Isosterism and bioisosterism in drug design

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J. A. Salmon et al., *Progress in Drug Research / Fortschritte der Arzneimittelforschung / Progrès des recherches pharmaceutiques* © Birkhäuser Verlag Basel 1991

1 Methods of drug discovery

1.1 The discovery of "lead" compounds

In every scientific undertaking that is to break new ground, one has to have a goal, a working hypothesis, or a leading idea or fact. This will encourage research and help in the pursuit of a worth-while objective, be it the enrichment of knowledge or the elaboration of a practical purpose. A medicinal scientist who hopes to find a new biologically active drug will have to be guided by such considerations.

When Nature presented itself to primitive human societies as a mysterious and clouded vision, people had no choice but to try its offerings, one after another, to discover some material that would help them to alleviate pain, heal diseases, and delay inevitable death, meanwhile maintaining an individual in tolerable health. Components of rocks, soils and minerals were tried by self-appointed medicine men and healers, and plants and animal tissues were applied or consumed as potential therapeutic materials. This random and wasteful process of trial and error was conducted both for the treatment of reasonably well-defined illnesses or, in the last two centuries, in biological model experiments with a bearing on the pathological condition that was to be halted, alleviated, or cured.

As more and more natural materials from the animal and plant kingdoms were screened and tested, and the successes and failures of these trials were recorded in ancient philosophical treatises, a therapeutic folklore developed as a tentative, selective guide for future medicinal experimentation. Some plant families could be recommended as sources of medicines for certain diseases, and other members of these families could then be expected to provide similar therapeutic powers. It was not a reliable method because ancient diagnoses of diseases could not often be trusted. At least there was a possibility of taking totally aimless randomness out of searches for natural drugs. Interestingly, the pharmacognocists of our day still screen plants and crude plant extracts and soil samples randomly if the biological test method is informative, quick, and inexpensive. Exotic botanical materials can now be transported rapidly from previously inaccessible regions. They can be purified by new isolation techniques and their structure elucidated quickly. They offer the never-ending hope that they may cure hitherto untreatable diseases, and provide novel sources of unexpected "lead" structures.

With the ascendency of synthetic organic chemicals, screening processes turned to available samples of such compounds. If a compound shows promise in a given test, structurally related substances will be chosen as test candidates. They will often also be screened for side effects or other activities that might enhance their therapeutic potential. Once in a while, an unexpected biological effect may turn up during such trials and call attention to a possible activity quite different from the original screening purpose. At this point the biological discovery assumes some aspects of drug design. A classical case of such a study was the discovery of significant antihypertensive activities of certain heterocyclic sulfonamides and sulfonylureas that had been conceived and tested as antibacterials. The design portion involved molecular modifications such as the displacement of the *p*-amino group, which is essential for bacteriostasis, by alkyl or halogen and incorporation of the sulfamyl groups into hetero rings. These measures suppress antibacterial activity and allow the new activity to come to the fore.

Other examples of useful side effects include the observation that propranolol, which was designed to block β-adrenergic impulses and to counteract angina, can be used to lower blood pressure. Phenobarbital was conceived as a hypnotic but became important in controlling epilepsy. Imipramine, originally planned to be a neuroleptic, was found to be an antidepressant in clinical trials. The local anesthetic, lidocaine, can stabilize cardiac arrhythmias. In some of these cases no molecular modification was needed to emphasize the new useful activity. In other instances, extensive molecular modification was required to convert a former side effect to a desirable therapeutic activity.

An important biochemically-based search for "lead" compounds arises from a study of metabolites. Thousands of metabolites of animals are known and some of them have been recognized as protecting the body against disease aberrations. By contrast, metabolites of invasive or infectious organisms are causative agents of the invasive or infectious diseases. They may be counteracted by metabolite analogs produced by molecular modification.

1.2 Molecular modification

The second stage of drug development involves the fine-tuning of a "lead" structure by molecular modification. Analogs resulting from

molecular modification must still be screened for biological behavior, and in most cases must be modified further to maximize the desired biological activity and specificity. The time of random structural variations in molecular modification ("molecular roulette") prevalent throughout the first three decades of the twentieth century is past. Molecular modification now always involves drug design as far as the state of the art permits. This article centers on one widely used approach to drug design with ramifications to other contributions in our understanding of rationality in medicinal chemistry. This does not rule out serendipity and empirical trials guided by "experience".

1.3 What is biological activity?

One of the most important goals of chemistry is the elucidation of the relationships of chemical structure and various physical properties, such as solubility, electrical conductance, plasticity, optical properties, etc. The relationships between chemical structure and color and dyestuff properties have been studied extensively; colored compounds as well as dyestuffs must contain a chromophore, commonly an unsaturated system of conjugated double bonds. Biological activity has been correlated with the presence of pharmacophores, i. e. sections of a molecule containing atoms and linkages responsible for the activity. Great caution is necessary not to generalize too much about such pharmacophores, because biological activity is much too diffuse a term. It may depend upon the presence of ionizable groups of atoms, or on unsaturated or potentially unsaturated functions, and on the shape and chirality of a molecule. Indeed, since biologically active substances must interact with active sites of flexible macromolecules such as enzymes, bioreceptors and nucleic acids, the shape of biosubstrates and drugs is of greatest importance. If it is complementary to a section of an active site, it offers functional atoms or groups of the approaching molecule a close fit at macromolecular sites, and thereby an opportunity to ligate to the macromolecule and rapidly interact with it.

In most cases, biological activity is much more complex than the biochemical reaction between an enzyme, receptor or polynucleotide with a usually relatively small-sized substrate or drug molecule. In order to produce a biological effect (motion, sound, pain, etc.) a cascade of interdependent reactions must take place which amplifies the en-

ergy liberated by each participating system until an overt action can be registered. The "biological activity" of a substrate therefore depends on some reaction that contributes a small increment to such biochemical cascades, at best setting in motion a sequence of biochemical events [1].

It is therefore necessary to understand what is meant by "biological activity" in each case. Studies of structure-activity relationships (SAR) are meaningful only if the same biological, and preferably biochemical phenomenon or reaction is affected by two compounds to be compared. Most drugs are inhibitors of enzyme systems or substrate antagonists. All too often the ability of a test substance to "lower blood pressure", to "affect diuresis", to "increase the pain threshold", and to produce similar pharmacological phenomena serves as a basis for comparison with another structurally similar chemical without referring to the very mechanism of the observed overall results. It is even worse to compare structurally unrelated compounds using qualitative pharmacological observations because such substances have a good chance of producing these test data by different biochemical mechanisms. That means, they may react at different active sites of the same or different receptors, or they may interfere with different enzymes in cascade systems which manifest themselves in the same biological end-occurrence.

The ideal comparison of the biochemical activity of two compounds should therefore be based on the effect of the compounds on the same mechanisms in enzymatic, receptor-ligating and other reactions with the biomacromolecules of the same organism. This goal is difficult to attain in therapeutic areas in which the causative biochemical agents have not yet become known. In tests involving invasive or infectious conditions, one will search for differences between isozymes of the host and those of the disease-causing cells. For some infectious and invasive pathogens, isozymes of dihydrofolate reductase have been purified and candidate drugs could be screened for cytostatic activity against the isozyme of the pathogen. For some viruses, their unique retropolymerases have become the target of drug tests. Bioisosteres of metabolites of such pathogens have become effective drugs for the inhibition of life processes of the damaging cells.

The requirement of searching for a drug that will act exactly like a known prototype is often relaxed for practical reasons. Especially in the pharmaceutical industry where the vast majority of therapeutic

agents is developed, one tries almost always to design compounds with enhanced potency, selectivity, and fewer side effects. In other words, one digresses voluntarily from a strictly scientific search for mechanistically matching drugs to a search for compounds with improved *in vitro* and *in vivo* properties. Needless to say such investigations dilute comparative knowledge and complicate the ultimate aims of biochemically based drug design.

Working with macromolecular biochemical and with cellular and tissue-oriented systems, one encounters complexities which stretch our contemporary understanding of biological phenomena to the limit. If such knowledge is to be heightened, one has to reduce these complexities radically. This does not often appeal to experimental biologists in the industry who confront tantalizing complex questions in their chosen sciences and hope to dent these uncertainties at perhaps one point in their lifetime. Physical chemists realize that even in such simple systems as diatomic molecules, let alone the much more complicated and biologically unavoidable molecules of water, $(H_2O)_n$, enough problems of intra- and interatomic forces have remained unsolved to provide fundamental research topics for a long time to come. If biological mechanisms in drug design are to be elucidated on the molecular or ionic levels, one had better revert first to very simple chemical structures in which molecules have been reduced to a bare minimum and the complexities of larger or very large molecules have been stripped off as far as possible.

1.4 Isosterism

An early attempt to compare chemical and physical properties of diatomic molecules such as N_2 and CO was made by Moir [2, 3]. His reasonings about "isomerism" of these two gases are no longer compatible with present-day views; nevertheless, his ideas were expanded by Hinsberg [4] who applied them to partial structures of organic compounds. He defined groups which can be exchanged for each other in aromatic ring systems without considerable change of the physical properties of the resulting compounds as ring equivalents, citing benzene, thiophene and pyridine as examples. A -CH=CH- group in benzene is replaced by divalent sulfur, -S-, in thiophene, or its -CH= by trivalent nitrogen, -N=, in pyridine. Indeed, benzene boils at 81.1° (760 mm), thiophene at 84.4° (760 mm), but pyridine digresses from

these similar data, boiling at 115–116° (760 mm). In those early days, it was actually difficult to fractionate benzene and thiophene from coal tar benzene. Hinsberg suggested that sulfur must resemble -C=C- in its external shape. The bothersome hydrogen atoms were disregarded as unimportant compared to the much larger aromatic carbon atoms. Hückel [5] then advanced other examples of ring equivalents, comparing the imino group, -NH-, to oxygen, -O-, in organic compounds such as pyrrole and furan. He also equated methyl, CH_3, with fluorine, and methylene, $=CH_2$, with nitrogen as seen in diazomethane, CH_2N_2, which was compared to ethylene, $CH_2=CH_2$.
The physicist Grimm [6–8] called groups such as OH and NH_2 "pseudoatoms". He assumed that a proton (H^+O-; H^+NH-) penetrated the electron shell of the larger atom and was "submerged" in it and did not have much effect on the interatomic ligating power of the heavier atom (O, N). This submergence of hydrogen – the smallest atom – appeared to be allowed [9] for small atoms. It could be interpreted as a combination with the orbitals of the outer valence electrons. The inner orbitals must not be affected. Of course, hydrogen bridging was not yet understood.
Compounds that contained pseudoatoms were predictably similar physically although quite different in their chemical reactivity, provided that their molecular size and overall charge distribution were similar and not affected by dipole moments. In an effort to imitate the periodic table of the elements and apply analogous ideas to molecules, Grimm arranged a number of elements in a Table which lists progressive increases in proton attachments, and which he called the "hydride displacement law". The last word must be regarded as excessive, because it is an illustration rather than a law of physics. As one descends diagonally from the left to the right in Table 1 representing this idea, one H is added after another, and the elements and groups in the vertical columns become "pseudoatoms". For the chemist searching for lines of comparison between functional groups, columns 2, 3, and 4 are particularly instructive.

Table I
Grimm's "hydride displacement law"

C	N	O	F	Ne
	CH	NH	OH	FH
		CH_2	NH_2	OH_2
			CH_3	NH_3

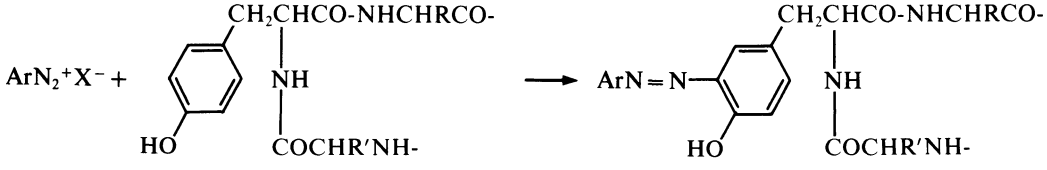
Among simple compounds which contain pseudoatoms would be such pseudohalogen molecules as hydrogen peroxide (HOOH), hydrazine (H_2NNH_2), and ethane (CH_3CH_3). The actual location, motion, and resonance of electrons in orbitals was not considered in this early picture.

In 1919, the American Nobelist, Irving Langmuir, published two papers [10] that were designed to develop the then emerging octet rule for electron shell stability. The second of these articles was entitled "Isomorphism, Isosterism and Covalences". The concept of electron orbitals had not yet taken hold, but it was known that the elements in the vertical groups of the periodic table owed their similarities to the identity of their outer electron shells. In molecules with similar physical properties, an analogous arrangement could be expected. Langmuir coined the term isosteres (*ἰσός* = like, *στερεός* = shape) for such pairs of molecules and offered 21 examples for this concept. He quoted some inorganic isosteres, among them the negative ions, ClO_4^-, SO_4^{2-}, and PO_4^{3-}. Such groups are not isoelectric, they do not have the same electric charge. Some others are isoelectric, however, and they are the ones that are most similar to one another. Among them, three pairs were listed: CO and N_2; N_2O and CO_2; and azide, N_3^-, and isocyanate (NCO^-) ions. Diazomethane was now compared to a proposed molecule, ketene ($CH_2=C=O$) which had not yet been discovered, and not to ethylene [5]. The prediction of the similarity of diazomethane and ketene was confirmed 18 years later [11].

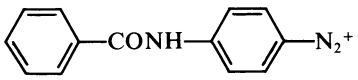
The definition of isosterism underwent a number of changes as the picture of electron orbitals emerged. It became necessary to include other properties that were to be compared, especially those that could be measured more easily at different stages of instrumental evolution. To begin with, they included similarities in boiling points, densities of liquids, melting points, molecular weights, and critical pressures (C_p), temperatures (C_t), and volumes (C_v). The more such physical properties could be measured and compared, the more accurate did such comparisons become. For example, C_p/C_v values for diphenylmethane and diphenyl ether ($=CH_2$ versus $O=$) had approximately the same collision areas [12–14], i.e. the same shape and spatial volume. Additional observations concerned similar mobilities of isosteric ions [15, 16], densities of complex ions [17], ionic radii [18], and parachors [19].

If one of the main characteristics of isosteres is equality of molecular

volumes and great similarity of shape, two such compounds should be isomorphic, that is, similar in crystal habits and probably their ability to form mixed crystals. A study of the behavior of about 200 pairs of compounds demonstrated that only one-fourth of these formed mixed crystals over the whole range of percentage composition, and only an additional 17 % over part of this range. In other words, isomorphism does not necessarily imply mixed crystal formation [20–23]. It seems that mixed crystal formation may require also similarity of polarization, and this property is not always found in isosteres. Therefore, requiring mixed crystal formation is unrealistic to qualify for isosterism.
Hans Erlenmeyer of the University of Basel [22, 24] suggested an extension of the concept of pseudoatoms, principally of pseudohalogens, to include groups mentioned by Birckenbach [15, 16, 25–27] such as -CN, -OCN, $-N(CN)_2$, $-C(CN)_3$, -SCN, and -SeCN. A number of other possibilities such as the comparison of C_6H_5COCl, $C_6H_5N_2{}^+Cl^-$, and C_6H_5MgCl, did not capture the attention of organic chemists either for synthetic purposes or other applications.
Erlenmeyer whose career included some time in the pharmaceutical industry before he returned to academic pursuits was quite naturally interested in the biological properties of organic compounds and the relationships of the structure of these substances to their biological activities. This interest was compounded by the research then flourishing all around him, especially in Switzerland and Germany, on the chemistry of vitamins, hormones and various drugs. The biological manifestations of almost all organic compounds were gross descriptions of activities in various pharmacological test systems at that time and were not yet based on biochemical reactions affected by the test substances. Trying to apply rigorous criteria to his explanations of biological observations, Erlenmeyer turned to a test system known for its specificity. The immunologist Karl Landsteiner [28] had shown that artificial antigens, formed by coupling aromatic diazonium ions to proteins, could be injected into animals and there provoked formation of specific antibodies as seen in combination and/or precipitation of the azoprotein. The immune reaction is highly specific, de-



pending on the structure of the hapten portion, i.e. the aromatic azo moiety. Even minor changes in this portion of the antigen molecule prevent reaction with a given antibody. In Erlenmeyer's experiments [21, 29], the aromatic portion was represented by p-C_6H_5-O-C_6H_4-, p-C_6H_5-NH-C_6H_4-, and p-C_6H_5-CH_2-C_6H_4-, the groups linking the aromatic nuclei corresponding to the third vertical column of Grimm's Table of pseudoatoms. All three azoproteins containing these coupling components showed similar antigenic properties. This demonstrated the application of isosteric replacements in a demanding biochemical situation. Similarly, the azoprotein haptens derived from p-benzamidophenyldiazonium *(1)* and p-(2-thienyl) carboxamidophenyldiazonium *(2)* ions were serologically indistinguishable and so were



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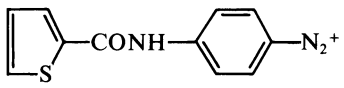

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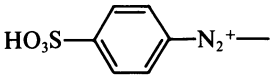
the azoproteins containing aryl groups with p-sulfonic *(3)* and p-selenoic acid *(4)* substituents [29, 30]. When the strongly polar substituents



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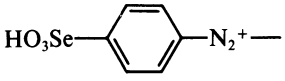

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were replaced by less polar groups such as p-$HO_2SC_6H_4N_2$, the resulting azoproteins did not overlap with the antibody of the p-sulfonic acid derivative. Similarly, the azoproteins synthesized from p-aminobenzenephosphonic and -arsonic acid had the same antigen activity [30–32] but the corresponding stibionic acid analog behaved differently, perhaps because stibionic acids exist only in a polymeric form.

When it became apparent that comparisons of one or two physical properties might not be adequate to classify compounds as isosteres in the classical sense proposed by Grimm [6–8] and by Erlenmeyer [33], other additional properties were contemplated even though some of them were harder to measure. In all these studies, too many exceptions were noted to use the comparison as a firm basis of classification. For example, only a few functional derivatives of benzene and thiophene, respectively, have similar boiling points [34, 35]. The ionization potentials and the decomposition voltage of compounds containing pseudohalogens (F, OH, NH_2, CH_3) do not show steady trends

[36]. When the conductivity of compounds containing pseudohalogens was measured, differences arose because of an unequal degree of hydration [16]. The acid dissociation constants of isosteric heterocyclic acids, which depend on the dipole moments of the compounds, cannot be compared readily either [37]. Such dissociation constants increase with an increase of electron-attracting (electronegative) groups present, as observed for aryl and heteroaryl-substituted sulfanilamides, p-$H_2NC_6H_4SO_2NH$-R, where R represents such substituents [38]. Some regularities have been encountered when wavelength absorptions were compared but only to some extent such as the occurrence of maxima and minima in otherwise non-overlapping curves [39]. The optical activity of the thiophene isostere of cocaine is similar to that of the natural alkaloid [40].

All the difficulties in arriving at binding directives that make two compounds isosteric show that molecules cannot be compared as strictly as elementary particles. Every nuance of analogy of shells of outer electrons with the accompanying accumulation of regions of high and low electron density can be fitted into the views proposed by Langmuir and elaborated by Grimm, Erlenmeyer and others. Similarity of overall molecular shape and stereochemistry must be observed as a prime prerequisite. The most important practical application of the concept of isosterism, diluted by the uncertainties just presented, has been in drug design. Even though regretfully it does not provide an accurate guide, it has led to the design of compounds which should have, and often do have, affinity to a given biochemical reaction system. Such compounds either activate such biochemical reactions, i.e. they are agonists of recognized substrates, or else deactivate the same biochemical system by reacting with it at rates faster than the substrate or by interdicting access of the substrate to catalytic sites by bulky steric blockade of the reactive biochemical. In that case they are antagonists. A steady transition from agonists to antagonists remains a weakness in designing a biologically active molecule on the basis of isosterism.

Agonists have a high intrinsic activity; competitive antagonists have low or no intrinsic activity whereas non-competitive antagonists lack structural features that are needed for a close fit at the receptor. They therefore attach themselves to non-binding sites of the receptor protein or polynucleotide. Agonist molecules are usually smaller than competitive antagonists which cover areas larger than the active site.

1.4.1 Bioisosterism

To deal with all these divergent possibilities and difficulties in interpretation, Harris L. Friedman [41] coined the term bioisosterism for the relationship of compounds "which fit the broadest definition of isosteres and have the same type of biological activity". In other words, bioisosterism should account for structure-activity relationships (SAR) of biologically active chemicals. The purpose of SAR studies is the elucidation of the reasons for equivalence or nonequivalence of biologically active compounds.

Friedman's definition is less restrictive than Erlenmeyer's postulate that isosteres are atoms, ions or molecules in which the peripheral layers of electrons can be considered to be identical. This becomes untenable if "identical" is enforced in the light of modern electron theory, and makes it less predictive and explanatory in medicinal drug design. The "broadest definition of isosteres" as suggested by Friedman provides such wide interpretation of SAR that it becomes scientifically too vague, but in another sense it is of great help: it permits the medicinal chemist to roam towards structures that are somewhat similar to a prototype.

Hansch [42] tried to extricate this vagueness of language by defining bioisosteres as "compounds causing identical biochemical or pharmacological response in a standard test system. The system might be an enzyme, membrane, mouse or man". This would probably hold if the test uses an enzyme as a target, but for biological activity measured in isolated tissues, not to speak of whole animals including man, too many variables could interfere. Absorption, metabolism [43], attack at different stages of a multireactional cascade of events, and especially different mechanisms of action which may present an overall identical therapeutic effect would have to be factored into the definition.

We would like to suggest an expanded statement which takes into account biochemical views of biological activity. "Bioisosteres are compounds or groups that possess near-equal molecular shapes and volumes, approximately the same distribution of electrons, and which exhibit similar physical properties such as hydrophobicity. Bioisosteric compounds affect the same biochemically associated systems as agonists or antagonists and thereby produce biological properties that are related to each other."

A subdivision of bioisosteres is now also in use. The cases that satisfy the conditions set forth by Langmuir, Grimm, and Erlenmeyer are called classical bioisosteres if biochemical or pharmacological interactions are concerned. Nonclassical bioisosterism [44–46] refers to a more widely applicable set of compounds which cause qualitatively similar agonistic or antagonistic biochemical or pharmacological responses at the molecular level. Hansch recommended for such compounds the term partial bioisosteres but "nonclassical bioisosteres" is used more often.

Bioisosteres are also found abundantly in nature. Many alkaloid-bearing plants contain series of chemically related alkaloids whose individual structures and biological activities overlap or differ only marginally. In animals, the many closely related adrenergic, steroid, thyroid, oxytocic, and other hormonal families can be classified as bioisosteres. Inter-species bioisosteres have also been observed, for example, the insulins isolated from the pancreases of different animals.

1.4.2 Physical properties

With the improvements in many branches of instrumentation, it has become possible to measure physical properties of chemicals which could barely have been attempted a few decades ago. This holds for many analytical methods and particularly for spectroscopic determinations (UV, IR, NMR, mass spectra, Raman spectra, X-ray diffraction spectra) and computational devices, often coupled with spectroscopic methods. The resulting savings in time and effort have made most of these measurements available for routine laboratory work. In another area, chromatographic distribution and separation procedures can be used rapidly by technicians to determine partition coefficients of chemicals between different solvent systems. Largely through the work of Hansch and his school, the lipophilicity, hydrophobicity and hydrophilicity of biologically active substances have been documented extensively [47–50]. The partition coefficients of thousands of substances have been determined conveniently in a 1-octanol – water system [51–53] and have probably become the most widely quoted physical property of biological test substances. They are criteria of the transport of a test compound through body liquids and cell membranes to their receptors, an important aspect of drug action. The value of hydrophobicity in explaining the action of a drug at its receptor is less certain.

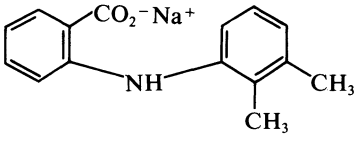
Interactions of substrates or drugs at biomacromolecules could be electrostatic, steric (blockade or repulsion), and hydrophobic and dispersive. Since partition coefficients are so easily determined, they can indicate what other compounds with similar numerical values one should choose in planning molecular modifications. Sometimes, agreement between partition coefficients has been calculated only after a series of molecular analogs had been prepared and tested intuitively. In any event, if two compounds exhibit the same degree of hydrophobicity, isosterism could be involved.

Despite all this work, the calculation of *log P* still involves many empirical data and variables. For aromatic compounds, should one use π values from benzene and σ values for regression equations? If benzene and pyridine isosteres are to be compared, the hydrogen-bonding nitrogen of pyridine will have to be incorporated in the equation. Moreover, the *log P* values will differ in their σ values for each of the isomeric positions of the pyridine ring, and thus lead to a large number of variables.

1.4.3 QSAR methods

Quantitative structure-activity relationships (QSAR) try to transform the chemical structure of a compound into quantitative numerical values that describe properties relevant to a given biological activity. Hansch [42] felt that organic formulas are "a terrible hindrance to progress in relating structure to activity" because "there is no dynamic message for those who are interested in rate processes". As pointed out above, the detailed organic reaction scheme of a drug with its receptor – be that the active site of an enzyme, an antibody [54, 55], or a high-molecular weight polynucleotide receptor region – makes good use of organic formulas and conveys to an experienced organic biochemist a set of properties, reaction possibilities, and steric effects that would require extensive correlation of physical data to summarize the same information.

An example of the advantage of emphasizing rate processes over comparisons of structures has been described by Hansch [42]. Two pairs of compounds, *5* and *6*, and *7* and *8*, respectively, increase the membrane potential of the mollusc basal ganglion by 20 mV [56]. In spite of irreconcilable differences in structure, identical concentrations (log 1/C; C is the molar concentration causing the 20 mV increase in membrane



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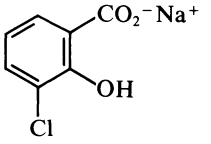

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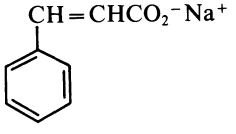
log 1/C	3.48	3.48
log P	0.28	0.23



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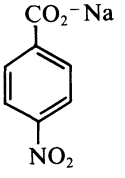

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log 1/C	1.48	1.48
log P	−2.23	−2.21

potential) for pair *5–6*, and the pair *7–8* produce identical biological responses in the test system, that is, they are isosteric on the basis of Hansch's definition.

The SAR is shown in equation (1):

$$\log 1/C = 0.839\,(\pm 0.07)\log \text{P-ion} + 3.308\,(\pm 0.10) \quad \overset{n}{30}\ \overset{r}{0.979}\ \overset{s}{0.177} \quad (1)$$

where P-ion is the 1-octanol/water partition coefficient of the sodium salts *5* to *8*, n is the number of measured data points, r is the correlation coefficient, and s is the standard deviation. Figures in parentheses are the 95 % confidence intervals; the slope of near 1 may be expected for membrane perturbation [49]. But although the pairs have the same log P, to be sure, this was discovered by screening various test compounds, and not by prediction based on these calculations [56]. This does not mean that once the predictive value of a partition coefficient has been confirmed that this measurement should not be used in every other case to support and explain biological evaluation.

Another illustration of the Hansch equation is a variation of a simple substituent, such as a group on an aromatic ring. This substituent will have several parameters, leading to equation (2) where *C* again is the concentration of the compound

$$\log(1/C) = X(\pi) + Y(\sigma) + Z(E_s) \quad (2)$$

needed for a given biological effect; π is Hansch's partition coefficient from which the lipophilic character can be determined; σ is Hammett's value for the electronic property of the substituent; and E_s is Taft's steric parameter which indicates the size of the substituent.

If Y and Z are zero for a given structural series, then the potency would be a function of π only, and the old and new substituent on the ring would be truly bioisosteric. If these substituents are structurally unimportant, even a significant numerical size of X, Y, and Z will not affect selective activity too much. If the values for σ, E_s, hydrogen bonding, pK_a and other measurable parameters are taken into account, then structural groups with analogous π values could be called isolipophilic [42].

Tables of σ, π, and E_s values have been published by Topliss [57] and Hansch and his associates [58, 59].

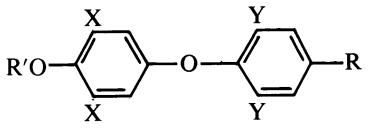
The interpretation of quantitative numerical data to express biological activity will be colored by the principal inclination of the investigating scientists. Physical and physical-organic chemists will approach the problem by studying physical measurements of the chemicals involved and arranging them in summarizing equations. Organic and especially medicinal chemists who think in terms of reaction mechanisms, likely transformations, and also the biological potential of their compounds will try to make generalizations that reflect their main expertise. This may explain the approach taken by James W. Wilson, a medicinal organic chemist, in collaboration with S. M. Free, a biostatistician. The Free-Wilson method [60] has the advantage that one does not need physicochemical measurements of the compounds, but of course one can therefore not make predictions based on such properties. The procedure assumes that introducing a given substituent at a particular position of the molecule always produces a quantitatively similar effect on the biological potency of the rest of the molecule [61]. In equation (3), i is the number of the position of substitution, j is the number of the substituent

$$\log(1/C) = x + \sum^{m,n} a_{ij} G_{ij} \tag{3}$$

at that position, m is the total number of substituted positions, and n is the number of substituents. The value a_{ij} represents the presence (1.0) or absence (0.0) of the substituent ij. The values G_{ij} are group contributions; they are obtained by multiple regression analysis. Examples may be found in a monograph by Yvonne Martin [62] and a review by Kubinyi [63].

The proposal that there is a skeletal center of a molecule (a pharmacophore) equipped with substituents and that these substituents make additive quantitative contributions to the biological activity has been in the minds of medicinal chemists for a long time. Indeed, it can be read into the 1868 statement by Crum Brown and Frazer [64] that the physiological activity of a compound is a function of its chemical structure. Later, Meyer [65], Overton [66] and others suggested that partition coefficients, solubility data, the vapor pressure and other physical properties had an effect on toxicity, narcotic, microbicidal and hemolytic activities. They thought first of general anesthetics such as ether and chloroform whose action must be based on their partition coefficients between blood (i.e. water) and nervous tissue lipids. Even the element xenon is a safe and potent clinical anesthetic, and since it is chemically inert these actions must be related to its partition coefficient.

Bruice et al. [67] correlated the thyromimetic activity of 47 analogs *(9)* of thyroxine with their sustituent constants f of R, R′, X and Y which



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were derived from Hammett σ constants. The correlation was expressed by equation (4). Other examples can be found in reference [63].

$$\log \text{ of \% of thyromimetic activity} = k.\Sigma f + c \qquad (4)$$

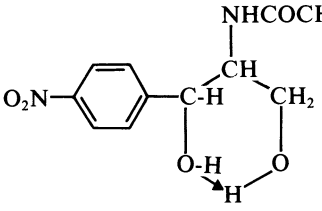
A simplification of the Free-Wilson equation was proposed by Fujita and Ban [68]. Many investigators now combine their modified Free-Wilson method with the extrathermodynamic approach by Hansch to arrive at mathematical formulations of QSAR. These ideas and other developments leading up to present-day usage have been summarized by Tute [69].

No attempt will be made here to describe all aspects of QSAR, its successes and failures. The goal of QSAR studies is to delineate more precisely the reasons for the equivalence or nonequivalence of biological activity than is possible by intuitive experience. This can decrease the average number of analogs one may have to test by 2.5–3-fold during the inevitable process of molecular fine-tuning. Since the concept of

bioisosterism relies so heavily on SAR, quantitation of SAR inevitably must sharpen up our view of bioisosteres.
Additional understanding of bioisosterism can be gained by the emerging application of X-ray diffractometry to the identification of hydrophobic regions of a drug molecule, especially in the process of binding to a macromolecular site. This can involve the measurement of likely hydrogen bonds and the adaptation of a sterically flexible molecule to a sterically compatible macromolecule. If one remembers the postulate that similar shape is a precondition of isosterism, any change in the shapes of molecules participating in mutual recognition, attraction and ligating can modify our concept of bioisosterism. Uncertainty about molecular conformation impairs the predictability of potency of a drug but could be removed by X-ray diffraction studies. It is not surprising that the most successful mapping of drug-receptor interactions, and hence a better understanding of the biological activity of a drug, has been achieved in series of relatively rigid drug molecules [70–72]. NMR spectroscopy can complement X-ray diffraction to reveal conformational changes of substrate and receptor protein during ligating processes in solution.

1.4.4 Stereochemical differences

The effect of steric differences is seen most clearly in stereoisomers. They may differ quantitatively in biological activity, occasionally also qualitatively. This includes rigid geometric and also chiral RS-isomers. Since in such pairs even antagonism has been observed occasionally, recommendations have been adopted that at least future drugs that can exist as optical isomers will have to be resolved and administered to the patient in a single steric conformation.
Non-chiral flexible compounds such as acetylcholine can assume a preferred and essential conformation during complexation with muscarinic receptors. In the case of *L*(−)*threo*-chloramphenicol *(10)* NMR coupling constants indicate that the hydrogen atoms of the alcoholic hydroxyls are in the gauche position. Apparently, there is hydrogen bonding between these groups. This conformation corresponds to that of uridine 5′-phosphate *(11)* in size, orientation of the hydroxyl groups, and distribution of negative charges.



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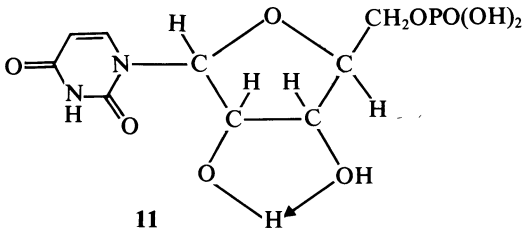

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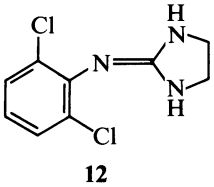
In bioisosteres, compounds with the same absolute configuration or with steric relationships are compared most meaningfully. The importance of a uniform test method for compounds of divergent structure and steric arrangements is illustrated in *12–16* [73]. All these substances, clonidine *(12)*, lofexidine *(13)*, guanabenz *(14)*, lidamidine *(15)*, and rolgamidine *(16)*, are potent antidiarrheal drugs when administered subcutaneously. Both inhibition of the propulsive activity of the gut, and of hypersecretion play a role in their action, the latter being mediated by an adrenergic α_2-agonist mechanism. There is a formal chemical relationship between *12*, *13*, and *14*, and also between *15* and *16*. The best separation of the two antidiarrheal mechanisms was achieved in *17*, a bioisostere of *14* with an α_2-agonist activity, by introducing a phenolic hydroxyl group.



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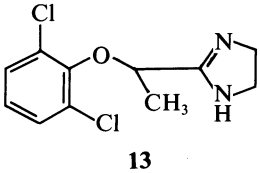

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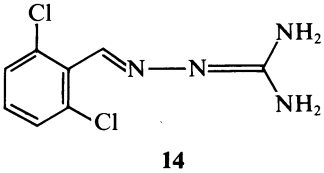

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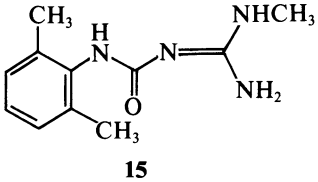

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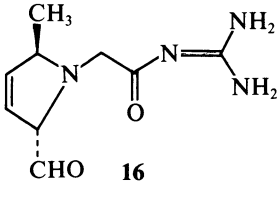

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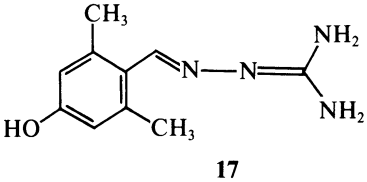

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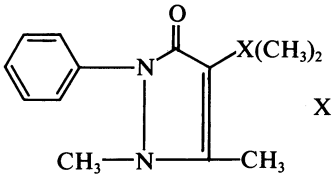
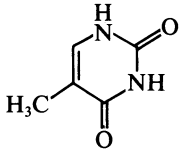
1.5 Examples of classical bioisosteres

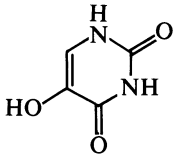
Many examples of bioisosterism from the early chemical and pharmacological literature suffer from a lack of measurements of the biological activity of the reported compounds. Terms such as "similarity of activity", or "both compounds possess a given activity" would no longer be adequate for meaningful comparisons. Methods for quantitative biological evaluations developed slowly over the years, and biochemical comparisons lagged behind even further. One must commend the pharmaceutical industry for creating so many useful drugs during those days.

Classical bioisosterism is seen in aminopyrine and its analog *18* in which $-CH_2CH(CH_3)_2$ replaces $-CH_2N(CH_3)_2$ to give great similarity of antipyretic action and toxicity. The isopropyl derivative *18* was potent enough to be tested clinically for a short while [30, 31, 33, 74, 75].



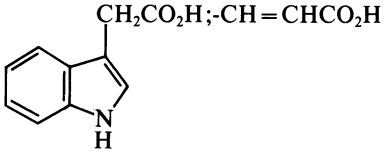
X = N , CH

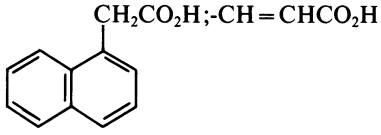




18 **19** **20**

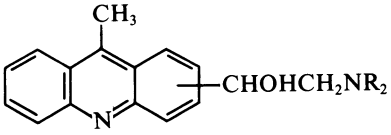
Replacement of OH by CH_3 or vice versa was seen in nicotinic acid (niacin) whose action is antagonized by 3-acetylpyridine [76]. The same relationship holds for thymine *(19)* ($-CH_3$) and isobarbituric acid *(20)* (-OH) [77]. Another comparison was made with indole-3- *(21)* and naphthalene-1-acetic and -acrylic acids *(22)* which exhibit similar heteroauxin properties but are antagonistic to tryptophan [78].





21 **22**

Amino alcohols derived from 5-methylacridine *(23)* and from phenanthrene *(24)* are low-grade experimental analgesics (79). 6-Aminonicotinic acid and *p*-aminobenzoic acid (-N= → -CH=) exhibit a similar antagonism to sulfanilamide [80], and adenine and aminobenz-



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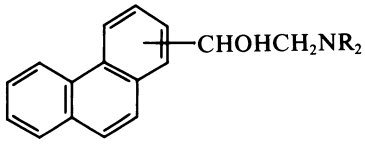

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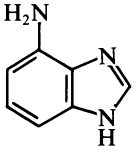
imidazole *(25)* are also antagonists in some test systems [74]. The same type of bioisosterism is found in the pair, guanine, and 5-amino-7-oxo-1-v-triazolo(d)pyrimidine *(26)* which are antagonists [81], and in thiamine *(27)* and the antagonistic oxythiamine *(28)* [82].



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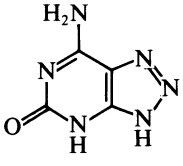

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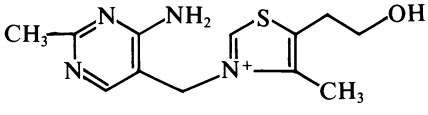

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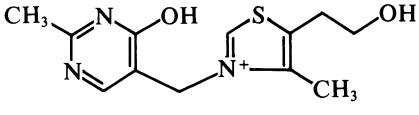

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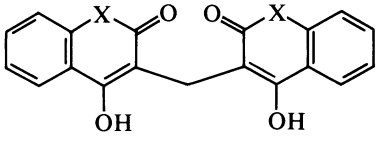
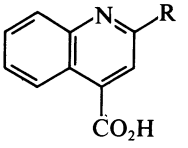
An ether oxygen → imino analogy lead to greatly diminished hemorrhagic activity in the quinolone analog *30* of dicumarol *(29)* [83, 84]. Furan analogues of benzenoid compounds were studied [85] to illus-

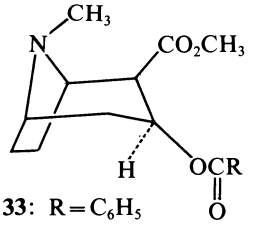


X = O: **29**
X = NH: **30**

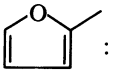
trate -O- versus -CH = CH- isosterism. The pairs phenethylamine – 2-furylethylamine; N-methylaniline – 2-furyl-N-methylamine; and 2-methylamino-3-phenylpropanol – 2-methylamino-3-(2-furyl)propanol exhibited the same relative effect on the blood pressure. Cinchophen *(31)* and 2-(2-furyl)-4-carboxyquinoline *(32)* behaved similarly in tests for analgesia and antipyresis and cocaine *(33)* and its furan analogue *34* acted similar in local anesthetic tests.

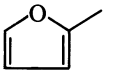


R = C_6H_5: **31**



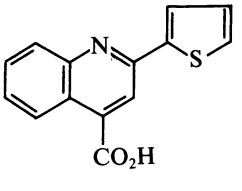
33: R = C_6H_5

R =  : **32**

34: R = 

Local anesthetic tests were also compared for diethylaminoethyl esters of aromatic acids such as *p*-aminobenzoic acid and 2-furanoic acid, as well as 2-furylacrylic acid [86] because vinylogous conjugation of the carboxyl with an ethylenic or acetylenic linkage often increased local anesthetic potency [87]. All these analogies suggest the similarity of chemical and physical properties of true aromatic and pseudoaromatic systems. In their effect upon local anesthetic activity, the relative contribution of various nuclei appear to be as follows: C_6H_5 > 2-pyrrolyl > 2-thienyl > 2-furyl, but there are exceptions to this sequence depending on the character of the acid and the tertiary amino group.

The most popular bioisostere of the phenyl group has been the thienyl radical, usually but not necessarily the 2-thienyl group because of the availability of thiophene-2-derivatives. 2-(2-Thienyl)-4-carboxyquinoline *(35)*, the thiophene isostere of cinchophen *(31)*, has similar analgesic and antiphlogistic properties [88] as *31* but it dyes the tissues of laboratory animals a deep purple. The thiophene bioisostere *36* of cocaine *(33)*, called an isologue by the authors [40], showed the same potent local anesthetic activity as cocaine in the rabbit eye test; the thio-



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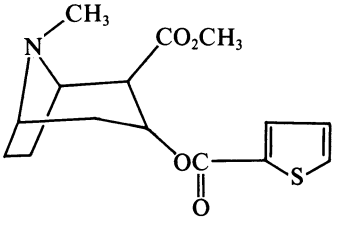

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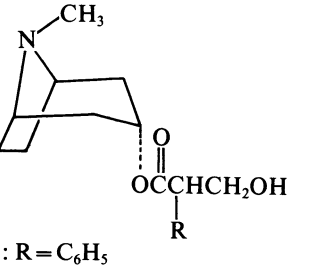
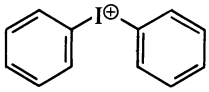


37: R = C_6H_5

38: R =

phene isostere *38* of atropine *(37)* was not tested biologically for lack of material [89]. Eucaine A and its thienyl bioisostere also behaved similarly [90]. By contrast, phenylalanine and 2-thienylalanine were antagonists in promoting the growth of yeast [91].
Diphenyliodonium sulfate *(38a)* inhibits the macrophage and endothelial cell nitric oxide synthase, and so does its di-2-thienyl bioisostere *38b* [91a].



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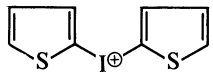


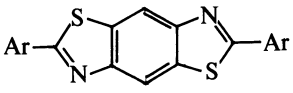
38b

Of the many other thiophene analogues of phenyl-containing prototypes, a few are listed in Table II. Their biological action has been reported only as "similar".

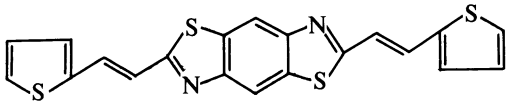
Table II
Some classical thiophene isosteres with "similar" biological activities

Benzene isostere	Thiophene isostere	References
p-Aminobenzanilide	*p*-Amino-2-thenoylanilide	[30–32]
Benzoic Acid	Thiophene-2-carboxylic acid	[92, 93]
Amylocaine	Thiophene isostere	[90]
Phenacetin	Thiophene isostere	[90]
O-Benzoylquinine	O-(2-Thenoyl)quinine	[90]
Hippuric acid	2-Thenoylglycine	[93]
N-Ethylaniline	N-Ethyl-(2-thienyl)amine	[94]

The electronic aspect of benzene-thiophene isosterism is not restricted to medicinal chemistry. In nonlinear optical properties which are used in computer data storage and communication systems based on light instead of electricity, hyperpolarizability can be manipulated to increase the frequency of an infrared laser. The second hyperpolarizability, γ, involves interactions of these photons in materials. Fused ring structures such as the benzothiazole *39* in which Ar is phenyl in-



39: Ar = C_6H_5



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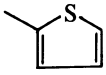
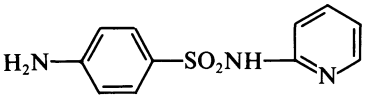
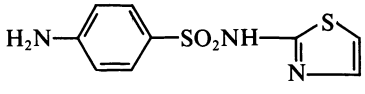
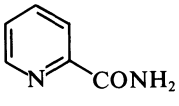
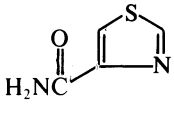
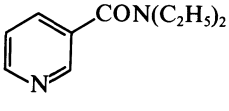
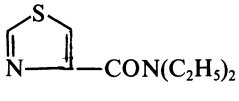
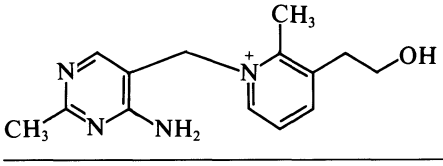
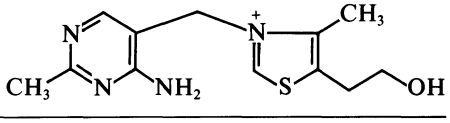


crease delocalizable π electrons. Exchange of phenyl for 2-thienyl further increases effective conjugation and electron density. Insertion of double bonds between the thiophene rings *(40)* and the central system further raises the number of conjugated π electrons [95].

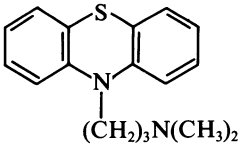
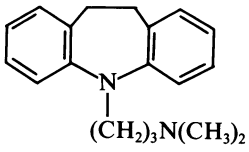
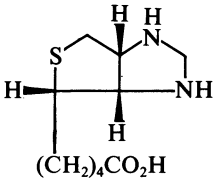
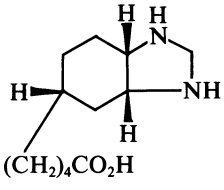
Another series of classical bioisosteres involving the replacement of -CH=CH- by sulfur is found in the comparison of pyridine and thiazole. This has furnished a number of instructive examples as well as useful drugs. The synthesis of sulfathiazole *(41)*, a potent and well-tolerated antibacterial agent, was undertaken to improve upon the effective but much more toxic sulfapyridine *42* [96].

Table III
Some classical thiazole bioisosteres of pyridine derivatives

Sulfapyridine *(42)*	Sulfathiazole *(41)*	[96] Bacteriostatic
		
Nicotinamide (niacinamide)	Thiazole-4-carboxamide	[97]
		
α-Picolinic acid	Thiazole-4-carboxylic acid	0.1 % activity [98]
Nikethamide	Thiazole-4-(N,N-diethyl)carboxamide	[99] Analeptic
		
Neopyrithiamine *(43)*	Thiamine *(27)*	Antagonistic in several bacterial growth systems [98, 100–102]
		

Sulfur has also been regarded as a nonclassical isostere of the group, $-CH_2-CH_2-$ (see Table IV).

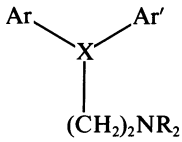
Table IV
Bioisosteres in which sulfur replaces $-CH_2CH_2-$

 Promazine *(44)*	 Imipramine *(45)*	Analogous activity in several tests [103, 104]
 Biotin *(46)*	 Cyclohexane Analogue *(47)*	Antagonistic [105]

1.5.1 Bioisosterism involving O, NH, CH_2, S

Steric factors account for many of the differences and analogies when oxygen, sulfur, imino and methylene replace each other. The valence angles of these atoms and groups are similar, at least in uncrowded aliphatic compounds, in ether, secondary amine, hydrocarbon and dialkyl sulfide linkages. They are approximately $108° \pm 3°$ for -O-; $112° \pm 2°$ for -S-; and $111.5° \pm 3°$ for $-CH_2-$ [106]. Ruling out connections of these atoms and groups to hydrogen (-OH, -SH, $-NH_2$, $-CH_3$), all other groups to which they are linked are larger and provide more shielding from other molecules which would compete for reactive sites. This effect, usually perceived as a means of preventing the approach of a damaging pathogen such as histamine or excessive concentrations of a neurohormone (e.g. epinephrine or acetylcholine) to an active site of an involved enzyme or receptor is seen in the structures of antihistaminics (histamine H_1 antagonists) *48* and *49* in which X can be any of the trivalent groups, -N<, -CH<, or -C=. Antihistaminic or anti-neurohormone activity can be fine-tuned by further bioisosteric variation of the Ar or $ArCH_2$ substituents such as C_6H_5,

$C_6H_5CH_2$, $C_6H_5CH(CH_3)$, pyridyl or other heterocyclic groups. Many of the classical blocking agents (H_1 receptor antagonists, and anticholinergics including blocking sedatives and anti-motion sickness drugs) have been conceived on this basis [107–109].



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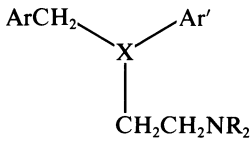

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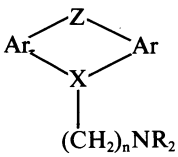
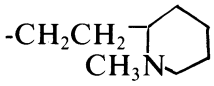
The two bulky blocking groups can be tied together by oxygen, sulfur, nitrogen, or carbon atoms or bridges. In such derivatives,



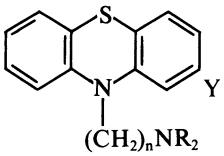
Z = O, S, NH, NR, CH_2, CH=CH (classical sulfur isostere), CH_2CH_2, CH_2S, CH_2O, CH_2-NH etc.

CNS depressant activities become more pronounced. Promazine *(44)* derivatives with more or less electronegative ring substituents (Y = Cl : chlorpromazine; Y = CF_3 : triflupromazine; Y = SO_2CH_3 with side chain



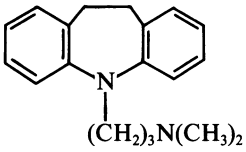
(sulforidazine)

are neuroleptics, while the bioisosteric drugs, imipramine *(45)* and amitriptyline *(50)* and many of their analogues are antidepressants. This transition to antidepressant properties has been attributed to the steric effect of the central seven-membered ring which tilts the two aromatic rings out of a planar conformation.



n = 3, R = CH_3, Y = H

44



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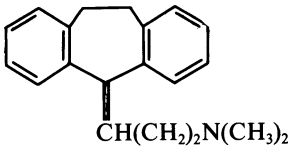
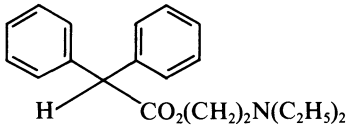


50

If the basic side chain of these compounds incorporates a dialkylaminoalkyl ester grouping such as $-CO_2(CH_2)_2NR_2$, the structures acquire

more affinity to the receptor of the neurohormone, acetylcholine [$CH_3CO_2CH_2CH_2N^+(CH_3)_3$] and become candidates for anticholinergic (antispasmodic) properties. Again, the two blocking moieties may be separate as in adiphenine, *51*, or joined by a linkage as in carbofluorene aminoester, *52*, or by a heteroatom as in propantheline *(53)*. In the design of basic derivatives of diphenylmethane, phenothiazine, dibenz[b,f]azepine, dibenzo[a,d]cycloheptene, fluorene, xanthene and similar systems, bioisosterism has played an initial important role to choose overall principles of structures that would furnish blocking compounds. The sheer number of hundreds of such compounds indicates that bioisosteric thinking takes a backseat to intuitive trials at molecular modification, and to the immense patience required to cull out a pharmacologically and toxicologically appropriate member of these series for therapeutic purposes.



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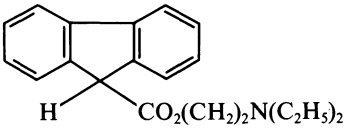

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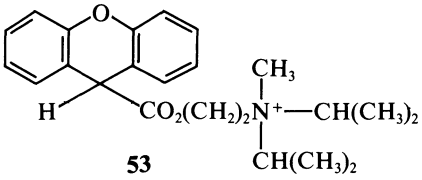
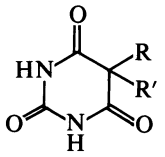


53

None of the antihistaminic H_1 receptor antagonists inhibited HCl secretion by the mast cells of the stomach which contributes to stomach and duodenal ulcers. Chemically, the antiallergic antihistaminics possessed blocking structures [48, 49] and not one of them resembled histamine or contained an imidazole moiety. When bioisosterism was invoked for imidazole derivatives, slow molecular modification of this heterocycle and opportunistic attachment of functional side chains laboriously led to the first H_2 receptor antagonists which are useful as antisecretory and antiulcer agents. The elegant story of the role of isosterism in elaborating their side chain will be detailed later, and so will the belated finding that the sacrosanct imidazole was finally not essential for high and useful activity.

1.5.2 Esters, ketones, thioloesters, amides and thioamides

Bioisosterism in esters, ketones, amides (-COOR, -CO-R, -CONH-R), their sulfur analogues, and compounds containing reversed ester and amide groups (-OCOR, -NHCOR) has been observed in local anesthetics [$ArCOO(CH_2)_nNR_2$; $ArCOS(CH_2)_nNR_2$] and blocking agents such as anticholinergics [$Ar_2CHCO\text{-}X\text{-}(CH_2)_nNR_2$; X = O, S, NH]. Amide-type agents are often inferior to esters, but thioloesters (X = S) may be more potent, depending on the pharmacological test method used [110–112]. Decreased resistance to hydrolysis by unspecific esterases may cloud the evaluation of these results. More important are differences between barbiturates *(54)*, thiobarbiturates *(55)*, iminobarbiturates *(56)*, and related ring systems which contain urea or thiourea



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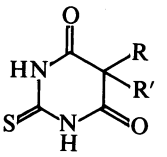

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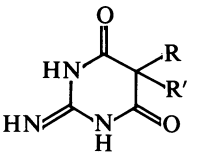
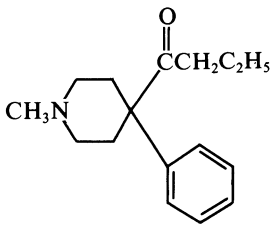


56

groupings. The thiourea-type compounds are often short-acting but more potent hypnotics and general anesthetics [113–115]. In H_2-receptor antagonists [116] containing thiourea moieties, the thiourea sulfur atom cannot be replaced by = NH, because the resulting guanidine derivatives are too basic to be bioisosteric with the thiourea derivatives. Activity returns readily if the basicity caused by the NH = analogues is weakened by electron-withdrawing groups, for instance, = NCN or = NNO_2 [117, 118]. Iminobarbiturates [58] corresponding to thiobarbiturates are similarly related; they were inactive [119], perhaps for the same reason, and moreover, they are too easily hydrolyzed.

Any biological differences of ester-type compounds in which the single-bonded oxygen atoms in R-O-R′ or R-C(=O)-O-R′ are replaced by -S- or -NH- may well be due to hydrolytic effects caused by the more polar properties of sulfur and imino linkages. Replacement of ester oxygens by methylene [R(C=O)OR′. . .R(C=O)CH_2R′] gives rise to ketones which are isosteric with esters in the classical sense. For instance, the propyl ketone *57* corresponding to meperidine *(58)* has about the same analgesic activity as the ethyl ester *(58)* drug [120].



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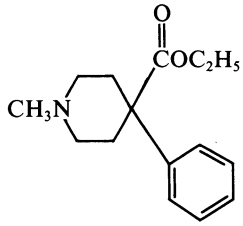


58

Extension of this ketone to branched and cyclic homologs weakens the analgesic properties but does not abolish it.

1.6 Transition from classical to nonclassical bioisosteres

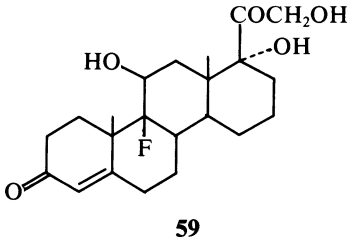
One of the most disappointing aspects of even the time-honored methods as we see them in Grimm's hydride displacement rules [6–8] is the finding that instead of an analogue with similar biological activity, one has created an antagonist to one's prototype. Even in the simplest case of bioisosterism, that of Na^+ and K^+, one encounters biological antagonism. The explanation that the new compound has at least *some* effect on a given biological behavior provides little comfort for additional rational plans in molecular modification. To the medicinal chemist, the finding of antagonistic properties must await quantitative biological test results and forces him to start all over in his SAR projections. Of course, measurements of several physical properties can precede biological testing and permit some evaluation of probable bio-properties before such tests are performed. The almost universal observation of side effects in biological tests only confirms the need for animal experiments and the failure of even the most carefully planned mathematical calculations to meet the complexity of living, biosynthesizing and biodegrading animals.

Among steroidal hormones, minor structural alterations can produce a range of overlapping, agonistic, antagonistic or widely different biological consequences. Androgens never have an aromatic A-ring, estrogens always do, but otherwise their structures are not so different. Some of the cortical hormones exhibit some major or minor sex hormone activities, and androgens have long been subdivided into anabolic and non-anabolic types. Substitution of hydrogen by fluorine [121] or oxidation of secondary alcohol groups to carbonyl enhances some hormonal activities and suppresses others. Elimination of angu-

lar methyl groups and substitution by acetylenic groups at C-17 can change one hormonal activity into another (estrogen to progestational etc.). Most of these compounds are not isomers; should at least some of these structurally overall similar agents many of which act at related receptors be called bioisosteres? Such a claim would have to be supported by studies of the lipophilicity and other physical properties of paired series of steroid hormones.

1.6.1 Isosteric halogens

Fluorine differs from the other halogens by its smaller size which leans more towards the volume of hydrogen, and by its stable shorter bond to carbon. These properties have been taken advantage of in 9α- (or 12α)-fluorocortisols *(59)* which are several times as active in many respects as the parent hormones [121], but other modifications such as the introduction of methoxyl lead to the same results; yet fluorine and methoxy are not isosteric in the classical sense. The firm bond between fluorine and carbon also favors stability in trifluoromethyl compounds. Thus trifluoperazine *(60)* is a more potent neuroleptic than prochlorperazine *(61)* but the more pronounced electronegative



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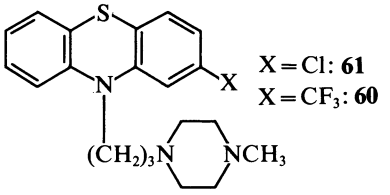
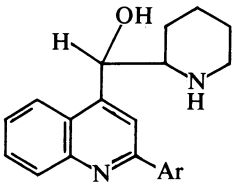


X = Cl: **61**
X = CF_3: **60**

character of trifluoromethyl probably accounts principally for this difference. Other highly electronegative groups (NO_2, SO_2CH_3) share this tendency, while electron-donating groups such as methyl are dystherapeutic. In many cases CF_3 substitution yields compounds that are less toxic than compounds with other electronegative substituents, and this has influenced the choice of test drugs to be prepared.
The trifluoromethyl group has played a role in molecular modification of quinine analogs. The antimalarial alkaloid, quinine, is deactivated by metabolic oxidation at position 2 of the quinoline ring.



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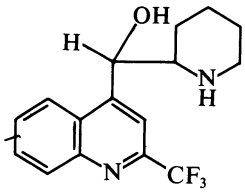

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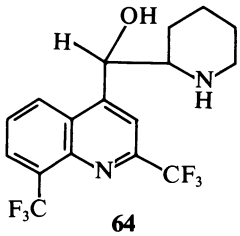
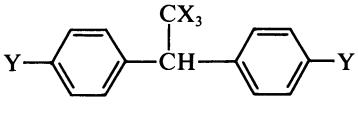


64

Blockade of this position by a variety of aromatic groups *(62)* regularly caused phototoxicity in the resulting derivatives and their analogues with a stripped-down piperidine ring [122]. It appeared possible that aryl substitution should be avoided and replaced by substitution with some other electron-rich but less conjugated structurally unrelated group. Trifluoromethyl was chosen as a test case *(63)* [123]. Although phototoxicity encountered in *62* was not eliminated completely, further substitution with CF_3 was undertaken because 3,6-bis(trifluoromethyl) substituted phenanthrene amino alcohols had shown a considerable increase in antimalarial activity over monotrifluoromethyl derivatives [124]. Empirical substitution in all benzenoid positions of the quinoline ring yielded the 2,8-bis (trifluoromethyl)quinoline piperidyl alcohol *64* (mefloquine) which has become an important antimalarial drug [125].

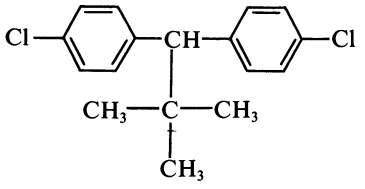
The relationship of fluorine, hydrogen, and other halogens is illustrated in a series of analogues of the contact insecticide, chlorophenothane (DDT) *(65)* in which the five chlorine atoms were systematically



65

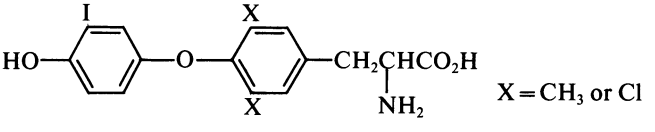
X = Y = Cl

exchanged for fluorine, bromine, and methyl. Here activity usually depends on the size of X, neurotoxic contact insecticidal activity being registered when X = Cl, Br or CH_3 with Y = Cl. No activity is found if X = F or H, but if Y = F and X = Cl, activity is comparable to Y = Cl or Br. However, if Y = F and X = CH_3, activity is lost [126], even though chlorine and methyl occupy comparable volumes [127]. This prediction is, however, confirmed in the bis(*p*-chlorophenyl)-*t*-bu-



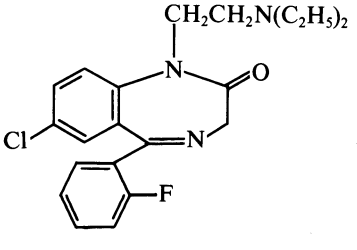
66

tylmethane analogue *66* which is as neurotoxic as chlorophenothane *(65)*. Possibly, steric factors are also involved, the trichloromethyl group of *65* and the *t*-butyl group of *66* bending the two aromatic nuclei out of the tetrahedral angle one expects for diphenylmethane.
In the non-phenolic ring of thyronine analogues, 3,5-dichloro substitution of *L*-T_3 in lieu of iodine produces an appreciable thyromimetic effect, and so does the 3,5-dimethyl derivative *(67)*. These substituents



67

contribute to the hydrophobicity of the ring [128]. Binding free energy values of the dimethyl analogue of *L*-T_3 and the dichloro analogue of *L*-T_4 are almost identical [129]. It must be kept in mind, however, that methyl groups can be oxidized metabolically, especially if they are attached to an aromatic ring. This may affect the half-life in tests *in vivo*.
The overall effects of fluorine substitution are hard to predict. Flurethyl, bis(trifluoromethyl)ether, is a central stimulant with an action similar to the initial phase of diethyl ether. In the replacement of the *o*-chlorine atom in chlorazepam by fluorine, flurazepam *(68)* deepens sedative-hypnotic activity [130]. On the other hand, while chloroform ($CHCl_3$) is a general anesthetic, dichlorodifluoromethane (CCl_2F_2)

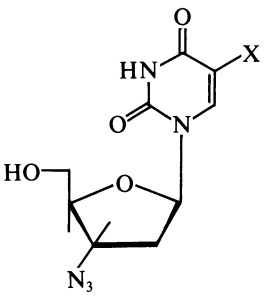


68

has little, if any, depressing or toxic properties. Here the lipophilic distribution may be the principal influence on activity.

3′-Azido-3′-deoxythymidine (AZT, zyvudine) *(69)* is a potent inhibitor of the replication of the human immunodeficiency virus (HIV) [131]. It is phosphorylated to the triphosphate which then inhibits the utilization of dTTP by reverse transcriptase and thereby prevents the elongation of viral DNA. Various 3′-azido nucleosides have been prepared and tested against HIV-1 in cell cultures; a few are listed in Chart 1. Of these modifications, AZT remained the most active drug. Trifluoromethyl substitution *(69 A)* that could be thought of as leading to a bioisostere, abolishes activity, whereas the halogen deriva-

Chart 1 [132]



X =	X =
H	$OCH_2CH=CH_2$ **(70)**
CH_3 **(69)**	$OCH_2C=CH$
CF_3 **(69 A)**	OCH_2CN
	SCH_3
F, Br, I **(69 B)**	SCN
OH	NH_2, $NHCH_3$
OCH_3	$N(CH_3)_2$
OC_2H_5	

tives (F, Br, I) *(69 B)* and the amino and hydroxyl compounds (other classical isosteres) retain significant antiviral potency. The allyl ether *70* analogue was highly active.

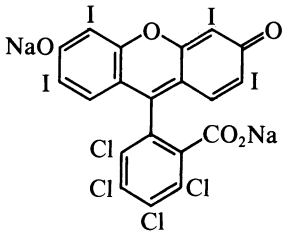
No clear relationship between antiviral activity and the electron-donating or electron-withdrawing capacities of the 5-substituents emerged from this study.

The replacement of chlorine by bromine in some drug structures generally, but not inevitably, deepens some biological actions, including toxicity. Bromide ion had been used as a calming sedative in the 19th century, and perhaps as a memory of this application some aliphatic bromo compounds such as diethylbromoacetylurea, $(C_2H_5)_2CBrCONHCONH_2$, and bromoisovalerylurea, $(CH_3)_2CHCHBrCONHCONH_2$, are still prescribed sporadically in some countries for this purpose. Chloral hydrate, $Cl_3CCH(OH)_2$, and chloroform are general anesthetics but the corresponding bromo compounds cannot be used. Here again, lipophilicity is the most import-

ant – at least the most widely studied – determinant in SAR. Force of inhibition of CNS enzymes may well shed more light on these questions. Thus, isosterism can be claimed for all the halogens but bioisosterism which postulates the similarity of biological properties cannot be predicted easily.

Two types of agents appear to depend on the presence of iodine in their molecules for useful activity. In the case of thyroid hormones, the several iodinated thyronines have not been rivalled by the corresponding bromo and chloro analogues. Enzymatic uptake of iodide and subsequent oxidative biosynthesis of the hormones is a process that has evolved over millions of years in aquatic and later in terrestrial animals. There is no reason to assume that other halogens could not participate in these natural reactions, but apparently this does not happen. That iodine is not unique in producing hormonal properties has been shown by synthesizing *D,L*-3,3′-diiodo-5-bromothyronine which increased the oxygen consumption of thyroidectomized rats and reduced the size of the thyroid gland in antigoiter tests [133]. *DL*-3,3′,5-Triiodo-5′-fluorothyronine, an H...F isostere of triiodothyronine (T_3), shows about one-third the effect of thyroxine by the goiter prevention method [134].

The other case is that of organic iodinated radiopaque agents. Iodine, in organic linkage, endows compounds with a greater capacity for x-ray opaqueness than do the other halogens. Nevertheless, tetrabromophenolphthalein was used briefly in cholecystography until the more effective isomeric tetraiodo compounds could be prepared. Phenoltetrachlorophthalein has also been used in hepatography [135], and so has Rose Bengal, the disodium salt of tetraiodotetrachlorofluorescein *(71)*.



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1.7. Limits of bioisosterism

By the early 1960's, some uncertainties had surfaced in the explanation and applications of bioisosterism. One apparent shortcoming was that what seemed to be a solid rule in one series of structurally related compounds failed to work out in a series with different overall structural characteristics. In such cases one could not predict whether one would end up with an agonist, a mixed agonist – antagonist, or an antagonist. Ariens [136] and Korolkovas [137] tried to list those structural moieties which often turn an agonist into an antagonist. However, they did not include the possibility that the bioisosteric planning process, i.e. experiments to rationally design a drug structure, may have disturbed one of several parameters that had proved to be necessary for inducing a given biological activity. Such parameters include size and bond angles (shape), ability to form hydrogen bonds at active sites of enzymes or drug receptors, pK_a, chemical reactivity, hydrophilicity, lipophilicity (i.e. maintenance of the same or at least similar partition coefficients), and also the metabolic fate of the compounds to be prepared. There are limits concerning the number of these parameters that can be changed unpunished. Yet the hope for potential success in bioisosteric exchanges has intrigued many medicinal chemists, even if they or their physical chemistry colleagues have not measured all the constants and values involved. This has led to a silent understanding that at least in early research in a given structural series one might postpone adhering to the more rigid requirements of classical isosterism.

The reasons for this apparent self-deception are practical realities of daily work. No two manufacturers of automobiles, computers, breakfast cereals, swimwear and other necessities of the good life will copy every feature of their competitors. Instead, they will try to improve some aspects of the original product and delete, or at least play down, any point that had elicited justified criticism. In order to achieve these guidelines, the properties of the product to be modified would have to be analyzed and understood as far as the state of the art permits.

It is the same with medicinal agents. One will rarely seek to obtain a drug with completely identical properties but almost always try to improve potency and specificity and decrease side effects and toxicity. Bioisosterism provides descriptions of electronic, steric, lipophilic,

and distribution characteristics of a compound, and these data help us to understand mechanisms of action and the role of a compound in producing measurable biological consequences. If the purpose of a research project is to come up with a drug that exhibits modifications of one or several properties and does not wholly match the model "lead" compound, then the description and definition of this "lead" compound will not suffice. At this point, human inventiveness and intuition and our gambling spirit as to which facet of the necessary properties we should change, will have to be brought into play. Experience in a structural field will be helpful in prompting our decision making. If we want to create a bioisosteric drug with some desirable changes, we will have to be aware of the fact that sooner or later we may step outside the limits of permissible classical bioisosteric alterations. As we transgress these limits we stand to lose activity altogether or sometimes, conjure up a totally new set of biological properties, some wanted, some undesirable, and usually baffling. The rules which delineate bioisosterism will point to compromise structures which may be just still acceptable as candidates. Such chemical structures are spoken of as non-classical bioisosteres. Their study and use has expanded steadily over the last several decades.
It will come as no surprise that reviewers of the subject of bioisosterism have given preference to either more biochemical or more physical aspects of the phenomenon [138, 139].

1.7.1 Interactions at receptor sites

One guideline how far we may venture in designing new drugs at the border of bioisosterism is provided by advances in our understanding of biochemical mechanisms at receptor sites. Receptors are glyco- and lipoproteins or, in some cases, nucleic acids. The protein receptors may be enzymes or membrane-spanning macropolypeptides. Active sites of enzymes are situated in the protein chains of these biocatalysts and are part of molecular bays formed by these chains. A drug or substrate has to fit more or less snuggly onto such harboring polypeptide bays and will try to make contact with the amino acid side chains or the amide groups of the peptide backbone. The side chains are SH groups, alcoholic and phenolic OH groups, amino and guanidino groups, carboxyls and carboxamide groups, and disulfide bridges which are partly responsible for the stereostructure of the enzyme.

Quite commonly, carbonyl and other groups of the enzyme's polypeptide chain attract drug molecules by way of hydrogen bonding. Since the angle of regions which permit hydrogen bonding can be as much as 60°, drug molecules differing considerably in atom-to-atom overlap but capable of hydrogen bonding may be attracted and bind to the same region of an enzyme protein. This type of combination will be favored if the approaching small molecule can assume a conformation complementary to the shape of the protein bay. In some cases, quite remote regions of the polypeptide will pass one atop of another, but at a distance that can be spanned by two or more hydrogen bonds of the drug molecule. In other cases, attractive van der Waals interactions or London dispersion forces will come into play between drug or substrate and polypeptide. If an enzyme contains a relatively small coenzyme or cofactor moiety such as some vitamin or metal atom, a substrate or drug may react with these active portions by ionic or covalent interaction.

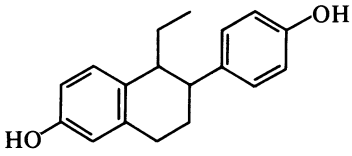
Similar conditions will affect recognition of foreign chemicals by trans-membrane receptor proteins. Here again, the usually heavily folded polypeptide chains will offer ample opportunity for attracting and ligating molecules that pass by in the circulation in search of a safely binding harbor. The need for steric fit cannot be overemphasized and emerges as the most important condition of bioisosteric requirements.

If a drug acts by intercalating between turns of the helix of a nucleic acid, steric fit to the available space in such turns will again be the precondition for intercalation. Hydrogen bonds plus van der Waals forces will be required to stabilize the hold of the polynucleotide onto the drug. These bonds may form with different groups of the polynucleotide and provide some latitude to details of the intruding drug molecule. That means, they will stretch the requirements of bioisosterism beyond classical limits.

1.7.2 Steric similarities

When distances between functional groups, and also molecular shapes are of prime importance to enable a compound to be recognized at a receptor, it becomes possible to interchange substituents more freely provided they are spatially compatible and have more or less similar bond angles. The insertion of -OCO- in lieu of $-CH_2CH_2-$

in the (weak) estrogens *72* and *73* is an example of such design [140, 141].



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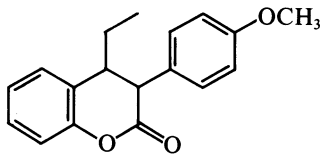
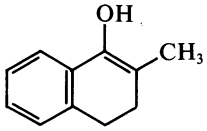


73

Another case of the replacement of $-CH_2CH_2-$ by a lactone group is the comparison of 3,4-dihydro-2-methyl-1-naphthol *(74)* with the α, β-unsaturated lactone *75* [140]. The naphthol derivative has considerable vitamin K-type coagulant activity (in the range of 1 γ) probably because it can be oxidized to a naphthoquinone. The coumarin *75* has only very weak activity in the same test [142, 144].



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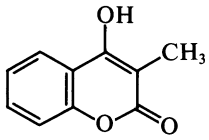

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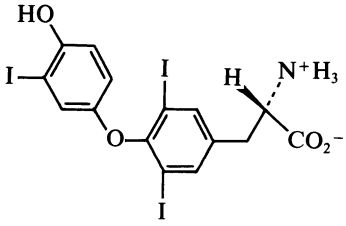
In another study of the significance of spatial analogy, the thyroid hormone, 3,3′,5-triiodo-*L*-thyronine (T_3) *(76)* was modified. A large number of compounds in which the 3′-iodine atom was replaced by all kinds of saturated, unsaturated, small, large, hydroxyalkyl, carbonyl-containing and other functional groups were tested *in vitro* for hormone-receptor binding to intact nuclei, and *in vivo* thyromimetic activity. Analysis of these data using conformational and QSAR structure-affinity methods revealed that the 3′-substituent recognition site on the T_3 receptor is hydrophobic and limited in depth to the length of the natural iodine substituent, but sufficiently wide to accommodate a phenyl or cyclohexyl group. These two groups may therefore be



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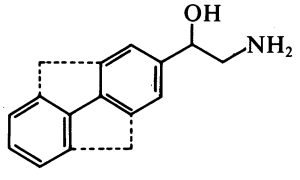
regarded as bioisosteric with iodine in this particular instance, but this suggestion could not be applied to unrelated situations with quite different receptor functions [143].

A flat hydrophobic surface at least 11 Å long, 4 – 5 Å wide, and approximately 2 Å above or below the plane of the aromatic ring has been calculated for the active site of phenylethanolamine N-methyltransferase (PNMT) (from bovine adrenals) whose catalytic properties are inhibited by conformationally defined analogues of amphetamine and phenylethanolamine. If the aromatic rings of the biphenyl systems *(77, 78)* are conformationally restricted by incorporation in



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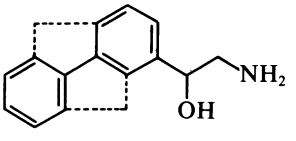

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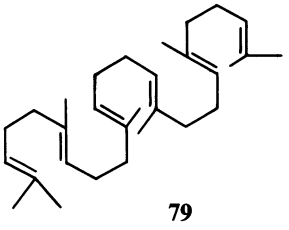
fluorene structures, dotted lines), the affinity for PNMT is enhanced. Thus, either the *p*- or *m*-biphenyl derivatives *(77, 78)* as well as the corresponding fluorenes may be regarded as bioisosteres in this enzyme system [144].

Potential bioisosteres of squalene *(79)* may be seen in some antifungal allylamines [145]. Squalene is a substrate of squalene epoxidase which is the first enzyme requiring molecular oxygen in the biosynthesis of



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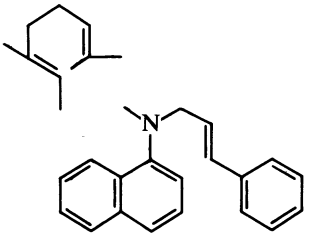

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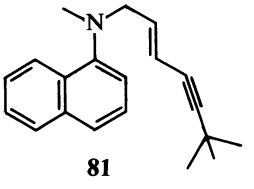

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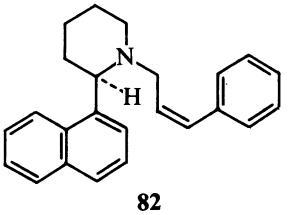

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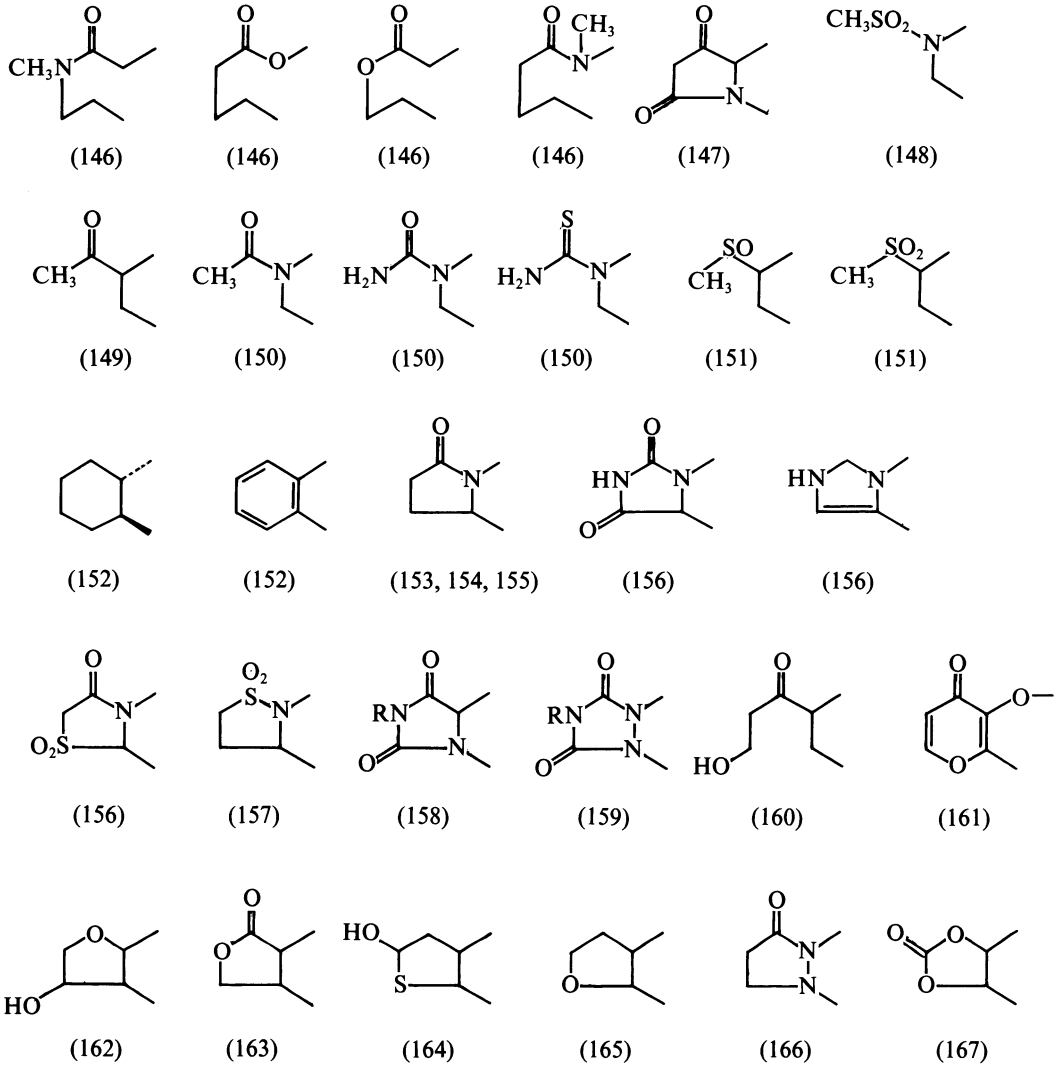
sterols. The N-1-naphthyl-N-allylamines *80* and *81*, and also the piperidino analogue *82* are specific inhibitors of squalene epoxidase and seem to exert their antimycotic activity by this mechanism. The formal similarity of squalene and these inhibitors lends credence to this interpretation. The acetylenic derivative *81* acts by noncompetitive kinetics at an inhibition constant of 3×10^{-8} M.

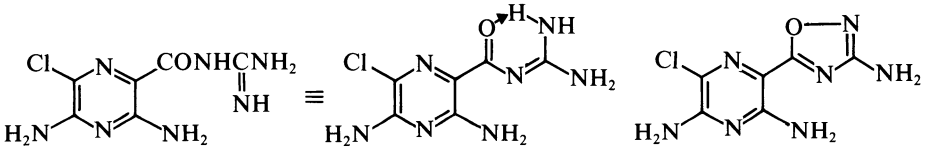
Thornber [138] has listed and referenced 32 variations of the central cyclopentane ring of the prostaglandins, some of which may be re-

Chart 2. Analogues of prostaglandins [138]



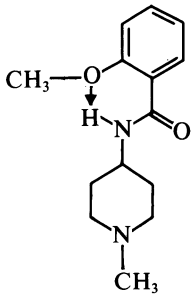
garded as bioisosteres of the dihydroxycyclopentane and hydroxycyclopentanone structures of the natural hormones. Carbon has been replaced by oxygen and nitrogen, the keto group has given way to amide, ureide, sulfonamide, methylsulfoxide, methylsulfone and cyclic oxygen (tetrahydrofuran) and sulfur (tetrahydrothiophene) functions, and the cyclopentane ring has been expanded to cyclohexane, benzene, pyranone and related systems. These derivatives may be a source of information on how far nonclassical bioisosterism can be carried. In these derivatives, the groups listed (Chart 2) represent the turning point of the long unsaturated carboxylic acid chains of the prostaglandins.

Even a cursory survey of these groups which are to mimic or systematically alter the central cyclopentane ring of the natural prostaglandins suggests that shape and molecular volume are the decisive attributes of bioisosterism in this series barring polar differences. Similar conditions are found in a series of analogues of the potassium-sparing diuretic, amiloride *(83)*. In this study the early application of ring equivalents in isosterism [4] has included hydrogen-bonded rings, specifically a hydrogen-bonded acylguanidine *(84)*. By slightly shortening the distance between the amide oxygen and the guanidine nitrogen, the 3-aminooxa-2,4-diazole *85* was designed; it had very similar diu-



83 **84** **85**

retic properties as behooves a bioisostere [168]. In an analogous fashion, incorporation of the amide group of the benzamide neuroleptic *86* into a hydrogen-bonded ring could explain the binding of this compound to a dopamine receptor [169].



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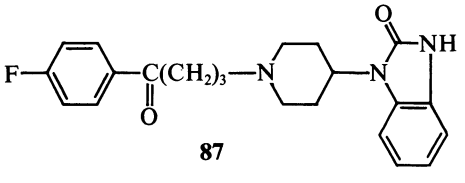
1.8 Non-classical bioisosteres of carbonyl-type structures

1.8.1 Carbonyl-type structures

This structural segment comprises ketones ($R_2C=O$), both saturated, enolic tautomers (=C-OH) and α, β-unsaturated carbonyl compounds [=CH-C(=O)R], carboxylic acids [RC(=O)OH], esters [R-C(=O)OR], amides [R-C(=O)NHR′], amidines [R-C(=NH)NHR′], guanidines [R-NHC(=NH)NHR′], and their cyclic and hetero analogues. Any of these groups can accept hydrogen bonds from receptor sites, or extend such bonds to macromolecular points. This happens also if the functions are inverted as in reversed esters [-C(=O)OR, -O-C(=O)R] which often exhibit almost identical biological properties. Hydrogen bonds can thus be diverted to neighboring positions. Bioisosteric incorporation of keto groups into heterocyclic arrangements is seen in oxazole analogues *(89)* [170] of the neuroleptics, benperidol *(87)* and haloperidol *(88)*. Similar examples are encountered in bioisosteres *(91)* [171] of diuretic 4-acylphenoxyacetic acids *(90)*.



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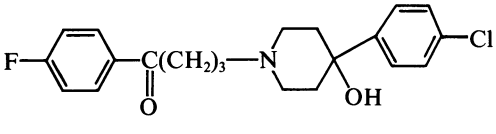

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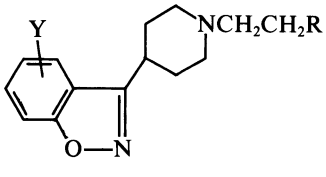

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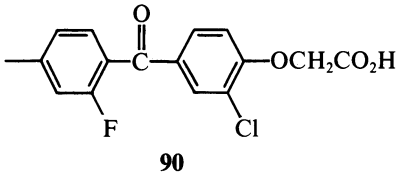

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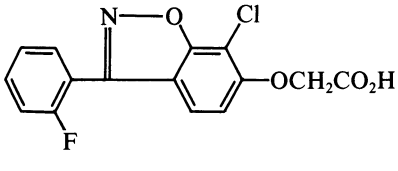
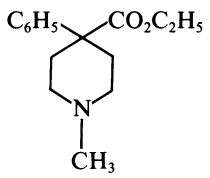


91

The keto carbonyl has been replaced by sulfoxide (> SO) and sulfone (> SO_2) *(93)* in methadone *(92)*; the sulfone isostere *93* is slightly more active and less toxic than methadone [172, 173].

$$(C_6H_5)_2 > \underset{\displaystyle CH_2CH(CH_3)N(CH_3)_2}{\overset{\displaystyle C\text{-}X\text{-}C_2H_5}{|}} \qquad \begin{array}{l} X = CO: 92 \\ X = SO, SO_2 : 93 \end{array}$$

Similar exchanges have been studied for meperidine *(58)* [174, 175].



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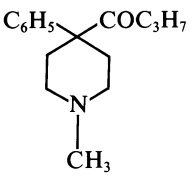

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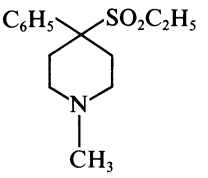

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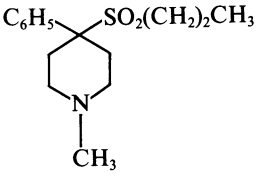
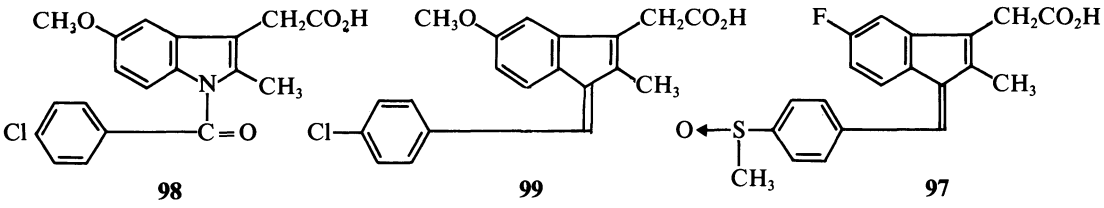


96

The ketone analogue, *94*, and sulfone derivatives *95* and *96*, are potent analgesics.

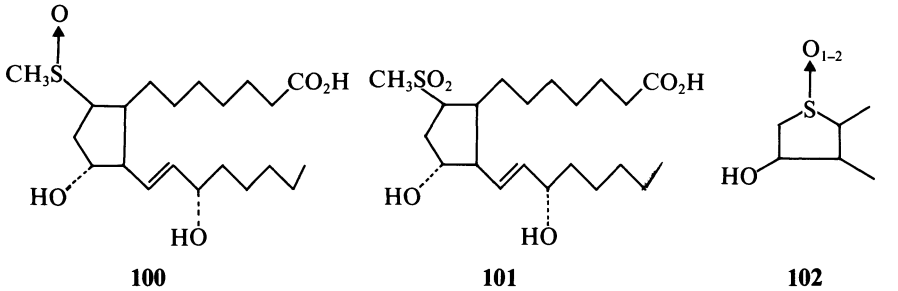
1.8.2 Sulfoxides and sulfones

Sulfoxides should resemble carbonyl compounds but they are more polar [41]. The sulfoxide, sulindac *(97)*, is a nonsteroidal antiinflammatory arylacetic acid. The *p*-methylsulfinyl group causes the compound with this substituent to become much more soluble and to provide a center of metabolism to minimize the accumulation of any single metabolite in the kidney. Sulindac was developed in the course of molecular modification of the *p*-chlorobenzoyl indole derivative indomethacin *(98)* [176, 177]. The indene isostere *(99)* of indomethacin, a typical application of -C(=O)-N< → -CH=C< bioisosterism, had fewer CNS side effects. Considerable and laborious molecular modification led to sulindac *(97)*. The active metabolite of *97* is its CH_3S- derivative. Bioisosterism of Cl and CH_3S had been observed in the indomethacin series. The indole → indene, and >N-C(O)- → >=CH- isosteric replacements could be anticipated, and the choice of a sulfoxide group to increase solubility was a logical plan. To complete the picture, the methylsulfonyl (CH_3SO_2) oxidation product is a metabolite of sulindac.



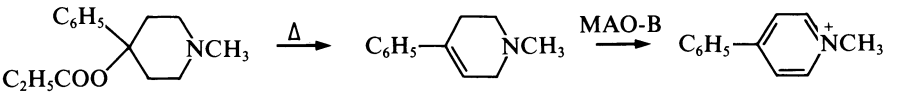
Another case of bioisosterism involving indole and carbocyclic systems is the pair, 2,4-dichlorophenoxyacetic acid (2,4-D), a herbicide, and 3-indoleacetic acid, a plant growth hormone (auxin). The herbicide is much more potent than 3-indoleacetic acid and more slowly degraded in plants. It accumulates in treated plants, causing them to grow much too rapidly. Thus young plant cells do not mature in a normal way. Parts of the plant's food-transporting vessels are plugged up by clusters of cells, inhibiting the movement of nutrients. 2,4-D is believed to damage plant respiration, cell division, hormone balance and protein synthesis.

Along with the extensive molecular modifications of the cyclopentanolone ring of the prostaglandins E_1 and E_2 listed in Chart 2, replacement of the 9-carbonyl by methylsulfoxide *(100)* and methylsulfone *(101)* has been recorded [178]. In addition, several derivatives incorporating the sulfoxide *(102)* [179] and sulfone groups [180] in the 5-membered ring have been prepared.



1.8.3 Reversal of functional groups

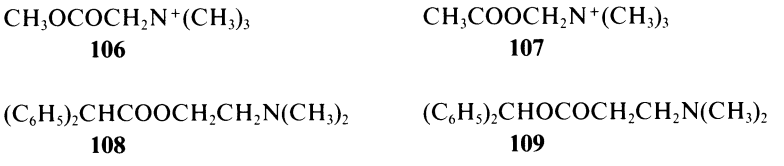
Reversal of the ester group of meperidine produces 1-methyl-4-phenyl-4-propionoxypiperidine (MPPT) *(103)* which is said to be five times more potent than meperidine in analgesic tests (181, 182). If too much heat is applied in the esterification of 1-methyl-4-phenyl-4-pip-



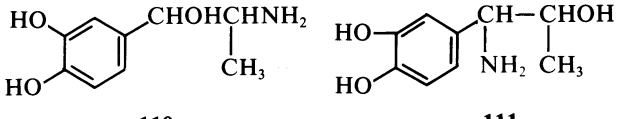
103 **104** **105**

eridinol which yields *103*, propionic acid is eliminated *(104)* and this unsaturated compound can be dehydrogenated biochemically by the action of monoamine oxidase-B to 1-methyl-4-phenylpyridinium (*105*, MPP^+), a dangerous hallucinogenic neurotoxin referred to as a "designer drug". It also causes severe extrapyramidal symptoms.

Reversal of functional groups in cholinergic and anticholinergic agents also results in very similar activity in the respective pairs. The muscarinic betaine *106* and its isomer *107*, as well as the antispasmodic diphenylacetate ester *108* and its benzhydryl isomer *(109)* may serve as examples [183–185].



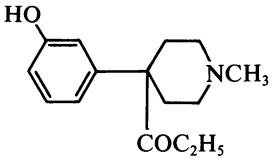
Not all kinds of isomeric exchanges and reversals are permissible in structures that are related to certain adrenergic hormones. Thus, reversal of the alcoholic hydroxyl and the amino groups in the norepinephrine homologue *110*, a pressor agent, leads to loss of activity *(111)* [186, 187]. Perhaps the genetic memory of biochemical evolution overrides bioisosteric considerations.



110 **111**

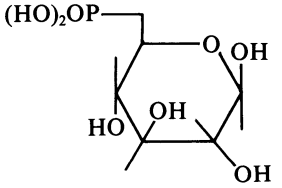
1.8.4 Deletion of oxygen

A ketonic analogue *(94)* of meperidine *(58)* has already been mentioned in connection with bioisosteric exchanges of CO with other electron-withdrawing groups. A lower homologue of *94*, with a *m*-phenolic hydroxyl group, is called ketobemidone *(112)* [188, 189]. It



112

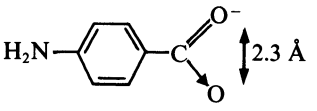
illustrates another suggestion that biological activity may be retained sometimes if an ether oxygen is deleted in nonclassical bioisosteres [190]. However, this is not a general rule and should be used with misgivings. A replacement of ether oxygen in esters and ethers by methylene as a spacer group offers a safer approach to the construction of bioisosteric compounds because at least the distance from other points in the molecule has been maintained in an -O-⟶-CH_2- exchange. An example in which the deletion hypothesis failed is the removal of the ether oxygen in *D*-glucopyranose 6-deoxy-6-phosphonic acid *(113)* [191]. This did not cause inhibition of several enzymes which require glucose 6-phosphate for catalytic performance.



113

1.8.5 Bioisosterism of carboxylic acids

Some of the most clearcut bioisosteric relationships have been established for carboxylic acids. The carboxylate ion, RCO_2^-, resembles the sulfonamide ion, RSO_2N^- [38]. This nonclassical isosterism explains the antagonism of *p*-aminobenzoic acid (PABA) and sulfanilamide [200, 201]. At a concentration of 3.03 X 10^{-4}M, sulfanilamide action is reversed by a molar concentration of 1.2–5.7 X 10^{-8} of PABA [200]. The distances between the two oxygen atoms of the sulfanilamide *(115)* and PABA *(114)* anions are virtually the same. Also, at phy-



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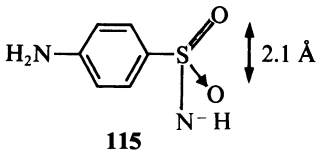


115

Table V (190)
Effects of Omissions and Replacements of Ether Oxygens on Biological Activities

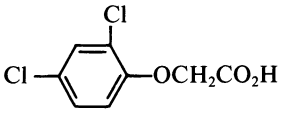
Chemical Structure	Biological Test	X=O	X=CH_2	X absent	Ref.
HO—C6H4—$OCH_2CH_2XC_2H_5$	Phenol Coefficient *Staph. aureus*	5	30	9	(192)
C6H4(XC2H5)(OCH2CHOHCH2OH)	ED_{50} mg/kg for muscular paralysis	1.4	5.8	1.8	(193)
$[(C_2H_5)_3N^+CH_2CH_2X]_2$—C6H4—	Curaremimetic Activity	100%	0	100%	(194)
$(CH_3)_3N^+CH_2CH_2]_2X$	Ganglionic Blockade	10%	100%	33%	(195)
$[(CH_3)_3N^+CH_2CH_2X]_2(CH_2)_n$	Curaremimetic PD_{50} micromoles/kg n=1 n=2 n=3	 91 50 0.3	 1.5 0.25 0.01	 60 61 0.25	(196)
C3H3N2(CH2XC6H5)(NH)	Effect on Blood Pressure	Pressor	Weak depressor	Strong depressor	(197)
$C_6H_5XCH_2CH_2NH_2$	Effect on Blood Pressure	Depressor	Weak depressor	Strong depressor	(198) (199)

siological pH the SO_2 group of the anion of sulfanilamide abstracts electrons from the nitrogen atom and thereby acquires a higher electron density than in the non-ionized state. Substituents which further attract electrons and strengthen the ionization of the sulfonamide ion have been shown to increase the bacteriostatic activity of sulfanilamide derivatives.

Bacteria which cannot biosynthesize tetrahydropteroylglutamic acid from available PABA will be inhibited by competition of SA at the step at which PABA is incorporated into the pteroylglutamate structure, and thereby lose the fundamental avenue to ribonucleic acid and hence to protein biosynthesis. Sulfanilamide must be present at concentrations higher than those of PABA to inhibit PABA incorporation. So great is the biochemical similarity of PABA and its antagonist that bacteria susceptible to SA can become resistant to this drug by acquiring the ability to synthesize unusually large amounts of PABA and thus overcome the inhibition of their reproduction.

Other acid functions such as phosphonic acids have also been studied as bioisosteres of carboxylic acids. Phosphanilic acid,

p-$H_2NC_6H_4PO(OH)_2$ [202], *p*-aminobenzenephosphonous acid, *p*-$H_2NC_6H_4P(OH)_2$ [203], and phosphanilamide, *p*-$H_2NC_6H_4PO(NH_2)_2$ [204], are bacteriostatic and the action of the two acids is antagonized by PABA [203–205]. Other phosphonic acids with useful biological activities include phosphonoacetic acid, $(HO)_2POCH_2CO_2H$, a bioisostere of malonic acid, and some of its carboxylate esters [206]. These compounds have antiherpes activity *in vivo* by inhibiting DNA polymerase induced by herpes and by cytomegalo viruses [207–210]. Another phosphonate-carboxylate analogy is that of the plant growth regulator, 2,4-dichlorophenoxyacetic acid (2,4-D) *(116)* and its monoethyl phosphonate bioisostere *(117)*. The latter has slight but appreciable auxin activity [211].



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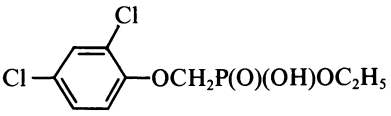
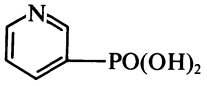


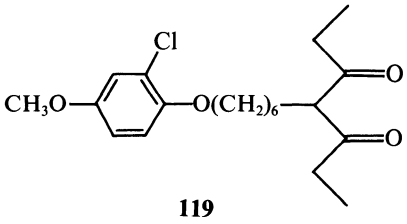
117

On the other hand, 3-pyridylphosphonic acid *(118)*, an analogue of niacin, did not inhibit the multiplication of *Lactobacillus arabinosus* which requires niacinamide, nor did it interfere with the incorporation of niacin into coenzymes or with the biosynthesis of niacin by two mycobacteria [212].



118

Unesterified phosphonic acids are more acidic than carboxylic acids and therefore monoesters can be expected to show a lower degree of acidity, approaching the range of carboxylic acids, i.e. to be more isoelectric. Phosphonate diesters may be more bioisosteric to β-diketones rather than to carboxylate esters. The antiherpetic arildone *(119)* has been compared to its diethyl phosphonate bioisostere *(120)* [213].



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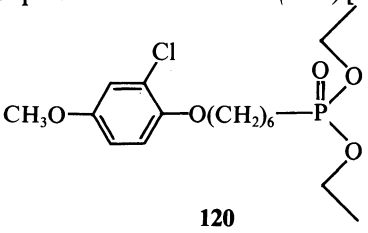

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Phosphate ester groups as they occur in nucleotides cannot be replaced successfully because nucleotides cannot enter cells but must be hydrolyzed to nucleosides which can penetrate cell walls and are then rephosphorylated intracellularly. The unique role of phosphate esters as agonists in many biochemical reactions has been detailed by Westheimer [214]. Analogues with ester groups other than phosphates will suffer the same fate and be converted to nucleotides inside the cell. However, a few hopeful variations have been recorded. Replacement of the terminal phosphate ester oxygen X of ATP (*121*, X = O) by NH, CF_2, CCl_2, CHF and CH_2 leads to an amide (X = NH) and

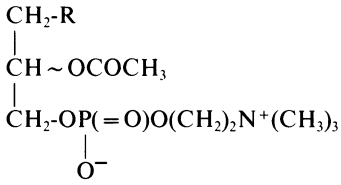
Adenine-$CH_2OP(O)(OH)$-O-$P(O)(OH)$-X-$P(O)(OH)_2$

121

to phosphonate-type compounds whose similarity to ATP decreases from NH to CH_2 [215]. The amide, AMP-PNP, is used widely in biochemical mechanistic studies.

In another nucleotide, a diphosphate ester was replaced synthetically by $OSO_2NHC(=O)O$- and the resulting bioisostere inhibited HSV-1 virus *in vitro* [216].

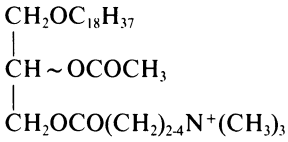
Platelet-activating factor (PAF-acether) *(122)* is a phospholipid mediator of anaphylaxis. Isosteric replacement of the phosphate ester by



$R = C_{18}H_{37}$ or $C_{16}H_{33}$

122

carboxylate ester groups *(123)* and also introduction of an ethyl ether group at C-2 *(124)* as well as changes in the ammoniumalkyl at C-3 *(125)* gave antagonists which provided 77–100 % protection against PAF-acether effects [217].



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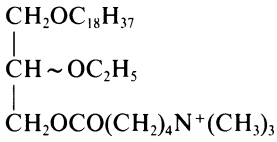

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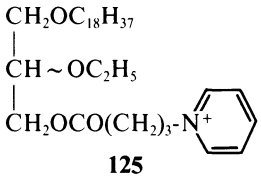
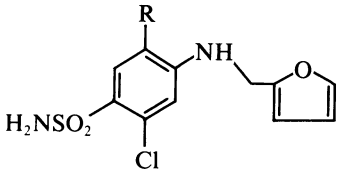
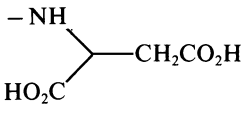


125

Similarly, the carboxyl group of furosemide *(126)* can be replaced by SO_3H *(127)* without loss of diuretic activity [218]. GABA has been imitated in the gabaergic sulfonic acid, $H_2N(CH_2)_4SO_3H$ [223].



126: $R = CO_2H$
127: $R = SO_3H$



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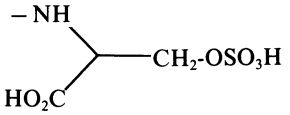
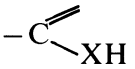
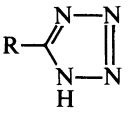


129

When the C-terminal aspartic acid residue *(128)* of gastrin was replaced by the electronically equivalent serine O-sulfate ester *(129)*, activity was retained [219]. In a number of enzyme systems, exchange of amino acid carboxyl for O-phosphate [220], phosphinate or phosphonate [221] or even for boronic acid groups [222] led to inhibitors of the catalytic activities.

Cyclic systems which contain the  arrangement can be classified as non-classical isosteres. Among them are especially tetrazoles *(130)* which resemble the corresponding carboxylic acids *(131)* in



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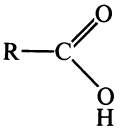

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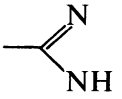
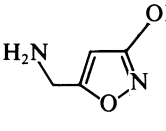
dissociation constants and can be interpreted as carboxylates in which -NH takes the place of -OH and in which the double and single bonds are stabilized by participating in the heteroring. One could even picture N_2 being discarded to unveil the isosteric 

moiety [224]. This system has also been extended by vinylogy, for example for GABA (γ-aminobutyric acid) analogues. The heterophen-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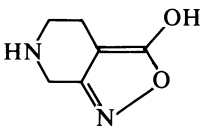
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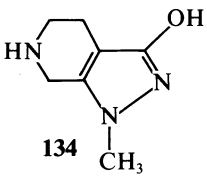
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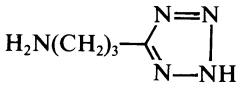
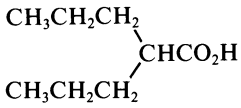

135

olic analogues *133* and *134* of the GABA agonist, muscimol *(132)*, have an acidity range of 3.0 *(133)* to 7.1 *(134)* and inhibit the binding of GABA to its receptor [225]. The tetrazole bioisostere *135* of GABA inhibited GABA transaminase [226]. In modifications of valproic acid *(136)* both the tetrazole *(137)* and 3,5-dioxo-1,2,4-oxadiazolidine *(138)* analogs inhibited SSA-DH [227]. On the other hand, branched analogues *(139)* where X is CH_2 or NH, were convulsants [228].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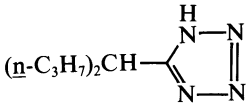
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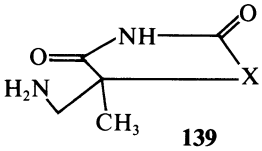
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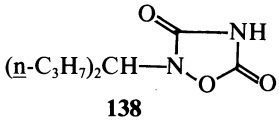
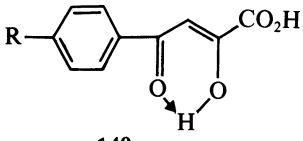
X = CH_2, NH


138

The inhibitory action on aromatic ring glycolic oxidase by the α, β-unsaturated α-hydroxy-γ-keto acid *140* and the 3-hydroxypyrroleine-2,5-dione *141* shows two partially superimposable compounds with the same biochemical behavior [229].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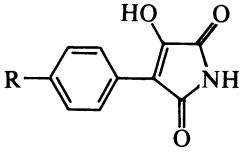
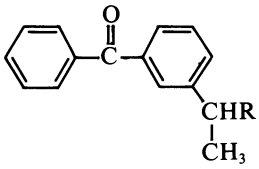
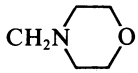

141

Not all replacements of carboxyl need to be actually or potentially acidic. The typically analgesic-antiinflammatory 2-arylpropionic acid,

ketoprofen *(142)* can have its carboxyl exchanged by the basic morpholinomethylene group without loss of cyclooxygenase inhibitory activity *(143)* [230].



142: R = CO_2H

143: R = 

1.8.6 Amide and peptide bioisosteres

Bioisosteric changes of carboxamides follow most of the alterations studied with carboxylic acids. The group -C(=O)NH- permits such permutations as $-SO_2NH-$, -C(=S)NH-, $-CH_2NH-$, and reversal of such structural fragments as in -NH-CO- (procaine versus lidocaine-type amides), or insertion of a spacer ring such as phenylene in a process referred to as arenology. Other spacer groups are -CH=CH- and other conjugating systems. In recent years, such changes have assumed intriguing aspects for the bioisosteric treatment of peptides, polypeptide antibiotics, hormones, and even biocatalytic enzymes and antibodies [231–233]. Apart from disulfide bridges, the conformation of a peptide is affected by the side chains of the participating amino acids and by resulting hydrogen bonds which tie overlaying but sequentially separate portions of the peptide chain together. This leads to the globular rolling-up of most biologically involved peptides with their ring formation, bay-like structures and other architectural characteristics which lend biological specificity to these peptides.
It is therefore of interest to alter both the side chains and the backbone of the peptides and to investigate any biochemical changes that can be attributed to such modifications. Such compounds have been called peptidomimetics [234], and can be regarded as bioisosteres of peptides. They can be synthesized by solid-phase methods using bioisosteric bifunctional acids or unnatural, heterocyclic, or enantiomeric amino acids at proper intervals. For example, phenyllactic acid, $C_6H_5CH_2CHOHCO_2H$ (OH→ NH_2 exchange), will give rise to esters, $-NHCHRCOOCH(CH_2C_6H_5)CO_2H$, which can then be used in additional peptide construction. Such isosteric peptidomimetics fulfill two functions. One is increased resistance to hydrolysis by peptidases,

although this may be counterbalanced by a greater sensitivity to esterases in this example. But monofunctional carboxylic acids such as cinnamic acid ($C_6H_5CH=CHCO_2H$) can initiate a peptide chain at position 1 and gain resistance to peptidases.
The second function of bioisosteric peptides would be improved conformational complementarity at active sites of enzymes or receptors, which might facilitate docking of the peptide drug. This is still in early phases of investigation because the three-dimensional structures of only few active sites have been determined. Taking into account the exquisite specificity of antibodies, and the genetic availability of vast numbers of antibodies, peptide modification of antigens and antibodies should hold great promise.

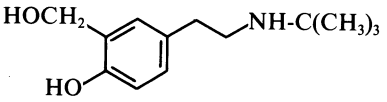
1.8.7 Coordination isosteres [235]

Coordinating agents or chelating agents are formed from primary, secondary and tertiary amines, imines, oximes, thioethers, ketones and thioketones, alcohols, and other hydrogen-bond forming groups. Many natural biologically active materials, e.g. insulin, hemoglobin, myoglobin, chlorophyll, cobalamine, and many enzymes are chelates. They have many vital functions: catalysis of redox reactions, oxygen transport, the biosynthesis of proteins, proteolysis, CO_2 transport by carbonic anhydrase, reactions in which pyridoxal phosphate participates, etc. Chelating agents can destroy parasitic microbes by removing metal ions essential for microbial life (isoniazid, tetracycline), or they can withdraw harmful metal ions from tissues as seen in dimercaprol, penicillamine, and such sequestering agents as ethylenediaminetetracetic acid (EDTA). Compounds which enhance or antagonize these activities by affecting ligating ability can be regarded as coordination isosteres. Antibiotics of the valinomycin family which transport metal ions across membranes by enclosing them in macrocycles, and other complexes formed by synthetic molecular hosts [236] could be interpreted in this manner.

1.8.8 Phenols

Phenols are weak acids but their ionization is stepped up by the presence of electron-attracting groups. Phenolic compounds have been varied by substituting other weakly ionized groups for the phenolic

hydroxyl. The most popular bioisosteric substituents are methanesulfamide (CH_3SO_2NH-), hydroxymethyl ($HOCH_2$-) or hydroxyisopropyl [$HOC(CH_3)_2$-], various amide groups (-NHCHO, -$NHCOCH_3$, -$NHCOC_6H_5$) etc., methanesulfamidomethyl ($CH_3SO_2NHCH_2$-), dimethylaminosulfonamide, $(CH_3)_2NSO_2NH$-, and others with an ionizable proton next to, or near an aromatic ring. These groups should be not much larger than hydroxyl, they should have the same approximate acidity range, form hydrogen bonds and, on occasion, redox systems, in conjunction with a *p*-OH. To some extent, these requirements are fulfilled by -NHCN and -$CH(CN)_2$ in which the electron-withdrawing nitrile groups increase protonation [224]. Examples of such drugs are R-albuterol *(144)*, a *β*-selective adrenergic bronchodilator [237], and the *β*-receptor stimulant, soterenol *(145)* [238].



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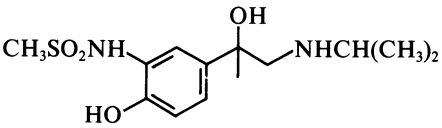
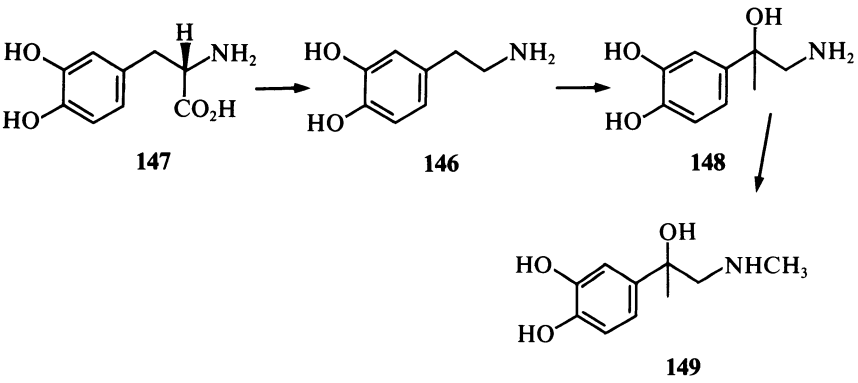


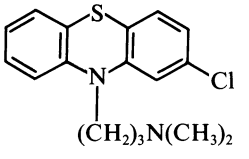
145

Catechols have been the targets of many attempts to place one or two other groups in their *o*-$(OH)_2$ positions. The reason for these experiments has been the potential oxidizability of catechols to *ortho* quinones, with a concomitant loss of their original biological purpose. Another reason is found in the frequent and annoying gastrointestinal side effects of catecholic drugs which limit their medicinal utility. Dopamine *(146)* is the neurohormone responsible for normal motor activity. It is formed in the corpus striatum in the brain by decarboxylation of *L*-dopa *(147)* under the influence of aromatic *L*-amino acid



decarboxylase. The same biosynthesis occurs also in peripheral tissues, especially in the kidney. Dopamine is an intermediate in the biosynthesis of norepinephrine *(148)* and thereby also of epinephrine *(149)*. The pK_a values of dopamine are 9.06, 10.60 and 12.05. Its log *P* in 1-octanol buffer at pH 7.4 is -2.36.

Only agonists and antagonists of the catecholamines will be contemplated here which have, in the widest sense, a bioisosteric relationship to the natural prototype structures. Compounds which, by virtue of divergent mechanisms, act as agonists or antagonists of catecholamines will be excluded because their chemical relationship is tenuous. At least two dopamine receptors, DA_1 and DA_2 receptors, are being distinguished. Among antagonists to these receptors, there are several neuroleptics of the phenothiazine and butyrophenone types [239],



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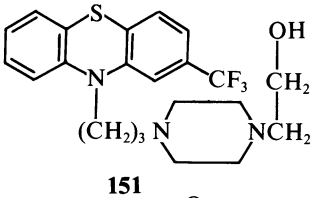

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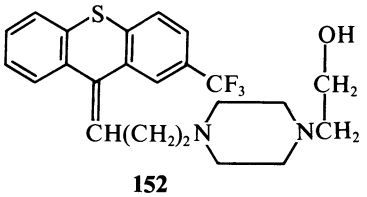

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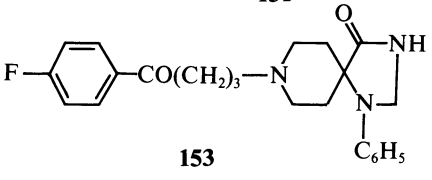
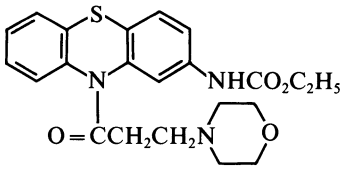


153

among them chlorpromazine *(150)*, fluphenazine *(151)*, and the thioxanthene, *cis*-α-flupenthixol *(152)* as well as butyrophenone neuroleptics such as haloperidol *(88)* and spiperone *(153)*. It has been suggested that the N.....N chains of the phenothiazine neuroleptics can span the HO.....NH distance of dopamine and thus induce receptor blockade. The same could be true for the C=O.....N stretch of butyrophenones but this picture of bioisosterism has remained unclear. The available data on central and peripheral adrenergic neurotransmitters and their autocoid receptors have been reviewed [240].

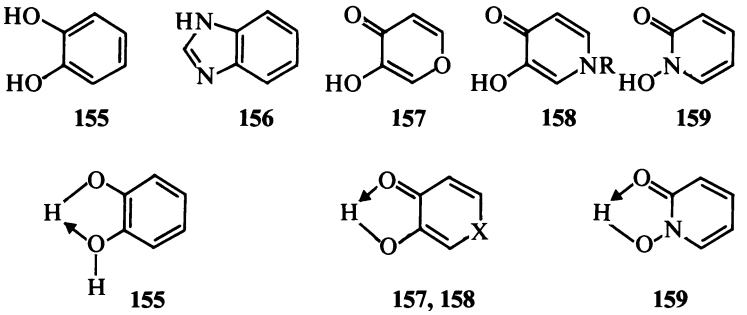
The antiarrhythmic drug, moricizine *(154)* apparently defies bioisosterism. This phenothiazine derivative contains a nuclear carbamate substituent but otherwise does not differ too widely from phenothiazine neuroleptics. However, its biological activity is different. It slows the initial bursts of electrical firing in Purkinje fiber cells and speeds



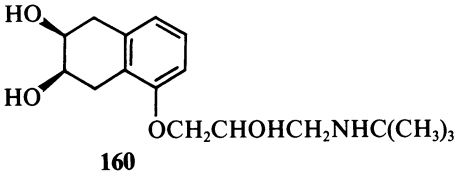
154

up the re-setting of the cells for re-firing. This is valuable in suppressing ventricular arrhythmias.

The structure of catechol *(155)* itself can be replaced by analogous heterocycles in various derivatives *(156, 157, 158, 159)* [138]. All these compounds share the ability to chelate metal atoms and to form hydrogen-bonded second rings; the benzimidazole *156* imitates this by way of a covalent ring structure. Such ring potentials are not restricted

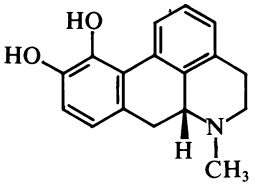


to aromatic catechol analogues. The β-adrenergic blocking agent, nadolol *(160)*, contains an alicyclic diol arrangement.



The interplay of medicinal chemistry and pharmacology has been particularly fruitful in the study of catecholamine receptors, of agonists and antagonists of these multiple receptor systems, the delineation of SAR and bioisosteres, and the discovery of new medicinal agents of adequate specificity. One of the structural characteristics that has held up well in these investigations is the steric restriction of the -C-C-N chain by incorporating it in inflexible rings. This is shown best in

apomorphine *(161)*, the most widely studied standard of dopamine agonists. Some more or less related analogues in which some of the phenolic hydroxyls have been transferred to cyclic imino functions include pergolide *(162)* in which the rigid -C-C-N group has the same conformation as in apomorphine [241]. Many other bioisosteric variations with the same conformational rigidity are cited in reference [138], p. 569.



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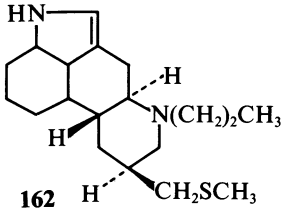
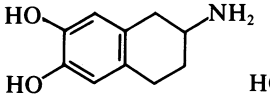


162

The ring compounds, 2-amino-6,7-dihydroxytetralin *(163)* (242, 243), 2-amino-4,5-dihydroxyindanes *(164)* [244], 6-amino-1,2-dihydroxybenzocycloheptene *(165)* [245], (+)*trans* N-propyloctahydrobenzo *(f)*hydroxy-oxaquinoline *(166)* [246], ergoline *(167)*, 4-aminobenz*[c, d]*indole *(168)* [247], fenoldopam *(169)* [244, 247], nomifensin *(170)* [244], and [1R, 3S] 7-aminomethyl-5,6-dihydroxy-3-phenylisochroman *(171)* [248] illustrate additional cases of conformational similarity in the catecholamine series.



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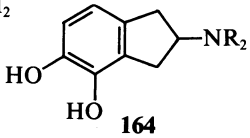

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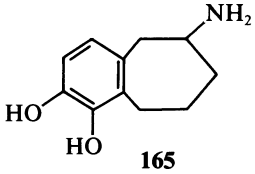

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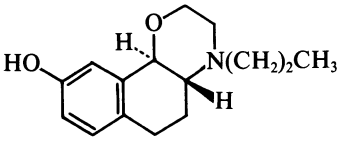

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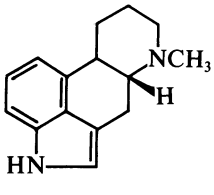

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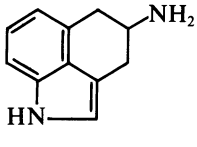

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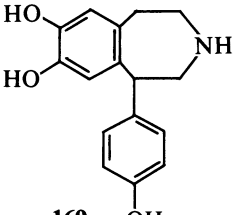

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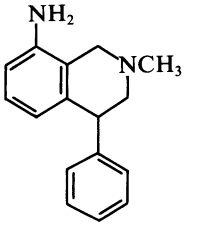

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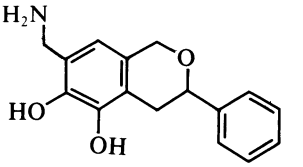


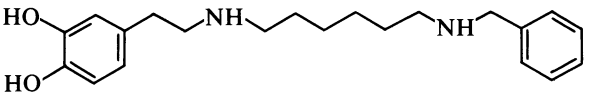
171

In ganglionic blocking agents an old procedure to increase biological activity has been the doubling of functional groups and connecting them through a "spacer" chain as in hexamethonium *(172)*, decamethonium *(173)*, their homologs and sulfonium bioisosteres. In the dopamine series, such "bissing" resulted in the selection of the unsymmetrical dopexamine *(174)* as a selective dopamine$_1$ receptor and adrenoceptor antagonist [249, 250].

$(H_3C)_3N^+\text{-}(CH_2)_{\underline{n}}\text{-}N^+(CH_3)_3$

$\underline{n}$ = 6: **172**

$\underline{n}$ = 10: **173**

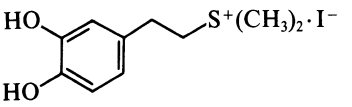


174

As seen in these examples, the phenolic hydroxyls are not required for dopaminergic activity. However, in all compounds that are active at dopamine receptors, the nitrogen atom must be protonated.

1.8.9 Divalent and sulfonium sulfur

The charged nitrogen of dopaminergic compounds can be replaced by permanently charged sulfur *(175, 176, 177)* with retention of activity



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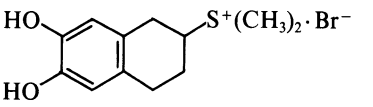

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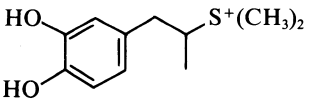

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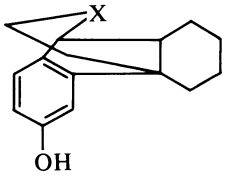
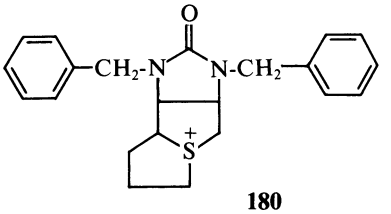
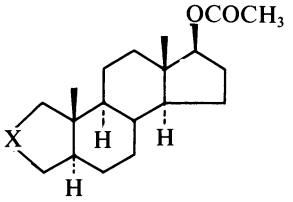


X = NCH_3 : **179**
X = S^+CH_3: **178**

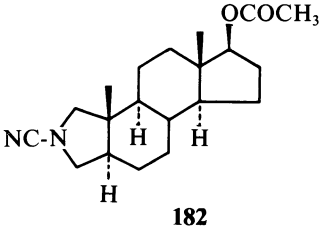


180

[248, 251]. The sulfonium bioisostere *178* of the potent analgesic, isolevorphanol *(179)* has also been prepared and tested [252].
Ganglionic blocking agents traditionally contain ammonium ions but sulfonium achieves the same activity in trimethaphan *(180)* [253].
Divalent sulfur has been used repeatedly to replace oxygen and methylene ($-CH_2-$), and two-carbon bridges can also be exchanged for thioether groups bioisosterically. This is seen in the relationship of imipramine *(45)* to promazine *(44)* and in the tetrahydrofuran and tetrahydrothiophene rings of ring-A modified androgens *(181)* [254]. Other groups such as $>NCN$ *(182)* can take the place of sulfur.



181: X = O,S



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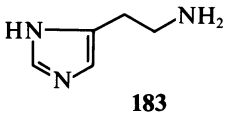
It is the steric, rather than the electronic, properties of the oxygen or sulfur ring atoms that are the determinants of pharmacological activity. In aza steroids the basic nitrogen often gives rise to biologically inactive compounds either by virtue of its high electron density or by formation of a cationic center. These effects are cancelled out in the cyanoazo derivative *182*, which is truly bioisosteric with the thia compound *181*; its androgenic activity is only slightly weaker than that of *181* (X = S).
Replacement of methylene by divalent sulfur (thioether) has been probed extensively in histamine H_2 receptor antagonists. These researches have been reviewed comprehensively [255]. Starting with the premise that such inhibitors should have a component containing imidazole like histamine *(183)* itself, some likely derivatives with modi-

fied side chains *(184, 185)* were examined. These side chains were to bind to the receptors more strongly than histamine and for this purpose were equipped with a guanidine group *(184)* [256] or its thiourea isostere in place of the amino group of histamine.



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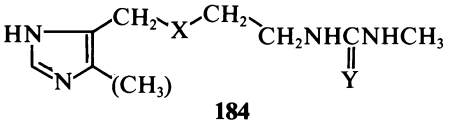
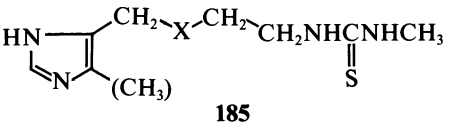
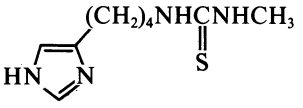


184
X = CH_2
Y = NH



185
X = CH_2, O, S

Even in these carefully planned modifications some 200 structural analogues were prepared and tested before burimamide *(186)* was chosen for clinical trials. The 4-carbon side chain was selected because systematic molecular modifications revealed that potency increased when the side chain was lengthened. Burimamide was not potent enough in the clinic and further modifications were contemplated. At this point it was remembered that in another series, that of β-adrenergic receptor inhibitors, interspersion of the traditional adrenergic side chain, $ArCHOHCH_2NHR$, by oxymethylene, $ArOCH_2CHOHCH_2NHR$, had led to highly active inhibitors such as propranolol *(187)*.



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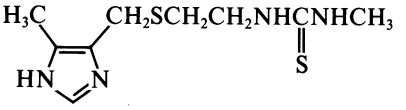

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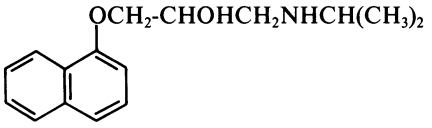

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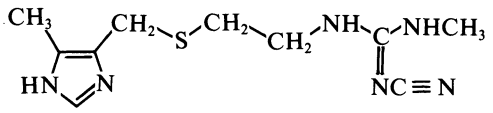
A similar change of the $-(CH_2)_4NHC(=Y)NHR$ chain was applied to an isosteric replacement of a methylene by oxygen, and then by sulfur

which furnished more potent derivatives (metiamide, *188*). This again produced some unacceptable clinical side effects which were attributed to the thiourea group. A guanidine group was tried, especially-with electron-withdrawing substituents such as NH-C-(=NCN)-NHR and NH-C-(=NNO_2)-NHR; the group =$CHNO_2$ was also examined. In separate experiments the classical isosterism of thiourea and cyanoguanidine was established by comparing their proton dissociation (weakly amphoteric), polarity (dipole moments), partition log *P* between octanol and water, solubility characteristics, and planar geometry measurements [255, p. 387]. On the basis of these encouraging data, the cyanoguanidine derivative, cimetidine *(189)* was developed as an antiulcer drug.



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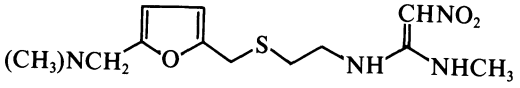
Subsequently, the need for a C-methyl-substituted imidazole moiety, originally thought de rigeur, had to be abandoned when H_2 receptor antagonism was discovered in compounds with other ring systems, for example, isothiazole, thiazole, oxazole, pyridine, furan and even benzene. In all these cases, ether or thioether-containing side chains with basic functions weakened by electron-withdrawing groups are optimal, and in the furan and benzene analogues the nitrogen functionality can be exocyclic. The four drugs *(190–193)* are used in medicine but represent only hundreds of analogues synthesized during their development. They are ranitidine *(190)*, famotidine *(191)*, nizatidine *(192)*, and roxatidine *(193)*. Roxatidine *(193)* is an example of



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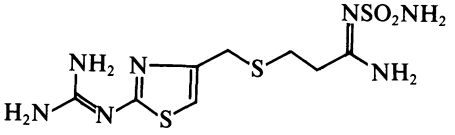

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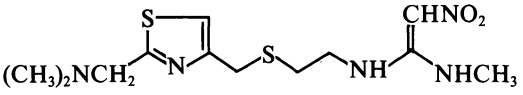

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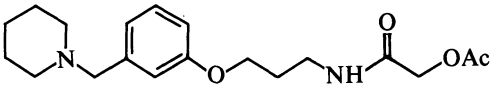


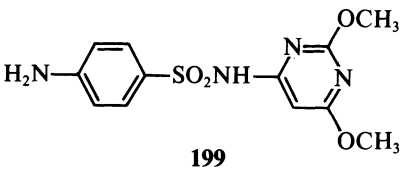
193

bioisosterism of *192*. The benzene ring carries a methylpiperidino group, and the phenoxy-attached side chain contains an amide group of acetyl glycolate.

1.8.10 Methine-nitrogen

Bioisosteres (agonists as well as antagonists) have been found in many ring systems in which -CH= is exchanged for -N=. That is the relationship of benzene and pyridine, but also of pyridine and diazines, triazines and similar aromatic heterocycles. The ring nitrogen atoms strongly attract electrons which have to be furnished by the aromatically shared ring electrons and, if possible, by substituents such as $-NH_2$, -OH, $-OCH_3$, halogens, etc. This localization of electrons progressively weakens the aromatic character and that in turn will be manifest in changes in biological properties that depend on ring-substituent tautomerism.

One such property of altered reactivity may underlie the ability of certain sulfanilamides to withstand metabolic attack. Two isomeric dimethoxypyrimidyl sulfanilamides, sulfadimethoxine *(199)* and sulfadoxine *(200)*, remain antibacterially active for 60–100 hours before they are metabolized or excreted. Since the detoxifying removal of many other sulfanilamides is initiated by N^4-acetylation, steric hindrance cannot be invoked for these two drugs but rather an effect propagated through the aminopyrimidine system must be involved in the longevity of the compounds [257–259]. Sulfadimethoxine is acetylated to about 10 %, excreted intact to about 15 %, and is principally conjugated as the glucuronide [260].



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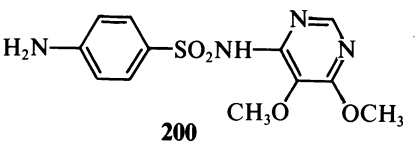

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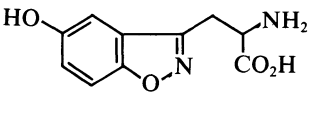
In a series of bioisosteres of 5-hydroxytryptophan, the 1,2-oxazine analogue *201* weakly inhibited 5-hydroxytryptophan decarboxylase. In



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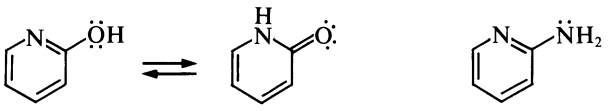
classical isosteres of 5-hydroxytryptamine (5-HT, serotonin) the indole-imino group was shown to be essential for several biochemical properties. Replacement of the cyclic NH by CH_2 inhibited the oxidation of 5-HT and norepinephrine by the enzyme, ceruloplasmin. Exchange of NH for oxygen caused only inhibition of 5-HT oxidation, and the benzothiophene isostere (S for NH) was quite inactive [262].

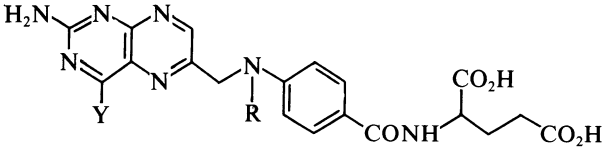
1.8.11 Amino – hydroxyl isosteres

The hydroxyl and amino groups are in column 4 of Grimm's Table and should be classical isosteres. They are both electron donors and both orient primarily *ortho-para* in aromatic rings. In heterocyclic systems containing electron-donating atoms such as N, C-OH will tautomerize to C=O *(194)* while amino nitrogen will remain largely in the C-NH_2 state *(195)*. On the other hand, nitrogen (-N, -NH_2, -NHR, -NR_2) forms hydrogen bonds and coordinates metal ions more easily than oxygen (-O-, -OH, -OR); this ability decreases if bulky substituents crowd the N or O atoms, but may be important biochemically in the existence of coordination isosteres [218].



194 **195**

The alcoholic hydroxyl dissociates less than the phenolic hydroxyl, and the aromatic amino group is less basic than its aliphatic counterpart. Nevertheless, phenol and many of its homologs destroy microbes and proteinogenous tissues, whereas aniline is a relatively nondescript, perhaps mutagenic base. On the whole, OH and NH_2, or OR and NR_2 analogues are more often bioantagonistic than bioisosteric in a positive sense. Aminopterin *(197)* and by extension methotrexate *(198)* are powerful antagonists of pteroylglutamic (folic) acid *(196)* and interfere with the role of folate in nucleoside synthesis,

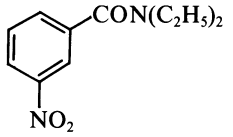


196: Y=OH, R=H
197: Y=NH_2, R=H
198: Y=NH_2, R=CH_3

1.9 Other nitrogen isosteres

1.9.1 Cyclic nitrogen compounds

Pyridine is sometimes compared to nitrobenzene in chemical substitution characteristics because electrons of both rings are drawn away, causing positions 2, 4 und 6 to be more electron-deficient than the *meta* 3- and 5-positions. An application to bioisosterism can be seen in the comparison of N,N-diethyl-3-nitrobenzamide *(199)* and niketh-amide *(200)*. These two amides have similar analeptic properties [263] while N,N-diethyl-*p*-nitrobenzamide is inactive.



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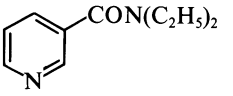

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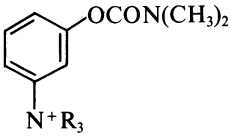

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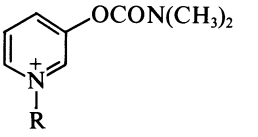

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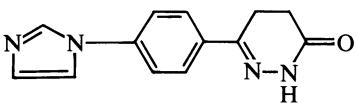
If the pyridinium nitrogen is quaternized, the resulting compounds *(202)* resemble the corresponding anilinium ions *(201)* in cholinergic properties [264, 265].

Ring size may be less important if other molecular distances become more significant. In a comparison of the cardiotonic agents, *203* and *204*, pyridine is equated with imidazole. Imazodan *(203)* is a dihydro-



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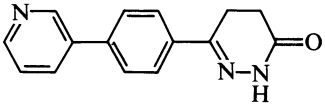

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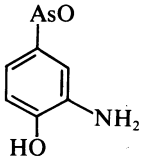
pyridazinone carrying a hydrogen bond acceptor site (an imidazole nitrogen) three atoms removed from the benzene ring; the *p*-(3-pyridyl)phenyldihydropyridazinone *204* possesses a nitrogen (in the pyridine ring) at the same distance but the molecular framework through which it is connected (-N=CH-C(=)–) versus that of *203* (-N=CH-N(|)-) has been altered. Both compounds exhibit a high degree of inotropic activity [266]. Bioisosterism of imidazole and pyridine has also been noted in the development of thromboxane synthetase inhibitors [267–269].

The comparison of pyridine and aniline carries over to some arsenical drugs. Oxophenarsine *(205)* and its pyridine analogue *(206)* are both



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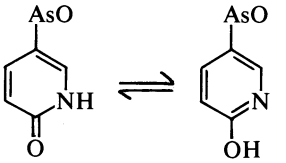

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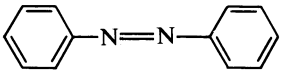

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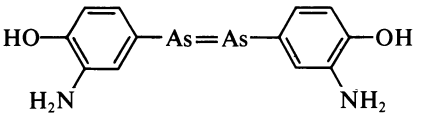

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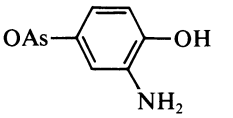
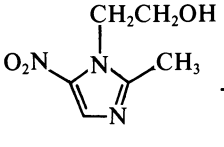


208

treponecidal [41, 270, 271]. It is not clear, however, whether this analogy is to be explained by electronic factors or by the presence of the arsenoxy group.

Arsenic is a classical bioisostere of nitrogen. Arsenoxides are usually spirocheticidal, and many nitroso compounds, especially nitrosoureas, inhibit the multiplication of malignant cells. On the other hand, many azo dyestuffs are carcinogens while arsenic isosteres such as arsphenamine *(207)* are spirocheticidal. However, this activity is probably due to their metabolic oxidation products, the arsenoxides *(208)*. Azobenzene itself *(209)* kills ascarides and is used to keep greenhouses free of these nematodes.

Nitroimidazoles such as metronidazole *(210)* and nitrofurans are drugs of value in the treatment of several protozoal diseases as well of infections due to anaerobic bacteria. Reduction of the nitro group is obligatory for the emergence of therapeutic activity. Obviously, reduction of the nitro group to NH_2 proceeds stepwise, and it may be surmised that intermediate reduction products such as the corresponding nitroso derivatives *(211)* should be closer to the end products of the reduction sequence *(212)*. Using *Escherichia coli* strain SR 58 whose defective DNA repair system makes it sensitive to metronidazole, it has been found indeed that the nitroso compounds are



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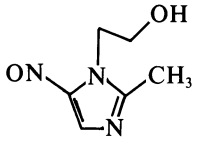

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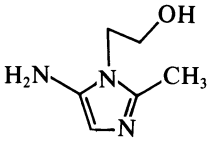

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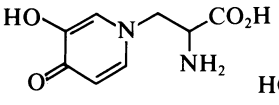
significantly more bactericidal than the corresponding nitro derivatives [272], perhaps due to the ability of the mutant to reduce *211* to *212*.

The great interest created by the therapeutic success of *L*-dopa as a pro-drug for dopamine in the treatment of Parkinsonism stimulated searches for dopa isosteres that might have fewer of the limiting side effects of dopa. Among such analogues were hydroxypyridine and pyridinequinoid-type compounds *(213, 214, 215)* with aminopropionic acid chains attached to isomeric positions of the ring [273–276].



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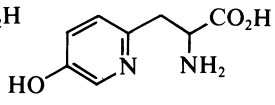

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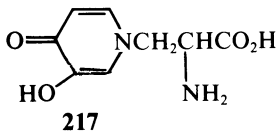

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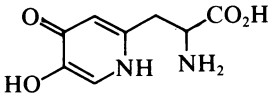

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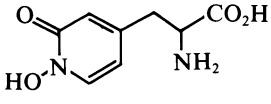

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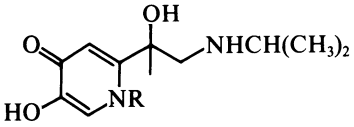
The tyrosine analogue *216* is a natural product obtained from *Streptomyces* species [274]. Mimosine (leucenol, *217*) is an amino acid [273] found in the seeds and foliage of legume genera *Mimosa* and *Leucena* [277]. It causes loss of hair growth in mice [278].

A hydroxypyridine ring has been constructed for a bioisostere *(218)* of adrenergic hormone agonists [243].



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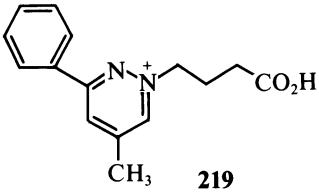


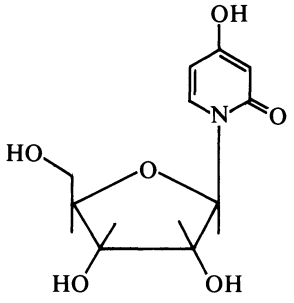
219

Introduction of a second nitrogen in pyridine leads to isomeric diazines. Among early diazine drugs were sulfonamides which carried a diazine ring, substituted or non-substituted, on the sulfonamide group. In other series of bioactive diazines was *219* in which a substituted pyridazinium ion took the place of ammonium in a GABA chain. This compound is a GABA agonist [279].

The most widely studied diazines are the pyrimidines and their con-

densed-ring companions, the purines, which constitute the basic portions of nucleotides. Many analogues of the nucleic acid constituents are bioisosteric antagonists of enzymes in natural nucleoside and nucleotide chemistry. See section 2.1.

The mesoionic pyridazine analogue *221* of 3-deazauridine *(220)* exhibits antibacterial properties *[280]* but here the phenolic OH has also been altered.



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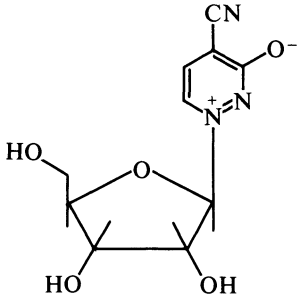
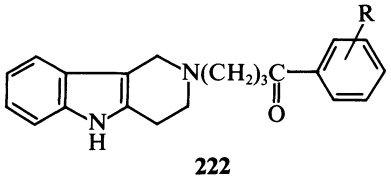


221

This particular change (OH to CN) was ineffective when the amide carbonyl was maintained in the pyridone ring (> N-CO-) or changed to > N-CH= [281]. Other replacements of carbonyl by $>C=C(CN)_2$ and -CH(CN)- bear some similarity to the 3-deazauridine case [282]. Additional changes involved replacement of the ring-CO by SO, SO_2, or even by CHCN. This latter change is exemplified in the neuroleptic piperidinoindoles *222* and *223* [283].



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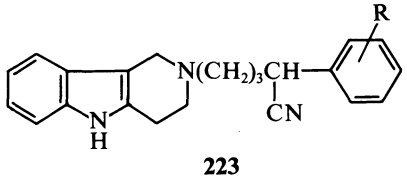
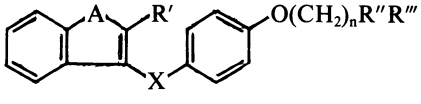


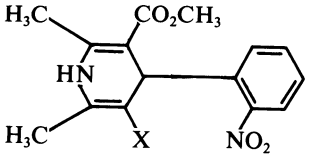
223

Vasodilatory activity has been found in benzofuryl phenyl ketones and sulfones with basic side chains *(224)* [284–286] and in the nitrophenyl-substituted dihydropyridines *225* and *226* [287, 288].



224

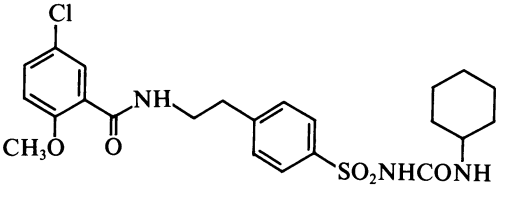
A = O or S	X = SO_2	(284)
A = O	X = CO	(285)
A = S	X = CO	(286)



X = $COOCH_3$: **225**

X = SO_2CH_3: **226**

Similarly, the benzamide function of the hypoglycemic sulfonylurea drug, glyburide *(227)* could be replaced by sulfonamide *(228)* [289] but the carboxylic acid *229* also had similar antihyperglycemic activity [290]. This suggests a bioisosteric relationship of SO_2NHCO and COOH groups.



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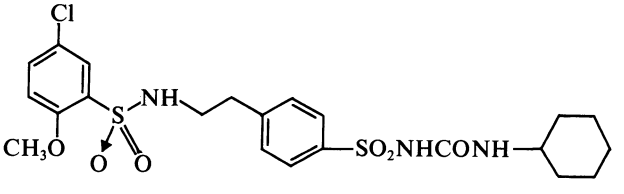

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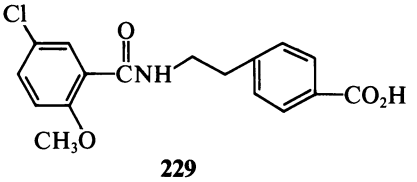

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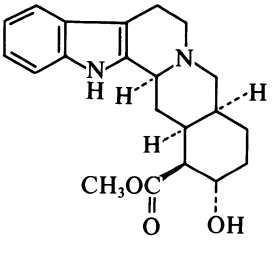

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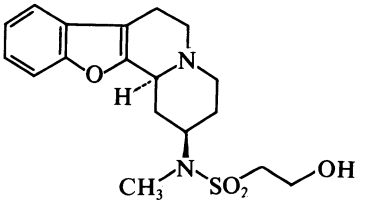


231

Farther afield is the replacement of the ester group of α-yohimbine (rauwolscine, *230*) by a sulfonamide in which the 17-hydroxyl of the alkaloid has been translocated into a side chain. The sulfonamide analogue *231* is an enantioselective α_2-adrenoreceptor antagonist but there were too many changes in the ring skeleton to assert defendable bioisosterism [291].

2 Metabolite analogues

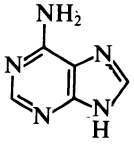
Metabolites are chemicals needed or discarded by cells in the course of their metabolism. Some of them are biosynthesized by a cell from available chemicals, while others that cannot be produced in this manner have to be furnished by cellular nutrients from the environment. The chemistry, biochemistry and physiological significance of many metabolites has been reviewed repeatedly in monographs on amino acids, carbohydrates, hormones, lipids, nucleotides, steroids, vitamins and other essential and non-essential substances that play a role in the chemical support and chemical reactions which we call cellular life. All these materials represent prototype structures which can be altered by chemical reactions within the organism, or by experimentation in the laboratory. If the reaction products support or enhance the biochemical transformations of the metabolites, they are agonists of such biochemical missions. Other reaction products, whether obtained from metabolic events or by laboratory chemistry, slow down, halt or even reverse reactions of the metabolites; they are metabolite antagonists (antimetabolites). Both agonists and antagonists, regardless of their source, often but not always, are structurally related to the metabolites; if so, they are metabolite analogues. Some representative metabolite analogues will be assembled in this section. Some others have already been mentioned on the preceding pages.

The often complex biochemical relationship of metabolite and metabolite analogue requires a knowledge of the behavior at enzymatic sites and of the reactions of these reagents. For this purpose, some structural similarity will be needed. Emil Fischer compared the relationship of the reactions of metabolite and metabolite analogue to the relationship of a key to a keyhole. This rather static picture is improved in a more adaptable analogy of a glove and a hand. This allows for flexibility of reactions and for steric changes involving reagents and reaction sites. Even so, the structural segments of reagents fitting the reac-

tion site "glove" will have to be highly similar. Segments adjacent to the structurally demanding sites can be much more varied, and therefore the differences between metabolite and metabolite analogue will be more pronounced in groups near but not in the pharmacophoric core of these agents. Bioisosterism will be most visible in those parts of the molecule which need to be as similar as possible in a metabolite and its analogue. Differences between such analogues will have to be placed in adjacent sections of the molecules. This idea has been promoted by Baker [282].

2.1 Nucleoside base isosteres

Biosisosteric replacements have been applied in every structural series of metabolites. Classical bioisosterism has been most successful in designing antagonists to metabolites in therapeutic areas where toxic metabolites of infectious and invasive cells have to be counteracted. Antimicrobial (antibacterial, antiprotozoal, antiviral) antimetabolites, and chemotherapeutic antitumor agents have been of great interest to medicinal chemists. Even the best planned molecular modifications in these series demand further systematic variations to cull out pharmacologically suitable members of widely spaced analogues. One such systematic search has been crowned by a Nobel prize [293]. The purine derivatives, adenine and guanine, and the pyrimidines, cytosine, thymine and uracil, form the basic components of the nucleic acids. In rapidly dividing cells, they must be resynthesized continually to replenish the supply of chemicals for the new cell nuclei. Adenine *(232)* contains an amino group, guanine *(233)* a tautomerizable hydroxyl and an amino group, and uracil *(234)* has two tautomerizable oxo groups. Thymine *(235)* carries a methyl function, and cytosine *(236)* an amino and a tautomerizable oxo group. These bases participate in many enzymic reactions in the form of their nucleosides (ribosides) or deoxynucleosides (2′-deoxyribosides) and the mono-, di-, or triphosphate esters, the nucleotides or 2′-deoxynucleotides.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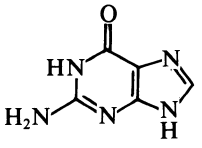
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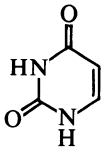
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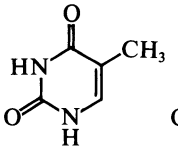
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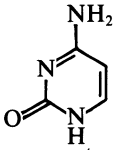
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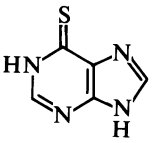

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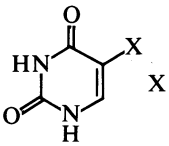

X = Cl, Br, I

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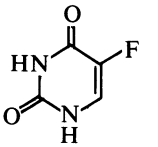

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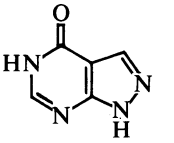
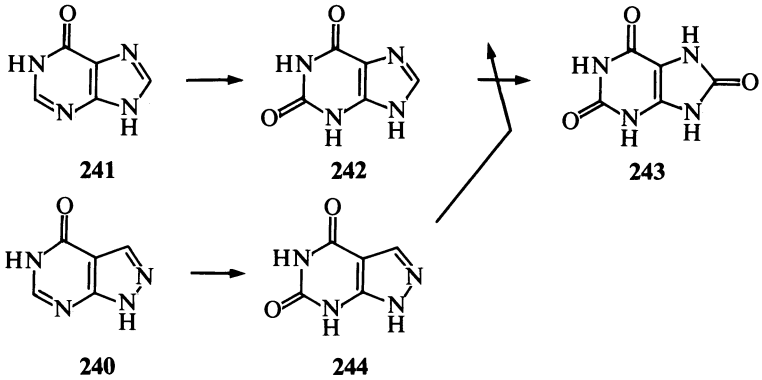


240

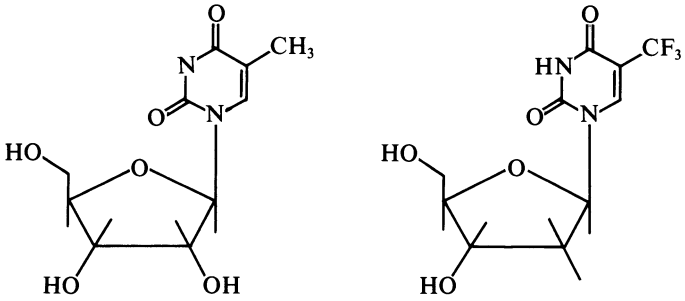
These NH_2 and tautomeric OH groups are amenable to bioisosteric replacement by halogens or SH; the reactive 5-hydrogen of uracil which normally can be biomethylated is an isostere of small halogens. Work on metabolite analogues of nucleoside bases started in the 1940's when the impact of isosterism was popular, and in short order the first wave of bioisosteric metabolite analogues was prepared and tested. They were 6-mercaptopurine (6-MP) *(237)* and its 2-amino and hydroxy derivatives, 5-halogenouracils *(238)* and especially 5-fluorouracil (5-FU) *(239)*. The complicated biochemistry of these compounds had to be unravelled and the enzymes they inhibited identified. 6-MP *(237)* and 5-FU *(239)* have become useful as antitumor drugs.

Another step in applying classical bioisosteric modifications to the purine and pyrimidine bases was to replace -CH= by -N=, -N= by -CH=, and -O- and -S-. Virtually every ring position has been subjected to such changes, and a few of these modifications have made it to clinical use. An example of the -CH= ⟶ -N= exchange is allopurinol *(240)* which inhibits the oxidation of hypoxanthine to uric acid and, since urate deposits in the joints are the cause of gout, can be used in this condition.

The relationship of allopurinol to uric acid biosynthesis illustrates some of the conditions imposed on metabolites of bioisosteres of purine and pyrimidine bases [294–296]. Hypoxanthine *(241)* is oxidized to xanthine (2,6-dioxopurine) *(242)* by the enzyme, xanthine oxidase, and xanthine is further oxidized to uric acid (2,6,8-trioxopurine, *243*). Allopurinol *(240)* can also be oxidized at its 2-position by the same enzyme, but its nitrogen at position 8 slows down recognition by the enzyme, and the initial oxidation to the isostere *244* of xanthine *(242)* is slow. Although allopurinol is thus a substrate of xanthine oxidase, albeit a poor one, it binds to the enzyme more tightly than hypoxanthine and thereby inhibits its oxidation. The next step, the oxidation of xanthine to uric acid, cannot be duplicated for the isosteric 2,4-dioxopyrazolopyrimidine *244* since this compound has N in place of CH at position 8 (purine numbering); *244* is therefore no longer a substrate but



because of its stronger complexation with the enzyme, becomes an inhibitor of the oxidation of xanthine, i.e. an antimetabolite.
Another example of positive bioisosterism as compared to metabolite antagonism is the replacement of the methyl group of thymidine *(245)* in the form of its triphosphate nucleotide, by halogen.



Thymidine triphosphate is a substrate of DNA polymerase. Replacement of the 5-methyl group by (the equivoluminous) chlorine atom, or the larger bromine or iodine atoms, furnishes analogues which increasingly lose the ability to substitute as substrates for the enzyme. At the end of this sequence, 5-iodouracil (*238*, X = I) (as the 2′-deoxynucleoside or nucleotide) whose iodine atom replaces CH_3, can no longer bind to DNA polymerase as a substrate but it is an inhibitor of the enzyme. It has antiviral activity in the topical therapy of ocular herpes diseases [297]. 5-Fluorouracil *(239)* [298, 299] is best interpreted as a bioisostere of uracil *(234)* since the fluorine atom has a van der Waals radius of 1.35 Å compared to hydrogen with a radius of 1.2 Å. It is a selective cancer chemotherapeutic drug, best in the form of its 2′-de-

oxynucleoside. Normal cells readily degrade 5-FU while cancer cells cannot reduce and thereby detoxify it [300].

5-Trifluoromethyl-2-deoxyuridine *(246)* is an isostere of 2-deoxythymidine with fluorine substituting for the methyl hydrogens. It is useful as a topical agent for herpes zoster-infected eyes, especially in cases resistant to iodouridine [301].

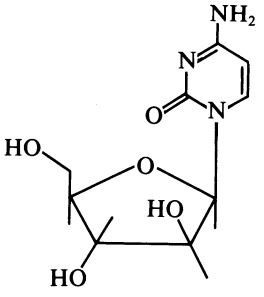
The most important therapeutic advances with purine and pyrimidine bioisosteres and metabolite antagonists have been made through increased selectivity for isozymes of invasive and malignant cells. The biochemical paths followed to bring selectivity to fruition may be found in reviews by Elion and by Montgomery et al. [293, 300].

Antiviral chemotherapy proceeded hesitantly as a sequel to cancer chemotherapy. Almost all antitumor agents were tested against experimental virus infections *in vitro* in infected egg membranes and in laboratory animals. When antiviral testing methods were firmly established, screening was extented to many structurally divergent chemicals. Within the discussion of bioisosterism, nucleoside analogues with altered carbohydrate moieties deserve attention.

The nucleic acids of viruses are similar to those of normal host cells. Ribotides are present in ribonucleic acids and therefore in RNA viruses. 2′-Deoxyribotides are found in 2′-deoxyribonucleic acids (DNA) and consequently in DNA viruses. Adenoviruses, Herpes 1 and 2, smallpox, vaccinia and polyoma viruses are DNA viruses. Many other viruses are RNA viruses, among them those that cause influenza, mumps, Newcastle disease, measles, distemper, polio, rabies, infectious bronchitis, yellow fever, encephalitis, rubella, some leukemias, and many other infections [302].

With the difference in the chemistry of RNA and DNA viruses concentrated in the carbohydrate moieties of their nucleic acids, bioisosteric changes of these moieties have been undertaken. The first modifications were made with carbohydrates isomeric with ribose, then with other sugar moieties that featured, if possible, furanose rings. Thus, 1-*β-D*-arabinofuranosylcytosine (cytarabine, Ara-C, *247*) was synthesized [303–305]. It can be applied in herpes zoster and other herpes infections and also as an antitumor agent.

Similarly, 9-*β-D*-arabinofuranosyladenine (vidarabine, Ara-A, *248*) was introduced. It can also be of value in herpes infections of the eye, brain and skin. Both these compounds contain arabinose rings, and inhibit the conversion of ribo- to deoxyribonucleotides.



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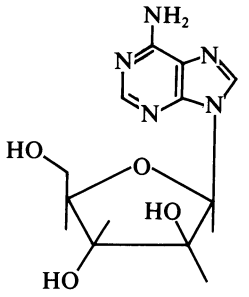

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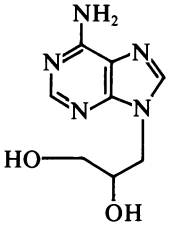

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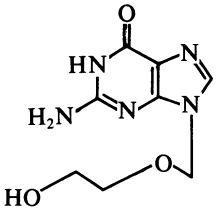

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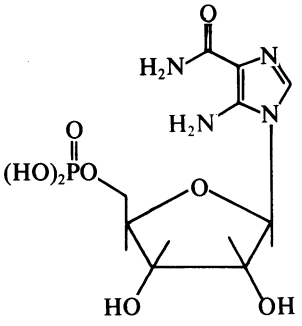
The next step was to probe whether a carbohydrate group was necessary for biological activity and enzyme binding. Instead of the glycosidic rings, aliphatic groups with two to five carbon atom chains ($HOCH_2CHOHCH_2$-, in (S)-9-(2,3-dihydroxypropyl)adenine, *249*), or ether group-containing chains to simulate the oxygen bridge of riboside groups ($HOCH_2CH_2$-O-CH_2-, in 9-(2-hydroxyethoxymethyl) guanine, acycloguanosine, acyclovir, *250*) [306] were designed bioisosterically. Acyclovir *(250)* has become the drug of choice for the control of herpes infections [307–309].



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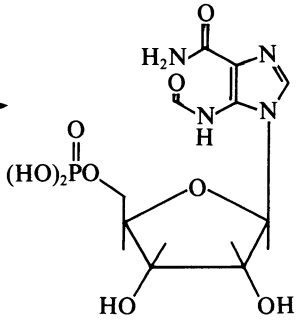

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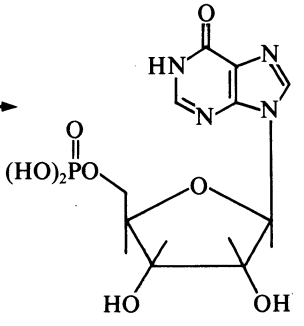

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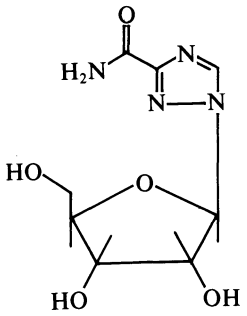
Since the biosynthesis of purine nucleotides *(253)* proceeds through 1-*β*-*D*-ribofuranosylphosphate)-5-aminoimidazole-4-carboxamide *(251)*, the missing carbonyl group for *252* being furnished by formyltetrahydrofolate, the imidazole ring of *251* and *252* was modified (=C<→=N- replacement) in ribavirin *(254)* [310, 311], which inhibits herpes 1 and 2, vaccinia, influenza, parainfluenza, rhino and certain RNA tumor viruses *in vitro* and *in vivo*.



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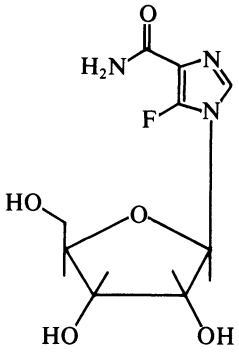
Many other bioisosteric replacements based on purines or their biosynthetic precursors have been reported, for example, a derivative of the imidazole amide *251* in which the 5-amino group was exchanged for fluorine *(255)* or other halogens [312].

Alternately, the pyrimidine portion of the purine nucleosides was stripped of one nitrogen and functional groups, in 3-*β*-*D*-ribofuranosylimidazole [4,5-*b*] pyridine *(256)* [313, 314] which showed activity against parainfluenza virus. A similar deazapyrimidine analogue *(221)* has already been described.



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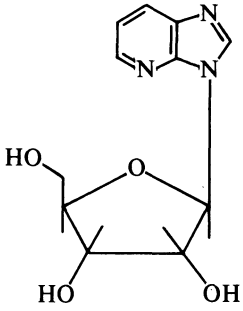

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With the exception of the hydroxyalkyl-chain derivatives of nucleosides, with their principally anti-herpes activities *(249, 250)* and of zyvudine *(69)* and its congeners, almost all the analogues described here are classical bioisosteres of their prototypes. Non-classical changes with their more far-reaching modifications have not yet been contemplated widely. With interest in antiviral and antitumor drugs at a high level in view of the epidemic HIV infection (AIDS), non-classical bioisosteres will undoubtedly be investigated.

Metabolite analogues, especially metabolite antagonists, have been encountered in many structurally and functionally divergent types of compounds. Some of them, such as analogues of several B-vitamins and amino acids are mentioned in Baker's review [292]. Many others, arranged by structure rather than function, may be found in this review.

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