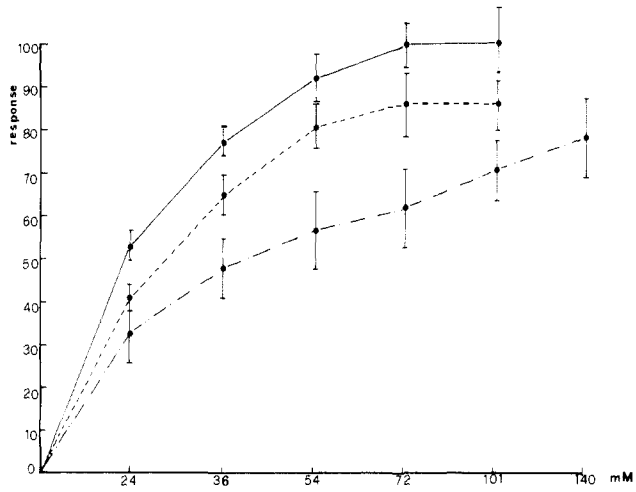

Figure 1. Variation of the maximal force of contraction of rabbit aorta with KCl (—) alone and in the presence of 1×10^{-5} M **8a** (- - -) and 1×10^{-6} M of verapamil (– · · –). $p < 0.05$ for the first two points, and $p < 0.01$ for the other ones of Verapamil. The variation in presence of **8a** is not significative.

strips 5 cm long that were suspended (at 3-g resting tension) in a muscle chamber containing Krebs solution gassed as above at pH 7.38 and 37 °C. Isometric tension was measured and recorded on a recording microdynamometer, Model, 7003 (U. Basile).

After 60-min stabilization the strips were stimulated cumulatively with elevated K^+ concentrations (24, 36, 54, 72, 101, 140 mM), and cumulative dose–response curves were obtained in the presence and absence of **8a** (10^{-5} M) and verapamil (10^{-6} M).

Each curve was obtained on five different preparations. Means and standard errors of the means were calculated and significance was tested by means of a Student's t-test.

Registry No. **6**, 97634-44-1; **6a**, 97634-45-2; **7**, 97634-26-9; **7a**, 97634-27-0; **8**, 97634-10-1; **8a**, 97634-11-2; **8b**, 97634-12-3; **9**, 97634-49-6; **9a**, 97634-50-9; **10**, 97634-35-0; **10a**, 97634-36-1; **11**, 96275-88-6; **11a**, 97634-13-4; **12**, 97634-41-8; **12a**, 97634-42-9; **13**, 97634-47-4; **13a**, 97634-48-5; **14**, 97634-07-6; **15**, 1705-77-7; **16**, 97634-08-7; **17**, 1705-68-6; **18**, 97634-09-8; **19**, 14078-27-4; **20**, 97634-14-5; **21**, 97634-28-1; **22**, 97634-15-6; **23**, 97634-29-2; **24**, 97634-16-7; **24b**, 97634-17-8; **25**, 97634-30-5; **26**, 97634-18-9; **27**, 97634-31-6; **28**, 97634-23-6; **29**, 97634-32-7; **30**, 97634-24-7; **31**, 97634-33-8; **32**, 97634-25-8; **33**, 97634-34-9; **34**, 97634-19-0; **35**, 97634-20-3; **36**, 97634-21-4; **36a**, 97634-22-5; **37**, 97634-37-2; **38**, 17024-04-3; **39**, 95156-69-7; **40**, 4440-26-0; **41**, 97634-39-4; **42**, 39633-87-9; **43**, 97634-40-7; **44**, 90033-35-5; **44** (carboxylic acid), 97634-43-0; **45**, 25789-95-1; **46**, 85322-70-9; **47**, 97634-46-3; **50**, 10347-28-1; **51**, 56740-71-7; **52**, 36265-55-1; **53**, 72936-07-3; **54**, 7498-88-6; **55**, 76694-24-1; **56**, 6078-95-1; 9-cyanofluorene, 1529-40-4; 3-bromo-1-propyl acetate, 592-33-6; *N*-methyl-*N*-homoveratrylamine, 3490-06-0; diphenylacetonitrile, 86-29-3; fluorene-9-carboxylic acid ethyl ester, 26878-12-6; isopropyl iodide, 75-30-9; triphenyl(carbethoxymethylene)phosphorane, 1099-45-2; ethyl diphenylacetate, 3468-99-3; 3-(hydroxymethyl)fluorene, 24324-17-2; bis(9-fluorenyl)methyl sulfite, 97634-38-3; phenanthrene, 85-01-8; 9-(chloromethyl)fluorene, 36375-77-6; 9-formylfluorene, 20615-64-9; 9-fluorenone, 486-25-9; cyclopropyl bromide, 4333-56-6; diphenylacetaldehyde, 947-91-1.

Pseudosymmetry and Bioisosterism in Biaryl Pyridyl Competitive Histamine H_2-Receptor Antagonists

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A process of drug design has previously been described that led to the synthesis of 3-amino-5-[2-(ethylamino)-4-pyridyl]-1,2,4-triazole (**4**), a competitive histamine H_2-receptor antagonist structurally unrelated to, but more potent than, cimetidine. A QSAR study on a subset of analogues closely related to **4** showed that gastric acid antisecretory activity increased with decreasing lipophilicity. An SAR study about **4** focused on (1) pyridine substitution compatible with both unidentate and bidentate hydrogen bonding, (2) exploration of the pseudosymmetry of **4**, and (3) examination of triazole and imidazole bioisosterism. This SAR study led to a definition of the minimum structural features required for antagonist activity. The pyridylamino group is not essential for activity since replacement with a methyl group results in a decrease but not loss of activity. The triazole amino group is also not essential since replacement of the triazole amino group by methyl results in very similar activity. A triazole ring nitrogen N-1 can be replaced by a CH as in imidazole **20**. The same methylimidazole in **20** when appended to a methyl pyridine as in **22** produces a competitive antagonist with Schild plot slope of unity. In summary compound **22** displays the minimum features required for antagonist activity, namely a 4-substituted pyridine appended to a 4(5)-substituted imidazole ring with single nitrogen to amidine nitrogen pair distances of 5.16 and 6.42 Å.

The discovery of biaryl pyridyltriazoles that are competitive histamine H_2-receptor antagonists by a process of bioisosteric drug design has been previously described.[2] Exploration of the structure–activity relationships of derivatives related to the original lead was influenced by the rationale used to discover the original lead. Accordingly, in addition to the standard techniques of modifying aromatic substitution according to the Topliss operational scheme and a QSAR study that we describe here, we focused particular attention on substituent effects on hydrogen bonding, the possibility that the pseudosymmetry of the lead might extend to the SAR pattern and the likelihood that imidazoles might function as triazole bioisosteres. This report provides data allowing us to define the minimum structural features required for activity in this series of histamine H_2-receptor antagonists, indicates that these derivatives exhibit a pseudosymmetrical SAR pattern, and illustrates that extended lipophilic side chains are consistent with in vitro activity but lead to antisecretory reduction at one position of the histamine H_2-receptor site.

Chemistry

The syntheses of the various 3-amino-5-(4-pyridyl)-1,2,4-triazole derivatives were straightforward. Four general routes were employed (Scheme I). In method A the appropriate isonicotinic acid hydrazide is condensed with *S*-methylpseudothiourea sulfate to give the intermediate amidinohydrazide, which was thermally closed to afford

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(2) Lipinski, C. A. *J. Med. Chem.* **1983**, *26*, 1–6.

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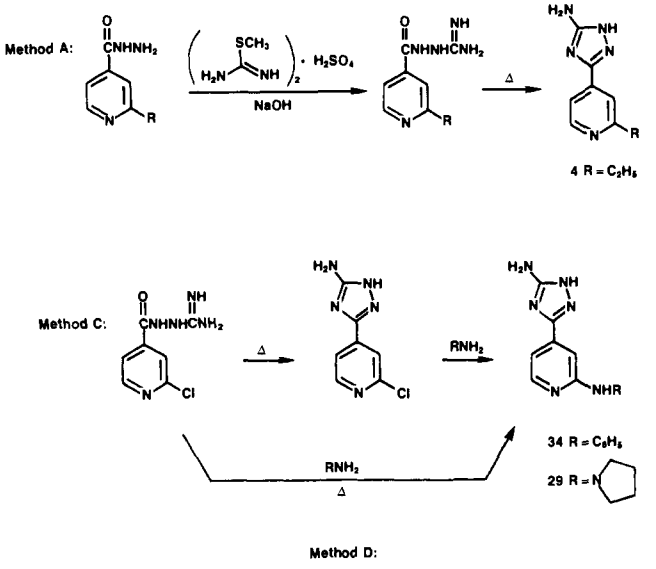
Scheme I. 3-Amino-5-(4-pyridyl)-1,2,4-triazoles



the final product. Compounds **1–4** were prepared in this fashion. A variation of this approach was applied in method B, in that the isonicotinic acids were fused with aminoguanidine sulfate to give the 3-aminotriazoles (**5–7**, **24**, **29** and **33**) directly.

2-Chloroisonicotinic acid 2-amidinohydrazide was the pivotal starting material for methods C and D. In the former, this material was closed thermally to yield 3-amino-5-(4-chloropyridyl)triazole, which can, in turn, be treated with a variety of amines under high-temperature/pressure conditions to afford the halogen-displacement products **34**, **36**, and **37**. For method D, 2-chloroisonicotinic acid 2-amidinohydrazide was treated directly with an excess of amine in H_2O also at high temperature/pressure to afford the desired products directly. By this method, compounds **23**, **25–28**, **30–32**, and **38–41** were prepared.

3-Alkyl-5-(4-pyridyl)-1,2,4-triazoles were prepared by methods E and F (Scheme II). For 2-(ethylamino)pyridyl derivatives, the imino ester of 2-chloroisonicotinonitrile was reacted with the appropriate acid hydrazide under basic conditions to effect ring closure to the 3-alkyl-5-(2-chloro-4-pyridyl)triazoles. These in turn were treated with ethylamine at high temperature/pressure to afford the halogen-displacement products **10–13**, **16**, and **17**. The 3-methyl-5-(2-alkylpyridyl)-1,2,4-triazoles **14** and **15** were prepared by radical alkylation of 3-methyl-5-(4-pyridyl)-1,2,4-triazole under acidic conditions by a literature procedure.[3]

2-Substituted 4-(4-imidazolyl)pyridines, **18–22**, in which an imidazole ring replaces the triazole moiety, were prepared by method G (Scheme III). This approach is based on cyclization of an appropriately substituted α-amino ketone (or α-amino ketal) with the proper reagent. The synthesis of **19** and **20** using this method has already been described.[4]

Results and Discussion

SAR Study Approach. The process of bioisosteric drug design when applied to histamine leads to the 2-unsubstituted pyridine **1** and 2-amino and ethylamino derivatives **2** and **4** (Table I).[2] Much of the over 100-fold activity increase in this process results when the 2-unsubstituted pyridine moiety as in **1** is replaced by the 2-aminopyridine moiety as in **2**. This modification arose in part from literature suggesting that 4-methyl-2-aminopyridine could be viewed as an amidine derivative.[5] In considering rational approaches to the exploration of structure–activity relationships about **2**, the combination of the amidine derivation of **2** coupled with the increase in activity of **2** over **1** suggested that compounds designed to incorporate a physicochemical property common to both amidines and pyridines and retained or enhanced in 2-aminopyridine over pyridine itself could lead to active compounds. This first approach was based on the following argument.

(1) Amidines and aminopyridines such as **2** are capable of both unidentate and bidentate hydrogen bonding. The bidentate character of amidine hydrogen bonding as described by Walker[6] can involve a simultaneous strong interaction between protons on each nitrogen of a charged amidinium group with the oxygen atoms sharing a negative charge as in a carboxylate anion. Such an interaction could also occur for a protonated 2-aminopyridine moiety. However, it is important to note that, as described by DelBene,[7] a bidentate interaction between a hydroxylic moiety and a neutral aminopyridine moiety can take place in which the neutral pyridine nitrogen is a hydrogen-bond acceptor and the pyridine amino N–H is a proton donor. This may have considerable biological relevance since the 2-aminopyridines in this study are 95% in the neutral form at pH 7.4. By way of contrast, the pyridine ring of compound **1** is only capable of unidentate hydrogen bonding. This reasoning led to the relatively unobvious approach of replacing a pyridine 2-amino substituent with alkyl groups having similar electron-donating and steric effects since these changes would retain the unidentate hydrogen-bond-accepting properties of **1**[8] but would eliminate the two possible types of bidentate hydrogen bonding that could be exhibited by **2** in either its neutral or protonated form.

(2) A second approach was suggested by the pseudosymmetry of **2**. This fact, coupled with the parallelism between pseudosymmetry in small effector molecules and pseudosymmetry in receptors,[9] suggested that there might be an element of symmetry in the histamine H_2 receptor.[2] If this speculation were valid, it should be reflected in the SAR about **2** and this in turn led to modifications of **2** that were not suggested by histamine H_2-receptor agonist SAR.

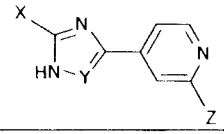
(3) Finally, since 3-amino-1,2,4-triazole can be viewed as an imidazole surrogate, the possible bioisosterism of imidazoles and triazoles was explored.

Biological Results

Table I shows that the sequence of pyridine substituent changes from 2-unsubstituted (**1**) to 2-amino (**2**), methylamino (**3**), and ethylamino (**4**) results in a progressive increase in competitive histamine H_2-receptor antagonist activity and gastric acid antisecretory activity. Replacement of the 2-amino substituent by an electron-donating alkyl substituent so as to retain the unidentate hydrogen-bonding properties of **1** results in 2-methyl, ethyl, and propyl derivatives **5–7** with activity improved over **1**; the

(3) Minisci, F.; Bernardi, R.; Bertini, F.; Galli, R.; Perchinummo, M. *Tetrahedron* **1971**, *27*, 3575–9.
(4) LaMattina, J. L. *J. Heterocycl. Chem.* **1983**, *20*, 533.
(5) Fastier, F. N. *Pharmacol. Rev.* **1962**, *14*, 37–90.
(6) Walker, J. *J. Chem. Soc.* **1949**, 1996–2002.
(7) Del Bene, J. E. *J. Am. Chem. Soc.* **1979**, *101*, 7146–7151.
(8) Barton, S. S.; Kraft, J. P.; Owens, T. R.; Skinner, L. J. *J. Chem. Soc., Perkins Trans. 2* **1972**, 339–341.
(9) Goodford, P. "Medicinal Chemistry", Proceedings of the 6th International Symposium on Medicinal Chemistry, Brighton, England, Sept 4–7, 1978; Simkins, M. A., Ed.; Cotswold Press: Oxford, 1979; pp 225–263.

Table I. Pyridine Histamine H_2-Receptor Antagonists



compd	X	Y	Z	mp, °C	mol formula[a]	H_2-recep antag act.: guinea pig atrium KB 10^{-6} M (±SD)[b]	Schild plot slope[c]	acid antisec act.: max inhibn in dogs at (dose) iv[d]
1	H_2N	N	H	279–281	$C_7H_7N_5$[e]	41.9 (±28.8)	1.22	43% (10), 73% (23)
2	H_2N	N	NH_2	246–247	$C_7H_8N_6 \cdot H_2O$[e]	2.22 (±0.18)	0.91 (±0.11)	ED_{50} = 2.35 (r = 0.95, n = 7)
3	H_2N	N	$NHCH_3$	265–266	$C_8H_{10}N_6$[f]	0.562 (±0.195)	0.84	50% (1)
4	H_2N	N	NHC_2H_5	255–260	$C_9H_{12}N_6$[e]	0.235 (±0.056)	1.00 (±0.10)	ED_{50} = 0.87 (r = 0.97, n = 22)
5	H_2N	N	CH_3	266–267	$C_8H_9N_5 \cdot {}^1/_4H_2O$[g]	12.9 (±11.9)	0.66	26% (1), 60% (5)
6	H_2N	N	C_2H_5	218–220	$C_9H_{11}N_5$[g]	1.82 (±1.10)	1.04	55% (5)
7	H_2N	N	C_3H_7	202–203	$C_{10}H_{13}N_5$[g]	3.98 (±1.72)	0.83	0% (5)
8	H_3CNH	N	NHC_2H_5	273–276	$C_{10}H_{14}N_6 \cdot {}^1/_4H_2O$[h]	1.27 (±0.38)	0.77	35% (1), 72% (10)
9	H_5C_2NH	N	NHC_2H_5	224–226	$C_{11}H_{16}N_6$[h]	4.65 (±1.72)	1.18	21% (0.5), 38% (1.0)
10	H	N	NHC_2H_5	153–155	$C_9H_{11}N_5$[i]	4.96 (±2.62)	1.15	29% (10)
11	H_3C	N	NHC_2H_5	226–228	$C_{11}H_{13}N_5$[i]	0.55 (±0.08)	0.97	35% (1), 72% (10)
12	H_5C_2	N	NHC_2H_5	142–144	$C_{11}H_{15}N_5 \cdot {}^1/_2H_2O$[i]	1.28 (±0.55)	0.90	17% (5)
13	H_7C_3	N	NHC_2H_5	146–148	$C_{12}H_{17}N_5$[i]	11.3 (±9.16)	1.86	45% (10)
14	CH_3	N	CH_2OH	245–247	$C_9H_{10}N_4O$[j]	22.7 (±10.4)	0.96	7% (10)
15	CH_3	N	$CH_2C_6H_5$	185–187	$C_{15}H_{14}N_4$[j]	not act.		31% (10)
16	$HOCH_2$	N	NHC_2H_5	226–228	$C_{10}H_{13}N_5O$[i]	4.51 (±2.05)	1.00	20% (1), 53% (10)
17	$H_5C_6CH_2$	N	NHC_2H_5	191–193	$C_{16}H_{17}N_5$[i]	not act.		19% (10)
18	H_2N	CH	NHC_2H_5	200–202	$C_{10}H_{13}N_5 \cdot HCl$[k]	3.29 (±2.81)[l]	1.96	22% (1), 74% (10)
19	H	CH	NHC_2H_5	135–137	$C_{10}H_{12}N_4$[k]	0.59 (±0.59)[l]	1.03	27% (10)
20	CH_3	CH	NHC_2H_5	214–216	$C_{11}H_{14}N_4$[k]	1.38 (±0.79)[l]	1.19	55% (10)
21	CH_3	CH	H	152–153	$C_9H_9N_3$[k]	36.2 (±10.6)	0.99	56% (25)
22	CH_3	CH	CH_3	115–117	$C_{10}H_{11}N_3$[k]	4.63 (±1.48)	1.07	24% (10)
	cimetidine[m]					0.630 (±0.360)	0.87 (±0.31)	ED_{50} = 1.02 (r = 0.97, n = 22)

[a] All compounds were analyzed for C, H, and N. [b] The dissociation constant (K_B) was calculated from the equation $K_B = B/(DR - 1)$, where DR is the respective ratio of concentrations of histamine needed to produce half-maximal responses in the presence and absence of different concentrations (B) of antagonists. [c] Slope of the plot of log (DR – 1) on log B. [d] Maximum percent inhibition of acid output in single experiments in pentagastrin-stimulated Heidenhain pouch dogs at a dose in milligrams per kilogram, given intravenously. [e] Reference 2. [f] Prepared by method A. [g] Method B. [h] Synthesis described in the Experimental Section. [i] Method E. [j] Method F. [k] Prepared by method G. [l] Determined using 30-min equilibration time for drug with atria (see the Experimental Section). [m] Data based on comparative testing of compounds **2**, **4**, and cimetidine: Lipinski, C. A. *J. Med. Chem.* **1982**, *26*, 1–6.

optimum derivative **6** has reduced antagonist and antisecretory activity relative to 2-methylamino derivative **3**.

The role of pseudosymmetry in activity was probed by preparation of (methylamino)- and (ethylamino)triazole derivatives **8** and **9** while retaining the optimum pyridine ethylamino substituent found in **3**. This change led to retention of appreciable activity, a pattern similar to that observed when these changes were applied in the pyridine moiety.

Histamine H_2-receptor agonist literature teaches that 2-amino substitution in 4(5)-(2-aminoethyl)imidazole (histamine) and 3-amino substitution in its 1,2,4-triazole counterpart lead to selective histamine H_2-receptor agonist activity while the corresponding alkyl substitution leads to selective histamine H_1-receptor agonist effects.[10] Therefore, a priori replacement of the 1,2,4-triazole 3-amino group by alkyl might not be anticipated to result in retention of histamine H_2-receptor antagonist effects. However, the pseudosymmetry of **2** and its bioisosteric origin via a symmetrical theoretical intermediate coupled with the activity of pyridine 2-alkyl substituents suggested that activity might be retained in 2-alkyltriazole derivatives.

Data on derivatives **10–13** illustrate that this proposal is correct and that, with triazole substituents varied from hydrogen through *n*-propyl, activity is retained and is optimum in methyl derivative **11**. Retaining the methyltriazole moiety as in active derivative **11** and introducing a pyridine substituent having additional hydrogen-bonding capability as in 2-(hydroxymethyl)pyridyl derivative **14** results in reduction of activity but a large lipophilic substituent as in 2-benzylpyridyl analogue **15** results in complete loss of activity. A pseudosymmetrical SAR pattern is further supported when these pyridine substituents as in **14** and **15** are placed on the triazole moiety as in **16** and **17** while retaining the optimum (ethylamino)pyridyl group. Activity is decreased in (hydroxymethyl)triazole derivative **16** and lost in benzyltriazole **17**.

The bioisosterism of imidazole and 1,2,4-triazole was investigated via imidazoles **18–22**. Although somewhat less active than the most active related triazoles, a similar SAR pattern was observed. As with the triazoles, amino (**18**), hydrogen (**19**), and methyl (**20**) imidazole substitution pattern combined with the optimum 2-(ethylamino)-pyridine substitution led to good activity. Compounds lacking amino substitution on either the imidazole or pyridine ring as in **21** and **22** also retained activity. Direct comparisons can be made between imidazoles and triazoles in the case of **18** and **4**, **19** and **10**, and **20** and **11**. Imidazole **18** is clearly less antisecretory than triazole **4** while antisecretory activity in the other two pairs of imidazoles and triazoles is approximately comparable. In vitro, imidazoles **18–20** equilibrated more slowly with atria than did corresponding triazoles. When a 30- rather than 3-min equilibration period was used, **19** and **20** exhibited Schild plot slopes of unity; however, **18** still exhibited an apparent Schild plot slope significantly greater than unity (see the Experimental Section). Comparing competitive histamine H_2-antagonist pairs, unsubstituted imidazole **19** is more active than triazole **10** while methylimidazole **20** is slightly less active than corresponding triazole **11**.

Table II shows the effects of variation in the pyridyl amino group while the triazole amino group is kept constant. In vitro histamine H_2-receptor antagonist activity improves through the sequence of change from amino (**2**) to methylamino (**3**) and ethylamino (**4**) and remains fairly constant from *n*-propyl (**23**) to *n*-hexyl (**25**). Appreciable in vitro activity is still found with very lipophilic substituents such as *n*-decyl (**26**) and with nitrogen substituents containing unsaturated functionality (**30**), polar functionality (**31**, **32**), or chain branching (**33**). Pyridine amino nitrogen disubstitution, however, results in a rapid decrease in activity. The dimethylamino derivative **27** is less active than monomethyl derivative **3**, and further elaboration such as to methylethylamino (**28**) or a pyrrolidino derivative (**29**) results in total loss of activity. An *N*-phenyl substituent (**34**) leads to loss of activity, which is likely due to an electronic rather than steric factor since *N*-isopropyl substitution (**33**) is compatible with activity. Activity was present in *N*-benzyl (**35**) and *N*-phenethyl substituents (**36**).

The effect of aromatic ring substitution was explored in the benzyl series according to the Topliss Tree approach. Activity within error limits was unchanged by 4-chloro substitution (**38**), and hence the 4-methyl (**39**) derivative was prepared that exhibited equal or more activity. The preparation of the very active 3,4-dimethyl (**40**) and 4-*tert*-butyl (**41**) derivatives clearly differentiates the direction of the branch point in the Topliss operational scheme.[11]

In summary, the receptor site recognizing the pyridylamino moiety can bind a variety of basic monosubstituted amino analogues having chain lengths at least as long as *n*-hexyl as well as benzyl and phenethyl without significant in vitro activity loss.

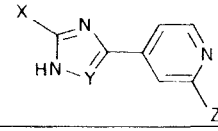
Structural requirements for acid antisecretory activity are quite narrow in contrast to the rather loose SAR pattern relating in vitro histamine H_2-receptor antagonist activity to pyridylamino monosubstitution. Inspection of Table II shows that antisecretory activity is at an optimum in 2-(ethylamino)pyridyl derivative **4** and declines with longer chain length (**23–26**). The good antisecretory activity of *N,N*-dimethyl derivative **27**, despite only moderate receptor antagonist activity, was shown to be due to metabolic demethylation to more active *n*-monomethyl dervative **3**. Although not measured, a similar metabolic process is likely responsible for the antisecretory activity of *N*-methylethyl derivative **28** that lacks receptor antagonist activity. All compounds were tested for antisecretory activity by the intravenous route so that the divergence between in vitro histamine H_2-receptor antagonist activity and antisecretory activity is not due to differences in compound absorption.

Qualitatively the data in Tables I and II suggest that gastric acid antisecretory activity declines with increasing lipophilicity. We were interested in trying to quantitate this effect and so attempted to correlate in vitro histamine H_2-antagonist activity with in vivo antisecretory activity by a QSAR study correlating acid antisecretory activity with histamine H_2-receptor dissociation constants and lipophilicity. In this respect the main purpose of this QSAR study differs from that usually seen in which one attempts to correlate intrinsic activity with terms describing physical properties of substituents. To optimize the probability of detecting a correlation between partitioning behavior and in vivo antisecretory activity, a structurally closely related set of compounds was examined which included all members having a 3-amino-1,2,4-triazole appended to a 4-pyridyl group bearing an unbranched 2-monoalkylamino substituent. Only compound **37**, which

(10) Grechishkin, L. L.; Goldfarb, V. L.; Gavrovskaya, L. K.; Brovtsyna, N. B. *Gen. Pharmacol.* **1980**, *11*, 33–36.

(11) Topliss, J. G. *J. Med. Chem.* **1972**, *15*, 1006–1011.

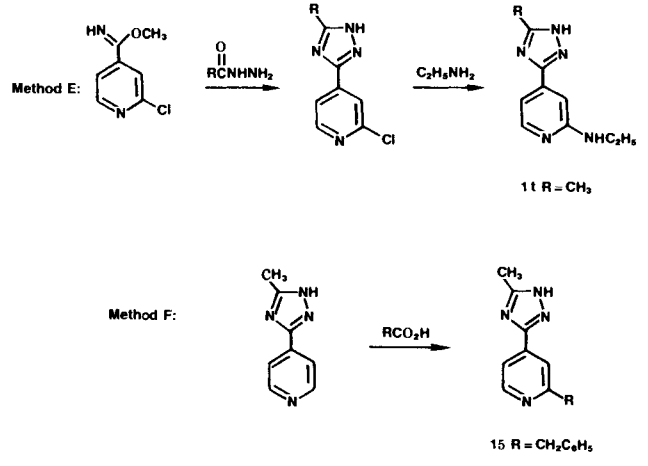
Table II. Pyridine Histamine H_2-Receptor Antagonists



compd	X	Y	Z	mp, °C	mol formula[a]	H_2 recep antag act.: guinea pig atrium KB 10^{-6} M (±SD)[b]	Schild plot slope[c]	acid antisec act.: max inhibn in dogs at (dose) iv[d]
2	NH_2	N	NH_2	246–247	$C_7H_8N_6 \cdot H_2O$[e]	2.22 (±0.18)	0.91 (±0.11)	ED_{50} = 2.35 (r = 0.95, n = 7)
3	NH_2	N	$NHCH_3$	265–266	$C_8H_{10}N_6$[f]	0.562 (±0.195)	0.84	50% (1)
4	NH_2	N	NHC_2H_5	255–260	$C_9H_{12}N_6$[e]	0.235 (±0.056)	1.00	ED_{50} = 0.87 (r = 0.97, n = 22)
23	NH_2	N	NH-n-C_3H_7	188–191	$C_{10}H_{14}N_6$[g]	0.768 (±0.376)	1.21	33% (1), 64% (5)
24	NH_2	N	NH-n-C_4H_9	262–264	$C_{11}H_{16}N_6$[h]	0.671 (±0.433)	0.89	29% (1), 69% (9)
25	NH_2	N	NH-n-C_6H_{13}	154–157	$C_{13}H_{20}N_6$[g]	0.274 (±0.075)	0.99	40% (10)
26	NH_2	N	NH-n-$C_{10}H_{21}$	152–154	$C_{17}H_{28}N_6$[g]	4.78 (±2.35)	0.84	14% (10)
27	NH_2	N	$N(CH_3)CH_3$	254–257	$C_9H_{12}N_6$[g]	3.19 (±2.15)	0.68	48% (1), 80% (5)
28	NH_2	N	$N(CH_3)C_2H_5$	199–201	$C_{10}H_{14}N_6$[g]	not act.		42% (1)
29	NH_2	N	$N(CH_2)_4$	252–255	$C_{11}H_{14}N_6$[h]	not act.		0% (1)
30	NH_2	N	$NHCH_2CH{=}CH_2$	198–200	$C_{10}H_{12}N_6$[g]	0.480 (±0.093)	0.91	31% (1)
31	NH_2	N	$NH(CH_2)_2OH$	223–225	$C_9H_{12}N_6O$[g]	2.05 (±0.84)	0.87	34% (10)
32	NH_2	N	$NH(CH_2)_2OCH_3$	154–156	$C_{10}H_{14}N_6O$[g]	3.47 (±1.60)	0.99	50% (10), 11% (1)
33	NH_2	N	$NHCH(CH_3)_2$	181–183	$C_{10}H_{14}N_6$[h]	3.58 (±4.56)	0.60	52% (5)
34	NH_2	N	NHC_6H_5	198–200	$C_{13}H_{12}N_6 \cdot H_2O$[i]	not act.		0% (10)
35	NH_2	N	$NHCH_2C_6H_5$	115–116	$C_{14}H_{14}N_6$[g]	0.71 (±0.288)	.81	72% (10), 21% (1)
36	NH_2	N	$NH(CH_2)_2C_6H_5$	182–184	$C_{15}H_{16}N_6$[i]	1.88 (±1.37)	1.09	50% (10)
37	NH_2	N	$NH(CH_2)_3C_6H_5$	80–82	$C_{16}H_{18}N_6 \cdot {}^1/_2H_2O$[i]	not act.		30% (10)
38	NH_2	N	$NHCH_2C_6H_4$-4-Cl	176–177	$C_{14}H_{13}ClN_6$[g]	0.301 (±0.089)	1.08	21% (1)
39	NH_2	N	$NHCH_2C_6H_4$-4-CH_3	162–163	$C_{15}H_{16}N_6$[g]	0.127 (±0.042)	1.13	33% (5)
40	NH_2	N	$NHCH_2C_6H_4$-3,4-$(CH_3)_2$	125–127	$C_{16}H_{18}N_6 \cdot {}^1/_2H_2O$[g]	0.114 (±0.056)	1.32	49% (5)
41	NH_2	N	$NHCH_2C_6H_4$-4-t-C_4H_9	306–307	$C_{18}H_{23}N_6 \cdot HCl$[g]	0.145 (±0.080)	1.58	0% (8)
			cimetidine			0.630 (±0.360)	0.87 (±0.31)	ED_{50} = 1.02 (r = 0.97, n = 22)

[a] Footnote *a*, Table I. [b] Footnote b, Table I. [c] Footnote c, Table I. [d] Footnote d, Table I. [e] Reference 2. [f] Prepared by method A. [g] Method D. [h] Method B. [i] Method C.

Scheme II. 3-Alkyl-5-(4-pyridyl)-1,2,4-triazoles



Scheme III. 2-Substituted 4-(4-Imidazolyl)pyridines

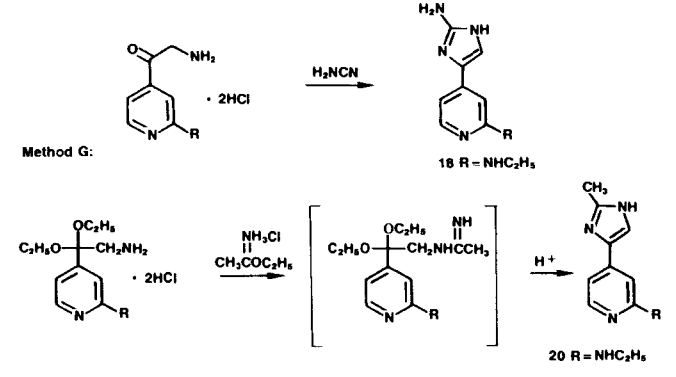
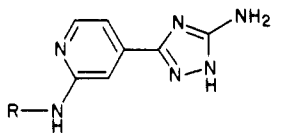


Table III. QSAR Parameters



compd	R	measd log K'[a]	log P from log K'[b]	calcd log P[c]	−log KB[d]	−log l_{50}[e]
2	H	−0.906	0.11		5.65	−0.306
3	CH_3	−0.592	0.694	0.61	6.25	0.000
4	C_2H_5	−0.358	1.13		6.63	−0.028
23	n-C_3H_7	−0.099	1.61	1.63	6.11	−0.379
24	n-C_4H_9	0.190	2.15	2.13	6.17	−0.500
25	n-C_6H_{13}	0.810	3.30	3.13	6.56	−1.195
26	n-$C_{10}H_{21}$	1.22	4.07	5.13	5.32	−1.701
30	$CH_2CH{=}CH_2$	−0.288	1.26	1.33	6.32	−0.370
31	CH_2CH_2OH	−0.836	0.24	−0.03	5.69	−1.312
32	$CH_2CH_2OCH_3$	−0.460	0.94	0.65	5.46	−0.880
35	$CH_2C_6H_5$	0.188	2.15	2.74	6.15	−0.568
36	$(CH_2)_2C_6H_5$	0.450	2.63	3.24	5.73	−1.000
37	$(CH_2)_3C_6H_5$	0.673	3.05	3.74	not act.	
38	$CH_2C_6H_4$-4-Cl	0.566	2.85	3.44	6.52	−0.428
39	$CH_2C_6H_4$-4-CH_3	0.479	2.69	3.24	6.90	−1.030
40	$(CH_2)_2C_6H_4$-3,4$(CH_3)_2$	0.689	3.08	3.74	6.94	−0.718
41	$CH_2C_6H_4$-4-t-C_4H_9				6.38	not act.

[a] log HPLC capacity factors. [b] log P calculated from log K' by using experimental log P values of 0.11 (compound **2**) and 1.13 (compound **4**) as described in the Experimental Section. [c] log P values calculated from standard Π values according to ref 12 using compounds **2** and **4** as starting points. [d] log dissociation constant guinea pig atrium. [e] log antisecretory dose in mg/kg iv required for 50% inhibition in dogs calculated from dose–response curves of **2**, **4**, **23**, **24**, **32**, and **35**.

lacked histamine H_2-antagonist activity, and **41**, which lacked acid antisecretory activity, were excluded.

Table III presents a summary of compounds studied and the parameters used in the QSAR study. Acid antisecretory dose–response data on compounds **2**, **4**, **23**, **24**, **32**, and **35** were used to calculate antisecretory 50% inhibition (I_{50}) values for other members of this series using single-point experimental values by assuming that the dose–response curves for all members of this structurally closely related series were parallel. log P values were calculated from reversed phase HPLC capacity factors. Since log P is linearly related to the log of the capacity factor (eq 2 experimental Section) an error in calculation of log P will change the coefficient of log P and the constant term in a QSAR equation but will not affect the fit of the equation to the experimental data.

Table IV presents three QSAR equations. There is only a very poor correlation between the antisecretory I_{50} and in vitro KB data (Table IV), eq 1). However, inclusion of a lipophilicity term results in a significantly better equation in which increasing antisecretory activity is correlated with increasing in vitro activity and lower lipophilicity (Table IV, eq 2). This equation, however, only accounts for about 50% of the variance in the data suggesting that the fit is moderate either because of uncertainty in the antisecretory I_{50} calculation or because another major factor such as metabolic half-life is not being accounted for.

In contrast to a previous histamine H_2-antagonist series[12] only a very poor correlation was observed between in vitro

Table IV. QSAR Equations

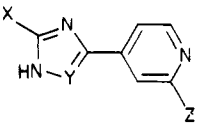
(eq 1) $-\log I_{50} = 0.348\ (\pm 0.496)^a\ (-\log \mathrm{KB}) - 2.838^b$
$n = 15,^c\ S = 0.459,^d\ F = 1.962,^e\ R^2 = 0.13^f$

(eq 2) $-\log I_{50} = -0.267\ (\pm 0.166)\ \log P + 0.504\ (\pm 0.392)\ (-\log \mathrm{KB}) - 3.283^g$
$n = 15,^c\ S = 0.359,^d\ F = 6.810,^e\ R^2 = 0.53^f$

(eq 3) $-\log \mathrm{KB} = 0.106\ (\pm 0.228)\ \log P + 5.956^h$
$n = 15,^c\ S = 0.507,^d\ F = 0.853,^e\ R^2 = 0.06^f$

[a] 95% confidence levels (2 standard deviations) in brackets. [b] Significance level of F value is 0.1848. [c] Number of compounds in data set. [d] Standard deviation of regression. [e] F value for entire equation. [f] Variance accounted for. [g] Significance level of F value is 0.010 56. [h] Significance level of F value is 0.373.

Table V. Minimum Structural Features and Histamine H_2-Receptor Antagonist Activity



compd	X	Y	Z	H_2 recep antag act.: guinea pig atrium KB 10^{-6} M (±SD)[a]	Schild plot slope[b]
2	H_2N	N	NH_2	2.22 (±0.18)	0.91
5	H_2N	N	CH_3	12.9 (±11.9)	0.66
4	H_2N	N	NHC_2H_5	0.235 (±.056)	1.00
11	H_3C	N	NHC_2H_5	0.55 (±0.08)	0.97
20	H_3C	CH	NHC_2H_5	1.38 (±0.79)	1.19
22	H_3C	CH	CH_3	4.63 (±1.48)	1.07

[a] Footnote *a*, Table I. [b] Footnote *c*, Table I.

H_2-antagonist activity and lipophilicity (Table IV, eq 3).

log P values computed for compounds **35–40** by addition of 0.5 unit per methylene chain length[13] to the parent molecule exceed the log P values calculated from HPLC capacity factors by about 0.5 unit while the computed log P value of **26** exceeds the value calculated from experimental data by 1.0 unit. A single side chain fold reduces log P by about 0.5 unit,[13] suggesting that in **35–40** a fold may be present in the side chain and that in the n-decylamino derivative **26** up to two folds in the side chain may be present.

Table V presents data that allow an estimate as to which structural features are the minimum required for histamine H_2-receptor antagonist activity. The pyridylamino group as in **2** is not essential for activity since replacement with a methyl group as in **5** results in a decrease but not loss of activity. Nor is the triazole amino group essential since replacement of the triazole amino group in **4** by a methyl as in **11** results in very similar activity. A triazole ring nitrogen N-1 as in **11** can be replaced by a CH as in imidazole **20**. The same methylimidazole in **20** when appended to a methyl pyridine as in **22** produces a competitive antagonist with Schild plot slope of unity.

In summary, compound **22** displays the minimum features required for antagonist activity, namely a 4-substituted pyridine appended to a 4(5)-substituted imidazole ring with single nitrogen to nitrogen amidine like pair distances of 5.16 and 6.42 Å.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Microanalyses were performed by the Analytical Development of Pfizer Inc., and analytical values were within ±0.4% of theoretical values unless otherwise noted. NMR and/or mass spectra were obtained on

(12) Ganellin, C. R. *J. Med. Chem.* **1981**, *24*, 913–920.

(13) Leo, A.; Hansch, C.; Elkins, D. *Chem. Rev.* **1971**, *6*, 525–554.

all compounds and were consistent with structures and assignments. NMR spectra were recorded on a Varian T-60 spectrometer, and the mass spectra were obtained on a Hitachi Perkin-Elmer RMU-6E spectrometer.

General Procedures. Method A. 3-Amino-5-[2-(*N*-methylamino)-4-pyridyl]-1,2,4-triazole (3). A mixture of 6.5 g (39 mmol) of 2-(*N*-methylamino)isonicotinic acid hydrazide,[14] 10.5 g (76 mmol) of *S*-methylpseudothiourea sulfate, 1.5 g (37 mmol) of sodium hydroxide, and 100 mL of water was stirred at room temperature for 24 h. the resulting precipitate was collected, washed well with water, and dried to constant weight, thereby affording 2.9 g (35%) of 2-(*N*-methylamino)isonicotinic acid 2-amidinohydrazide. This material was placed in a 100-mL round-bottomed flask and heated at 230 °C for 10 min, during which time a melt occurred, water was given off, and the mixture resolidified. This solid was recrystallized from ethyl acetate/methanol to afford 1.1 g (38%) of **3**, mp 261-263 °C.

Method B. 3-Amino-5-[2-(*N*-isopropylamino)-4-pyridyl]-1,2,4-triazole (33). A mixture of 4.0 g (22 mmol) of 2-(*N*-isopropylamino)isonicotinic acid and 5.5 g (44 mmol) of aminoguanidine sulfate was heated at 200 °C under nitrogen for 9 h. The mixture was cooled, and the resulting residue was diluted with water prior to adjusting the pH of the mixture to 7.0 with 1 N aqueous sodium hydroxide. The aqueous mixture was extracted with ethyl acetate (3 × 100 mL), and the combined extracts were dried (Na_2SO_4), filtered, and evaporated, leaving a solid residue. Recrystallization from ethyl acetate afforded 1.07 g (22%) of **33**, mp 185–186 °C.

Method C. Synthesis of 3-Amino-5-[2-[(2-phenylethyl)amino]-4-pyridyl]-1,2,4-triazole (36) from 3-Amino-5-(2-chloro-4-pyridyl)-1,2,4-triazole. A mixture of 35.8 g (0.21 mol) of 2-chloroisonicotinic acid hydrazide[15] and 58.4 g (0.42 mol) of *S*-methylpseudothiourea sulfate was suspended in a solution of 8.4 g (0.21 mol) of sodium hydroxide dissolved in 250 mL of water. The resulting slurry was stirred at room temperature for 20 h and then filtered. The solid was washed with water and then diethyl ether and subsequently dried to constant weight, thereby affording 44.5 g (95%) of 2-chloroisonicotinic acid 2-amidinohydrazide, mp 198–200 °C.

A 10-g (46.8 mmol) sample of 2-chloroisonicotinic acid 2-amidinohydrazide was placed in a sublimation apparatus and heated at 210 °C under high vacuum (0.05 torr) for 14 h. The small amount of sublimate was discarded, and the solid residue was triturated with methanol. Any insoluble material was removed and the filtrate concentrated, leaving 4.8 g (53%) of 3-amino-5-(2-chloro-4-pyridyl)-1,2,4-triazole as a white solid, mp 232–235 °C. This material was sufficiently pure to use directly. However, analytically pure material could be obtained in the form of a white powder, mp 237–238 °C, by chromatography over silica gel using 4:1 ethyl acetate/methanol as eluent.

A mixture containing 1.0 g (5.1 mmol) of 3-amino-5-(2-chloro-4-pyridyl)-1,2,4-triazole, 5 mL of 2-phenylethylamine, and 15 mL of water was placed in a steel bomb and heated at 175 °C for 15 h. The bomb was cooled; the contents were removed and then partitioned between ethyl acetate and water. The ethyl acetate layer was separated, and the aqueous mixture was extracted twice more with ethyl acetate. The combined organic extracts were dried (Na_2SO_4), filtered, and evaporated leaving an oil, which solidified after trituration with toluene and brief scratching. Recrystallization of this solid from water afforded 0.69 g (49%) of 3-amino-5-[2-[*N*-(2-phenylethyl)amino]-4-pyridyl]-1,2,4-triazole (**36**), mp 182–184 °C.

Method D. 3-Amino-5-[2-[(*p*-chlorobenzyl)amino]-4-pyridyl]-1,2,4-triazole (38). A mixture of 1.20 g (5.6 mmol) of 2-chloroisonicotinic acid 2-amidinohydrazide, 8 mL of *p*-chlorobenzylamine, and 20 mL of water was heated at 75 °C for 60 h. The mixture was cooled and then extracted 4x with 20-mL portions of ethyl acetate. The combined ethyl acetate extracts were dried (Na_2SO_4), filtered, and evaporated, leaving a crude oil. Chromatography over silica gel using 19:1 chloroform/methanol as eluent afforded a white solid. Recrystallization from acetonitrile/toluene gave 720 mg (43%) of **38** as a white solid, mp 176–177 °C.

Method E. Synthesis of 3-Methyl-5-[2-(*N*-ethylamino)-4-pyridyl]-1,2,4-triazole (11) from 3-Methyl-5-(2-chloro-4-pyridyl)-1,2,4-triazole. 2-Chloroisonicotinonitrile (6.9 g, 49.8 mmol) was stirred with sodium methoxide (5.2 mmol) from 120.2 mg of sodium in 100 mL of methanol. After 1 h at 25 °C, 3.7 g (49.9 mmol) of acetic acid hydrazide was added to give after several minutes a clear solution. The reaction was heated at reflux for 20 h at which time a precipitate began to form. Reflux was continued for an additional 120 h during which time the reaction precipitate went into solution. The reaction was cooled, and the initially formed precipitate was removed by filtration. The mother liquors were concentrated in vacuo with formation of a second precipitate. This was collected by filtration and dried to give 1.8 g (19%) of 3-methyl-4-(2-chloro-4-pyridyl)-1,2,4-triazole, mp 215–218 °C.

3-Methyl-5-(2-chloro-4-pyridyl)-1,2,4-triazole (1.6 g, 8.22 mmol) was combined with 50 mL of 70% aqueous ethylamine and the resultant mixture placed in a 150-mL stainless-steel reaction vessel and heated at 170 °C for 16.5 h. By a similar procedure, 4.4 g (22.6 mmol) of 3-methyl-5-(2-chloro-4-pyridyl)-1,2,4-triazole was combined with 50 mL of 70% aqueous ethylamine and heated at 170 °C for 16.5 h. On cooling, the contents of the reaction vessels were combined and concentrated in vacuo to a crude brown solid. Water was removed by repeatedly concentrating in vacuo with methanol to give a crude brown solid. This material was crystallized from acetonitrile/ethanol to give a pale brown solid, mp 223–227 °C. This material was recrystallized from acetonitrile/ethanol to give 1.63 g (26%) of **11** as an off-white solid, mp 226–228 °C.

Method F. 3-Methyl-5-(2-benzyl-4-pyridyl)-1,2,4-triazole (15). To 40 mL of 2 N sulfuric acid were added 6.04 g (40 mmol) of 3-methyl-5-(4-pyridyl)-1,2,4-triazole, 676 mg (4.0 mmol) of silver nitrate, and 27.2 g (200 mmol) of phenylacetic acid. The mixture was warmed to 90 °C, and a solution of 9.12 g (40 mmol) of ammonium peroxydisulfate in 20 mL of warm water was added over 20 min. After addition was complete, the mixture was warmed at 100 °C for 1 h. The mixture was cooled and then brought to pH 10 with ammonium hydroxide, and the resulting solid was removed by filtration. The aqueous solution was extracted with chloroform, dried (Na_2SO_4), filtered, and evaporated, leaving an oil. This was chromatographed over silica gel with 49:1 chloroform/ethanol as eluent to afford 950 mg (10%) of **15** as a white solid. Analytically pure **15**, mp 185–187 °C, could be obtained by recrystallization from ethyl acetate.

Method G. 4-(4-Pyridyl)-2-methyl-1*H*-imidazole (21). A mixture of 1.05 g (5.00 mmol) of 2-(4-pyridyl)-2,2-diethoxyethylamine,[16] 0.70 g (5.00 mmol) of ethyl acetimidate hydrochloride, and 30 mL of absolute ethanol was heated at reflux for 1 h, during which time the mixture became homogeneous. The mixture was concentrated and the residue taken up into 10 mL of concentrated hydrochloric acid and then heated on a steam bath for 1 h. The mixture was concentrated, and the residue was dissolved in 15 mL of H_2O and neutralized with solid potassium carbonate. The aqueous mixture was evaporated to dryness, the residue was triturated with 2-propanol, and the insolubles were removed by filtration. Evaporation of the filtrate afforded a crude solid that was purified over silica gel with 19:1 chloroform/methanol as eluent to give 0.32 g (40%) of **21** as a white solid. Analytically pure material, mp 152–153 °C, could be obtained by recrystallization from acetone.

3-(Methylamino)-5-[2-(ethylamino)-4-pyridyl]-1,2,4-triazole (8). A mixture consisting of 8.0 g (0.0444 mmol) of 2-(ethylamino)isonicotinic acid hydrazide[2] and 10.3 g (0.0444 m) of *N*-methyl-*S*-methylpseudothiourea hydriodide was placed in a solution of 1.77 g (0.0444 m) of sodium hydroxide in 50 mL of water. The resulting mixture was then heated at the reflux point for a period of 22 h, cooled to room temperature (~25 °C), and filtered. The recovered solid product was subsequently dried in vacuo to constant weight, and there was ultimately obtained 1.9 g (19%) of pure **8**, mp 273–276 °C.

(14) Lipinski, C. A. German Patent 3 022 302, Jan 8, 1981; *Chem. Abstr.* **1981**, *94*, 175124 *m*.

(15) Baumler, J.; Sorkin, E.; Erlenmeyer, E. *Helv. Chim. Acta* **1951**, *34*, 496.

(16) LaMattina, J. L. *Synthesis* **1980**, 329.

3-(Ethylamino)-5-[2-(ethylamino)-4-pyridyl]-1,2,4-triazole (9). 2-Chloroisonicotinic acid 2-amidinohydrazide (60 g, 0.963 mmol) and 600 mL of 70% aqueous ethylamine were reacted at 160 °C for a period of 40 h. Upon completion of the reaction, the contents of the tube were cooled to room temperature and the reaction mixture was concentrated in vacuo to afford a thick syrup that was chromatographed on silica gel with chloroform/methanol as the eluant. The first fractions collected were saved and concentrated in vacuo to a crude solid. Trituration with ethyl acetate/methanol, followed by filtration, then gave a white solid on the filter funnel and a dark organic solution as the filtrate. The filtrate was saved and subsequently evaporated to dryness while under reduced pressure to yield 36.1 g of a crude oil. The latter (oil) was then chromatographed on silica gel with ethyl acetate/methanol (95:5 by volume) as the eluant to afford a crude white solid. Trituration of the latter product with ethyl acetate/diethyl ether and then with methanol finally gave 369 mg (0.165%) of **9**, mp 224–226 °C.

Partition Coefficient Measurements. Compounds listed in Table III were analyzed for relative lipophilicity by reversed-phase high-performance liquid chromatography (RP HPLC). Mobile phase for this isocratic assay consisted of 50% v/v methanol and 50% of a mixed buffer containing 0.05 M KH_2PO_4 and 0.01 M NaAc at pH 7.4. At this pH and cosolvent level, one would expect all of this series of compounds to be at least 90–95% in the neutral form; hence, there was no attempt to correct the HPLC data for this minor extent of ionization.

Stationary phase for this assay consisted of a C-18 μ-Bondapak column (Waters Associates, 10-μm packing, 30 cm × 3.9 mm i.d.). Flow was set at 1 mL/min with detection at 254 nm. Competitive (n = 4) analysis of compound **32** gave an average retention time of 3.69 min with standard deviation of 0.2%.

Retention times (t) for each analogue were converted to HPLC capacity factors (k') by considering the system void column (t_0) in time units as shown in eq 1. These capacity factors (k') were

$$k' = (t - t_0)/t_0 \quad (1)$$

linearly correlated with two manually measured octanol/water partition coefficients ($P_{o/w}$) as described in eq 2 where m is the

$$\log P_{o/w} = m \log k' + C \quad (2)$$

slope and C the intercept for this relationship. The remaining capacity factors were then converted via this linear equation to measured partition coefficients (measured log P). Since this lipophilicity relationship was established with only two compounds over the log P range of 0.1–1.1, the absolute but not relative log P values in excess of about 1.5 may contain significant error.

Histamine H_2-Antagonist Activity. The procedure is a modification of that described by Black.[17] Guinea pigs were killed rapidly with a blow to the head, the heart was removed, and the right atria were dissected free. Atria were suspended, isometrically, in a temperature-controlled (32 ± 2 °C) tissue bath (10 mL) containing oxygenated (95% O_2/5% CO_2) Krebs–Henseleit buffer (pH 7.4) and allowed to stabilize approximately 1 h, during which time the tissue bath was flushed several times. Individual atrial contractions were followed with a force-displacement transducer connected to a cardiotachometer and Grass polygraph recorder. After a dose–response curve to histamine was obtained, the bath containing each atrium was flushed several times with fresh buffer, and the atria were reequilibrated to basal rates. Following the return to basal rate, test compounds were added at selected final concentrations, and the histamine dose–response curve was again determined in the presence of antagonist. Dose ratios (DR) were calculated as the ratio of histamine concentrations required to produce half of maximal stimulation in the presence and absence of antagonist concentration, B. The arithmetic means plus or minus standard deviation of the Schild plots in n atria of log (DR − 1) vs. log B unless specifically stated were not significantly different from unity. Most of the compounds studied, like cimetidine, equilibrated rapidly with the atria within a 3-min time period. Accordingly, experimental conditions for equilibration times were selected with the expectation that these properties would be encountered for all analogues. Some compounds, however, although inducing a clear parallel shift to the right of the histamine dose–response curves and a surmountable antagonism exhibited Schild plot slopes greater than 1.00 that could be attributable to insufficient time for equilibration with drug. Compounds **18–20** were tested using both short (3-min) and long (30-min) equilibration times. The $K_{B_{app}} \times 10^{-6}$ and Schild plot slope for these compounds using 30- and 3-min equlibration times were as follows: **18**, 3.29 (±2.81), slope 1.96; 1.05 (±0.31), slope 1.56; **19**, 0.59 (±0.59), slope 1.03; 3.92 (±3.18), slope 1.69; **20**, 1.38 (±0.79), slope 1.19; 2.88 (±3.34), slope 1.72. For 30-min equilibration time experiments, 9–12 points and four atria were used. Dissociation constants ($K_{B_{app}}$) were calculated with the theoretical value of 1.00 required for competitive antagonism.

Gastric Acid Antisecretory Activity. Compounds were tested for their ability to inhibit gastric acid secretion in fasted unanesthetized Heidenhain pouch dogs. Animals were first administered pentagastrin in order to stimulate acid output by continuous infusion of drug into a superficial leg vein at doses earlier determined to stimulate near maximal acid output from the gastric pouch. Gastric juice was then collected at 30-min intervals following the start of a pentagastrin infusion, and volume was measured to the nearest one-tenth of a millimeter (0.1 mL). Ten collections were taken for each dog during an experiment. Acid concentration was determined by titrating 0.1 mL of gastric juice to a pH value of 7.4 with 0.1 N aqueous sodium hydroxide, using an Autoburette and a glass electrode pH meter (Radiometer). Animals were adminstered the test compounds at a specified dose in milligrams per kilogram, respectively, or the control vehicle alone, via the intravenous route of administration, at 90 min following the starting of the pentagastrin infusion. Gastric antisecretory effects were calculated by comparing the lowest acid output after drug administration with the mean acid output immediately prior to drug administration. The results obtained in this manner were expressed in terms of percent inhibition. The dose required for 50% inhibition (ID_{50}) for **2**, **4**, and cimetidine was calculated from the linear regression of log dose on percent inhibition in n dogs. The mean percent inhibition in pentagastrin stimulated dogs given 1 mg/kg iv. of cimetidine was 46 ± 11%.

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Registry No. 1, 3652-17-3; 1(Z = Cl), 77314-55-7; **2**, 77314-75-1; **3**, 77314-76-2; **4**, 77314-77-3; **5**, 98087-86-6; **6**, 98087-87-7; **7**, 98087-88-8; **8**, 77314-83-1; **9**, 77314-68-2; **10**, 86649-16-3; **10** (Z = Cl), 86649-15-2; **11**, 86649-14-1; **11** (Z = H), 57980-40-2; **11** (Z = Cl), 86649-12-9; **12**, 86649-17-4; **12** (Z = Cl), 98087-99-1; **13**, 98087-89-9; **13** (Z = Cl), 98088-00-7; **14**, 98087-90-2; **15**, 98087-91-3; **16**, 86649-19-6; **16** (Z = Cl), 86649-18-5; **17**, 98087-92-4; **17** (Z = Cl), 98088-01-8; **18**·HCl, 98087-93-5; **21**, 98087-94-6; **22**, 80882-69-5; **23**, 77314-58-0; **24**, 77314-79-5; **25**, 77314-59-1; **26**, 77314-60-4; **27**, 77314-65-9; **28**, 77314-66-0; **29**, 98087-95-7; **30**, 77314-61-5; **31**, 77314-62-6; **32**, 77314-64-8; **33**, 77314-80-8; **34**, 98087-96-8; **35**, 77314-71-7; **36**, 77314-69-3; **37**, 98087-97-9; **38**, 77314-72-8; **39**, 77314-73-9; **40**, 77314-84-2; **41**·HCl, 98087-98-0; $H_2NC(SCH_3)$=$NH \cdot Y_2H_2SO_4$, 867-44-7; $H_2NNHC(NH_2)$=$NH \cdot {}^1/_2H_2SO_4$, 1068-42-4; $C_6H_5(CH_2)_2NH_2$, 64-04-0; 4-$ClC_6H_4CH_2NH_2$, 104-86-9; $CH_3CONHNH_2$, 1068-57-1; $C_2H_5NH_2$, 75-04-7; $C_6H_5CH_2CO_2H$, 103-82-2; $CH_3C(OC_2H_5)$=NH·HCl, 2208-07-3; HN=$C(SCH_3)N$-HCH_3·HI, 41306-45-0; $C_6H_5NH_2$, 62-53-3; $C_3H_7NH_2$, 107-10-8; n-$C_6H_{13}NH_2$, 111-26-2; n-$C_{10}H_{21}NH_2$, 2016-57-1; $(CH_3)_2NH$, 124-40-3; $C_2H_5NHCH_3$, 624-78-2; CH_2=$CHCH_2NH_2$, 107-11-9; $HO(CH_2)_2NH_2$, 141-43-5; $CH_3O(CH_2)_2NH_2$, 109-85-3; $C_6H_5CH_2$-NH_2, 100-46-9; $C_6H_5(CH_2)_3NH_2$, 2038-57-5; 4-$CH_3C_6H_4CH_2NH_2$, 104-84-7; 3,4-$(CH_3)_2C_6H_3CH_2NH_2$, 102-48-7; 4-t-$C_4H_9C_6H_4CH_2NH_2$, 39895-55-1; $HCONHNH_2$, 624-84-0; C_2H_5C-$ONHNH_2$, 5818-15-5; $C_3H_7CONHNH_2$, 3538-65-6; $HOCH_2CON$-HNH_2, 3530-14-1; $C_6H_5CH_2CONHNH_2$, 937-39-3; $HOCH_2CO_2H$, 79-14-1; H_2NCN, 420-04-2; 2-(N-methylamino)isonicotinic acid hydrazide, 77314-45-5; isonicotinic acid hydrazide, 54-85-3; 2-aminoisonicotinic acid hydrazide, 58481-01-9; 2-(N-ethylamino)isonicotinic acid hydrazide, 77314-47-7; 2-(N-methylamino)isonicotinic acid 2-amidinohydrazide, 77314-46-6; iso-

(17) Black, J. W.; Duncan, W. A. M.; Durant, G. J.; Ganellin, C. R.; Parsons, M. E. *Nature (London)* **1972**, *236*, 385–390.

nicotinic acid 2-amidinohydrazide, 4427-16-1; 2-aminoisonicotinic acid 2-amidinohydrazide, 77314-44-4; 2-(*N*-ethylamino)isonicotinic acid 2-amidinohydrazide, 77314-48-8; 2-methylisonicotinic acid, 4021-11-8; 2-ethylisonicotinic acid, 3376-96-3; 2-propylisonicotinic acid, 57663-82-8; 2-(*N*-butylamino)isonicotinic acid, 77314-78-4; 2-(*N*-isopropylamino)isonicotinic acid, 77314-51-3; 2-pyrrolidinoisonicotinic acid, 98088-04-1; 2-chloroisonicotinic acid hydrazide, 58481-04-2; 2-chloroisonicotinic acid 2-amidinohydrazide, 77314-43-3; 2-chloroisonicotinonitrile, 33252-30-1; 2-(4-pyridyl)-2,2-diethoxyethylamine, 74209-44-2; 2-(2-methyl-4-pyridyl)-2,2-diethoxyethylamine dihydrochloride, 98088-03-0; 2-(*N*-ethylamino)-4-glycylpyridine dihydrochloride, 98088-02-9.

Dog Coronary Artery Adenosine Receptor: Structure of the N^6-Alkyl Subregion

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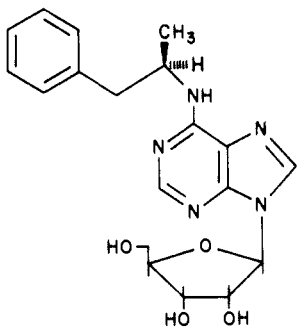
The moderately potent and stereoselective coronary vasoactivity of N^6-[1-phenyl-2(*R*)-propyl]adenosine (1) is the basis for the present study that maps the N^6 region of the coronary artery adenosine receptor by means of the structure–coronary vasoactivity relationships of 81 analogues of 1 in the open-thorax dog. Stereoselectivity is a general property of N^6-substituted adenosines that have a chiral center adjacent to N^6. The activity ratio of 1 to its *S* diastereomer is 10, the result of the positive interaction with the receptor of the propyl C-3 group of the *R* diastereomer in combination with the steric hindrance exerted by this group of the *S* diastereomer. Replacing the benzyl moiety of 1 by an ethyl, phenyl, phenethyl, or naphthyl group lowers potency of the *R* diastereomer and, accordingly, the *R*/*S* ratio. Propyl C-1 of 1 interacts with a receptor region large enough to accommodate three methylene residues and the propyl C-3 residue with a separate region large enough to accommodate two. The receptor subregion that interacts with the propyl C-1 of 1 is more tolerant of bulk and of polar substituents than the subregion that interacts with propyl C-3. Evidence bearing on the possible contribution of N^6 to activity, e.g. through hydrogen bonding, is ambiguous. These results support a provisional model of the N^6-alkyl subregion.

A large body of evidence indicates that the adenylate cyclase stimulatory (A2 or R_a) type of purinergic receptor mediates the coronary vasoactivity of adenosine.[1] One line of evidence is the order of coronary vasodilator potency of adenosine receptor-selective analogues in the open-chest dog: *N*-ethyladenosine-5′-uronamide > *N*-cyclopropyladenosine-5′-uronamide ≫ 2-chloroadenosine > N^6-[1-phenyl-2(*R*)-propyl]adenosine (1) > adenosine > N^6-[1-phenyl-2(*S*)-propyl]adenosine (**2**).[2] Although such an order of potency is unusual for an A2 receptor in that 1 is more active than adenosine, it does have precedent in tracheal smooth muscle[3] and in the adenylate cyclase A2 receptors of guinea pig thyroid epithelium[4] and rat aorta smooth muscle cells.[5] While the preeminent vasoactivity of the *N*-alkyladenosine-5′-uronamides implies that the ribose domain of the A2 receptor contains the "ethyl pocket" that seems to be characteristic of A2 receptors,[6,7] the potencies of 2-chloroadenosine and 1 suggest the existence of additional regions of specialized structure in the purine domain.



1

A previous survey of 128 adenosine analogues as agonists at the A2 receptor of a transformed human fibroblast line[7] included 18 purine ribosides modified at C-6. Although the number of N^6-alkyladenosines was too limited to define structure–activity rules, the potency of these analogues showed that an N^6-alkyl substituent makes a modest contribution to activity.

The stereoselective coronary vasoactivity of 1[2,8] is the basis for the hypothesis tested in this study, namely, that the coronary A2 receptor contains a specialized N^6 region. Deriving the structure–activity rules of the N^6-substituted adenosines constitutes one test of this hypothesis. The experimental design, i.e. the selection of analogues for testing, is based on a model of the N^6 receptor region[9] derived from the structure of 1. This model (Figure 1) posits that each of several chemical attributes of the purine C6 substituent could contribute to potency: 1, the absolute configuration at the propyl C-2 chiral center; 2, the size and hydrophobicity of propyl C-1 and C-3; 3, the chemistry of N^6, especially its potential to act as either a donor or acceptor in hydrogen bonding; 4, the length of the alkyl chain separating the phenyl moiety from N^6; and 5, the chemical attributes of the phenyl moiety e.g. aromaticity, planarity, size, and its angulation and torsion relative to the axis of the propyl C-1 to C-2 bond. For convenience we consider the N^6 region of the coronary A2 receptor as composed of alkyl and phenyl subregions. The experiments described here concern only the structure of the alkyl subregion and thus deal with attributes 1–3 of the model depicted in Figure 1.

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(1) Kusachi, S.; Bugni, W. J.; Olsson, R. A. *Circ. Res.* **1984**, *55*, 116.
(2) Kusachi, S.; Thompson, R. D.; Olsson, R. A. *J. Pharmacol. Exp. Ther.* **1983**, *227*, 316.
(3) Brown, C. M.; Collis, M. G. *Br. J. Pharmacol.* **1982**, *76*, 381.
(4) Fradkin, J. E.; Hardy, W. C.; Wolff, J. *Endocrinol.* **1982**, *110*, 2018.
(5) Anand-Srivastava, M. B.; Franks, D. G.; Cantin, M.; Genest, J. *Biochem. Biophys. Res. Commun.* **1982**, *108*, 213.
(6) Stein, H. H.; Somani, P.; Prasad, R. N. *Ann. N.Y. Acad. Sci.* **1975**, *255*, 380.
(7) Bruns, R. F. *Can. J. Physiol. Pharmacol.* **1980**, *58*, 673.
(8) Vapaatalo, H.; Onken, D.; Neuvonen, P. J.; Westerman, E. *Arzniem.-Forsch.* **1975**, *25*, 407.
(9) Olsson, R. A. *Trends Pharmacol. Sci.* **1984**, *5*, 113.

0022-2623/85/1828-1636$01.50/0