The Histamine H_2 Receptor Agonist Impromidine: Synthesis and Structure-Activity Considerations[1]

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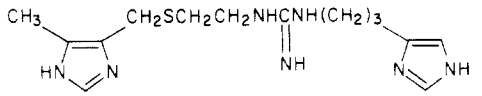
Impromidine (1) is a potent and selective histamine H_2 receptor agonist and its structure comprises a strongly basic guanidine group containing two different imidazole-containing side chains. In this paper we report the synthesis of analogues in which both of the side chains and the guanidine group are modified and tested as agonists or antagonists at histamine H_2 receptors on guinea pig atrium. A protonated amidine group linked by a chain of three carbon atoms to a tautomeric imidazole ring appears to be an essential feature for agonist activity and it is suggested that the second imidazole-containing side chain in impromidine mainly contributes toward affinity for histamine H_2 receptors.

N-[3-(1*H*-Imidazol-4-yl)propyl]-*N'*-[2-[[(5-methyl-1*H*-imidazol-4-yl)methyl]thio]ethyl]guanidine has the WHO-recommended international nonproprietary name impromidine (1) and is a highly potent and selective agonist for histamine H_2 receptors.[2] Unlike previously described

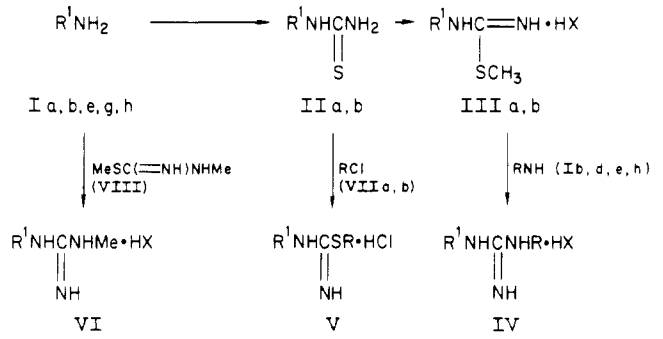


impromidine (1)

H_2 receptor agonists including 4-methylhistamine[3,4] and dimaprit,[5] impromidine is noteworthy in being considerably more potent than the natural agonist molecule histamine. The potency of impromidine relative to histamine on isolated tissue preparations or in stimulating gastric acid secretion in vivo varies by a factor of between 9 and 50.[2] On the isolated rat uterus and rat stomach[6] preparations however, although impromidine is considerably more potent than histamine, it elicits submaximal responses, and it was therefore suggested that impromidine has reduced "efficacy" compared with histamine and that the increased potency of impromidine results from increased "affinity" for histamine H_2 receptors.[2]

We have previously[2] observed that the chemical structure of impromidine poses interesting questions in relation to its pharmacological activity. Impromidine (1) possesses a guanidine group $R_1NHC(=NH)NHR_2$ with two different imidazole-containing substituents (where R_1 = 2-[[(5-methylimidazol-4-yl)methyl]thio]ethyl and R_2 = 3-imidazol-4-ylpropyl). The monosubstituted guanidine bearing this substituent R_2 (SK&F 91486[7]) is a weak partial agonist at H_2 receptors, whereas substituent R_1 is a structural feature of many H_2-receptor antagonists, e.g., metiamide,[8] cimetidine,[9] and oxmetidine.[10] Furthermore,

Scheme I. Synthesis of Impromidine and Related Compounds via Isothiouronium Salts (Method A)[a]



[a] Structures a-h are included in Table III.

the *N*-methylguanidine derivative (**20**, Table II) containing this substituent R_1 is an antagonist exhibiting no agonist activity.[9] We suggested[2] that R_1 may be associated more with affinity than with efficacy, but that introducing this affinity-contributing group into a weak partial agonist results in a molecule, i.e., impromidine, with increased affinity and also increased efficacy for H_2 receptors. These observations prompted us to synthesize various analogues of impromidine and here we report the synthesis and pharmacological activity at histamine H_2 receptors on guinea pig right atrium in vitro of compounds in which structural modifications are introduced into the substituents R_1 and R_2 and the guanidine group. These include (Table I) examples (**2–6**) in which the substituent R_1 is replaced by alternative imidazole- or thiazole-containing substituents while R_2 is unchanged, examples (**7–12**) in which R_2 is replaced by imidazole- or thiazole-containing substituents while R_1 is unchanged, and additional analogues (**13–19**) in which the substituents R_1 and R_2 of impromidine are unchanged while the guanidine group is modified as indicated (Table I). For comparison, included in Table II are analogues of the guanidinium or isothiouronium compounds of Table I in which one of the substituents R_1 and R_2 is replaced by methyl.

The compounds in Tables I and II were assayed for H_2-receptor agonist activity on the isolated guinea pig atrium preparation.[3,4] Details of the methodology used are included in the Experimental Section. Agonist potencies expressed relative to histamine (=1) and the percentage maximum response achieved relative to histamine are included in Tables I and II.

Synthesis of Impromidine and Analogues

Three general routes were used for the synthesis of impromidine and analogous disubstituted guanidinium de-

(1) A preliminary account of these studies was presented at the 8th Meeting of the European Histamine Research Society, Stockholm, Sweden, May 1979.
(2) Durant, G. J.; Duncan, W. A. M.; Ganellin, C. R.; Parsons, M. E.; Blakemore, R. C.; Rasmussen, A. C. *Nature (London)* **1978**, *276*, 403.
(3) Black, J. W.; Duncan, W. A. M.; Durant, G. J.; Ganellin, C. R.; Parsons, M. E. *Nature (London)* **1972**, *236*, 385.
(4) Durant, G. J.; Ganellin, C. R.; Parsons, M. E. *J. Med. Chem.* **1975**, *18*, 905.
(5) Parsons, M. E.; Owen, D. A. A.; Ganellin, C. R.; Durant, G. J. *Agents Actions* **1977**, *7*, 31.
(6) Parsons, M. E.; Sykes, K. *Br. J. Pharmacol.* **1980**, *69*, 6.
(7) Parsons, M. E.; Blakemore, R. C.; Durant, G. J.; Ganellin, C. R.; Rasmussen, A. C. *Agents Actions* **1975**, *5*, 464.
(8) Black, J. W.; Duncan, W. A. M.; Emmett, J. C.; Ganellin, C. R.; Hesselbo, T.; Parsons, M. E.; Wyllie, J. H. *Agents Actions* **1973**, *3*, 133.
(9) Durant, G. J.; Emmett, J. C.; Ganellin, C. R.; Miles, P. D.; Parsons, M. E.; Prain, H. D.; White, G. R. *J. Med. Chem.* **1977**, *20*, 901.
(10) Blakemore, R. C.; Brown, T. H.; Durant, G. J.; Emmett, J. C.; Ganellin, C. R.; Parsons, M. E.; Rasmussen, A. C. *Br. J. Pharmacol.* **1980**, *70*, 105P.

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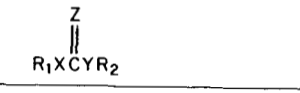
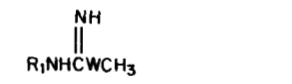
Table I. Structures and H_2-Receptor Activities of Impromidine Analogues



										H_2-receptor activities on guinea pig atrium[a]					
										agonist activity			antagonist activity		
no.	R_1[d]	R_2[d]	X	Y	Z	salt	molecular formula	mp, °C	cryst solvent	potency[b] rel to histamine = 1, mean (with 95% limits)	% max response (mean ± SEM)	n[c]	pA_2 (95% limits)	slope (mean ± SEM)	n[c]
1[f]	a	b	NH	NH	NH	3HCl	$C_{14}H_{23}N_7S·3HCl$	195–197	EtOH						
						3 picrate	$C_{14}H_{23}N_7S·3C_6H_3N_3O_7$	183–185	$Me_2CO–H_2O$	48.1 (37.6–60.1)	98.7 ± 3.6	10			
						2 oxalate	$C_{14}H_{23}N_7S·2C_2H_2O_4$	125–127	EtOH–H_2O						
2	c	b	NH	NH	NH	3 picrate	$C_{13}H_{21}N_7S·3C_6H_3N_3O_7$	173–175	$MeNO_2$–MeOH	5.38 (4.55–6.24)	95	3			
3	d	b	NH	NH	NH	3 picrate	$C_{14}H_{23}N_7·3C_6H_3N_3O_7$	206–208	$Me_2CO–H_2O$	12.35 (10.92–13.82)	85.5	4			
4	b	b	NH	NH	NH	3 picrolonate	$C_{13}H_{21}N_7·3C_{10}H_8N_4O_5$	273–275		4.83	100	2			
5	g	b	NH	NH	NH	3 picrate	$C_{13}H_{20}N_6S_2·3C_6H_3N_3O_7$	157–158	MeCN–EtOH	24.40 (19.8–31.1)	91	5			
6	h	b	NH	NH	NH	3HCl	$C_{13}H_{20}N_6S·3HCl$	174–175	EtOH–Et_2O	26.65 (15.38–46.50)	87	3			
7	a	e	NH	NH	NH	3 picrolonate	$C_{13}H_{21}N_7S·3C_{10}H_8N_4O_5$	257–259	DMF–EtOH	1.93[e]	100	1			
8	a	d	NH	NH	NH	3HCl	$C_{15}H_{25}N_7S·3HCl$	170–172	EtOH–Et_2O	0.83	20		5.94	1.3	2
9	a	a	NH	NH	NH	3HCl	$C_{15}H_{25}N_7S_2·3HCl$	214–216	MeOH–*i*-PrOH				6.67 (6.51–6.90)	0.79 ± 0.17	13
10	a	f	NH	NH	NH	3 picrolonate	$C_{15}H_{25}N_7S·3C_{10}H_8N_4O_5$	148–149	DMF–H_2O	0.007	11	2	5.80 (5.60–5.97)	0.99 ± 0.2	4
11	a	h	NH	NH	NH	3 picrate	$C_{14}H_{22}N_6S_2·3C_6H_3N_3O_7$	149–151					6.50 (6.30–6.81)	0.88 ± 0.29	4
12	a	g	NH	NH	NH	2 picrate	$C_{14}H_{22}N_6S_2·2C_6H_3N_3O_7$	101–102	$MeNO_2$				6.11	1.30	5
13	a	b	NH	NH	NCN		$C_{15}H_{22}N_8S$	140–142	*i*-PrOH–Et_2O				6.24	1.0	4
14	a	b	NH	NH	S		$C_{14}H_{22}N_6S_2·1.5C_2H_2O_4$	134–135	MeOH				5.92	1.28	3
15	a	b	NH	NH	O		$C_{14}H_{22}N_6OS$	173–174	EtOH–Et_2O				5.2 (4.40–5.73)	0.87 ± 0.33	
16	a	b	S	NH	NH	3 picrate	$C_{14}H_{22}N_6S_2·3_6H_3N_3O_7$	121–123	EtOH–H_2O	6.85 (4.54–11.82)	83	4			
17	a	b	NH	S	NH	3HCl	$C_{14}H_{22}N_6S_2·3HCl·H_2O$	113–114	EtOH–*i*-PrOH				5.48	1.08	3
18	a	b	NH	NH	NCH_3	3 picrolonate	$C_{15}H_{25}N_7S·3C_{10}H_8N_4O_5$	150–152		0.062	91	4			
19	a	b	NH	NH	SCH_3	3HCl	$C_{15}H_{24}N_6S_2·3HCl$			0.01	17	1	4.75 (3.88–5.34)	0.95 ± 0.29	5

[a] See Experimental Section for pharmacological methodology. [b] Values with confidence limits determined by 2 + 2 assay; other values estimated from cumulative dose response curves. [c] Number of determinations. [d] See Table III for structures of groups a–h. [e] Potency relative to histamine of compound 7 reported to be 0.126 (ref 16). [f] Impromidine

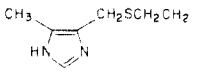
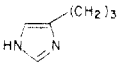
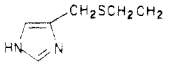
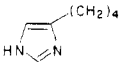
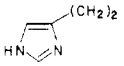
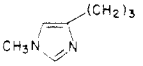
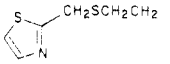
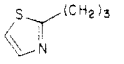
Table II. Structure and H_2-Receptor Activities of *N*-Methylguanidine and Isothioureas



								H_2-receptor activities on guinea pig atrium[a]					
								agonist activity			antagonist activity		
no.	R_1[d]	W	salt	molecular formula	mp, °C	cryst solvent	potency[b] rel to histamine = 1	% max response	n[c]	pA_2	slope	n[c]
20	a	NH	2HCl	$C_9H_{17}N_5S·2HCl$	205–206	EtOH–Et_2O			3	4.79 (4.49–5.09)	0.99 ± 0.14	3
21	c	NH	2HCl	$C_8H_{15}N_5S·2HCl$						4.38	0.9	3
22	g	NH	2 picrate	$C_8H_{14}N_4S_2·2C_6H_3N_3O_7$	137–138					4.96	0.75	2
23	h	NH	HI	$C_8H_{14}N_4S·HI$	163–165	*i*-PrOH				4.20	1.05	2
24	e	NH	H_2SO_4	$C_7H_{13}N_5·H_2SO_4$	241–245	H_2O–EtOH	0.01	62	1	<3.3		3
25	b	NH	HI	$C_8H_{15}N_5·HI$	169–171	*i*-PrOH–Et_2O	0.20	62		4.9		
26	d	NH	2HCl	$C_9H_{17}N_5·2HCl$	130–131	*i*-PrOH–$CHCl_3$	0.074	46	1	4.0	1.6	2
27	a	S	HI	$C_9H_{16}N_4S_2·HI$	128–131	*i*-PrOH–pet. ether				5.16 (4.13–5.86)	0.90 ± 0.35	6
28	b	S	2HI	$C_8H_{14}N_4S·2HI$	107–109	*i*-PrOH–Et_2O	0.10	23		4.6	1.3	

[a–d] See footnotes to Table I.

Table III. Imidazole- and Thiazole-Containing Side Chains

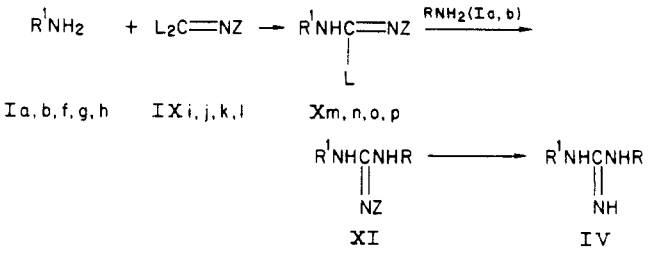
	side chain
a	
b	
c	
d	
e	
f	
g	
h	

rivatives (Table I) from appropriate primary amines. The synthesis of previously undescribed amines is included in the Experimental Section. The side chains utilized in these synthetic studies are indicated in Table III. The first method (Scheme I, method A), which is based upon the original Rathke guanidine synthesis,[11] requires the reaction of a primary amine with an isothiouronium salt derived from a second primary amine. In the original synthesis of impromidine, the amine precursor (Ia) for cimetidine was converted into the thourea IIa by initial reaction with benzoyl isothiocyanate followed by alkaline hydrolysis of the resulting benzoylthiourea. Further reaction with methyl iodide afforded the *S*-methylisothiouronium iodide IIIa (**27**), which, following conversion into the sulfate salt by ion-exchange chromatography, provided a useful synthesis of guanidines IV including impromidine (**1**) and the analogues **7**, **8**, and **11** by reaction with the primary amines Ib,d,e,h. The *S*-methylisothiourea IIIb (**28**) was synthesized analogously. The *N*-methylguanidines VI (**22–25**, Table II) were prepared in a similar way from primary amines Ib,e,g,h and *N*,*S*-dimethylisothiourea VIII (Scheme I). The remaining *N*-methylguanidines **20**, **21**, and **26** were prepared by acid hydrolysis of previously described[9] *N*-cyano-*N'*-methylguanidines. Isothiourea analogues V (**16** and **17**) of impromidine were prepared by reaction of appropriate thioureas II and halides VII (Scheme I). In the synthesis of impromidine and related guanidines, product isolation and purification were generally troublesome and guanidines were frequently isolated and characterized as picrate or picrolonate salts prior to conversion into solutions of hydrochloride salts for pharmacological testing.

A second method (Scheme II, method B) utilized for the synthesis of impromidine (**1**) and the analogous guanidines (**5**, **6**, **10**, and **12**) involved imidocarbonate or thiocarbonate derivatives (IX) and sequential displacement by primary amines $R'NH_2$ and RNH_2 to form the "protected" N,N'-disubstituted guanidine derivatives XI, which were cleaved by acid hydrolysis to guanidines IV. The method was an improvement over method A in that neutral and more readily isolable and purifiable intermediates were used. However, a disadvantage in the synthesis of impromidine by this method from the cimetidine precursor Xm and

(11) Rathke, B. *Chem. Ber.* **1881**, *14*, 1774.

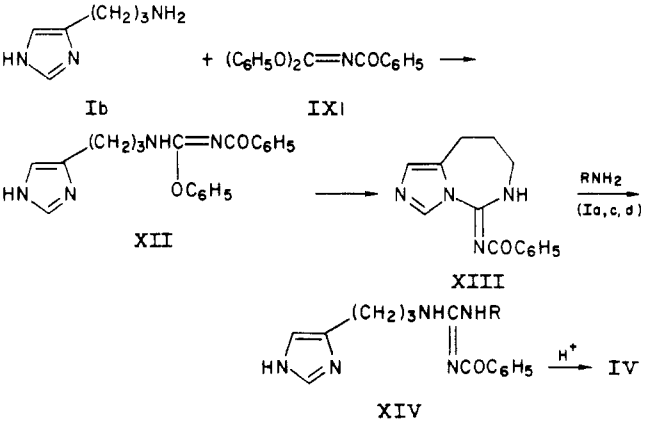
Scheme II. Synthesis of Impromidine and Related Compounds from Imidates by Sequential Displacement by Primary Amines (Method B)[a]



Ia, b, f, g, h IXi, j, k, l Xm, n, o, p XI IV

[a] i, L = MeS, Z = CN; j, L = PhO, Z = CN; k, L = MeS, Z = COPh; l, L = PhO, Z = COPh; m, R^1 = a, L = MeS, Z = CN; n, R^1 = g, L = MeS, Z = COPh; o, R^1 = h, L = MeS, Z = CN; p, R^1 = f, L = MeS, Z = CN; q, R^1 = g, L = MeS, Z = CN; structures a–h are included in Table III.

Scheme III. Synthesis of Impromidine and Analogous (3-Imidazol-4-ylpropyl)guanidine Derivatives from 5-(Benzoylimino)-5,6,8,9-tetrahydro-7*H*-imidazo-[1,5-*c*][1,3]diazepine



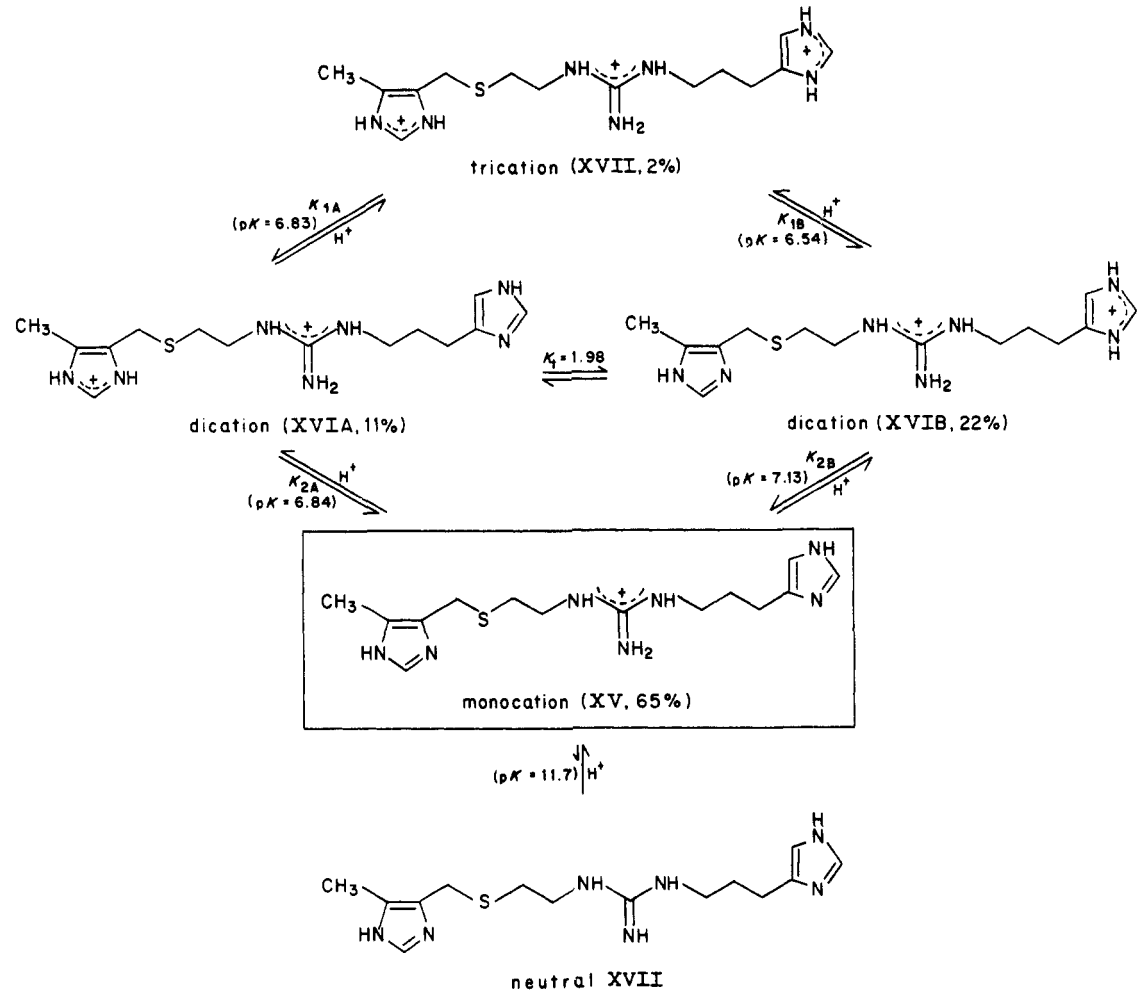
Ib IXI XII XIII XIV IV

3-imidazol-4-ylpropylamine (Ib) is that this second amine displacement to form the *N*-cyanoguanidine XI (**13**) requires forcing conditions that lead to problems of product isolation and the method was not amenable to scale-up. Additionally, the second amine displacement from the intermediate X also leads to the concomitant formation of the symmetrically substituted *N*-cyanoguanidine derivatives $(RNH)_2C = Z$ and $(R'NH)_2C = Z$, particularly when L = PhO. The preparation of guanidine derivatives free from impurities derived by this means was generally troublesome. The method however was particularly useful for the synthesis of the symmetrically disubstituted guanidine derivatives **4** and **9**.

A preferred method[12] (method C) that was developed for the synthesis of impromidine (**1**) and also utilized for analogous (3-imidazol-4-ylpropyl)guanidine derivatives (**2**, **3**) is illustrated in Scheme III. The method utilized the reaction of IXl with 3-imidazol-4-ylpropylamine (Ib) to form the *O*-phenylisourea XII, which underwent base-catalyzed cyclization to 5-(benzoylimino)-5,6,8,9-tetrahydro-7*H*-imidazo[1,5-*c*][1,3]diazepine (XIII); this was readily purified and subsequently underwent ring opening with primary amine Ia to form the *N*-benzoylguanidine XIV, which upon acid hydrolysis afforded impromidine in the form of its trihydrochloride salt (**1**) in an acceptable state of purity and containing minimal levels of the symmetrically disubstituted guanidines **4** and **9**.[13] Guanidines

(12) Hills, D. W.; White, G. R. U.S. Patent 4 375 435, 1983.
(13) Hills, D. W.; White, G. R.; Darken, D. SK&F Research Limited, unpublished results.

Scheme IV. Ionic Equilibria of Impromidine and Species Populations at pH 7.4 (37 °C)



2 and 3 were prepared analogously from XIII and the primary amines Ic,d followed by acid hydrolysis.

Urea (15), thiourea (14), *S*-methylisothiourea (19), and *N*-methylguanidine (18) analogues of impromidine were synthesized by common procedures and details are included in the Experimental Section.

Discussion

Impromidine is a highly polar molecule that contains three basic centers, namely, the guanidine group and two imidazole rings. Impromidine has macroscopic pK_a values (determined potentiometrically) of 6.41, 7.26, and 11.6.[14] The pK_a value of 11.66 is due to the guanidine group, which is the site exclusively protonated by 1 equiv of acid. The remaining pK_a values are due to the two imidazole rings and are overlapping, and the ionic behavior of impromidine is therefore relatively complex. Thus two different dications exist in equilibrium in solution. An NMR method (described in the Experimental Section) to derive the microionization constants indicated in Scheme IV provided an estimate of the species populations at pH 7.4 (37 °C) as indicated. The predominant ionic species of impromidine at a physiological pH of 7.4 is likely to be the monocation (XV, 65%). Significant populations of the dications XVIA and XVIB (total 33% in a ration of approximately 2:1 as indicated in Scheme IV) are also likely to be present and a much smaller population of trication (XVII, 2%). There is a further complication in that the monocation XV and the dications XVIA and XVIB exist as equilibrating mixtures of tautomeric species. One purpose of this present study is to investigate whether these ionic and tautomeric properties of impromidine are associated with its activity at histamine H_2 receptors.

Compounds 1–6 in Table I, in which the substituent R_2 is retained as 3-imidazol-4-ylpropyl (as present in impromidine), are all potent agonists exerting a maximal or near-maximal response relative to histamine on guinea pig right atrium, being between 5 and 50 times the potency of histamine. Compounds 5 and 6 possess a thiazol-2-yl ring system in place of the imidazole ring present in substituent R_1 of impromidine (1). From these results it may be deduced that the imidazole ring present in substituent R_1 is not essential for agonist activity at histamine H_2 receptors; since the thiazole ring is nontautomeric, it is also evident that the presence of a tautomeric ring system in substituent R_1 is not essential for agonist activity. Furthermore, compounds 20–23 (Table II), namely, those guanidinium structures in which the 3-imidazol-4-ylpropyl substituent (R_2) of impromidine (1) and analogues 2, 5, and 6 is replaced by methyl, do not elicit an agonist response on guinea pig right atrium, but all are histamine antagonists, with pA_2 values in the range 4.2–5.0. These data are consistent with the view that these substituents R_1 are associated with affinity for histamine H_2 receptors. Furthermore, the symmetrical guanidinium structure 9, the 4-imidazol-4-ylbutyl analogue 8, and the thiazole-containing side-chain analogues 11 and 12 are also H_2-receptor antagonists of considerably greater potency than the *N*-methylguanidine structure (20), which may reflect the marked H_2-receptor affinity contributions of these sub-

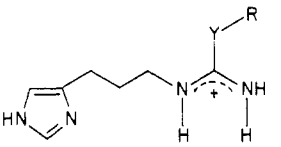
(14) Graham, M. J. SK&F Research Limited, unpublished results.

stituents in disubstituted guanidinium structures. Consideration of impromidine and compounds **7–12**, i.e., structures in which R_1 is held constant while R_2 is modified, indicates that H_2-receptor agonist activity is highly sensitive to the properties of substituent R_2. The lower homologue of impromidine (**7**, R_2 = 2-imidazol-4-ylethyl) is a full agonist on the atrium but about 20 times less potent.[15,16] The higher homologue of impromidine (**8**, R_2 = 4-imidazol-4-ylbutyl) is predominantly an H_2-receptor antagonist that elicits a submaximal agonist response (20% maximum) on the atrium. Furthermore, as previously noted, the symmetrical guanidine (**9**) containing two "cimetidine" side chains ($R_1 = R_2$ = a) is a potent antagonist. The agonist activity of impromidine is clearly highly sensitive to the chain length separating the imidazole ring in R_2 from the guanidine residue, suggesting that drug conformation is critical for activity. The analogues of impromidine in which the imidazole ring present in R_2 is replaced by the nontautomeric 1-methylimidazol-4-yl (**10**) or thiazol-2-yl (**11**) are both H_2-receptor antagonists with a barely detectable level of agonist activity on guinea pig atrium. These results suggest that the tautomeric imidazole ring present in the substituent R_2 in impromidine may be important for the initiation of an agonist response at H_2 receptors and furthermore that the prototropic property of this imidazole ring may be required for the efficacy of impromidine.

A further property of impromidine that appears to be important for its agonist activity is a basic amidine group in its cationic form. The analogues **13–15** in which the basic guanidine group is replaced by the neutral groups, cyanoguanidine, thiourea, or urea are H_2-receptor antagonists apparently devoid of agonist activity. It is additionally instructive to compare isothiourea analogues (**16** and **17**) of impromidine, i.e., compounds in which the NH groups containing the substituents R_1 or R_2 are separately replaced by sulfur. The isothiourea group resembles guanidine in being a strong base (*S*-methylisothiourea is reported to have pK_a = 9.78 at 20 °C)[17] which will exist predominantly in the protonated form at physiological pH. However, whereas the isothiourea **16** resembles impromidine in acting as a strong agonist on guinea pig atrium, the isomer **17** is essentially an antagonist devoid of agonist activity. These results suggest that, for agonist activity, it is important for the atom bearing the 3-imidazol-4-ylpropyl side chain (R_2) to possess a proton, whereas a proton on the atom bearing the 2-[[(5-methylimidazol-4-yl)methyl]thio]ethyl substituent (R_1) is not essential for the agonist activity of impromidine and related compounds. Comparison of compound **16** with the *S*-methylisothiourea **28**, which is a weakly active partial agonist on guinea pig atrium, indicates that the effect of replacing an *S*-methyl substituent in this isothiourea by the "affinity-contributing" cimetidine side chain is qualitatively similar to that of replacing an *N*-methyl substituent in the corresponding guanidine **25**, namely, a large increase in agonist potency and additionally an increase in efficacy. The isothiourea **17** should be compared with the *S*-methylisothiourea **27**. Both are H_2-receptor antagonists of similar potency and devoid of agonist activity. It would therefore appear that the 3-imidazol-4-ylpropyl substituent R_2 in the isothiourea **17** is not involved to a significant extent in its interaction with the H_2 receptor on guinea pig atrium.

Introduction of a methyl substituent on to the primary guanidine nitrogen atom of impromidine to form the trisubstituted guanidinium structure **18** causes a marked reduction in agonist activity. Although this analogue elicits a near-maximal response, agonist potency on guinea pig atrium is reduced by a factor of nearly 1000, and clearly this methyl substituent is having a profound effect on the ability of the molecule to function as an H_2-receptor agonist. This methyl-substituted guanidine analogue (**18**) of impromidine could be a useful compound as a chemical control for studying further biological effects of impromidine since it closely resembles impromidine in most of its chemical properties but is essentially devoid of its agonist activity at H_2 receptors. The corresponding *S*-methylisothiourea analogue **19** is a weakly active partial agonist at H_2 receptors on guinea pig atrium. The weak agonist activity of **18** and **19** is consistent with the view that an amidinium group containing an NH_2 group is important for the agonist activity of impromidine-like compounds at H_2 receptors on guinea pig atrium. Thus, of the N–H bonds in the guanidine group in impromidine, it appears that the NH_2 group and the NH bearing the 3-imidazol-4-ylpropyl are associated with its agonist activity whereas the NH bearing the substituents R_1 is not essential.

One extrapolation from the results of the limited series of guanidine group modifications in Tables I and II is that the 3-imidazol-4-ylpropyl amidinium fragment XIX is important for H_2-receptor agonist activity in impromidine congeners, where Y = S or NH and R, is a typical side chain that contributes affinity in H_2-receptor antagonist structures. This fragment may be compared with the structure of histamine and the chemical criteria that have been considered necessary for its agonist action at H_2 receptors.[4,18] In attempts to correlate structure and H_2-receptor agonist activity, conformational space and molecular surfaces of the monocations of the H_2-receptor agonists histamine, dimaprit,[5] and impromidine have been compared.[19]



XIX

Experimental Section

Melting points (°C) were generally determined on an Electrothermal apparatus using a thermometer corrected for stem exposure. Melting points and recrystallization solvents are included in Tables I and II. Microanalyses for elements listed are within ±0.4 of calculated values unless indicated otherwise. Compounds **2–5**, **7**, **10–12**, **16**, **18**, and **22**, which were isolated and characterized as picrate or picrolonate salts, were converted into aqueous solutions of hydrochlorides for pharmacological assay. HPLC assays were conducted on a Perkin-Elmer LC instrument linked to a water pump.

(15) Buyuktimkin and Schunack[16] report that compound 7 has 12.6% of the agonist potency of impromidine on guinea pig atrium. These authors also report that branching of the C(2) atom side chain by introducing a methyl substituent adjacent to the guanidine group yields chiral compounds with interesting differences in pharmacological activity. The *R* enantiomer is reported to have 7.4 times the agonist activity of histamine on guinea pig atrium whereas the *S* enantiomer is an H_2-receptor antagonist.

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Primary Amines. The amines 4-[[(2-aminoethyl)thio]-methyl]-5-methylimidazole[9] (Ia), 4-(3-aminopropyl)imidazole[20] (Ib), 4-[[(2-aminoethyl)thio]methyl]imidazole[9] (Ic), and 4-(4-aminobutyl)imidazole[21] (Id) have been reported previously. Histamine (Ie) was obtained as the dihydrochloride from Koch Light & Co. The remaining amines were prepared as follows.

1-Methyl-4-(3-aminopropyl)imidazole (If). A mixture of Ib (16.0 g, 0.13 mol) and Ac_2O (30 mL) was heated under reflux for 1 h, cooled, diluted with H_2O (60 mL), and concentrated under reduced pressure. Further dilution with H_2O and evaporation afforded the *N*-acetyl derivative as an oil, which was directly dissolved in 10% NaOH (100 mL), and the stirred solution was treated dropwise with Me_2SO_4 (7.6 mL) at 20–30 °C. Further quantities of NaOH (100 mL) and Me_2SO_4 (7.6 mL) were added as before, and the solution was heated for 45 min on a steam bath. After cooling, saturation with Na_2SO_4, and extraction with $CHCl_3$ (9 × 100 mL), the extract was concentrated and the residue dissolved in 6 N HCl (300 mL) and the mixture heated overnight under reflux. Following evaporation the residual hydrochloride was dissolved in H_2O and treated with ethanolic picric acid. The crude picrate (33 g) was recrystallized three times from H_2O to give the isomerically pure dipicrate of If (16.4 g, mp 190–191 °C). Further recrystallization afforded an analytically pure sample, mp 194–196 °C. Anal. ($C_7H_{13}N_3 \cdot 2C_6H_3N_3O_7$) C, H, N. The dihydrochloride had mp 258–259 °C. Anal. ($C_7H_{13}N_3 \cdot 2HCl$) C, H, N, Cl.

2-[[(2-Aminoethyl)thio]methyl]thiazole (Ig). Bromoacetal (416 g, 2.1 mol) was added to phenoxythioacetamide[22] (318 g, 1.9 mol) dissolved in warm Me_2CO (1.9 l) over 5 min. Following a moderately exothermic reaction, the solution was heated under reflux for 3 h, cooled, and diluted with Et_2O (750 mL). The product, 2-(phenoxymethyl)thiazole hydrobromide (400 g, 77%, mp 127–129 °C) was collected and dried in vacuo at 50 °C. Anal. ($C_{10}H_9NOS \cdot HBr$) C, H, N, S, Br. A solution of 2-(phenoxymethyl)thiazole hydrobromide (2090 g, 7.7 mol) and cysteamine hydrochloride (905 g, 8.0 mol) in 48% HBr (11 L) was heated overnight under reflux, cooled, washed with toluene (7.5 L), and evaporated under reduced pressure: a solution of the residue in hot MeOH–EtOH was treated with charcoal. The title compound crystallized on cooling as the dihydrobromide (1635 g, 64%, mp 143–144 °C). Anal. ($C_6H_{10}N_2S_2 \cdot 2HBr$) C, H, N, S, Br.

2-(3-Aminopropyl)thiazole (Ih). A solution of 4-phthalimidothiobutyramide (19.0 g, 0.08 mol) and bromoacetaldehyde diethyl acetal (19.8 g, 0.1 mol) in Me_2CO (250 mL) was heated under reflux for 7 h. Cooling afforded *N*-(3-thiazol-2-ylpropyl)phthalimide hydrobromide (12.0 g, 44%, mp 195–197 °C). Anal. ($C_{14}H_{12}N_2O_2S \cdot HBr$) C, H, N, S. The phthalimido derivative (11.0 g, 0.031 mol) was converted to the base with K_2CO_3, dissolved in HCl (120 mL), heated under reflux for 18 h, cooled, and recrystallized (EtOH) to afford Ih as the dihydrochloride (6.2 g, 93%, mp 165–167 °C). Anal. ($C_6H_{10}N_2S \cdot 2HCl$) C, H, N, S, Cl.

Synthesis of Impromidine and Related Compounds (Method A, Scheme I). *N*-[2-[[(5-Methyl-1*H*-imidazol-4-yl)methyl]thio]ethyl]thiourea (IIa). A solution of Ia (5.0 g, 0.03 mol) and benzoyl isothiocyanate (23.8 g, 0.15 mol) in $CHCl_3$ was heated under reflux for 1 h and concentrated to afford *N*-benzoyl-*N'*-[2-[[(5-methyl-1*H*-imidazol-4-yl)methyl]thio]ethyl]-thiourea (7.5 g, 75%). The benzoylthiourea (22 g) was hydrolyzed with K_2CO_3 at 60 °C and acidified to form the thiourea IIa (3.8 g, 32%, mp 110–112 °C, *i*-PrOH–ether). Anal. ($C_8H_{14}N_4S_2$) C, H, N, S.

***S*-Methyl-*N*-[2-[[(5-Methyl-1*H*-imidazol-4-yl)methyl]-thio]ethyl]thiourea Hydriodide (IIIA, 27)** . The thiourea IIa (2.3 g, 0.01 mol) and MeI (1.6 g, 0.011 mmol) in Me_2CO (45 mL) containing MeOH (5 mL) was set aside at room temperature for 18 h, concentrated, and recrystallized from *i*-PrOH–petroleum ether to afford IIIa (2.3 g, 62%), mp 128–131 °C. Anal. ($C_9H_{16}N_4S_2 \cdot HI$) C, H, N, S, I.

***N*-[3-(1*H*-Imidazol-4-yl)propyl]-*N'*-[2-[[(5-methyl-1*H*-imidazol-4-yl)methyl]thio]ethyl]guanidine (Impromidine, 1).** The hydroiodide of IIIa (2.3 g, 0.006 mol) was converted into the corresponding sulfate by ion exchange (Amberlite IRA 401, SO_4^{2-}). A solution of the sulfate salt (2.93 g, 0.0087 mol) and Ib (1.25 g, 0.01 mol) in H_2O (10 mL) was heated under reflux for 3 h and concentrated and the residue converted into the free base with an ion-exchange resin (Amberlite IRA 401, OH^-), applied to ion-exchange resin C 650, H^+, and eluted with HCl. The eluate was converted into the tripicrate of impromidine (1; 1.8 g, 21%, mp 183–185 °C (Me_2CO–H_2O)). Anal. ($C_{14}H_{23}N_7S \cdot 3C_6H_3N_3O_7$) C, H, N, S. The tripicrate was converted into the free base by ion exchange (IRA 401, OH^-) and subsequently converted into a dioxalate, mp 125–127 °C (EtOH–H_2O). Anal. ($C_{14}H_{23}N_7S \cdot 2C_2H_2O_4$) C, H, N, S.

***N*-[2-(1*H*-Imidazol-4-yl)ethyl]-*N'*-[2-[[(5-methyl-1*H*-imidazol-4-yl)methyl]thio]ethyl]guanidine (7).** By use of the above procedures, the reaction of the sulfate salt of IIIa (2.93 g, 0.0087 mol) and Ie (1.1 g, 0.01 mol) afforded the guanidine **7**, which was isolated and characterized as the tripicrolonate salt (2.7 g, mp 257–259 °C (DMF–EtOH). Anal. ($C_{13}H_{21}N_7S \cdot 3C_{10}H_8N_4O_5$) C, H, N, S.

***N*-[4-(1*H*-Imidazol-4-yl)butyl]-*N'*-[2-[[(5-methyl-1*H*-imidazol-4-yl)methyl]thio]ethyl]guanidine (8).** By use of the above procedures, the reaction of the sulfate salt of IIIa (2.93 g, 0.0087 mol) and Id (1.39 g, .01 mol) afforded the title compound as the trihydrochloride (1.9 g, 50%), mp 170–172 °C (EtOH–ether). Anal. ($C_{15}H_{25}N_7S \cdot 3HCl$) H, N, S; C: calcd, 40.5; found, 39.2.

***N*-[2-[[(5-Methyl-1*H*-imidazol-4-yl)methyl]thio]ethyl]-*N'*-[3-(thiazol-2-yl)propyl]guanidine (11).** The reaction of the hydriodide salt of IIIa and Ih in H_2O afforded **11**, which was isolated as the tripicrate, mp 149–151 °C. Anal. ($C_{14}H_{22}N_6S_2 \cdot 3C_6H_3N_3O_7$) C, H, N, S.

Synthesis of Impromidine and Related Compounds (Method B, Scheme II). *N*-Cyano-*N'*-[3-(1*H*-imidazol-4-yl)-propyl]-*N'*-[2-[[(5-methyl-1*H*-imidazol-4-yl)methyl]thio]-ethyl]guanidine (13). A mixture of Ib (5.0 g, 0.04 mol) and *N*-cyano-*S*-methyl-*N'*-[2-[(5-methyl-1*H*-imidazol-4-yl)methyl]-thio]ethyl]isothiourea[7] (Xm; 3.5 g, 0.013 mol) was heated at 120–130 °C for 1.25 h and the product was chromatographed on silica gel with EtAc–*i*-PrOH (1:1) as eluent and subsequently recrystallized from *i*-PrOH–Et_2O to afford XI (**13**; mp 140–142 °C, 1.7 g, 38%). Anal. ($C_{15}H_{22}N_8S$) C, H, N, S.

A mixture of Ib (29 g, 0.23 mol) and Xm (51.1 g, 0.19 mol) was heated at 100 °C for 14 h, cooled, diluted with H_2O (150 mL) with stirring, and set aside overnight. The precipitated solid (ca. 60 g of XI, **13**) was hydrolyzed in concentrated HCl (750 mL) for 8 h at 100 °C and evaporated to dryness and the residue extracted with hot *i*-PrOH (to remove NH_4Cl) and filtered, and the residue was crystallized from a minimum volume of EtOH to yield impromidine (**1**) as the trihydrochloride (mp 195–197 °C, 23.3 g, 28%); HPLC (camphorsulfonic acid) 98.8%. Anal. ($C_{14}H_{23}N_7S \cdot 3HCl$) C, H, N, S, Cl.

***N*-[3-(1*H*-Imidazol-4-yl)propyl]-*N'*-[2-[(thiazol-2-ylmethyl)thio]ethyl]guanidine (5).** A mixture of Ig (3.36 g, 0.01 mol), IXk[23] (2.25 g, 0.01 mol), and anhydrous K_2CO_3 (1.38 g, 0.01 mol) in MeOH (25 mL) was stirred for 24 h at room temperature and evaporated to dryness. The residue was extracted with Et_2O to give Xn (2.1 g, 60%, mp 64–65 °C (Et_2O). Anal. ($C_{15}H_{12}N_3OS_3$) C, H, N, S. A mixture of Xn (8.0 g, 0.023 mol) and Ib (from 6.5 g (0.023 mol) of the dihydrobromide) in pyridine (10 mL) was heated at 100 °C for 4 h and evaporated to an oil, which afforded a solid (7.55 g) on trituration with Et_2O. Chromatography on silica gel with EtOAc–*i*-PrOH as eluent afforded the *N*-benzoyl derivative of **5** (XI, R^1 = g, R = b, Z = COPh, 4.76 g, 48%, mp 127–178 °C).

Hydrolysis of the above compound (4.5 g, 0.01 mol) and concentrated HCl (40 mL) for 6 h at 100 °C, cooling, filtration, and treatment with excess ethanolic picric acid afforded the title compound (**5**) as the tripicrate, mp 157–158 °C (MeCN–EtOH). Anal. ($C_{13}H_{20}H_6S_2 \cdot 3C_6H_3N_3O_7$) C, H, N, S.

***N*-[3-(1*H*-Imidazol-4-yl)propyl]-*N'*-(3-thiazol-2-ylpropyl)guanidine (6).** A mixture of amine Ih (10.0 g, 0.07 mol) and IXi[24] (15.5 g, 0.11 mol) in EtOH (50 mL) was heated at 100

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°C for 30 min and added to Et_2O (30 mL) and the deposited solid filtered, washed with Et_2O, and recrystallized from EtOH to afford Xo (14.1 g, 84%), mp 121–122 °C. A mixture of Xo (7.85 g, 0.033 mol) and amine Ib (7.0 g, 0.049 mol) was heated at 100 °C for 6 h and the reaction mixture was chromatographed on silica gel with $CHCl_3$ containing 5% MeOH, affording the *N*-cyano derivative of **6** (XI; R^1 = h, R = b, Z = CN) (6.5 g, 63%), mp 104–105 °C (from MeCN). Anal. ($C_{14}H_{19}N_7S$) C, H, N, S. Hydrolysis of the cyanoguanidine (3.0 g, 0.01 mol) with concentrated HCl and with use of the conditions described above afforded **6** as the trihydrochloride (2.0 g, 53%), mp 174–175 °C (EtOH–Et_2O). Anal. ($C_{13}H_{20}N_6S$·3HCl) C, H, N, S.

***N*-[3-(1-Methylimidazol-4-yl)propyl]-*N'*-[2-[[(5-methyl-1*H*-imidazol-4-yl)methyl]thio]ethyl]guanidine (10).** A solution of the dihydrochloride salt of If (3.0 g, 0.014 mol) and IXi (3.2 g, 0.022 mol) in H_2O (30 mL) and *i*-PrOH (60 mL) containing K_2CO_3 (1.93 g, 0.014 mol) was heated under reflux for 4 h, concentrated, redissolved in EtOH to remove inorganic material, and treated with Et_2O to afford crude isothiourea Xp (2.4 g). This material (2.1 g, 0.009 mol) and Ia (4.53 g, 0.027 mol) was heated at 120 °C for 2.5 h and the product chromatographed on silica gel with $CHCl_3$ containing 8% MeOH as eluent, affording the *N*-cyano derivative of **10** as a white solid (1.15 g, 36%). This material was hydrolyzed directly with HCl and the product treated with picrolonic acid to afford **10** (2.2 g, 63%) as a tripicrolonate, mp 148–149 °C (DMF/H_2O). Anal. ($C_{15}H_{25}N_7S$·$3C_{10}H_8N_4O_5$) H, N, S; C: calcd, 47.9; found, 47.2.

***N*-[2-[[(5-Methyl-1*H*-imidazol-4-yl)methyl]thio]ethyl]-*N'*-[2-[(thiazol-2-ylmethyl)thio]ethyl]guanidine (12).** A mixture of IXq (4.0 g, 0.015 mol from IXi and Ig) and Ia (8.0 g, 0.046 mol) was heated at 100 °C for 3 h and the product chromatographed on silica gel with EtOH containing 20% *i*-PrOH as eluent to afford the *N*-cyano derivative of **12** as a white foam (5.0 g, 86%). This was hydrolyzed directly with HCl and the product treated with sodium picrate to afford **12** as the dipicrate (2.84 g, 27%), mp 101–102 °C ($MeNO_2$). Anal. ($C_{14}H_{22}N_6S_3$·$2C_6H_3N_3O_7$) H, N, S; C: calcd, 37.7; found, 37.0.

***N,N'*-Bis[3-(1*H*-Imidazol-4-yl)propyl]guanidine (4).** A solution of Ib (2.50 g, 0.02 mol) and IXk (2.25 g, 0.01 mol) in pyridine was heated at 100 °C for 3 h, diluted with H_2O, evaporated to dryness, and reevaporated to dryness with H_2O followed by EtOH. The residue was chromatographed on silica gel with *i*-PrOH as eluent and the product recrystallized from H_2O to afford the *N*-benzoyl derivative of **4** (0.84 g, 22%, mp 115–117 °C). Anal. ($C_{20}H_{25}N_7O$) C, H, N.

Hydrolysis of the above (0.60 g, 0.0016 mol) with concentrated HCl (10 mL) for 7 h at 100 °C, dilution with H_2O, extraction with Et_2O, and evaporation followed by treatment of the product with picrolonic acid afforded the title compound as the tripicrolonate (1.1 g, 65%), mp 273–275 °C dec. Anal. ($C_{13}H_{21}N_7$·$3C_{10}H_8N_4O_2$) C, H, N.

***N,N'*-Bis[2-[[(5-Methyl-1*H*-imidazol-4-yl)methyl]thio]ethyl]guanidine (9).** By use of the above procedure, Ia (13.6 g, 0.08 mol) and IXk (10.0 g, 0.04 mol) afforded the *N*-benzoyl derivative of the title compound (12.0 g, 64%), mp 163–164 °C (H_2O–EtOH). Anal. ($C_{22}H_{29}N_7OS_2$) C, H, N, S. Hydrolysis of the benzoyl derivative (4.3 g, 0.009 mol) with HCl as above afforded the title compound as the crystalline dihydrochloride (3.5 g, 81%), mp 214–216 °C (MeOH–*i*-PrOH). Anal. ($C_{15}H_{25}N_7S_2$·3HCl) C, H, N, S, Cl.

Synthesis of Impromidine and Related Compounds (Method C, Scheme III). 5-(Benzoylimino)-5,6,8,9-tetrahydro-7*H*-imidazo[1,5-*c*][1,3]diazepine. A solution of Ib in EtOH (from the dihydrochloride 4.95 g, 0.025 mol) was added dropwise with stirring over 15 min to a cooled solution of IX1 (XI, 7.93 g, 0.025 mol) in CH_2Cl_2 at 5–7 °C. The reaction mixture was stirred for 30 min at room temperature, evaporated to low bulk, added to H_2O, and chilled to afford XII (6.1 g, 70%). A 5.0-g (0.014 mol) sample of XII was added to a stirred suspension of NaH (0.5 g, as 50% dispersion in oil and washed before use with 40–60 °C petroleum ether) in dry DMF over 10 min at 5–10 °C. Stirring was continued until the reaction appeared complete on TLC (S_iO_2, EtOAc–MeOH–NH_4OH, 5/1/1). Addition to H_2O and recrystallization from EtOH afforded XIII (2.31 g, 65%), mp 158–160 °C. Anal. ($C_{14}H_{14}N_4O$) C, H, N.

Impromidine (1). Compound XIII (2.54 g, 0.010 mol) was added to a solution of Ia (from the dihydrochloride, 2.53 g, 0.010 mol) in EtOH and heated under reflux for 20 h, cooled, filtered, and evaporated to give the benzoyl derivative of impromidine (XIV, R = a) as a white solid: mp 98.5–100 °C; yield 2.67 g (89%). Anal. ($C_{21}H_{27}N_7OS$) C, H, N.

Hydrolysis of the benzoyl derivative (2.37 g, 0.056 mol) with HCl as previously described afforded impromidine as the trihydrochloride (1.93 g, 80%): mp 200–201 °C; HPLC (camphorsulfonic acid) 98.4%.

***N*-[4-(1*H*-Imidazol-4-yl)butyl]-*N'*-[3-(1*H*-imidazol-4-yl)-propyl]guanidine (3).** The reaction of XIII (2.54 g, 0.010 mol) and Id by the above procedure afforded XIV (R = d), which was directly hydrolyzed with HCl to form **3** as the tripicrate, mp 206–208 °C (aqueous acetone). Anal. ($C_{14}H_{23}N_7$·$3C_6H_3N_5O_7$) C, H, N.

***N*-[3-(1*H*-Imidazol-4-yl)propyl]-*N'*-[2-[[(1*H*-imidazol-4-yl)methyl]thio]ethyl]guanidine (2).** The reaction of XIII (2.54 g, 0.01 mol) and Ic (from the dihydrochloride, 2.53 g, 0.011 mol) by the above procedure afforded XIV (R = c) (1.7 g), which was hydrolyzed with HCl to the title compound (**2**) as the tripicrate, mp 173–175 °C ($MeNO_2$–MeOH). Anal. ($C_{13}H_{21}N_7S$·$3C_6H_3N_3O_7$) C, H, N, S.

Synthesis of *N*-Methylguanidine Derivatives 20–26. The synthesis of the *N*-methylguanidine (**20**) as a dihydrochloride salt by acid hydrolysis of cimetidine has been described previously.[9] The analogues **21** (obtained as a glass; anal. ($C_8H_{15}N_5$·2HCl) C, H, N, S, Cl) and **26** (mp 130–131 °C, *i*-PrOH–$CHCl_3$); anal. ($C_9H_{17}N_5$·2HCl) C, H, N, Cl) were prepared analogously from previously described *N*-cyanoguanidines.[9]

***N*-[3-(1*H*-Imidazol-4-yl)propyl]-*N'*-methylguanidine (25).** A solution of Ib (2.50 g, 0.02 mol) and *N,S*-dimethylisothiourea hydriodide (4.64 g, 0.02 mol) in EtOH (50 mL) was heated under reflux for 18 h and concentrated and the residue crystallized from *i*-PrOH–Et_2O to afford **25** as the hydroiodide salt (2.0 g, 32%), mp 169–171 °C. Anal. ($C_8H_{15}N_5$·HI) C, H, N, I.

***N*-Methyl-*N'*-(3-thiazol-2-ylpropyl)guanidine (23).** An analogous synthesis from Ih afforded **23** as the hydriodide, mp 163–165 °C (*i*-PrOH). Anal. ($C_8H_{14}N_4S$·HI) C, H, N, S, I.

***N*-Methyl-*N'*-[2-[(thiazol-2-ylmethyl)thio]ethyl]guanidine (22).** An analogous synthesis from Ig afforded **22**, which was isolated and characterized as a dipicrate, mp 137–138 °C. Anal. ($C_8H_{14}N_4S_2$·$2C_6H_3N_3O_7$) C, H, N, S.

***N*-[2-(1*H*-Imidazol-4-yl)ethyl]-*N'*-methylguanidine (24).** An analogous synthesis from Ie afforded **24**, which was isolated as a sulfate (mp 241–245 °C, H_2O–EtOH) following ion-exchange chromatography. Anal. ($C_7H_{13}N_5$·H_2SO_4) C, H, N, S.

Synthesis of Ureas, Thiourea, and Isothioureas (14–19). *S*-[3-(1*H*-Imidazol-4-yl)propyl]-*N*-[2-[[(5-methyl-1*H*-imidazol-4-yl)methyl]thio]ethyl]isothiourea Trihydrochloride (17). A solution of 4-(3-chloropropyl)imidazole hydrochloride[25] (1.8 g, 0.01 mol) and IIa (2.3 g, 0.01 mol) in EtOH (10 mL) containing 0.82 mL of concentrated HCl was heated at 140 °C for 2 h. The reaction mixture was dissolved in EtOH and treated with charcoal and the filtrate reevaporated with *i*-PrOH to a volume of 25 mL. A solid (1.3 g) deposited, which was recrystallized from EtOH–*i*-PrOH to afford **17** (1.1 g, 25% theory, mp 113–114 °C). Anal. ($C_{14}H_{22}N_6S_2$·3HCl) C, H, N, S, Cl.

***N*-[3-(1*H*-Imidazol-4-yl)propyl]thiourea (IIb).** A solution of benzoyl isothiocyanate (65.2 g, 0.41 mol) in $CHCl_3$ was added slowly to Ib (50.0 g, 0.040 mol) in $CHCl_3$ (1.5 L) and the resultant solution heated under reflux for 2 h, concentrated, and added to H_2O, affording *N*-benzoyl-*N'*-[3-(1*H*-imidazol-4-yl)propyl]thiourea (44 g, 38%), mp 145–148 °C. Anal. ($C_{14}H_{16}N_4OS$) C, H, N, S. The benzoylthiourea (47.0 g, 0.16 mol) was added, with stirring, to a solution of K_2CO_3 (13.8 g) in H_2O (800 mL) at 60–70 °C, heated for 1 h at this temperature, and concentrated to low bulk to afford IIb (23 g, 84%), mp 149–150 °C (H_2O). Anal. ($C_7H_{12}N_4S$) C, H, N, S.

***N*-[3-(1*H*-Imidazol-4-yl)propyl]-*S*-[2-[[(5-methyl-1*H*-**

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imidazol-4-yl)methyl]thio]ethyl]isothiourea (16). A solution of the thiourea IIb (0.92 g, 0.005 mol) and 5-methyl-4-[[(2-chloroethyl)thio]methyl]imidazole hydrochloride (VIIa; 1.13 g, 0.005 mol) in concentrated HCl (25 mL) was heated under reflux for 6 h, evaporated to dryness, and reevaporated with H_2O and EtOH. The residue was treated with sodium picrate to afford **16** (2.1 g, 41%, mp 121–123 °C, H_2O–EtOH). Anal. ($C_{14}H_{22}N_6S_2 \cdot 3C_6H_3N_3O_7$) C, H, N, S.

***S*-Methyl-*N*-[3-(1*H*-imidazol-4-yl)propyl]isothiourea (IIIb, 28).** Aqueous HI (64/66%, 12 mL) was added slowly to a stirred and cooled suspension of IIb (20.0 g, 0.116 mol) in EtOH, which following the addition of Et_2O afforded the monohydriodide salt (29.2 g), mp 135–136 °C as a yellow solid. The addition of MeI (21 g, 0.148 mol) to a methanolic solution of this hydriodide, followed by heating under reflux for 1 h and concentration, afforded IIIB (**28**) as the dihydriodide (35.0 g, 96%), mp 107–109 °C (*i*-PrOH–Et_2O). Anal. ($C_8H_{14}N_4S \cdot 2HI$) C, H, N, S, I.

***N*-[3-(1*H*-Imidazol-4-yl)propyl]-*N'*-[2-[[(5-methyl-1*H*-imidazol-4-yl)methyl]thio]ethyl]urea (15).** A solution of amine Ia (1.71 g, 0.01 mol) and diphenyl carbonate (2.14 g, 0.01 mol) in EtOH (25 mL) was stirred overnight at room temperature, added to H_2O, and recrystallized from EtOH to afford phenyl *N*-[2-[[(5-methyl-1*H*-imidazol-4-yl)methyl]thio]ethyl]carbamate (2.4 g, 84%).

The carbamate (1.2 g, 0.004 mol) was added to a solution of amine Ib (from the dihydrochloride, 0.82 g, 0.004 mol and 0.19 g of sodium (0.008 mol)) in EtOH (25 mL) and the resultant solution heated under reflux for 4 h, concentrated, and triturated with Et_2O. The solid obtained was recrystallized from EtOH–Et_2O to afford the title compound (0.73 g, 55%), mp 173–174 °C (EtOH–Et_2O). Anal. ($C_{14}H_{22}N_6OS$ + 1.3% H_2O) H, N, S; C: calcd, 51.5; found, 50.8.

***N*-[3-(1*H*-Imidazol-4-yl)propyl]-*N'*-[2-[[(5-methyl-1*H*-imidazol-4-yl)methyl]thio]ethyl]thiourea (14).** A solution of Ia (10.2 g, 0.006 mol) in EtOH (75 mL) was added slowly with stirring to CS_2 (200 mL) and the resultant mixture set aside overnight at room temperature. The solid formed was collected and recrystallized from aqueous *i*-PrOH to afford *N*-[2-[[(5-methyl-1*H*-imidazol-4-yl)methyl]thio]ethyl]dithiocarbamic acid (9.8 g, 66%), mp 127–129 °C. Anal. ($C_8H_{13}N_3S_3$) C, H, N. Methyl iodide (4.0 g, 0.0028 mol) was added to a suspension of the dithiocarbamic acid (7.0 g, 0.0028 mol) in CH_3OH and a solution ensued after stirring for 1.5 h at room temperature. Concentration followed by recrystallization from *i*-PrOH–ether afforded the *S*-methyldithiocarbamate hydriodide (XV, R^1 = a; 8.6 g, 78%), mp 167–169 °C. A solution of this material (7.14 g, 0.02 mol) and amine IIb (3.96 g, 0.02 mol, as the dihydrochloride) and K_2CO_3 (4.14 g, 0.03 mol) in water (50 mL) and *i*-PrOH (50 mL) was heated under reflux for 5 h. The product was chromatographed on silica gel with EtOAc–*i*-PrOH (60:40) as eluent and the product in EtOH was treated with oxalic acid in EtOH, affording **14** as an oxalate, mp 134–135 °C (CH_3OH). Anal. ($C_{14}H_{22}N_6S_2 \cdot 1.5(COOH)_2$ C, H, N, S.

***N*-[3-(1*H*-Imidazol-4-yl)propyl]-*N'*-[2-[[(5-methyl-1*H*-imidazol-4-yl)methyl]thio]ethyl]-*S*-methylisothiourea (19).** The oxalate salt of **14** (4.0 g, 0.008 mol) was converted into the free base with NaOEt in EtOH and treated with aqueous HI (2 mL), ether was added to precipitate the hydriodide salt, which was washed with ether dissolved in MeOH, and MeI (5 mL) was added. After 24 h at room temperature, the reaction mixture was evaporated, treated with IRA 400 (Cl^-), and acidified to pH 3 with HCl and evaporated to generate the title compound as a hygroscopic trihydrochloride. Anal. ($C_{15}H_{24}N_6S_2 \cdot 3HCl$) C, H, N, Cl.

***N*-[3-(1*H*-Imidazol-4-yl)propyl]-*N'*-[2-[[(5-methyl-1*H*-imidazol-4-yl)methyl]thio]ethyl]-*N''*-methylguanidine (18).** The hydriodide salt of **19** (2.6 g, 0.0034 mol) in 33% $MeNH_2$–EtOH (20 mL) was set aside overnight at room temperature and the residue following evaporation was dissolved in H_2O (150 mL) and treated with ion-exchange resin IRA 401 (OH^-) to pH 11. Following filtration and evaporation, the residue was treated with excess picrolonic acid to afford **18** as the tripicrolonate (2.5 g, 65%), mp 150–152 °C. Anal. ($C_{15}H_{25}N_7S \cdot 3C_{10}H_8N_4O_5$ + 2% w/v $(CH_3)_2NCHO$) H, N, S; C: calcd, 47.9; found, 47.2.

NMR Procedure for Determining Microionization Constants of Impromidine. The pK_a values of impromidine trihydrochloride determined potentiometrically in water at 25 °C were 6.41 ± 0.3, 7.26 ± 0.02, and 11.7. This latter figures is at the upper limit of measurement by this method and may be an underestimate. In the NMR procedure, impromidine trihydrochloride (86 mg, ca. 0.02 mol), MeOH (15 μL), and KCl (78 mg), contained in a Radiometer Autoburette ABU12 titration vessel, were dissolved in H_2O (9.75 mL) and D_2O (0.25 mL) and the mixture maintained at 25 °C. The D_2O was added for NMR spectrometer lock and MeOH was included as a chemical shift reference. Titration was carried out with KOH (0.40 M). The volume after addition of 1 equiv of alkali was 10.5 mL, at which point the ionic strength was arranged to be 0.1 M, in order to approximately match the conditions used in a normal potentiometric titration. During NMR titration, aliquots of alkali were added to raise the pH by about 0.25 unit at a time. After the addition of each aliquot, a sample (ca. 0.5 mL) was withdrawn from the titration vessel and transferred to a dry NMR sample tube and its proton spectrum recorded on a JEOL PFT100P spectrometer operating at 100 MHz. Each sample was returned to the titration vessel in order to maintain volume and titration was continued over the pH range 3.5–12.0. Titration curves were plotted of chemical shifts for ring protons at carbon-2 against pH for both the mono- and disubstituted imidazole rings.

If the ratio of dications XVIB/XVIA is defined as K_t (Scheme IV), it can be shown that microscopic pK_a values are given by the following equations:

$$pK_{1A} = pK_1 + \log (1 + K_t)$$

$$pK_{1B} = pK_1 + \log (1 + K_t) - \log K_t$$

$$pK_{2A} = pK_2 - \log (1 + K_t)$$

$$pK_{2B} = pK_2 - \log (1 + K_t) + \log K_t$$

For the C(2) protons in each of the two imidazole rings, a computer was used to generate a set of theoretical curves of average chemical shift aginst pH for various values of K_t, using as starting points previously measured macroscopic pK_a values. The weighted averaged observed chemical shifts ($\delta_{obsd}{}^D$ and $\delta_{obsd}{}^M$) of the C(2) protons are given by the following equations:

$$\delta_{obsd}{}^D = \delta_{XVII}{}^D[XVII] + \delta_{XVIA}{}^D[XVIA] + \delta_{XVIB}{}^D[XVIB] + \delta_I{}^D[XV]$$

$$\delta_{obsd}{}^M = \delta_{XVII}{}^M[XVII] + \delta_{XVIA}{}^M[XVIA] + \delta_{XVIB}{}^M[XVIB] + \delta_I{}^M[XV]$$

where δ values are chemical shifts of individual subscripted species and superscripts D and M refer to disubstituted and monosubstituted imidazole rings.

The chemical shifts for C(2) protons in charged rings were assumed to be the same for tri- and dications. Similarly, the chemical shifts for C(2) protons in uncharged rings were assumed to be the same for dications and monocations. Hence $\delta_{XVIA}{}^D = \delta_{XVII}{}^D$ and $\delta_{XVIB}{}^M = \delta_{XVII}{}^M$. Also $\delta_{XVIA}{}^M = \delta_{XV}{}^M$ and $\delta_{XVIB}{}^D = \delta_{XV}{}^D$. The guanidine group was assumed to be fully protonated over the pH range of interest, since its pK_a of 11.7 was so much higher than those of the imidazole rings.

Observed chemical shifts, with respect to the methanol methyl singlet, were $\delta_{XVII}{}^D$ 516.8, $\delta_{XV}{}^D$ 419.9, $\delta_{XVII}{}^M$ 521.5, and $\delta_{XV}{}^M$ 430.1 Hz at 100 MHz.

Parameters pK_1, pK_2, and K_t were varied to find a good fit between observed titration curves and calculated curves and a satisfactory fit with both observed curves was found with pK_1 = 6.53, pK_2 = 7.39, and K_t = 1.98 (Table IV).

Since potentiometric titration is expected to produce more accurate macroscopic pK_a values than NMR titration, microscopic pK_a values were calculated by using potentiometric macroscopic pK_a values and K_t values were derived from NMR titration. When this is carried out, since pK_{1A} slightly exceeds pK_{2A}, the sum of the macroscopic potentiometric pK_a values was increased by 0.1 pK_a unit.

Pharmacological Assays. Guinea Pig Atrium. Guinea pigs weighing between 400 and 700 g were killed, and a triangular piece of right atrium (including the sinoatrial node) was removed as quickly as possible. This atrial strip, mounted in an acrylic holder, was suspended in a 15-mL bath containing McEwen's solution at 34 °C and gassed with 95% O_2–5% CO_2. The contraction frequency was recorded continuously on a potentiometric chart

Table IV. Microscopic pK_a Values Calculated from (a) NMR Data Alone, (b) Potentiometric pK_a Values and K_t from NMR, (c) Potentiometric pK_a Values and K_t from NMR plus Correction (See Text; K_t = 1.98)

	pK_1	pK_2	pK_{1A}	pK_{1B}	pK_{2A}	pK_{2B}
(a)	6.53	7.39	7.00	6.71	6.91	7.21
(b)	6.41	7.26	6.88	6.58	6.79	7.08
(c)	6.36	7.31	6.83	6.54	6.84	7.13

recorder; the signal was the smoothed output of an instantaneous (reciprocal of interval) rate meter, which had been triggered by a force transducer attached to the muscle. The muscle was loaded with 400-mg tension. Agonists and antagonists were added to the bath by micrometer syringe. Compounds were tested in this in vitro preparation in the presence of propanolol (0.5 μM).

Agonist Activity. Agonist activity was assayed up to a concentration of 781 μM (0.1% histamine), and relative activities were assessed from cumulative dose–response curves. Construction of complete dose–response curves to histamine and test compounds were used to determine maximum responses obtainable and relative potencies were determined from concentrations required to elicit 50% of maximal responses. For selected compounds indicated in Table I, parallel line assays using a 2 + 2 Latin square design were used to compare agonist potency with that of histamine.

Antagonist Activity. The dissociation constant (K_B) was calculated from the equation $K_B = B/(x = 1)$, where x is the respective ratio of concentrations of histamine needed to produce half-maximal responses in the presence and absence of different concentrations (B) of antagonist and $-\log K_B = pA_2$.

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Registry No. 1, 55273-05-7; 1·3$C_6H_3N_3O_7$, 55273-06-8; 1·2$C_2H_2O_4$, 63169-77-7; 1·3HCl, 65573-02-6; 2·3$C_6H_3N_3O_7$, 97043-24-8; 3·3$C_6H_3N_3O_7$, 81282-31-7; 4·3$C_{10}H_8N_4O_5$, 97043-26-0; 4 (benzoyl derivative), 97043-27-1; 5·3$C_6H_3N_3O_7$, 63169-82-4; 6, 97043-29-3; 6·3HCl, 97043-28-2; 6 (cyano derivative), 97043-35-1; 7·3$C_{10}H_8N_4O_5$, 97043-30-6; 8, 97043-31-7; 8·3HCl, 60078-40-2; 9, 55272-99-6; 9·3HCl, 55273-01-3; 9 (benzoyl derivative), 97059-56-8; 10·3$C_{10}H_8N_4O_5$, 97043-33-9; 10 (cyano derivative), 97043-34-0; 11·3$C_6H_3N_3O_7$, 63779-37-3; 12·2$C_6H_3N_3O_7$, 97043-37-3; 12 (cyano derivative), 63779-32-8; 13, 97043-38-4; 14·$^3/_2C_2H_2O_4$, 97059-58-0; 15, 97043-39-5; 16·3$C_6H_3N_3O_7$, 97043-41-9; 17, 97043-43-1; 17·3HCl, 97043-42-0; 18·3$C_{10}H_8N_4O_5$, 97043-45-3; 19, 97043-47-5; 19·3HCl, 97043-46-4; 19·3HI, 97043-48-6; 20, 70172-53-1; 20·2HCl, 58726-90-2; 21, 97043-49-7; 21·2HCl, 52568-77-1; 22·2$C_6H_3N_3O_7$, 97043-51-1; 23, 97043-53-3; 23·HI, 97043-52-2; 24, 97043-54-4; 24·H_2SO_4, 97043-55-5; 25, 97043-57-7; 25·HI, 97043-56-6; 26, 97043-59-9; 26·2HCl, 97043-58-8; 27, 55272-97-4; 27·HI, 55272-96-3; 27·H_2SO_4, 55272-98-5; 28, 33551-01-8; 28·2HI, 40836-60-0; Ia, 38585-67-0; Ib, 40546-33-6; Ib (acetyl derivative), 97043-64-6; Ic, 38585-66-9; Id, 40546-47-2; Ie, 51-45-6; If, 34034-74-7; If·2HCl, 33544-95-5; Ig, 38603-99-5; Ih, 63779-34-0; Ih·2HCl, 33545-16-3; IIa, 38603-54-2; IIb, 34970-64-4; IIb·HI, 40836-59-7; VIIa, 60588-77-4; IXi, 10191-60-3; IXk, 24786-18-3; IXl, 81282-38-4; Xl, 97043-60-2; Xm, 52378-40-2; Xn, 63169-79-9; Xo, 97043-61-3; Xp, 97043-62-4; Xq, 63809-73-4; XII, 81282-23-7; XIII, 97043-66-8; XIV (R = a), 81282-28-2; XIV (R = c), 97043-63-5; XV (R′ = a)·HI, 55272-82-7; bromoacetaldehyde diethyl acetal, 2032-35-1; phenoxythioacetamide, 35370-80-0; cysteamine hydrochloride, 156-57-0; 4-phthalimidothiobutyramide, 41306-76-7; 2-(phenoxymethyl)-thiazole hydrobromide, 97043-65-7; *N*-(3-thiazol-2-ylpropyl)-phthalimide hydrobromide, 33545-15-2; benzoyl isothiocyanate, 532-55-8; *N*-benzoyl-*N*′-[2-[[(5-methyl-1*H*-imidazol-4-yl)-methyl]thio]ethyl]thiourea, 38603-53-1; *N*,*S*-dimethylisothiourea hydriodide, 41306-45-0; 4-(3-chloropropyl)imidazole hydrochloride, 51722-01-1; *N*-benzoyl-*N*′-[3-(1*H*-imidazol-4-yl)propyl]thiourea, 33550-99-1; diphenyl carbonate, 102-09-0; phenyl *N*-[2-[[(5-methyl-1*H*-imidazol-4-yl)methyl]thio]ethyl]carbamate, 97059-59-1; *N*-[2-[[(5-methyl-1*H*-imidazol-4-yl)methyl]thio]ethyl]dithiocarbamic acid, 55317-80-1.

N^{α}-(Diphenoxyphosphoryl)-L-alanyl-L-proline, N^{α}-[Bis(4-nitrophenoxy)phosphoryl]-L-alanyl-L-proline, and N^{α}-[(2-Phenylethyl)phenoxyphosphoryl]-L-alanyl-L-proline: Releasers of Potent Inhibitors of Angiotensin Converting Enzyme at Physiological pH and Temperature

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The rate of loss of phenol or 4-nitrophenol from N^{α}-(diphenoxyphosphoryl)-L-alanyl-L-proline (**2**), N^{α}-[bis(4-nitrophenoxy)phosphoryl]-L-alanyl-L-proline (**5**), and N^{α}-[(2-phenylethyl)phenoxyphosphoryl]-L-alanyl-L-proline (**12**) was determined spectrophotometrically at pH 7.5 and 37 °C in both Tris and phosphate buffers. These moderately potent inhibitors of angiotensin converting enzyme ($K_i > 0.8$ μM) all hydrolyze, losing 1 mol of phenol to yield highly potent inhibitors (K_i = 0.5–18 nM). The half-times for loss of 1 mol of phenol in Tris buffer are 22 days (**2**), 3.4 h (**5**), and 21 days (**12**). The half-times in phosphate buffer were not significantly different. The mono(4-nitrophenoxy) ester **6** (K_i = 18 nM) loses its 1 mol of nitrophenol with a half-time of 35 h to yield N^{α}-phosphoryl-L-alanyl-L-proline **16** (K_i = 1.4 nM), which hydrolyzes at the P–N bond with a half-time of 2.2 h. Hydrolysis of the P–N bond in **2** and **12** was not observed during the time course of the kinetic experiments. The two phosphoramidate diesters **2** and **5** and the phosphonamidate monoester **12** thus release powerful inhibitors of angiotensin converting enzyme with a known time course at physiological pH and temperature in vitro. A time-dependent increase in inhibitory potency against converting enzyme that paralleled the kinetics of phenyl ester hydrolysis was confirmed in vitro.

Phosphoramidates, phosphonamidates, and phosphonic and phosphinic acids have been shown to be powerful inhibitors of angiotensin converting enzyme.[1–6] In these inhibitors the tetrahedral phosphorus atom is thought to

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