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Medicinal Chemistry and Dynamic Structure–Activity Analysis in the Discovery of Drugs Acting at Histamine H_2 Receptors‡

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Applications of physical organic chemistry and dynamic structure–activity analysis are illustrated by studies made during the development of specific drugs acting as antagonists of histamine at H_2 receptors. Imidazolylalkylguanidines, isothioureas, and carboxamidines were found to be partial agonists, and attempts were made to remove the agonist component by lengthening the side chain and replacing the strongly basic group in these molecules by nonbasic groups. This approach furnished thioureas and cyanoguanidines as competitive antagonists, e.g., burimamide and cimetidine. Thoughts about molecular interactions led to the discovery of other nonbasic chemical groups for antagonist structures, in particular 1,1-diamino-2-nitroethenes and 2-amino-pyrimidin-4-ones (isocytosines). Such moieties are incorporated, respectively, in the recently described antagonist drugs ranitidine and oxmetidine.

It is a great honor and extremely flattering to be singled out this year (1980) as the first recipient of the new ACS Division of Medicinal Chemistry Award, and I am especially impressed by the great generosity of spirit demonstrated by the Division in looking abroad when there is so much outstanding medicinal chemistry practiced in the United States. Luck is a well-known determinant of drug research and it has been my good fortune to work with a remarkable pharmacologist, Sir James Black, whose perceptive questions opened my eyes to medicinal chemistry, and to be involved with a drug discovery which has been an outstanding therapeutic success. I am extremely appreciative of receiving this award because I do have great enthusiasm for medicinal chemistry and for drug research, but I am particularly mindful that many people contributed to the work that I shall describe and I must identify for special mention my two colleagues in medicinal chemistry, Dr. Graham Durant and Dr. John Emmett. The three of us have had a very close and most exciting collaboration and, in accepting this award, it is clear that they too are being honored.

Structure–activity analysis in new drug discovery is invariably told retrospectively, especially when the research comes from an industrial laboratory. One is apparently forced into this position by the need for confidentiality; as a consequence, one loses the excitement of the immediacy of the discovery and is faced with the temptation of editing out all one's mistakes and stupid actions, thereby making oneself out to be really clever: I have tried hard to resist this temptation. Much structure–activity work is, in any case, concerned with the rationalization of discoveries after the event. However, does predictive structure–activity analysis really exist? In our attempts to discover drugs acting as antagonists of histamine at H_2 receptors we have tried to use chemical properties to provide a link between chemical structure and the biological properties and have drawn heavily upon physical-organic chemistry. This led naturally into the dual exercise of determining the chemical properties of drug molecules and of trying to discern which properties were most critical for biological activity. There is a continuous process, viz., one is continuously analyzing for relationships between chemical properties and biological activity, then predicting the next compounds to be made, and, finally, finding out how to synthesize them.

Molecular interactions between molecules are determined fundamentally by molecular size, shape, and charge distribution, but when one inspects molecules in these terms one becomes aware that they rarely have a unique description and there may be several different forms or species in equilibrium. Thus, although molecules are usually represented on paper as rigid structures, they may in fact be quite different in solution and their dynamic nature should be recognized. I shall be mentioning examples of conformational equilibria and prototropic equilibria, from which an interesting question arises: If one changes drug structure to alter the equilibrium, can one relate the consequences to changes in biological activity? If one can, one may obtain some insight into the mechanism of drug action and provide a method for drug design; one may call this approach[1] *dynamic structure–activity analysis* (DSAA) (Scheme I).

In 1966, Ash and Schild[2] published an explicit definition of histamine H_1 receptors (i.e., those actions of histamine competitively and specifically blocked by the antihistamine drugs, e.g., stimulation of contraction of smooth muscle from the guinea pig ileum or bronchi) and indicated the need for specific antagonists to classify other receptors for histamine. The problem had also been recognized by other researchers; indeed, in 1964 Dr. James Black had initiated

‡Award address delivered at the 17th National Medicinal Chemistry Symposium, Rensselaer Polytechnic Institute, Troy, NY, June 18th, 1980.

(1) C. R. Ganellin, in "Drug Action at the Molecular Level", G. C. K. Roberts, Ed., Macmillan, London, and University Park Press, Baltimore, 1977, pp 1–39.
(2) A. S. F. Ash and H. O. Schild, *Br. J. Pharmacol. Chemother.*, **27**, 427 (1966).

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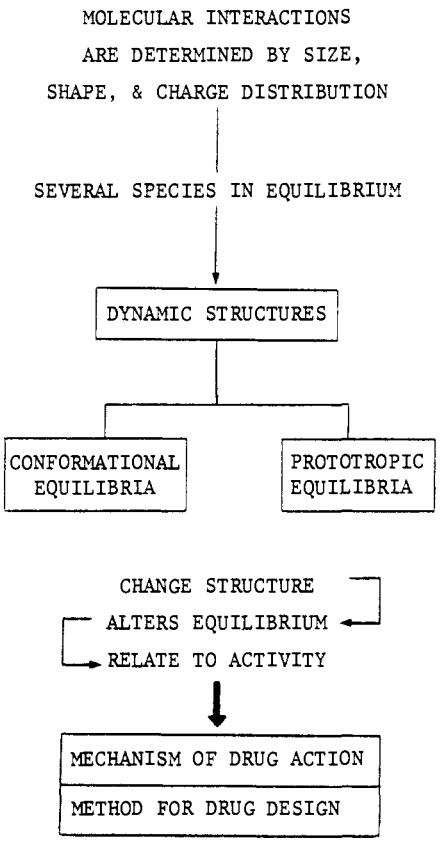
Scheme I. The Dynamic Nature of Molecules Is Accommodated in the Technique of Dynamic Structure-Activity Analysis (DSAA)

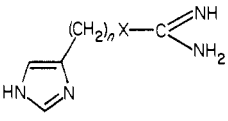


such a search in our laboratories at Smith Kline and French (Welwyn Garden City, U.K.) and, together with Michael Parsons, had set up as the primary screening assay, the examination of compounds for their effect on histamine-stimulated gastric acid secretion in the anesthetized rat, measuring the pH of the perfusate from the lumen of the stomach.[3]

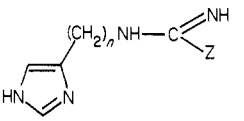
I will not dwell on our early attempts to find an antagonist other than to say that our simple-minded approach was to go back to first principles and to take histamine as our starting point (e.g., see ref 4). We thought in terms of an antagonist having some chemical similarity to histamine to aid receptor recognition, being sufficiently different so as not to stimulate a response, and possessing additional groups to assist receptor binding. We spent 4 years and made some 200 compounds before uncovering a lead, viz., N^{α}-guanylhistamine (**1a**) (Table I), which was very weakly active as an inhibitor of histamine stimulation; it had indeed been synthesized at the beginning of the research by my colleague Dr. Graham Durant but it had been missed in the initial testing because it also acted as a stimulant. It was in fact later shown to be a partial agonist.[5] Within a few days, another early compound synthesized by my colleague Dr. John Emmett was found to be more active, viz., the analogous isothiourea (**1b**). These compounds were our first real leads, and the task was to synthesize a much more potent antagonist. The structures are obviously closely related, being simple isosteric N and S analogues.

(3) M. E. Parsons, Ph.D. Thesis, University of London, 1969.
(4) C. R. Ganellin, G. J. Durant, and J. C. Emmett, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, **35**, 1924 (1976).
(5) G. J. Durant, M. E. Parsons, and J. W. Black, *J. Med. Chem.*, **18**, 830 (1975).

Table I. Structures and Antagonist Activity[a] of Some Imidazolylalkylisothioureas, -guanidines, and -amidines



1a-d

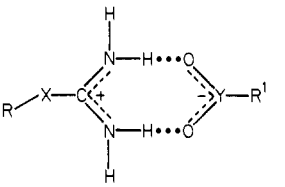


3a-d

no.	n	X	act.[a]
1a	2	NH	+
1b	2	S	+ +
1c	3	NH	+ + +
1d	3	S	±
		Z	
3a	2	SMe	±
3b	2	Me	±
3c	3	SMe	+ + +
3d	3	Me	+ + +

[a] Tested for inhibition of histamine-stimulated gastric acid secretion in the lumen-perfused anesthetized rat. Results represented semiquantitatively as: ±, detectable; +, $ID_{50} > 500$ μmol/kg; ++, $ID_{50} \approx 200$ μg/kg; +++, $ID_{50} = 100$–50 μmol/kg. ID_{50} is the intravenous dose which reduces a near maximal secretion to 50%.

Structural variables initially identified for study were the amidine groups, amidino N-substituents, side-chain length, and alternatives to imidazole. The amidine group differs from the ammonium group in histamine in being planar and in having more opportunities for H bonding. We envisaged that it might act as an antagonist through the type of H bonding suggested by Walker for the "doublet ion pairing" between amidines and oxy acids as in formula **2**. Walker drew attention[6] to the special sta-



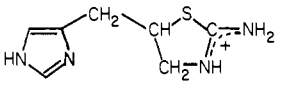
2, amidinium oxy acid salt
(H-bonded ion pair envisaged by Walker[6])

bility of isothiouronium salts of carboxylic and sulfonic acids, stating that "anion and cation hold each other electrostatically and rigidly in a preferred orientation, and this behavior may well underlie the activities of amidines, guanidines, isothioureas, and isoureas as drugs". We made analogues of **1a,b**, most of which turned out to be less active; at that time, unsubstituted imidazole appeared to be the best ring, alkyl substitution on the amidine N gave inconsistent results, and lengthening the side chain gave another breakthrough but threw up an apparent contradiction.

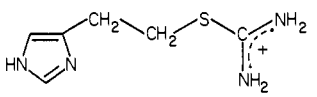
For the guanidine structure, increasing the chain length led to a compound (**1c**) showing an increase in antagonist activity. However, for the isothiourea, the reverse result was obtained; i.e., increasing the chain length gave a compound (**1d**) of reduced antagonist activity. Clearly, N and S could no longer be regarded as simple isosteric replacements and, in an attempt to rationalize these differences, various related amidines were examined. The activities of these compounds are expressed semiquantitatively in Table I, reflecting the data available at the time.

(6) J. Walker, *J. Chem. Soc.*, 1996 (1949); O. Kennard and J. Walker, *ibid.*, 5513 (1963).

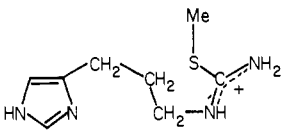
It was found that the reversed isothioureas **3a,c** (side chain on N instead of S) resembled the guanidines in chain-length requirements, as did carboxamidines, e.g., **3b,d**. These results indicated that we should reappraise our view of amidine interactions. The initial analysis had suggested H bonding of the terminal NH groups; in the reversed isothiourea and carboxamidine, H bonding would have to be lateral. Could these differences be reconciled? Comparison of space-filling molecular models suggested that a folded form of the *N*-(imidazolylpropyl)isothiourea (**3c**) may resemble the extended form of *S*-(imidazolylethyl)-isothiourea (**1b**) with respect to the relative positions of imidazole rings, S atoms, and amidine NH groups. The comparison reinforced the idea of a bidentate H-bonding interaction and also predicated a new structure, viz., the aminothiazoline (**4**). Eventually, **4** was made but it proved



4, 5-(imidazolylmethyl)-2-aminothiazoline
(a composite of structures 1b and 3c)



1b



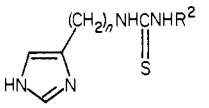
3c

to be only weakly active, from which we concluded that the side chain probably needed flexibility. It was clear that if there were any substance to these ideas on ion pairing, then the effect of substituents on S or N could be dramatic, since they would alter conformational preference; this led to NMR studies of the conformation of simple model compounds.

The apparent nonadditivity between structural change and biological effect posed a typical problem familiar to all practicing medicinal chemists; viz., with so many structural variables to study (e.g., ring, side-chain length, amidine system, amidine substituents), there are many hundreds of structures incorporating different combinations of these variables, and one cannot make and test them all. What then should govern the selection? It is my belief that an essential feature of the discipline in medicinal chemistry is to find logical bases for defining the boundary conditions for the selection of structures for synthesis. In our case, we continuously searched for useful physicochemical models for studying the chemistry of these compounds and used the inconsistencies in the structure–activity pattern to challenge our model or to reexamine the meaning of the biological test results. This dialogue, a search for self-consistency between the chemistry and the biology, is vital to new drug research where no precedent exists.

We continued exploring amidines and substituents, but it became clear that we were not making progress. The problem seemed to be that the compounds had mixed activities, although to varying degrees. In the main they acted both as agonists and as antagonists; i.e., they appeared to be partial agonists.[7] This meant that the compounds could block histamine, but they could not block acid secretion, since they acted as stimulants. This appeared to impose a limitation on the potential of this type of structure for providing antagonists and seemed to be hindering our progress. Thus, we reached a critical stage in the need for selectivity: we had to achieve a separation between agonist and antagonist activities. It occurred to us that these compounds might act as agonists by mimicking histamine chemically. Like histamine, they are imidazoles, and we noted another common feature in the side chain; viz., at physiological pH it carries a positive charge. It seemed likely that these features permitted receptor recognition and provided binding for a competitive antagonist but allowed the molecule to mimic histamine and act as an agonist. This posed a considerable dilemma because the chemical groups which appeared to be required for antagonist activity were the same groups that seemed to confer the agonist effect. In an attempt to separate these activities, the strongly basic amidine group was replaced by nonbasic groups which, though polar, would not be charged. Some nonbasic analogues were made, and these were found to lack stimulant activity but, unfortunately, they were not antagonists!

Eventually a thiourea analogue (**5a**) was found to have



5a, $n = 3$; R^2 = H
b, $n = 4$; R^2 = H
c, $n = 4$; R^2 = Me (burimamide)

weak antagonist activity without being a stimulant, but it was not until the side chain was lengthened still further that the significance became clear and the desired aim was achieved, i.e., a pure competitive antagonist without agonist effects. This compound (**5b**, SK&F 91863) paved the way for the synthesis of substituted analogues, and in a short while the *N*-methyl analogue burimamide (**5c**) was obtained. Burimamide was shown to be an effective inhibitor of histamine-stimulated gastric acid secretion in the rat, cat, dog, and man. It was also shown to be a highly specific and competitive antagonist of histamine on two in vitro non-H_1 test systems, viz., histamine stimulation of the rate of the spontaneously beating guinea pig right atrium and histamine inhibition of electrically evoked contractions of the rat uterus, thereby defining histamine H_2 receptors and allowing burimamide to be classified as an H_2-receptor histamine antagonist.[8]

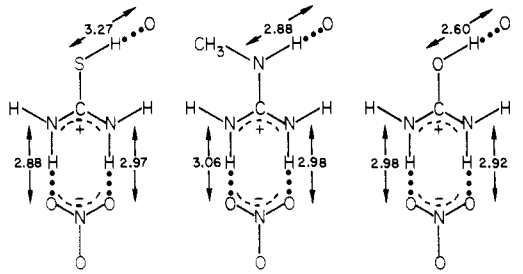
The activity of burimamide posed a structure–activity problem. If the amidine antagonists acted by ion pairing and H bonding, where did nonbasic thioureas fit in? The rats and guinea pigs saw a similarity, but the question remained: Could the medicinal chemist see one? A search of the literature revealed that thiourea will form crystalline salts with some carboxylic acids, e.g., oxalic and trichloroacetic. Furthermore, with HNO_3, the nitrate will even crystallize out from water, suggesting that the molecular interaction is sufficiently strong to permit desolvation. In the crystal,[9] protonation occurs on S, and the conjugate cation then H bonds to the oxyanion (**6a**), just as with amidinium oxy acid salts (e.g., methylguanidine nitrate,[10] **6b**). Thus, in principle the amidines and thiourea groups might resemble one another when undergoing a bidentate H-bonding interaction. It is interesting to note

(7) C. R. Ganellin, *J. Appl. Chem. Biotechnol.*, **28**, 183 (1978).

(8) J. W. Black, W. A. M. Duncan, G. J. Durant, C. R. Ganellin, and M. E. Parsons, *Nature (London)*, **236**, 385 (1972).

(9) D. Feil and W. Song Loong, *Acta Crystallogr., Sect. B*, **24**, 1334 (1968).

(10) R. M. Curtis and R. A. Pasternak, *Acta Crystallogr.*, **8**, 675 (1955).



6a 6b 6c

that there are no close H-bonding contacts from the SH, whereas there are with NH and OH; indeed, in the analogous urea nitrate,[11] OH···O is the shortest H bond in the crystal (**6c**). The urea analogue of burimamide was found, however, to be much less active as an antagonist, and this difference between urea and thiourea made us wonder whether the S atom was contributing to hydrophobicity (desolvation). I will return to this latter.

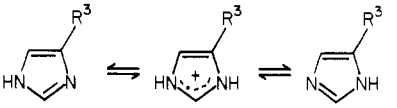
Thoughts about molecular interactions led to a paper by Gordon and Jencks[12] on "the relationship of structure to the effectiveness of denaturing agents for proteins". They examined over 100 small molecules for the effect on denaturing bovine serum albumin, as indicated by changes in optical rotation, and reported that in addition to the well known agents urea and guanidine hydrochloride, molecules such as methylisothiourea, methylisourea, acetamidine, and thiourea were effective denaturants. Thus, it appeared that there were many structural similarities between the compounds which functioned in denaturation and the groups found to confer activity in histamine antagonist structures. There were also some differences, so the parallelism was only partial. There is no intention to suggest that this is how the antagonists work. However, it does seem likely that a similar underlying chemical property is contributing. Gordon and Jencks suggested that effectiveness as denaturants may be explained by bifunctional H bonding, and this of course comes close to our working model of bidentate H bonding. The list of denaturants also includes biguanide, aminotriazole, and cyanoguanidine, and these were subsequently incorporated into antagonist structures but in each case for additional reasons.

Burimamide was the first H_2 antagonist to be tested in man and it confirmed the transferability of the animal pharmacology to humans. However, it lacked adequate oral activity needed for exploring its therapeutic potential, and it appeared that a more potent compound was required. Of various attempts made to produce a more suitable drug, a successful approach was based on the realization that burimamide in aqueous solution is a mixture of many chemical species in equilibrium (Scheme II). At physiological pH there are three main forms of the imidazole ring (**7a–c**), three planar configurations of the thioureido group [**7d,e,g**; a fourth (**7f**) is theoretically possible but is disfavored by internal steric hindrance], and various trans and gauche rotamer combinations of the side-chain CH_2–CH_2 bonds. This means that at any given instant only a small proportion of the drug molecules would be in a particular form.

The existence of a mixture of species leads one to question which may be biologically active and whether

Scheme II. Burimamide Species Equilibria in Solution

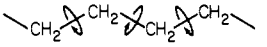
(a) Imidazole Ring (Ionization and Tautomerism)



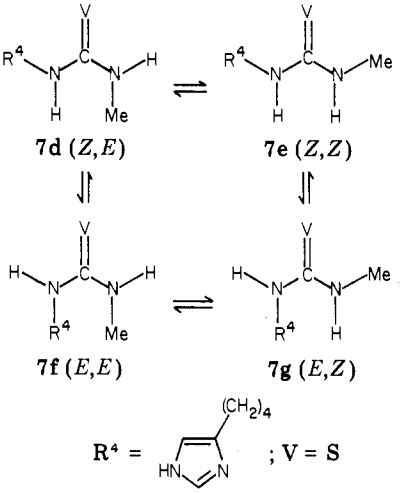
7a **7c** **7b**

$R^3 = (CH_2)_4NHC(=S)NHMe$

(b) Alkane Chain (C-C Bond Rotation Gives Trans and Gauche Conformers)



(c) Thiourea Group (V = S) (Configurational Isomerism)



7d (*Z,E*) **7e** (*Z,Z*)

7f (*E,E*) **7g** (*E,Z*)

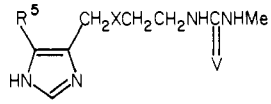
$R^4 =$; V = S

altering drug structure to favor a particular species would alter drug potency. There are substantial energy barriers to interconversion between the species of burimamide so that it is quite likely that a drug molecule, presenting itself to the receptor in a form unfavorable for drug–receptor interaction, might diffuse away again before having time to rearrange into a more favorable form. The relative population of favorable forms might therefore determine the amount of drug required for a given effect. The various species do not interconvert instantaneously, but whereas the rotamers of the side chain and thioureido groups are interconverted simply by internal rotation of a C–C or C–N bond, interconversion of the ring forms probably involves a water-mediated proton transfer. If a molecule presents itself to the receptor with the ring in an unfavorable form it might not readjust, unless there were suitably orientated water molecules (or other hydrogen donor–acceptors) present. The form of the ring therefore merits special consideration.

The above arguments led to a study of the population of imidazole species in burimamide in comparison with histamine.[4,13] At physiological pH the main species are the cation (**7c**) and two uncharged tautomers (**7a** and **7b**), and their populations were estimated qualitatively from the electronic influence of the side chain using pK_a data and the Hammett equation:[14] $pK_{a,R} = pK_{a,H} + \rho\sigma_m$. For burimamide, the ring pK (7.25) is greater than that of unsubstituted imidazole, indicating that the side chain is mildly electron releasing. In contrast, for histamine the

(11) J. E. Worsham and W. R. Busing, *Acta Crystallogr., Sect. B*, **25**, 572 (1969).
(12) J. A. Gordon and W. P. Jencks, *Biochemistry*, 2, 47 (1963).
(13) J. W. Black, G. J. Durant, J. C. Emmett, and C. R. Ganellin, *Nature (London)*, **248**, 65 (1974).
(14) M. Charton, *J. Org. Chem.*, **30**, 3346 (1965).

ammonium ethyl side chain was seen to be electron withdrawing, since it lowers the pK_a of the imidazole ring. Thus, although both histamine and burimamide are monosubstituted imidazoles, the structural similarity is misleading in that the electronic properties of the respective imidazole rings are different. If the active form of the antagonist were tautomer **7a**, the form most preferred for histamine, then increasing its relative population might increase activity; e.g., incorporating an electronegative atom into the antagonist side chain should convert it into an electron-withdrawing group and favor species **7a**. This would not be the only requirement for activity and it would be necessary to minimize disturbance to other biologically important molecular properties such as stereochemistry and lipid–water interactions. For reasons of synthesis, the first such substitution to be made was the replacement of a methylene group ($-CH_2-$) by the isosteric thioether linkage (–S–) at the carbon atom next but one to the ring, to afford "thiaburimamide" (**8a**) which was

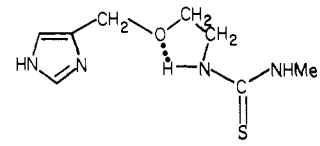


	R^5	X	V
8a (thiaburimamide)	H	S	S
b (metiamide)	Me	S	S
c (oxaburimamide)	H	O	S
d (urea isostere)	Me	S	O
e (guanidine isostere)	Me	S	NH
f (nitroguanidine isostere)	Me	S	NNO_2
g (cimetidine)	Me	S	NCN
h (SK&F 92456)	Me	S	$CHNO_2$

found to be more active as an antagonist. It was argued that a further stabilization of tautomer **7a** might be obtained by incorporating an electron-releasing substituent in the vacant 4(5) position of the ring. A methyl group was selected, since it was thought that it should not interfere with receptor interaction, 4-methylhistamine having been shown to be an effective H_2-receptor agonist.[8] This approach was successful, and introduction of a methyl group into the ring of the antagonist furnished the more potent drug metiamide[13] (**8b**). The two ring substituents appeared to have electronic effects of equal magnitude but of opposite direction, combining to favor tautomer **7a** but opposing in their effect on ring pK_a.

Although the above molecular manipulations were made through consideration of the electronic effects of substituents and the first compounds prepared were those most accessible synthetically, evidence has subsequently accrued to suggest that conformational effects are probably more important. Crystal structure studies indicate that the thioether linkage may increase molecular flexibility and the ring-methyl group may assist in orientating the imidazole ring.[15] Furthermore, the oxygen (ether) analogue (**8c**) which should fulfill the electronic requirements is less potent than burimamide, possibly by encouraging a different conformation through intramolecular H bonding (e.g., **9**).[16,17]

Metiamide represented a major improvement, being 10 times more potent than burimamide in vitro and a potent inhibitor of stimulated acid secretion in man. It was investigated for potential use in peptic ulcer therapy and was shown to produce a significant increase in the healing rate of duodenal ulcers and marked symptomatic relief. However, out of 700 patients treated there were a few cases of granulocytopenia, which, although reversible, limited the amount of clinical work.[18]



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The possibility existed that the granulocytopenia associated with metiamide was caused by the thiourea group in the molecule and this led to the need to examine another compound. Fortunately, we had continued to explore other possible structures and, in particular, had sought alternatives to the thiourea group. One approach taken was to examine isosteric replacement of the thiourea sulfur atom (=S) of metiamide. Replacement by carbonyl oxygen (=O) gave the urea analogue (**8d**), but this was much less active. We returned to the idea of guanidine derivatives which had provided the original breakthrough. Replacement by imino nitrogen (=NH) afforded the guanidine (**8e**) which, interestingly, was not a partial agonist but a fairly active antagonist. However, in vitro, the urea and guanidine isosteres were both about 20 times less potent than metiamide, and other ways were investigated for removing the positive charge. It came to our attention that nitroguanidine was not basic, and further investigation revealed a publication by Charton[19] on the Hammett relationship between σ and pK_a for substituted amidines and guanidines. Guanidine basicity is markedly reduced by electron-withdrawing substituents, and Charton demonstrated[19] a high correlation between the inductive substituent constant σ_I and pK_a for a series of monosubstituted guanidines. The cyano and nitro groups are sufficiently electron withdrawing to reduce the pK_a by over 14 units, to values <0; indeed, the ionization constants of cyanoguanidine ($pK_a = -0.4$) and nitroguanidine ($pK_a = -0.9$) approach that of thiourea (–1.2). The nitroguanidine (**8f**) and cyanoguanidine (**8g**) analogues of metiamide were synthesized and found to be active antagonists comparable with metiamide.[20] Of these two compounds, the cyanoguanidine (cimetidine, **8g**) is slightly more potent and was selected for development.[21]

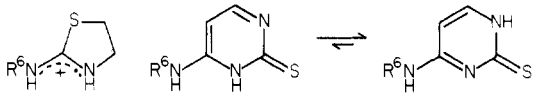
The guanidinium cation can lose a proton from each of the three nitrogen atoms to give three different forms of the conjugate base. Powerful electron-withdrawing substituents favor the imino tautomer over the amino tautomers, since the proton on the adjacent nitrogen in the cation is more acidic than the protons on the more distant terminal nitrogen atoms. Thus, cyanoguanidines exist predominantly in the cyanoimino form, and in cimetidine, the cyanoimino group (=NCN) replaces the thione (=S) sulfur atom of metiamide. Cyanoguanidine and thiourea have many chemical properties in common. They are planar structures of similar geometries; they are weakly amphoteric (very weakly basic and acidic), so that in the

(15) S. R. Critchley, K. Prout, C. R. Ganellin, and R. C. Mitchell, *J. Chem. Soc., Perkin Trans. 2*, 68 (1977).
(16) R. C. Mitchell, in ref 17.
(17) G. J. Durant, in Proceedings of the Sixth International Symposium on Medicinal Chemistry, M. A. Simkins, Ed., Cotswold, Oxford, 1979, pp 189–202.
(18) J. A. H. Forrest, D. J. C. Shearman, R. Spence, and L. R. Celestin, *Lancet*, 1, 392 (1975).
(19) M. Charton, *J. Org. Chem.*, **30**, 969 (1965).
(20) G. J. Durant, J. C. Emmett, C. R. Ganellin, P. D. Miles, H. D. Prain, M. E. Parsons, and G. R. White, *J. Med. Chem.*, **20**, 901 (1977).
(21) R. W. Brimblecombe, W. A. M. Duncan, G. J. Durant, J. C. Emmett, C. R. Ganellin, and M. E. Parsons, *J. Int. Med. Res.*, **3**, 86 (1975).

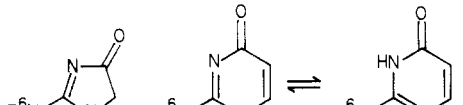
pH range 2–12 they are un-ionized; they are very polar and hydrophilic. The similar behavior of cimetidine and metiamide as histamine H_2-receptor antagonists and the close similarity in physicochemical characteristics of thiourea and cyanoguanidine permit the description of the thiourea and cyanoguanidine groups in the present context as bioisosteres.[20]

There is indeed a true chemical isosterism between the thiourea and cyanoguanidine groups, as revealed by the almost identical crystal structures of metiamide[15] and cimetidine.[22,23] However, there is a very important difference in the conformational properties of the two compounds. N,N'-Disubstituted thioureas assume three stable configurations (viz., **7d,e,g**, V = S), whereas N,N'-disubstituted cyanoguanidines appear to assume only two (**7d,g**, V = NCN) (Scheme II); NMR studies have confirmed that in solution that same holds true for the antagonist molecules.[20] Cimetidine assumes only the two staggered configurations (**7d,g**, V = NCN), which are in equilibrium and interconvert by C–N bond rotation. This has implications for the proposed working model of bidentate H-bonding interactions; since cimetidine is unlikely to adopt the requisite *Z,Z* configuration (**7e**), it follows that either several H-bonding modes may be permitted for interaction or the model must be modified. Conformational effects might partly account for the weaker activity of ureas, since NMR analysis indicates that in deuteriomethanol the *Z,Z* configuration (**7e**, V = O; R^4 = Me) is the preferred form for *N,N'*-dimethylurea;[24] if the *E,Z* or *Z,E* conformers were the pharmacologically active forms and if these are energetically unfavorable for the urea antagonists, then this property may contribute to the reduced effectiveness of ureas.[17]

Concurrent with these developments, in an alternative approach, the effect was being examined of introducing lipophilic groups at the end of the side chain. However, because of the difficulty of predicting the conformational consequences, the possibility was investigated of incorporating the amidine NH groups rigidly in a ring system. This approach introduced other problems. For example, the aminothiazoline (**10**) system was still sufficiently basic



10 **11a** **11b**



12 **13a** **13b**

to be protonated and positively charged; by contrast, thiocytosine (**11**) is neutral at physiological pH and may be regarded as a vinologous thiourea. The incorporation of thiocytosine into an antagonist structure, however, gave only a weakly active compound, probably because of tautomerism, since the tautomer (**11b**) does not retain the 1,3-amidine arrangement of NH groups. These complications were avoided by using the glycocyamidine (**12**) and isocytosine (**13**) systems, which are formally cyclic acylguanidines, neutral at physiological pH, and in which both tautomers (**13a,b**) satisfy the structural requirement with respect to the amidine NH system. This work was mainly effected by my colleague Dr. John Emmett, and I am indebted to his generosity for permission to quote it.

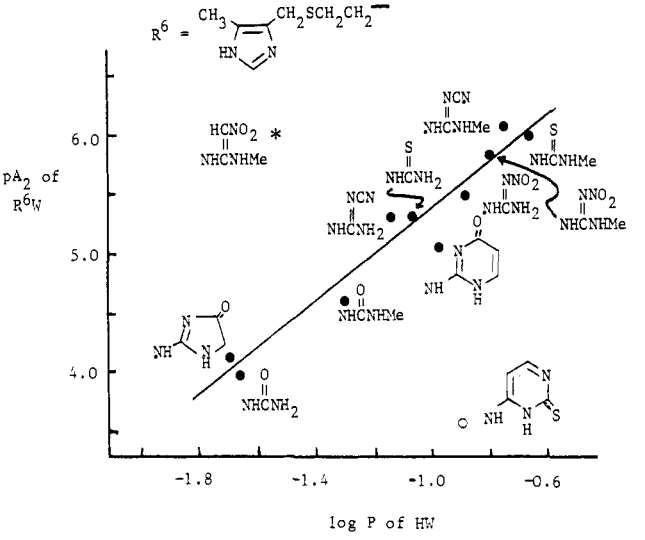


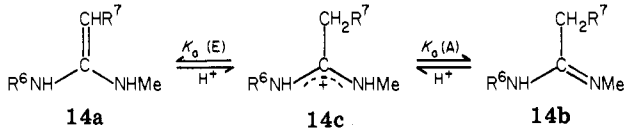
Figure 1. Least-squares fit ($pA_2 = 2.0 \log P + 7.4$) correlation for the series R^6W (●) between H_2-receptor histamine antagonist activity pA_2, determined on the isolated, beating, guinea pig right atrium, and log *P*, the octanol–water partition coefficient for the group HW. The structure of W is shown on the plot for each point, respectively, and R^6 is given above the figure.

Having generated a number of antagonists with different types of side-chain groups, we searched for some type of structure–activity correlation. We still had it in mind that the greater activity of thioureas compared with ureas might be related to desolvation effects (see above). Judged by octanol–water partition or water solubility, thiourea is less hydrophilic than is urea. Analysis of alkyl substituent contributions to octanol–water partition did not appear to give a simple correlation with activity. A correlation was found, however, between in vitro activity for a series R^6W of H_2-receptor histamine antagonists (measured on the guinea pig atrium) and the octanol–water partition of the group HW (Figure 1), within the limitations imposed by the selection of a group of compounds where close structural homogeneity is maintained.[25]

The selection rules for the structure R^6H are as follows: (1) R^6 is constant, viz., 2-[(5-methylimidazol-4-yl)-methylthio]ethyl, (2) W retains the planar 1,3-amidine NH system and is neutral at physiological pH, (3) alkyl substitution on C is excluded (to avoid introducing new steric effects), but replacement of NHMe by NH_2 is permitted since this is of obvious importance to consideration of hydrophilicity. Within these severe constraints upon structural variation, it appears that activity is inversely related to hydrophilicity. Since these compounds were evaluated in vitro, partition is unlikely to represent a distribution property, but it might reflect access; however, it most probably reflects a property concerned with receptor interaction. If one may speculate from these results, it seems that antagonist interaction with the receptor

(22) E. Hädicke, F. Frickel, and A. Franke, *Chem. Ber.*, 111, 3222 (1978).
(23) K. Prout and C. R. Ganellin, in "Structural Studies on Molecules of Biological Interest", G. Dodson, J. P. Glusker, and D. Sayre, Eds., Oxford University Press, Oxford and New York, 1981, pp 176–197.
(24) E. S. Pepper in ref 17.
(25) G. J. Durant, J. C. Emmett, C. R. Ganellin, R. C. Mitchell, and H. D. Prain, Communicated to European Histamine Research Society, 9th Meeting, Visegrad, Hungary, May 7–9, 1980.

Scheme III. 1,1-Diaminoethene and Amidine Equilibria



14a 14c 14b

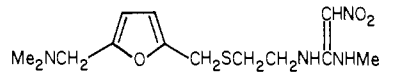
probably involves H bonding, and this is facilitated by desolvation, as might occur in an environment where electrostatic interaction and the strength of the H bond is of importance.

The above correlation identified that it should be possible to increase antagonist potency by reducing the inherent hydrophilicity of the group W to below that of cyanoguanidine. It seemed likely that the N atoms in the guanidine assist water solvation through H bonding and, therefore, it might be possible to decrease hydrophilicity by substituting C for N, e.g., replace N–CN to give a 1,1-diaminoethene (**14a**). There is, however, a problem in making this substitution, since the resulting ethene is merely a tautomer of a substituted acetamidine (**14b**), the two forms being related by equilibration via the conjugate amidinium cation (**14c**) (Scheme III). Most substituted acetamidines would be sufficiently basic to be protonated at physiological pH and would therefore not fulfill the requirement for a neutral drug.

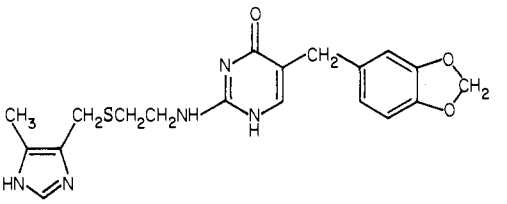
To select a suitable substituent (R^7) for the ethene (**14a**) to predominate, one must consider the equilibria in Scheme III and seek to lower pK_a (E) below that of pK_a (A); i.e., the methane CH must be more acidic than the amidinium NH. For substituted amidines, pK_a is linearly related to the Hammett σ_m substituent constant[19] from which it was predicted that for CH_2CN as a substituent ($\sigma_m = 0.18$),[19] $pK_a = 9.1$, and for CH_2NO_2 as a substituent [σ_m (CH_2NO_2) $\simeq 0.36\sigma_I$ (NO_2) = 0.27],[26,27] $pK_a = 7.8$. For substituted 1,1-diaminoethenes, the relationship is more complex, since the pK_a of carbon acids is affected both by the inductive effect of a substituent and also by the ability to stabilize a negative charge, mesomerically, in the conjugate anion; e.g., nitromethane is much more acidic than is cyanomethane.[28] It is, however, difficult to predict the quantitative effect of the amidinium cation on CH pK_a, but obviously a second electron-withdrawing group would cause a marked reduction.[28] Our prediction was that NO_2 should favor the ethene tautomer, but CN probably would not. A literature search revealed that a diaminonitroethene system had been described by Gompper and Schaefer[29] and that the spectral data supported the ethene formula. The corresponding antagonist structure (**8h**, SK&F 92456) was therefore synthesized[30] but found to be only equiactive with the nitroguanidine (**8f**), i.e., not the hoped for increase in activity through reduced hydrophilicity. The partition coefficient was then measured, and it was discovered that the 1,1-diaminonitroethene was more hydrophilic than the corresponding nitroguanidine, not less!

1,1-Diaminonitroethene and nitroguanidine are isosteric, and in the present context of H_2-antagonism they function as bioisosteres. The position of the 1,1-diaminonitroethene in the log P vs. pA_2 plot indicates that the correlation has broken down, since the compound is much more active than is predicted by partition, and it clearly suggests that some other chemical feature must be making a marked contribution to activity. An important feature of this type of analysis is that it draws attention to potential anomalies which on investigation may provide further structure–activity insight.

These studies have led to further new drug developments. Glaxo have paid us the compliment of using our diaminonitroethene discovery and has extended the knowledge of antagonist requirements by showing that in the drug ranitidine (**15**), a nitrogen heterocycle (such as



15, ranitidine (AH 19065)



16, oxmetidine (SK&F 92994)

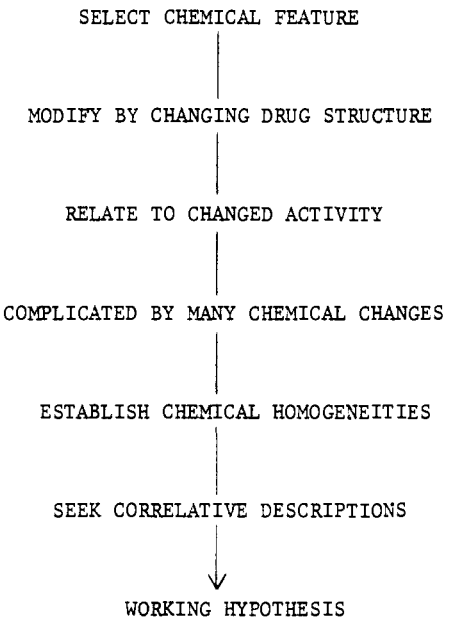
imidazole) is not needed.[31] Oxmetidine (SK&F 92994, **16**) is a development of the isocytosines where we have discovered that potency can be increased by using the correct type of lipophilic substituent.[32]

The above is a description of some of the structure–activity studies conducted during our search for H_2-receptor histamine antagonists. It will be seen to have a largely intuitive element which has drawn heavily from physical-organic chemistry as a means of describing chemical properties of molecules. Starting with a small group of compounds, in practice one can only really progress if it becomes apparent that activity is determined primarily by a particular chemical feature or property. Thus, one is continually seeking the dominant properties that may determine biological activity. To do this, one may select a particular chemical characteristic for investigation and then modify it by changing drug structure while attempting to minimize alterations in other chemical properties. Thus, one builds a series of compounds, as chemically homogeneous as possible, in which one seeks to establish whether a given property may dominate in a structure–activity correlation. Rarely will a unique description be found, and one may have to sustain concurrently several lines of analysis. Each description becomes a working hypothesis to be tested, refined, modified, or discarded, depending upon the result obtained from the next compound tested. This analytical procedure is represented somewhat simplistically in Scheme IV. As the series expands one may find that it divides into subgroups of compounds in which different properties appear to predominate in correlating activity. At this stage it clearly becomes worthwhile to examine the series using modern computational methods of analysis, searching for possible underlying controlling factors.

Finally, I would like to end on a philosophical note. I

(26) R. W. Taft in "Steric Effects in Organic Chemistry", M. S. Newman, Ed., Wiley, New York, 1956, p 592.
(27) M. Charton, *J. Org. Chem.*, **29**, 1222 (1964).
(28) R. G. Pearson and R. L. Dillon, *J. Am. Chem. Soc.*, **75**, 2439 (1953).
(29) R. Gompper and H. Schaefer, *Chem. Ber.*, **100**, 591 (1967).
(30) G. J. Durant, J. C. Emmett, C. R. Ganellin, and H. D. Prain, British Patent 1 421 792 (1976).
(31) J. Bradshaw, R. T. Brittain, J. W. Clitherow, M. J. Daly, D. Jack, B. J. Price, and R. Stables, *Br. J. Pharmacol.*, **66**, 464P (1979).
(32) R. C. Blakemore, T. H. Brown, G. J. Durant, J. C. Emmett, C. R. Ganellin, M. E. Parsons, and A. C. Rasmussen, *Br. J. Pharmacol.*, **70**, 105P (1980).

Scheme IV. Analytical Procedure for Seeking the Dominant Chemical Properties That May Determine Biological Activity



am often asked whether we obtained any real benefit from the "Hansch approach" to correlation analysis or from studies of conformation. I find it difficult to separate cause and effect, but it seems to me that the whole business of medicinal chemistry is one of "knowing your molecules". I think there is a lot to be gained from studying chemical properties. It means that one has to use the literature and also be prepared to make measurements. If nothing else, it is thought provoking and increases one's chemical awareness, which I am sure is very worthwhile. One can of course still progress without such an approach; one can be more empirical and examine substituent effects per se, but that seems to me to leave too many options open at the beginning. I believe that the strength of the approach which I have outlined is that it gives structure to one's thinking and ultimately this increases one's perception and leads to economy of effort.

Acknowledgment. The work described here was a collaborative effort between Sir James Black, F.R.S., Dr. G. J. Durant, Dr. J. C. Emmett, Dr. M. E. Parsons, Dr. G. R. White, and myself. Major contributions were also made by R. C. Blakemore and A. C. Rasmussen (pharmacology), M. J. Bull, D. B. Farrer, Dr. M. E. Footit, A. J. Hayter, D. W. Hills, D. R. Hollyman, F. H. Holloway, R. J. King, J. M. Loynes, P. D. Miles, D. F. Pipe, H. D. Prain, W. Tertiuk, C. S. Whyatt, and C. J. Woolston (chemical synthesis), M. J. Graham, Dr. R. C. Mitchell, and Dr. E. S. Pepper (physicochemical studies), and Dr. R. C. Young (correlation studies).