Smith et al.[15] reported that for the cysteine proteinase cathepsin B the inhibitory constants of a series of peptidyl ketones (with the exception of the trifluoromethyl ketone) correlate with the equilibrium constants $K_{RSD,app}$ for the addition in aqueous medium of the thiol group of mercaptopropionic acid to the ketones CH_3COR, where R corresponds to the group flanking the electrophilic carbonyl in the inhibitor. A similar analysis of our inhibitors using the data of Burkey and Fahey[16] predicts that the α-diketone ($K_{RSD,app}$ = 33 M^{-1}) should be more potent than the α-keto ester ($K_{RSD,app}$ = 20 M^{-1}) and the aldehyde ($K_{RSD,app}$ = 29 M^{-1}). Thus, it would appear that the simple thermodynamically driven equilibrium system of Smith et al.[15] has poor predictive value for the inhibition of calpain by peptidyl α-diketones. It is also noteworthy that the K_i values for calpain of the fluoromethyl ketone derivatives in the mono- and diamino acid series are greater than 1000 and 180 μM, respectively. This finding corroborates the observation of Smith et al.[15] that Cbz-Phe-Ala-CF_3[17] was 4 orders of magnitude less potent than the corresponding aldehyde in inhibiting cathepsin B. Electronic factors not taken into account by the model of Smith et al.[15] could be important for inhibition.

In summary, peptides containing an α-dicarbonyl unit, such as an α-diketone or an α-keto ester, are potent inhibitors of the cysteine and serine proteinases calpain and α-chymotrypsin. These new calpain inhibitors may offer significant therapeutic utility.[18] Preliminary results indicate that peptidyl α-diketones also inhibit other cysteine and serine proteinases.

Registry No. **1a**, 1161-13-3; **1b**, 19542-51-9; **2a**, 114744-85-3; **2b**, 121253-52-9; **3a**, 59830-60-3; **3b**, 88191-84-8; **4a**, 111491-96-4; **5a**, 121253-53-0; **5b**, 121253-54-1; **6a**, 121253-55-2; **6b**, 121253-56-3; **7a**, 121253-57-4; **7b**, 121253-58-5; Bz-Phe-H, 35593-57-8; Bz-Phe-COOMe, 123540-99-8; Bz-Phe-CHF_2, 123541-00-4; Bz-Phe-CF_3, 123620-04-2; Cbz-Val-Phe-COOMe, 118943-12-7; Cbz-Val-Phe-CF_3, 123541-01-5; Cbz-Val-D-Phe-CF_3, 123541-02-6; $EtOCH{=}CH_2$, 109-92-2; Bz-Phe-OH, 2566-22-5; calpain, 78990-62-2; α-chymotrypsin, 9004-07-3; cysteine proteinase, 37353-41-6; serine proteinase, 37259-58-8.

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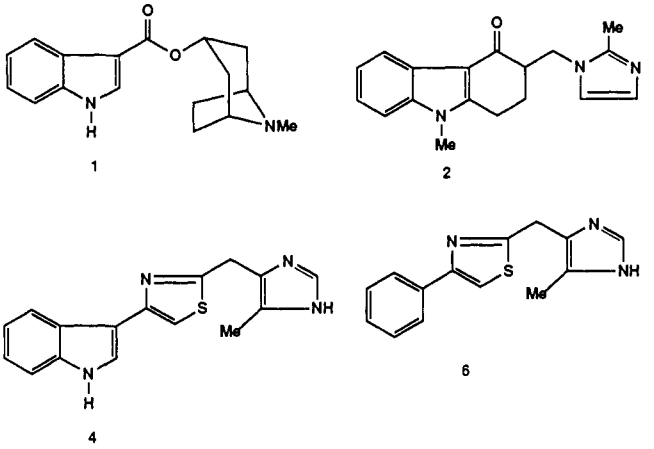
Aromatic Thiazole Derivatives: Structurally Novel and Selective Serotonin-3 Receptor Antagonists

Sir:

Antagonists of the serotonin-3 (5-HT_3) receptor subtype fall into two broad structural classes: the aromatic ester/amide series, represented by ICS-205-930 (**1**),[1] MDL-72,222,[2] BRL-43,674,[3] LY-278,584,[4] and zacopride,[5] and the indole-3-ketone series typified by the carbazole derivatives ondansetron (**2**)[6] and GR-65,630.[7] Because the 5-HT_3



receptor exists in both the peripheral[1] and central nervous systems,[7,8] 5-HT_3 antagonists exhibit a variety of pharmacological effects. Several 5-HT_3 receptor antagonists have demonstrated potent antagonism of chemotherapy- or radiation-induced emesis in man,[9] and various animal models also suggest that these drugs may have utility in the treatment of psychosis,[10] anxiety,[11] substance abuse

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(16) Burkey, T. J.; Fahey, R. C. *J. Am. Chem. Soc.* **1983**, *105*, 868.
(17) Cbz-Phe-Ala-CF_3 is a very slow-binding inhibitor of cathepsin B. The autolysis of calpain in the presence of activating calcium precluded the detection of a very slow onset of inhibition with our fluorinated ketones. However, the cysteine proteinase papain was not significantly inhibited when incubated for 6 h with 0.3 mM of Bz-Phe-CF_3 whereas the corresponding aldehyde **3a** inhibited papain with a K_i of 5 μM.
(18) Calpain has been implicated in important biological roles such as the modification of hormonal binding receptors,[19] the development of long-term memory,[20] the breakdown of neurofilaments at axon terminals,[21] muscle-protein turnover,[22] and metabolism of neuropeptides.[23]
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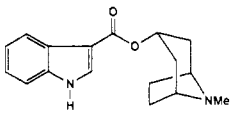
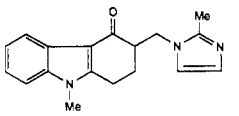
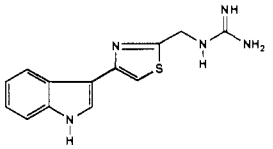
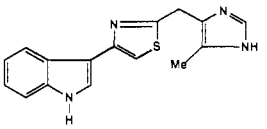
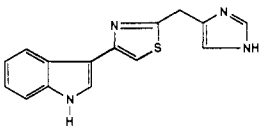
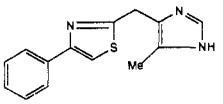
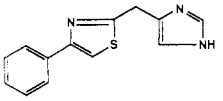
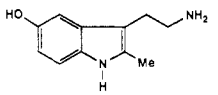
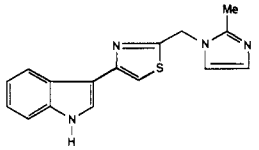
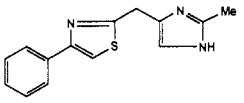
Table I. 5-HT_3 in Vitro Binding Affinity and in Vivo IV Activity in the von Bezold–Jarisch Reflex in Rats

structure	compd	in vitro receptor affinity: [^{3}H]ICS-205-930 displacement[a] K_i, nM (±SEM)	antagonist activity: % inhibn of serotonin-induced B-J reflex[b] (μg/kg dose) (±SEM)	agonist activity: initial bradycardia as % response to 100 μg/kg serotonin (±SEM)
	1	2.7 ± 0.3	76 ± 6 (2)	0
	2	16.2 ± 5.7	43 ± 9 (2) 90 ± 4 (20)	0
	3	3.3 ± 1.3	51 ± 13 (2) 82 ± 12 (20)	60 ± 8
	4	10.4 ± 2.7	45 ± 7 (100)	0
	5	14.2 ± 3.6	63 ± 9 (100)	14 ± 6
	6	1.5 ± 0.6	11 ± 12 (2) 86 ± 1 (20)	0
	7	0.99 ± 0.19	41 ± 13 (2)	80 ± 73
	8	1271 ± 635.8	23 ± 3 (10) 66 ± 14 (50)	41 ± 9
	9	>1.0 μM	7 ± 2 (100)	0
	10	226	7 ± 6 (100)	0

[a] Procedure; ref 16b. Average of a minimum of two determinations. [b] Procedure; ref 1. Iv injections of test compounds as well as serotonin were made through a cannula placed in the right femoral vein.

withdrawal,[12] migraine,[13] and pain (peripheral analgetic[1]). In this communication, we describe a structurally novel series of selective 5-HT_3 receptor antagonists (represented by prototypical derivatives **4** and **6**) containing a thiazole ring system which, on the basis of computer modeling studies,[14] appears to be functioning as an isostere for the carbonyl moiety common to the previous series of selective 5-HT_3 antagonists.

Using both an in vitro binding assay (displacement of [^{3}H]-**1**[15] from 5-HT_3 sites in cultured NG-108-15 glioma cells[16]) and the von Bezold–Jarisch reflex (B-J reflex) paradigm (blockade of serotonin-induced bradycardia in

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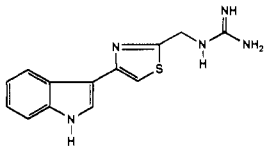
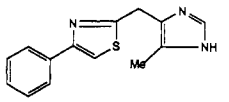
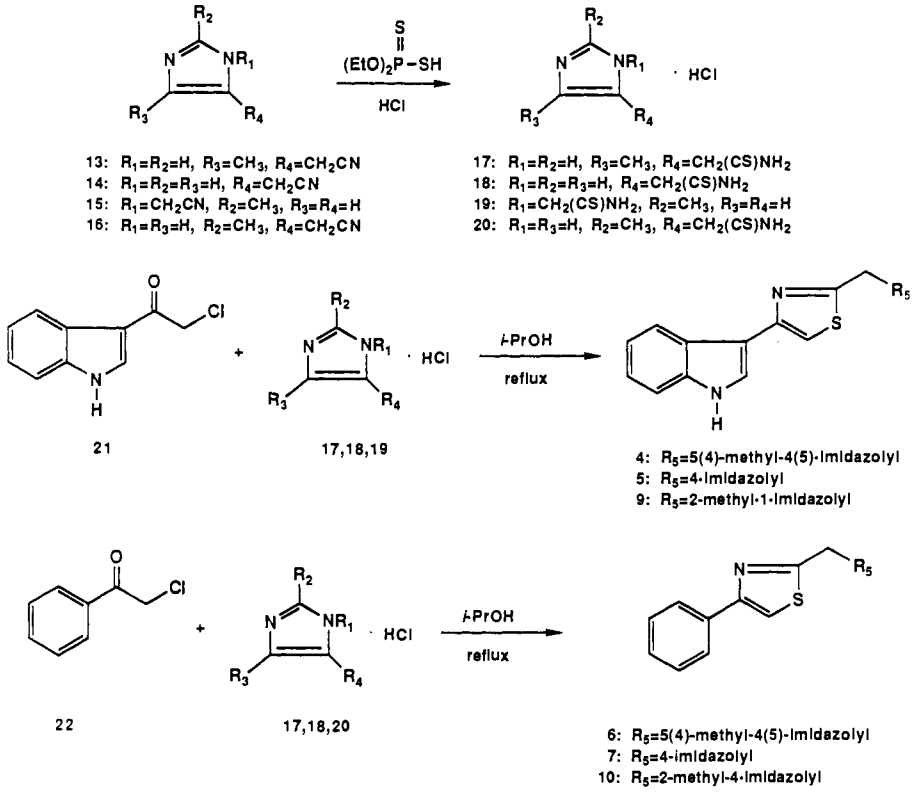
Table II. In Vivo Inhibition of the von Bezold-Jarisch Reflex in Rats[a]

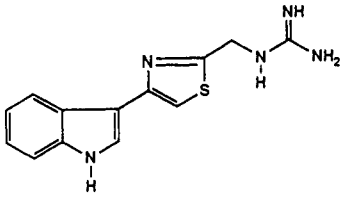
structure	compd	dose (mg/kg po)	% inhibn of B-J reflex 30 min postadministration
	3	1.0	0
	6	1.0	67 ± 29

[a] Procedure; ref 4a. Average of a minimum of three determinations. Test compounds were administered by oral gavage in unfasted, urethane anesthetized animals in a total volume of 1.5 mL.

Scheme I



an anesthetized rat[1]), the structurally novel guanidine derivative **3**[17] was found to possess potent 5-HT_3 receptor activity (Table I). Guanidine **3** is particularly interesting



3

because it lacks the potentially labile ester/amide linkage found in **1** and related congeners, instead linking the aryl and basic moieties (structural components present in all reported selective 5-HT_3 receptor antagonists) with a thiazole ring system.

Unlike other reported 5-HT_3 receptor antagonists, intravenous (iv) injection of guanidine derivative **3** has the property of inducing a transient bradycardia when examined in the B-J reflex assay, while blocking the bradycardia response induced by a subsequent injection of serotonin. These data indicate that compound **3** is a mixed agonist/antagonist or partial agonist at 5-HT_3 receptors. The observation that an iv dose of 4 μg/kg of selective 5-HT_3 antagonist **1** totally blocked the bradycardia normally produced by an iv injection of 20 μg/kg compound **3** supports this conclusion. Furthermore, bradycardia produced by compound **3** is not likely to result from an interaction with cholinergic or adrenergic receptors since compound **3** is relatively inactive (IC_{50} > 1 μM) at displacing [3H]QNB, [^{3}H]clonidine, or [^{3}H]prazosin binding to neuronal membranes. Since guanidine **3** also lacks oral activity in this functional paradigm (Table II), synthetic modification of structure **3** was initiated with the simultaneous goals of obtaining a full antagonist and an agent with improved oral activity.

Table I presents a series of aromatic thiazole derivatives in which various substituted imidazole groups replace the guanidine function in structure **3**. Aromatic thiazole derivatives **4**–**7**, **9**, and **10** were synthesized convergently[18]

(17) Zawistoski, M. *J. Heterocycl. Chem.* In press.

by condensation of an α-halomethyl ketone with an appropriate thioamide as outlined in Scheme I.[19] The required α-halomethyl ketones **21**[20] and **22**[21] and (cyanomethyl)imidazoles **13**,[22] **14**,[24] **15**,[23] and **16**[22] were prepared by known procedures. Thioamides **17–20** were generated from the corresponding nitrile derivatives with either diethyl dithiophosphonate[25] or thioacetamide and hydrochloric acid.[26]

Crucial to this investigation was the discovery that replacement of the guanidine portion of compound **3** with a 5(4)-methyl-4(5)-imidazolylmethyl functionality affords an agent (derivative **4**) that retains both 5-HT_3 receptor binding affinity and serotonin antagonism in the B-J reflex assay. Most importantly, this structural modification eliminates the initial bradycardia that is observed with guanidine **3** in the B-J reflex paradigm, providing full antagonist activity. In addition, both 5-HT_3 receptor binding affinity and antagonism of the serotonin induced B-J reflex are significantly enhanced by substituting a phenyl group for the indole moiety in derivative **4**. As can be seen in Table I, imidazole derivative **6** is a potent and selective 5-HT_3 antagonist (IC_{50} values for displacement of radioligand binding to other serotonin, cholinergic, adrenergic, and dopamine receptors are >1 μM). Derivative **6** also demonstrates robust antagonism of the serotonin induced B-J reflex upon oral administration (Table II).

It should be noted that desmethylimidazole derivatives **5** and **7** also possess nanomolar 5-HT_3 receptor binding affinity, but analogous to guanidine derivative **3**, cause initial bradycardia upon iv injection when evaluated in the B-J reflex paradigm. This response is similar to that observed with the known 5-HT_3 receptor agonist 2-methylserotonin (**8**)[1] (Table I). This observation suggests that the 4(5)-methyl substituent on the imidazole functionality is necessary to confer full antagonist properties to this series. Interestingly, the substitution pattern of the imidazole substituent appears to be not only important for full antagonist activity but also for 5-HT_3 receptor affinity as well. As shown in Table I, both ondansetron-related N-substituted imidazole derivative **9** and 2-methyl-4(5)-imidazolylmethyl derivative **10** are considerably less effective in the displacement of **1** from 5-HT_3 receptor binding sites.

In summary, a series of potent, orally active and structurally novel 5-HT_3 receptor antagonists in which a thiazole ring system replaces the amide/ester linkage in the ICS-205-930 series and the ketone functionality in the ondansetron series is described. As such, this series of compounds represents the first group of selective and potent 5-HT_3 receptor antagonists lacking a carbonyl-containing side chain between the aryl and basic portions of the molecule. Within the thiazole series, agonist activity, observed with the guanidine derivative **3**, can be eliminated by placing a 5(4)-methyl substituent on the 4(5)-imidazolylmethyl functionality. This strategy has resulted in the preparation of the imidazole derivative **6**, which is a potent and selective 5-HT_3 antagonist. The related desmethyl derivative **7**, based on its activity in the B-J reflex assay, would appear to possess 5-HT_3 receptor agonist character.

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(19) All new compounds have spectral data (^{1}H NMR, high-resolution mass spectra, and/or C, H, N analysis) supporting their structural assignment. Compound/melting point (ethanol): **4** > 200 °C (hydrochloride salt); **5**, 167–169 °C (hydrochloride salt); **6**, 138–143 °C; **7** 234–236 °C (hydrochloride salt); **9**, 198–202 °C; **10**, 224–227 °C (hydrochloride salt).
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