Molecular Conformations of Aminophenylimidazoles Exhibiting Antiulcer Activities

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To experimentally clarify a possible stereostructure–activity relationship proposed for H_2-receptor antagonists, three 5-aminophenylimidazoles (1, 2 and 3), in which respective amino groups are located on the *ortho*, *meta* and *para* positions of the benzene ring, were synthesized and examined for their conformational characteristics using X-ray diffraction and proton nuclear magnetic resonance (^{1}H-NMR) methods, and for antiulcer activities on rats and H_2-receptor antagonist activities in guinea pig. The *ortho* isomer 1, which preferentially formed an intramolecular N–H (amino)···N (imidazole) hydrogen bond, showed the highest antiulcer activity with half the efficacy of cimetidine. On the other hand, none of 1, 2 and 3 showed significant H_2-receptor antagonist activity. Based on these results, the conformational characteristic for the exhibition of antiulcer activity has been discussed.

Keywords aminophenylimidazole; antiulcer activity; H_2-receptor binding; molecular conformation; X-ray analysis; ^{1}H-NMR study

Introduction

The therapeutic success of cimetidine as a clinically useful drug for the treatment of peptic ulcers and associated gastrointestinal disorders has provided a stimulus in research for the development of more potent and long-lasting drugs. It has also aided in the evaluation of the structure–activity relationship of the H_2-receptor antagonist.

In a previous paper[1] we described a possible relationship between the molecular conformation and inhibitory activity as the H_2-receptor antagonist, based on X-ray structural and proton nuclear magnetic resonance (^{1}H-NMR) spectroscopic analyses. For the emergence of the H_2-receptor antagonist activity, it is advantageous for the compound to have a folded conformation, so that an intramolecular hydrogen bond is formed between the acceptor atom of the central heteroaromatic ring and the donor group of a side chain, as shown in Chart 1. This has also been derived by Michell[2] and Gilman *et al.*[3]

The effective design of potent drugs requires identification of the key structural features responsible for the exhibition of antiulcer activity. As part of the experimental approach to confirm the above hypothesis, we report here the syntheses of aminophenylimidazoles and their molecular conformations, antiulcer activities and H_2-receptor antagonist activities (chronotropic response on guinea pig right atrium).

Results

Chemical Syntheses The syntheses of aminophenylimidazoles **1**, **2** and **3** used for this study are shown in Chart 2. The syntheses were straightforward through two general routes: the bromination of 3-nitroacetophenone followed by the condensation with formamide[4] and by reduction of the nitro group to obtain 5-(3-aminophenyl)-3*H*-imidazole (**2**) as a dihydrochloride salt.[5] Nitration of 5-phenyl-3*H*-imidazole produced a mixture of *ortho*- and *para*-nitro derivatives, which were then separated from water by fractional recrystallization.[6] The reduction of the nitro group, respectively, afforded pure 5-(2-aminophenyl)-3*H*-imidazole (**1**) and 5-(4-aminophenyl)-3*H*-imidazole (**3**) as the dihydrochloride salts. The free forms of compounds **1**, **2** and **3** were prepared by ion-exchange chromatography.

X-Ray Structure Analyses Crystals of compound **1** (free form and dihydrochloride salt) and **2** (dihydrochloride salt) were used for X-ray structure analyses. The stereoscopic representations of the observed molecular conformations, viewed perpendicular to the imidazole ring, are shown in Fig. 1, where two crystallographically independent molecules existing in the crystal structure of **1** dihydrochloride salt are represented by the suffix letters A and

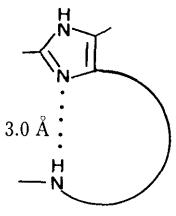

Chart 1

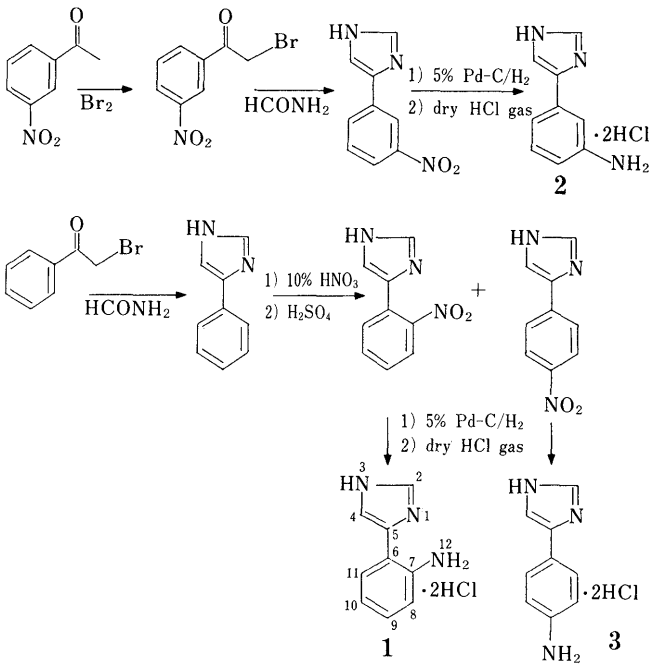

Chart 2

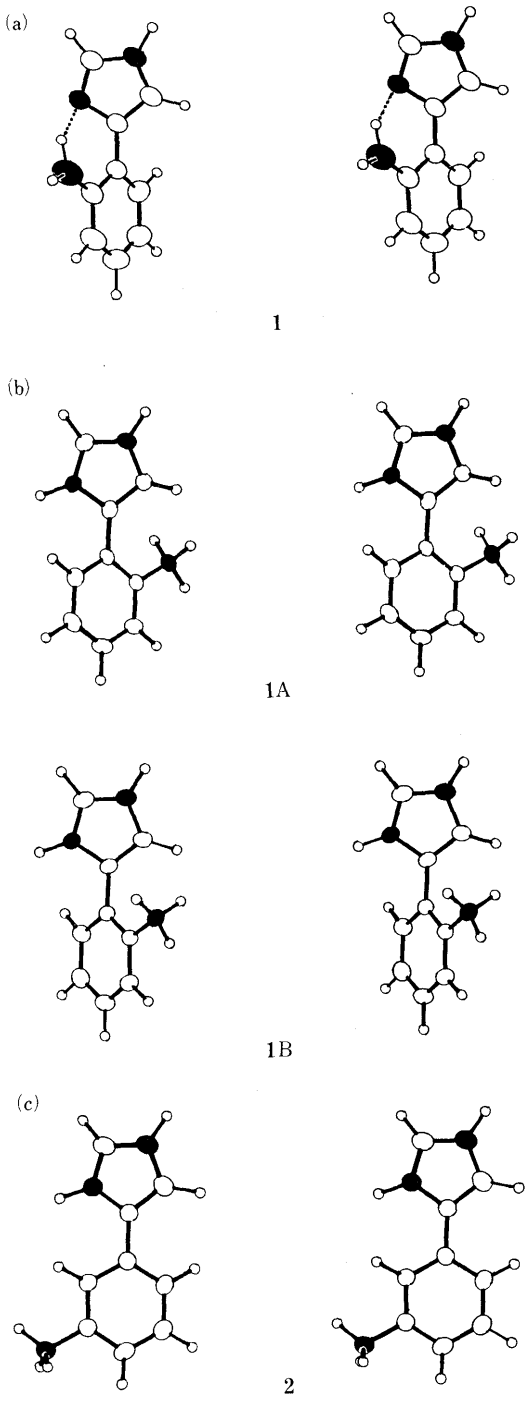

Fig. 1. Stereoscopic Views of Molecular Conformations Observed in the Crystals of (a) Compound **1** Free Form, (b) Compound **1** Dihydrochloride Salt and (c) Compound **2** Dihydrochloride salt

The dotted lines in **1** free form compound represent the hydrogen bonds. The filled circles represent nitrogen atoms.

B (Fig. 1(b)).[7] The bond lengths and angles are listed in Table I; all are in normal range. The imidazole and benzene rings are almost planar. The imidazole N1 and amino N12 atoms are assumed to be in a protonated state in the crystals of both the **1** and **2** dihydrochloride, and this causes the N12 atom to part from the N1 atom in compound **1** because of electrostatic repulsion. The torsion angle θ (N1–C5–C6–C7) takes a value larger than 90° [147.1 (4)° for **1A** and −127.0 (5)° for **1B**] (Fig. 1(b)), whereas those of the **1** free form and **2** dihydrochloride compounds are −50.1 (6)° and −6.8 (6)°, respectively. The molecular conformation of the **1** free form contains an intramolecular hydrogen bond [N12···N1 = 2.978 (6) Å, H···N1 = 2.11 (5) Å and ∠N12–H···N1 = 137 (4)°].

TABLE I. Bond Lengths (Å) and Angles (°) with Their e.s.d.s in Parentheses

(a) Bond Length

Bond	**1** free	**1** dihydrochloride **1A**	**1B**	**2** dihydrochloride
N(1)–C(2)	1.318 (6)	1.323 (6)	1.324 (5)	1.327 (6)
N(1)–C(5)	1.375 (5)	1.387 (5)	1.384 (5)	1.372 (5)
C(2)–N(3)	1.343 (6)	1.311 (6)	1.317 (5)	1.334 (6)
N(3)–C(4)	1.355 (6)	1.367 (5)	1.389 (5)	1.366 (6)
C(4)–C(5)	1.362 (6)	1.356 (5)	1.346 (6)	1.358 (6)
C(5)–C(6)	1.470 (6)	1.465 (5)	1.470 (5)	1.474 (6)
C(6)–C(7)	1.402 (6)	1.411 (5)	1.392 (5)	1.388 (6)
C(6)–C(11)	1.389 (6)	1.394 (5)	1.395 (5)	1.392 (6)
C(7)–C(8)	1.406 (7)	1.399 (5)	1.386 (5)	1.378 (6)
C(7)–N(12)	1.389 (6)	1.468 (5)	1.448 (5)	—
C(8)–C(9)	1.373 (8)	1.371 (6)	1.386 (6)	1.371 (6)
C(8)–N(12)	—	—	—	1.476 (5)
C(9)–C(10)	1.381 (8)	1.386 (6)	1.387 (6)	1.377 (7)
C(10)–C(11)	1.386 (7)	1.411 (6)	1.388 (6)	1.389 (6)

(b) Bond Angle

Bond	**1** free	**1** dihydrochloride **1A**	**1B**	**2** dihydrochloride
C(2)–N(1)–C(5)	105.7 (3)	108.0 (3)	108.4 (2)	109.8 (3)
N(1)–C(2)–N(3)	111.4 (2)	108.7 (3)	108.9 (2)	107.3 (3)
C(2)–N(3)–C(4)	107.2 (3)	110.1 (3)	109.0 (3)	109.6 (3)
N(3)–C(4)–C(5)	106.8 (3)	106.0 (2)	106.3 (2)	106.9 (3)
N(1)–C(5)–C(4)	108.8 (3)	107.1 (2)	107.3 (2)	106.5 (3)
N(1)–C(5)–C(6)	121.8 (2)	121.1 (2)	121.7 (2)	123.3 (2)
C(4)–C(5)–C(6)	129.4 (3)	131.7 (2)	130.9 (2)	130.2 (3)
C(5)–C(6)–C(7)	121.6 (3)	122.5 (2)	121.5 (2)	120.6 (2)
C(5)–C(6)–C(11)	119.7 (3)	119.4 (2)	119.8 (2)	119.5 (3)
C(7)–C(6)–C(11)	118.7 (3)	118.1 (2)	118.7 (2)	119.8 (3)
C(6)–C(7)–C(8)	119.0 (3)	121.3 (2)	121.0 (2)	118.5 (3)
C(6)–C(7)–N(12)	120.9 (3)	120.8 (2)	121.1 (2)	—
C(8)–C(7)–N(12)	120.1 (3)	117.9 (2)	117.8 (2)	—
C(7)–C(8)–C(9)	120.9 (3)	119.7 (2)	119.8 (2)	122.7 (3)
C(7)–C(8)–N(12)	—	—	—	118.1 (2)
C(9)–C(8)–N(12)	—	—	—	119.2 (3)
C(8)–C(9)–C(10)	120.4 (3)	120.2 (3)	119.6 (3)	118.4 (3)
C(9)–C(10)–C(11)	119.2 (3)	120.6 (3)	120.5 (3)	120.6 (3)
C(6)–C(11)–C(10)	121.8 (3)	120.0 (2)	120.1 (2)	119.8 (3)

The crystal packings of **1** (free and dihydrochloride salt) and **2** (dihydrochloride salt) are shown in Fig. 2, where the filled and dotted circles represent chloride ions and water molecules, respectively. Possible intermolecular hydrogen bonds and short contacts less than 3.5 Å are given in Table II. The polar N(1) and/or N(3) atoms of the imidazole ring participate in the hydrogen bond formation. Chloride ions and waters of crystallization locate in the cavities formed by the molecular packing and stabilize respective crystal structures by hydrogen bond formations and van der Waals contacts.

Theoretical Conformational Analyses of 1 and 2 Neutral Forms Figure 3 shows the potential curves of the **1** and **2** neutral forms as a function of the torsion angle θ, which were obtained by CNDO/2 calculations; no noticeable variation was observed for the **3** neutral form. Compound **1** overwhelmingly assumes a *cis* orientation ($\theta = 0°$) of the

amino group of a phenyl ring with respect to the imidazole N1 atom; its energy difference from the second stable conformer ($\theta = 145°$) is -5.69 kcal/mol. Compound **2** does not show such a large energy variation; the energy difference between the most stable ($\theta = -20°$) and unstable ($\theta = 90°$) conformations is 0.711 kcal/mol. In addition to the resonance effect between the imidazole and benzene rings, this *cis* preference of compound **1** could, therefore, be mainly due to an intramolecular N–H···N hydrogen bond formation.

^{1}H-NMR Study The temperature dependence of the amino proton chemical shifts (δ) is shown in Fig. 4, where

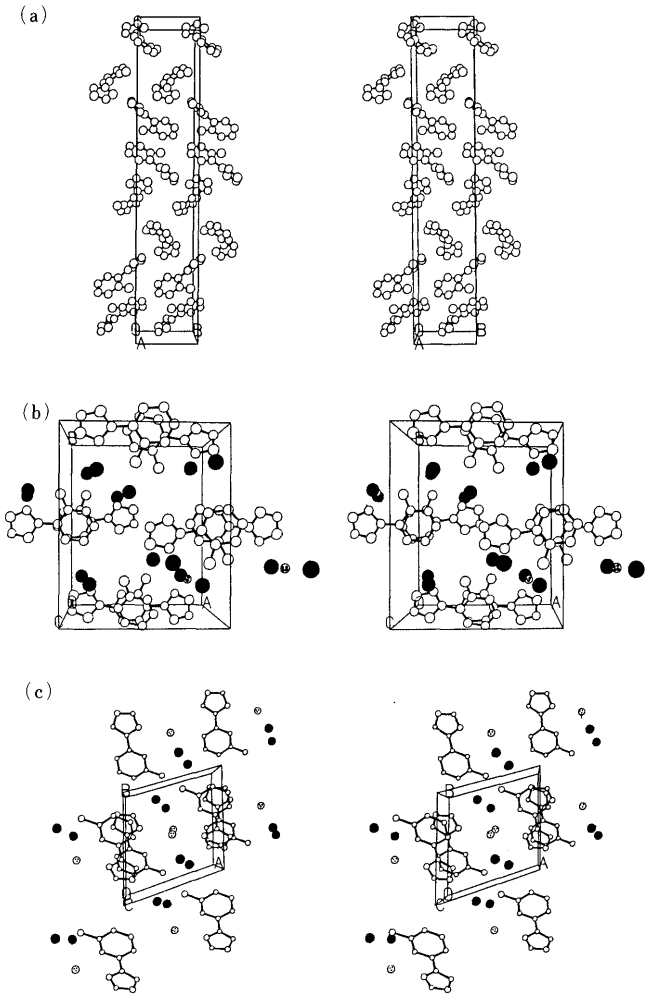

Fig. 2. Stereoscopic Views of Crystal Packing of **1** Free Form (a), **1** Dihydrochloride Salt (b) and **2** Dihydrochloride Salt (c)

The filled and dotted circles indicate the chloride ions and water molecules of crystallization, respectively.

TABLE II. Intermolecular Hydrogen Bond Distances and Short Contacts Less Than 3.5 Å with Their e.s.d.s in Parentheses

(a) Hydrogen Bond

Donor at x, y, z	Accceptor at symmetry operation		Distance (Å)
1 free			
N(3)	N(1)	$y, x-1, -z+2$	2.938 (5)
1 dihydrochloride			
N(3)A	Cl(2)B	$x+1, y, z$	3.085 (3)
N(3)B	Cl(1)B	$x-1, y, z$	3.149 (3)
N(1)A	Cl(1)B	$-x+1.5, y-0.5, -z+0.5$	3.095 (3)
N(1)B	O(W1)	$-x+1, -y, -z+1$	2.727 (4)
N(12)A	Cl(2)A	$x-0.5, -y+0.5, z-0.5$	3.122 (3)
N(12)B	Cl(2)A	$x-0.5, -y+0.5, z-0.5$	3.005 (3)
N(12)A	Cl(1)A	$x+0.5, -y+0.5, z-0.5$	3.042 (3)
N(12)B	Cl(2)B	$x+0.5, -y+0.5, z+0.5$	3.177 (3)
O(W1)	Cl(2)B	$x+0.5, -y+0.5, z+0.5$	3.134 (3)
2 dihydrochloride			
N(3)	Cl(1)	$x-1, y+1, z$	3.079 (4)
N(12)	Cl(2)	$x, y, z-1$	3.117 (4)
O(W1)	Cl(2)	$-x+1, -y+1, -z+2$	3.299 (4)

(b) Short Contact

Atom at x, y, z	Atom at symmetry operation		Distance (Å)
1 free			
N(3)	C(10)	$x-1, y, z$	3.491 (6)
C(4)	C(4)	$y+1, x-1, -z+2$	3.391 (6)
1 dihydrochloride			
C(4)A	C(10)A	$-x+1, -y, -z$	3.471 (6)
N(1)B	Cl(2)A	$-x+1, -y, -z+1$	3.400 (3)
C(7)B	C(9)B	$-x+1, -y, -z+1$	3.458 (5)
C(2)A	Cl(2)A	$-x+2, -y, -z+1$	3.498 (4)
C(8)A	O(W1)	$x-0.5, -y+0.5, z-0.5$	3.490 (5)
2 dihydrochloride			
C(2)	C(6)	$x-1, y, z$	3.491 (6)
C(2)	C(11)	$-x, -y+1, -z+1$	3.273 (6)
N(3)	C(6)	$-x, -y+1, -z+1$	3.496 (5)
C(5)	C(5)	$-x, -y+1, -z+1$	3.438 (5)
N(12)	Cl(1)	$-x+1, -y, -z+1$	3.357 (4)
N(12)	Cl(2)	$-x+1, -y, -z+1$	3.225 (4)
N(1)	Cl(1)	$-x+1, -y+1, -z+1$	3.457 (3)
O(W1)	C(7)	$-x+1, -y+1, -z+1$	3.453 (5)

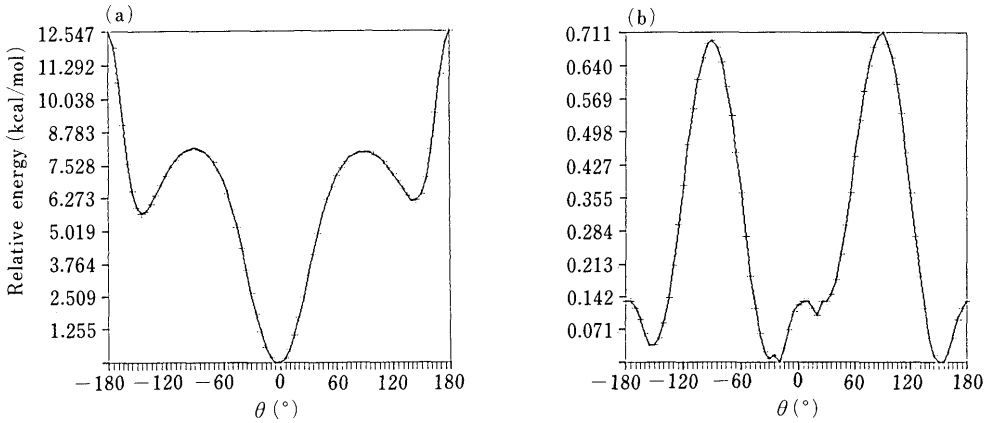

Fig. 3. Relative Energy Variation of **1** (a) or **2** (b) Free Form Compounds as a Function of Torsion Angle θ

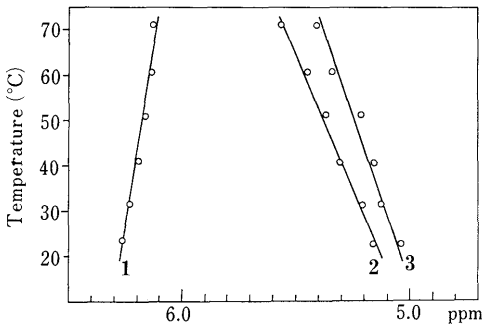

Fig. 4. Temperature-Dependences of Amino Proton Chemical Shifts of **1**—**3** Free Form Compounds in $(CD_3)_2SO$ Solution

The chemical shift of **2** is slightly inaccurate because of its broad peak.

TABLE III. Effect of Compounds **1**—**3** on Stress-Induced Ulceration in Rats

Dose, mol/kg × 10^4	Mean area of ulcer (mm^2)	Inhibition rate (%)
Control	19.3±4.1	
Cimetidine		
1.0	1.1±0.7	92.2[a]
1		
1.0	15.1±5.9	21.8
2.0	3.7±1.5	85.5[b]
2		
1.0	16.3±5.0	15.5
2.0	6.5±1.4	65.9[c]
3		
1.0	17.0±4.4	11.9
2.0	12.1±3.1	37.3

The values of the mean area of the ulcer represent the mean±S.E. Number of data is 6. *a*) $p<0.005$, *b*) $p<0.01$, and *c*) $p<0.025$ compared with control.

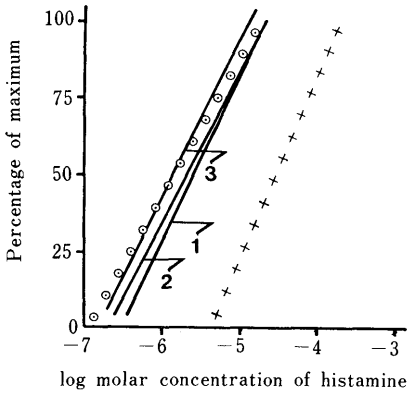

Fig. 5. Effect of **1** (1.5×10^{-5} M), **2** (2.0×10^{-5} M), **3** (2.5×10^{-5} M) and Cimetidine (5.0×10^{-6} M, +) on the Chronotropic Dose–Response Curve to Histamine in Guinea Pig Atrium

⊙, without antagonist. For the sake of clarity, not all data were plotted. Regression lines were calculated by the least squares method.

the neutral forms of compounds **1**, **2** and **3** (30 mM) were measured in $(CD_3)_2SO$ solution. The temperature coefficient of the chemical shift ($=d\delta/dT$) is −0.0027 ppm/°C for compound **1**, 0.0079 ppm/°C for **2** and 0.0074 ppm/°C for **3**. A similar tendency is also observed at lower concentration (*ca.* 10 mM).

Antiulcer Activity Table III shows the effects of compounds **1**, **2** and **3** on rat gastric ulcers produced by restraint and water-immersion stress. Although the inhibitory effect of compound **1** was about half that of cimetidine, **1** was significantly more effective than **2** and **3** at 2×10^{-4} mol/kg.

Chronotropic Response on Guinea Pig Right Atrium As shown in Fig. 5, the histamine dose–response curve on the atrium was not influenced by the presence of compounds **1** (1.5×10^{-5} M), **2** (2.0×10^{-5} M) or **3** (2.5×10^{-5} M), while a clear parallel displacement to the right without change in the maximum response was observed for the cimetidine (5×10^{-6} M). Since it is known that the H_2 receptor mediates the stimulation of the spontaneous beating rate of the guinea pig atrium,[8] this result shows that none of compounds **1**, **2** and **3** interacts with the H_2-receptor.

Discussion

Based on the conformational studies of histamine derivatives,[9] it has been considered that a folded conformation of histamine is probably responsible for the interaction with the H_2-receptor; the amino group of the side chain takes a *gauche* orientation with respect to the imidazole N1 atom, and the NH···N interatomic distance is close to 3.6 Å. Various physicochemical studies[1-3,10] showed that a potent H_2-receptor antagonist such as cimetidine or famotidine can take the folded conformation, in which the donor atom of the side chain is able to form an intramolecular hydrogen bond with the acceptor atom of imidazole or other hetero aromatic ring. Therefore, the folded conformation of the H_2-receptor agonist or antagonist appears to have relevance to the biological activity.

Compounds **1**, **2** and **3** were synthesized to experimentally ascertain the importance of the folded conformation with an intramolecular hydrogen bond for the exhibition of antagonist activity; to our knowledge, there is no direct evidence to substantiate this hypothesis. Compound **1** was designed to be able to mimic a *gauche* conformation of the histamine molecule. The N1 (imidazole)···N12 (amino) distance varies from 2.61 to 4.26 Å accompanying the rotation of θ. If θ is in the range of −60—60°, compound **1** can form an intramolecular N–H···N hydrogen bond. For compounds **2** and **3**, such a hydrogen bond formation is impossible: the minimum N···N distance is 5.22 Å for **2** and 6.50 Å for **3**.

When the molecular conformations found in the crystal structures of the free form and dihydrochloride salt of compound **1** are compared (Figs. 1(a) and (b)), it is obvious that the conformation is largely dependent on the environmental circumstance (see also Figs. 2(a) and (b)). It could be thought in the solution, as well as in the solid state, under the acidic condition that compound **1** takes a dicationic form and the imidazole N1 and amino N12 atoms are protonated by the proton-transfer from hydrochloride. The protonations cause electrostatic repulsion between these nitrogen atoms. Thus, the conformation of compound **1** shows a large deviation from 0° for θ, as was observed in the crystal structure of **1** dihydrochloride salt. Under neutral conditions, it is conceivable that compound **1** would take a conformation with an intramolecular N–H···N hydrogen bond, as is seen in Fig. 3(a). On the other hand, the dicationic form of **2** appears to take a coplanar conformation of imidazole and benzene rings (Fig. 1(c)); generally, two aromatic rings connected by a C–C single bond prefer to

take a coplanar conformation by the resonance effect between them.

Since the imidazole ring of histamine exists predominantly as the N3-H tautomer at pH 7.4,[11] and the pK_a values for imidazole N1 and amino N12 atoms are, respectively, 6.0 and 4.63,[12] it is quite reasonable to consider that compounds **1**, **2** and **3** exist as neutral forms with the N3-H tautomer under a physiological condition. Thus, it becomes important to elucidate the molecular conformations, especially of compound **1**, at the neutral states in order to consider the stereostructure–antiulcer activity relationship. The solid conformation of compound **1** neutral form shown in Fig. 1(a) suggests the importance of an intramolecular hydrogen bond for its stability.

The rotational potential curve of torsion angle θ (Fig. 3) showed a large energy variation for **1**; the conformation at $\theta=0°$ is more stable by 5.69 or 6.17 kcal/mol than that at $\theta=145°$ or $-140°$, respectively; the deviation of molecular conformation ($\theta=-50.1°$) observed in compound **1** crystal (free form) from its most stable form is probably due to the effect of crystal packing (Fig. 2(a)). In contrast, it is interesting to note that compounds **2** and **3** showed no noticeable potential variation accompanying the rotation of θ; the variations were within 0.711 kcal/mol for **2** and 0.415 kcal/mol for **3**. Thus, the strong preference of compound **1** for the orientation of $\theta=0°$, which is stabilized by an intramolecular hydrogen bond, was shown by complete neglect of differential overlap (CNDO)/2 energy calculations.

The existence of this intramolecular N–H···N hydrogen bond of compound **1** was further suggested in the solution state from the temperature-dependence of amino protons (Fig. 4). As the temperature is increased, the amino protons of **1** show a characteristic upfield shift, whereas those of **2** and **3** shift proportionally to the downfield side. Provided that the circumstance around the NH_2 proton of compound **1** in solution is the same as those of **2** and **3**, the downfield shift with the temperature rise could be expected for compound **1** as well. Therefore, the upfield shift observed only for compound **1** is explained as follows: the chemical shift of a proton which participates in a hydrogen bond can be expected to become temperature-sensitive; the breaking of the hydrogen bond by an increase in temperature should give rise to a significant upfield shift of the proton. Thus, Fig. 4 strongly suggests the existence of an intramolecular N–H···N hydrogen bond in the neutral form of compound **1**.

A correlation between the molecular conformations of compounds **1**, **2** and **3** and their antiulcer activities can be derived from Table III. The antiulcer activity of compound **1** is significantly higher than those of compounds **2** and **3** in the order **1**>**2**>**3**. The chronotropic response on guinea pig right atrium (Fig. 5), however, clearly showed no significant interactions of compounds **1**, **2** and **3** with the H_2-receptor. This means that the antiulcer activity of compound **1** does not occur through its H_2-receptor antagonist activity, but through an other mechanism.

In conclusion, the present study can be summarized as follows: 1) antiulcer activity can be stimulated through the cooperation of the imidazole ring and the amino group of side chain, where the amino group is able to form an intramolecular hydrogen bond with the imidazole acceptor atom, and 2) antiulcer activity does not occur as a result of H_2-receptor antagonist activity. These findings, though not definitive, deny the hypothesis put forth in the Introduction, *i.e.*, the importance of the intramolecularly hydrogen-bonded conformation of the H_2-receptor antagonist for the exhibition of antiulcer activity. In this regard, it appears worthwhile to note that the roxatidine molecule, which is unable to form an intramolecular hydrogen bond because of its nonpolar benzene ring, has recently been reported to be a potent H_2-receptor antagonist.[13]

Experimental

Chemistry All melting points of compounds **1**—**3** were determined with a Yanagimoto micromelting point apparatus and were uncorrected. The chemical structures of compounds were confirmed by their infrared (IR), NMR and mass (MS) spectra. The IR spectra were measured on a Hitachi IR (Model 260-10) spectrometer. The ^{1}H-NMR spectra were measured on a Gemini 200 (Varian) spectrometer and MS spectra were taken on a JEOL JMS-DX 300 mass spectrometer. Microanalyses for the elements indicated were identical within 0.5% of their theoretical values.

General Procedure for the Preparation of 5-Aminophenylimidazoles (1—3) 5-Nitrophenyl-3*H*-imidazole (2 g) was hydrogenated with 5% Pd–C (0.7 g) in MeOH (20 ml) for 3 h using a Skita apparatus (2 kg/cm^2). After removal of the catalyst by filtration, the filtrate was condensed *in vacuo* to give a viscous oil, which was dissolved in EtOH (10 ml). Dry HCl gas was subbled into the solution under ice-cooling, and the resulting precipitate was collected by filtration and purified by recrystallization from EtOH.

X-Ray Structure Analyses Three crystal structures of the compounds, **1** free form, **1** dihydrochloride salt and **2** dihydrochloride salt, were determined by X-ray diffraction experiments. Crystallographic data were as follows: **1** free form, crystallization from acetonitrile, plates, $C_9H_9N_3$, F.W.=159.19, tetragonal, space group $P4_32_12$, $a=b=6.861$ (4) Å, $c=35.813$ (13) Å, $V=1685.7$ (16) Å^3, $Z=8$, $D_{obsd.}=1.254$ (2), $D_{calcd.}=1.254\,g\cdot cm^{-3}$, μ(Cu K_α)$=5.99\,cm^{-1}$, $F(000)=672$; **1** dihydrochloride, crystallization from water, needles, $2(C_9H_9N_3\cdot 2HCl)\cdot H_2O$, F.W.=482.24, monoclinic, space group $P2_1/n$, $a=11.249$ (2), $b=13.510$ (2), $c=15.039$ (2) Å, $\beta=93.92$ (1), $V=2280.3$ (7) Å^3, $Z=4$, $D_{obsd.}=1.395$ (2), $D_{calcd.}=1.403\,g\cdot cm^{-3}$, μ(Cu K_α)$=49.67\,cm^{-1}$, $F(000)=1000$; **2** dihydrochloride, crystallization from a water–methanol–butanol mixture (1:2:4, v/v/v), $C_9H_9N_3\cdot 2HCl\cdot H_2O$, F.W.=250.13, triclinic, space group $P\bar{1}$, $a=9.239$ (2), $b=8.657$ (2), $c=8.648$ (2) Å, $\alpha=100.34$ (2), $\beta=116.31$ (1), $\gamma=69.95$ (2)°, $V=582.1$ (2) Å^3, $Z=2$, $D_{obsd.}=1.420$ (2), $D_{calcd.}=1.427\,g\cdot cm^{-3}$, μ(Cu K_α)$=49.52\,cm^{-1}$, $F(000)=260$.

A computer-controlled diffractometer (Rigaku AFC-5, with Cu K_α radiation, $\lambda=1.5418$ Å) with an incident beam graphite-monochromator was used for data collection. A total of 940 (for **1** free form), 4044 (for **1** dihydrochloride) and 1978 (for **2** dihydrcholoride) independent reflections were measured in an $\omega-2\theta$ scan mode to $2\theta_{max}=130°$ at 18 °C. The intensities were corrected for the Lorentz and polarization effects. Corrections of the absorption effects were also carried out for compounds **1** and **2** dihydrochloride crystals using an empirical method based on the ϕ scan near $\chi=90°$. The structures were solved by a direct method with the aid of the program SHELX-86[14] or by the heavy atom method, and then refined by the least-squares method. The 145 (for **1** free form), 359 (for **1** dihydrochloride) and 189 (for **2** dihydrochloride) parameters were refined including the coordinates and anisotropic thermal parameters for all nonhydrogen atoms. Ideal positions of hydrogen atoms, calculated on the basis of stereochemical considerations, were verified on a difference Fourier map and refined isotropically. The final R and R_w values for the 849 (for **1** free form), 3617 (for **1** dihydrochloride) and 1904 (for **2** dihydrochloride) observed reflections ($|F_o|>3\sigma|F_o|$) were 0.065 and 0.078 (for **1** free form), 0.078 and 0.091 (for **1** dihydrochloride) and 0.087 and 0.101 (for **2** dihydrochloride), respectively; goodness of fit, S, for the respective crystals was 1.68, 2.20 and 0.89. The atomic coordinates of nonhydrogen atoms are given in Table IV.[15] For crystallographic computations, the UNICS programs[16] were used. The atomic scattering factors were taken from ref. 17.

Semiempirical Energy Calculation Total energy variations of **1**, **2** and **3** neutral forms as a function of the torsion angle θ were calculated by the CNDO/2 method. The changes of respective electronic energies were

TABLE IV. Final Atomic Coordinates with Their e.s.d.s in Parentheses

Atom	x	y	z	B_{eq}[a]
1 free form				
N(1)	0.5358 (5)	0.1067 (5)	0.98548 (9)	3.8 (2)
C(2)	0.4033 (6)	0.0012 (6)	1.0029 (1)	4.0 (2)
N(3)	0.4110 (5)	−0.1862 (5)	0.9922 (1)	3.9 (2)
C(4)	0.5538 (7)	−0.2012 (6)	0.9662 (1)	3.9 (2)
C(5)	0.6324 (6)	−0.0201 (6)	0.9622 (1)	3.2 (2)
C(6)	0.7962 (5)	0.0436 (6)	0.9388 (1)	3.2 (2)
C(7)	0.7825 (6)	0.2087 (6)	0.9158 (1)	3.6 (2)
C(8)	0.9442 (8)	0.2627 (7)	0.8941 (1)	4.5 (2)
C(9)	1.1158 (7)	0.1603 (8)	0.8961 (1)	4.7 (2)
C(10)	1.1311 (6)	−0.0009 (8)	0.9190 (1)	4.4 (2)
C(11)	0.9720 (6)	−0.0566 (7)	0.9403 (1)	3.9 (2)
N(12)	0.6118 (7)	0.3174 (6)	0.9142 (1)	5.6 (2)
1 dihydrochloride				
Cl(1)A	0.15640 (9)	0.17735 (7)	0.55048 (8)	3.42 (4)
Cl(2)A	0.87271 (8)	0.19166 (7)	0.77711 (7)	2.91 (4)
N(1)A	0.7682 (3)	−0.0741 (2)	0.1533 (2)	3.0 (1)
C(2)A	0.8811 (3)	−0.0508 (3)	0.1447 (3)	3.6 (2)
N(3)A	0.8867 (3)	0.0419 (2)	0.1194 (2)	3.1 (1)
C(4)A	0.7747 (3)	0.0815 (3)	0.1095 (3)	2.7 (2)
C(5)A	0.6991 (3)	0.0082 (3)	0.1302 (2)	2.5 (1)
C(6)A	0.5688 (3)	0.0035 (2)	0.1265 (2)	2.2 (1)
C(7)A	0.4980 (3)	0.0851 (3)	0.1481 (2)	2.2 (1)
C(8)A	0.3735 (3)	0.0794 (3)	0.1417 (3)	2.8 (2)
C(9)A	0.3186 (3)	−0.0076 (3)	0.1161 (3)	3.2 (2)
C(10)A	0.3859 (3)	−0.0895 (3)	0.0959 (3)	3.3 (2)
C(11)A	0.5113 (3)	−0.0837 (3)	0.0991 (3)	2.9 (2)
N(12)A	0.5530 (3)	0.1782 (2)	0.1799 (2)	2.5 (1)
Cl(1)B	0.79595 (8)	0.20817 (7)	0.30768 (7)	3.20 (4)
Cl(2)B	0.07772 (9)	0.18555 (8)	0.05778 (9)	4.09 (5)
N(1)B	0.1424 (2)	−0.0263 (2)	0.3938 (2)	2.5 (1)
C(2)B	0.0333 (3)	0.0085 (3)	0.3768 (3)	3.1 (2)
N(3)B	0.0394 (3)	0.0934 (3)	0.3343 (2)	3.0 (1)
C(4)B	0.1582 (3)	0.1153 (3)	0.3233 (3)	3.0 (2)
C(5)B	0.2222 (3)	0.0406 (3)	0.3616 (2)	2.3 (1)
C(6)B	0.3514 (3)	0.0229 (3)	0.3697 (2)	2.3 (1)
C(7)B	0.4303 (3)	0.0929 (3)	0.4075 (2)	2.3 (1)
C(8)B	0.5523 (3)	0.0777 (3)	0.4108 (3)	2.9 (2)
C(9)B	0.5971 (3)	−0.0094 (3)	0.3775 (3)	3.3 (2)
C(10)B	0.5193 (3)	−0.0817 (3)	0.3436 (3)	3.2 (2)
C(11)B	0.3971 (3)	−0.0655 (3)	0.3383 (3)	2.8 (2)
N(12)B	0.3877 (3)	0.1847 (2)	0.4436 (2)	2.7 (1)
O(W1)	0.8140 (3)	0.2027 (2)	0.5222 (2)	4.0 (1)
2 dihydrochloride				
Cl(1)	0.6696 (1)	0.1269 (1)	0.5884 (1)	3.35 (4)
Cl(2)	0.5491 (1)	0.2338 (1)	1.0036 (1)	3.60 (4)
N(1)	0.1517 (4)	0.6394 (4)	0.5174 (4)	2.8 (1)
C(2)	0.0899 (5)	0.7743 (4)	0.5965 (5)	3.2 (2)
N(3)	−0.0755 (5)	0.8311 (4)	0.4988 (5)	3.4 (1)
C(4)	−0.1198 (5)	0.7287 (5)	0.3573 (5)	3.3 (2)
C(5)	0.0239 (4)	0.6073 (4)	0.3682 (4)	2.6 (1)
C(6)	0.0498 (4)	0.4627 (4)	0.2531 (5)	2.5 (1)
C(7)	0.2115 (4)	0.3593 (4)	0.2822 (4)	2.6 (1)
C(8)	0.2290 (4)	0.2222 (4)	0.1752 (4)	2.6 (1)
C(9)	0.0935 (5)	0.1837 (5)	0.0415 (5)	3.1 (2)
C(10)	−0.0665 (5)	0.2879 (5)	0.0120 (5)	3.4 (2)
C(11)	−0.0897 (5)	0.4270 (5)	0.1174 (5)	3.0 (2)
N(12)	0.4016 (4)	0.1142 (4)	0.2059 (4)	3.0 (1)
O(W1)	0.4845 (4)	0.4806 (4)	0.6950 (4)	4.6 (1)

a) $B_{eq}=4/3\sum_i\sum_j a_i a_j B_{ij}$.

used to verify the convergency in the iteration calculations. The atomic coordinates of the **1**—**3** neutral forms were derived from the present crystal structures. The computation was carried out for each conformation varying θ from 0° to 360° at 10° increments.

Temperature-Dependence of Amino Proton The amino protons of the **1**, **2** and **3** neutral forms were monitored on a Varian XL-300 spectrometer equipped with fast Fourier transform and temperature control units (accuracy, ±1 °C). The proton chemical shift was measured as a downfield shift from the internal tetramethylsilane. Concentration of the solution prepared using $(CD_3)_2SO$ solvent was approximately 30 mM. Spectra were recorded in the range of 20—70 °C at intervals of 10 °C.

Inhibitory Activity for Stress-Induced Ulcer Wistar strain male rats, weighing 180—200 g were deprived of food for 15 h before the experiments and given access to water only. At the start of the experiment, the powdered compounds **1**, **2** and **3** were orally administered with 0.8 ml water at dosages of 1.0×10^{-4} or 2.0×10^{-4} mol/kg through a catheter. A gastric ulcer was induced in the rat by restraint and water immersion stress, according to the procedure of Okabe *et al.*[18] The rats were placed in stress cages and immersed to the level of the xiphoid in a water bath at 20 °C for 7 h. After removed from the cages, they were injected with Evan's blue dye *via* the tail vein to enhance the contrast of the gastric lesions. After 10 min, the stomach was removed, slightly inflated with 1% (w/v) formalin solution, and immersed in 10% (w/v) formalin solution for 10 min to fix the inner and outer layers of the gastric walls. The area (mm^2) of each stress-induced ulcer was measured under a dissection microscope. The inhibitory rate of compounds **1**, **2** and **3** for a stress-induced ulcer was calculated as follows:

$$\text{inhibition rate (\%)}=\frac{M_c-M_t}{M_c}\times100$$

where M_c and M_t are the means of the total ulcer area in the control group (not administered inhibitor) and in the test group, respectively. The Student's t-test was used to determine the statistical significance of data obtained.

Chronotropic Response on Guinea Pig Right Atrium Male Hartley guinea pigs weighing between 500 and 650 g were stunned by a blow to the head and killed by exsanguination. The right atrium was dissected and suspended at 0.7 g tension in a 25 ml organ bath containing Tyrode's solution[19] at 38±0.5 °C and with a bubbled gas mixture of 95% O_2 and 5% CO_2. Individual contractions were recorded with a force-displacement transducer through a strain gauge, and contraction frequency was obtained. The tissue was allowed to stabilize in frequency and height of contraction before experimental procedures began.

Cumulative concentration–response curves were constructed following sequential additions of the H_2-agonist, histamine (10^{-7} to 3×10^{-4} M). This procedure was done 3 times for each preparation: at the first and the third without test drug and at the second after the equilibration with compound **1**, **2**, **3** or cimetidine. The second concentration–response curve was then compared with those of the first and the third. Five preparations were used for each concentration of test drug.

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