Bioisosterism: A Rational Approach in Drug Design

George A. Patani and Edmond J. LaVoie*

Department of Pharmaceutical Chemistry, College of Pharmacy, Rutgers, The State University of New Jersey, Piscataway, New Jersey 08855-0789

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George Patani graduated with a B.Pharm. in 1992 from the College of Pharmaceutical Sciences, Mangalore University at Manipal, India. In 1996, he received his M.S. in Pharmaceutical Science at Rutgers University under the direction of Professor Edmond J. LaVoie. He is presently pursuing graduate studies in pharmaceutics. His current research interests are focused on drug design and controlled drug delivery.

Edmond J. LaVoie received his B.S. in Chemistry from Fordham University in 1971 and his Ph.D. in Medicinal Chemistry from S.U.N.Y. at Buffalo under the direction of Dr. Wayne K. Anderson. After postdoctoral study with Dr. S. Morris Kupchan at the University of Virginia, he joined the American Health Foundation in Valhalla, NY. In 1988, he was appointed Professor of Medicinal Chemistry in the College of Pharmacy at Rutgers University. His current research interests are in the design and synthesis of cancer chemotherapeutics and in the elucidation of mechanism(s) of carcinogenesis.

I. Introduction

Years of cumulative research can result in the development of a clinically useful drug, providing either a cure for a particular disease or symptomatic relief from a physiological disorder. A lead compound with a desired pharmacological activity may have associated with it undesirable side effects, characteristics that limit its bioavailability, or structural features which adversely influence its metabolism and excretion from the body. Bioisosterism represents one approach used by the medicinal chemist for the rational modification of lead compounds into safer and more clinically effective agents. The concept of bioisosterism is often considered to be qualitative and intuitive.[1]

The prevalence of the use of bioisosteric replacements in drug design need not be emphasized. This topic has been reviewed in previous years.[2−5] The objective of this review is to provide an overview of bioisosteres that incorporates sufficient detail to enable the reader to understand the concepts being delineated. While a few popular examples of the successful use of bioisosteres have been included, the

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present review is focused primarily upon specific examples from current literature. The emphasis in this review was to outline bioisosteric replacements which have been used to advance drug development. No attempt was made to be exhaustive or to illustrate all of the specific analogues represented within a single study.

The ability of a group of bioisosteres to elicit similar biological activity has been attributed to common physicochemical properties. In this review an attempt has been made to quantitate, in specific instances, physicochemical effects such as electronegativity, steric size, and lipophilicity and to correlate these values to the observed biological activity. Thus, an additional objective of this review was to demonstrate the opportunities that one has in employing bioisosteres to gain more specific insight into the quantitative structure−activity relationships (QSAR) associated with a specific class of drugs. While in some instances such associations were detailed by the authors of these literature examples, others were developed on the basis of evident correlations. To further explain and rationalize the biological activity observed with nonclassical bioisosteric groups, the observed biological activity has also been correlated with some substituent constants commonly employed in QSAR studies. These observations are consistent with the fact that bioisosteric replacements often provide the foundation for the development of QSAR in drug design.[4,6] Recent advances in molecular biology, such as cloning of the various receptor subtypes, have enabled a clearer definition of the pharmacophoric sites. Bioisosteric replacements of functional groups based on this understanding of the pharmacophore and the physicochemical properties of the bioisosteres have enhanced the potential for the successful development of new clinical agents.

The bioisosteric rationale for the modification of lead compounds is traced back to the observation by Langmuir in 1919 regarding the *similarities of various physicochemical properties* of atoms, groups, radicals, and molecules.[7] Langmuir compared the physical properties of various molecules such as N_2 and CO, N_2O and CO_2, and N_3^- and NCO^- and found them to be similar. On the basis of these similarities he identified 21 groups of *isosteres*. Some of these groups are listed in Table 1. He further deduced from the octet theory that the number and arrangement of electrons in these molecules are the same. Thus, isosteres were initially defined as those compounds or groups of atoms that have the same number and arrangement of electrons. He further defined other relationships in a similar manner. Argon was viewed as an isostere of K^+ ion and methane as an isostere of NH_4^+ ion. He deduced, therefore, that K^+ ions and NH_4^+ ions must be similar because argon and methane are very similar in physical properties. The biological similarity of molecules such as CO_2 and N_2O was later coincidentally acknowledged as both compounds were capable of acting as reversible anesthetics to the slime mold *Physarum polycephalum*.[8]

Table 1. Groups of Isosteres as Identified by Langmuir

groups	isosteres
1	H^-, He, Li^+
2	O^{2-}, F^-, Ne, Na^+, Mg^{2+}, Al^{3+}
3	S^{2-}, Cl^-, Ar, K^+, Ca^{2+}
4	Cu^{2-}, Zn^{2+}
↓	↓
8	N_2, CO, CN^-
9	CH_4, NH_4^+
10	CO_2, N_2O, N_3^-, CNO^-
↓	↓
20	MnO_4^-, CrO_4^{2-}
21	SeO_4^{2-}, AsO_4^{3-}

A further extension to this concept of isosteres came about in 1925 with Grimm's Hydride Displacement Law.[9,10] This law states: "Atoms anywhere up to four places in the periodic system before an inert gas change their properties by uniting with one to four hydrogen atoms, in such a manner that the resulting combinations behave like pseudoatoms, which are similar to elements in the groups one to four places respectively, to their right." Each vertical column as illustrated in Table 2, according to Grimm, would represent a group of isosteres.

Table 2. Grimm's Hydride Displacement Law

C	N	O	F	Ne	Na
	CH	NH	OH	FH	−
		CH_2	NH_2	OH_2	FH_2^+
			CH_3	NH_3	OH_3^+
				CH_4	NH_4^+

Erlenmeyer[11] further broadened Grimm's classification and redefined isosteres as atoms, ions, and molecules in which the peripheral layers of electrons can be considered identical (Table 3).

Table 3. Isosteres Based on the Number of Peripheral Electrons

no. of peripheral electrons				
4	5	6	7	8
N^+	P	S	Cl	ClH
P^+	As	Se	Br	BrH
S^+	Sb	Te	I	IH
As^+		PH	SH	SH_2
Sb^+			PH_2	PH_3

The widespread application of the concept of isosterism to modify biological activity has given rise to the term *bioisosterism*. As initially defined by Friedman,[2] bioisosteres were to include all atoms and molecules which fit the broadest definition for isosteres and have a similar type of biological activity, which may even be antagonistic. More recently this definition has been broadened by Burger as "Compounds or groups that possess near-equal molecular shapes and volumes, approximately the same distribution of electrons, and which exhibit similar physical properties...".[5] The critical component for bioisosterism is that bioisosteres affect the same pharmacological target as agonists or antagonists and, thereby, have biological properties which are related to each other.

Bioisosteres have been classified as either classical or nonclassical.[12] Grimm's Hydride Displacement Law and Erlenmeyer's definition of isosteres outline a series of replacements which have been referred to as classical bioisosteres. Classical bioisosteres have been traditionally divided into several distinct categories: (A) monovalent atoms or groups; (B)

divalent atoms or groups; (C) trivalent atoms or groups; (D) tetrasubstituted atoms; and (E) ring equivalents.

Nonclassical isosteres do not obey the steric and electronic definition of classical isosteres. A second notable characteristic of nonclassical bioisosteres is that they do not have the same number of atoms as the substituent or moiety for which they are used as a replacement. Nonclassical bioisosteres can be further divided into groups: (A) rings vs noncyclic structures; and (B) exchangeable groups.

This approach to classifying bioisosteres will be used to review literature examples of those bioisosteric replacements that have provided useful information on the structure−activity relationships associated with various pharmacologically active compounds.

II. Classical Bioisosteres

A. Monovalent Atoms or Groups

Similarities in certain physicochemical properties have enabled investigators to successfully exploit several monovalent bioisosteres. These can be divided into the following groups: (1) fluorine vs hydrogen replacements; (2) amino−hydroxyl interchanges; (3) thiol−hydroxyl interchanges; (4) fluorine, hydroxyl, amino, and methyl group interchanges (Grimm's Hydride Displacement Law); (5) chloro, bromo, thiol, and hydroxyl group interchanges (Erlenmeyer's Broadened Classification of Grimm's Displacement Law).

1. Fluorine vs Hydrogen Replacements

The substitution of hydrogen by fluorine is one of the more commonly employed monovalent isosteric replacements. Steric parameters for hydrogen and fluorine are similar, their van der Waal's radii being 1.2 and 1.35 Å, respectively.[13] Thus, the difference in the electronic effects (fluorine being the most electronegative element in the periodic table) is often the basis for the major differences in the pharmacological properties of agents where fluorine has been substituted for hydrogen. Due to its electronegativity, fluorine exerts strong field and inductive effects on the adjacent carbon atom. Fluorine substitution, in general, exerts a diminished electron-withdrawing effect at distal sites. However, fluorine can donate a lone pair of electrons by resonance. This is commonly referred to as its mesomeric effect. The opposing resonance and field effects can nearly cancel. The pharmacological differences can be attributed to the influence of the electron-withdrawing effect that the fluorine substitution causes on interaction with either a biological receptor or enzyme, as well as its effect on the metabolic fate of the drug.

The antineoplastic agent 5-fluorouracil (5-FU) represents a classical example of how fluorine substitution of a normal enzyme substrate can result in a derivative which can alter select enzymatic processes. In this instance, 5-FU is biochemically transformed *in vivo* into 5-fluoro-2′-deoxyuridylic acid. Its close similarity to uracil allows this fluoro derivative to be a successful mimetic. This biochemically altered form of 5-FU, 5-fluoro-2′-deoxyuridylic acid, is ultimately responsible for the inhibition of thymidylate synthase, an enzyme involved in the conversion of uridylic acid to thymidylic acid and critical for DNA synthesis (Figure 1). The increased reactivity of 5-fluoro-2′-deoxyuridylic acid relative to 2′-deoxyuridylic acid is due to the inductive effect of fluorine which results in its covalent binding to thymidylate synthase.

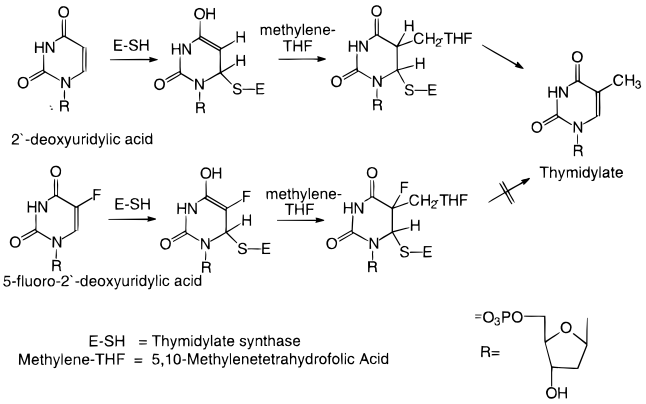

Figure 1.

The application of the monovalent substitution of a fluorine atom for a hydrogen atom can also be seen in a more recent study with naphthyl-fused diazepines, which were employed as agonistic probes of the pharmacophore of benzodiazepine receptors.[14] Replacement of the hydrogen with fluorine at the *ortho* position of the pendent phenyl group of either naphthyl-fused diazepines, as illustrated in Figure 2, resulted in enhanced affinity and efficacy for both naphthyl isomers (Table 4). This greater receptor binding affinity could again be attributed to the inductive effect of the fluorine atom facilitating a stronger interaction with the receptor.

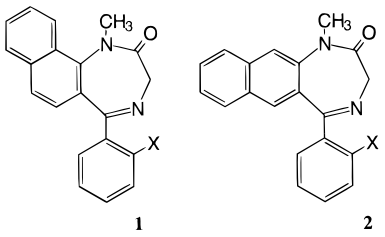

Figure 2.

Table 4. Benzodiazepine Receptor Binding Affinity for Naphthyl-Fused Diazepines

compound	X	IC_{50} (nM)[a]
1a	H	1000
1b	F	260
2a	H	1000
2b	F	55

[a] *In vitro* potency of the compound to displace [^{3}H]flunitrazepam from the benzodiazepine receptor.

Another good illustration of this monovalent bioisosteric replacement is observed in a recent series of anti-inflammatory corticosteroid analogues (**3**, Figure 3).[15] In this study, the topical anti-inflammatory activity of two pairs of structurally similar corticosteroids were compared. Their relative anti-inflammatory activity was normalized to fluocinolone acetonide, which was assigned a potency of 100. Table

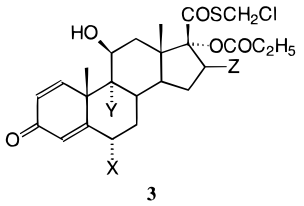

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Figure 3.

Table 5. Biological Activities of Halomethyl Androstane-17β-carbothionates

compound	X	Y	Z	topical anti-inflammatory activity[a]
3a	H	F	$=CH_2$	42
3b	F	F	$=CH_2$	108
3c	H	H	β-CH_3	27
3d	H	F	β-CH_3	41

[a] Topical anti-inflammatory activity was measured in mice by modifications of the croton oil ear assay.[16] Fluocinolone acetonide served as a positive control and is assigned a relative potency index of 100.

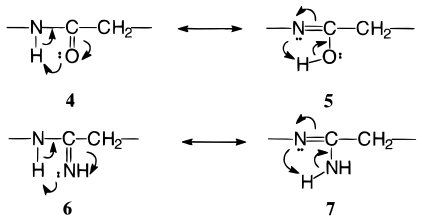
5 shows that, in the case of the pair of compounds possessing a 16-methylene substituent, the presence of an additional fluorine atom at the 6α position results in a derivative with greater activity than **3a** or fluocinolone acetonide. With the pair of corticosteroids with a 16-methyl substituent (Z = CH_3), replacement of hydrogen with fluorine at the 9α position, **3d**, also increased anti-inflammatory activity relative to **3c**.

Thus, the ability of fluorine to replace hydrogen is an effective method of exploring the affinity of an agent to the target site (receptor or enzyme) by virtue of its greater electronegativity while other parameters such as steric size and lipophilicity[17] are maintained.

2. Interchange of Hydroxyl and Amino Groups

The monovalent interchange of amino and hydroxyl groups is well known and has been successfully employed in the development of various pharmacological agents. The similar steric size (Table 7), spatial arrangement, and the ability of these functional groups to act as either *hydrogen bond acceptors or donors* is likely responsible for their successful use as bioisosteres.

Several medicinal agents under investigation as potential clinical agents carry heteroaromatic moieties. Many of these heteroaromatic compounds are capable of tautomerization. The prototropic tautomerism of heteroaromatic compounds includes all agents wherein a mobile proton can move from one site to another within the heteroaromatic molecule. Figure 4 illustrates one of the more common types of tautomerization involving the movement of a proton between a cyclic nitrogen atom and a substituent on the neighboring carbon atom within the ring. Tautomerism in heterocyclic molecules has been extensively studied.[18] In the presence of electron-donating atoms such as nitrogen in heterocyclic systems, it is known that there will be substantial tautomerization where a neighboring C—OH will tautomerize to C=O.[19] In the case of a neighboring

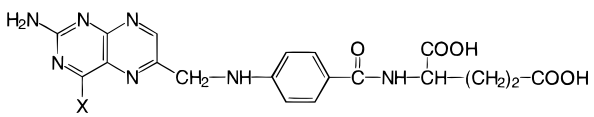


4 5 6 7

Figure 4.

carbon containing C—NH_2 (**7**, Figure 4), the preferred tautomer is the C—NH_2 form.

Perhaps the best known example of classical isosteric substitution of an amino for a hydroxyl group is illustrated by aminopterin (**8b**) wherein the hydroxyl substituent of folic acid (**8a**) has been substituted by an amino group (Figure 5). As previously noted, this represents a monovalent bioisosteric substitution at a carbon atom adjacent to a heterocyclic nitrogen atom. Thus, this bioisosteric replacement has the capability of mimicking even the tautomeric forms of folic acid. The similarity as well as the capability of the amino group to hydrogen bond to the enzyme are two important factors that facilitate the binding of aminopterin to the enzyme dihydrofolate reductase.



8a X = OH Folic Acid
8b X = NH_2 Aminopterin

Figure 5.

Interchange of an amino group with a hydroxyl moiety in the case of 6,9-disubstituted purines (Table 6) has been shown to result in the development of agents with similar benzodiazepine receptor binding activity.[20] This example further substantiates the ability of the amino group to mimic the hydroxyl group at the receptor site. In this study a series of 6,9-disubstituted purines were tested for their ability to bind to the benzodiazepine receptor in rat brain tissue. The relative activity of the 9-(3-aminophenyl)-methyl derivative (**9a**) was compared to the 9-(3-hydroxyphenyl)methyl analogue (**9b**) (Figure 6). In contrast to aminopterin where a dramatic difference in binding affinity was observed relative to the normal substrate, these bioisosteric 6,9-disubstituted

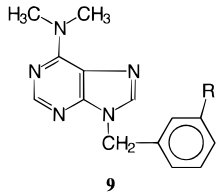


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Figure 6.

Table 6. Benzodiazepine Receptor Binding Activity of Substituted 6-(Dimethylamino)-9-benzyl-9*H*-purines

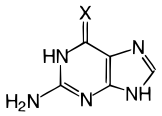
compound	R	IC_{50} (μM)[a]
9a	NH_2	0.9
9b	OH	1.2

[a] Concentration of compound that decreased specific binding of 1.5 nM [^{3}H]diazepam to rat brain receptors by 50%.

purines exhibited similar activity with regard to their affinity for the benzodiazepine receptor. In this example of bioisosteric replacement, pharmacological activity was retained. It is important to note that retention of biological activity based on *in vitro* data can be critical in those instances where differences between bioisosteric analogues exist with regard to *in vivo* parameters which may include absorption, distribution, metabolism, or elimination. While one may only observe retention of activity associated with interaction of drug with the pharmacophore, bioisosteres may differ dramatically in their *in vivo* efficacy. Additional examples of this bioisosteric replacement will be discussed in the next section on monovalent replacement of hydroxyl and thiol groups.

3. Interchange of Hydroxyl and Thiol Groups

The interchange of thiol for hydroxyl can be considered as an extension of the amino−hydroxyl replacement and has been used extensively in medicinal chemistry. This replacement is based on the ability of both these functional groups to be *hydrogen bond acceptors or donors*. A classical illustration of this replacement being guanine (**10a**) and 6-thioguanine (**10b**, Figure 7).[21]



10a X = O Guanine

10b X = S 6-Thioguanine

Figure 7.

As discussed in the previous section, when part of a heteroaromatic ring, these functional groups can exist in different tautomeric forms. Figure 8 illustrates the most common example wherein a mobile proton on a nitrogen atom in the aromatic ring can be transferred to the heteroatom attached to the adjacent carbon resulting in the different tautomers.

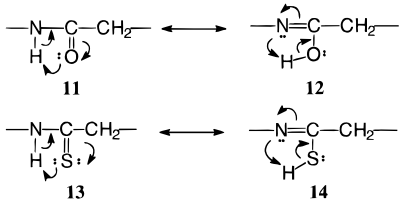


Figure 8.

In the case of 6-thioguanine, the ability of this bioisosteric analogue to be viewed as a substrate by the salvage pathway associated with purine biosynthesis, allows for its transformation into 6-thioguanylic acid by hypoxanthine-guanine phosphoribosyltransferase (HGPRT). However, the significance of this "fraudulent" nucleic acid with respect to its lethality to neoplasms is uncertain.[22] It is as this phosphoriboside that either the *de novo* synthesis of nucleic acids is inhibited or incorporation into deoxyribonucleic acid occurs.

In an attempt to enhance the calcium channel blocking capacity of certain dihydropyrimidine agents, a number of isosteric analogues with the general structure **15** (Figure 9) were synthesized.[23] Substitution of the hydroxyl with an amino resulted in

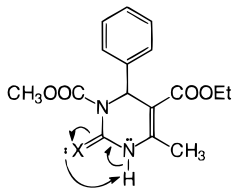


Figure 9.

Table 7. Calcium Channel Blocking Activity of 1,4-Dihydropyrimidines

compound	X	van der Waal's radius[24] (Å)	IC_{50} (nM)[a]
15a	=O	1.40	140
15b	=NH	1.50	160
15c	=S	1.85	17

[a] Concentration that produced 50% inhibition and determined for the vasorelaxant activity with potassium-depolarized rabbit thoracic aorta.

analogues with similar potency. However, substitution with the thiol resulted in enhanced potency (Table 7). This could be explained by the fact that the size of the substituents, described here as the van der Waal's radii, and the ability to hydrogen bond were the important factors influencing retention of activity. Therefore, replacement with the amino group, which has a similar size, resulted in similar potency. However, replacement with the sterically optimal thiol resulted in an analogue which was an order of magnitude more potent.

The use of this replacement in the design of novel anti-inflammatory agents substantiates its utility as a monovalent bioisostere. Long term use of nonsteroidal anti-inflammatory drugs (NSAIDs) for the treatment of rheumatoid arthritis and other inflammatory diseases has been associated with side effects such as gastrointestinal ulceration, bleeding, and nephrotoxicity.[25,26] With a view to designing new drugs with an improved safety profile, certain thiazoles (**16**, Figure 10 and Table 8) that are dual

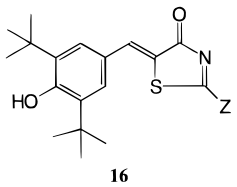


Figure 10.

Table 8. Anti-inflammatory Activity of Benzylidene Derivatives in Intact Rat Basophilic Leukemia (RBL-1) Cells

compound	Z	electronegativity[29]	IC_{50} (μM)[a] 5-LO	IC_{50} (μM)[a] CO
16a	OH	3.51	1.4	0.35
16b	NH_2	2.61	0.77	0.39
16c	SH	2.32	0.38	0.012

[a] Concentration of the test compound causing 50% inhibition of 5-LO or CO formation.

inhibitors of both cyclooxygenase (CO) and 5-lipoxygenase (5-LO) are being studied as potential anti-inflammatory agents.[27] The beneficial effects of NSAIDs have been attributed to the inhibition of the enzyme cyclooxygenase, thereby preventing production of pro-inflammatory prostaglandins.[28] Leuko-

trienes produced by the 5-lipoxygenase enzyme pathway may also contribute to both inflammation and NSAID-induced effects. Table 8 summarizes the concentrations of test compounds required to cause a 50% inhibition of 5-LO and CO formation.

Replacement of the hydroxyl with an amino group resulted in more potent activity toward 5-LO while the potency toward CO remained the same. However, replacement with a thiol resulted in enhanced potency toward both 5-LO and CO. Comparison of the electronegativity values of oxygen, nitrogen and sulphur (Table 8) suggests that this could be a factor that modulates the degree of inhibition of 5-LO. Size, however, may play a significant role with regard to inhibition of CO (Table 7). Thus, the thiol group may be a suitable and informative bioisostere for the amino and hydroxyl groups in several different series of medicinal agents by the virtue of its size, lower electronegativity, and ability to hydrogen bond.

4. Fluorine and Hydroxyl, Amino, or Methyl Groups as Replacements for Hydrogen (Grimm's Hydride Displacement Law)

This monovalent group of isosteres is a result of the direct adaptation of Grimm's Hydride Displacement Law. The basis for the fluorine−hydrogen interchange and the hydroxyl−amino interchange was discussed previously. The existence of this larger group of isosteres might be attributable to a greater tolerance of the different physicochemical parameters of these functionalities within a particular series of agents. However, in the studies outlined in this section, an attempt was made to correlate a physicochemical parameter of this group of bioisosteres with the observed effect on biological activity.

In designing agents for the treatment of cardiovascular diseases, it may be beneficial to associate the hypotensive effects resulting from the inhibition of angiotensin II formation with the diuretic and natriuretic responses. Diuretic and natriuretic effects can be mediated by protection of the endogenous atrial natriuretic peptide (ANP) from inactivation by inhibition of epithelial neutral endopeptidase (NEP). Inhibition of angiotensin II formation may be brought about by inhibition of endothelial angiotensin-converting enzyme (ACE). A series of dual metallopeptidase inhibitors have been designed on the basis of the characteristics of the active sites of both enzymes. Monovalent substitution by fluorine, hydroxyl, and amino in place of hydrogen has recently been used in the design of these metallopeptidase inhibitors (Figure 11, Table 9).[30]

In this study optically pure *N*-[2-(mercaptomethyl)-3-phenylbutanoyl] amino acids (**17**) were evaluated as dual inhibitors of NEP and ACE. Substitution with isosteres (−F, −OH, $-NH_2$) as described by Grimm's Hydride Displacement Law (Table 2) resulted in retention of activity. It was observed within this series, however, that the increase in the effective van der Waal's radii of the isosteric substituents resulted in a decrease in activity (Table 9). In this instance, no significant alteration in preferential activity with either of the peptidases, ACE or NEP, was observed for these bioisosteres.

The empirical approach used to advance the structure−activity relationships with these peptidase

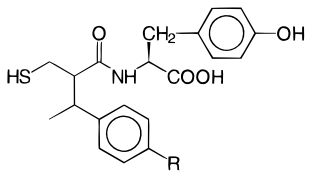


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Figure 11.

Table 9. *In Vitro* Inhibition of NEP and ACE by *N*-[2-(Mercaptomethyl)-3-phenylbutanoyl] Amino Acids

			IC_{50} (nM)	
compound	R	effective van der Waal's radii (Å)[31]	angiotensin converting enzyme	neutral endopeptidase
17a	H	1.20	4.3	2.5
17b	F	1.47	6.9	5.9
17c	OH	1.53	7.9	9.0
17d	NH_2	1.79	12.0	16.0

inhibitors is useful despite the fact that a more selective ACE inhibitor was not developed. Retention of activity within this series of bioisosteres permits an assessment of the validity of a possible correlation with one or more specific physicochemical parameters. This study, for example, did provide insight into structural features which were critical to their activity as inhibitors of these peptidases.

Recently, several 8-substituted O^6-benzyl guanines (**18**, Figure 12) were evaluated for their ability to inactivate the human DNA repair protein, O^6-alkylguanine-DNA alkyltransferase (AGT) (Table 10).[32] Inactivation of the human DNA repair protein O^6-alkylguanine-DNA alkyltransferase by exposure to compounds such as O^6-benzylguanine leads to a dramatic enhancement in the cytotoxic response of human tumor cells and tumor xenografts to chemotherapeutic drugs. This effect is principally observed for chemotherapeutic agents whose mechanism of action involves modification of DNA guanine residues at the O^6-position. In this study the effect of the interchange of NH_2, OH, as well as CF_3 (a bioisostere for a methyl group based on the replacement of hydrogen with fluorine) on activity was assessed. Analogues possessing electronegative groups at the 8-position were more effective as inactivators of AGT in human HT29 colon tumor cell extracts. The relative activities of these bioisosteres based on the dose required for 50% inhibition (ED_{50}) along with their electronegativities are outlined in Table 10.

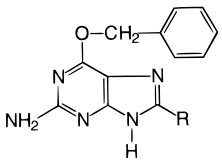

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Figure 12.

Table 10. Alkyl Guanine Transferase Inactivating Activity of 6-(Benzyloxy)purine Derivatives

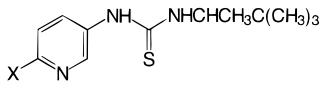
compound	R	electronegativity[29]	ED_{50} (μM)[a]
18a	NH_2	2.61	2.0
18b	CF_3	3.46	0.25
18c	OH	3.51	0.15

[a] Effective dose required to produce 50% inactivation in HT-29 cells upon incubation for 4 h.

Again, the retention of activity within a series of bioisosteres provides the basis for the discovery of a possible correlation between pharmacological activity and the physicochemical properties of specific agents.

As an extension to the above group defined by Grimm's Hydride Displacement Law, the widespread use of the chlorine atom as a bioisostere has been observed in several different series of biologically active compounds. This could be attributed to the similarity in size between these atoms, a comparison of which is made in Table 11. Further, there exists similarity in the lipophilicity of the methyl group with that of chlorine which may be responsible for its suitability as a monovalent bioisosteric replacement.

N-(Substituted-3-pyridyl)-*N*′-alkylthioureas (**19**, Figure 13), which have been evaluated as novel potassium channel openers,[33] are among the more recent illustrations of the replacement of chlorine with isosteres from Grimm's Hydride Displacement Law (Table 11). Potassium channel openers cause vasorelaxation in vascular smooth muscle through hyperpolarization of the cell membrane. There is an increased interest in these compounds based on their therapeutic potential in the treatment of cardiovascular diseases. Substitution at the 6-position with monovalent isosteres ($-NH_2$, $-CH_3$, $-Cl$) results in analogues with similar biological activity. It was observed that substituents with similar biological activity had comparable effective van der Waal's radii (Table 11). The methyl group, which has a lower electronegativity, elicited a weaker pharmacological response, suggesting an additional correlation between activity and a physicochemical property.



19

Figure 13.

Table 11. Inhibition of Spontaneous Mechanical Activity in Rat Portal Vein (*in vitro*)

compound	X	maximum fall in SBP[a] (%)	electronegativity[29]	effective van der Waal's radii[31] (Å)
19a	NH_2	29	2.61	1.79
19b	CH_3	18	2.27	1.80
19c	Cl	27	3.0	1.73

[a] Antihypertensive activity measured as maximum % fall in systolic blood pressure in anesthetized normotensive rat by iv injection.

Table 12 lists the relative potency of a group of bioisosteres which act as inhibitors of thymidylate synthase. Each of these benzo[*f*]quinazolin-1(2*H*)-ones (**20**, Figure 14), inhibit thymidylate synthase by virtue of their structural relation to its cofactor, 5,10-methylenetetrahydrofolic acid.[34] They are, therefore, referred to as folate-based thymidylate synthase inhibitors. These analogues differ from other folate-based thymidylate synthase inhibitors as the absence of a glutamate residue suggests that they are not dependent upon active folate transport and polyglutamylation for activity, the two mechanisms of resistance that have been observed with agents such as methotrexate. Within this series, it was observed that hydrogen bond donors were more potent than the unsubstituted parent compound. These analogues, however, were less active than compact lipophilic groups in elevating thymidylate synthase inhibition. Thus, at the 9-position, optimal size and lipophilicity appear to be critical factors associated with their ability to inhibit thymidylate synthase.

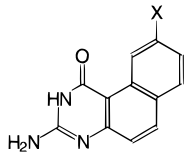


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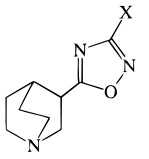
Figure 14.

Table 12. Thymidylate Synthase Enzyme Inhibition Data for Benzo[f]quinazolin-1(2*H*)-ones

compound	X	thymidylate synthase inhibitory activity IC_{50} (μM)[a]
20a	Cl	0.025
20b	CH_3	0.178
20c	OH	0.48
20d	NH_2	0.63
20e	H	1.08

[a] Inhibitor concentration producing 50% inhibition and determined by the tritium release assay of Roberts[35] as modified by Dev et al.[36]

In another study aimed at designing cholinergic agents which would be capable of penetrating the central nervous system and displaying high efficacy at the cortical muscarinic receptors, a series of oxadiazole-based tertiary amines **21** (Figure 15, Table 13) were tested. The assay used was designed to measure affinity and predict cortical efficacy from the antagonist−agonist (i.e. NMS/OXO-M) binding ratio in rat cortical membranes.[37] The log of this ratio has been shown to correlate with the ability of the ligand to stimulate the hydrolysis of cortical phosphatidylinositol. It is known that at least three muscarinic receptor subtypes m1, m3, and m5 may be capable of positively stimulating phosphatidylinositol hydrolysis. In this series it was observed that replace-



21

Figure 15.

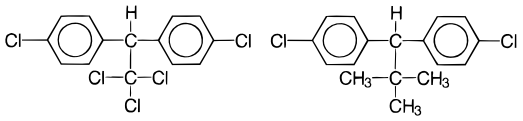
Table 13. *In Vitro* Binding Data for Substituted Oxadiazoles

compound	X	K_a(app) (μM) [^{3}H]NMS[a]	K_a(app) (μM) [^{3}H]OXO-M[b]	ratio[c]
21a	CH_3	0.44	0.0009	490
21b	Cl	0.19	0.00048	400
21c	NH_2	0.60	0.0005	1200

[a] Displacement of [^{3}H]-*N*-methylscopolamine. [b] Displacement of [^{3}H]oxotremorine-M. [c] Ratio of K_a(app) for NMS to K_a(app) for OXO-M.

ment of the methyl with the less lipophilic amino group resulted in an increase in this antagonist−agonist binding ratio. However, replacement with a chloro substituent which is isosteric and isolipophilic to the methyl group resulted in a compound which had lower binding affinity, but a very similar antagonist−agonist binding compared to the methyl-substituted analogue.

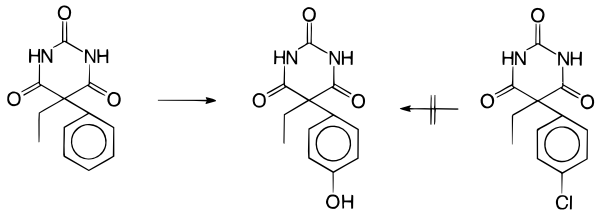
While the chlorine atom is often viewed to be isosteric and isolipophilic with the methyl group, it is very often selected as a bioisosteric replacement because of its ability to alter the metabolism. Previous research has very effectively illustrated the feasibility of altering the metabolism of an agent by the interchange of chlorine with one of the bioisosteres from Grimm's Hydride Displacement Law. Replacement of a chloro atom with a methyl substituent can facilitate metabolism of a xenobiotic. Lipid-soluble chemicals tend to be distributed into adipose tissue where, unless they are metabolized, they tend to accumulate for long periods of time, e.g. DDT (**22**, Figure 16). The replacement of the trichloromethyl moiety with a *tert*-butyl group (**23**, Figure 16) results in diminished persistence of this pesticide.[38] The methyl substituents provide a site which is susceptible to metabolic degradation.



22 23

Figure 16.

Alternatively, the addition of a chloro substituent can be effective in inhibiting metabolic oxidation. Phenobarbital (**24**) is metabolized by aromatic hydroxylation at the *para* position to produce **25** (Figure 17), which then forms a glucuronide conjugate. Replacement of the hydrogen at the *para* position with chlorine (**26**) prevents this route of metabolism. Thus, in this instance the chlorine atom blocks the metabolism of phenobarbital and thereby increases its duration of action.



24 25 26

Figure 17.

2-(Diethylamino)ethyl benzoates (**27**, Figure 18), which are used as local anesthetics provide yet another example where classical bioisosteric replacement affects the observed duration of action by altering metabolism.[39] On the basis of the results outlined in Table 14, it appears that the decreased half-lives ($t_{1/2}$) associated with those analogues having electron-withdrawing groups is likely associated with their increased susceptibility to hydrolysis.

27

Figure 18.

Table 14. Biological Half-Lives of *Para*-Substituted 2-(Diethylamino)ethyl Benzoates

compound	X	σ_p	$t_{1/2}$
27a	NH_2	−0.66	96.29
27b	OCH_3	−0.26	26.75
27c	Cl	0.23	4.94
28d	CN	0.63	1.28

5. Monovalent Substitutions Involving Chloro, Bromo, Thiol, and Hydroxyl Groups (Erlenmeyer's Broadened Classification of Grimm's Displacement Law)

The last class of monovalent isosteres involves chloro, bromo, thiol, and hydroxyl group interchanges. While we have already rationalized the hydroxyl−thiol interchanges, the classification of the chloro, bromo and thiol group together is based on their similarity in their number of peripheral electrons as defined by Erlenmeyer. Use of these monovalent isosteric replacements is illustrated for certain C8-substituted guanosine analogues (**28**, Figure 19). To evaluate the efficacy of C8-substituted guanosine analogues in the enhancement of primary antibody response *in vitro*, these analogues were cultured with antigen and adjuvant.[40] B lymphocytes were incubated with 1.0 mM of the guanosine analogues in presence or absence of type 2 antigen TNP-Ficoll and after 3 days the cells were processed, collected, and then evaluated for the number of antibody-forming cells. The results of this study demonstrated that the bromo-, hydroxyl-, and thio-substituted analogues could stimulate polyclonal immunoglobulin secretion and in the presence of antigen enhanced the magnitude of the anti-TNP plaque-forming cell (Anti-TNP PFC) antibody response to antigen TNP-Ficoll as summarized in Table 15. The thiol bioisostere was more potent as compared to the hydroxyl and the bromo isosteres.

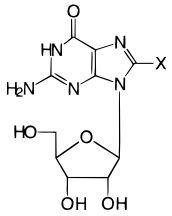


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Figure 19.

Table 15. Enhancement of Antibody Responses of DBA/2 B Cells by Guanosine Analogs

		antigens: no. of anti TNP plaque-forming cells/culture	
compound	X	medium	TNP-Ficoll
28a	medium	14	8
28b	medium + Br analogue	78	198
28c	medium + OH analogue	118	200
28d	medium + SH analogue	80	296

Dihydrotestosterone is a potent androgen known to be essential for male differentiation, growth, and function of androgen-sensitive sex organs and is involved in the pathophysiology of many diseases

such as benign prostatic hyperplasia and prostrate cancer. Inhibition of steroid 5α-reductase is of recent pharmaceutical interest in view of its role in the conversion of testosterone to dihydrotestosterone. Recent research has isolated and characterized two types of human 5α-reductases, namely type I and type II. Therefore, a rational approach for treatment of these androgen-sensitive disease states can be envisioned by the inhibition of either one or both enzymes with specific inhibitors. 4-Substituted *N*-(1,1-dimethylethyl)-3-oxo-4-androsten-17β-carboxamide analogues (**29**, Figure 20) with hydroxy, thiol, chlorine, or bromo substituents at the 4-position were evaluated and found to show intermediate inhibitory activity on human type II 5α-reductase activity.[41] From the data obtained (Table 16), it could be inferred that the enzyme has steric and electronic preferences at this position, resulting in significantly enhanced potency for the hydroxy and chloro substituted compounds.

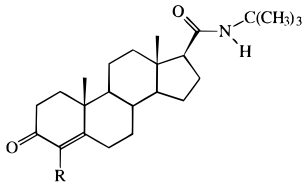

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Figure 20.

Table 16. *In Vitro* Inhibition of Human Type II 5α-Reductase of 4-Substituted *N*-(1,1-Dimethylethyl)-3-oxo-4-androstene-17β-carboxamides

compound	R	effective van der Waal's radii[31] (Å)	electro-negativity[29]	IC_{50} (nM)
29a	OH	1.53	3.51	172
29b	Cl	1.73	3.00	192
29c	SH	1.80	2.32	437
29d	Br	1.86	2.80	387

[a] IC_{50} values represent the concentration of compounds required to inhibit 5α-reductase activity by 50%.

B. Divalent Isosteres

Divalent isosteres can be classified into two subgroups: (1) Those divalent bioisosteres which involve the interchange of atoms that are involved in a double bond, such as in the series; C=**C**, C=**N**, C=**O**, and C=**S**. And (2) those divalent isosteres where substitution of a different atom results in the alteration of two single bonds such as in the series; C−**C**−C, C−**NH**−C, C−**O**−C, and C−**S**−C. Both of these types of bioisosteric substitutions have been used extensively in the study of the structure−activity relationships of various pharmacologically active agents.

1. Divalent Replacements Involving Double Bonds

This subclass includes replacements such as C=S, C=O, C=NH, and C=C. As we have already discussed in the earlier sections, the presence of heterocyclic systems in most of the lead compounds under study as medicinal agents facilitates the tautomerization of these groups. As mentioned earlier, for the purpose of this review, the classification of such analogues as divalent bioisosteres requires the absence of a mobile proton which can migrate within the ring system.

The replacement of C=S with C=O in Tolrestat (**30a**, Figure 21), an aldose reductase inhibitor currently under study in human subjects for the treatment of diabetic neuropathy, resulted in oxo-Tolrestat (**30b**), which retained activity both *in vitro* and *in vivo* (Table 17).[42]

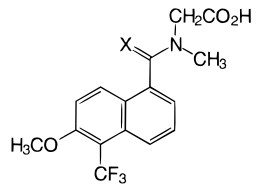

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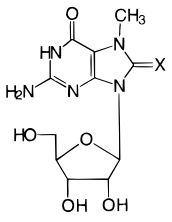
Figure 21.

Table 17. Aldose Reductase Inhibitory Activity of Tolrestat and Oxo-Tolrestat

		aldose reductase inhibition	
compound	X	*in vitro*[a]	*in vivo*[b]
30a	S	94	53
30b	O	86	56

[a] Inhibition of enzyme activity in partially purified bovine lens preparation. [b] Inhibition of galactitol accumulation in the sciatic nerves of rats fed 20% galactose for 4 days.

Another illustration of the use of this class of divalent isosteres is with certain novel purine nucleoside analogues (**31**, Figure 22) that were tested *in vivo* for antiviral activity against Semliki Forest virus (SFV) infection in a mouse model.[43] Table 18 outlines the percentage of mice that survived for 21 days for the various bioisosteric analogues compared to the absence of any survivors in control infected mice. Replacement of the sulfur atom at C-8 with the oxygen or selenium atom, another well-known divalent isosteric replacement, resulted in weaker activity relative to the thio analogue.



31

Figure 22.

Table 18. Activity of Guanosine Bioisosteric Analogues against Semliki Forest Virus

compound	X	% total survivors
31a	S	83 (10/12)
31b	O	67 (8/12)
31c	Se	58 (7/12)

2. Divalent Replacements Involving Two Single Bonds

The second major class of divalent bioisosteres represents those atoms or groups which are attached to different substituents. As these divalent bioisosteres are attached to two different substituents, the chemical and polar differences are less pronounced.

The bond angle or the conformation associated with the use of these divalent bioisosteres may be an important factor associated with retention of biological activity. Table 19 shows a comparison of the antiallergy activity and bond angles of several divalent bioisosteric substitutions which have been investigated for a series of 4-(diarylhydroxymethyl)-1-[3-(aryloxy)propyl]piperidines (**32**, Figure 23).[44]

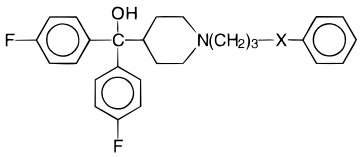


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Figure 23.

Table 19. Oral Antiallergy Activity in the Passive Foot Anaphylaxis Assay of Analogues Containing Varied Heteroatoms

compound	X	electronegativity[29]	bond angle[45] (deg)	passive foot anaphylaxis assay (10 mg/Kg)[a]
32a	−O−	3.51	108.0	+++
32b	−S−	2.32	112.0	+
32c	$-CH_2-$	2.27	111.5	+
32d	−NH−	2.61	111.0	+

[a] Aminophylline orally at 100 mg/kg was used as a positive control and assigned a biological response of (++); (−) not significantly different from negative control group at $p < 0.05$ as determined by the Dunnett's t test; (+) activity between positive and negative groups; (++) activity equivalent to positive control group; (+++) activity greater than positive control group.

These compounds were tested using the passive foot anaphylaxis assay which is an IgE-mediated model useful in the detection of compounds possessing antiallergic activity. A significant correlation between biological activity and electronegativity was observed for these analogues.

Another illustration of divalent bioisosteric linkers is observed in the study of inhibitors of the nuclear factor of activated T cells (NFAT)-mediated transcription of β-galactosidase.[46] T cells are essential components of the immune response. They are activated upon contact with foreign substances, or antigens, present on invading organisms. One of the earlier events that occurs after T cells recognize a foreign antigen is the induction of the interleukin-2 (IL-2) gene. IL-2 is an essential autocrine growth factor for T cells and its appearance marks the commitment of the T cell toward activation. These activated cells release a variety of bioactive molecules which initiate a cascade of events which initiate an immune/inflammatory response. The region 257−286 base pairs upstream of the IL-2 structural gene binds to a protein, the nuclear factor of activated T cells-1 (NFAT-1), prior to IL-2 gene transcription. NFAT-1 is expressed in relatively few cells besides T cells and is markedly upregulated upon stimulation of the T cell receptor. This makes it a highly specific target within activated T cells. When the cell is activated, the NFAT-1 protein binds to the DNA at its recognition site and induces the transcription of β-galactosidase. This study evaluated some of the bioisosteric analogues of quinazolinediones (**33**, Figure 24) as potential immunosuppressive agents by their ability to inhibit β-galactosidase expression as summarized in Table 20. Here again the similar bond angles and electronegativities (Tables 19 and 20) of the −NH− and $-CH_2-$ bioisosteric linkers result in analogues which retain activity. In this study, the use of an oxygen atom as a bioisosteric linker, which has a marginally smaller bond angle and much greater electronegativity, results in an analogue with increased potency.

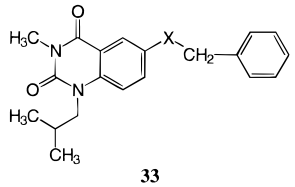

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Figure 24.

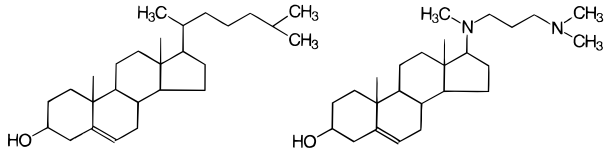
Table 20. Regulation of NFAT-1-Regulated β-Galactosidase Activity by Quinazolinediones

compound	X	IC_{50} (μM)
33a	−NH−	4.47
33b	$-CH_2-$	4.03
33c	−O−	2.5

Other divalent linkers that have been obtained as modifications of the above classical isosteres include higher oxidation states of the thioether linker resulting in sulfoxide and sulfone derivatives. These types of replacements will be discussed in the section on nonclassical isosteres.

C. Trivalent Atoms or Groups

A classical trivalent bioisosteric replacement is −CH═ with −N═. This replacement has been widely used in the drug discovery process and has been further discussed among the ring equivalent class of classical bioisosteres. This replacement when applied to cholesterol (**34**) resulted in 20,25-diazacholesterol (**35**, Figure 25) which is a potent inhibitor of cholesterol biosynthesis.[47] The greater electronegativity of the nitrogen atom could be responsible for the biological activity of this bioisostere.



34 **35**

Figure 25.

Another trivalent substitution based on Erlenmeyer's definition of the similarity in the number of peripheral electrons would be the replacement of −P═ (bond angle C−P−C = 100 ± 4°) with −As═ (bond angle C−As−C = 96 ± 5°).[48] Arsenic is a classical bioisostere of nitrogen (Table 3). Arsenicals have received considerable attention due to their therapeutic significance. The oxidation of arseno compounds to arsenoxides is important in the bioactivation of a number of chemotherapeutic arsenicals. One of the first drugs used clinically was arsphenamine (**36**, Figure 26). The activity of arsphenamine against the syphilis organism was attributed to its oxidized metabolite oxophenarsine. However,

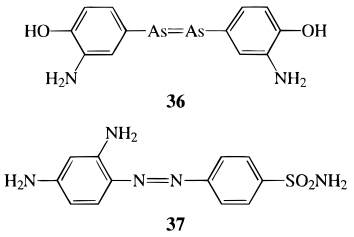


Figure 26.

the lack of selective toxicity associated with these arsenicals has led to a reduced interest in such replacements in current research. Analogy is drawn to prontosil (**37**, Figure 26) which is found to be metabolized to *p*-aminobenzenesulfonamide. Prontosil is inactive against microorganisms *in vitro* but active *in vivo*.

D. Tetrasubstituted Atoms

One of the more widely used tetravalent replacements has been the interchange of a quaternary charged nitrogen atom with a tertiary carbon atom. C_2-C_{20} acyl groups of acyl-CoAs are reversibly transferred to the β-hydroxyl group of (*R*)-carnitine by carnitine acyltransferases. The selective inhibition of individual carnitine acyltransferases may be useful in the therapy of diabetes and heart disease. The determination of relative substrate specificity and the development of specific inhibitors for individual carnitine acyltransferases has been of considerable interest because of its possible therapeutic implications. Certain simple acylcarnitine analogues are potent carnitine acyltransferase (CAT) inhibitors.[49] Structure−activity studies in this series have included the bioisosteric replacement of the hydroxyl group of carnitine (**38**) with an amino (**39**) and replacement of the tetravalent trimethylammonium group with a tertiary butyl group (**40**, Figure 27). Table 21 lists the similar kinetic constants that were obtained for these bioisosteres.

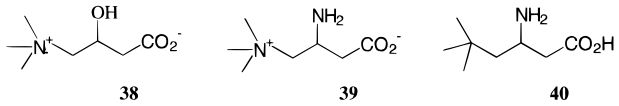


Figure 27.

Table 21. Rate Constants for Carnitine and Synthetic Analogues with Pigeon Breast Carnitine Acyltransferase

X	K_i (mM)[a]
39	4.0
40	2.6

[a] K_i values were obtained from a plot of apparent K_m vs [inhibitor].

Other tetravalent bioisosteric replacements have been investigated which use members of the same group of the periodic table (group IVB), i.e. the silicon or germanium atom. These atoms are also tetravalent and have similar hydrophobicity, but possess different electronic and steric properties from carbon. The ability of trimethylsilyl- or trimethylgermyl-containing retinobenzoic acids (**41**, Figure 28) to induce differentiation of the human promyelocytic leukemia cell line HL-60 to mature granulocytes was examined as a measure of retinoidal activity.[50] Compounds with a trimethylsilyl or trimethylgermyl group at the *meta* positions showed similar activities as the corresponding retinobenzoic acids with a *tert*-butyl group. Results in Table 22 summarize the differentiation-inducing activity observed for one such series of retinobenzoic acids.

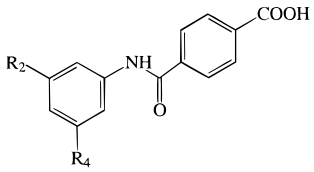


Figure 28.

Table 22. Differentiation-Inducing Activity of Trimethylsilyl- or Trimethylgermyl-Containing Retinoids

compound	R_2	R_4	ED_{50}[a]
41a	*tert*-butyl	*tert*-butyl	3.6×10^{-8}
41b	trimethylsilyl	trimethylsilyl	3.0×10^{-8}
41c	trimethylgermyl	trimethylgermyl	4.2×10^{-8}

[a] Effective dose required to cause differentiation-inducing activity in 50% of human promyelocytic leukemia cells.

A classical illustration of tetrasubstituted isosteres involves replacement of the quaternary ammonium group in case of cholinergic agonists (**42**, Figure 29) with the phosphonium and arsonium analogues.[51] In this study, it was observed that such replacements resulted in less potent analogues with greater toxicity. Activity was found to decrease as size of the onium ion increased. The decreased potency and greater toxicity of these higher elements has diminished interest in replacements of this type for the development of direct-acting cholinergic agonists.

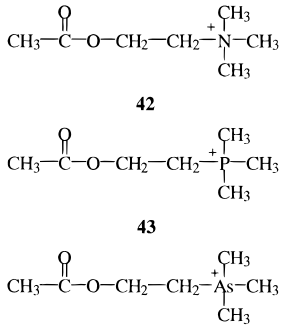
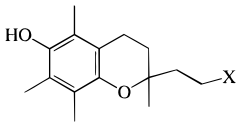


Figure 29.

A more recent illustration of retention of activity within a series of charged isosteres, as described by Erlenmeyer (Table 3) was observed for a series of α-tocopherol analogues (**45**, Figure 30) that were found to scavenge lipoperoxyl and superoxide radicals *in vitro* and accumulate in heart tissue as demonstrated by measurement of *ex vivo* inhibition of lipid peroxidation in mouse heart homogenates.[52] Table 23 illustrates that all the bioisosteric analogues of α-tocopherol were found to elicit similar biological activity.



45

Figure 30.

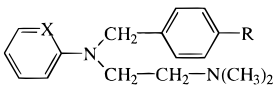
Table 23. *In Vitro* and *ex Vivo* Inhibition of Lipid Autooxidation in Mouse Heart Homogenate

compound	X	*in vitro*[a] IC_{50} (μM)	*ex vivo*[b] ID_{50} (μM/kg)	IC_{50}/ID_{50}
45a	$-N^+(CH_3)_3$	19	11	1.7
45b	$-P^+(CH_3)_3$	10	8	1.3
45c	$-S^+(CH_3)_2$	7	6	1.3

[a] Concentration that inhibits thiobarbituric acid reactive substances (TBARS) formation by 50%. [b] Dose that inhibits TBARS formation by 50% 1 h after sc administration.

E. Ring Equivalents

Ring equivalent bioisosteres represent the final subclass of classical bioisosteres that will be reviewed. Classical isosteric substitutions when applied within ring systems result in different heterocyclic analogues which can be effective bioisosteres. The use of the classical bioisosteres benzene, thiophene, and pyridine resulted in analogues with retention of biological activity within different series of pharmacological agents. One of the successful uses of this replacement resulted in the potent antihistamine mepyramine (**47**, Figure 31) which evolved by the replacement of the phenyl moiety in antegran (**46**) by a pyridyl group.

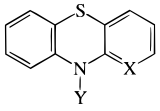


46 X = CH , R = H Antergan

47 X = N , R =OCH_3 Mepyramine

Figure 31.

Substitution of benzene by pyridine also improved activity in the tricyclic antihistamines [promethazine (**48a**) and isothipendyl (**48b**)] and neuroleptics [promazine (**48c**) and prothipendyl (**48d**)] (Figure 32) and resulted in a reduction of both sedative and extrapyramidal effects. In reviewing some more recent studies, this subclass of bioisosteres will be divided into (1) divalent ring equivalents and (2) trivalent ring equivalents.



48a Y = $CH_2CH(CH_3)N(CH_3)_2$ X = CH

48b Y = $CH_2CH(CH_3)N(CH_3)_2$ X = N

48c Y = $CH_2CH_2CH_2N(CH_3)_2$ X = CH

48d Y = $CH_2CH_2CH_2N(CH_3)_2$ X = N

Figure 32.

1. Divalent Ring Equivalents

Some of these heterocyclic rings obtained by the replacement of a heteroatom within different ring systems will be discussed among the nonclassical category of bioisosteres on the basis of their ability to mimic the spatial conformation of certain other functional groups.

A copper-containing monooxygenase, dopamine β-hydroxylase, present in a variety of mammalian tissues, catalyses the benzylic hydroxylation of dopamine. Since dopamine β-hydroxylase plays an important role in the biosynthetic production of noradrenaline, it could be a target for the design of inhibitors as potential therapeutic agents for the modulation of adrenergic activity *in vivo*. Isosteric divalent ring replacements as obtained from Grimm's Hydride Displacement Law (Table 2) resulted in retention of activity within a series of indane derivatives (**49**, Figure 33) evaluated as inhibitors of dopamine β-hydroxylase[53] (Table 24).

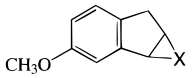


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Figure 33.

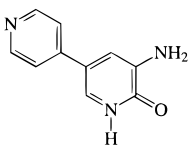
Table 24. Kinetic Inhibition Parameters for Dopamine β-Hydroxylase by Indan Derivatives

compound	X	K_i (mM)[a]
49a	$-CH_2-$	1.12
49b	$-O-$	0.65
49c	$-NH-$	0.4

[a] Apparent binding constant K_i estimated by plotting $1/k_{obs}$ as a function of 1/[I].

The applicability of divalent ring-equivalent isosteres has also been demonstrated in other five- and seven-membered ring systems. The search for novel cardiotonic agents resulted in the successful development of two clinically useful agents, amrinone[54] (**50**) and milrinone[55] (**51**) (Figure 34). It is known that the positive vasodilatory actions of amrinone and milrinone are related to the inhibition of adenosine 3′,5′-cyclic phosphate phosphodiesterase III (cAMP PDE III).

During SAR studies on amrinone (**50**), it was observed that a free amino group was not necessary for *in vitro* cAMP PDE III activity.[56] This prompted the design and synthesis of analogues with general structure **52**.[57] Table 25 summarizes the *in vitro* cAMP PDE III activity of the divalent ring-equivalent



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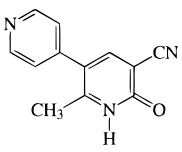

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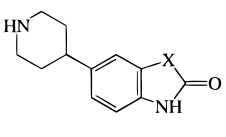

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Figure 34.

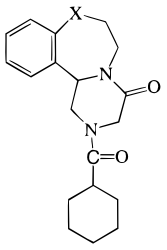
Table 25. *In Vitro* cAMP PDE III Activity of Ring-Equivalent Bioisosteres of 5-(4-Pyridinyl)benzoxazol-2(3*H*)-one

compound	X	cAMP PDE III IC_{50} (μM)[a]
50	amrinone	28
51	milrinone	0.36
52a	$-O-$	9.8
52b	$-CH_2-$	5.3
52c	$-NH-$	1.3
52d	$-S-$	0.54

[a] Concentration required to cause 50% inhibition of cAMP PDE III *in vitro*.

isosteres that were evaluated in this study. No clear correlation with physicochemical parameters could be extrapolated from this series. However, cAMP PDE inhibitory activity was retained by all the bioisosteric analogues of this series.

Divalent isosteric ring substitutions of the pyrazino-[2,1-*a*][2]benzazepine system (**53**, Figure 35) resulted in derivatives containing different heterocyclic systems. All these bioisosteres exhibited anthelmintic activity.[58] Table 26 shows a relative comparison of the MIC values of the various divalent isosteres. Replacement of the methylene with a sulfur or oxygen atom resulted in analogues with decreased potency relative to the carbocyclic analogue.



53

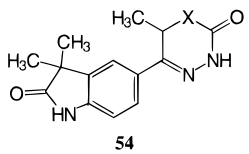
Figure 35.

Table 26. Anthelmintic Potency of Derivatives of the Pyrazino[2,1-*a*][2]benzazepine System

compound	X	minimum inhibitory concentration,[a] *Taenia crassiceps*
53a	$-CH_2-$	+++
53b	$-O-$	++
53c	$-S-$	+

[a] Concentration required to prevent all movement of cysts and evaginated scoleces: ++++ = MIC < 0.1 μg/mL; +++ = 0.1 μg/mL < MIC < 1.0 μg/mL; ++ = 1.0 μg/mL < MIC < 10 μg/mL; + = MIC > 10 μg/mL.

Replacements using selenium have been less commonly used as a divalent ring equivalent. However ring substitution of $-Se-$ has frequently resulted in retention of their respective biological activities. Replacement with selenium has been shown to introduce a potential risk of toxicity. Substitution with the different divalent isosteres including selenium in a novel class of cardiotonic agents **54** (Figure 36 and Table 27) resulted in retention of activity.[59]



54

Figure 36.

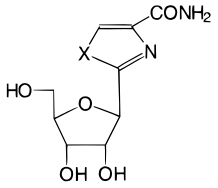
Table 27. Biochemical Properties of Heterocyclic Indolones

compound	X	IC_{50}[a] (μM)
54a	$-NH-$	0.17
54b	$-CH_2-$	0.24
54c	$-S-$	0.33
54d	$-Se-$	0.54
54e	$-O-$	0.96

[a] Concentration required to produce 50% inhibition of the sarcoplasmic reticulum bound low-K_m cAMP phosphodiesterase (SR-PDE).

The inhibition of cardiac phosphodiesterase, which is related to the development of cardiotonic agents, was assessed on the canine cardiac sarcoplasmic reticulum-bound, cGMP-inhibited, high-affinity cAMP-PDE (SR-PDE). Table 27 shows a comparison of the inhibition of phosphodiesterase (PDE) by the various divalent bioisosteric ring substitutions.

C-Glycosyl nucleosides which are analogues of nicotinamide nucleoside are expected to be converted to the analogues of NAD coenzyme and to inhibit NAD-dependent inosine monophosphate dehydrogenase (IMPD). Inhibition of this enzyme produces the accumulation of IMP and the depletion of guanine nucleotides, which is linked to DNA synthesis inhibition. Both 2-β-D-ribofuranosylthiazole-4-carboxamide (tiazofurin, **55b**) and 2-β-D-ribofuranosylselenazole-4-carboxamide (selenazofurin, **55c**) (Figure 37) are metabolized to analogues of NAD and have pronounced antitumor activity in animals and broad spectrum antiviral activity.[60] Selenazofurin is about 10 times more active than tiazofurin with a similar spectrum of antitumor activity (Table 28).



55

Figure 37.

Table 28. Bioisosteres of 2-β-D-Ribofuranosyl-4-carboxamides

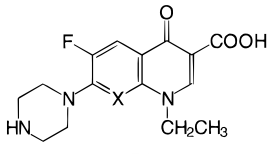
compound	X	antitumor agent	relative activity, IC_{50}[a] (μM)
55a	$-O-$	oxazofurin	inactive
55b	$-S-$	tiazofurin	1.0
55c	$-Se-$	selenazofurin	0.1

[a] Estimated relative potency against P388 and L1210 leukemias. Inactive until a maximum tested concentration of 4×10^{-3} M.

Replacement with oxygen in 2-β-D-ribofuranosylthiazole-4-carboxamide (tiazofurin) resulted in 2-β-D-ribofuranosyloxazole-4-carboxamide (oxazofurin, **55a**). Oxazofurin lost the ability to inhibit the growth of P388 and L1210 murine leukemia and HL 60 human promylelocytic leukemia.[61] This may be attributed to the lower basicity of the oxazole moiety compared to that of the thiazole moiety of tiazofurin.[62]

2. Trivalent Ring Equivalents

The trivalent substitution of $-CH=$ with $-N=$ is commonly used in modern drug design. Trivalent ring substitution of $-CH=$ with $-N=$ in the antibacterial agent norfloxacin (**56a**) resulted in enoxacin (**56b**, Figure 38) which is also in clinical use for its antibacterial activity[63] (Table 29).



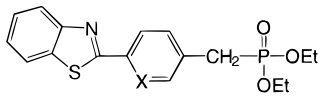
56

Figure 38.

Table 29. Isosteres of Clinically Significant Quinolone Antibacterials

compound	X	antibacterial agent
56a	−CH−	norfloxacin
56b	−N−	enoxacin

Another example of such a replacement is illustrated for the (benzothiazolylbenzyl)phosphonate derivatives (**57**, Figure 39) which retained vasodilatory activity.[64] Table 30 outlines the percent increase in coronary flow obtained by replacement of the benzene ring with pyridine.



57

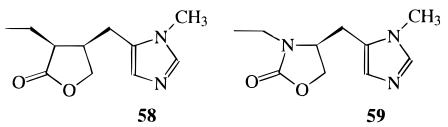
Figure 39.

Table 30. Effect of Phosphonates on Coronary Flow of Isolated Guinea Pig Heart

compound	X	maximum increase in coronary flow[a] (%)
57a	−CH−	79.9
57b	−N−	94.0

[a] Langendorff's method in isolated guinea pig heart.

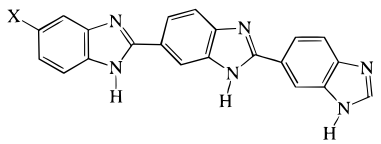
Pilocarpine (**58**, Figure 40) is widely employed as a topical miotic for controlling the elevated intraocular pressure associated with glaucoma. The duration of lowering of intraocular pressure by pilocarpine lasts only for about 3 h. This short duration of action is mainly due to the hydrolytic cleavage of the lactone ring, resulting in the formation of pilocarpic acid and rapid elimination and/or epimerization to form isopilocarpine.[65] In an attempt to study the influence of the carbamate nitrogen on physiological properties, certain carbamate analogues corresponding to pilocarpine were synthesized and evaluated.[66] Replacement of the carbon atom on the oxazolidinone ring with nitrogen resulted in the carbamate analogue **59** which was equipotent with pilocarpine (ED_{50} = 1 μM). This analogue bypasses the problem associated epimerization as observed in the case of pilocarpine. In addition, this cyclic carbamate would be expected to be less susceptible to hydrolysis than the lactone in pilocarpine.



58 **59**

Figure 40.

A more recent illustration of this replacement was observed in the evaluation of terbenzimidazoles as topoisomerase I inhibitors.[67] DNA topoisomerases are nuclear enzymes that control and modify the topological states of DNA by catalyzing the concerted breaking and rejoining of DNA strands.[68] Topoisomerases represent effective pharmacological targets for the development of cancer chemotherapeutics. Replacement of the phenyl (**60a**) with 2-pyridyl (**60b**), 3-pyridyl (**60c**), and 4-pyridyl (**60d**) resulted in retention of cytotoxicity and topoisomerase I inhibitory activity (Figure 41, Table 31).



60

Figure 41.

Table 31. Topoisomerase I-Mediated DNA Cleavage and Cytotoxicity of Terbenzimidazoles

compound	X	Topo I-mediated DNA cleavage[a]	cytotoxicity IC_{50} (μM) RPMI 8402
60a	phenyl	2	0.09
60b	2-pyridyl	3.3	0.16
60c	3-pyridyl	2	0.035
60d	4-pyridyl	2	0.035

[a] Topoisomerase I cleavage values are reported as REC, relative effective concentration, i.e., concentrations relative to Hoechst 33342, whose value is arbitrarily assumed as 1, that are able to produce the same cleavage on the plasmid DNA in the presence of calf thymus topoisomerase I.

III. Nonclassical Bioisosteres

This major class of isosteres includes all those replacements that are not defined by the classical definitions of bioisosteres. These isosteres are capable of maintaining similar biological activity by mimicking the spatial arrangement, electronic properties, or some other physicochemical property of the molecule or functional group that is critical for retention of biological activity.

A. Cyclic vs Noncyclic Nonclassical Bioisosteric Replacements

This subclass includes all those nonclassical replacements wherein a noncyclic functional moiety mimics a cyclic group sterically or electronically resulting in retention of biological activity. Comparison of the various structural analogues of estradiol is a classical illustration of this phenomena. Here the ability of the structure to hold the critical functionality in a particular spatial configuration was essential for activity. It was found that the central bond of diethylstilbestrol (**2**, Figure 43) was important for the correct orientation of the phenolic and ethyl groups for binding to the estrogenic receptor.[69] This is evidenced by the observation that the *cis* isomer of **2** is only $^1/_{14}$ as active as the *trans* isomer.[70] Structurally or conformationally rigid analogues (compounds **2**−**4**) were equipotent as estradiol (**1**, Figure 43) when injected. Nonrigid analogues (compounds **5**−**7**), however, were found to have little or no estrogenic activity.[71,72]

This concept was extended by the use of the methyleneaminoxy methyl moiety (C═$NOCH_2$, MAOMM) (Figure 42) as a bioisostere of aryl and other aromatic groups.

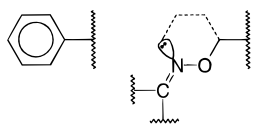


Figure 42.

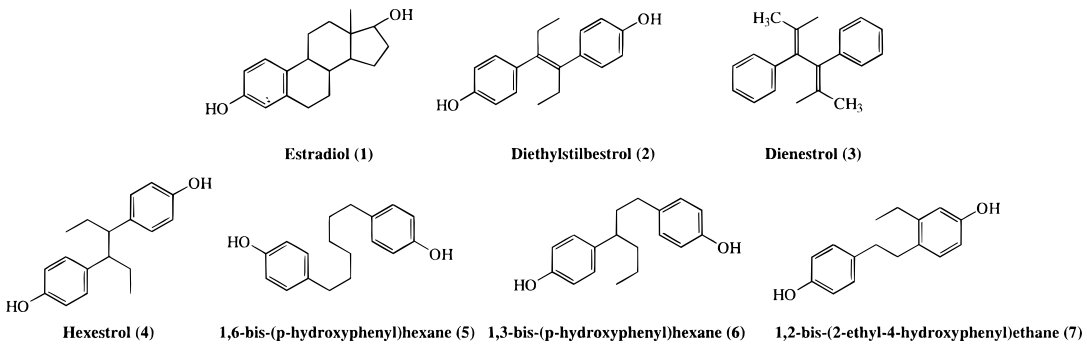


Figure 43.

Various studies associated with the development of β-adrenergic drugs have supported the utility of this nonclassical bioisostere.[73−75] Chemically synthesized analogues of biological catecholamines were among the first agents used to interact with β-adrenergic receptor sites. Analogues of the biological catecholamines often incorporated within their general structure a 1-phenyl-2-aminoethanol moiety (**8**, Figure 44). Closely related to these agents is a series of the 1-(aryloxy)-3-amino-2-propanol derivatives (**9**, Figure 44) which are β-adrenergic antagonists.[76−77] With regard to these adrenergic drugs, it has been observed that the aminoethanol portion, $-CH(OH)-CH_2NHR$ is present in both agonists and antagonists. Thus, it could be inferred from these data that this portion of these drug molecules plays a significant role in the ability of these compounds to bind with the receptor. On the other hand, it has been observed that the nature of the aromatic nucleus can significantly influence their agonistic or antagonistic properties. Results from theoretical studies[78,79] noted that the $C_3-O_2-C_4-C_5$ moiety of the class B type of β-blocking adrenergic drugs (**9**, Figure 44), can electronically and sterically simulate a portion of the aromatic ring. Data from these studies showed that the electronic distribution (based on the trend of the electrostatic molecular potential, EMP) generated by the $C_3-O_2-C_4-C_5$ was similar to that generated by the aromatic ring of antagonists such as **9**. This suggested that the electronic distribution essential for interaction with the β-adrenergic receptor may not require an aromatic ring.

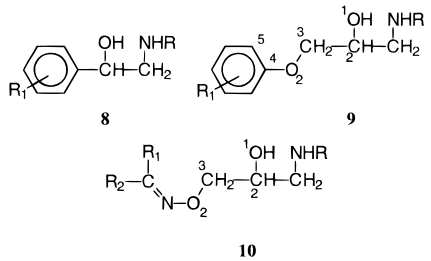


Figure 44.

These results led to the development of the aliphatic oxime ether derivatives (**10**, Figure 44) which showed marked and competitive antagonism at the β-adrenoceptors *in vitro* in pharmacological tests. This success led to the application of this bioisosteric replacement to other categories of drugs. One such application has been its use in the aryl acetic acids which are one of the most widely developed and investigated class of anti-inflammatory agents. Agents in this class include ibufenac (**11**), aclofenac (**12**), and diclofenac (**13**) which are widely used clinically (Figure 45).

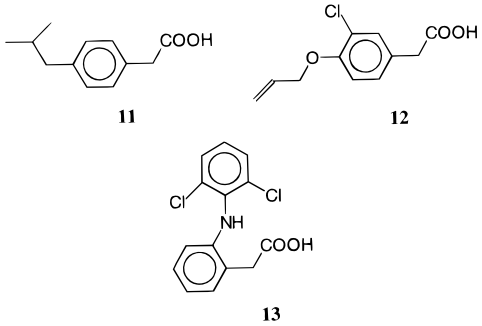


Figure 45.

Several β-aminooxypropionic acids were evaluated as analogues of arylacetic acids wherein the aryl moiety was substituted by MAOMM.[80] Of the various analogues evaluated, the (*E*)-3-[(benzylideneamino)oxy]propionic acid (**14**, Figure 46) showed similar potency to that of diclofenac (Table 32). The results of the conformational studies performed confirmed the existence of bioisosterism, at least from a steric point of view. Although the EMP trend, a measure of the electronic distribution, calculated for one of the β-aminooxypropionic acids and ibufenac showed that the MAOMM had a similar reactivity to that of the aryl moiety of ibufenac, it was observed in additional studies that this EMP trend was not essential for anti-inflammatory activity.

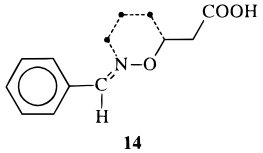


Figure 46.

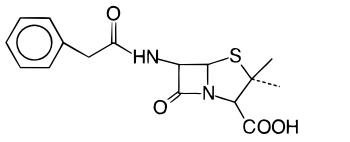
Table 32. Pharmacological Data of β-Aminooxypropionic Acid Derivatives

compound	% inhibition in the carrageenan paw edema assay
13 (Diclofenac)	67
14 (*E*)-3-[(benzylidene-amino)oxy]propionic acid	66

Further, as an extension to these studies, certain β-aminooxypropionyl penicillins and cephalosporin analogues were synthesized and evaluated for their antimicrobial properties.[81] It has been recognized that changes on the side chain linked to the β-lactam

nucleus of β-lactam antibiotics exert an influence on acid stability, resistance to enzyme inactivation, potency, and the spectrum of antimicrobial activity. It is observed among classical β-lactam antibiotics that those compounds widely used in therapeutic practice, such as ampicillin, cefalexin, and penicillin G (**15**), all have side chains of an acetamido type, substituted with an aromatic or a heteroaromatic ring.

A comparison of the MIC values outlined in Table 33 shows that replacement of the phenyl group of penicillin G (**15**) with a β-aminooxypropionyl group (**16**, Figure 47) results in retention of biological activity. The potency against both Gram-positive and Gram-negative bacteria, however, was reduced by approximately 50%.



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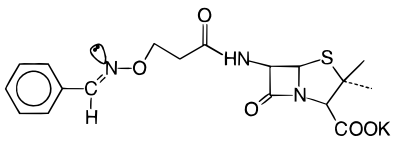

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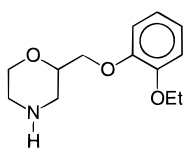
Figure 47.

Table 33. Minimum Inhibitory Concentration (MIC) of β-Aminooxypropionyl Penicillins

	MIC (μg/mL)	
compound	Gram-positive bacteria	Gram-negative bacteria
15	0.05	71
16	0.10	136

Another recent application of this replacement has been documented for the morpholine class of antidepressants.[82] In this study, 2-[(methyleneamino)oxy]-methyl morpholines (**18**, Figure 48) were synthesized as analogues of viloxazine (**17**). Here the (aryloxy)-methyl group of viloxazine is substituted by a [(methyleneamino)oxy]methyl moiety (MAOMM). *In vivo* and *in vitro* tests showed that some of these morpholine derivatives possess a pharmacological profile similar to that of viloxazine (**17**).



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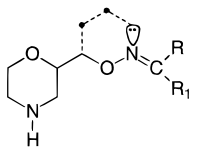

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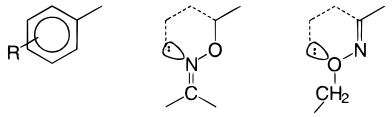
Figure 48.

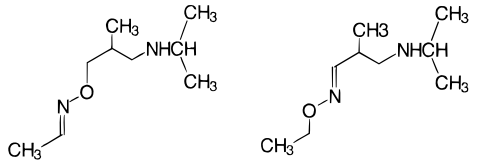
These earlier studies have shown that the [(methyleneamino)oxy]methyl moiety is a suitable bioisostere for the aryl moiety. It was noted however, that the carbon of the $-CH_2-$ linked to the oxygen (**20**, Figure 49) is of different hybridization (sp^3) than the corresponding aryl carbon (**19**, Figure 49) which is sp^2 hybridized. Further, in its preferred conformation, the unsaturated portion (C=N) of the MAOMM, is situated spatially in an area which does not exactly correspond to the area occupied by the aryl group. An attempt to optimize these differences resulted in the formal inversion of the atomic sequence $C{=}NOCH_2$ of the MAOMM (**20**, Figure 49) which lead to a different bioisostere, the [(methyloxy)imino]methyl moiety ($CH_2ON{=}C$, MOIMM) (**21**, Figure 49).[83] This moiety, in the *E* configuration, presents greater steric and electronic analogies to an aryl group as compared to MAOMM by virtue of similarities in hybridization and geometry.



19 **20** **21**

Figure 49.

This similarity explains the retention of β_1-adrenergic binding affinity and activity (Table 34) on replacement of the MAOMM (**22**, Figure 50) with MOIMM (**23**, Figure 50). Results from the conformational studies and an analysis of molecular electrostatic potentials have shown a high degree of homology between these pharmacophoric groups and no significant differences in the chemical reactivity. Applications of this replacement (MOIMM) beyond this class of drugs have not yet been reported.



22 **23**

Figure 50.

Table 34. β-Adrenergic Activity and Radioligand Binding Affinity of MOIMM and MAOMM as Bioisosteric Replacements

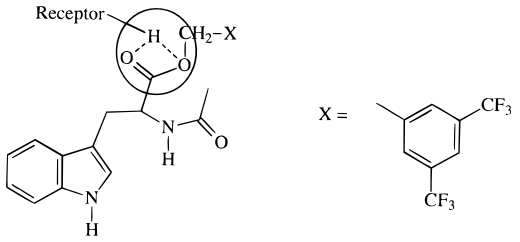
compound	β_1-adrenergic binding affinity[a] K_i (nM)	β_1-adrenergic activity[b] $-\log IC_{50}$
22	1480	4.52
23	1040	4.91

[a] β_1-Adrenergic binding affinity was measured using rat cortical membranes. [b] Isolated guinea pig atria was used to determine β_1-adrenergic activity.

A number of medicinal agents in clinical use contain an ester moiety as part of the molecule. The distribution of esterases in the body is ubiquitous and several types can be found in the blood, liver, and other organs and tissues. In many instances, the prevalence of these esterases causes these molecules to be highly labile *in vivo*. Bioisosteres of the ester moiety have been developed wherein such substitution can lead to an increase in the hydrolytic stability of the drug. One such replacement of the ester group is a heterocycle. The use of a ring in place of a noncyclic moiety is a common approach used to increase structural rigidity. When applied to ester moieties, such bioisosteric replacements can provide additional insights into the structural requirements of a specific receptor as well as result in the development of analogues with greater stability.

The role of the neuropeptide substance P in the endogenous response to pain and inflammation has

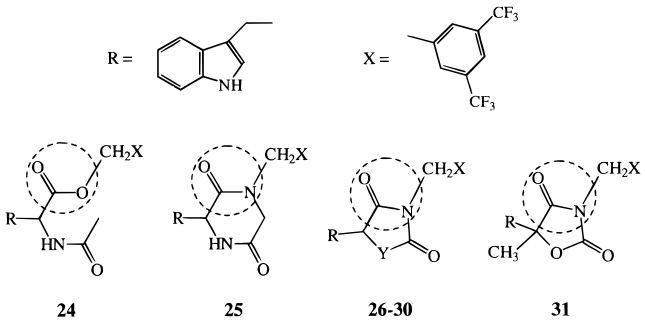
received considerable attention in recent years. These observations have suggested that suitable substance P receptor antagonists (NK_1 antagonists) may be of therapeutic use in the treatment of several clinical conditions including arthritis, migraine, postoperative pain, and nausea. (*S*)-Tryptophan benzyl esters such as L-732,138 (**24**, Figure 51) represent a new structural class of potent and selective NK_1 antagonists.[84] In this molecule the ester linkage is known to contribute significantly to receptor binding, presumably via hydrogen bonding.[85]



24, L-732,138

Figure 51.

A series of analogues in which 6-membered heterocyclic rings served as bioisosteric replacements for the ester linkage have been evaluated with the intent of improving their *in vivo* stability.[86] Although replacement of the ester by various substituted piperazines (**25**, Figure 52) and a variety of five-membered ring heterocycles (**26**−**30**) resulted in a loss of binding affinity (Table 35), the oxazolidinedione moiety (**31**, Figure 52) was identified as a useful bioisostere for the ester linkage and had the greatest potency with respect to NK_1 binding affinity. This analogue, however was less active than L-732,138 (**24**).



24 **25** **26-30** **31**

Figure 52.

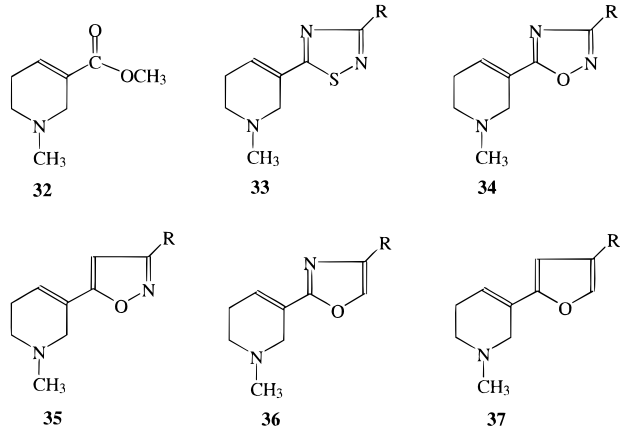
Table 35. NK_1 Antagonist Activity of Five- and Six-Membered Ring Heterocyclic Templates

bioisosteric ring (Figure 52)	Y	human NK_1 receptor binding affinity[a] (nM)
24 (L-732,138)	–	2.5
25	–	4333
26	NH	317
27	*N*-CH_3	153
28	*N*-$CONHCH_3$	63
29	CH_2	203
30	O	22
31	–	11

[a] Human NK_1 receptor binding affinity measured by displacement of [^{125}I]Tyr8-substance P.

Although this study failed to identify a more potent bioisostere because of the inability to attain optimal binding geometry, presumably the rings listed in Figure 52 could represent useful bioisosteric replacements for an ester moiety within another series of compounds. Unlike the ester moiety, these bioisosteres would not be subject *in vivo* to the action of esterases.

Another area in which bioisosteric replacements for the ester moiety have been investigated is in the design of centrally active muscarinic agents based on arecoline (**32**, Figure 53). Replacement of the ester group with either 3-alkyl-1,2,4-thiadiazoles (**33**) or 3-alkyl-1,2,4-oxadiazoles (**34**) have produced very potent muscarinic agonists.[87−92] Systematic removal of the heteroatoms in 3-methyl-1,2,4-oxadiazole gives isoxazoles and furans (**35**−**37**) with a decrease in affinity for the binding site. Thus, the electronic effects associated with these heterocyclic rings appear to be essential for muscarinic activity.

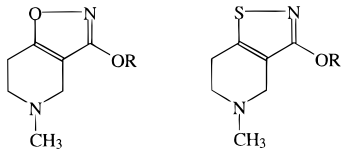


32 **33** **34**

35 **36** **37**

Figure 53.

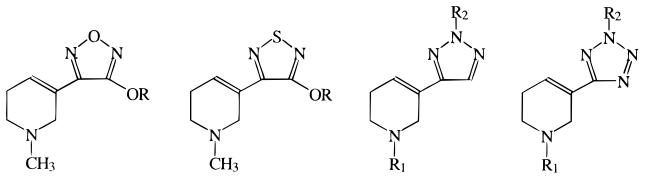
Certain other conformationally restricted compounds based on the bioisosteric replacement of the methyl ester groups with the 3-alkoxyisoxazoles (**38**) and the 3-alkoxyisothiazoles (**39**) (Figure 54) have also been found to result in muscarinic agonists with activity similar to arecoline.[93−94]



38 **39**

Figure 54.

On the basis of the structural resemblance of the 1,2,5-oxadiazoles and the 1,2,5-thiadiazoles with the 3-alkoxyisoxazoles (**38**) and the 3-alkoxyisothiazoles (**39**), several 1,2,5-oxadiazole (**40**, Figure 55) and 1,2,5-thiadiazole (**41**, Figure 55) analogues have been evaluated *in vitro* and found to be selective M_1 muscarinic agonists.[95] Recently a series of arecoline



40 **41** **42** **43**

Figure 55.

bioisosteres, where the ester group was replaced by a 1,2,3-triazol-4-yl (**42**) or a tetrazol-5-yl group (**43**), has been evaluated *in vitro* for affinity and efficacy at the muscarinic receptors and *in vivo* for cholinergic side effects.[96] The structure−activity relationships within these series are similar. Two compounds exihibited activity as M_1 agonists/M_2 antagonists, a profile of particular interest for drugs in the treatment in Alzheimer's disease. Further optimization showed that the position of the substituent in the 1,2,3-triazole or tetrazole ring has a significant influence on the affinity for muscarinic receptors. The 2-alkyl derivatives exerted high affinity, while 1- and 3-alkyl substituents resulted in analogues with very low affinity for the muscarinic recptors. This example clearly demonstrates the effective use of nonclassical bioisosterism in the development of new drugs which are significantly different from the lead compound.

Modulators of the central neurotransmitter GABA represent another class of compounds in which this concept of cyclic and noncyclic bioisosteres has played a major role. 4-Aminobutanoic acid (GABA, **44**, Figure 56) is the major inhibitory neurotransmitter in the mammalian central nervous system. This neurotransmitter functions at $GABA_A$, $GABA_B$, and probably $GABA_C$ receptors.[97−99] In order to pharmacologically characterize these receptors, a number of $GABA_A$ agonists bioisosterically derived from GABA, such as muscimol (**45**, Figure 56),[100−101] thiomuscimol (**46**, Figure 56),[101] and the much weaker $GABA_A$ agonist, isomuscimol (**47**, Figure 56),[101] have been developed. The establishment of specific $GABA_A$ agonists is of major therapeutic importance. It is anticipated that such research may represent the initial steps in the development of new types of antiepileptics, analgesics, and memory-improving drugs. The success of bioisosteric modifications in the development of GABA agonists is dependent on the ability of these receptors to tolerate bioisosteric modifications. Minor structural modifications in these agonistic molecules has frequently resulted in a marked or complete loss of activity.[102−103]

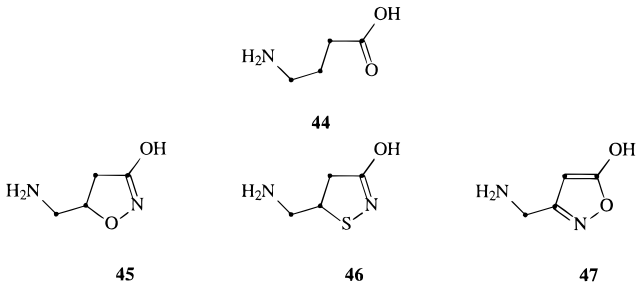


Figure 56.

The bioisosteric bicyclic analogues of these compounds, 4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol (THIP, **48**, Figure 57),[101,104] 4,5,6,7-tetrahydroisothiazolo[5,4-*c*]pyridin-3-ol (thio-THIP, **49**, Figure 57),[105] and 4,5,6,7-tetraisoxazolo[3,4-*c*]pyridin-3-ol (iso-THIP, **50**, Figure 57),[106] as well as isoguvacine (**51**, Figure 57),[101,104] and isonipecotic acid (**52**, Figure 57),[104] have been characterized as $GABA_A$ receptor ligands.

A further extension of the series of $GABA_A$ agonists described above led to another bicyclic series. On the

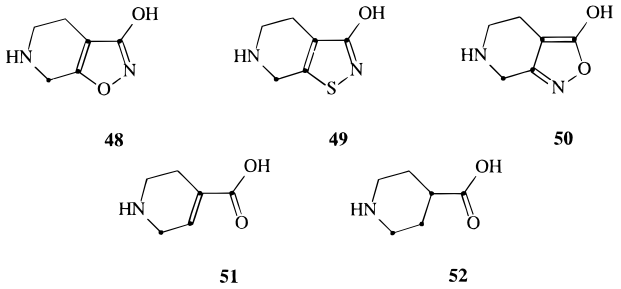


Figure 57.

basis of the lead that 5-(4-piperidyl)isoxazol-3-ol (4-PIOL, **53**, Figure 58) is a specific low-efficacy partial $GABA_A$ agonist,[107−109] several additional analogues of **53** were synthesized. The SARs for the 4-PIOL analogues showed no conspicuous relationships between pK_a values, receptor affinity, and agonist efficacy.[110]

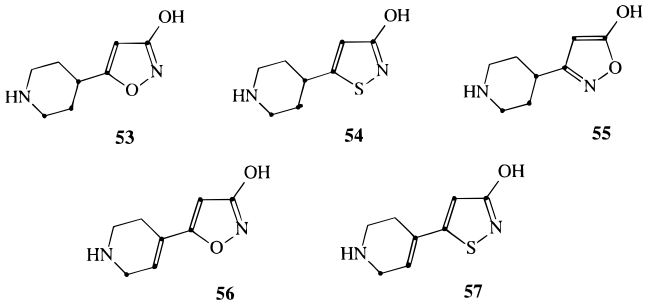


Figure 58.

Although these bioisosteric replacements did not provide an analogue with substantially greater potency, these studies helped characterize several chemically distinct $GABA_A$ agonists which exhibited a range of efficacies. These bioisosteric replacements did provide insight into the spatial geometry required for these agents to maintain their specificity for a given pharmacological target.

The concept of cyclic and noncyclic bioisosteres has also been successfully applied to the development of several peptidomimetics. One of the most important aspects in the development of potent bioactive peptides and peptidomimetics is the process of elucidating the active conformation of the peptide. Advances in molecular biology have led to the identification and characterization of different subtypes of the same receptor, each of which are now known to be related to different physiological phenomena. The ability of a peptide to bind to a specific receptor subtype may require a particular conformation of the peptide. For this purpose, constrained peptidomimetics based on cyclic structures, constrained amino acids, and mimetics of peptide secondary structures have been used in efforts to duplicate the conformation that will bind to the specific receptor subtype and elicit a specific physiologic effect.

The tripeptide Pro-Leu-Gly-NH_2 (PLG, **58**) selectively enhances the binding of dopamine receptor agonists to dopamine receptors in the mammalian central nervous system.[111−113] Several lactam analogues **59**, where X was varied to give different sized or functionalized lactam rings, were synthesized and found to enhance the binding of the agonist 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (ADTN) to dopamine receptors.[114,115] On the basis of this

study it was postulated that the bioactive conformation of PLG is a type-II β-turn. In order to test this hypothesis conformationally restricted bicyclic thiazolidine lactams (**60**, **61**, Figure 59), that restrict the torsion angles to values which are close to those of an ideal type-II β-turn, were synthesized and were more effective than PLG in enhancing the binding of the dopamine receptor agonist ADTN to dopamine receptors.[116]

This example demonstrates how cyclic bioisosteres could be used in place of noncyclic structures in peptides to restrict the torsion angles and evaluate the bioactive conformation essential for physiological activity.

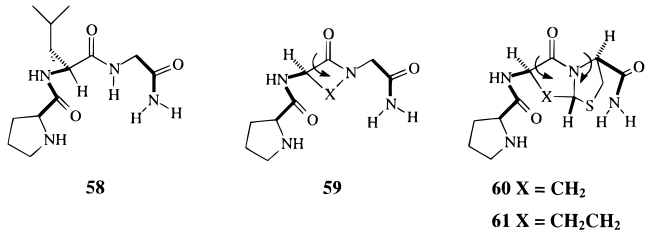


Figure 59.

B. Nonclassical Bioisosteric Replacements of Functional Groups

In this section possible nonclassical bioisosteric replacements for different functional groups will be discussed. As can be the case for any bioisostere, not all of these replacements will necessarily result in a compound with comparable biological activity to the template drug. However, in those instances where such replacements have resulted in retention of biological activity, the examples as outlined in this section may encourage the use of these isosteres in future structure activity studies.

1. Hydroxyl Group Bioisosteres

Nonclassical bioisosteres for phenolic hydroxyl groups generally do not resemble this functional group in terms of size or potential as a strong electron-donating group. Several nonclassical bioisosteres for the phenolic hydroxyl group are listed in Table 36. Molar refractivity is an index of relative size. While the hydroxymethyl bioisostere most closely approximates the size of the phenolic hydroxyl group, it remains significantly different. In terms of these nonclassical bioisosteres, only the urea bioisostere is an electron-donating substituent. Thus, these nonclassical bioisosteres are unlikely to be suitable in those instances where biological activity is adversely affected by increased molecular size or is strongly dependent on electronic parameters. These nonclassical bioisosteres tend to be most effective, in those instances where the role of the phenolic hydroxyl group is to act as either a hydrogen bond acceptor or donor. Such bioisosteres are also effective when moderate hydrophilicity is correlated with improved biological activity.

Table 36. Aromatic Substituent Constants for Hydroxyl Group Bioisosteres[117]

bioisosteres	σ_p	π	MR
OH	−0.37	−0.67	2.85
CH_2OH	0.00	−1.03	7.19
$NHCONH_2$	−0.24	−1.30	13.72
$NHCOCH_3$	0.00	−0.97	14.93
$NHSO_2CH_3$	0.03	−1.18	18.17
NHCN	0.06	−0.26	10.14

A widely used nonclassical bioisosteric replacement for the hydroxyl group is the alkylsulfonamido group. The acidity of the exchangeable proton of the phenolic hydroxyl group and an alkylsulfonamide is comparable. Further, the −N−H portion of the sulfonamide is able to align itself, in relation to the receptor, in a manner closely approximating the phenyl −O−H grouping (Figure 60). An illustration of the successful use of this replacement is in its use in a series of compounds that possess a similar biological profile to that of structurally related phenolic ethanolamines.[118]

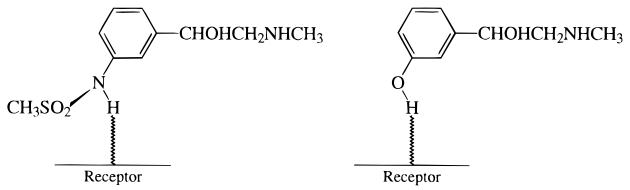


Figure 60.

Notable examples of β-adrenoceptor agonists in which a 3-hydroxyl group has been replaced with bioisosteric groups (Figure 61) include albuterol (**63**, 3-CH_2OH), soterenol[119] (**64**, 3-$NHSO_2CH_3$), and carbuterol[120] (**65**, 3-$NHCONH_2$). Isoproterenol (**62**, Figure 61), the prototype of the β-adrenergic agonists, is widely used clinically as a bronchodilator; however, it is not a β_2-selective agent. In addition this catechol is rapidly metabolized by catechol *O*-methyl transferase (COMT) which catalyzes the methylation of the *m*-hydroxy group.[121] Attempts to increase the duration of action of this class of agents by preventing this route of metabolism has resulted in the replacement of the *m*-hydroxyl with bioisosteres such as the methanesulfonamido, hydroxymethyl, and ureido groups, resulting in agents with potent and selective activities (Figure 61).

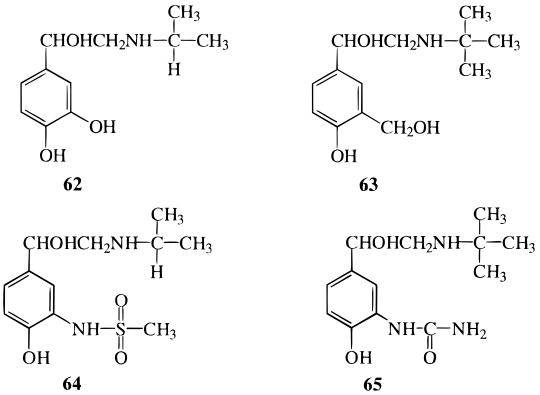


Figure 61.

A more recent illustration of bioisosteric replacements for a phenol was observed in a study designed to evaluate the D_1- and D_2-receptor affinity for a series of *N*,*N*-di-*n*-propyldopamine (DPDA) analogues (Figure 62).[122] In this study, a number of analogues in which a hydroxyl group was substituted with nonclassical bioisosteric replacements were evaluated

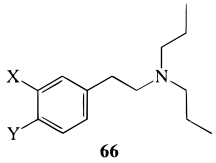


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Figure 62.

Table 37. Ligand Binding Data for *N,N*-Di-*n*-propyldopamine (DPDA) Cogeners

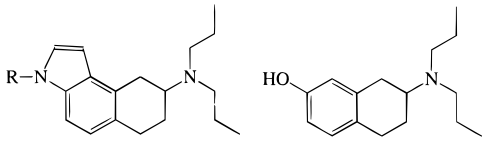
compound	X	Y	Hill coefficient, pIC_{50}[a]
66a	OH (DPDA)	OH	7.65
66b	NHCHO	OH	6.94
66c	$NHCOCH_3$	OH	5.10
66d	$NHCONH_2$	OH	5.15
66e	$NHSO_2CH_3$	OH	7.87
66f	$NHCOCH_3$	H	4.69
66g	$NHCONH_2$	H	4.87
66h	$NHSO_2CH_3$	H	5.45

[a] Negative logarithm of the concentration required to produce 50% inhibition in EDTA-washed rat strital membranes using [^{3}H]spiperone

(Table 37) for their ability to bind to the D_2-receptor in rat striatal membranes.

In this illustration, it is evident (Table 37) that the methanesulfonamide (**66e**) appears to be a suitable bioisosteric replacement for the 3-hydroxyl group of DPDA. The formamide group (**66b**) also serves as a bioisostere in this series, but this analogue exhibited lower affinity for the D_2-receptor than DPDA. Both of these bioisosterically derived compounds also demonstrated *in vivo* cardiovascular and renal profiles that were consistent with selective D_2-receptor agonism.

Certain nitrogen heterocycles can serve as potential bioisosteres for the phenolic moiety. Heterocycles, such as pyrrole, indole or benzimidazoles, that have a proton attached to a nitrogen atom and whose lone pair of electrons is involved in maintaining aromaticity, have proved particularly effective. Benz[*e*]-indole (**67**, Figure 63), the pyrrolo analogue of the dopaminergic agonist (**69**),[123−125] displays potent dopaminergic properties, is orally active and has a

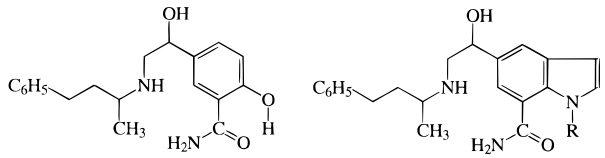


67 R = H
68 R = CH_3 **69**

Figure 63.

longer duration of action than **69**. The effectiveness of the pyrrolo ring of **67** as a bioisosteric replacement for the phenolic hydroxyl group has been ascribed to the ability of both groups to hydrogen bond to a common acceptor nucleus on the dopamine receptor.[126] Evidence for the involvement of the hydrogen bond formation being related to the success of this bioisosteric replacement was provided by the lack of activity observed with the *N*-methylpyrrolo analogue **68**.

Examination of the antihypertensive activity of the pyrrolo analogue **71** of labetalol (**70**, Figure 64), an α- and β-adrenergic receptor antagonist[127−128] provides further evidence for the successful use of the indole phenol bioisosteric replacements.[129]

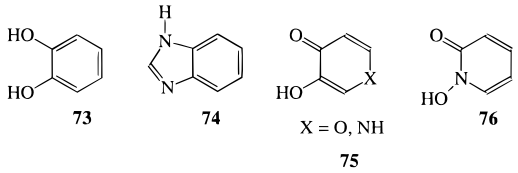


70 **71** R = H
72 R = CH_3

Figure 64.

The indolo analogue **71** of labetalol (**70**) was found to reduce blood pressure in spontaneously hypertensive rats, without causing a decrease in heart rate. The antihypertensive activity of these compounds is related to their ability to hydrogen bond to the adrenergic receptors. The importance of a hydrogen atom on the indole nitrogen atom is again evidenced by the fact that the N-methylated analogue **72** is inactive. While both **70** and **71** are antihypertensive agents, there are differences between these molecules with respect to their lipophilicities and pK_a values. While the phenol derivative is acidic, the indole analogue is almost neutral. These differences may partially explain the subtle differences in pharmacological activity between these compounds. Labetalol, in contrast to **71**, shows no significant β_1-ISA and therefore exhibits negative chronotropic activity.

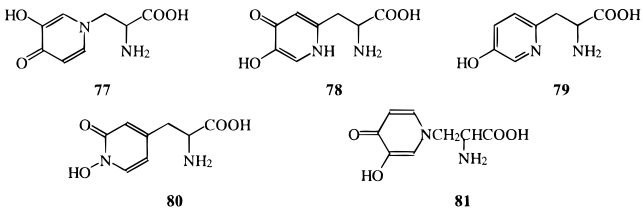
Bioisosteres have also been developed as replacements of both hydroxyl groups of catechols. Several examples are illustrated in Figure 65.[3]



73 **74** X = O, NH **75** **76**

Figure 65.

The success of L-dopa in the treatment of parkinsonism resulted in the search for analogues of dihydroxyphenylalanine which would exhibit fewer side effects. Some of these hydroxypyridine and pyridinequinoid-type compounds are listed in Figure 66.[130−133]



77 **78** **79** **80** **81**

Figure 66.

2. Carbonyl Group Bioisosteres

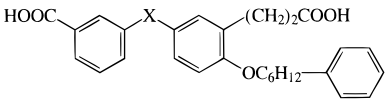
Carbonyl group bioisosteres for the purposes of this review refer to replacements which have been used primarily for the aldehyde or ketone moiety (Table 38). The difference in the electronegativity between oxygen and carbon associated with these functional groups results in a partial positive charge on the carbon atom while the oxygen acquires a partial negative charge. Comparison of the substituent

constants associated with isosteres for the carbonyl group illustrate that these nonclassical bioisosteric replacements are generally electron-withdrawing moieties that are relatively large in size.

Table 38. Aromatic Substituent Constants for the Carbonyl Group Bioisosteres[134]

bioisosteres	σ_p	π	MR
$SOCH_3$	0.49	−1.58	13.70
SO_2CH_3	0.72	−1.63	13.49
CH=NOH	0.10	−0.38	10.28
$CH=NOCH_3$	0.30	0.40	14.93

In the section on monovalent isosteres (hydroxyl−thiol interchanges), the pharmacological basis leading to the development of different classes of antagonists of Leukotriene B_4 (LTB_4) was discussed. LTB_4 has been implicated as a potential mediator of inflammation. Elevated amounts of LTB_4 have been found in human psoriatic plaque[135−136] and in the colonic mucosa of patients with inflammatory bowel disease.[137−138] Levels of LTB_4 in these tissues appear to correlate with the extent of disease. The design of potent and selective antagonists of LTB_4 has been proposed for the development of new therapeutic approaches for the treatment of these diseases or any condition where LTB_4 may play a role as a pathological mediator. A series of benzophenone dicarboxylic acids (Figure 67) were synthesized as potential inhibitors of LTB_4 to its receptors in intact human neutrophils.[139] Replacement of the carbonyl with a variety of polar and nonpolar bioisosteres led to marginal changes in binding affinity (Table 39).



82

Figure 67.

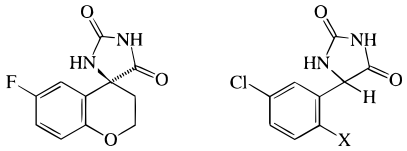
Table 39. Receptor Affinity of LTB_4 Receptor Antagonists with Modified Linking Groups

compound	X	percent inhibition of specific [^{3}H]LTB_4 binding (μM)
82a	C=O	85
82b	C=NOH	84
82c	(C=O)NH	73
82d	S	67
82e	SO	71
82f	SO_2	79

It was observed that the oxime (**82b**, Table 39) showed activity most similar to that of the corresponding benzophenone (**82a**, Table 39). The activity of the amide-linked (**82c**, Table 39) and the sulfur-linked derivatives (**82d**−**f**, Table 39) was also similar to that of the benzophenone. While the sulfoxides and sulfones are recognized as nonclassical bioisosteres suitable for replacement of a carbonyl group, thioethers such as **82d** are not. However, the lack of any significant difference in activity upon replacement of the carbonyl with either a thioether, sulfoxide or sulfone, which differ widely with respect to their polarities and hybridization, suggests that this portion of the molecule is not critically involved in LTB_4 receptor binding. This example illustrates how bioisosteric analogues can be used to identify those sites on the drug which have a major impact on the interaction that occurs with the pharmacophore.

The sulfoxide and the sulfone moieties have increasingly been used as nonclassical divalent bioisosteres of the carbonyl group.[140−145] In addition, metabolic oxidation of a pharmacologically active agent which has a thioether incorporated within their structure results in the formation of corresponding sulfoxide and sulfone analogues. In many instances, these are more potent than the corresponding sulfides. The greater size associated with the sulfone moiety has also been shown to be a factor that modulates biological activity. This was illustrated in the development of novel aldose reductase inhibitors related to sorbinil (**83**, Figure 68).

Sorbinil is a spirocyclic hydantoin that exhibits activity as a potent inhibitor of the enzyme aldose reductase.[146] On the basis of this property, a number of spirohydantoin aldose reductase inhibitors have been studied.[147] In an attempt to simulate the rigid nature of a spirocyclic hydantoin with a nonspirocyclic compound several analogues (**84**, Figure 68) with different substitutions at the 2-position were synthesized and evaluated for their ability to inhibit aldose reductase.[144] Comparison of their IC_{50} values in Table 40 reveals that the sulfone analogue is the most potent analogue, with significantly greater activity than the thioether and sulfoxide analogues. The sulfone moiety in this instance hinders the rotation of the hydantoin ring, forcing it to be aligned in a manner similar to that of the spirocyclic hydantoin sorbinil.



83 **84**

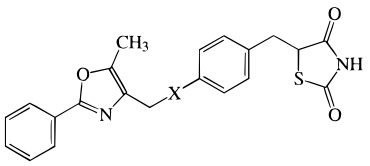
Figure 68.

Table 40. Biological Activity of Nonspirocyclic Hydantoin Aldose Reductase Inhibitors

compound	X	IC_{50}[a] (μM)
83	Sorbinil	0.12
84a	SO_2CH_3	0.05
84b	$SOCH_3$	0.25
84c	SCH_3	0.62

[a] Concentration that causes a 50% inhibition of human placental aldose reductase with glyceraldehyde as substrate.

Another example of the use of carbonyl bioisosteres is illustrated for a novel series of thiazolidine-2,4-diones that were evaluated as potent euglycemic agents. Non-insulin-dependent diabetes mellitus (NIDDM) is a metabolic disorder characterized by hyperglycemia as well as insulin resistance and/or impaired insulin secretion.[148,149] Most commmonly used agents in the therapy of NIDDM are the sulfonylureas.[150,151] CP-86,325 (**85a**, Figure 69) is representative of a relatively new class of glucose-lowering agents.[152] Several bioisosteres in which the ketone moiety of CP-86,325 has been replaced, have been synthesized and evaluated for their ability as glucose-lowering agents (Table 41). While these



85

Figure 69.

Table 41. Euglycemic Activity of Various Ketone Isosteres

compound	X	dose (mg/kg)	% glucose normalization in ob/ob mouse[a]
85a	$CH_2C{=}O$ (CP-86,325)	1	100
85b	CH_2SO_2	5	69
85c	CONH	5	64
85d	$CH_2C(NOH)$	5	100
85e	$CH_2C(NOMe)$	5	100

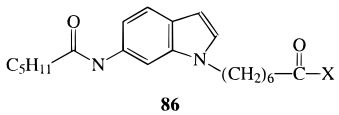
[a] Ciglitazone was dosed at 50 mg/kg as a positive control and results are reported as the percentage of glucose normalization compared to the standard ciglitazone-treated group (100% at 50 mg/kg) and the vehicle treated group (0%).

bioisosteres did retain activity as euglycemic agents, none of these analogues exhibited potency similar to **85a**[153] (Table 41).

3. Carboxylate Group Bioisosteres

Nonclassical bioisoteres for the carboxylate group consist of replacements which involve (a) only the hydroxyl portion or (b) both the hydroxy and carbonyl fragments of this functional group.

a. Replacements Involving Only the Hydroxyl Portion. The types of nonclassical bioisosteres used for the replacement of the acidic hydroxyl group of a particular carboxylic acid are similar to the nonclassical bioisosteres which have been previously outlined for phenolic hydroxyl groups. This subclass of bioisosteric replacements used in place of the carboxylate group will, therefore, not be reviewed in detail. The determination of suitable replacements for the carboxylate group is often based on the ability of the bioisostere to possess similar acidity and to exhibit similar physicochemical properties. Replacement of the hydroxyl moiety of a carboxylic acid with a phenyl sulfonamide, for example, results in the formation of a sulfonimide. The estimated pK_a values for sulfonimides are similar to that of an aryl carboxylic acid. A recent example of the use of this bioisoteric replacement is in the development of indole derived leukotriene antagonists (Figure 70).[154] As indicated in Table 42, there is little difference between the sulfonimide derivative **86b** and its parent compound



86

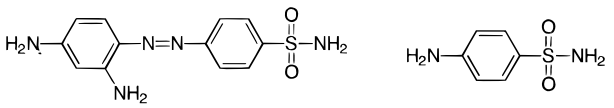
Figure 70.

Table 42. Biological Activity of 7-[6-Hexanamidoindol-1-yl]heptane Derivatives

compound	X	% antagonism of 8 nM LTE_4-induced contraction
86a	OH	53
86b	$NHSO_2Ph$	58

86a with respect to their ability to antagonize LTE_4-induced contraction.

b. Replacements Involving the Entire Carboxylate Functional Group. In 1932, it was discovered that Prontosil (**87**, Figure 71) was able to provide cures of streptococcal infections in mice.[155] The active form of Prontosil was later proven to be *p*-aminosulfanilamide (**88**).[156]



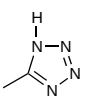
87 88

Figure 71.

The mechanistic basis for the antimicrobial activity of sulfonamides is their ability to act as competitive inhibitors of the incorporation of *p*-aminobenzoic acid associated with the formation of dihydropteroic acid, thereby, ultimately inhibiting the biosynthesis of dihydrofolic acid (Figure 72).[157] Thus, one of the earliest synthetic antibiotics developed consisted of an antimetabolite in which a nonclassical bioisostere replaced a carboxylic acid moiety.

While sulfonamides represent one of the first nonclassical bioisosteres used to replace the carboxyl group, there has been increased interest in the use of tetrazole as a nonclassical bioisosteric replacement for carboxylate groups. Table 43 outlines a comparison of the physicochemical properties of the carboxyl group and its anionic derivative with those of the tetrazole in both its neutral and anionic state. Replacement of the carboxylic acid moiety with a tetrazole is well known and has increasingly been used with success within different classes of medicinal agents. Comparison of these groups at physiological pH reveals that the tetrazole group is almost 10 times more lipophilic while having similar acidity, pK_a = 4.9, to that observed for carboxylic acids (pK_a 4.2−4.4).

Table 43. Aromatic Substituent Constants for Carboxylic Acid Bioisosteres[117]

bioisosteres	σ_p	π	MR
	0.45	−0.32	6.9
	0.00	−4.36	6.0
	0.56	−0.48	15.6
	0.35	−3.55	14.6

A recent study of second generation benzodiazepine CCK-B (cholecystokinin) antagonists (**89**, Figure 73) can serve as an illustration of the bioisosteric replacement of the carboxylate group with a tetrazole moiety resulting in enhanced potency due to the reduced hydrophilicity.[158] The effect of increasing lipophilicity, upon replacing a carboxylic acid with tetrazole, on CCK-B receptor binding can be seen in Table 44. Further replacement with an oxadiazolone ring in place of the carboxylic acid resulted in even

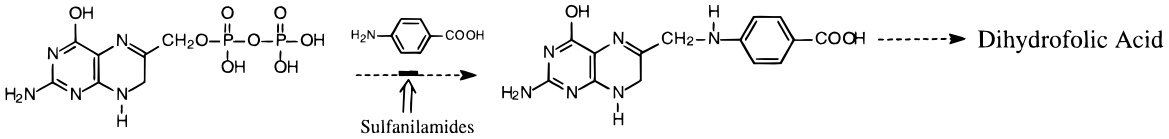
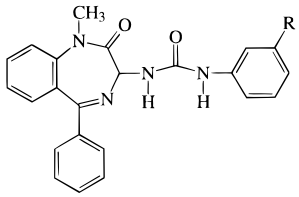


Figure 72.

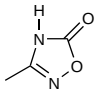
greater potency at the CCK-B receptor (Table 44). The greater activity of tetrazole and the oxadiazole is attributed to the optimal placement of the polar substituent facilitating a favorable interaction within the CCK-B receptor.



89

Figure 73.

Table 44. Receptor Binding Affinities and Solubility Properties 3-(Phenylureido)-1,4-benzodiazepines

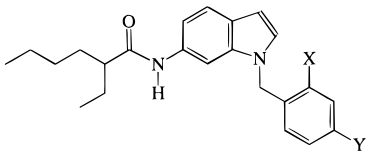
R	IC_{50}[a] (nM) CCK-B	solubility, mg/mL
	6.69	4.7
	1.02	3.74
	0.266	0.41

[a] Concentration of compound required for half-maximal inhibition of the binding of [^{125}I]BH CCK-8s to receptors in guinea pig cortical membranes.

Other commonly used isosteres for the carboxylic acid moiety, besides sulfonamides and tetrazoles, include sulfonate and phosphate. Among these bioisosteric replacements, both the sulfonates and phosphates are more hydrophilic than the carboxylate anion and are 100% ionized at physiological pH.

To illustrate the applicability of these replacements, the literature outlines a systematic structure–activity study of the carboxylic acid region in a series of indole- or indazole-derived leukotriene antagonists (**90**, Figure 74).[154] The peptidoleukotrienes are known to be the active components of the "slow reacting substance of anaphylaxis" (SRS-A).[159] SRS-A is believed to be an important biological mediator in several disorders, especially human allergic diseases. Thus, the design and development of selective antagonists of leukotrienes presents a potential target for the treatment of asthma.

In a series of leukotriene antagonists (Table 45), it was observed that the sulfonic acid analogue, (**90b**) was equipotent with the carboxylic acid (**90a**). Bioisosteric replacement with the sulfonimide moiety, which has a pK_a similar to that of the carboxylic acid,[160] resulted in a 100-fold increase in activity. Transposition of the carbonyl and sulfonyl groups resulted in a loss of activity which was similar to that of a carboxylic acid. The spatial relationship between the pendant phenyl group and the sulfone was noted as being responsible for the increased potency of analogue **90c** as compared to analogue **90d** (Table 45).



90

Figure 74.

Table 45. Biological Activity of Indole Acid Leukotriene Antagonists

compound	X	Y	test concentration (10^{-7} M)	% antagonism of LTE_4
90a	H	COOH	100	44
90b	H	SO_3H	100	48
90c	H	$CONHSO_2Ph$	1	42
90d	H	$SO_2NHCOPh$	100	37

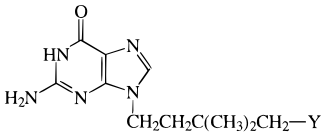
Table 46. LTE_4 Antagonism of Indole Acid Leukotriene Antagonists

compound	X	Y	test concentration (10^{-7} M)	% antagonism of LTE_4
90e	OCH_3	COOH	10	59
90f	OCH_3	1*H*-tetrazol-5-yl	10	50
90g	OCH_3	$CONHSO_2CH_3$	100	100
90h	OCH_3	$CONHSO_2Ph$	1	100

Among the methoxy analogues (**90e**–**h**, Table 46), it was also observed that the phenyl sulfonimide (**90h**) was 100 times more potent than the methylsulfonamide (**90g**). In this study, the tetrazole analogue was also shown to be equipotent to the carboxylic acid. The methylsulfonimide, however, was only one-tenth as active as the carboxylic acid derivative (**90e**, Table 46). The pK_a value of the methylsulfonamide is similar to that of the phenylsulfonamide and the carboxylic acid.[160] The increased potency of the phenylsulfonamide compared to the methylsulfonamide in this instance was attributed to a region of the receptor which specifically binds the phenyl of the sulfonamide (**90h**).

The phosphate, as previously noted, can be viewed as being interchangeable with the carboxylate moiety and other related bioisosteres. In a recent study, a number of functional end groups attached to the 9-position of guanine by an alkyl side chain (Figure 75), were evaluated as phosphate mimics on the basis of their ability to inhibit purine nucleoside phosphorylase (PNP).[161] This provided a sensitive method for the evaluation of the ability of these groups to occupy the phosphate binding site of PNP.

The data obtained (Table 47) reveal that the sulfonic acid interacts quite well with the phosphate binding site, while the carboxyl group does not



91

Figure 75.

Table 47. Inhibition of Purine Nucleoside Phosphorylase by Guanine Analogues

compound	Y	IC_{50} (μM)[a]
91a	PO_3H_2	0.04
91b	SO_3H	0.18
91c	SO_2NH_2	100.0
91d	COOH	8.0
91e	$CONH_2$	200.0

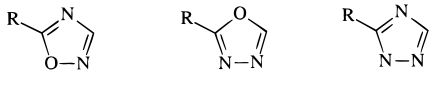
[a] Concentration required to cause 50% inhibition of calf spleen PNP.

interact as well. The sulfonamide (**91c**) and the carboxamide (**91e**), however, have little affinity for this binding site. X-ray crystallography studies show that the binding of the sulfonic acid analogue is almost identical to that of the phosphate analogues, as illustrated by the IC_{50} values. The loss of activity by substitution of the phosphate with the carboxy group was specifically related to the hydrogen bonding of the carboxyl group with the hydroxyl of serine 220 and the amide nitrogen atom of methionine 219 of PNP. These interactions pull the guanine moiety out of the position it normally occupies in the purine binding site of PNP.

4. Amide Group Bioisosteres

Bioisosteric replacements for the amide represents an area that is currently the center of focus because of its implications in peptide chemistry and the development of peptide mimetics. Peptide bonds and peptide fragments have been replaced with a wide variety of structural moieties in attempts to convert peptides into chemically stable and orally available molecules. In view of the fact that replacements for the peptide bonds have been reviewed extensively,[162−163] only some of the bioisosteric replacements of the amide will be discussed.

Esters have been widely used as replacements for amides. In the section on cyclic vs noncyclic bioisosteres, the use of heterocyclic rings as replacements for the ester moiety has been illustrated. Heterocyclic rings such as 1,2,4-oxadiazoles (**92**),[164−168] 1,3,4-oxadiazoles (**93**),[169−171] and triazoles, such as the 1,2,4-triazoles (**94**)[172] (Figure 76), have also been used as replacements for amide or ester bonds.

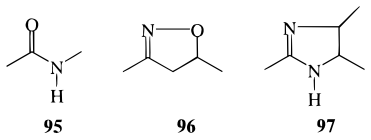


92 93 94

Figure 76.

Some of the other heterocyclic rings used in peptidomimetic chemistry include the 2-isoxazoline[173] (**96**, Figure 77) and imidazoline moieties[174] (**97**, Figure 77).

Another approach that has facilitated the design and development of HIV protease inhibitors has been



95 96 97

Figure 77.

the application of the "transition-state peptidomimetic" principle. This principle involves the replacement of the hydrolyzable peptide linkage with nonhydrolyzable transition-state isosteres.[175] Figure 79 outlines the hydrolysis of a peptide bond with its transition-state intermediate. A series of transition-state mimetics of the peptide bond is illustrated in Figure 78.

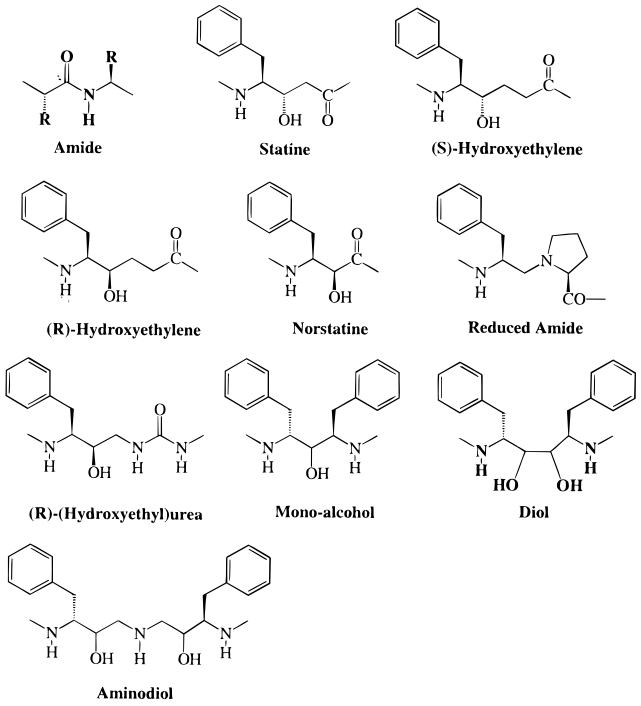


Figure 78.

Listed in Table 48 are possible bioisosteric replacements for the amide bond. The ability of these groups to be suitable bioisosteres depends on the role the amide group plays in eliciting the biological activity and the ability of the bioisostere to mimic this role as closely as possible. Two examples will be discussed, one in which the hydrogen donor and acceptor functionalities of an amide are required for maintaining biological activity and another in which the amide group functions only as a spacer between two functional groups.

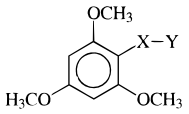
Table 48. Bioisosteres of the Amide Bond

bioisostere	formula
amide	−NHCO−
reversed amide	−CONH−
thioamide	−NHCS−
amide homologue	$-CH_2NHCO-$
ketomethylene	$-COCH_2-$
urea	−NHCONH−
methyleneamino	$-CH_2NH-$
carbamate	$-NHCO_2-$
thiocarbamate	−NHCOS−
ester	$-CO_2-$
sulfonamide	$-NHSO_2-$
hydroxyethylene	$-CH(OH)CH_2-$

Figure 79.

The enzyme acyl-coenzyme A:cholesterol acyltransferase (ACAT) catalyzes the formation of cholesteryl esters from cholesterol and fatty acyl-CoA. Accumulation of cholesterol and its esters in coronary arteries is a prominent feature observed in atherosclerotic patients. Further evidence suggests that ACAT is necessary for hepatic very low density lipoprotein secretion[176] and intestinal absorption of cholesterol.[177] Thus, inhibition of ACAT may be of therapeutic value in the treatment of atherosclerosis by reducing the amount of cholesterol absorbed in the intestine, reducing VLDL secretion, and retarding the accumulation of cholesteryl esters in the artery walls.[177] In an attempt to define the structural features for potent ACAT inhibition, a series of oleic and fatty acid analogues were synthesized with various bioisosteric replacements of the amide group. These were evaluated for their ability to inhibit ACAT *in vitro* (Table 49).[178]

Among compounds **98e**−**h** listed in Table 49 (Figure 80), it was observed that only the urea bioisostere **98e** retained ACAT inhibitory activity *in vitro*. Apparently, bioisosteric substitutions having both hydrogen bond donor and acceptor functionalities were required to maintain potent inhibitory activity as illustrated by the thioamide (**98d**), carbamate (**98f**), urea (**98e**), and thiocarbamates (**98g**) in Table 49. The location of the hydrogen bond donor and acceptor was important for activity since the reversed amide (**98b**) was less potent than the amide.



98

Figure 80.

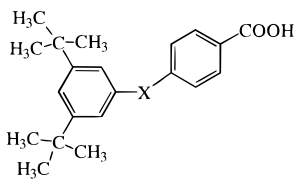
Table 49. *In Vitro* Activity of Bioisosteric Analogues of 2,4,6-Trimethoxy Anilides of Oleic and Stearic Acid

compound	X	Y	IC_{50}[a] (μM)
98a	NHCO	$C_{17}H_{33}$	0.044
98b	CONH	$C_{18}H_{35}$	9.4
98c	CH_2NH	$C_{18}H_{35}$	>100
98d	NHCS	$C_{17}H_{33}$	0.9
98e	NHCONH	*n*-$C_{18}H_{37}$	0.9
98f	$NHCO_2$	*n*-$C_{18}H_{37}$	7.1
98g	NHCOS	*n*-$C_{18}H_{37}$	5.7
98h	$CH_2NHCONH$	*n*-$C_{18}H_{37}$	>5

[a] ACAT inhibition *in vitro* in intestinal microsomes isolated from cholesterol-fed rabbits.

In contrast to the previous example, the main function of the amide group in retinobenzoic acids (**99**, Figure 81) appears to be in the positioning or spacing of the *m*-dialkylphenyl and the *p*-carboxyphenyl group.[179]

It was observed that amide (−NHCO−) group replacements[179,180] such as −CONH−,[180,181] $-SO_2NH-$, −COCH=CH−,[182,183] −N=N−[184,185] retained activity, regardless of their electronic properties or ability to act as hydrogen bond donors.

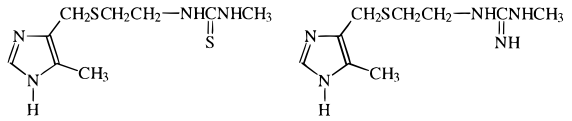


99

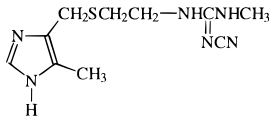
Figure 81.

5. Thiourea Bioisosteres

Thiourea bioisosteres have been successfully employed in the development of H_2-receptor antagonists. Histamine H_2-receptor antagonists have been widely used in the treatment of peptic ulcer disease. Side effects such as agranulocytosis observed in early clinical studies with the H_2-antagonist metiamide (**100**, Figure 82) was attributed to the thiourea moiety. Replacement with a guanidino group (**101**, Figure 82) resulted in absorption problems because of its high degree of ionization at physiological pH. Further, bioisosteric substitution with the cyanoguanidino derivative provided cimetidine (**102**, Figure 82), which was twice as active as metiamide as an inhibitor of gastric acid secretion. The presence of a polar nonbasic functional group attached to the secondary position of an (ethylthio)methyl substituent on an aromatic heterocycle exists for all four of the major H_2-antagonists which are in clinical use. These include cimetidine, famotidine, ranitidine, and nizatidine.



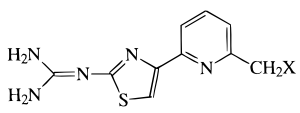
100 **101**



102

Figure 82.

In the continuing search for novel H_2-antagonists, a series of 2-guanidino-4-pyridylthiazole derivatives (**103**, Figure 83) were synthesized and evaluated for gastric antisecretory activity.[186] Replacement of the cyano group in the cyanoguanidine moiety with electron-withdrawing groups such as a sulfonylalkyl group or nitromethine resulted in retention of activity. However these analogues were less potent than cimetidine (Table 50).



103

Figure 83.

Table 50. Pharmacological Activities of 2-Guanidino-4-pyridylthiazoles

X	% inhibition of gastric secretion[a]
cimetidine	53
$NHC(=NCN)NHCH_3$	15
$NHC(=NSO_2CH_3)NHCH_3$	24
$NHC(=CHNO_2)NHCH_3$	18

[a] Inhibition of histamine-stimulated gastric acid secretion in the lumen-perfused stomachs of the anesthetized rats.

6. Halogen Bioisosteres

The last class of isosteres which will be discussed are the isosteres for the halogens. Halogens have been replaced by electron-withdrawing groups such as a cyano or trifluoromethyl group. Replacements of this type were observed in a series of 1-[(2-hydroxyethoxy)methyl]-5-benzyluracils (**104**, Figure 84) that were tested for inhibition of liver uridine phosphorylase (UrdPase).[187] Uridine phosphorylase is an enzyme that catalyzes the reversible phosphorolysis of pyrimidine nucleosides. Uridine phosphorylase is responsible for the degradation of chemotherapeutic agents such as 5-fluoro-2′-deoxyuridylic acid.[188] Thus, the development of agents belonging to this class has been of chemotherapeutic interest.[189] Within the series of 5-benzyluracils, it was suggested that electron-withdrawing groups at the 3-position decreased potency. This hypothesis was supported by the observation that replacement of the chloro atom with stronger electron-withdrawing groups such as the cyano or the trifluromethyl resulted in less potent analogues (Table 51).

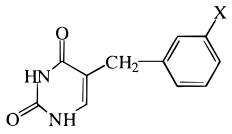


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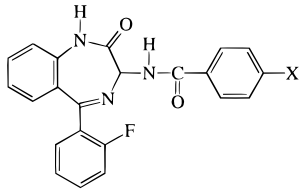
Figure 84.

Table 51. Uridine Phosphorylase Inhibition of 5-Benzyluracils

compound	X	IC_{50}[a] (μM)
104a	Cl	2.5
104b	CN	13.2
104c	CF_3	21.4

[a] Concentration of inhibitor that results in 50% decrease in reaction velocity.

Another illustration of such nonclassical replacements for halogen was illustrated in a structure−activity relationship study of cholescystokinin-A receptor antagonists (**105**, Figure 85).[190] In the section on carboxylate bioisosteres the pharmacological basis behind the design of CCK-A receptor antagonists has been discussed. Here again CCK receptor antagonists have been proposed as potentially useful in the treatment of appetite disorders, abnormalities of gastric motility, biliary tract disease, pain management, and psychiatric disorders.[191] As illustrated in Table 52 replacement with nonclassical bioisosteres of the halogens in the benzodiazepines resulted in retention of antagonistic activity at the CCK-A receptor.



105

Figure 85.

Table 52. Pancreas Receptor Binding Affinities for 3-(Benzoylamino)benzodiazepines

compound	X	$-\log(IC_{50})$[a]
105a	Cl	8.54
105b	CN	7.37
105c	CF_3	7.70

[a] Micromolar concentration of maximal inhibition of binding of [^{125}I]CCK-33 or [^{125}I]CCK-8 to rat pancreas.

IV. Conclusion

Bioisosteres modulate biological activity by virtue of subtle differences in their physicochemical properties. Systematic correlation of physicochemical parameters with observed biological activity has been very effective in highlighting subtle differences within bioisosteric groups which often increase activity. Of significance is the ability of these bioisosteric groups to define some of the essential requirements of the pharmacophore. This is especially important when the synthesis of a large number of drug candidates for evaluation is not economically feasible. A number of less known replacements have not been reviewed because of their inability to demonstrate bioisosterism in more than a single class of agents. In this review, an attempt has been made to explain the rationale behind the use of bioisosteric replacements using examples from current literature. It is hoped that this systematic approach will facilitate the use of bioisosteric replacements in future structure−activity studies.

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