesting thiophene isostere (**2**) in this study had a binding selectivity > 12-fold of ketanserin or ritanserin.

Résumé — Systèmes thiophéniques. 12. Analogues de la kétansérine et de la titansérine, antagonistes 5-HT_2 sélectifs. *Une série de dérivés de thiéno[3,2-*d*]- et [3,4-*d*]- et [2,3-*d*]pyriminedione a été préparée avec une substitution de* N*-3 contenant les chaînes latérales de la kétansérine et de la ritansérine. Les meilleurs de ces analogues thiophéniques étaient les isostères de kétansérine qui se sont montrés plus de 20 fois plus puissants que les étalons dans les tests de liaison 5-HT_2. Plus important, en plus de leur plus grande efficacité, ces dérivés se sont montrés plus sélectifs que les étalons en raison de leur moindre affinité de liaison avec les sites 5-HT_{1A} et α_1. Cette sélectivité est particulièrement notée par le rapport de α_1-HT_2 dans laquelle l'isostère thiophénique plus intéressant dans ces travaux a une sélectivité 12 fois supérieure à celle de la kétansérine et de la ritansérine.*

serotonin / ketanserin / ritanserin / thienopyrimidinedione

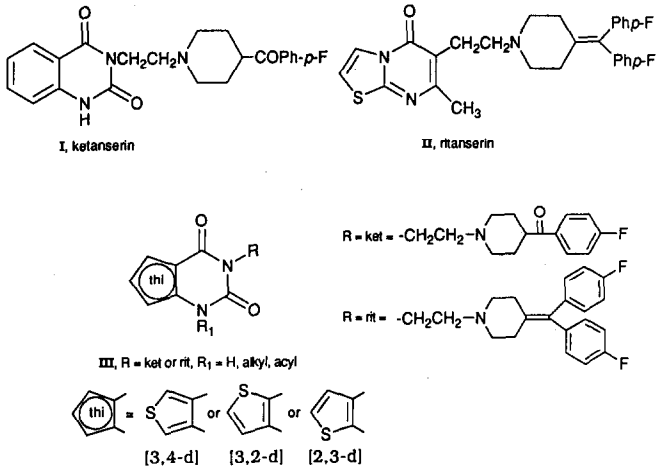
Introduction

Receptors for serotonin (5-hydroxytryptamine, 5-HT [1]) exist in the cardiovascular system and may play a role in the pathogenesis of certain cardiovascular disorders including hypertension, unstable angina, migrane, Raynaud's disease and other peripheral vasospastic phenomena [2]. A causal relationship is suggested since serotonin can constrict vascular smooth muscle, amplify the vasoconstrictor actions of norepinephrine, facilitate the release of catecholamines and induce platelet aggregation [3, 4]. These events are mediated almost exclusively by activation of the 5-HT_2 receptor subtype [5]. Hence, selective inhibition of 5-HT_2 receptors could, in principle, have therapeutic application in the treatment of cardiovascular diseases.

Ketanserin (**I**) is the prototypic 5-HT_2 receptor antagonist and is virtually devoid of 5-HT_1 receptor antagonist properties [6]. Ketanserin has been shown to reduce arterial blood pressure in patients with essential hypertension [7]. However, ketanserin possesses significant α_1-adrenoceptor antagonist activity which may well contribute to its antihypertensive effect [8]. Ritanserin (**II**), somewhat related to ketanserin, has also been reported to possess specific 5-HT_2 receptor blocking properties [9] but is under development as a CNS agent and lacks general cardiovascular activity. An orally effective, selective 5-HT_2 receptor antagonist, devoid of 5-HT_1 or alpha-receptor blocking properties and without the CNS properties of ritanserin, could help to clarify the pathophysiological importance of serotonin in the cardiovascular system which might lead to the development of new, more selective therapeutic agents.

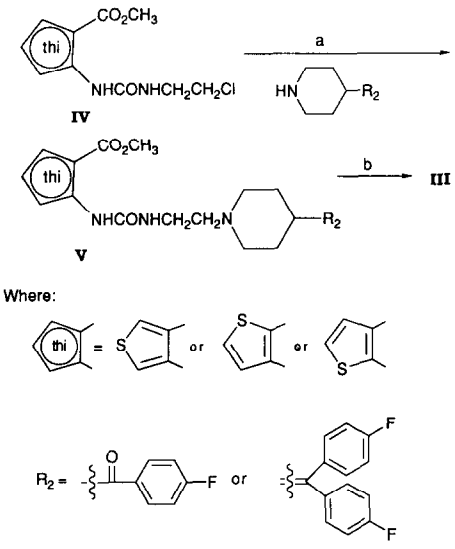
Our laboratories have had a long-standing interest in the thiophene isosteres of pharmacologically active agents [10]. In terms of drug design, bioisosteric replacement of benzene with thiophene frequently leads to compounds with improved potency and

selectivity. For example, we recently reported the synthesis and potent antihypertensive activity of a series of thienopyrimidinedione derivatives which had properties superior to those of the benzo analgoues [11]. For the targets of the current report, our previous synthesis of thienopyrimidinediones was easily applicable to the preparation of the thiophene isosteres of ketanserin and analogues of ritanserin (**III**).



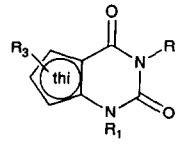
Chemistry

The compounds of this study were prepared according to scheme 1. The starting chloroethyl urea derivatives **IV** were obtained as described in our previous report [11] starting form related isomeric aminothiophene carboxylates [12–15]. The ureas were treated with either 4-(4-fluorobenzoyl)piperidine or bis(4-fluorophenyl)methyl-4-piperilydene in either THF, 2-propanol or DMF to give the amine substituted urea derivatives **V**. These intermediates were ring closed by the action of potassium hydroxide in methanol to give the thienopyrimidinediones **III** in excellent overall yields. To examine the effects of *N*-1 substitution on the SAR of these compounds, we prepared the 1-methyl, 1-butyl, 1-acetyl, 1-pivaloyl and 1-benzoyl derivatives in the thieno[3,4-*d*]series. The alkylations of the thienopyrimidine-2,4-diones were accomplished by deprotonation with NaH in either DMF or THF and treatment with the appropriate alkyl halides. Likewise, **III** was reacted with either an alkyl or aryl acid chloride in either CH_2Cl_2/TEA or DMF/NaH to cleanly produce the acyl derivatives. Similar to the results reported for the analogous piperazines [11], there was no evidence of *O*-alkylation or acylation. The compounds and their physical properties are summarized in table I.



Scheme 1. Reagents: a, THF/$NaHCO_3$/NaI/reflux for days or 2-propanol/$NaHCO_3$/NaI/reflux for 18 h; b, KOH/MeOH.

Table I. Physical properties of thienopyrimidinediones.



Cmpd.	Fusion	R_1	R_3	M.P.(°C)	Empirical Formula
KetanserinSeries:					
		R = ket = -CH2CH2-N, O, F			
1	[3,2-d]	H	H	141-143	$C_{20}H_{20}FN_3O_3S$
2	[3,4-d]	H	H	208-215 dec	$C_{20}H_{20}FN_3O_3S$
3	[2,3-d]	H	H	231-235	$C_{20}H_{20}FN_3O_3S$
4	[3,4-d]	H	5-Me	212-214 dec	$C_{21}H_{22}FN_3O_3S$
5	[3,2-d]	Me	H	171-174	$C_{21}H_{22}FN_3O_3S$
6	[3,4-d]	Me	H	176-178	$C_{21}H_{22}FN_3O_3S$
7	[3,4-d]	Ac	H	150-151	$C_{22}H_{22}FN_3O_4S$
8	[3,4-d]	Piv	H	110-111	$C_{25}H_{28}FN_3O_4S$
9	[3,4-d]	Bz	H	154-155	$C_{27}H_{24}FN_3O_4S$
10	[2,3-d]	Me	H	175-177	$C_{21}H_{22}FN_3O_3S$
11	[2,3-d]	Ac	H	137-141	$C_{22}H_{22}FN_3O_4S$
Ritanserin Series:					
		R = rit = -CH2CH2-N, F, F			
12	[3,2-d]	H	H	207-208	$C_{26}H_{23}F_2N_3O_2S$
13	[3,4-d]	H	H	204-206	$C_{26}H_{23}F_2N_3O_2S$
14	[2,3-d]	H	H	206-207	$C_{26}H_{23}F_2N_3O_2S$
15	[3,4-d]	H	5-Me	137-139	$C_{27}H_{25}F_2N_3O_2S$
16	[3,2-d]	Me	H	146-148	$C_{27}H_{25}F_2N_3O_2S \cdot 0.25H_2O$
17	[3,4-d]	Me	H	152-153	$C_{27}H_{25}F_2N_3O_2S$
18	[3,4-d]	Ac	H	67-74	$C_{28}H_{25}F_2N_3O_3S$
19	[3,4-d]	Bz	H	148-151	$C_{33}H_{27}F_2N_3O_3S$

Results and discussion

The compounds prepared for this study were initially evaluated in receptor binding assays for 5-HT_{1A}, 5-HT_2 and α_1-adrenergic activity. These results provided a means of analyzing both the potency and selectivity of test compounds vis-à-vis reference standards ketanserin and ritanserin. Both of these reference standards have high affinity for 5-HT_2 with ritanserin approximately 3-fold more potent than ketanserin. These IC_{50} values correlate very closely with those reported by Leysen *et al* [16]. On the other hand, the affinity for α_1 receptors is much weaker than that for 5-HT_2 receptors. The α_1 IC_{50} value for ritanserin is > 100-fold greater than that for central 5-HT_2 receptors, while the binding constant for ketanserin is 47-fold greater for α_1 than for 5-HT_2 receptors. These data are also qualitatively very similar to those reported by Leysen *et al* [16] and demonstrate the selectivity of these agents for 5-HT_2 receptors. Selected compounds were further evaluated for antagonism of vascular smooth muscle contraction *in vitro* and inhibition of pressor responses to 5-HT_2 *in vivo* to gain a measure of functional activity. These results are summarized in tables II and III.

Examination of the 5-HT_2 binding data for all of the derivatives (**1–19**) reveals that almost all of them have high affinity for this receptor (table II). In the ketanserin isostere series (**1–11**), most notable are the 3 unsubstituted thiophene [3,2-*d*], [3,4-*d*] and [2,3-*d*] isomers (**1–3**) which are all more potent than ketanserin. The most potent of this group is thieno-[3,4-*d*]pyrimidine **2** which is 20-fold more potent than the standard. In the ritanserin isostere series (**12–19**), the unsusbstituted isosteres (**12–14**) are 5–15-fold less potent than ritanserin. In comparing the 2 series, it is interesting that the potencies of the unsubstituted ketanserin isosteres are significantly better than ketanserin while those of unsubstituted ritanserin analogues are significantly worse than ritanserin. Obviously, the pyrimidinediones fused with a thiophene are similar (but better) for binding at the ketanserin binding site, but replacement of the thiazolo-pyrimidine of ritanserin with a pyrimidinedione re-

Table II. Binding affinity for various receptor sites and inhibition of 5-HT induced muscle contractions of kitanserin/ritanserin analogues.

	Binding Data					Tissue Strip Data
Cmpd No.	5-HT_2[a]	5-HT_{1A}[a]	α_1[a]	5-HT_{1A}/5-HT_2[b]	α_1/5-HT_2[b]	5-HT[c]
Ketanserin	6.2	2405	290	388	47	3
1	1.1	1850	95	1682	87	5
2	0.33	1600	200	4848	606	5
3	4.3	6500	261	1511	61	23
4	13	1600	1300	123	100	NT
5	15	900	7800	60	520	NT
6	11	9500	8400	864	764	3
7	12	NT[d]	5800	----	483	NT
8	16	NT[d]	6800	----	425	NT
9	24	900	8000	37	333	NT
10	4.4	1000	280	227	64	NT
11	2.4	400	200	167	83	NT
Ritanserin	2.1	810	230	386	109	2
12	10	6000	485	600	49	100
13	30	3300	900	110	30	16.5
14	26	40,000	3800	1538	146	9
15	71	5000	330	70	5	NT
16	4.5	600	500	133	111	NT
17	1.8	3100	240	1722	133	NT
18	5.1	4650	4800	912	941	NT
19	211	710	3500	3	17	NT

[a]Binding affinities in rat cortical membranes were determined as described in the *Experimental protocols*. Data are expressed as IC_{50} values in nM concentrations. Each value is the mean of 2-3 determinations. [b]Ratios of binding affinities. [c]Muscle strip data are DR_{10} values expressed in nM concentrations as described in the *Experimental protocols*. Each value is the mean of duplicate determinations. [d]NT = not tested.

Table III. *In vivo* 5-HT_2 and α_1 antagonism of selected kitanserin/ritanserin analogues.

Cmpound	5-HT_2[a] (ED_{50}, µg/kg)	α_1[b] (ED_{50}, µg/kg)	α_1/5-HT_2[c] ratio
Ketanserin	13	1080	83
1	9	3080	342
2	8	2880	360
4	121		
6	18		
7	29		
9	>300		
11	40		
Ritanserin	9	5050	561
12	17		
13	159		
14	18		
16	28		
17	20		
18	24		

Compounds were studied in pentobarbital anesthetized, ventilated, spontaneously hypertensive rats in which arterial pressure was recorded from a carotid artery and compounds were administered into a jugular vein. Vasopressor responses to serotonin (5-HT_2) using alpha-adrenergic and ganglion blocked rats, or phenylephrine (α_1) using vagotimized rats were evaluated.

[a]5-HT_2 antagonist potency (ED_{50}) is reported as the dose (µg/kg, iv) causing 50% inhibition of a serotonin (100 µg/kg, iv) pressor response in anesthetized, ganglion-blocked rats. Each value is the mean of 2-5 rats. [b]α_1-Adrenergic antagonist potency is reported as the ED_{50} (µg/kg, iv) which is the dose causing 50% inhibition of the phenylephrine pressor response in anesthetized, vagotomized rats. Each value is the mean of 2-7 rats. [c]Ratio of ED_{50} values.

duces the ability of these compounds to bind at the ritanserin binding site. It is also quite interesting that the [3,4-*d*] isomer in the ketanserin series is the most potent while the [3,2-*d*] isomer is best in the ritanserin series.

Several *N*-1 substitutions were examined to test whether alkylation or acylation would enhance binding activity either by steric or electronic interaction. In the ketanserin series, *N*-1 methylation reduces (**5** < **1**, **6** < **2**) 5-HT_2 binding activity in the thieno[3,2-*d*]- and [3,4-*d*]-systems with little effect (**10** ≈ **3**) in the [2,3-*d*]-system. In contrast, *N*-1 methylation enhances (**16** > **12**, **17** ≫ **13**) 5-HT_2 binding in the ritanserin series. Acylation at *N*-1 in the thieno[3,4-*d*]-ketanserin series also significantly reduces binding affinity (**7**–**9** < **2**) but has little effect in the [2,3-*d*]-system (**11** ≈ **3**). In the ritanserin series, acetyl derivative **18** is better than both the benzoyl derivative **19** and the unsubstituted compound **13**. In both series, methyl substitution on the thiophene (**4**, **15**) significantly reduces 5-HT_2 binding activity (compared to **2** and **13**, respectively). In general, the ketanserin isosteres are more potent that the ritanserin analogues with hydrogen substitution at *N*-1 required for the most potent 5-HT_2 bonding affinity. These compounds are highly selective for 5-HT_2 receptors with little 5-HT_{1A} binding affinity (table II). The best compounds in this study (**1**, **2**, **3**, **14**, **17**) were at least 4 times more selective for 5-HT_2 than either ketanserin or ritanserin.

One of the factors complicating the reported clinical activities of ketanserin is its α_1-blocking activity [8]. The development of a selective 5-HT_2 antagonist depends on the reduction of the α_1-binding component within the series. As may be seen from the data in table II, almost all of the compounds in this report have equivalent or less α_1-binding activity than the standards. This is especially noteworthy in view of the increased 5-HT_2 potencies of these thiophene isosteres; comparison of the ratio of α_1/5-HT_2 underscores this selectivity. While the ratios for ketanserin and ritanserin are ≈ 50, the best compounds in our study (**2**, **6** and **18**) are at least 10 times more selective than ketanserin. Such selectivity is required to address the physiological importance of specific 5-HT_2 antagonism.

The primary goal of our program was to evaluate the thiophene analogues of ketanserin and ritanserin to find how such structural modifications might enhance 5-HT_2 binding affinity. Compounds **1**, **2**, **3**, **10**, **11**, **16**, **17** and **18** have greater affinity for the 5-HT_2 receptor than ketanserin and hence meet our first research objective. When selectivity for 5-HT_2 *vs* 5-HT_{1A} and α_1 receptors is considered, ketanserin isosteres **1**, **2** and **3** are especially interesting (table II). Further evaluation of selected compounds in smooth muscle strips for antagonism of 5-HT induced contractions (table II) show that only ketanserin isosteres **1**, **2** and **6** have potencies similar to ketanserin while the ritanserin analogues **12**, **13** and **14** are less interesting.

Most of these compounds were tested for *in vivo* 5-HT_2 antagonism as shown in table III. As predicted from the *in vitro* binding data, compounds **1** and **2** have potent antagonist activity. Compounds **6**, **12** and **14** are less interesting while the other compounds tested are significantly less potent than the standards. For the most interesting compounds (**1** and **2**), *in vivo* α_1 antagonism was also determined. In keeping with the 2 to 13-fold greater binding selectivity of these compounds *in vitro*, **1** and **2** are ≈ 4-fold more selective as 5-HT_2 antagonists than ketanserin *in vivo*. Thus, these *in vivo* results show that the best compounds in this study achieve incrementally better selectivity than ketanserin as selective 5-HT_2 antagonists.

In this study, compounds **1** and **2** appear to have the necessary improvement in biological properties that would warrant their evaluation as selective 5-HT_2 antagonists. Replacement of benzene with thiophene causes subtle electronic changes which enhance binding for the isosteres of ketanserin. *N*-substitution attenuates this enhancement. Our data suggest that the thiazolopyrimidine nucleus is important for optimal activity since the thiophene analogues of this study were uninteresting. The fact that *N*-substitution in our ritanserin analogues enhances activity is interesting and worthy of further study. It is important to emphasize that thiophene effectively replaces benzene at least in the ketanserin series; as noted previously [11], different thiophene isomers produce different (but unpredictable) biological profiles. While the current study focused only on binding affinity to HO-DPAT, prazosin and ketanserin labelled sites, both ketanserin and ritanserin bind with moderate to high affinity to other receptors including those for dopamine and histamine. The affinity and intrinsic activity of the title compounds at other receptor sites still require study.

Experimental protocols

Melting points were taken on a Thomson–Hoover melting point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin–Elmer 283 or 1430 instrument. ^{1}H NMR spectra were recorded on a Varian T-60 or EM 390 instrument with the chemical shifts reported in δ downfield from tetramethylsilane as internal standard. All spectra were in agreement with the structures cited. The elemental analyses were run on a Perkin–Elmer 240C instrument. Standard flash column techniques were employed to purify crude reaction mixtures using 230–400 mesh E Merck silica gel under positive nitrogen pressure.

General procedures for the synthetic steps shown in scheme I are exemplified by the preparation of **1**, **6**, **7** and **14**. Methyl 4-aminothiophene-3-carboxylate [12, 13], methyl 4-amino-2-methylthiophene-3-carboxylate [14] and ethyl 2-

aminothiophene-3-carboxylate [15] were prepared by literature procedures whereas all the other aminothiophene carboxylates were available commercially.

N-(2-Carbomethoxythien-3-yl)-N-[2-[4-(4-fluorobenzoyl)piperidin-1-yl]ethyl]urea
A mixture of *N*-(2-carbomethoxythien-3-yl)-*N*-(2-chloroethyl)-urea [11] (4.0 g, 15.2 mmol), 4-(4-fluorobenzoyl)piperidine hydrochloride (9.24 g, 38.0 mmol), sodium bicarbonate (4.47 g, 53.2 mmol) and sodium iodide (0.50 g, 3.3 mmol) in tetrahydrofuran (100 ml) was heated to reflux for 4 days. The solvent was evaporated *in vacuo*, and the resultant residue was treated with water (100 ml). The product was extracted into CH_2Cl_2 (2 x 75 ml) and dried over magnesium sulfate. The product was purified by flash chromatography on silica gel 60 (250 g) using 2% methanol in CH_2Cl_2 as the eluant to give the product (4.07 g, 62%) as a colorless solid. IR (KBr): 1540, 1560, 1585, 1660, 2770, 2920, 3300 and 3360 cm^{-1}; MS (DCI), *m/z* 434 (MH^+); ^1H NMR ($CDCl_3$): δ 1.6–2.4 (m, 6H), 2.53 (m, 2H), 2.8–3.6 (m, 5H), 3.85 (s, 3H), 5.5 (br s, 1H, exchanges with D_2O), 6.9–7.1 (m, 2H), 7.26 (d, *J* = 5 Hz, 1H), 7.80–8.07 (m, 3H) and 9.45 (br s, 1H, exchanges with D_2O). Anal $C_{21}H_{24}FN_3O_4S$ (C, H, N, F, S).

3-[2-[4-(4-Fluorobenzoyl)piperidin-1-yl]ethyl]thieno[3,2-d]-pyrimidine-2,4-dione ***1***
A solution of *N*-(2-carbomethoxythien-3-yl)-*N*-[2-[4-(4-fluorobenzoyl)-piperidin-1-yl]ethyl]urea (1.2 g, 2.8 mmol) and 50% sodium hydroxide (0.213 g, 2.7 mmol) in methanol (30 ml) was stirred at room temperature for 24 h. The solution was acidified with acetic acid (0.5 ml) and the solvent was evaporated *in vacuo*. The residue was treated with water (75 ml) and neutralized with sodium bicarbonate. The resultant solid was collected by filtration, washed with water, air dried and triturated in hot acetone to give the product (1.04 g, 94%) as a colorless solid, mp = 141–143°C. IR (KBr): 1580, 1640, 1695, 2790, 2940, 3200 and 3240 cm^{-1}; MS (DCI), 402 *m/z* (MH^+); ^1H-NMR (DMSO-d_6): δ 1.47–1.60 (m, 2H), 1.74 (d, *J* = 11.3 Hz, 2H), 2.12–2.20 (m, 2H), 2.52–2.55 (m, 2H), 2.97 (d, *J* = 6.7 Hz, 2H), 3.32–3.38 (m, 1H), 4.00 (t, *J* = 6.7 Hz, 2H), 6.95 (d, *J* = 5.2 Hz, 1H), 7.32–7.39 (m, 2H), 8.02–8.08 (m, 3H) and 11.85 (br s, 1H, exchanges with D_2O). Anal $C_{20}H_{20}FN_3O_3S$ (C, H, N).

3-[2-[4-[Bis-(4-fluorophenyl)methylene]piperidin-1-yl]ethyl]-thieno[2,3-d]pyrimidine-2,4-dione ***14***
A mixture of *N*-(3-carboethoxythien-2-yl)-*N*-(2-chloroethyl)-urea [11] (2.10 g, 7.6 mmol), bis-(4-fluorophenyl)methyl-4-piperilydene (3.2 g, 11.4 mmol), sodium bicarbonate (2.55 g, 30.4 mmol) and sodium iodide (0.57 g, 3.81 mmol) in isopropanol (15 ml) was heated to reflux for 16 h. The solvent was evaporated *in vacuo* and the residue was treated with water (50 ml). The resultant solid was collected by filtration, washed with water and air dried. The product was purified by flash chromatography on silica gel 60 (250 g) using 4% methanol in CH_2Cl_2 to give the product (0.937 g, 26%) as a colorless solid, mp = 205–207°C. Ir (KBr): 1510, 1650 and 1715 cm^{-1}; MS (DCI), *m/z* 480 (MH^+); ^1H-NMR (DMSO-d_6): δ 2.21 (br s, 4H), 2.51 (br s, 6H), 3.45 (br s, 1H, exchanges with D_2O), 3.97 (t, *J* = 6 Hz, 2H) and 7.06–7.20 (m, 10H). Anal $C_{26}H_{23}F_2N_3O_2S$ (C, H, N, F, S).

1-Acetyl-3-[2-[4-(4-fluorobenzoyl)piperidin-1-yl]ethyl]thieno-[3,4-d]pyrimidine-2,4-dione ***7***
The title compound was prepared by suspending 3-[2-[4-(4-fluorobenzoyl)piperidin-1-yl]ethyl]thieno[3,4-*d*]pyrimidine-2,4-dione (2.0 g, 5 mmol) in CH_2Cl_2 (50 ml) and adding acetyl chloride (469 mg, 6 mmol) and triethylamine (1 equivalent). After stirring at room temperature for 24 h, the organic phase was washed with water and brine and dried over $MgSO_4$. Solvent removal produced a crude product which was purified by flash silica gel chromatography using EtOAc/hexane (1/1). The product was crystallized from CH_2Cl_2/hexane to give a white solid (337 mg, 15%), mp = 150–151°C. IR (KBr): 599, 783, 848, 854, 960, 980, 1031, 1050, 1680 and 1732 cm^{-1}; ^1H-NMR ($CDCl_3$): δ 1.72 (m, 4H), 2.14 (br s, 2H), 2.58 (t, *J* = 6 Hz, 2H), 2.65 (s, 3H), 3.05 (m, 3H), 4.1 (t, *J* = 6.3 Hz, 2H), 7.04 (t, *J* = 8.6 Hz, 2H), 7.8 (d, *J* = 4.9 Hz, 1H), 7.9 (d, *J* = 4.4 Hz, 2H) and 8.2 (d, *J* = 3 Hz, 1H). Anal $C_{22}H_{22}FN_3O_4S$ (C, H, N).

3-[2-[4-(4-Fluorobenzoyl)piperidin-1-yl]ethyl]-1-methyl-thieno[3,4-d]pyrimidine-2,4-dione ***6***
The title compound was prepared by dissolving 3-[2-[4-(4-fluorobenzoyl)piperidin-1-yl]ethyl]thieno[3,4-*d*]pyrimidine-2,4-dione (1.8 g, 4.6 mmol) in DMF (10 ml) at 0°C. After treating this solution with NaH (203 mg, 5 mmol) followed by 720 mg (5 mmol) of MeI, the mixture was stirred at room temperature for 24 h. The reaction was poured into water and extracted with CH_2Cl_2. The combined extracts were washed with water and brine and dried over $MgSO_4$. Solvent removal produced a crude product which was purified by flash silica gel chromatography using 2% methanol in CH_2Cl_2. The title compound was crystallized from CH_2Cl_2/hexane to give a white solid (1.04 g, 54%), mp = 176–178°C. IR (KBr): 618, 640, 762, 862, 978, 1658, 1681, and 1700 cm^{-1}; ^1H-NMR ($CDCl_3$): δ 1.74 (m, 4H), 2.15 (br s, 2H), 2.6 (t, *J* = 6.3 Hz, 2H), 3.05 (m, 3H), 3.4 (s, 3H), 4.1 (t, *J* = 6.7 Hz, 2H), 6.5 (br s, 1H), 7.03 (t, *J* = 7.5 Hz, 2H), 7.87 (m, 2H), 7.9 (d, *J* = 4.4 Hz, 2H) and 8.15 (d, *J* = 3 Hz, 1H). Anal $C_{21}H_{22}FN_3O_3S$ (C, H, N).

Brain serotonin (5-HT_2) radioreceptor assay
A crude membrane fraction of the frontal cortex of rats was prepared essentially by the method of Leysen *et al* [16]. Membrane fragments (350–450 μg protein), ^3H-ketanserin (1 nM, specific activity 60–90 Ci/mmol, New England Nuclear, Boston, MA) and an unlabeled test compound were incubated for 15 min at 37°C in 50 mM Tris and 2.5 mM $CaCl_2$ (pH 7.4). The reaction was terminated by the addition of 3.0 ml of ice-cold buffer and the contents were filtered rapidly through glass fiber filters. The ability of a non-labeled compound to complete with ^3H-ketanserin for binding sites is a measure of the compound's affinity for the 5-HT_2 receptor. Specific binding was determined by the difference between counts (cpm) bound in the presence and absence of 1 x 10^{-6} M methysergide. The determinations were made in duplicate or triplicate.

Data are presented as the IC_{50}, the concentration of non-labeled compound required to displace 50% of specifically bound ^3H-ketanserin.

Brain serotonin (5-HT_{1A}) radioreceptor assay
Crude membrane fragments of the frontal cortex of rats were prepared and the 5-HT_{1A} sites were labeled with ^3H-8-hydroxy-2-dipropylaminotetralin (8-OH-DPAT, Research Products International, Mount Prospect, IL; specific activity 70–85 Ci/mmol) as previously described [17]. Membrane fragments (350–450 μg protein), ^3H-8-OH-DPAT (nM), Tris buffer (50 mM, 2.5 mM $MgCl_2$, pH 7.4) and unlabeled compound were incubated for 15 min at 37°C. Separation of bound from unbound ^3H-8-OH-DPAT was performed by vacuum filtration. The ability of a compound to compete with ^3H-8-OH-DPAT for binding sites is a measure of the affinity of the compound

for the 5-HT_{1A} receptor. Specific binding was determined by the difference between counts (cpm) bound in the presence and absence of 1 x 10^{-5} M 5-hydroxytryptamine. The determinations were made in duplicate or triplicate.

Data are presented as the IC_{50} (concentration required to displace 50% of specifically bound ^{3}H-8-OH DPAT).

[^{3}H]Prazosin α_1-adrenergic binding assay

The affinity of test compounds for α_1-adrenergic binding sites was determined using homogenates of the frontal cortex of rats [18]. A crude membrane fraction was incubated with a test compound at various concentrations in borosilicate glass tubes on a shaking metabolic incubator at 37°C for 10 min. The assay medium, in order of addition, consisted of: 50 mM Tris (pH 7.4); 2.5 mM $MgCl_2$ and 2 nM [^{3}H]prazosin (specific activity 10–30 Ci/mmol, New England Nuclear, Boston, MA). The binding reaction was initiated by the addition of 350–450 µg of protein. The final incubation volume was 250 µl and nonspecific binding was defined with 1 x 10^{-5} M phentolamine. The reaction was terminated by the addition of 3.0 ml ice-cold rinse buffer (20 mM Tris, pH 7.4) and the contents were rapidly filtered through glass fiber filters.

In each of the radioligand binding experiments, 13–15 concentrations of each drug were used in the competition experiments and the data were analyzed by nonlinear regression using LUNDON. All binding data are reported as IC_{50} values.

Protein determinations

The method of Lowry *et al* [19] was used to analyze the amount of protein.

Inhibition of serotonin-induced and phenylephrine-induced smooth muscle contractions

To test 5-HT antagonism, rabbits were sacrificed by T-61 injection and the thoracic aorta was removed immediately [20]. The aorta was placed in warmed, oxygenated Krebs–Henseleit buffer of the following composition (mM): NaCl, 121; KCl, 5.4; $CaCl_2$, 125; $MgSO_4$, 0.6; NaH_2PO_4, 12; $NaHCO_3$, 15.0; glucose, 11.5. After the tissue was cleaned, a 2–3 mm wide strip was cut helically from each aorta. A strip of ≈ 5 cm was suspended in Krebs–Henseleit buffer at 37°C in a 25-ml tissue bath and the tissues were equilibrated at an initial resting tension of 4 g for 90–120 min. An initial concentration response curve to serotonin (0.01–10 µM) was conducted for each tissue, followed by a 60-min washout and reequilibration. The test drug was then added for a 10 min preincubation and another serotonin dose curve was completed using 0.01–100 µM. Determinations were performed in duplicate and 3–4 concentrations of test compound were evaluated. In a similar manner but in separate experiments, α_1-antagonism of smooth muscle contraction was determined except that phenylephrine was used as the agonist.

All experimental compounds produced competitive and parallel shifts of the agonist dose response curves. Limited availability of sample caused the study of only 3–4 concentrations of test compound. As a consequence, the data are expressed as DR_{10} values rather than pA_2 values which require more data points. The DR_{10} is the concentration of the compound required to shift the serotonin or phenylephrine dose curve 10 times to the right, *ie*, the concentration of antagonist at which the concentration of serotonin or phenylephrine required for 50% maximal contraction is increased 10-fold.

In vivo antagonism of serotonin-induced pressor responses

Compounds were evaluated for antagonism of serotonin-induced pressor activity in anesthetized spontaneously hypertensive rats by adapting a previously published method in the dog [21]. Spontaneously hypertensive rats (Charles River, 275–400 g) are anesthetized with sodium pentobarbital (50 mg/kg, ip). A heparin-filled catheter (PE 50) is inserted into the right carotid artery and connected to a Gould pressure transducer to record arterial blood pressure while a right jugular vein is cannulated for drug administration. The trachea is cannulated and rats are placed on a small animal respirator and ventilated with room air at 50–60 breaths/min. Rats are then pretreated with prazosin hydrochloride (1 mg/kg) and pentolinium tartrate (5 mg/kg) to block α_1-adrenergic receptors and sympathetic reflex activity, respectively. Ten minutes later, animals are challenged with a bolus of serotonin (100 µg/kg iv) which transiently elevates mean arterial pressure by 60–100 mmHg. Nonresponsive rats are discarded. After blood pressure returns to baseline, the antagonist compound is administered. A bolus of serotonin (100 µg/kg) is repeated 10 min after each dose of antagonist. Up to 3 cumulative doses of antagonist are given to a single animal. Blood pressure responses are continuously monitored using a Gould recorder.

The percent inhibition of the serotonin-mediated increase in arterial blood pressure is calculated as a function of the cumulative dose of antagonist. Antagonist potency is reported as the dose (µg/kg) causing 50% inhibition of the serotonin pressor response (ED_{50}). Each value is the mean of 2–5 rats.

In vivo antagonism of phenylephrine-induced pressor responses

Spontaneously hypertensive rats are anesthetized and ventilated in the manner described above. In addition, rats are bilaterally vagotomized. Catheters are implanted in a carotid artery and jugular vein to record arterial blood pressure and to administer drugs, respectively. Phenylephrine is administered as a bolus (30 µg/kg, iv) to elicit an α_1-adrenoceptor mediated elevation in arterial blood pressure. Test compounds with potential alpha-antagonist activity are then given iv and animals are rechallenged with phenylephrine (30 µg/kg, iv). Up to 3 cumulative doses of the antagonist are administered.

Inhibition of α-adrenergic responses is quantitated by measuring the percent inhibition of the pressor response to phenylephrine before and after cumulative doses of the test drug. Data are reported in mg/kg as the $ED_{50\%}$ (*ie* the dose causing 50% inhibition of the phenylephrine pressor response). Each value is the mean of 2–7 rats.

Test drugs were solubilized using dimethylformamide or polyethylene glycol and diluted in 0.9% saline or 5% dextrose and administered as a bolus in a volume of 1 ml/kg. Phenylephrine, serotonin, and pentolinium were obtained from Sigma Chemical Co (St Louis, MO).

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