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An approach to the design of brain-penetrating histaminergic agonists

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Summary — Known and novel histaminergic H_1- and H_2-receptor agonists were investigated as potentially potent and selective brain-penetrating compounds. Structural modifications were introduced in an attempt to favour passive diffusion across the blood–brain barrier by reducing hydrogen-bonding ability according to a previously developed model. While no novel compound was identified which satisfied our requirements for a brain-penetrating agonist, betahistine **14** and 2-(thiazol-2-yl)ethylamine **16** can be regarded as H_1-receptor agonists with moderate brain-penetrating ability, of potential value as pharmacological tools. A novel histamine analogue, *N,N*-bis-{2-[4(5)-imidazolyl]ethyl}amine **25** is reported which, although unlikely to be brain penetrant, was found to be equipotent with histamine at H_1- and H_1-receptors.

histamine / H_1 receptor / H_2 receptor / agonist / blood–brain barrier

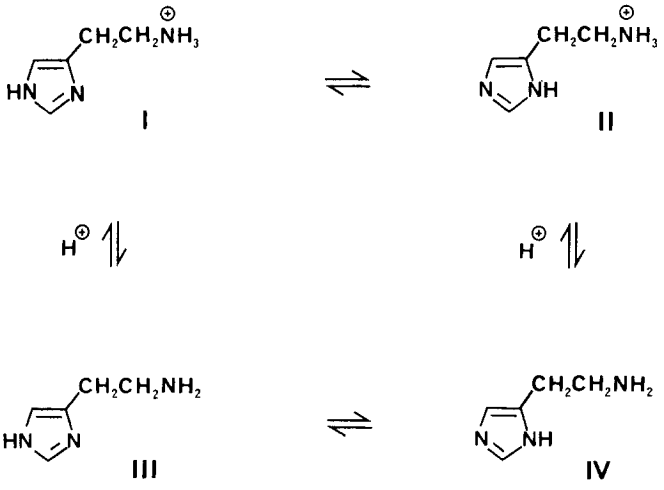
Introduction

Although there is abundant circumstantial evidence that histamine has a neurotransmitter role in mammalian brain [1, 2], its function remains largely unknown. Further insight into central histaminergic mechanisms might be gained using potent, selective receptor stimulants capable of crossing the blood–brain barrier. Because of its high polarity and basicity, histamine does not readily cross the blood–brain barrier [3]. At pH 7.4 it exists predominantly (97%) as a mixture of monocations [4] I and II, while the neutral but still very polar species III and IV, which are most likely to cross the blood–brain barrier, only comprise about 1% (scheme 1).

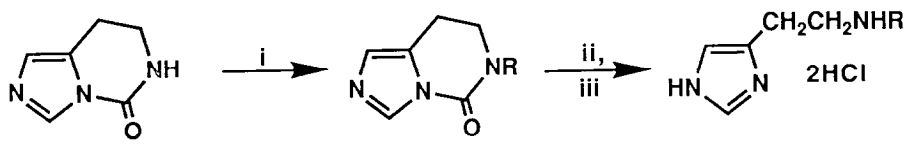
Here we consider the possibilities for introducing structural modifications into histamine and related histaminergic agonists to promote a desirable profile of affinity, efficacy and H_1- or H_2-receptor selectivity.

Chemistry

The N^{α}-substituted histamines, **10** and **12**, were prepared by the known method [5], shown in scheme 2 by alkylation of **28** followed by base-catalyzed hydrolysis, and the products were isolated as their hydrochloride salts. Compound **17** was prepared by cyclization of the thioamide **31** with bromoacetal, using the method of Goldberg and Kelly [6], as shown in scheme 3, to give the benzamide **32**, which was hydrolyzed to **17** using conc HCl. The *N*-dimethylated analogue, **18**, was prepared starting from 2-methylthiazole **33** by the sequence shown in scheme 4. Heating 2-methylthiazole in a sealed glass tube with paraformaldehyde gave the intermediate alcohol, **34**, in low yield. This was converted into the alkyl



Scheme 1. Histamine monocations I and II and neutral species III and IV.



28 29a R = $(CH_2)_2Ph$ 10

29b R = $(CH_2)_4Ph$ 12

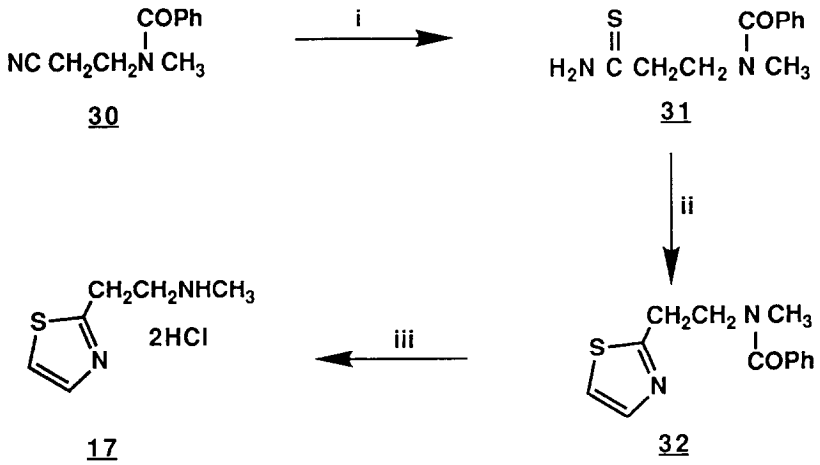
Scheme 2. *Method A*: i: RBr, NaH, DMF; ii: aq KOH, Δ; iii: HCl, EtOH.

chloride **35** and finally aminated with dimethylamine in ether. The imidazo[2,1-*a*]isoquinoline derivative **22** was synthesized using the method reported for the preparation of **20** [7]. Condensation of 1-aminoisoquinoline with the bromoketone, **36**, in the presence of sodium bicarbonate gave **37**, which was hydrolyzed using hydrochloric acid to **22**, as shown in scheme 5. Reaction of 2-vinylbenzothiazole **38** with potassium phthalimide, as shown in scheme 6, gave **39**, which was hydrolyzed with hydrochloric acid to give the known [8] compound, **23**. Treatment of **38** with ammonia in ethanol resulted in formation of *N*,*N*-bis-2-[2-(benzothiazolyl)ethyl]amine **27** together with some of the *N*,*N*,*N*-tris-2[2-(benzothiazolyl)-ethyl]amine product **40**. These products were readily separated by chromatography.

The radiolabelled compounds required for brain penetration studies were prepared either by direct tritiation of the unlabelled compounds ([^{3}H] **14** and [^{3}H] **15**) or by specific syntheses incorporating a ^{14}C label ([^{14}C] **16**[9], [^{14}C] **19** and [^{14}C] **20**). Thus, Friedel–Crafts acetylation [10] of benzene with sodium [1-^{14}C]acetate gave [carbonyl-^{14}C]acetophenone **41** which was brominated, and the bromoketone **42** condensed with β-benzamido thiopropionamide to give **43**. The latter was finally hydrolyzed with hydrochloric acid to give [^{14}C] **19** by the route shown in scheme 7. Reaction of β-phthalimidopropionyl chloride, **44**, with [^{14}C]diazomethane and hydrogen bromide gave [^{14}C] **36**, which was condensed with 2-aminopyridine to give **45**, as shown in scheme 8. Subsequent hydrolysis with hydrochloric acid afforded the desired compound, [^{14}C] **20**.

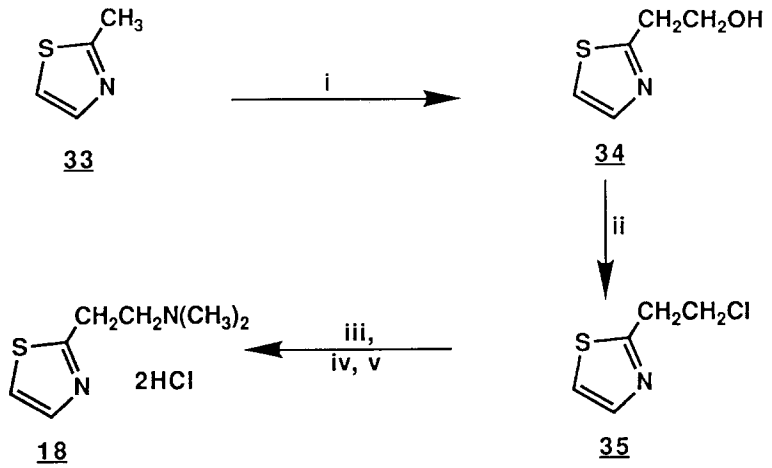
Results and discussion

In attempting to design suitable agonists capable of crossing the blood–brain barrier, we have investigated the scope for structurally modifying histamine using, as a guide, a model for brain penetration which was developed and successfully applied to designing



30 31 32 17

Scheme 3. *Method B*: i: $(NH_4)_2S$, MeOH; ii: $BrCH_2CH(OEt)_2$, MeOH; iii: conc HCl, Δ.



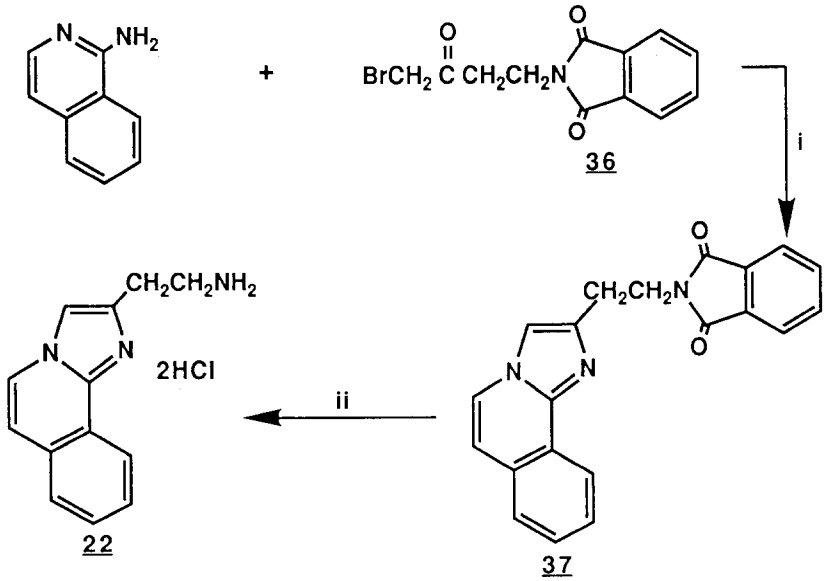
Scheme 4. *Method C*: i: $(HCHO)_n$, Δ; ii: $SOCl_2$, CH_2Cl_2, Δ; iii: Me_2NH, EtOH, Δ; iv: aq NaOH; v: HCl, EtOH.

brain-penetrating H_2-receptor antagonists [11]. The model, shown in equation 1, relates the degree of brain penetration in a structurally diverse series of small molecules to the lipophilicity parameter, $\Delta\log P$. This parameter, which is defined as the difference between the logarithms of the partition coefficients, P_{oct} and P_{cyh}, measured in the octanol/water and cyclohexane/water systems, respectively (equation 2), is considered to be an approximate measure of the overall hydrogen-bonding ability of a molecule [12].

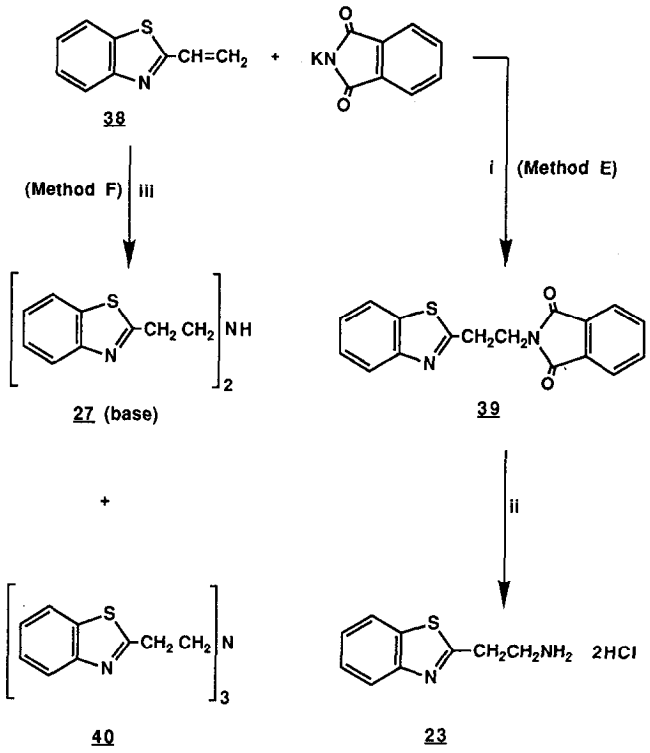
$$\log(C_{brain}/C_{blood}) = -0.485\ \Delta\log P + 0.889 \quad [1]$$

$$\Delta\log P = \log P_{oct} - \log P_{cyh} \quad [2]$$

In order to promote the passive diffusion of a histamine analogue across the blood–brain barrier, it is thus necessary to substantially reduce the value of $\Delta\log P$, and hence, its H-bonding potential. This can be achieved either by removing polar groups not essential for agonist activity or by reducing the



Scheme 5. *Method D*: i:$NaHCO_3$, DMF, Δ; ii: conc HCl, Δ.



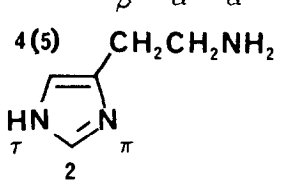
Scheme 6. i: Δ; ii: conc HCl, Δ; iii: NH_3, EtOH.

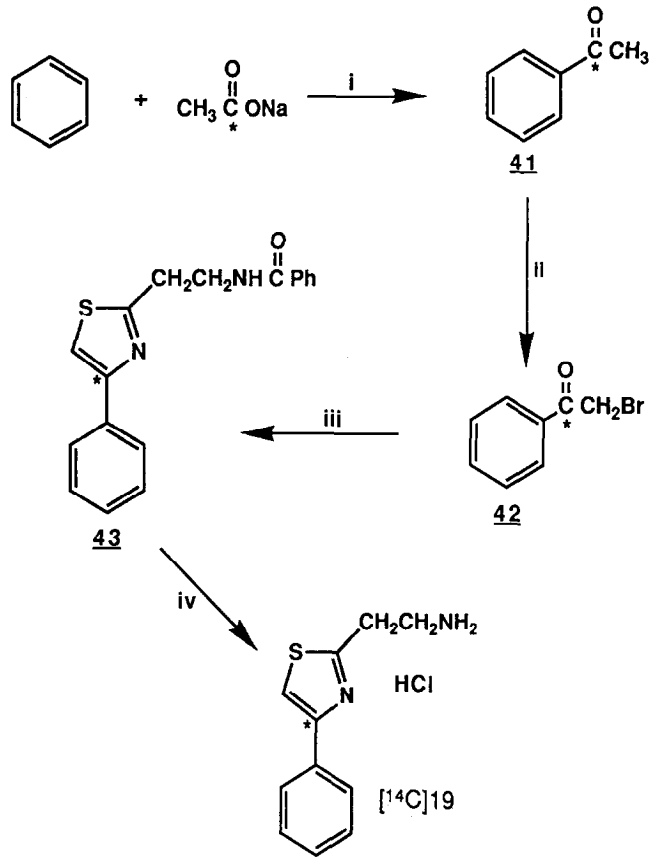
polarity of those groups considered necessary for retaining agonist activity, for instance, by encouraging intramolecular H-bonding or discouraging H-bonding through steric inhibition by a lipophilic group [11].

Substitution at the terminal nitrogen (N^{α})

Reduction of both the basicity and H-bonding potential of the primary amino group in histamine was considered to be a priority in attempting to increase brain penetration. N^{α}-alkylation (see molecule V) should not only reduce H-bonding ability but the introduction of 2 alkyl groups should also lead to a significant reduction in basicity through steric inhibition of hydration of the conjugate acid. Histamine may be N^{α}- mono- or dimethylated to give derivatives which are moderately potent, non-selective agonists [13], but all other examples of N^{α}-substitution investigated showed reduced potency and partial agonism (table I).

V



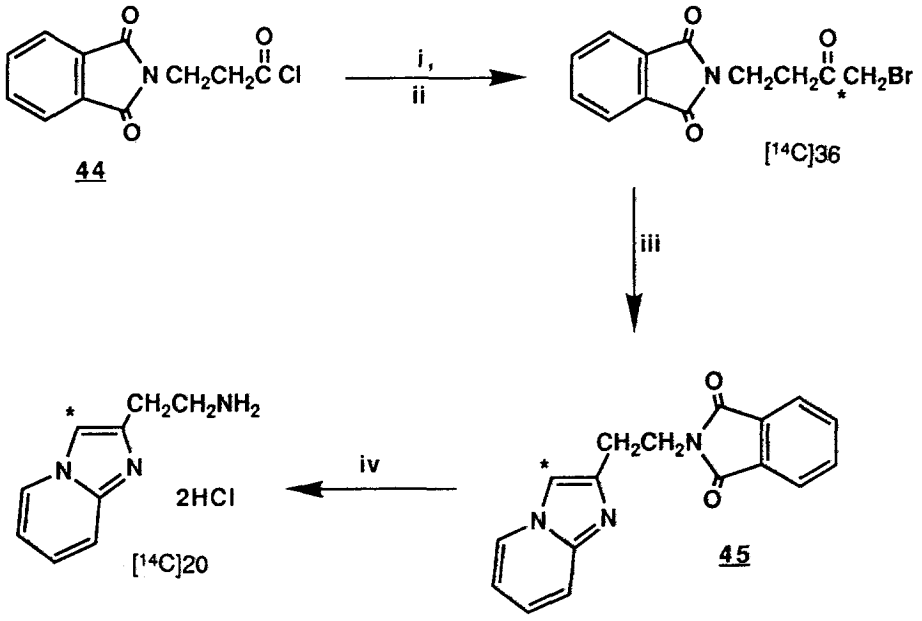


Scheme 7. i: $AlCl_3$; ii: Br_2, $AlCl_3$; iii: $PhCONHCH_2CH_2C(=S)NH_2$; iv: conc HCl, Δ; *denotes position of ^{14}C label.

Substitution in the imidazole ring

The H-bonding ability of the imidazole ring in histamine might be significantly reduced by introducing large lipophilic substituents at the 2- or 4(5)-positions. Previous studies have shown, however, that the scope for substitution is severely limited. While methylation at the 4(5)-position results in the polar selective H_2-agonist, 4(5)-methylhistamine [15], larger groups are not well accommodated [16]. Moreover, lipophilic, electron-withdrawing groups such as bromo greatly reduce H_2-agonist potency, apparently because of their undesirable effect on the tautomerism of the ring [17].

Methylation at the imidazole 2-position of histamine results in the relatively selective, polar H_1-agonist, 2-methylhistamine [15], while substitution of larger alkyl groups leads to large decreases in H_1-agonist potency [16]. A phenyl substituent is moderately well tolerated at this position both in terms of



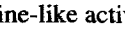
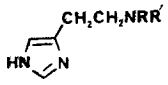
Scheme 8. i: $^*CH_2N_2$; ii: HBr; iii:  , Na_2CO_3, DMF; iv: conc HCl, Δ; *denotes position of ^{14}C label.

Table I. Histamine-like activities of N^{α}-substituted histamines.



Compd	*NRR'*	*Formula* [a]	*mp (°C)*	*Solvent*	*Method* [b]	*Guinea pig ileum (H_1)* [c]	*Guinea pig atrium (H_2)* [c]
Histamine	NH_2					100	100
1	NHMe					72[d]	74[d]
2	NMe_2					44[d]	51[d]
3	$NHPr^n$	$C_8H_7N_3 \cdot 2HCl$	184–185	$EtOH/Et_2O$	e	0.75 (45% max)	1.0 (76% max)
4	$NHPr^i$	$C_8H_7N_3 \cdot 2HCl$	198–200	$EtOH/Et_2O$	f	0.09	0.23 (25% max)
5	$NHBu^n$	$C_9H_9N_3 \cdot 2HCl$	206–208	*n*-BuOH	e	0.19 (88% max)	0.25
6	$NHBu^t$	$C_9H_9N_3 \cdot 2HCl$	286–288	$MeOH/iPrOH/Et_2O$	e	0.33 (7% max)	$pA_2 = 2.7$
7	NHHex	$C_{11}H_{13}N_3 \cdot 2HCl$	230–232	*n*-BuOH	e	0.25 (12% max)	$pA_2 = 4.6$
8	NHPh	$C_{11}H_{13}N_3 \cdot 2C_4H_4O_4$	131–132	EtOH	e	0.84 (10% max)	g
9	$NHCH_2Ph$	$C_{12}H_{15}N_3 \cdot 2HCl$	225–227	$EtOH/Et_2O$	e	0.42	0.4 (11% max)
10	$NH(CH_2)_2Ph$	$C_{13}H_{17}N_3 \cdot 2HCl$	242–243	*i*-PrOH	A	0.68 (75% max)	$pA_2 = 3.9$
11	$NH(CH_2)_3Ph$	$C_{14}H_{19}N_3 \cdot 2HCl$	209–211	*i*-PrOH	e	< 0.12	73 (14% max)
12	$NH(CH_2)_4Ph$	$C_{15}H_{21}N_3 \cdot 2HCl$	220–221	*i*-PrOH/EtOH	A	1.3 (72% max)	$pA_2 < 4.3$

[a]All compounds were analyzed for C, H, N and Cl and are within ± 0.4% of theoretical values; [b]see *Chemistry* section; [c]histamine-receptor-agonist activities expressed relative to histamine = 100 (100% maximal stimulation) as the molar concentration ratio, [histamine]/[compound] necessary to produce equal effects; [d]Ganellin *et al* [13]; [e]Durant *et al* [5]; [f]Huebner *et al* [14]; [g]not determined.

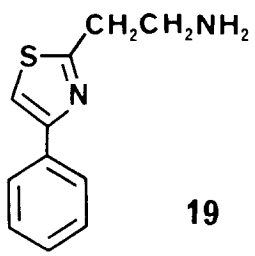
H_1- and H_2-receptor stimulation, but the resulting 2-phenylhistamine is a non-selective partial agonist [18, 19]. Furthermore, substitution in the benzene ring of 2-phenyl histamine has been reported to give analogues which retain both the H_1-agonist activity and potency of histamine (*eg* 2-(*m*-chlorophenyl)-histamine [20]), while others are less potent or have no detectable agonist activity [18, 21]. N^{α}-dimethylation, which may be required to overcome the high polarity of the basic amino group in these analogues, however, appears to be poorly tolerated [18].

Imidazole replacements

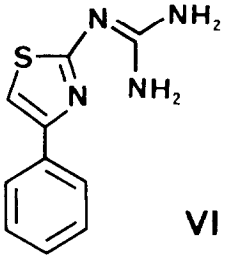
The polar imidazole ring in histamine can be replaced by other heterocycles and, in some cases, retain reasonable levels of H_1-agonist activity (table II). Thiazole and pyridine are useful replacements as they are both less basic and have lower H-bonding abilities than imidazole. 2-(Pyridin-2-yl)ethylamine **13** is a fairly weak, moderately selective H_1-receptor agonist [16]. The N^{α}-monomethyl analogue is betahistine **14**, whose potencies at H_1- and H_2-receptors are similar to those of **13** [16]. The neutral form of this compound has a relatively low $\Delta\log P$ value of 1.76, for which equation 1 predicts a brain/blood concentration ratio of 1.08. Experimentally, betahistine has been found to cross the blood–brain barrier in anaesthetized rats moderately well, with a brain/blood ratio of 0.50, in reasonable agreement with the predicted value, and has been found to block [^{3}H]-mepyramine binding in the brain of the mouse *in vivo* when administered at a dose of 150–300 mg/kg ip [22]. Further reduction of $\Delta\log P$ can be achieved by side-chain *N*-dimethylation of **13** to give **15**, and, as predicted, brain penetration is also increased. The brain/blood ratios estimated from equation 1 and determined experimentally are 1.83 and 0.88, respectively, again in reasonable agreement. *N*-Dimethylation, however, also led to a marked fall in H_1-agonist potency and maximal stimulation (table II), and the combination of potency and brain penetration in **15** was considered to be insufficient for its use as a pharmacological tool.

2-(Thiazol-2-yl)ethylamine **16** is a moderately potent and selective H_1-receptor agonist [4] which has also been found to enter rat brain. The experimental brain/blood ratio for this compound (0.38) compares fairly well with the predicted value of 1.04. Mono- and di-*N*-methylation of the side-chain amino group in this compound to give **17** and **18**, respectively, however, led only to undesirable losses of affinity and efficacy (table II).

Introduction of a phenyl substituent at the thiazole 4-position of **16** (by analogy with the imidazole 2-position of histamine discussed above) led to some loss of both H_1-receptor affinity and efficacy (table II) but not the complete loss reported by Lee and Jones [23]. The resulting compound **19**, which was predicted to have a brain/blood ratio of 1.50 on the basis of its low $\Delta\log P$ value of 1.47, was surprisingly found not to cross the blood–brain barrier of the rat. This result contrasts with that for the structurally analogous H_2-receptor antagonist, **VI**, which crosses the blood–brain barrier moderately well [11]. Although the $\Delta\log P$ value of this compound (3.65) is considerably higher than that of **19**, its basic pK_a is much lower, and ionization, if important, will not be so great a problem. Moreover, the brain penetration of **VI** was significantly greater than predicted by eq 1 [11].



19



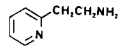
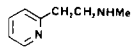
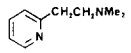
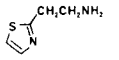
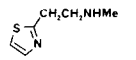
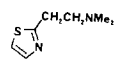
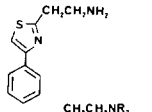
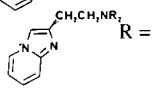
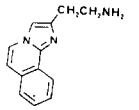
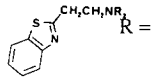
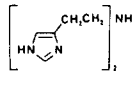
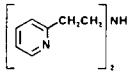
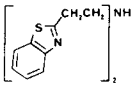
VI

Attempts to reduce polarity while maintaining reasonable agonist potency by introducing more lipophilic bicyclic and tricyclic replacements for imidazole were largely unsuccessful. The imidazopyridine derivative **20** [7] was found to be a weak, partial H_1-receptor agonist, and its $\Delta\log P$ value of 3.30 indicates that it still has moderate hydrogen-bonding ability. Equation 1 predicts a brain/blood ratio for this compound of 0.19, which compares with the experimental ratio of 0.04.

Terminal *N*-dimethylation of this compound to give **21**, as a way of further reducing $\Delta\log P$, however, led to complete abolition of agonist activity, to result in a moderately potent H_1-receptor antagonist (table II). The imidazo-isoquinoline analogue, **22**, in which a second benzene ring was introduced in a position analogous to that in 2-phenylhistamine, showed no stimulatory properties, but was found to be a weak, competitive H_1-receptor antagonist. Benzo-ring fusion in 2-(thiazol-2-yl)ethylamine, **16**, interestingly, led to an H_1-receptor agonist, **23**, of greater potency, but capable of only about 50% maximal stimulation of histamine (table II). Here again, side-chain *N*-dimethylation abolished the agonist properties, giving an H_1-receptor antagonist, **24**. Methylation of the side-chain primary amino group as a means of reducing H-bonding ability, which was well accommodated in terms of agonist activity in histamine, has thus not been generally beneficial amongst analogues.

An alternative modification to histamine, which is tolerated, is to introduce a second imidazolylethyl substituent at N^{α}. The resulting compound, **25**, is effectively equivalent to histamine on both guinea pig ileum and atrium (table II). Symmetrical bis-hetero-

Table II. Histamine-like activities and physicochemical data for histamine analogues.

Comp	Structure	Formula [a]	mp (°C)	Solvent	Method [b]	Ileum (H_1) [c]	Atrium (H_2) [c]	log P_n oct	log P_n cyh	Δlog P	C brain / C blood
13		$C_7H_{10}N_2$				5.6[d]	2.5 (50% max)				
14		$C_8H_{12}N_2$•2HCl				8.0[e]	1.5 (40% max)	0.68[f]	−1.08[f]	1.76	0.50
15		$C_9H_{14}N_2$•2HCl	176–178	EtOH/EtOAc		2.8 (83% max)	< 0.13	1.12[f]	−0.17[f]	1.29	0.88
16		$C_5H_8N_2S$•2HCl	156–158	MeOH/Et_2O	g	26[d]	2.2	0.16[h]	−1.64[h]	1.80	0.38
17		$C_6H_{10}N_2S$•1.9HCl[n]	140–141	EtOH/Et_2O	B	10 (78% max)					
18		$C_7H_{12}N_2S$•2HCl	169–170	EtOH	C	8.2 (67% max)	1.6 (19% max)				
19		$C_{11}H_{12}N_2S$•1.4HCl	192–201	EtOH	i	8.3 (78% max)	< 0.13	2.22[f]	−0.75[f]	1.47	0.05
20	 R = H	$C_9H_{11}N_3$•2HCl	285–290	EtOH	j	7.0 (52% max)[k]	[l]	0.59[f]	−2.71[m]	3.30	0.04
21	R = Me	$C_{11}H_{15}N_3$•2HBr	262–264	MeOH	j	$pA_2 = 6.9$					
22		$C_{13}H_{13}N_3$•2HCl[n]	295 dec	MeOH	D	$pA_2 = 5.4$					
23	 R = H	$C_9H_{10}N_2S$•1.9HCl	162–165	EtOH/MeOH	E	48 (51% max) $pA_2 = 6.1$	[l]				
24	R = Me	$C_{11}H_{14}N_2S$•1.2HCl	139–141	EtOH	o	$pA_2 = 6.9$					
25		$C_{10}H_{15}N_5$ •$3C_6H_3N_3O_7$	224	H_2O	p	104	94 (94% max)				
26		$C_{14}H_{17}N_3$•3HCl	177–180	EtOH	q	2.7 (66% max)	< 0.13				
27		$C_{18}H_{17}N_3S_2$•2HCl	149–153	MeOH	F	8.2 (41% max)					

[a]All compounds were analyzed for C, H, N and Cl and are within ± 0.4% of theoretical values unless stated; [b]see *Chemistry* section; [c]see footnote d, table I; [d]Durant *et al* [4]; [e]Ganellin [16]; [f]measured at pH 12.2; [g]Behringer *et al* [24]; [h]measured at pH 12.0; [i]Jones *et al* [25]; [j]Durant *et al* [7]; [k]$pA_2 = 5.8$; [l]inactive; negative chronotropic effect observed at high doses; [m]measured at pH 11.8; [n]contains 1.5% (w/w) water; [o]Kiprianov *et al* [26]; [p]Durant *et al* [5]; [q]Uhlig *et al* [27].

cyclylethylamines derived from 2-(pyridin-2-yl)ethylamine and 2-(benzothiazol-2-yl)ethylamine were therefore prepared. These compounds, **26** and **27**, were found to be only relatively weak partial agonists on the guinea pig ileum, comparing unfavourably with the parent compounds, and inactive on the atrium.

Conclusion

In choosing histamine as a starting point, structural modifications were explored with a view to markedly reducing hydrogen-bonding ability, introducing receptor subtype selectivity and retaining adequate potency. For agonists, receptor affinity and efficacy are notoriously sensitive to relatively minor structural changes, and this has been borne out in the present study, where no novel modification resulted in a compound possessing an acceptable combination of brain penetration and receptor activity. This has been especially true in attempting to retain H_2-receptor agonist activity. Two known compounds, betahistine, **14**, and 2-(thiazol-2-yl)ethylamine, **16**, however, which are moderately potent and relatively selective H_1-receptor agonists, were found to cross the blood–brain barrier of the anaesthetized rat reasonably well, and might therefore be useful in studying central H_1-receptor function. It should, however, be recognized that **14** is a moderately potent histamine H_3-receptor antagonist [22], and this might complicate the interpretation of data obtained.

The hitherto unreported compound, *N,N*-bis-{2-[4(5)-imidazolyl]ethyl}amine, **25**, although unlikely to cross the blood–brain barrier, is worthy of special mention, as it is almost identical to histamine in its agonistic effects on both the guinea pig ileum and atrium.

Experimental protocols

Chemistry

Proton magnetic resonance (^{1}H-NMR) spectra were obtained using a Jeol PFT-100P (100 MHz) spectrometer, with tetramethylsilane for reference. Elemental analyses were within ± 0.4% of theoretical values except where noted; melting points were determined using a Buchi 510 capillary apparatus and are uncorrected.

Compounds **13** and **14** were purchased from Aldrich Chemical Co, and **15** was obtained form Croda Synthetic Chemicals Ltd. Both **13** and **15** were subsequently converted into the dihydrochloride salts. Reported methods were used for the preparation of compounds **3–9**, **11** and **25** [5]; **16** [24]; [^{14}C] **16** [9]; **19** [25]; **20** and **21** [7]; **24** [26]; and **26** [27].

*4(5)-[2-(4-Phenylbutylamino)ethyl]imidazole dihydrochloride **12***

To a stirred suspension of sodium hydride (50%, 1.68 g, 0.035 mol) in dry dimethylformamide (DMF) (50 ml) under an atmosphere of nitrogen was added 5-oxo-5,6,7,8-tetrahydroimidazo[1,5-*c*]pyrimidine (**28**; 4.81 g, 0.035 mol) in dry DMF (150 ml), and the mixture was stirred for 1.5 h. A solution of phenylbutyl bromide (7.50 g, 0.035 mol) in DMF (50 ml) was added, and stirring was continued for a further 3 h at room temperature. Solvent was removed by azeotroping with *p*-xylene, and the crude mixture was purified by chromatography (SiO_2; CH_2Cl_2/EtOH) to give **29b** (6.11 g, 65%), which solidified upon refrigeration, mp: 57–58°C. The above product was mixed with potassium hydroxide (18.2 g, 0.324 mol) in water (300 ml) and heated under reflux for 1 h. The oily product was extracted with ethyl acetate (3 x 100 ml), the extract dried ($MgSO_4$), and the solvent evaporated leaving an oil. The latter was dissolved in isopropanol, treated with ethanolic HCl in excess and allowed to stand, to yield **12** as a white crystalline solid (5.58 g, 77%), mp: 220–221°C. Anal $C_{15}H_{21}N_3$·2HCl (C, H, N, Cl). ^{1}H-NMR (D_2O) δ 1.50–1.95 (m, 4H), 2.51–2.84 (m, 2H), 2.90–3.55 (br m, 6H), 7.16–7.60 (m, 6H), 8.66 (d, 1H).

*4(5)-[2-(2-Phenylethylamino)ethyl]imidazole dihydrochloride **10***

Alkylation of **28** (10.3 g, 0.075 mol) with phenylethyl bromide (15.9 g, 0.086 mol) and sodium hydride (60%, 3.03 g, 0.075 mol) in dry DMF by the method described for **12** gave **29a** (2.80 g, 16%), mp: 92–93°C.

Hydrolysis of **29a** (2.56 g, 0.011 mol) with potassium hydroxide (10.4 g, 0.19 mol) in water, followed by conversion into the hydrochloride salt and recrystallization from isopropanol furnished **10** (1.42 g, 49%) mp: 242–243°C. Anal $C_{13}H_{17}N_3$·2HCl (C, H, N, Cl). ^{1}H-NMR (D_2O) δ 2.90–3.54 (br m, 8H), 7.38 (m, 6H), 8.68 (d, 1 H).

*2-(2-Methylaminoethyl)thiazole dihydrochloride **17***

N-Methyl-*N*-cyanoethyl benzamide (**30**; 9.21 g, 0.049 mol) was treated with a slurry of ammonium sulphide (excess) in methanol and heated in a steel bomb at 40°C for 5 days. The vessel was carefully opened and the contents evaporated to dryness and dissolved as far as possible in 10% aqueous methanol (100 ml). Insoluble sulphur was filtered off, and the filtrate was reduced in volume and left to stand, to yield **31** (6.11 g, 55%) mp: 140–142°C. The above product (4.00 g, 0.018 mol) was mixed with bromoacetal (3.64 g, 0.021 mol) in methanol (50 ml) and the mixture was heated under reflux for 5 h. Methanol was evaporated and the crude residue was hydrolyzed without further purification by heating it with conc HCl (25 ml) on a steam bath for 3 h. Benzoic acid formed in the reaction was filtered off, and the filtrate was washed with chloroform, basified with aq sodium bicarbonate and extracted with chloroform. The dried ($MgSO_4$) extract was evaporated to dryness, dissolved in ethanol and treated with ethanolic HCl, then ether to yield a brown solid, which was recrystallized from ethanol/ether to give **17** (0.35 g, 10%) mp: 140–141°C. Anal $C_6H_{10}N_2S$·1.94HCl (C, H, N, Cl). ^{1}H-NMR (D_2O) δ 2.79 (s, 3H), 3.30–3.80 (m, 4H), 7.80 (d, 1H), 7.98 (d, 1H).

*2-(2-Dimethylaminoethyl)thiazole **18***

2-Methylthiazole (**33**; 21.6 g, 0.22 mol) and paraformaldehyde (9.95 g, 0.11 mol) were heated together in a sealed glass tube at 165°C for 3 h. After cooling, the reaction mixture was removed carefully and purified by chromatography (SiO_2; EtOAc) to give **34** (2.80 g, 10%) as a yellow oil. ^{1}H-NMR ($CDCl_3$) δ 3.23 (t, 2H), 3.99 (t, 2H), 4.52 (br.s, 1H), 7.21 (d, 1H), 7.66 (d, 1H).

The above product (1.40 g, 0.011 mol) was dissolved in dichloromethane (60 ml), treated with redistilled thionyl chloride (1.31 g, 0.011 mol) and heated under reflux for 1.5 h. Solvent was removed *in vacuo* to leave **35** as a crude oil, which was taken up in an ethanolic solution of dimethylamine (30%, 25 ml) and heated in a sealed glass tube at 100°C for 7 h. After

cooling, the mixture was carefully removed, evaporated to dryness, neutralized with aqueous sodium hydroxide and extracted with dichloromethane. Purification by chromatography (SiO_2; EtOAc/MeOH) yielded a crude product which was dissolved in ethanol and treated with ethanolic HCl. On adding ether, a solid was formed, a sample of which was recrystallized from ethanol to give **18** as a pale brown solid, mp: 169–170°C. Anal $C_7H_{12}N_2S$·2HCl (C, H, N, Cl). ^{1}H-NMR (D_2O) δ 3.00 (s, 6H), 3.68 (s, 4H), 7.75 (d, 1H), 7.93 (d, 1H).

2-(2-Aminoethyl)imidazo[2,1-a]isoquinoline dihydrochloride ***22***
The bromoketone **36** (2.96 g, 0.010 mol), sodium bicarbonate (0.84 g, 0.010 mol) and 1-aminoisoquinoline (1.44 g, 0.010 mol) were mixed in dry DMF (15 ml) and heated on a steam bath for 1 h. After cooling, the green mixture was poured into water (200 ml) to give a green oil which solidified. The latter was recrystallized from ethanol to give **37** (2.61 g, 77%), ^{1}H-NMR (DMSO–d_6) δ 3.05 (t, 2H), 3.93 (t, 2H), 7.15 (d, 1H), 7.45–7.62 (br m, 2H), 7.72 (s, 1H), 7.80 (s, 5H), 8.00–8.17 (br m, 1H) 8.25 (d, 1H).

Compound **37** (2.5 g, 0.0073 mol) was treated with conc HCl and heated on a steam bath for 36 h. The resulting mixture was evaporated to dryness and recrystallized from methanol to give pale beige crystals of **22** (0.90 g, 43%), mp: 295°C dec. Anal $C_{13}H_{13}N_3$·2HCl (C, H, N, Cl). ^{1}H-NMR (D_2O) δ 3.29–3.72 (br m, 4H), 7.48 (d of d, 1H), 7.60–8.25 (br m, 6H).

2-(2-Aminoethyl)benzothiazole dihydrochloride ***23***
A mixture of 2-vinylbenzothiazole (7.20 g, 0.045 mol) and potassium phthalimide (10.8 g, 0.058 mol) in 150 ml of DMF was heated on a steam bath for 3 h. After cooling, the solid was filtered off and the filtrate was treated with water to precipitate an oil which was extracted with ether. The aqueous phase was washed with ether then evaporated to dryness to a brown residue which was dissolved in ethyl acetate (100 ml) and extracted with 2 N HCl (5 x 100 ml) to give crude **39**. This product was hydrolyzed with an excess of conc HCl on a steam bath for 3 h and left to cool. Solid was filtered off and the filtrate was evaporated to dryness. The resulting yellow solid was shaken with a mixture of chloroform and water, and the aqueous phase was basified to pH 13 with aqueous sodium hydroxide to precipitate a solid. The latter was continuously extracted with chloroform, and the extracts were dried ($MgSO_4$) and evaporated to a pale yellow oil which was treated with ethanolic HCl to precipitate a solid. Recrystallization from ethanol/methanol gave **23** (0.73 g, 7%) mp: 162–165°C ([8] mp: 206°C). Anal $C_9H_{10}N_2S$·1.9HCl (C, H, N, S, Cl). ^{1}H-NMR (D_2O) δ 3.61 (m, 4H), 7.43–7.76 (m, 2H), 7.83–8.12 (m, 2H).

N,N-Bis-[2-(2-benzothiazolyl]ethyl]amine dihydrochloride ***27***
A solution of 2-vinylbenzothiazole (2.50 g, 0.016 mol) in ethanol (13 ml) was saturated with ammonia gas, while stirring and cooling in ice for 1.5 h. The mixture was then allowed to warm to room temperature and left to stand, securely stoppered for 6 days. After evaporating to dryness, the mixture was chromatographed (SiO_2; $CHCl_3$/EtOAc) to give a crude product which was treated with excess ethanolic HCl, evaporated to dryness and recrystallized from methanol to give **27** (0.50 g, 8%) mp: 149–153°C. Anal $C_{18}H_{17}N_3S_2$HCl (C, H, N, S, Cl). ^{1}H-NMR (DMSO–d_6) δ 3.60 (m, 8H), 7.34–7.70 (m, 4H), 7.75– 8.22 (m, 4H).

4-Phenyl-[4-^{14}C]-2-(2-aminoethyl)thiazole hydrochloride [^{14}C] ***19***
Sodium [1-^{14}C]acetate (54.7 mg, 0.67 mmol, 20 mCi) was converted into [carbonyl-^{14}C]acetophenone, **41**, by the usual method [10], in 58% yield (46.3 mg). The latter (46.3 mg, 0.39 mmol) was dissolved in diethyl ether (1 ml), cooled to 0°C in an ice bath, and treated with bromine (20 ml, 0.39 mmol) and anhydrous aluminium trichloride (10.0 mg, 0.075 mmol) for 1 h. The reaction mixture was evaporated to dryness *in vacuo*, dissolved in water (500 μl), and extracted with diethyl ether (3 x 1 ml). The extracts were dried ($MgSO_4$) and evaporated to dryness *in vacuo* to leave crude **42**. This product was mixed with β-benzamidothiopropionamide (80 mg, 0.39 mmol) in ethanol (2 ml) and heated under reflux with stirring for 7 h. Purification by preparative thin-layer chromatography (TLC) (SiO_2, EtOAc) gave **43** (5.44 mCi, 27% from sodium [1-^{14}C]acetate). Hydrolysis of **43** by heating with hydrochloric acid (5 ml, 5 N) under reflux for 24 h, followed by washing with diethyl ether (2 x 5 ml), basification with aqueous sodium hydroxide to pH 10 and extraction with diethyl ether (4 x 3 ml) gave a crude product, which was purified by preparative TLC (SiO_2, EtOAc/MeOH/NH_4OH, 10/2/1). The product was treated with ethanolic HCl to yield [^{14}C] **19** (17.2 mg, 0.071 mmol, 2.15 mCi, 11% from sodium [1-^{14}C]-acetate).

2-(2-Aminoethyl)-[3-^{14}C]imidazo-[1,2-a]pyridine dihydrochloride [^{14}C] ***20***
To [methyl-^{14}C]-*N*-methyl-*N*-nitrosourea (62.5 mg, 0.61 mmol, 10.6 mCi) was added diethyl ether (2 ml), and the mixture was cooled to 0°C in an ice bath. Sodium hydroxide solution (3 ml, 40%) was added dropwise over 15 min with gentle agitation. After a further 30 min at 0°C, the reaction was cooled to –76°C and the ether layer carefully decanted onto potassium hydroxide pellets and dried for 2 h. A solution of β-phthalimidopropionyl chloride (**44**; 72 mg, 0.30 mmol) in diethyl ether (2 ml) was added dropwise to the [^{14}C]-diazomethane solution at –20°C over 30 min. The mixture was allowed to warm to 0°C over 2 h and then hydrogen bromide (100 μl, 48%) was added, with stirring for 1 h. The resulting ethereal solution was washed with saturated aqueous sodium carbonate (3 x 5 ml), dried and evaporated *in vacuo* to leave crude [^{14}C] **36** (81 mg, 91%). This product (81 mg, 0.275 mmol) was heated with 2-aminopyridine (26.1 mg, 0.277 mmol) and sodium carbonate (23.1 mg, 0.218 mmol) in DMF (400 μl) at 100°C, with stirring for 6 h. The reaction mixture was cooled, water (1 ml) added, and the whole evaporated to dryness *in vacuo*. Purification by preparative TLC (SiO_2, $CHCl_3$/MeOH, 9/1) gave **45**, which was hydrolyzed by heating with 6 N HCl (2 ml) under reflux for 3 h. The mixture was cooled, evaporated to dryness *in vacuo* and the residue recrystallized from ethanol to yield a first crop of [^{14}C] **20** (9.0 mg, 0.038 mmol, 408 μCi).

Tritiation of unlabelled compounds

Compounds **14** and **15** were tritiated using the TR8 procedure by Amersham International Plc, in which approximately 5 mg of each compound was treated with ^3H_2O of high isotopic abundance, generally under neutral conditions, in a suitable solvent, such as DMF or acetonitrile. After tritiation the compounds were diluted with unlabelled material and purified by preparative TLC in a suitable system. The compounds were stored in ethanol at –25°C.

Biological methods

Brain penetration determinations
The extent to which compounds entered the brain of rats was estimated using the method described previously [11]. The radiolabelled agonists were dissolved in saline and administered to urethane-anaesthetized male rats initially as an intra-

Table III. Radiochemical and brain penetration data.

Compd	*Isotope*	*Specific activity*	*% Radiochemical purity*		c_{brain}/c_{blood}	*n*
		(μCi.mg⁻¹)	*System 1*	*System 2*	*(± SD)*	
14	^{3}H	106	> 96.5A[a]	>96.5B	0.50 ± 0.09	4
15	^{3}H	102	> 97C	> 97D	0.88 ± 0.04	4
16	^{14}C	180	97.8A	98.5E	0.38	1
19	^{14}C	125	> 98F	> 98G	0.05[b]	1
20	^{14}C	45.3	> 98A	> 98G	0.04[b]	1

[a]A: EtOAc/MeOH/NH_4OH (5/1/1); B: c-C_6H_{14}/$CHCl_3$/Et_2NH (5/4/1); C: EtOAc/MeOH/NH_4OH (8/1/1); D: $CHCl_3$/MeOH (9/2); E: $CHCl_3$/MeOH/NH_4OH (10/2/1); F: EtOAc/MeOH/NH_4OH (10/2/1); G: *n*-PrOH/NH_4OH (7/3); [b]ratio measured approaching equilibrium conditions.

venous (iv) bolus, followed by an iv infusion over 2–3 h, to establish a steady-state blood concentration. In the case of compounds **19** and **20**, near-equilibrium conditions were reached during this time. The rat was next exsanguinated and the brain removed. Brain tissues were weighed and solubilised in Soluene-100. Aliquots of this solution were treated with Dimilume-30 scintillant and glacial acetic acid and counted using a Searle Mk III liquid scintillation counter. Dose solutions and blood samples were counted in Pico-fluor 15 scintillant. Samples were quench-corrected using an automated external standard method. The degree of brain penetration for each compound was estimated as the ratio of radioactivity in brain to that in peripheral blood at the end of infusion, and the data are shown in table III.

Partition coefficient measurements

The partition coefficients were measured either by a filter–probe method similar to that described by Tomlinson [28] or by the 'shake–flask' procedure summarised below. An aliquot of the organic phase (either *n*-octanol or cyclohexane), presaturated with the aqueous phase, was added to an aliquot of a solution of the compound in the buffered aqueous phase, presaturated with the organic phase, contained in either a glass (when using *n*-octanol) or a Nalgene FEP (when using cyclohexane) screw-capped bottle. The bottle was tumbled in a water bath at 37°C for *ca* 1 h. The phases were then allowed to separate out, under gravity, for 1–2 h. Finally, the UV/visible spectra of samples of the aqueous phase before and after partitioning were recorded using a Perkin–Elmer 320 spectrometer. The partition coefficient (P) was calculated using the equation, $P = (A_i - A_f) \cdot V_w / A_f \cdot V_o$, where A_i and A_f are the absorbancies of the aqueous phase before and after partitioning, respectively, measured at the λ_{max} of the compound (261 nm for **14** and **15**; and 238, 254 and 295 nm for **16**, **19** and **20** respectively); and V_w and V_o are the volumes of the aqueous and organic phases, respectively.

For each compound, the pH of the buffered aqueous phase was at least 2 units above the pK_a of the compound.

Pharmacology

Histamine H_1- and H_2-receptor agonist activities were determined *in vitro* on guinea pig ileum and atrium, respectively. Guinea pigs of either sex weighing from 400–700 g were killed, and the terminal ileum was removed, washed and mounted in a 15 ml bath containing Tyrode's solution gassed with 95% O_2/5% CO_2 and maintained at 30°C. The tissue was loaded with 0.5 g tension and contractions were detected by a force transducer and displayed on a potentiometric recorder. Assays were performed in the presence of 10^{-6} M atropine. From the same animals, a triangular piece of right atrium (including the sinoatrial node) was removed quickly, mounted in an acrylic holder, and suspended in a 15-ml bath containing McEwen's solution, gassed with 95% O_2/5% CO_2 and maintained at 34°C. The contraction frequency was recorded continuously on a potentiometric chart 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 g tension. Agonists were added to the bath by a micrometer syringe, and assays were performed in the presence of 5 x 10^{-7} M propranolol.

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