Histamine H_2-Receptor Agonists. Synthesis, in Vitro Pharmacology, and Qualitative Structure–Activity Relationships of Substituted 4- and 5-(2-Aminoethyl)thiazoles[1]

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It is well known that both histamine and dimaprit show moderate histamine H_2-receptor agonistic activities on the guinea pig right atrium. Quantum chemical calculations on these two compounds showed similarities in electron distributions and molecular electrostatic potentials (MEP's), which could be extended to rigid analogues [2-amino-5-(2-aminoethyl)thiazoles] of the latter structure. On the base of these results a series of substituted 4- and 5-(2-aminoethyl)thiazoles was synthesized applying small alkyl substitution variations as reported for histamine. 2-Amino-5-(2-aminoethyl)-4-methylthiazole (Amthamine) proved to be the most potent full histamine H_2-receptor agonist on the guinea pig right atrium, being with a pD_2 value of 6.21 slightly more potent than histamine. This compound shows no affinity for H_1-receptors and is a full but weak agonist on the histamine H_3-receptor with a pD_2 value of 4.70, thus showing a marked specificity for histamine H_2-receptors. In the 5-(2-aminoethyl)thiazole series the presence of a 2-amino substituent proved to be not essential for stimulation of the histamine H_2-receptor, leading to the important conclusion that in contrast to histamine, for this series, acceptance of a proton by the thiazole nucleus of the agonist from the active site of the receptor is sufficient for the stimulation of the histamine H_2-receptor.

Introduction

Besides histamine (1) (Table I), which is able to stimulate histamine H_1-, H_2-, and H_3-receptors, only a few general classes of selective histamine H_2-receptor agonists are known in literature.

Dimaprit (2), an isothiourea derivative described by Parsons et al.,[2] has shown in vitro potency of 71% that of histamine[3] on the guinea pig right atrium (H_2), whereas the response on the guinea pig isolated ileum (H_1) proved to be less than 0.0001% of the activity of histamine.[3]

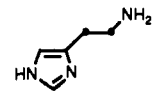
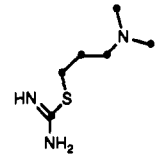
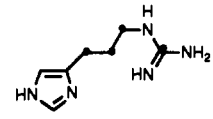
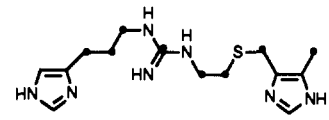
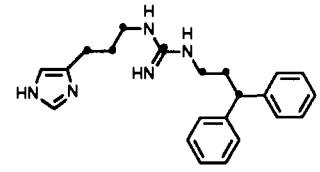
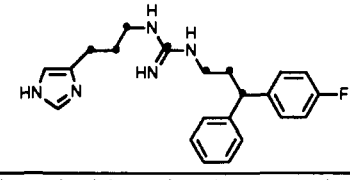
N-[3-[4(5)-Imidazolyl]propyl]guanidine (SK&F 91486, (3)), a partial agonist with an activity of only 4% that of histamine on the guinea pig right atrium, was prepared by Parsons et al.[4] and served as a lead structure for the development of impromidine (4). This *N'*-substituted-*N*-[3-[4(5)-imidazolyl]propyl]guanidine described by Durant et al.[5] was the first selective full histamine H_2-receptor agonist being more potent than histamine on the guinea pig right atrium. Quite remarkable is the fact that in vitro impromidine behaves as a partial agonist on histamine-sensitive adenylate cyclase,[6] although it is in functional tests much more potent than histamine.

Analogues of impromidine as described by Sterk et al.[7] (5) and Buschauer[8] (6) are potent histamine H_2-receptor agonists, and some of them show considerable histamine H_1- and H_3-receptor antagonistic properties on the isolated guinea pig ileum.

Histamine and impromidine and its analogues all contain a 4-(ω-aminoalkyl)imidazole fragment from which the imidazole moiety is supposed to trigger the histamine H_2-receptor according to the model as presented by Weinstein et al.[9] The second substituent on the guanidine moiety, the 3,3-di((hetero)aryl)propyl fragment enhances the activity on the histamine H_2-receptor as can be seen from the marked differences in activity on the histamine H_2-receptor (Table I) for SK&F 91486 (3) and impromidine and its analogues (4, 5, 6), but can also be used to introduce a second biological effect. In arpromidine (6) the 3-(4-fluorophenyl)-3-(2-pyridyl)propylamine fragment is held responsible for the considerable histamine H_1-receptor antagonistic property of this molecule.[8]

The effects of substitution in histamine itself on the selectivity toward histamine H_1- and H_2-receptors have almost fully been explored by Durant et al.,[10,10a] Lennartz et al.,[11] Hepp et al.,[12] and Vitali et al.[13] In general, introduction of a small alkyl group in position 5(4) of the imidazole ring in histamine increases the selectivity toward

Table I. Some Histamine H_2-Receptor Agonists

compd	structure	name	pD_2[a]
1		histamine	6.10[b]
2		dimaprit	5.95[c]
3		SK&F 91486	4.70[d]
4		impromidine	7.80[e]
5		impromidine analogue	7.70[f]
6		arpromidine	8.00[g]

[a] Guinea pig right *atrium* (chronotropic effect), calculated from relative potencies setting histamine $pD_2 = 6.10$. [b] Sterk et al. ref 7. [c] Parsons et al. ref 2. [d] Partial agonist $\alpha = 0.7$, Parsons et al. ref 4. [e] Durant et al. ref 5. [f] Sterk et al. ref 7. [g] Buschauer ref 8.

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(1) Presented in part at the Satellite Symposium of the XIth International Congress of Pharmacology of IUPHAR, New Perspectives in Histamine Research, July 6-8, 1990, Noordwijkerhout, The Netherlands.

(2) Parsons, M. E.; Owen, D. A. A.; Ganellin, C. R.; Durant, G. J. Dimaprit—[S-[3-(N,N-dimethylamino)propyl]isothiourea]—A Highly Specific Histamine H_2-receptor Agonist. Part 1. Pharmacology. *Agents Actions* 1977, 7, 31-37.

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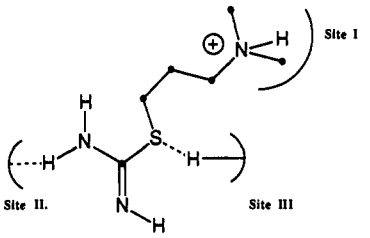
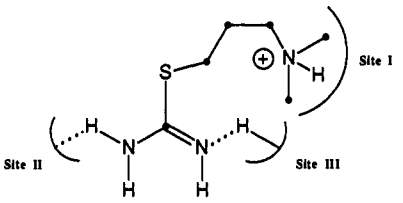
Chart I. Interaction of Dimaprit with the Histamine H_2-Receptor[a] (S-Fit)



[a] According to Green et al. ref 14.

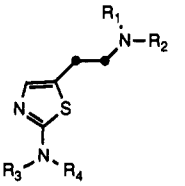
Chart II. Interaction of Dimaprit with the Histamine H_2-Receptor[a] (N-Fit)



[a] According to Durant et al. ref 15.

the histamine H_2-receptor, whereas introduction of these groups in position 2 of the imidazole part enhances selectivity toward histamine H_1-receptors; in both cases the activity on histamine H_1- and H_2-receptors is decreased as compared as with the activity of the unsubstituted histamine. Dimaprit, however, looked more promising as a lead structure for the development of selective histamine H_2-receptor agonists.

Chart III. Substituted 2-Amino-5-(2-aminoethyl)thiazoles: In Vivo and in Vitro Activity on Gastric Acid Secretion[a]



	in vivo pD_2[b]	in vitro pD_2[c]
7a, $R_1 = R_2 = R_3 = R_4 = H$	6.10[d]	
7b, $R_1 = R_2 = CH_3$, $R_3 = R_4 = H$	6.30[d]	5.81[e]
7c, $R_1 = CH_3$, $R_2 = R_3 = R^4 = H$	6.52[f]	6.02[e]
7d, $R_1 = R_2 = R_3 = R_4 = CH_3$	N.A.[f]	
7e, $R_1 = R_2 = R_3 = CH_3$, $R_4 = H$	N.A.[f]	

[a] Taken from Impicciatore et al. ref 17. [b] Gastric acid secretion in concious cat. [c] Gastric acid secretion on the isolated guinea pig stomach. [d] Partial agonist $\alpha = 0.8$. [e] Partial agonist $\alpha = 0.7$. [f] Not active.

As histamine is supposed to trigger the histamine H_2-receptor via a 1,3-prototropic shift[9] involving one proton from a proton donating group at the histamine H_2-receptor and the tele proton of the imidazole nucleus, the mechanism for dimaprit is less clear. Green et al.[14] suggested (Chart I) the formation of a hydrogen bond between a proton of the histamine H_2-receptor and the sulfur atom of dimaprit in the so-called S-fit and the formation of a second hydrogen bond between a proton of one of the nitrogen atoms of the isothiourea group and another site of the same receptor. As with histamine two protons are involved, one from the receptor and one from the agonist; however, in contrast to histamine a tautomeric proton shift is impossible in the same region of the active site of the receptor.

Durant et al.[15] considered a second possibility for the interaction of dimaprit with the histamine H_2-receptor, the so-called N-fit, placing the two nitrogen atoms of the isothiourea moiety of dimaprit in the same position as the two imidazole nitrogen atoms of histamine. As can be seen in Chart II, this mechanism supposes an interaction between the receptor proton (site III) and a nitrogen atom of the isothiourea group and a proton of the second nitrogen atom of this same group and site II of the receptor, thus leaving the possibility for a 1,3-prototropic shift as proposed for the mechanism of histamine.

Donné-Op den Kelder et al.[16] performed fit procedures on both the N- and S-fit of dimaprit, showing that the conformation of the N-fit is not likely to be the "active" conformation.

Calculation of the molecular electrostatic potential (MEP)[16] for this N-fit of dimaprit and histamine (N_τ-tautomer) reveals marked differences. Although both in histamine and dimaprit pronounced minima are observed near the double-bonded nitrogen atoms, the geometric positions of these minima do not show a significant overlap.

(3) Cooper, D. G.; Young, R. C.; Durant, G. J.; Ganellin, C. R. Histamine Receptors. In *Comprehensive Medicinal Chemistry*; Emmett, J. C., Ed.; Pergamon Press: Oxford, 1990; Vol. 3, p 357.
(4) Parsons, M. E.; Blackemore, R. C.; Durant, G. J.; Ganellin, C. R.; Rasmussen, A. C. 3-[4(5)-Imidazolyl]propylguanidine (SK&F 91486)—a Partial Agonist at Histamine H_2-Receptors. *Agents Actions* **1975**, *5*, 464.
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(6) See ref 3, p 361.
(7) Sterk, G. J.; Koper, J.; Van der Goot, H.; Timmerman, H. Studies on histaminergic compounds VIII. A new series of compounds showing H_1-antagonistic and H_2-agonistic properties; synthesis, in vitro pharmacology, and structure-activity relationships. *Eur. J. Med. Chem.* **1987**, *22*, 491–498.
(8) Buschauer, A. Synthesis and in vitro Pharmacology of Arpromidine and Related Phenyl(pyridyl)guanidines, a Potential New Class of Positive Inotropic Drugs. *J. Med. Chem.* **1989**, *32*, 1963–1970.
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(10) Durant, G. J.; Ganellin, C. R.; Parsons, M. E. Chemical Differentiation of Histamine H_1- and H_2-Receptor agonists. *J. Med. Chem.* **1975**, *18*, 905–909. (a) Durant, G. J.; Emmett, J. C.; Ganellin, C. R.; Roe, A. M.; Slater, R. A. Potential Histamine H_2-Receptor Antagonists. 3. Methylhistamines. *J. Med. Chem.* **1976**, *19*, 923–928.
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(12) Hepp, M.; Dziuron, P.; Schunack, W. 2-Substituierte Histamine mit selektive H_1-agonistischer Aktivität. *Arch. Pharm. (Weinheim)* **1979**, *312*, 637–639.
(13) Vitali, T.; Impicciatore, M.; Plazzi, P. V.; Bordi, F.; Vitto, M. Sintesi ed attività del diclorato di 2-(2-amino-4-imidazolil)-etilamina (2-aminoistaminia). *Il Farmaco Ed. Sci.* **1984**, *39*, 70–80.
(14) Green, J. P.; Johnson, C. L.; Weinstein, H. Histamine as a neurotransmitter. In *Psychopharmacology, A Generation of Progress*; Lipton, M. A., Di Mascio, A., Killam, K. F., Eds.; Raven Press: New York, 1978; 319–322.
(15) Durant, G. J.; Ganellin, C. R.; Parsons, M. E. Dimaprit, [S-[3-(N,N-dimethylamino)propyl]isothiourea]. A Highly Specific Histamine H_2-Receptor Agonist. Part 2. *Agents Actions* **1977**, *7*, 39–43.
(16) Donné-Op den Kelder, G. M.; Haaksma, E. E. J.; Timmerman, H. Theoretical Considerations concerning the H_2-receptor. In *Trends in Medicinal Chemistry '88*; Van der Goot, H., Domány, G., Pallos, L., Timmerman, H., Eds.; Elsevier: Amsterdam, 1989; 365–392.

Contrary, in the S-fit a rather sharp minimum is observed in the vicinity of the double-bonded nitrogen and a second minimum of about −2 kcal/mol is indicated near the sulfur atom of dimaprit, showing overlap with the minimum as observed in the neighborhood of the double-bonded nitrogen in histamine (N_τ-tautomer).

On the basis of these results and the inactivity of the S-[2-(*N,N*-dimethylamino)ethyl]isothiourea no definite conclusions about the mechanism of the stimulation of the histamine H_2-receptor can be drawn, leaving only geometric considerations in favor of the S-fit of dimaprit as originally postulated by Green et al.[14]

Impicciatore et al.[17] first considered substituted 2-amino-5-(2-aminoethyl)thiazole derivatives (**7a–e**) (Chart III) which are rigid analogues of dimaprit. A few of the substituted thiazole derivatives prepared by these authors (Chart III) stimulated in vitro dose-dependent gastric acid secretion in the guinea pig isolated stomach. No data on 2-amino-5-(2-aminoethyl)thiazole (**7a**) are mentioned, only in vivo data (concious cat). A $pD_2 = 6.10$ on gastric acid secretion is reported for this derivative. From the "pronounced differences in the ability of stimulation of the gastric acid secretion in in vivo and in vitro experiments", the authors[17] concluded that for this type of compound "a stimulating H_2-dependent activity, rather than a direct interaction with the H_2-receptor" occurs.

Vitali et al.[18] reported 2-amino-5-(2-aminoethyl)thiazole (**7a**) having an activity of 0.3% that of histamine on the guinea pig right atrium (inotropic effect) and 2-amino-5-[2-(dimethylamino)ethyl]thiazole (**7b**) being the most potent one with an activity equal to histamine. No data on chronotropic effects and H_2 specifity were presented.

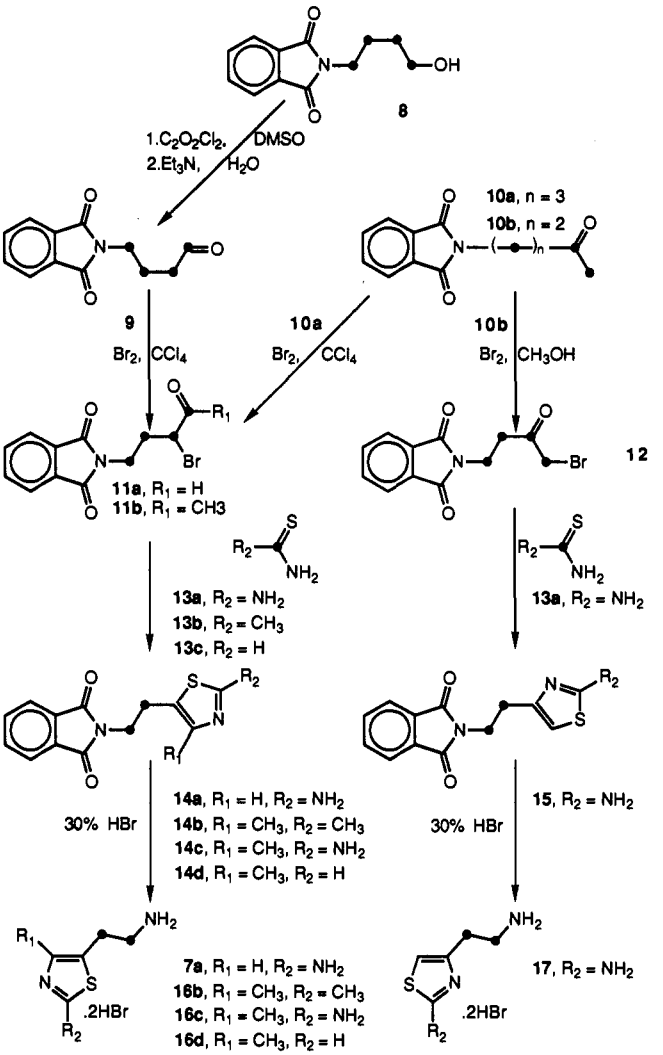
All former considerations, but especially the possible H_2-receptor agonistic properties of the "cyclic" dimaprit analogues, convinced us of the fact that dimaprit and its rigid analogues should provide information about histamine H_2-/H_1-receptor selectivity and possibly could lead to the development of new and specific histamine H_2-receptor agonists. We therefore decided to synthesize and test a series of "rigid" dimaprit analogues, applying comparable alkyl substitution patterns as in the histamine series.

Chemistry

Numerous thiazole derivatives have been prepared via the Hantzsch synthesis[19] by cycloaddition of thiourea and an α-halo ketone or an α-halo aldehyde. This general method is not limited to thiourea, but is extended by many authors using thioformamide and both aliphatic and aromatic thioamides as reported in an extended review by Vernin.[20]

Spare examples of 4- or 5-(2-aminoethyl)thiazoles do occur in literature. The unsubstituted 4-(2-aminoethyl)-thiazole has been prepared from 1-bromo-4-phthalimido-2-butanone and thioformamide in anhydrous ethanol

Scheme I



followed by hydrazinolysis in 76% overall yield by Jones et al.[21] Jonas[22] prepared 2-amino-4-(2-aminoethyl)thiazole from the same starting material and thiourea, followed by hydrazinolysis yielding only 8% of the desired compound. A few more examples have been claimed in patents,[23–25] but the methods of preparation are either described in general terms or no data on the desired compounds are mentioned. The substituted 2-amino-5-(2-aminoethyl)-thiazoles (**7a–7e**) (Chart III) as mentioned by Impicciatore et al.[17] were prepared via different routes; only the unsubstituted 4-[2-(dimethylamino)ethyl]thiazole was prepared by these authors via the Hantzsch synthesis.

As this Hantzsch synthesis affords ω-phthalimido aldehydes (**9**) (Scheme I) or ω-phthalimido-2-alkanones (**10**), we paid attention to the preparation of these key inter-

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(19) Hantzsch, A.; Traumann, V. Amidothiazole aus Sulfoharnstoff und halogenisirten Ketonen resp. Aldehyden. *Berichte* **1888**, *21*, 938–941.

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(21) Jones, R. G.; Kornfeld, E. C.; McLaughlin, K. C. The Synthesis of Some Analogs of Histamine and Histidine Containing the Thiazole Nucleus. *J. Am. Chem. Soc.* **1950**, *72*, 4526–4529.

(22) Jonas, J. Preparation of 2-amino-4-(ω-aminoalkyl)thiazoles. Spisy Prirodovedecke Fak. Univ. Brne., 1963, 447, 405–411. *Chem. Abstr.* **1964**, *60*, 15853a.

(23) Poittevin, A.; Derible, P. H.; Fournex, R. 5-(Aminomethyl)-thiazole derivatives with pharmaceutical activity. Ger. Offen. 2,641,129, Mar 24, 1977. Fr. Appl. 75/28.095, Sept 12, 1975. *Chem. Abstr.* **1977**, *87*, 39465b.

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(25) Sprague, J. M.; Land, A. H. 2-Aminothiazoles. US 2,636,037, Apr 21, 1953. *Chem. Abstr.* **1954**, *48*, 4008g.

mediates. Although 4-phthalimidobutanol (8) can be prepared according to Suguria et al.[26] from 4-chlorobutanol and potassium phthalimide in rather low yield (46%), preparation was either performed starting from 4-aminobutanol and phthalic anhydride to yield 91% of the desired compound or performed alternatively from phthalimide and chlorobutanol in DMF in the presence of anhydrous potassium carbonate in 95% yield. Both methods are generally suitable for the preparation of ω-phthalimido alcohols.[27a,b]

The 4-phthalimidobutyraldehyde (9) might be prepared by the reduction of 4-phthalimidobutyronitrile with stannous chloride as described by King et al.,[28] or via oxidation of 4-phthalimidobutanol (8) with chromium(III) oxide in pyridine according to Wada et al.[29] Preferably, 4-phthalimidobutyraldehyde (9) is obtained from 4-phthalimidobutanol (8) by reaction with oxalyl chloride/dimethyl sulfoxide, followed by proton abstraction with triethylamine and hydrolysis to the corresponding aldehyde as described by Omura and Swern.[30]

Bromination of the aldehydes was performed in carbon tetrachloride and after identification with NMR, the crude α-bromo aldehyde was used in the cyclization reaction (Scheme I).

The ω-phthalimido-2-alkanones (**10a,b**) were prepared according to a modified method of Sletzinger et al.[31] Selective bromination of these ketones in position 1 was performed according to the procedure described by Gaudry and Marquet,[32] which gave significantly higher yields of the desired bromo ketone[33] as compared with the method using dioxane/bromine complex as mentioned by Elz and Schunack.[34]

Ring closure of the α-bromo aldehydes or α-bromo ketones with the appropriate thioamides was performed in dimethylformamide giving high yields of the desired 4- or 5-(2-phthalimidoethyl)thiazoles (**14a–d, 15**).

The corresponding 4- or 5-(2-aminoethyl)thiazoles (**7a, 16b–d,** and **17**) were obtained either by hydrazinolysis according to known methods[22,23,35] or by hydrolyses with 30% aqueous hydrochloric or hydrobromic acid.

Pharmacology

Histamine H_2-receptor agonistic properties were determined on the isolated spontaneously beating guinea pig right atrium according to Sterk et al.[36] In contrast to these authors the pD_2 values from the chronotropic effects were not evaluated as relative activities toward histamine but for statistical reasons[37] the uncorrected pD_2 values (taken at 50% maximal response of the agonistic dose–response curves) of the investigated H_2-receptor agonists are reported. Intrinsic activities were evaluated from the ratio of maximal effect in the agonistic dose–response curves and the maximum effect in the corresponding histamine curves. Receptor binding at H_2-receptors was determined on guinea pig cerebral cortex preparations as described by Gajtkowski et al.[38] by the displacement of [^{3}H]tiotidine. The obtained displacement curves were analyzed with the nonlinear regression program Ligand.[39] Histamine H_1-receptor agonistic and antagonistic activities were tested on the isolated guinea pig ileum as described by Emmet et al.[40]

Receptor binding at H_1-receptors were determined on the rat cerebellum by displacement of [^{3}H]mepyramine according to Leurs et al.[41] Data were analyzed as mentioned under H_2-receptor binding studies.

Agonistic activities at H_3-receptors were obtained via electrically evoked contractions of guinea pig ileum segments to a modified procedure as described by Hew et al.[42]

Results and Discussion

Compounds **7a** and **16c** (Table II) which are both (substituted) cyclic analogues of dimaprit (**2**) show marked histamine H_2-receptor agonistic properties, the 2-amino-5-(2-aminoethyl)-4-methylthiazole (**16c**) [Amthamine, from 2-(2-amino-4-methyl**thi**azol-4-yl)ethyl**amine**] proved to be the most potent full agonist on the guinea pig right atrium in the obtained series, with a $pD_2 = 6.21$ being slightly more potent than histamine (1) ($pD_2 = 6.14$). Competitive displacement, with different concentrations of the histamine H_2-receptor antagonist cimetidine in the functional study on the guinea pig right atrium, causes significant parallel shifts of the agonistic curves of this compound. Calculation of the pA_2 of cimetidine from these curves

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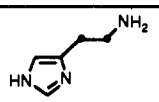
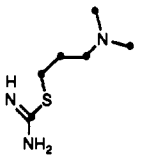
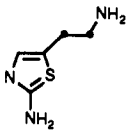
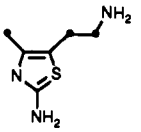
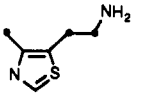
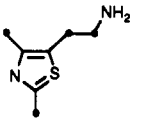
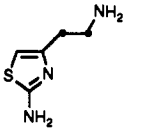
Table II. Binding and Functional Effects on Histamine H_2-Receptors

		pK_d[a]	n	pD_2[b]	α[c]	n
1		4.16 ± 0.08	3	6.14 ± 0.04	1.00	22
2		4.58 ± 0.11	2	5.67 ± 0.12	1.06 ± 0.03	4
7a		4.82 ± 0.10	3	5.51 ± 0.05	1.00 ± 0.05	4
16c		5.30 ± 0.08	3	6.21 ± 0.09	0.95 ± 0.02	7
16d		3.45 ± 0.12	3	4.67 ± 0.12	0.96 ± 0.03	4
16b		3.76 ± 0.21	2	3.78 ± 0.13[d]	0.97 ± 0.10	3
17		3.96 ± 0.07	4	4.60 ± 0.09	0.94 ± 0.04	4

[a] Guinea pig *cerebral cortex*. [b] Guinea pig right *atrium* (chronotropic effect). [c] Intrinsic activity. [d] Reported earlier[1] as being inactive due to improper experimental conditions.

resulted in a value of 6.26 ± 0.12 (n = 4) being very close to reported literature values using histamine as the agonist.[43]

Concentrations up to 10^{-4} M of **16c** did not show any significant contraction of the guinea pig ileum, indicating that compound **16c** does not possess histamine H_1-receptor agonistic properties. Agonistic curves of histamine in the presence of 10^{-4} M **16c** did not show significant shifts and reduction of the maximum effects toward the reference curves of histamine, indicating that this compound also does not possess antagonistic properties. The former results were confirmed by binding studies on the rat cerebellum by displacement of the H_1-selective antagonist [^{3}H]mepyramine. Only concentrations of **16c** above 10^{-3} M caused slight displacement of the applied histamine H_1-receptor antagonist, indicating that compound **16c** has no affinity towards the histamine H_1-receptor. On the histamine H_3-receptor **16c** proved to be a weak but full agonist with a pD_2 value of 4.70.

The high selectivity of amthamine (**16c**) toward the histamine H_2-receptor might be attributed to the presence of the 4-methyl substituent; a similar effect is observed for 4(5)-methylhistamine, which shows a considerable specificity for the histamine H_2-receptor as compared to histamine itself.[10,10a,11] In contrast to histamine however, where introduction of a methyl group in position 4(5) results in a decrease of the agonistic activity (both on right atrium and gastric acid secretion as determined by Lennartz et al.[11] and Durant et al.[10]) in the thiazole series, the introduction of a 4-methyl substituent (**16c**) results in an increase of the agonistic activity as compared with the "unsubstituted" 2-aminothiazole derivative **7a**. (pD_2 = 6.21 and 5.51, respectively.)

In the limited series of (substituted) 5-thiazolyl derivatives (**7a**, **16b–d**, and **17**) (Table II) both affinity toward and activity on the histamine H_2-receptor seems to be positively correlated with the basic properties of the heteroaromatic nucleus. Considering a series of substituted thiazoles as presented by Phan-Tan-Luu et al.,[44] the basicity is expected to increase in the series 4-CH_3, 2,4-diCH_3, 2-NH_2, and 2-NH_2-4-CH_3 substituted thiazole derivatives. Obtained proton association constants from pH values at half neutralization of the salts of **7a**, **16b–d**, and 17 of 4.85 ± 0.02, 3.97 ± 0.07, 5.25 ± 0.02 and 3.65 ± 0.13, respectively, are in good line with these expectations.

Affinity (pK_d cortex) and activity (pD_2 right atrium) are increased in a similar way in the series **16d** (4-CH_3), **7a** (2-NH_2), and **16c** (2-NH_2-4-CH_3), suggesting that the binding on the histamine H_2-receptor depends on the affinity of the nitrogen atom of the thiazole nucleus of the agonists for a proton-donating site at the receptor. A

(43) Van der Goot, H.; Bast, A.; Timmerman, H. Structural Requirements for Histamine H_2 Agonists and H_2 Antagonists; Histamine and Histamine Antagonists. In *Handbook of Experimental Pharmacology*; Uvnäs, B., Ed.; Springer-Verlag: Berlin Heidelberg, 1991; Vol. 97, pp 573–748.

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similar qualitative correlation can be observed with the histamine H_2-receptor agonistic activities of 4(5)-nitrohistamine, 4(5)-chlorohistamine, and histamine itself. Reported relative potencies of 0.6%, 12%, and 100% as determined from the stimulation of gastric acid secretion in the anesthetized rat by Durant et al.[10] increase with increasing basicity of the imidazole nucleus within this series.

Whereas the 2,4-diCH_3-derivative **16b** fits into the "correlation" with respect to affinity (pK_d cortex), in terms of activity (pD_2 right atrium) this derivative seems to be an outlier. Although in terms of molecular volume a methyl group does not differ very much from an amino group, the methyl group in the 2-position of the heteroaromatic nucleus is obviously not very well "tolerated" by the histamine H_2-receptor, a result which is in line with findings in the histamine series.[12] This phenomenon is supported by the marked differences between the efficacies of the observed compounds, which were calculated from pK_d and pD_2 values according to Stephenson.[45] The 2,4-dimethyl derivative **16b** shows only an efficacy of 2.0, whereas compounds **16d**, **16c**, and **7a** show efficacies of 17.5, 9.1, and 5.9, respectively.

Remarkable is the fact that the nontautomeric compounds **16b** and **16d**, which lack the amino group in position 2 of the heteroaromatic nucleus, are still full agonists at the guinea pig right atrium, suggesting that in the investigated series of thiazole derivatives tautomerism is not essential for the stimulation of the histamine H_2-receptor and supporting the doubts of Cooper et al.[46] "whether tautomerism is necessary involved in the action of the several types of agonists on the H_2-receptor". The weak histamine H_2-receptor agonistic activities of 5-(2-aminoethyl)thiazole and 2-(2-aminoethyl)thiazole as reported by Durant et al.[10] also support these doubts; moreover, the presented histamine H_2-receptor agonistic activities as determined from the stimulation of gastric acid secretion by these authors are in good agreement with our results.

Alternative mechanisms of stimulation of the histamine H_2-receptor based on quantum chemical calculations have been postulated by interalia Haaksma et al.[47] and Pardo et al.[48] suggesting an agonist-mediated proton shift for 2-aminothiazoles and dimaprit from site III to site II (Chart I) of the histamine H_2-receptor. Because these proton shifts were forced to occur in their calculational procedures, the extrapolation of these results toward more physiological conditions is yet very difficult.

Extremely interesting in this respect is the proposed proton shift for 2-amino-4-(2-aminoethyl)thiazole (**17**) by Haaksma et al.[47] where compared with dimaprit (**2**) and 2-amino-5-(2-aminoethyl)thiazole (**7a**) the positions of the nitrogen and sulfur atom toward the active sites are effectively interchanged, thus suggesting a proton shift against the existing dipole moment of the agonist. Although the mentioned proton shift might explain the histamine H_2-receptor agonistic properties of dimaprit and 4- and 5-thiazolyl derivatives, incorporation of structures like 2-(2-pyridyl)ethylamine, betahistine, betazole, and 2-(2-aminoethyl)thiazole[49] in these models raise severe problems. Thus further extensive research on "simple" heterocyclic analogues of histamine seems to be indicated.

Replacement of the imidazole nucleus by the 2-amino-4-methylthiazol-5-yl fragment proved not only to be successful in histamine resulting in compound **16c**, but similar results could be obtained in impromidine (**5**) and arpromidine (**6**) leading to highly potent and selective histamine H_2-receptor agonists.[27a,b]

From the former pharmacological results it is obvious that with Amthamine (**16c**) a new and powerful selective histamine H_2-receptor agonist is available which may be of great pharmacological importance for studies concerning the different subtypes of histamine receptors.

Experimental Section

Chemistry. All chemicals and solvents used are commercially available unless stated otherwise. Melting points were recorded on a Mettler FP5 melting point apparatus and are uncorrected. ^1H NMR spectra were recorded at 90 MHz on a Bruker WH-90 spectrometer. Chemical shifts are reported in parts per million (δ) downfield from tetramethylsilane for $CDCl_3$ and sodium 3-(trimethylsilyl)propionate for D_2O and DMSO-d_6 as internal standard. Mass spectra and exact masses were determined on a Mat 90 (Finnigan Mat, San José, CA) mass spectrometer (electron impact at 70 eV). The obtained thiazole derivatives were titrated potentiometrically under a N_2 atmosphere at 37 °C using 50.00 mL of an approximately 10^{-3} M solution of the hydrobromic salts in twice-distilled H_2O containing 0.1 M KNO_3, with a standardized NaOH solution (Titrisol, Merck) using a Mettler DV11 microburette. Measurements of the pH were performed with a Metrohm Herisau Ion-Activity-Meter E 580. Calibration of the electrode system (Metrohm, E 202 pH electrode) was performed with J. T. Baker Dilut-it pH buffers (pH = 4.00 and pH = 7.00, I = 0.05 and 0.1, respectively). Proton association constants (Log K_a) are reported on the heteroaromatic nucleus of the 4- or 5-(2-aminoethyl)thiazoles and were obtained from pH values at half neutralization. Microanalyses for elements indicated are within 0.4% of the theoretical values. TLC analyses of the obtained thiazole derivatives were carried out on Merck DC-Alufolien Kieselgel 60 F_{254} plates (0.2 mm) using a 2% solution of NH_3 in 100% EtOH. Spots were identified with ninhydrin.

4-Phthalimidobutanol (8). A mixture of 148 g (1 mol) of finely powdered phthalic anhydride and 89 g (1 mol) of 4-aminobutanol were slowly heated until approximately 80 °C with vigorous stirring under a N_2 atmosphere. After starting of the exothermic reaction the temperature rose to 140 °C and stirring was continued for an additional 3 h, and after cooling to 80 °C the reaction mixture was poured while stirring into 500 mL of ice-cold H_2O. The product was extracted three times with 250 mL of $CHCl_3$, and the combined $CHCl_3$ layers were washed with a 5% $NaHCO_3$ solution and three times with 100 mL of H_2O. After drying on anhydrous Na_2SO_4, the organic phase was filtered and the solvent removed in vacuo (20 mmHg, 80 °C) to give the crude 8 (199.3 g, 91%) which after identification with ^1H NMR was directly used for the preparation of **9**. ^1H NMR ($CDCl_3$) δ (ppm) 1.38–1.90 (br m, 4 H), 2.53 (br s, 1 H), 3.51–3.83 (br m, 4 H), 7.58–7.94 (m, 4 H). Alternatively 8 can be obtained by refluxing a solution of 147 g (1 mol) of phthalimide and 108.5 g (1 mol) of 4-chlorobutanol in 500 mL of anhydrous DMF under vigorous stirring in the presence of 138.0 g (1 mol) of finely powdered anhydrous K_2CO_3 during 12 h. After cooling, the inorganic materials are filtered off and the solvent is evaporated in vacuo (1 mmHg, 90 °C). The residue is dissolved in 250 mL of EtOAc, and after standing overnight at 5 °C, the solution is filtered and concentrated in vacuo (20 mmHg, 80 °C); the residue is taken up in 200 mL of $CHCl_3$. After extraction with 5% $NaHCO_3$ solution and H_2O, the solvent is removed in vacuo (20 mmHg, 80 °C) giving the crude 1 (208.0 g, 95%), which proved to be identical with product 8 as obtained by the first procedure. On standing for a prolonged period the viscous oil crystallized giving the solid 8, mp 47–49 °C (57–59 °C[26]).

(45) Stephenson, R. P. A modification of receptor theory. *Br. J. Pharmacol.* **1956**, *11*, 379–393.
(46) See ref 3, p 353.
(47) Haaksma, E. E. J.; Donné-Op den Kelder, G. M.; Timmerman, H.; Weinstein, H. Theoretical analysis of the activity of dimaprit derivatives on the H_2-receptor. *Agents Actions Suppl.* **1991**, *33*, 315–324.
(48) Pardo, L.; Mazurek, A. P.; Osman, R.; Weinstein, H. Theoretical Studies of the Activation Mechanism of Histamine H_2-Receptors: Dimaprit and the Receptor Model. *Int. J. Quantum Chem., Quantum Biol. Symp.* **1989**, *16*, 281–290.
(49) See ref 3, p 365.

4-Phthalimidobutanal (9). To a well stirred solution of 63.5 g (0.5 mol) of oxalyl chloride in 1000 mL of dry CH_2Cl_2 a solution of 82.7 g (1.06 mol) of anhydrous DMSO in 200 mL of CH_2Cl_2 is added under a N_2 atmosphere at −50 °C at such a rate that the temperature is maintained at −50 °C. After the addition is completed, stirring is continued for 15 min, after which a solution of 87.6 g (0.4 mol) crude 1 in 400 mL of dry CH_2Cl_2 is added while keeping the temperature at −50 °C. The reaction mixture is stirred for another 30 min at −50 °C, and 222.2 g (2.2 mol) of Et_3N is added. The mixture is allowed to warm to ambient temperature, and 1250 mL of H_2O is added and stirring continued for 30 min. The organic layer is separated and extracted with H_2O to almost neutral reaction, dried on anhydrous Na_2SO_4, filtered, and concentrated in vacuo (20 mmHg, 80 °C) to yield the crude **9** (69.4 g, 80.0%). The obtained viscous oil is stored under N_2 and used without further purification for the preparation of **11a**: ^{1}H NMR ($CDCl_3$) δ (ppm) 1.80–2.19 (m, 2 H), 2.56 (t, J = 7.4 Hz, 2 H), 3.74 (t, J = 6.8 Hz, 2 H), 7.52–7.93 (m, 4 H), 9.74 (s, 1 H).

5-Phthalimido-2-pentanone (10a). To a well-stirred solution of 735 g (5 mol) of phthalimide and 602.5 g (5 mol) of freshly distilled 5-chloro-2-pentanone (Janssen Chimica) in 2500 mL of anhydrous DMF is added under a N_2 atmosphere 345 g (2.5 mol) of finely powdered K_2CO_3 and 1.0 g of KI. The reaction mixture is slowly heated to 90 °C (moderate CO_2 production) after which an additional 345 g (2.5 mol) of finely powdered K_2CO_3 is added in small portions, and the mixture is heated for 3 h at 110 °C. After this period, TLC (Merck, DC-Alufolien Kieselgel 60 F_{254} (0.2 mm), eluent EtOAc/PE 100–140 °C, 90:10 v/v) shows a minor amount of the unreacted phthalimide. After cooling and standing overnight, the inorganic salts are filtered off and washed three times with 100 mL of anhydrous DMF. The combined filtrates are concentrated in vacuo (20 mmHg, 80 °C) followed by removal of the last traces of DMF in vacuo at 0.1 mmHg, 90 °C. The viscous oily residue is dissolved in 1250 mL of EtOAc and while stirring chilled to 5 °C. After 2 h stirring, the solid materials are filtered off and the filtrate is concentrated in vacuo (20 mmHg, 80 °C). To the warm residue is added 1500 mL of MeOH and this solution is warmed to 60 °C, after which 2.0 g of finely powdered **10a** (obtained from a previous preparation) is added and the solution chilled to 5 °C while stirring. After 2 h, the crystalline **10a** is filtered off and dried in vacuo (0.1 mmHg, 25 °C) to yield 625 g. The mother liquor is concentrated in vacuo (20 mmHg, 80 °C), the residue dissolved in 500 mL of MeOH, and the crystallization process repeated to yield another 94 g of product, thus giving 719 g (62.8%) of pure **10a**: ^{1}H NMR ($CDCl_3$) δ (ppm) 1.90–2.12 (m, 2 H), 2.15 (s, 3 H), 2.51 (t, J = 7.2 Hz, 2 H), 3.72 (t, J = 6.6 Hz, 2 H), 7.67–7.92 (m, 4 H); mp 72–74 °C (lit. 75–77 °C,[31] 71–72 °C[34]).

4-Phthalimido-2-butanone (10b). To a well-stirred suspension of 147.0 g (1 mol) of phthalimide and 70.0 g (1 mol) of 3-buten-2-one (Janssen Chimica) in 1000 mL of EtOAc is added a freshly prepared solution of 2.7 g (0.05 mol) of NaOEt in 250 mL of anhydrous EtOH under a N_2 atmosphere. After 2 h stirring at ambient temperature, the mixture is refluxed until an almost clear solution is obtained and refluxing is continued for an additional 2 h. After cooling, the solvent is removed in vacuo (20 mmHg, 60 °C) and the solid residue crystallized from hot 96% EtOH to yield **10b** (195.3 g, 90.0%): ^{1}H NMR ($CDCl_3$) δ (ppm) 2.22 (s, 3 H), 2.96 (t, J = 7.0 Hz, 2 H), 3.96 (t, J = 7.0 Hz, 2 H), 7.62–7.96 (m, 4 H); mp 108.5–110.0 °C (lit.[50] 111–113 °C).

2-Bromo-4-phthalimidobutanal (11a). To a stirred solution of 108.5 g (0.5 mol) of **2** in 500 mL of CCl_4 is added 80 g (0.5 mol) Br_2 in small portions under a N_2 atmosphere. The reaction mixture is stirred for 3 h at ambient temperature after which 100 mL of $CHCl_3$ and 500 mL of H_2O are added followed by stirring for 30 min. The organic phase is separated and extracted under N_2 with H_2O till neutral reaction. After drying on anhydrous Na_2SO_4 and filtration, the solvent is removed in vacuo (20 mmHg, 60 °C). The resulting viscous oil (containing 116.2 g of **11a**, 78.5% based on ^{1}H NMR) is used without further purification for the preparation of **14a**: ^{1}H NMR ($CDCl_3$) δ (ppm) 2.00–2.75 (m, 2 H), 3.87 (t, J = 6.8 Hz, 2 H), 4.40 (tt, J_1 = 7.2 Hz, J_2 = 1.8 Hz, 1 H), 7.59–7.96 (m, 4 H), 9.45 (d, J = 1.8 Hz, 1 H).

3-Bromo-5-phthalimido-2-pentanone (11b). As described for **11a** starting with 114.5 g of (0.5 mol) **10a**. The viscous oily residue was crystallized from hot MeOH giving **11b** (97.8 g, 63.5%): mp 99.0–100.7 °C; ^{1}H NMR ($CDCl_3$) δ (ppm) 2.22–2.70 (m, 2 H), 2.42 (s, 3 H), 3.82 (t, J = 6.6 Hz, 2 H), 4.34 (t, J = 7.2 Hz, 1 H), 7.69–7.97 (m, 4 H); mass spectrum, m/e (rel inten) 267 (20), 230 (99), 188 (47), 174 (52), 161 (94), 160 (100), 148 (23), 133 (20), 104 (43); exact mass M^+ = m/e 309.001, calcd for $C_{13}H_{12}BrNO_3$ 309.001.

1-Bromo-4-phthalimido-2-butanone (12). To a stirred solution of 231 g (1 mol) of **10b** in 1350 mL of anhydrous MeOH at 0 °C is added 160.0 g (2 mol) of Br_2 in portions of 40 mL. Stirring is continued for 24 h at ambient temperature. To the clear solution is added 200 mL of 10 M H_2SO_4, and the mixture is stirred overnight. The precipitate is filtered off and boiled for 15 min in 500 mL of anhydrous MeOH. The hot suspension is filtered and the solid material dried in vacuo (0.1 mmHg, 25 °C) to yield **12** (161.2 g, 52.0%): ^{1}H NMR ($CDCl_3$) δ (ppm) 3.13 (t, J = 7.2 Hz, 2 H), 3.92 (s, 2 H), 4.04 (t, J = 7.2 Hz, 2 H), 7.60–7.96 (m, 4 H); mp 120–122.4 °C (lit.[21] 119–120 °C).

General Procedure for the Preparation of 4- or 5-(2-Phthalimidoethyl)thiazole Hydrobromides (14a–d,15). To a solution of 0.05 mol of bromo aldehyde or bromo ketone (**11a**, **11b**, **12**) in 50.0 mL of anhydrous DMF is added under a N_2 atmosphere while stirring a solution of 0.05 mol of the appropriate thioamide (**13a–c**) in 50.0 mL of anhydrous DMF. A slightly exothermic reaction occurs, and the mixture is heated at 120 °C for 3 h. After cooling, the solvent is removed in vacuo (20 mmHg, 90 °C), the residue stirred with 50.0 mL of anhydrous EtOH and cooled to 5 °C, and the precipitate filtered off and recrystallized from an appropriate solvent and dried in vacuo (0.1 mmHg, 25 °C). By this procedure the following phthalimidothiazoles were prepared.

2-Amino-5-(2-phthalimidoethyl)thiazole Hydrobromide (14a). From 14.8 g (0.05 mol) of crude **11a** and 3.8 g (0.05 mol) of thiourea (**13a**). Recrystallization from anhydrous hot EtOH/MeOH yielded 11.2 g (63.3%) of **14a**: mp dec starting at 180 °C; ^{1}H NMR (DMSO-d_6) δ (ppm) 2.98 (t, J = 6.8 Hz, 2 H), 3.78 (t, J = 6.8 Hz, 2 H), 7.10 (s, 1 H), 7.86 (s, 4 H), 9.08 (br s, 2 H).

2,4-Dimethyl-5-(2-phthalimidoethyl)thiazole Hydrobromide (14b). From 15.5 g (0.05 mol) of **11b** and 3.75 g (0.05 mol) of thioacetamide (**13b**). Recrystallization from anhydrous hot EtOH yielded 10.6 g (58.0%) of **14b**, mp dec starting at 230 °C; ^{1}H NMR (DMSO-d_6) δ (ppm) 2.28 (s, 3 H), 2.89 (s, 3 H), 3.14 (t, J = 6.3 Hz, 2 H), 3.82 (t, J = 6.3 Hz, 2 H), 7.88 (s, 4 H).

2-Amino-4-methyl-5-(2-phthalimidoethyl)thiazole Hydrobromide (14c). From 15.5 g (0.05 mol) of **11b** and 3.8 g (0.05 mol) of thiourea (**13a**). Recrystallization from anhydrous hot EtOH/EtOAc yielded 10.1 g (55.3%) of **14c**: mp 270.3–272.4 °C dec; ^{1}H NMR (DMSO-d_6) δ (ppm) 1.98 (s, 3 H), 2.98 (t, J = 6.3 Hz, 2 H), 3.78 (t, J = 6.3 Hz, 2 H), 7.91 (s, 4 H), 9.23 (br s, 2 H).

4-Methyl-5-(2-phthalimidoethyl)thiazole Hydrobromide (14d). From 15.5 g (0.05 mol) of **11a** and 3.05 g (0.05 mol) of thioformamide (**13c**): recrystallization from anhydrous hot EtOH/Et_2O yielded 7.0 g (39.8%) of **14d**, mp dec starting at 180 °C; ^{1}H NMR (DMSO-d_6) δ (ppm) 1.99 (s, 3 H), 2.96 (t, J = 6.3 Hz, 2 H), 3.77 (t, J = 6.3 Hz, 2 H), 7.92 (s, 4 H), 9.23 (br s, 2 H).

2-Amino-4-(2-phthalimidoethyl)thiazole Hydrobromide (15). From 14.8 g (0.05 mol) of **12** and 3.8 g (0.05 mol) of thiourea (**13a**). Recrystallization from anhydrous hot EtOH yielded 15.9 g (89.8%) of **15**, mp dec starting at 195 °C; ^{1}H NMR (DMSO-d_6) δ (ppm) 2.95 (t, J = 6.3 Hz, 2 H), 3.87 (t, J = 6.3 Hz, 2 H), 6.57 (s, 1 H), 7.86 (s, 4 H), 8.98 (br s, 2.5 H).

2-Amino-5-(2-aminoethyl)thiazole Hydrobromide Hydrochloride (7a). A solution of 3.54 g (0.01 mol) of **14a** and 0.02 mol of $N_2H_4 \cdot H_2O$ in 40 mL of MeOH is refluxed for 5 h. After cooling in ice, the crystallized phthalhydrazide is filtered off and the filtrate concentrated in vacuo (1 mmHg, 80 °C). Last traces of $N_2H_4 \cdot H_2O$ are removed by coevaporation with xylene (20 mmHg, 80 °C) after which the residue is dissolved in 50.0 mL of anhydrous EtOH and acidified with concentrated 37% HCl solution to pH = 2.00, followed by removal of the solvent in vacuo (20 mmHg, 80 °C). The residue is crystallized from hot MeOH

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yielding 1.82 g (70.0%) of **16a**: mp 237.9–239.6 °C dec; ^{1}H NMR (DMSO-d_6) δ (ppm) 2.88–3.14 (br m, 4 H), 7.20 (s, 1 H), 8.29 (br s, 3 H), 9.35 (br s, 2 H); mass spectrum *m/e* (rel inten) 144 (5), 143 (10), 114 (100), 113 (42), 86 (35), 30 (94); exact mass *m/e* 143.051, calcd for $C_5H_9N_3S$ 143.052. Identical to mass spectrum in ref 18.

5-(2-Aminoethyl)-4-methylthiazole Dihydrobromide* (16d). As for compound **7a** from 3.54 g (0.01 mol) of **14d**. The obtained hydrochloric salt which did not crystallize was, after liberation of the free base with $NaOCH_3$ in MeOH, converted to the hydrobromic salt, yielding after crystallization from hot EtOH/EtOAc 2.7 g (88.8%) of **16d**: mp >300 °C; ^{1}H NMR (DMSO-d_6) δ (ppm) 2.12 (s, 3 H), 2.74–3.14 (br m, 4 H), 7.92 (br s, 3 H), 8.36 (s, 0.5 H), 8.76 (br s, 2 H). ^{1}H NMR (D_2O) δ (ppm) 2.21 (s, 3 H), 3.04 (t, J = 6.6 Hz, 2 H), 3.28 (t, J = 6.6 Hz, 2 H), ^{13}C NMR (D_2O) δ (ppm) 14.01, 28.53, 25.98, 42.19, 115.29, 136.96, 171.35; mass spectrum, *m/e* (rel inten) 143 (12), 142 (9), 126 (7), 113 (33), 30 (63); exact mass M^+ = 142.054, calcd for $C_6H_{10}N_2S$ 142.056. *Mixed salt 1.64 HBr, 0.36 HCl. Anal. ($C_6H_{12}Br_{1.64}Cl_{0.36}N_2S$) C, H, N, S, Br, Cl.

General Procedure for the Preparation of 4- or 5-(2-Aminoethyl)thiazole Dihydrobromides (16b–d, 17). A solution of 0.01 mol of the appropriate 4- or 5-(2-phthalimidoethyl)thiazole hydrobromide (**14b–d, 15**) is refluxed for 5 h in 50.0 mL of 30% HBr solution. After cooling, the mixture is concentrated in vacuo (20 mmHg, 80 °C). Last traces of H_2O and HBr are removed by coevaporation with toluene, after which the remaining solid is crystallized from an appropriate solvent. By this method the following thiazoles were prepared.

5-(2-Aminoethyl)-2,4-dimethylthiazole Dihydrobromide Hemihydrate (16b). From 3.67 g (0.01 mol) of **14b**, yielding after crystallization from hot EtOH/EtOAc 2.6 g (81.8%) of **16b**, mp dec starting at 200 °C; ^{1}H NMR (DMSO-d_6) δ (ppm) 2.39 (s, 3 H), 2.82 (s, 3 H), 2.97–3.13 (br m, 4 H), 8.09 (br s, 3 H), 10.30 (br s, 1 H); mass spectrum *m/e* (rel inten) 156 (4), 127 (100), 126 (62), 82 (52), 30 (79); exact mass M^+ = 156.073, calcd for $C_7H_{12}N_2S$ 156.072. Anal. ($C_7H_{15}Br_2N_2SO_{0.5}$) C, H, N, S, Br.

2-Amino-5-(2-aminoethyl)-4-methylthiazole Dihydrobromide (16c). From 3.69 g (0.01 mol) of **14c**, yielding after crystallization from hot EtOH/Et_2O 2.4 g (75.2%) of **16c**: mp dec starting at 275 °C; ^{1}H NMR (DMSO-d_6) δ (ppm) 2.16 (s, 3 H), 2.84–3.12 (br m, 4 H), 8.08 (br s, 3 H), 9.32 (br s, 2 H); mass spectrum *m/e* (rel inten) 158 (5), 157 (7), 127 (18), 104 (31), 30 (2); exact mass M^+ = 157.065, calcd for $C_6H_{11}N_3S$ 157.067. Anal. ($C_6H_{13}Br_2N_3S$) C, H, N, S, Br.

2-Amino-4-(2-aminoethyl)thiazole Dihydrobromide Hemihydrate (17). From 3.55 g (0.01 mol) of **15**, yielding after crystallization from hot MeOH 1.95 g (63.9%) of **17**, mp dec starting at 110 °C; ^{1}H NMR (DMSO-d_6) δ (ppm) 2.96 (t, J = 6.3 Hz, 2 H), 3.12 (t, J = 6.3 Hz, 2 H), 6.72 (s, 1 H), 8.04 (br s, 3 H), 9.27 (br s, 2 H), 9.80 (br s, 1 H); mass spectrum *m/e* (rel inten) 144 (47), 143 (4), 115 (42), 114 (100), 30 (16); exact mass M^+ = 143.052, calcd for $C_5H_9N_3S$ 143.052. Anal. ($C_5H_{12}Br_2N_3SO_{0.5}$) C, H, N, S, Br.

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Registry No. **7a**, 142437-63-6; **8**, 24697-70-9; **9**, 3598-60-5; **10a**, 3197-25-9; **10b**, 3783-77-5; **11a**, 133118-36-2; **11b**, 112357-34-3; **12**, 51132-00-4; **13a**, 62-56-6; **13b**, 62-55-5; **13c**, 115-08-2; **14a**, 136604-50-7; **14b**, 136604-56-3; **14c**, 136604-53-0; **14d**, 136604-55-2; **15**, 95914-09-3; **16b**, 142437-64-7; **16b** free base, 142437-68-1; **16c**, 142457-00-9; **16c** free base, 142437-67-0; **16d**, 142437-65-8; **16d** free base, 58981-35-4; **17**, 142437-66-9; **17** free base, 124458-10-2; phthalic anhydride, 85-44-9; 4-aminobutan-1-ol, 13325-10-5; 4-chloro-1-butanol, 928-51-8; phthalimide, 85-41-6; 5-chloro-2-pentanone, 5891-21-4; 3-buten-2-one, 78-94-4.

Synthesis, Characterization, and Ca^{2+} Antagonistic Activity of Diltiazem Metabolites[1]

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Diltiazem is a calcium antagonist widely used in the treatment of angina and hypertension. The contributions of metabolites of diltiazem to the vasorelaxant effects of diltiazem were investigated. The synthesis and spectroscopic characterization of eight major *cis*-diltiazem metabolites are described. Three of the compounds—N,O-didemethylated metabolite (**21**), O-demethylated metabolite (**22**), and diltiazem *N*-oxide (**27**)—have been recently reported and have not previously been synthesized. The identities of all eight synthetic metabolites have been verified with samples obtained from human urine using combined LC-MS/MS. The Ca^{2+} antagonistic activities of diltiazem and its metabolites (except **27**) were studied on hamster arota preparations depolarized with KCl. The order of potencies (IC_{50} ± SE, μM) is as follows: diltiazem (0.98 ± 0.47) > **17** (2.46 ± 0.38) ≥ **23** (3.27 ± 1.02) > **26** (20.2 ± 10.5) > **22** (40.4 ± 15.4) ≥ **25** (45.5 ± 18.1) > **21** (112.2 ± 33.2) ≥ **24** (126.7 ± 24.2). Structure–activity relationships are also discussed.

Introduction

Ca^{2+} antagonists, widely used in the treatment of angina pectoris, hypertension and certain cardiac arrhythmias, are classified structurally into three groups: dihydropyridines represented by nifedipine and nicardipine, phenylalkylamines represented by verapamil, and benzothaizepines represented by diltiazem.[2-4] Diltiazem is subject to sig-

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