were combined and allowed to stir at 25 °C for 18 h and then evaporated to dryness at 60 °C under vacuum. One-hundred grams of ice was added, and the contents were triturated. After the ice had melted, the gummy product was separated from water by decantation and hydrolyzed with 100 mL of 0.1 N NaOH and 25 mL of acetonitrile at 25 °C for 6 h. Most of the acetonitrile was removed by rotary evaporation under vacuum. The solution was adjusted to pH 7.5 with 1 N HCl, diluted to 450 mL with distilled water, and applied on a DEAE column. The unreacted pteroic acid **13** was eluted during the application. When the application was complete, the column was washed with 200 mL of 0.015 M NaCl in 0.005 M phosphate buffer, pH 7.0. The column effluents were pooled for the recovery of unreacted **13**. After removal of **13**, the gradient was started, and a clean, pure, UV-absorbing material was eluted. Fractions corresonding to this material were pooled, evaporated to 15 mL, chilled, and acidified to obtain the light yellow product **3**, which was separated by filtration, washed with ice-cold water at pH 4.0 (adjusted with HOAc) several times to remove inorganic materials, and dried: yield 180 mg (39%); mp >300 °C; NMR 220 MHz (TFA) δ 8.85 (s, 1 H, C^7 proton), 4.8 (t, 1 H, α proton of glutamate), 3.8 and 3.5 (2 t, bridge ethyl), 3.4 (c, 1 H, 1′-H or 4′-H), 2.8–1.6 (cyclohexane).

A small amount of this material (5 mg) was dissolved in 0.5 mL of 0.005 N NaOH, diluted to 50 mL, adjusted to pH 7.5 with 0.1 N HCl, and chromatographed on a 5 × 25 cm DEAE column with a linear NaCl gradient. Compound **3** eluted from the column at 0.03 M NaCl as a single, symmetrical, UV-absorbing peak, establishing its purity. Anal. ($C_{20}H_{27}N_7O_6 \cdot H_2O$) C, H, N, O.

Biological Evaluation. The methods of preparation of the 7,8-dihydro and *d,l*-tetrahydro analogues of **13** and **3** were identical with those employed previously in our laboratories for the preparation of such derivatives of 11-oxa- and 11-thiohomofolate. Details of these procedures have been published.[10,11] Thymidylate synthase assays were carried out according to the procedure of Wahba and Friedkin.[22] Microbiological and dihydrofolate reductase assays were performed as described.[23,24]

Acknowledgment. This investigation was supported by Grants CA 27101 (M.G.N.) and CA-10914 (R.L.K.) from the national Cancer Institute, National Institutes of Health, and Grant CH-53C from the American Cancer Society. We are also indebted to Dr. S. Y. Chen of New England Nuclear, Boston, MA, for the 220-MHz NMR spectrum of **3**.

Registry No. **3**, 83704-88-5; 7,8-dihydro-**3**, 83719-42-0; *dl*-dihydro-**3**, 83705-02-6; **4**, 6959-76-8; **5**, 83704-89-6; **6**, 83704-90-9; **6** acid chloride, 83704-91-0; **7**, 83704-92-1; **8**, 83704-93-2; **9**, 83704-94-3; **10**, 83704-95-4; **11**, 83704-96-5; **12**, 83704-97-6; **12** dithionite reduced, 83704-98-7; **13**, 83705-00-4; 7,8-dihydro-**13**, 83705-01-5; *dl*-dihydro-**13**, 83710-12-7; **13** ethyl pteroate analogue, 83704-99-8; **14**, 83710-11-6; diazomethane, 334-88-3; 2-amino-6-chloro-4-hydroxy-5-nitropyrimidine, 1007-99-4; diethyl L-glutamate hydrochloride, 1118-89-4; thymidylate synthase, 9031-61-2; dihydrofolate reductase, 9002-03-3.

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Conformational Requirements for Histamine H_2-Receptor Inhibitors: A Structure–Activity Study of Phenylene Analogues Related to Cimetidine and Tiotidine†

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Two series of compounds related to cimetidine and tiotidine were synthesized as part of a study to evaluate the importance of conformational parameters in binding at histamine H_2 receptors. The flexible methylthioethyl connecting chain was replaced by a conformationally restricting phenylene unit. These compounds were evaluated for antagonism of the dimaprit-stimulated chronotropic response in the guinea pig atrium and inhibition of histamine stimulated secretion of gastric acid in the dog. In both series, biological activity is markedly dependent on the *m*-phenylene regioisomers. Histamine H_2-receptor activity is retained in both series; however, in the tiotidine series, gastric antisecretory activity is significantly improved. Regardless of the end group, *N*-cyanoguanidine (**1b**), 1,1-diamino-2-nitroethene (**2b**), or 3,4-diamino-1,2,5-thiadiazole 1-oxide (**3b** and **4b**), each 3-(2-guanidino-4-thiazolyl)phenyl analogue was ca. 8 and 90 times more potent intraveneously than tiotidine and cimetidine, respectively. The electronic influences of the phenylene unit on biological activity were also evaluated. It was concluded that the geometric constraints imposed by the *m*-phenylene connecting element were more important than electronic factors in binding events at the histamine H_2 receptor.

The development of the clinically useful histamine H_2-receptor antagonist cimetidine as a gastric antisecretory drug has provided the stimulus in the search for more active agents.[1] Two highly potent histamine H_2-receptor inhibitors, ranitidine[2] [1-(methylamino)-1-[[2-[[[5-[(dimethylamino)methyl]-2-furanyl]methyl]thio]ethyl]-amino]-2-nitroethane] and tiotidine[3] [*N*-cyano-*N*′-methyl-*N*″-[[2-[[(2-guanidino-4-thiazolyl)methyl]thio]-ethyl]amino]guanidine] evolved from such endeavors.

† A preliminary account of this work has been presented: see "Abstracts of Papers", 183rd National Meeting of the American Chemical Society, Las Vegas, NV, Mar 28, 1982; American Chemical Society: Washington, DC, 1982; Abstr MEDI 60.

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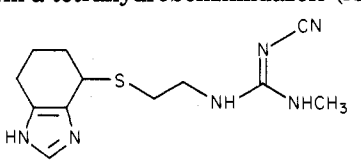
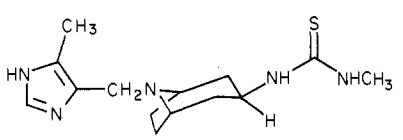
Recently, another class of potent antagonists was reported[4] in which the "urea equivalent" (i.e., cyanoguanidine or diaminonitroethene moieties) was replaced by a 3,4-diamino-1,2,5-thiadiazole 1-oxide or 1,1-dioxide group. A structure comparison reveals that while the heterocyclic components, i.e., methylimidazole, guanidinothiazole, and dimethylaminomethylfuran, and the urea equivalents of these antagonists can be modified to improve potency, the highly flexible methylthioethyl connecting chain has been maintained as a structurally constant element.

Mitchell has suggested that the intramolecular ordering present in the crystal lattice of cimetidine due to a unique 10-membered ring, hydrogen-bonded conformation may contribute to its biological activity.[5] In fact, the sulfur atom in the methylthioethyl connecting chain is required for adoption of this conformation. A folded or intramolecularly stacked conformation also has been reported for an example in the thiadiazole oxide class of histamine H_2-receptor antagonists.[4b] While alkyl substitution on the chain methylene adjacent to the imidazole ring in cimetidine is reported to diminish activity, this modification does not severely limit the chain flexibility.[6] Only two studies on histamine H_2-receptor inhibitors with conformationally restricted connecting chains have been reported. In one case, incorporation of the imidazole methyl substituent into a portion of the connecting chain of cimetidine to form a tetrahydrobenzimidazole (A) resulted in



A

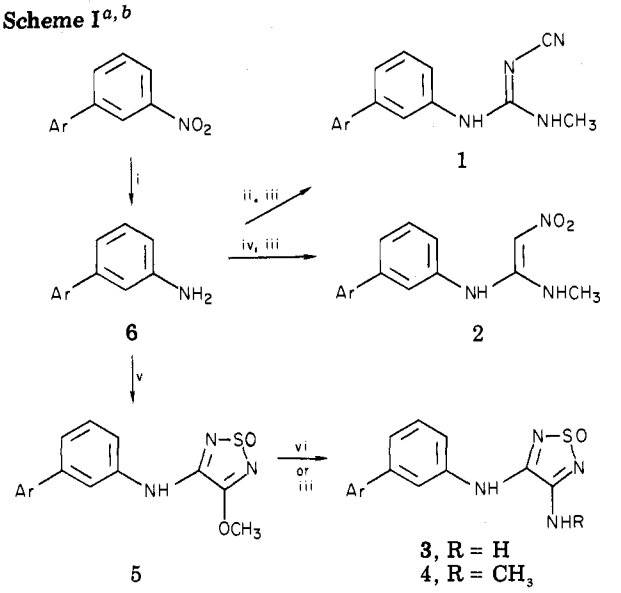


B

a complete loss of gastric antisecretory activity in rats and dogs.[7] In the other case, nortropane analogues of cimetidine and metiamide were prepared.[8] Only one epimer (B) of a metiamide analogue exhibited significant histamine H_2-receptor inhibition in the isolated guinea pig atrium. No antisecretory data were reported.

This report describes the preparation of histamine H_2-receptor inhibitors in which reduced flexibility is attained by the replacement of the methylthioethyl connecting chain with a phenylene unit. The interdependency of the heterocycle and "urea equivalent" as reflected by

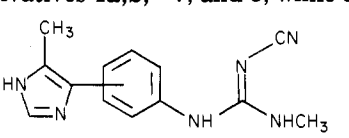
Scheme I[a,b]



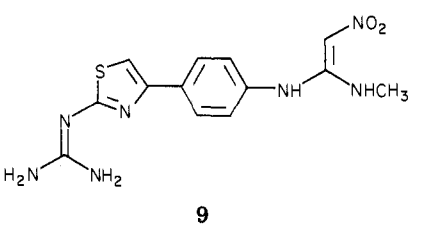
[a] a, Ar = 5(4)-methyl-4(5)-imidazolyl; **b**, Ar = 2-guanidino-4-thiazolyl. [b] i = H_2, 10% Pd/C, HCl/EtOH; ii = dimethyl *N*-cyanodithioimidocarbonate, ROH; iii = CH_3NH_2, EtOH; iv = 1,1-bis(methylthio)-2-nitroethene, CH_3CN; v = 3,4-bis(methylthio)-1,2,5-thiadiazole 1-oxide, CH_3OH; vi = NH_3, EtOH.

histamine H_2-receptor affinity and gastric antisecretory activity in this series are also discussed.

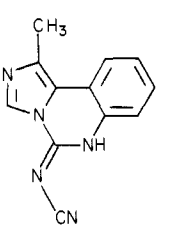
Chemistry. All compounds evaluated in this study were prepard by procedures similar to those outlined in Scheme I for the *m*-phenylene analogues. The sequential reaction[9] of dimethyl cyanodithioimidocarbonate and methylamine with an appropriate aniline, such as **6**, gave the cyanoguanidine derivatives **1a,b**,[10] **7**, and **8**, while a similar se-



7 (ortho)
8 (para)



9



10

quential reaction[11] with 1,1-bis(methylthio)-2-nitroethene and methylamine yielded the nitroethene analogues **2a,b**[10] and **9**. However, in the formation of ortho derivative **7**, the initial intermediate isolated before reaction with methylamine was not the expected *S*-methylisothiourea but a cyclic imidazoquinazoline, **10**. This intermediate readily reacted with methylamine to give **7**, but in refluxing methanol, **7** recyclized to **10** with elimination of methylamine.

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The thiadiazole analogues were prepared by a reaction between 3,4-bis(methylthio)-1,2,5-thiadiazole 1-oxide[12] and anilines **6a,b** to give intermediates **5a,b**, which in turn were reacted with ammonia or methylamine to give **3a,b** and **4b**, respectively. Instead of the expected methylthio intermediate, the methoxy analogue **5** was isolated as a result of the ready solvolysis of the methylthio intermediate in the methanol reaction solvent. The sluggish reaction between 3,4-diethoxy-1,2,5-thiadiazole 1-oxide[12] and aniline **6** strongly suggests that 3,4-bis(methylthio)-1,2,5-thiadiazole 1-oxide is not solvolyzed prior to reaction with the aniline. The decreased nucleophilicity of the anilines precluded the preparation of thiadiazole analogues **3a** and **3b** from the previously described 3-amino-4-ethoxy-1,2,5-thiadiazole 1-oxide.[4b]

The required aniline intermediates, such as **6**, were prepared by the catalytic reduction of the respective nitrobenzenes. 5(4)-Methyl-4(5)-(3-nitrophenyl)imidazole was reported in the literature;[13] however, the 2-nitro and 4-nitro isomers were unknown. These could be readily prepared by nitration of 5(4)-methyl-4(5)-phenylimidazole[14] and the isomers separated by a literature method.[15] 2-Guanidino-4-(3-nitrophenyl)thiazole was prepared by the condensation of amidinothiourea with 1-bromo-3′-nitroacetophenone, a procedure similar to that described for the para isomer.[16]

Biological Results and Discussion

The biological results are listed in Table I. All compounds were evaluated in vitro for histamine H_2-receptor inhibition using the dimaprit-stimulated chronotropic response of the guinea pig atrium.[17] In addition, these compounds were administered intravenously to dogs with a gastric fistula under histamine stimulation to determine gastric antisecretory activity.[4b] Compounds with sufficient activity were then administered intragastricly via fistula to determine oral potency.[18]

Although the meta isomer **1a** exhibited a higher affinity for the histamine H_2 receptor on the guinea pig atrium than the ortho and para isomers **7** and **8**, it was only about 0.01 times as potent as cimetidine. Similarly, on a dose basis, **1a** was more potent than **7** or **8** when administered intravenously to dogs, and the percent inhibition of acid output was determined. In this model, **1a** was approximately one-third as potent as cimetidine.

The low oral potency of **1a** prompted a search for other urea equivalents that might be incorporated into the phenylene substructure to improve activity. Two selected were the nitroethene unit of ranitidine and the thiadiazole oxide end group. Replacement of the cyanoguanidine group of **1a** with these urea equivalents resulted in enhanced in vitro activity for only **3a**. In the dog, potency was not similarly enhanced when these compounds were administered intravenously. However, the oral potency of **2a** did increase relative to **1a**, but was still only approximately one-seventh that of cimetidine.

Table I. Chemical and Biological Data on Phenylene Analogues

compd	mp,[c] °C	formula[d]	in vitro inhibn of H_2 receptor: [a] $-\log K_I$ (GP atrium)	inhibition of acid output in dogs [b]				
				intravenous			peroral	
				ED_{50}, mg/kg	dose, mg/kg	% [e]	ED_{50}, mg/kg	dose, mg/kg % [f]
8	244–246	$C_{13}H_{14}N_6$	inact[j]		5.0	0 ± 0		
1a	188–189[g]	$C_{13}H_{14}N_6 \cdot C_4H_4O_4$	4.4		5.0	48 ± 14		20 8 ± 4
7	241–244	$C_{13}H_{14}N_6$	inact[j]		5.0	4 ± 4		
2a	233 dec	$C_{13}H_{15}N_5O_2$	4.8		5.0	28 ± 20		20 54 ± 4
3a	261–263	$C_{12}H_{12}N_6OS$	6.1		5.0	5 ± 4		20 1 ± 1
1b	248 dec	$C_{13}H_{14}N_8S$	6.5	0.02 (0.01, 0.03)	0.03	79 ± 10	2.81 (0.7, 10.9)	10 73 ± 19
2b	236 dec	$C_{13}H_{15}N_7O_2S$	6.7	0.02 (0.01, 0.03)	0.03	59 ± 14	2.80 (0.8, 9.1)	10 70 ± 9
9	270 dec	$C_{13}H_{15}N_7O_2S$	4.5		3.0	55 ± 12		
3b	219–220[h]	$C_{12}H_{12}N_8OS_2 \cdot C_2H_6SO$	6.5	0.02 (0.01, 0.08)	0.03	58 ± 22		10 31 ± 15
4b	160–161[h]	$C_{13}H_{14}N_8OS_2 \cdot C_2H_6SO$	6.4	0.017 (0.01, 0.02)	0.03	85 ± 5		10 21 ± 9
cimetidine[i]			6.3	1.84 (1.37, 2.46)			2.86 (2.43, 3.38)	
tiotidine[i,g]			7.5	0.16 (0.15, 0.18)			0.12 (0.05, 0.28)	

[a] Chronotropic response to increasing concentrations of the H_2-receptor agonist dimaprit (10^{-7}–10^{-4} M); three to four atria per test concentration; $-\log K_I = \log (ED_{50}$ dose ratio $- 1) - \log$ (drug dose); see ref 23. [b] Gastric secretion evoked by a maximal stimulating dose of histamine (64 μg/kg, sc) in dogs with a chronic gastric fistula. [c] Uncorrected. [d] Analyses for C, H, and N were within 0.4% of theoretical values, and NMR were consistent with assigned structures. [e] Determination on three to four dogs per dose. [f] Determination on three to five dogs per dose. [g] Maleate salt. [h] Me_2SO solvate. [i] Synthesized in these laboratories. [j] Inactive at 8×10^{-5} M.

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Table II. Acidities of Some Phenylene Analogues[a]

compd	pK_a (het)[b]	pK_a (urea)[c]
1b	6.2	*d,e*
2b	6.0	9.2[f]
9	6.2	9.7
3b	6.2	8.9[g]
cimetidine	6.4	*d,e*
tiotidine	7.1	*d,e*

[a] Potentiometric determinations in 30% ethanol/water, since all compounds were not sufficiently soluble in water. Relatively small differences were observed when the pK_a's of **2b** (5.8 and 8.8) and tiotidine (6.8) were determined in water. [b] pK_a of heterocyclic component measured as proton gained. [c] pK_a of urea component measured as proton lost. [d] A reference pK_a for *N,N'*-dimethyl-*N''*-cyanoguanidine is ~14 in water.[22] [e] pK_a is >11, upper limit of potentiometric method with glass electrodes. [f] A reference pK_a for the diaminonitroethene group of ranitidine is ⩾11 in 30% ethanol/water. [g] A reference pK_a for the 3,4-diaminothiadiazole 1-oxide moiety is ~11 in water.[4b]

Since a higher affinity for the histamine H_2 receptor has been obtained by replacement of the imidazole ring of cimetidine (pA_2 = 6.3) with the guanidinothiazole ring of tiotidine (pA_2 = 7.5),[3] this modification was incorporated into the phenylene series. Up to 100-fold enhancement was realized in vitro with guanidinothiazole analogues **1b** and **2b** relative to imidazoles **1a** and **2a**, while guanidinothiazoles **3b** and **4b** were comparable to imidazole **3a**. All four guanidinothiazoles, **1b–4b**, however, exhibited unexpectedly high intravenous activity, being ~8 times more potent than tiotidine in the dog. Furthermore, N-methylation of the end group of **3b** as in **4b** did not lower activity as had been observed for other thiadiazole 1-oxide analogues.[4b] The para-isomer **9** was found to be less than 0.01 as potent as the corresponding meta-isomer **2b** both in vitro and in vivo, corroborating the requirement for the *m*-phenylene substructure as was demonstrated in the imidazole series. Orally, compounds **1b** and **2b** were comparable to cimetidine but only 0.04 times as potent as tiotidine. The thiadiazole oxide modifications (**3b** and **4b**) offered no advantage over the cyanoguanidine or nitroethene end groups.

In general, replacement of the imidazole ring with guanidinothiazole improved both histamine H_2-receptor inhibition and intravenous gastric antisecretory activity in the *m*-phenylene series. In contrast, however, biological activity was not markedly dependent on the choice of a urea equivalent when the imidazole ring was replaced by the guanidinothiazole group. In all examples, oral antisecretory activity was low.

The overall structural effect of the replacement of the methylthioethyl connecting chain by a *m*-phenylene ring is to impose a geometrical constraint on the close approach of the heterocycle and urea equivalent in order to prevent the formation of an effective intramolecular hydrogen bond as in cimetidine. This constraint alters conformational flexibility, as well, by removing several degrees of rotational freedom.

In addition, the phenylene modification introduces profound electronic effects on the heterocyclic and urea components. In an attempt to correlate these electronic effects with activity, the basicity of the heterocyclic element and acidity of the urea component were determined potentiometrically for each guanidinothiazole analogue and compared with that of cimetidine and tiotidine (Table II). The phenyl substitution increased the acidity of the urea components in those analogues for which a pK_a could be determined by at least a full pK_a unit relative to tiotidine and decreased the basicity of the guanidinothiazole moiety by nearly 1 pK_a unit to a value comparable to that of the imidazole ring of cimetidine. Most importantly, the electronic differences between the meta (**2b**) and para (**9**) analogues are small compared with their differences in biological activity.

Thus, it would appear that the selective geometric constraints introduced by the *m*-phenylene modification are of a more relative importance to biological activity than the concomitant electronic contributions. The possibility that the π ring may contribute to other receptor-site interactions that are unrelated to conformation cannot be excluded.

In conclusion, the successful replacement of the flexible methylthioethyl connecting chain common to potent histamine H_2-receptor antagonists with a more rigid phenylene unit has been achieved. In vitro receptor activity is retained, and in vivo gastric antisecretory activity, at least intraveneously, is improved. Furthermore, the biological activity is markedly dependent on the spatial relationship between the heterocycle and the urea equivalent and is uniquely optimized in the meta regioisomers of the phenylene series. These results offer the most definitive evidence yet presented for the steric requirement for binding to the histamine H_2 receptor.

Experimental Section

Chemistry. All melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Analyses indicated by symbols of the elements were within ±0.40% of the theoretical values. Routine NMR spectra, obtained on the Varian Associates spectrometers, Model T-60 and EM-390, were consistent with the structures indicated. Yields were not optimized.

***N*-Cyano-*N'*-methyl-*N''*-[4-[5(4)-methyl-4(5)-imidazolyl]-phenyl]guanidine (8).** 5(4)-Methyl-4(5)-(4-aminophenyl)-imidazole (5.9 g, 34 mmol) was added to a solution of dimethyl cyanodithioimidocarbonate[19] (5.4 g, 37 mmol) in absolute ethanol (30 mL). After stirring overnight, 8.0 g (87%) of ***N*-cyano-*S*-methyl-*N'*-[4-[5(4)-methyl-4(5)-imidazolyl]phenyl]isothiourea** (mp 217–219 °C) was collected by filtration and used without further purification.

This crude isothiourea (6.8 g, 25 mmol) was added to a solution of 30 wt % ethanolic methylamine (50 mL) cooled in an ice bath. The solution was allowed to gradually warm to room temperature overnight. The solvent was evaporated, and the residue was crystallized from methanol to give 3.7 g (58%) of 8, mp 243–245 °C. Anal. ($C_{13}H_{14}N_6$) C, H, N.

Compounds **1a** and **1b**[10] were prepared in 46 and 40% overall yields from the appropriate anilines by the same procedure; however, **1a** was obtained crystalline as a maleate salt. Their respective melting points are listed in Table I.

***N*-Cyano-*N'*-methyl-*N''*-[2-[5(4)-methyl-4(5)-imidazolyl]-phenyl]guanidine (7).** 5(4)-Methyl-4(5)-(2-aminophenyl)-imidazole (5.0 g, 28.9 mmol) was added to a solution of dimethyl cyanodithioimidocarbonate[19] (4.7 g, 32 mmol) in absolute ethanol (25 mL) and stirred for 2 days. The precipitate that formed was collected, washed with ethanol and diethyl ether, and air-dried to give 5.4 g (83%) of **5-(cyanoimino)-5,6-dihydroimidazo-[3,4-*c*]quinazoline (10)**, mp 277–279 °C.

This intermediate (4.9 g, 21.9 mmol) was added to a solution of methylamine (12 g) dissolved in ethanol (30 mL) and precooled in an ice bath. The reaction mixture was allowed to gradually warm to room temperature overnight, and the precipitate that formed was collected, washed with ethanol and diethyl ether, and air-dried to give 3.3 g (59%) of pure **7**, mp 241–244 °C. Anal. ($C_{13}H_{14}N_6$) C, H, N. During attempts to recrystallize **7** from refluxing methanol, **10** was regenerated.

1-[[3-(2-Guanidino-4-thiazolyl)phenyl]amino]-1-(methylamino)-2-nitroethene (2b). To a solution of 1,1-bis(methyl-

(19) Hantzch, A.; Wolvekamp, M. *Justus Liebigs Ann. Chem.* **1904**, *331*, 265.

thio)-2-nitroethene[20] (3.46 g, 21 mmol) in acetonitrile (40 mL) was added 2-guanidino-4-(3-aminophenyl)thiazole (4.6 g, 20 mmol). The mixture was refluxed for 2 h as the thiazole dissolved and the product precipitated. This precipitate was collected from the cooled reaction, washed with acetonitrile, and air-dried to give 5.8 g (82%) of **1-[[3-(2-guanidino-4-thiazolyl)phenyl]amino]-1-(methylthio)-2-nitroethene**,[10] mp 212–214 °C. Anal. ($C_{13}H_{19}N_6O_2S_2$) C, H, N.

This intermediate was added to a solution of methylamine (15 g) dissolved in methanol (60 mL) and precooled in an ice bath. The reaction mixture was allowed to gradually warm to room temperature overnight and was filtered and evaporated. The residue crystallized upon treatment with methanol/water (20:1). This product was dissolved in a minimum amount of DMF, and the solution was slowly diluted with 2-propanol until a dark gum ceased to precipitate. The decanted solution was then diluted with diethyl ether, and pure **2b**[10] slowly precipitated (1.5 g, 27%), mp 236 °C dec. Anal. ($C_{13}H_{15}N_7O_2S$) C, H, N.

Compounds **2a** and **9** were prepared in 21 and 28% overall yields from the appropriate anilines by this same procedure. Their melting points are listed in Table I.

3-Amino-4-[[3-[5(4)-methyl-4(5)-imidazolyl]phenyl]amino]-1,2,5-thiadiazole 1-Oxide (3a). To a solution of 5-(4)-methyl-4(5)-(3-aminophenyl)imidazole (1.9 g, 11 mmol) in methanol (25 mL) was added 3,4-bis(methylthio)-1,2,5-thiadiazole 1-oxide[12] (2.15 g, 11 mmol). This mixture was allowed to stir at room temperature for several days as the product slowly formed and precipitated. The crude solid (2.0 g) collected was chromatographed on silica gel and eluted with 10% methanol/chloroform to give 1.75 g (47%) of pure **3-methoxy-4-[[3-[4(5)-methyl-5-(4)-imidazolyl]phenyl]amino]-1,2,5-thiadiazole 1-oxide methanolate (5a)**, mp 165–195 °C. Anal. ($C_{13}H_{13}N_5O_2S\cdot CH_4O$) C, H, N.

This intermediate (1.5 g, 4.5 mmol) was added to a solution of 2 M ammonia/ethanol, and the suspension was stirred overnight at room temperature. The suspended solid was collected and dissolved in a minimum amount of warm DMF (60 °C), treated with charcoal, and filtered, and the filtrate diluted with diethyl ether. Pure **3a** (1.1 g, 84%) slowly crystallized, mp 261–263 °C. Anal. ($C_{12}H_{12}N_6OS$) C, H, N.

Compounds **3b** and **4b** were prepared similarly in 13 and 17% overall yields from aniline **6b** but were crystallized from Me_2SO/2-propanol and their melting points are listed in Table I as Me_2SO solvates.

Preparation of Substituted Anilines. All required anilines were prepared by the following procedure from their respective nitrobenzenes.

5(4)-Methyl-4(5)-(3-aminophenyl)imidazole (6a). A solution of 5(4)-methyl-4(5)-(3-nitrophenyl)imidazole[13] (17.5 g, 86 mmol) in ethanol (200 mL) containing concentrated HCl (20 mL) and 10% Pd/C (2.2 g) was hydrogenated in a Parr apparatus under about 50 psi of hydrogen until the theoretical amount was taken up (4 h). The reaction was filtered to remove catalyst and concentrated to remove the ethanol solvent. The resulting aqueous solution was cooled and made alkaline with sodium hydroxide. The precipitated product was collected, dried, and recrystallized from ethanol to give 11.1 g (75%) of **6a**, mp 200–202 °C. Anal. ($C_{10}H_{11}N_3$) C, H, N.

5(4)-Methyl-4(5)-(4-aminophenyl)imidazole: yield 89%; mp 214–217 °C. Anal. ($C_{10}H_{11}N_3$) C, H, N.

5(4)-Methyl-4(5)-(2-aminophenyl)imidazole: yield 73%; mp 158–160 °C. This compound was not analyzed.

2-Guanidino-4-(3-aminophenyl)thiazole (6b): yield 85%; mp 212–214 °C. Anal. ($C_{10}H_{11}N_5S$) C, H, N.

2-Guanidino-4-(4-aminophenyl)thiazole: yield 91%; mp 257 °C dec. Anal. ($C_{10}H_{11}N_5S$) C, H, N.

5(4)-Methyl-4(5)-(4-nitrophenyl)imidazole and 5(4)-Methyl-4(5)-(2-nitrophenyl)imidazole. 5(4)-Methyl-4(5)-phenylimidazole nitrate (44.0 g, 0.2 mol), mp 168–169 °C dec, which is obtained by crystallizing 5(4)-methyl-4(5)-phenylimidazole[14] from 10% HNO_3, was added portionwise to concentrated H_2SO_4 (80 mL) maintained below 10 °C. After 3 h, the reaction was diluted with ice–water and neutralized with sodium hydroxide. The collected precipitate was dried in a vacuum oven and crystallized from methanol. After several recrystallizations, pure **5(4)-methyl-4(5)-(4-nitrophenyl)imidazole** (32 g, 79%), mp 228–231 °C, was obtained. Anal. ($C_{10}H_9N_3O_2$) C, H, N.

The combined mother liquors were evaporated, and the residue was dissolved in hot 10% HNO_3. While the solution cooled to 20 °C, a residual amount of 5(4)-methyl-4(5)-(4-nitrophenyl)-imidazole precipitated as a nitrate salt and was removed by filtration. Further cooling in an ice bath afforded the nitrate salt of 5(4)-methyl-4(5)-(2-nitrophenyl)imidazole, which was collected and added to a sodium hydroxide solution. This collected solid was dried in a vacuum oven, but not recrystallized, to give 7 g (17%) of **5(4)-methyl-4(5)-(2-nitrophenyl)imidazole**, mp 180–185 °C.

2-Guanidino-4-(3-nitrophenyl)thiazole. This compound was prepared according to the literature procedure described for the preparation of 2-guanidino-4-(4-nitrophenyl)thiazole.[16] To a suspension of amidinothiourea hydrochloride (61.8 g, 0.4 mol) in methanol (420 mL) cooled to 5 °C was added a solution of triethylamine (55.5 mL, 0.4 mol) in methanol (180 mL). After the solution was stirred for 15 min, solid 2-bromo-3′-nitroacetophenone[21] (97.6 g, 0.4 mol) was added, and the reaction mixture was allowed to warm to room temperature and to stir overnight. The reaction was cooled, and the precipitated salt was collected, washed with 2-propanol, dissolved in water, and neutralized with ammonium hydroxide. This collected precipitate was digested in hot ethanol to give 62 g (59%) of product, mp 215–216 °C. Anal. ($C_{10}H_9N_5O_2S$) C, H, N.

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Registry No. **1a** maleate, 75814-88-9; **1b**, 72801-63-9; **2a**, 83831-27-0; **2b**, 72801-90-2; **3a**, 83831-28-1; **3b**, 81074-55-7; **4b**, 81074-37-5; **5a**, 83831-29-2; **6a**, 75815-17-7; **6b**, 72801-60-6; **7**, 75814-89-0; **8**, 75814-86-7; **9**, 83831-30-5; **10**, 83844-77-3; 5(4)-methyl-4(5)-(4-aminophenyl)imidazole, 75815-15-5; dimethyl cyanodithioimidocarbonate, 10191-60-3; *N*-cyano-*S*-methyl-*N*′-[4-[5(4)-methyl-4(5)-imidazolyl]phenyl]isothiourea, 75814-85-6; 5(4)-methyl-4(5)-(2-aminophenyl)imidazole, 75815-16-6; 1-[[3-(2-guanidino-4-thiazolyl)phenyl]amino]-1-(methylthio)-2-nitroethene, 83844-78-4; 3,4-bis(methylthio)-1,2,5-thiadiazole 1-oxide, 79844-65-8; 2-guanidino-4-(4-aminophenyl)thiazole, 83831-31-6; 5(4)-methyl-4(5)-(3-nitrophenyl)imidazole, 54887-79-5; 5(4)-methyl-4(5)-(4-nitrophenyl)imidazole, 75815-10-0; 5(4)-methyl-4(5)-(2-nitrophenyl)imidazole, 75815-13-3; 5(4)-methyl-4(5)-phenylimidazole nitrate, 75815-09-7; 5(4)-methyl-4(5)-phenylimidazole, 826-83-5; 5(4)-methyl-4(5)-(4-nitrophenyl)imidazole nitrate, 75815-12-2; 5(4)-methyl-4(5)-(2-nitrophenyl)imidazole nitrate, 75815-14-4; 2-guanidino-4-(3-nitrophenyl)thiazole, 83831-32-7; amidinothiourea hydrochloride, 52834-99-8; 2-bromo-3′-nitroacetophenone, 2227-64-7; 1,1-bis(methylthio)-2-nitroethene, 13623-94-4.

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(23) Data are presented as $-\log K_1$ rather than pA_2, since the slopes of all compounds were not parallel over the dose range tested and, thus, would not give unit slopes from a standard Schild plot. In addition, some $-\log K_1$ values increased as the incubation time was extended from our standard 10 min to 30 min. This may indicate slow receptor on-rates.