RELATIONS BETWEEN STRUCTURE AND BIOLOGICAL ACTIVITY OF SULFONAMIDES

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Thomas H. Maren
Department of Pharmacology and Therapeutics, University of Florida College of Medicine, Gainesville, Florida 32610

Five major and biologically diverse types of drugs have resulted from the discovery of sulfanilamide (*p*-aminobenzene sulfonamide). Each type has been the fulcrum for enormous intellectual and medical progress. It is not the prime purpose of this review to deal with these notable advances; such reviews are available in the separate fields, and will be cited. I wish rather to deal with the five related chemical types, primarily to show the specific structural features that yield activity in each of the cases. Nowhere in pharmacology can the theoretical organic chemist see more clearly the profound results of molecular change; nowhere can the biologist have more useful probes for physiological or biochemical mechanisms; nowhere can the physician have a group of drugs whose actions are more realistic and reliable.

Figures 1–5 show the basic structures. On the left for each category is shown the simplest stem structure; many molecules from such a stem are but weakly active. One of the main goals of this review is to show the progression from such stem compounds to those with much greater potency and medical utility, examples of which are shown at the right of the figures. The following classes of drugs are dealt with in this chapter: antibacterial sulfonamides and sulfones, carbonic anhydrase inhibitors, antidiabetic (insulin-releasing) sulfonamides, saluretics of the "thiazide" and high ceiling sulfonamide type, and certain antithyroid drugs.

ANTIBACTERIAL SULFONAMIDES AND SULFONES

An astonishingly complete and thoughtful monograph (1) covers the pioneering and most productive years (1935–1946) of sulfonamide chemistry, in which some 5000 compounds were made and tested. Newer drugs made in the next 20 years (2) showed variation in excretion, distribution, and metabolism, but contributed little to relations between antibacterial action and chemical structure. The principal

advances since 1946 are the knowledge that sulfonamides and sulfones interfere with the assembly of folic acid at the step that adds *p*-aminobenzoic acid (PABA) (3), and the development of cell-free enzyme systems to study the synthetic steps (4).

The stage was then set for a true evaluation of structure-activity relations. The Bell-Roblin theory of 1942 had been based on the activity of 50 sulfonamides against growth of *Escherichia coli* (5). This work emphasized the N'-substituted sulfanilamides, exemplified by sulfadiazine (Figure 1). This paper had tremendous intellectual impact and is still widely used as an example of how molecular forces are significant for chemotherapy. The theory was based on the known competition between PABA and the sulfonamides and the fact that, at pH 7, PABA yields the negative COO^- ion. Then, "the more negative the SO_2 group of an N' substituted sulfanilamide derivative, the greater the bacteriostatic activity of the compound." Experimental data yielded maximum activity for drugs with pK_a of 6–7, and their theoretical treatment suggested that such drugs had maximum negativity of the SO_2 group.

Two major problems arose with the Bell-Roblin formulation. First, the data are obtained in whole cells, so drug activity is a composite of true antibacterial effect

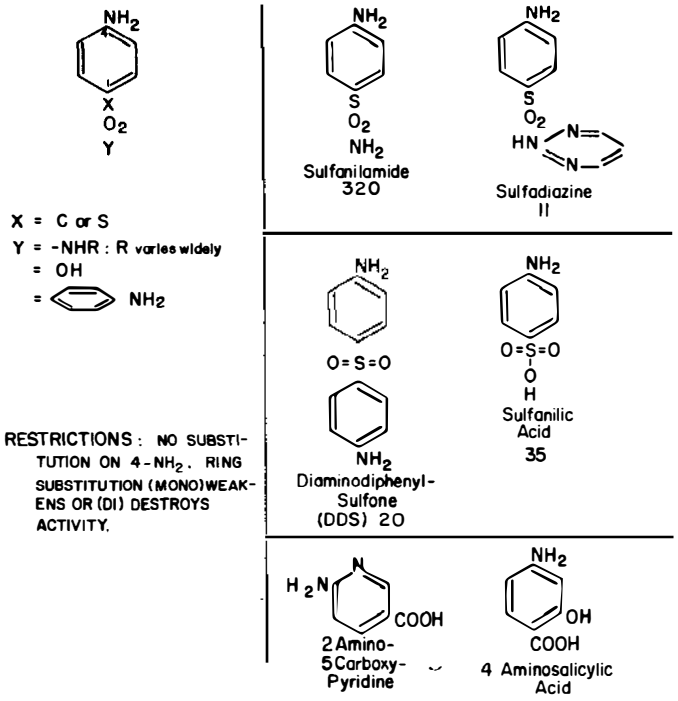


Figure 1 Antibacterial sulfonamides and sulfones. Concentration (μM) for 50% inhibition of synthesis of dihydropteroic acid in presence of 10 μM PABA (6).

and penetration. Highly ionized drugs are weakly active (5); but when sulfanilic acid (Figure 1) was tested in a cell-free system for folate synthesis, it proved almost equal to sulfadiazine (4). Second, there were numerous exceptions to the parabolic relation of activity vs pK_a; sulfaguanidine and diaminodiphenylsulfone (DDS) (Figure 1) have no acidic pK_a yet are highly active in either intact cells (5) or the cell-free system (6). Other exceptions included active compounds disubstituted on the $-SO_2N<$group, which have no acidic pK_a (7).

Early work in the field had shown that isosteres of benzene could also produce active compounds; the analog of PABA, 2-NH_2-5-COOH pyridine (Figure 1), was bacteriostatic for *E. coli* and reversed by PABA. Some activity was also found among thiophenes and pyrimidines with NH_2 and COOH arranged in homologous fashion to PABA (8).

Substituents in the ring of PABA yield active compounds, although on a weight basis they are relatively weak (8, 9). The most important member of this class is *p*-aminosalicylic acid (Figure 1), which appears somewhat specific for the tubercle bacillus, although active as a PABA antagonist for other organisms as well (10).

It is thus clear that the five different but related structures of Figure 1 are all active and may be regarded as subsets of an ideal molecular shape that interferes with the assimilation of PABA into folic acid. Before attempting to define this molecular shape, we may briefly set down the specific reactions involved.

The first organic step in the synthesis of folic acid is the condensation of 2-NH_2-4-OH-6 hydroxymethylpteridine (Pt) pyrophosphate with PABA to form dihydropteroic acid. The reaction occurs between the $-CH_2O-PO_3H-$ group of Pt pyrophosphate and the $-NH_2$ group of PABA to form a $-CH_2-NH-$ link. It is here that the sulfonamides are thought primarily to act by competing in some fashion with the condensation of PABA. A secondary site is the conjugation between Pt pyrophosphate and *p*-aminobenzoylglutamate. The same chemical link is involved, yielding $-CH_2-NH-$. Evidence for both of these is reviewed and diagrammed in (11), largely from the work of (4). From consideration of these pathways, it is not surprising that all inhibitors of these reactions (or of bacterial growth, reversed by PABA) contain an aromatic NH_2 group or radicals that are readily converted to such a group. It seems likely, but is not finally proven, that sulfonamides (and sulfones) act as false substrates for PABA in these reactions, forming a dihydropteroic sulfonamide or a false folate–incorporating sulfonamide (4). Such compounds remain to be identified.

The basic molecular shape of the "sulfonamide" or "sulfone" inhibitors is shown on the left of Figure 1. As suggested in the brief review of active compounds just given, a wider range of substituents on C^1 is permitted than is suggested by inspection of available sulfonamide drugs, in which the substituent is always $-SO_2NHR$. This is evident from the structures on the right of Figure 1. In the cell-free system, $-SO_3H$ is highly active (4), and even –COOH is inhibitory when modified by an adjacent OH group (10). Furthermore, ionization of the group on C^1 is not demanded, despite an enormous amount of work on this issue. DDS is virtually as active as sulfadiazine (6), and certain N'N' dialkyl compounds are as active as their monosubstituted analogs (12).

A recent paper attempts again to link activity with increased ionization of $-SO_2NHR$ (13) but neglects DDS and sulfaguanidine and the N'N' dialkyl compounds. It is likely that in a closely knit homologous series, or in studies of a single drug at different pH, ionization will increase activity, although not quantitatively.

There does not seem to be any measurable (as yet) property of the SO_2 group that confers activity, as had been postulated (5, 12). It is intuitively clear that reactivity of the N^4 amino group is essential for competition at the PABA steps, but that the properties of the group at C^1 directly bear on this. These properties remain elusive; however, I conclude this section by considering some papers that attempt to relate structure to activity, as a means of tentative identification of the requirements at C^1.

Structure-action relations cannot be obtained rigorously in bacterial growth studies because of the problem of drugs penetrating bacterial cells. Such studies might well yield highly effective drugs, as a composite of activity and access to cells, but will probably not give the key to synthetic or inhibitory mechanisms. In terms of sulfonamides inhibiting the condensation of Pt pyrophosphate + PABA, four papers using cell-free dihydropteroate synthetase from *E. coli* are relevant (4, 6, 12, 13). These data show, as indicated above, that ionization on substituents of C^1 is not a factor, and that drugs can be weak acids, weak bases, or carry no ionic charge. Indeed, the weak base, DDS, is virtually as potent as the weak acid sulfadiazine (6). A weakness of an interesting study purporting to show relation between pK_a and cell-free activity is the narrow range of pK_a and chemical type studied (13). The same criticism applies to correlations with NMR-measured shifts of primary (N^4) amine protons, with the additional caveat that these shifts are of the precursor amines used in the synthesis, not the sulfonamide itself (13).

Inspection of the 50-odd structures studied in these four papers conveys the general impression that any substituent that is electron-rich and attached to $-SO_2-$ at C^1 is associated with high activity; electron-poor substituents are weak. Thus the aromatic ring of DDS, the O atom of sulfanilic acid, and the heterocyclic rings of the drugs in common use (cf sulfadiazine) are potent, while sulfanilamide and its N^1 alkyl derivatives are weak. The potent compounds inhibit PABA incorporation into folate at concentrations about half that of PABA (inhibition index ranging from 0.3 to 0.8), while weak compounds have indices of 20–30 (4, 12). Figure 1 shows inhibitory concentrations for the several drugs, in the presence of a fixed concentration of PABA (6).

The effect of electron pressure on the N^4 amine is crucial, since this is the reactive part of the molecule. Nevertheless, no definitive property of N^4 has been shown to correlate with activity across the wide range of drug type shown on the right of Figure 1. Attempts to make such a correlation with either basic pK_a of the amine or chemical shift are unconvincing, since a very narrow range of pK_a or shift is covered, the precursor amine rather than the sulfonamide was studied, data are from a small homologous series of N^1 benzenesulfonamides, and activity was measured by cell growth (7).

The intimate nature of N^4 reactivity must also await more detailed knowledge of how sulfonamides and sulfones are interpolated in the folate pathway. Although

there are several points at which the drugs may replace PABA, it is not yet clear what products are formed; neither the postulated Schiff base (7) from Pt aldehyde nor the presumed "false folate"–incorporating sulfonamide (4) has been isolated. This matter is discussed in reference 11.

CARBONIC ANHYDRASE INHIBITORS

The criteria for activity in this class are the simplest in pharmacology; all unsubstituted aromatic sulfonamides (aryl SO_2NH_2) inhibit carbonic anhydrase, and no other class of organic compounds approaches these in activity. Figure 2 shows representative compounds. Several hundred chemicals of this class have been tabulated according to activity (relative to sulfanilamide) and structural type. It was not possible to make any general rules, but interesting insights within certain series of compounds were made (14). The dissociation constants (K_I) of some 60 drugs against red cell carbonic anhydrase of various species have been tabulated. K_I was a function of inhibition of the catalytic hydration of CO_2. In addition, twelve representative drugs are discussed in terms of K_I, physical and chemical properties, and their effects in vivo. The history of their development is also given, stemming

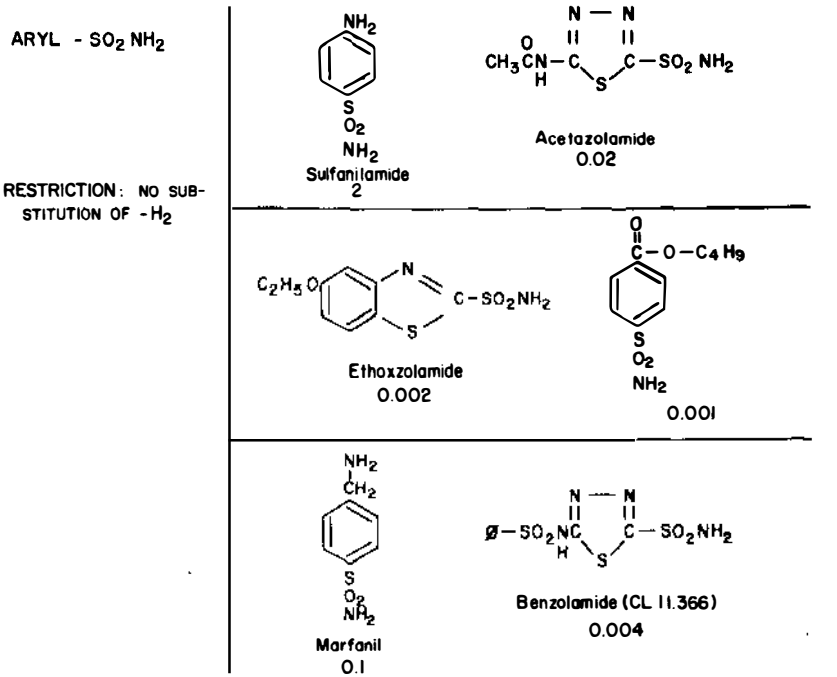
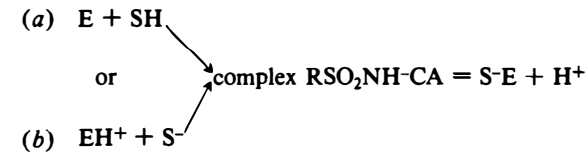


Figure 2 Carbonic anhydrase inhibitors. Concentration (μM) for K_I against human red cell carbonic anhydrase C (15).

from unexplained clinical and chemical findings with sulfanilamide when it was first used in 1935–1937 (15). As is evident from the structural requirements for carbonic hydrase inhibition, such findings never occurred when sulfanilamide gave way to the N^1-substituted compounds now used in antibacterial chemotherapy (Figure 1).

The nature of the reaction between sulfonamides and the active site of carbonic anhydrase has been reviewed (16, 17). An important element is the measurement of the overall association (k_a) and dissociation (k_d) rate constants (18) which are the bases for the net activity, by the relation $k_d/k_a = K_I$. As known for a long time, K_I varies greatly, some 10^5-fold, among all drugs studied (15). For the 24 compounds in which rate constants were measured, k_a varied 240-fold and k_d 80-fold. It then becomes relevant to discuss structure-activity relations in terms, largely, of k_a. Furthermore, the effect of the charge of the sulfonamide group (as judged by measuring k_a, k_d, or K_I at differing pH) is upon k_a (19).

The inquiry into structure-activity relations has naturally led into the question of the form in which the sulfonamides react, whether undissociated weak acid (SH) or anion (S^-). This has been studied by relating pH to either their rates of inhibition of CO_2 hydration (20, 21) or the actual rates of association between sulfonamide and enzyme (18, 19, 22). Analysis of the data has been complicated by the fact that the enzyme (as weak base) ionizes in the same pH range (7–8) as many of the drugs. When k_a is plotted against pH, bell-shaped curves are generated with k_a maximal at pH 7–9, depending on the drug used. Data were consistent with either



Ancillary evidence supported *b*, particularly the ultraviolet difference spectroscopy of the sulfonamide in the complex showing the form S^-, and the fact that the cyanate (pK_a 4) acts only as the anion. There are no sulfonamides in critical range (pK_a 6 or below) for an unequivocal test of activity of species S^- vs SH, but, if anions act by a mechanism similar to that of sulfonamides, the NCO^- experiment is important (16).

However, mechanism *b* fails when it is realized that several drugs of $pK_a = 10$, salicylazobenzenesulfonamide (17, 18), and esters of $-OOC\langle\bigcirc\rangle SO_2NH_2$ (23), are highly active when tested at pH 6.5. To act as the anion in the overall reaction (mechanism *b*), their k_a would have to be $> 10^{10}\ M^{-1}\ sec^{-1}$, essentially an impossibly great speed for collision between a drug and macromolecule (19).

It has therefore been suggested that "the initial formation of the complex is more or less independent of ionization state of the sulfonamide" (16, 17). If this is accepted, it means that *a* or *b* (or both) must apply in any given case, but we cannot have $E + S^-$ or $EH^+ + S$, since these would not generate the bell-shaped curves that all investigators have found for the sulfonamides and the weak anions (pK_a 7–10) HS^- and CN^-.

The matter now has been freshly investigated, with results that clarify the situation and resolve these dilemmas (23). Association rates between enzyme and drug for six homologous series of sulfonamides were studied with emphasis on esters and amides of benzene sulfonamide. Figure 2 shows the butyl ester, one of the most active inhibitors known. As noted above, activity was largely a function of the association rate constant k_a. Substitution of ester or amide on the para position of the benzene ring gave far greater activity than ortho or meta substitution. A wide range of activity was studied, with K_I from 4×10^{-5} to 4×10^{-10} M. The principal advance lay in considering the reaction of drug and human red cell carbonic anhydrase C as a two-step reaction, first to the apoenzyme [equivalent to an intermediate complex (En D_1)], followed by isomerization to a final coordinating complex (En D_2). For the first time it was possible to measure affinity of drugs to the apoenzyme (K_{apo}) and to compare them with overall dissociation constants (K_I) or affinity constants (K_{holo}). Finally, estimations could be made of the rate constants, according to the following scheme

$$\text{En} + \text{D} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [\text{En D}_1] \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} [\text{En D}_2] \lesssim\!\!=\!\!\gtrsim (\text{En D})_x.$$

In general, it was found that $k_1/k_{-1} = K_{apo}$ was about 10^3 M^{-1} while K_{holo} (reciprocal of K_I) was about $10^8 M^{-1}$. However, the initial "on" rate constant k_1, assumed from diffusion, is very fast (about 10^8 sec^{-1}) and is a significant factor in the overall activity.

We may now contrast the two reactions: the first, to form En D_1, is not dependent on the active Zn site, is not pH dependent, does not require an intact RSO_2NH_2 (binding is observed with RSO_2NH acetyl), and its affinity constant K_{apo} is proportional to lipid solubility. It is thus a typical hydrophobic reaction. The second or coordination step, to form En D_2, appears to involve the active site, is pH dependent, and probably involves the charged species of both enzyme and drug. As the authors point out (23), their earlier stricture (19) relative to rates that appeared faster than diffusion (discussed above) is irrelevant, since diffusion is not a factor in the second or isomerization step.

This study is a valuable contribution to molecular pharmacology. Specifically it enables us to understand, for the first time, how inhibition of carbonic anhydrase appearing similar in overall kinetics can be accomplished by either hydrophilic (i.e. anions and partially charged sulfonamides) or hydrophobic (uncharged organic sulfonamides) substances. Perhaps optimum activity occurs when important elements of both are present in the same molecule. The two-step system yields the latitude of interpretation demanded by the large body of data in the literature. It is evident that the hydrophobic (step 1) interaction ($K_{apo} = k_1/k_{-1}$) can vary at least 40-fold (almost certainly more if the chemical series were extended) and the second or isomerization stage ($k_2/k_{-2} = K_2$) some 2000-fold. Thus both the structurally nonspecific binding (step 1) and that concerned with active site, charge, and intact RSO_2NH_2 structure (step 2) are significant (23). Step 2 appears to link the activity of inorganic anions with that of the sulfonamides. Further work, hopefully, will include the heterocyclic sulfonamides represented by Figure 2, so that

precise structural relations for K_2 (independent of hydrophobic binding) can be found.

Certain structural relations may be tentatively established as a result of the studies described (14–23). These pertain to overall activity, since not enough data exist to characterize fully K_2, the specific drug-enzyme step.

The first "rule" is that activity of $K_I < 10^{-4}$M requires an unsubstituted group $R\text{–}SO_2NH_2$. As Figure 2 shows, a wide variety of structures has been studied, whose K_I range is almost 10^5. Clearly there is no simple guide to structure-action relations, and this is now explicable in terms of the two-step sequence just described (23). High activity has generally been associated with resonating heterocyclic structures (e.g. ethoxzolamide, benzolamide, acetazolamide; see reference 15); these may be particularly effective in the second or coordination step. The esters of *p*-sulfonamide benzoic acid appear to owe their high activity to the first or hydrophobic step, with a moderate affinity in the second step. It appears, however, that affinity in the first step alone could not yield $K_I < 10^{-4}$M (23), which shows why the specific RSO_2NH_2 structure, yielding k_{-2}/k_2 of 10^{-6} M and overall K_I to 10^{-9} M, is necessary for high activity.

Second, R should be aromatic; alkyl compounds (23a) are exceedingly weak ($K_I > 10^{-4}$M). Such activity may be due to contamination by aromatic sulfonamides during synthesis or, in terms of the above model, some affinity of the alkyl sulfonamide in the nonspecific first step.

Third, within homologous series of benzene sulfonamides, ester or amide substitution on the para position yields far more active compounds than ortho or meta substitution. This is due to superior affinity at both steps, but chiefly at the specific second (23).

Fourth, very large, or bulky, fused ring systems with multiple substituents seem to repress activity. Thus the "diuretic sulfonamides" or saluretics to be considered below are relatively weak inhibitors (15, 23a). Some of these compounds show unusual increments of activity during incubation with enzyme in absence of substrate; this merits further attention in terms of mechanism (23a). This phenomenon may be the basis for the curious pharmacology of 2-amino-4-phenylsulfonylbenzenesulfonamide (NSD 3004), which appears to bind carbonic anhydrase in red cells in vivo with much greater affinity than would be predicted from in vitro assays against the enzyme in high CO_2 (24 and papers cited). This type of dissociation (not evident in the drugs shown in Figure 2) (15) may be related to multiple ring systems or a sulfoxide group, and may indicate a degree of irreversible binding not generally seen in sulfonamide-carbonic anhydrase reactions.

Fifth, within a homologous series, activity increases with lipid solubility of the undissociated molecule (23). This appears to reflect increased affinity in the hydrophobic initial step.

Lastly, within a given group of drugs, introduction of an acidic group appears to increase activity, in degree relative to the strength of the acid group. Thus, in the series of thiadiazole-5-sulfonamides, there is increasing activity from $2\text{-}NH_2$ to 2-acetylamino (acetazolamide) to 2-benzenesulfonamido (benzolamide). The same relation is found between sulfanilamide and 4-acetylsulfanilamide (15). This appears reasonable in terms of ionic interactions in the coordination step 2, just described.

Attempts have been made to predict structure-activity relations of carbonic anhydrase inhibitors in terms of Hammett's factor, pK_a, chemical shift of $-SO_2NH_2$ protons, valence force of S = O bond, and hydrophobic factors. The correlations do not seem convincing, in part because very few of the highly active and diverse heterocyclic drugs were studied (25). Significantly, if the foregoing model is accepted, overall activity could never be related to a given set of forces, since at least four rate constants, which vary independently, are involved.

Thus there are reasonable guides to the strength of the highly specific group aryl–SO_2NH_2 as inhibitors of vertebrate carbonic anhydrase, but there are no single or set of molecular configuration(s) absolutely predicting degree of activity.

The nature of the inhibition of catalytic hydration (or dehydration) is outside the scope of this review. In brief, the sulfonamides appear to inhibit hydration (of CO_2) noncompetitively, and dehydration (of HCO_3^-) competitively (15). This agrees with the ionic nature of the dominant second step of sulfonamide enzyme binding in the above model.

Finally, it now appears that the dissociation constants for acetazolamide and human red cell enzymes may be 5- to 10-fold less when measured in the CO_2 hydration reaction, compared to HCO_3^- dehydration; further work is in progress on this critical point (25a).

INSULIN-RELEASING SULFONAMIDES

Unlike the two classes of sulfonamides that have just been discussed, the chemistry of interaction at the active site is unknown for the present class. The intriguing game of structural analogy cannot be played, and only empirical relations can be discussed. This class is defined physiologically by hypoglycemic action in animals and man whose pancreases contain cells capable of preparing and secreting insulin.

The discovery of these so-called sulfonylureas (26) was made accidentally when blood sugar was found to be low during treatment with the antibacterial sulfonamide, sulfathiadiazole (Figure 3). This compound has a thiourea skeleton, and later developments showed that most, if not all, aromatic sulfonylureas or thioureas reduced blood sugar in animals or humans with potential insulin stores. The standard drug for many years was tolbutamide (Figure 3), a sulfonylurea with no antibacterial activity (no free aryl NH_2) and no activity against carbonic anhydrase (no free aryl SO_2NH_2).

Further work, however, (27, 28) showed that the urea structure was not essential, and that C = O or C = S could give way to C–N, as in glycodiazine (Figure 3). The fundamental structure is simply R_1SO_2NH–, and there are few restrictions on R_1. Of course, not all compounds with this group are active, but it is worth noting that compounds in the classes of Figures 1, 2, and 4 have the possibility of lowering blood sugar.

During the past two decades, some 12,000 compounds, an extraordinary number, have been synthesized and tested, largely in the German pharmaceutical industry. Although the compounds have been duly recorded and divided into appropriate chemical classes (27, 28), this has not been accompanied by a quantitative measure of activity. For certain key compounds, we do have the parenteral dose needed to

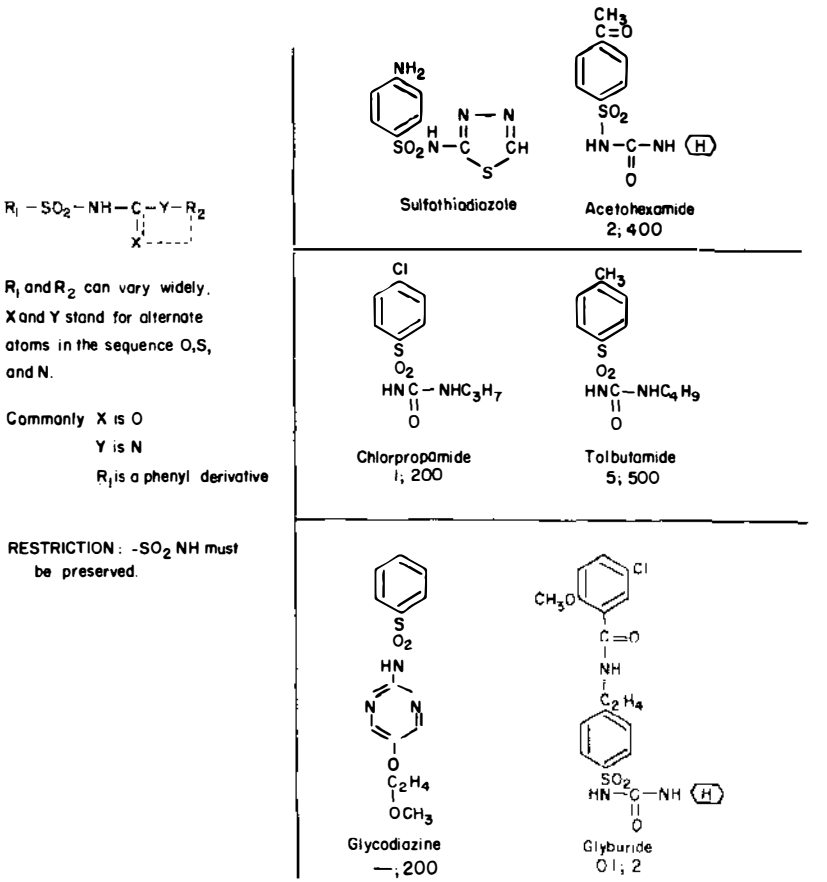


Figure 3 Insulin-releasing sulfonamides. Minimum oral dose mg/kg, for 10% reduction in blood glucose in rat; human single oral dose, mg (28).

lower blood sugar in standard tests in the rat, and these numbers, along with clinical doses, will be used as a measure of activity. It is important to note that in vivo activity has, in the few cases studied, been correlated successfully with the release of insulin in vitro from slices of pancreas (29). It is also important that quantitative potency of several drugs could be measured in dog and man by both fall in blood sugar and rise in insulin concentration after intravenous doses (30). Using these data, I attempt some generalizations about structure and degree of activity.

In the tolbutamide or chlorpropamide type (Figure 3), best activity results when R_2 is C_3H_7 to C_6H_{13}. If C = 2, activity declines; it is zero when C is 12. If R_2 is H (*p*-toluenesulfonylurea) the drug is inactive. The effect of *p*-Cl in chlorpropamide is to increase activity fourfold over tolbutamide. Other halogens or CF_3 in the para

position do not enhance activity. If R is cycloaliphatic, activity is enhanced, as in acetohexamide (Figure 3), and in such compounds the cycloalkyl C can be 7 or 8.

The changes so far described lead to a modest (about four to sixfold) increase in activity over tolbutamide, for instance, in the experimental drug V 14826, *p*-chlorbenzenesulfonyl-cycloheptyl urea. A further variation of this compound is the inclusion of N in the seven-membered cycloalkyl ring of R_2 to yield the semicarbazide link of azepinamide, which is stated to be about ten times as active as tolbutamide.

A truly striking change in activity is brought about by *p*-substitution with acylaminoalkyl groups; these findings resulted in glyburide (Figure 3), which is about 100 times as active as tolbutamide, either in vivo or in vitro (29). The basis for the increase in activity is *p*-C_2H_4NHCO–aryl–; the nature and substituents of the aryl ring may not be critical. This pragmatic finding has not resulted in any systematic or theoretical treatment. Again, as in the other classes of drugs under review, molecular changes relatively far from the core or basic structure essential to activity have a large influence on the potency.

Recent developments in the field show the subtlety of the problem. In glycodiazine (Figure 3) and congeners, there are no substituents on the benzene ring, and the sulfonyl urea link has given way to $-SO_2NH-C-N_2$, in which the nitrogens are incorporated into a pyrimidine ring, with alkoxy side chain. Despite elimination of two seemingly important elements, glycodiazine is about twice as active as tolbutamide. Yet when *p*-benzene substitution is made with the 5-chlor-2-methoxybenzamidoethyl chain (as in glyburide, Figure 3), very large activation occurs. Clearly, this acylamino-alkyl group is an important clue in structure-action relations.

Many other relations, particularly within the many subclasses of antidiabetic sulfonamides, have been indicated (28). However, there has been little attempt to correlate activity with any chemical or physical property, such as we have seen for the antibacterial and carbonic anhydrase inhibitory drugs. Such work should lie in the future, along with studies of the reaction by which these drugs effect the release of insulin.

THIAZIDE AND HIGH CEILING SALURETICS

Figures 4 and 4*A* show the structures of the saluretics. All have 1,3-disulfamyl or 1-sulfamyl-3-carboxy groups attached to a benzene ring. Pharmacologically, they cause increased renal excretion of sodium and chloride in roughly equimolar amounts. Their mechanism of action is not known. Although all the useful drugs of this class have unsubstituted R–SO_2NH_2 groups and so are carbonic anhydrase inhibitors in vitro, they are usually weak inhibitors. All (except dichlorphenamide, see below) are used in doses that do not inhibit renal carbonic anhydrase physiologically, i.e. they do not alkalinize the urine. This saluretic class is complicated by the existence of a most important subclass (Figure 4*A*) of high ceiling compounds; these have certain chemical and pharmacological properties in common with those of Figure 4. But at their maximal dose, the high ceiling compounds elicit about three times as much NaCl excretion as from maximal doses of drugs of Figure 4. There

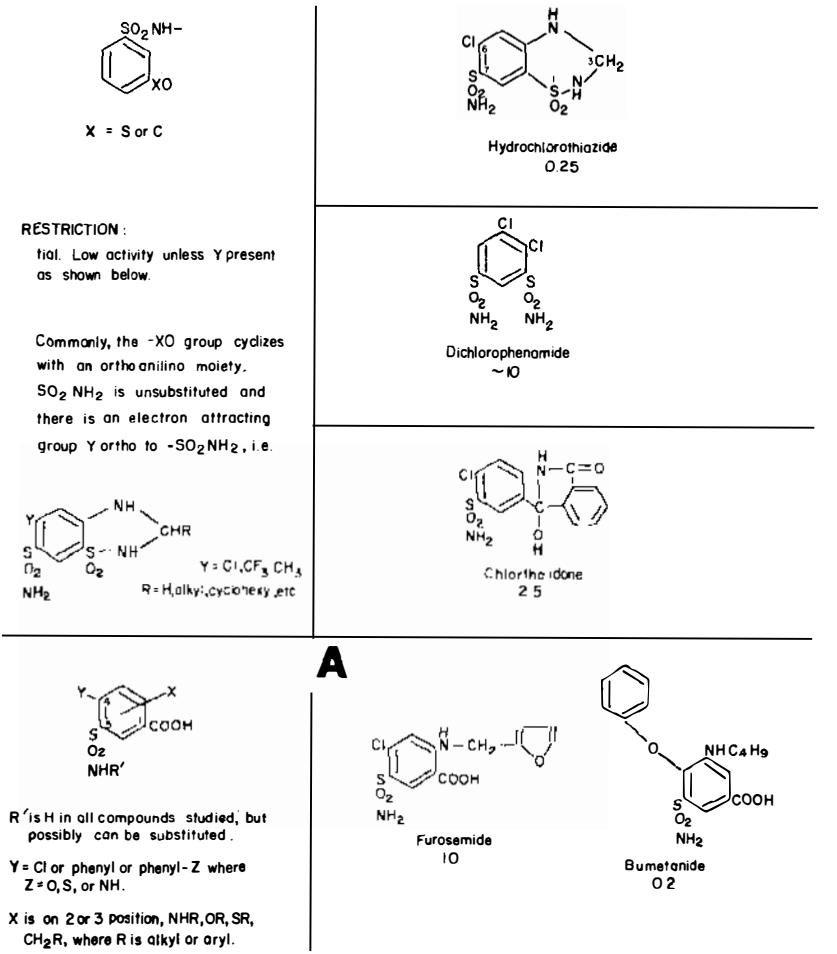


Figure 4 Thiazide saluretics and (*A*) High ceiling saluretics. Minimum i.v. dose for maximum Na^+ or Cl^- effect in dog, mg/kg (31).

are also differences in the renal mechanisms and structure. This section lists the basic structure requirements of both classes 4 (thiazide and related types) and 4A (high ceiling sulfonamides), as well as some of the elements that determine potency in these classes.

Thiazide and Related Types

The first few years of research in this field culminated in an important review by its leaders (31). Two additional, excellent monographs are available, emphasizing

the chemical (32) and pharmacological (33) sides of the structure-activity relation. The latter (33) includes an invaluable listing of the quantitative natriuretic potency of 50 of these drugs in animals and in man. Activity, based on dose, covers a 2000-fold range! From these papers, we may lay out the structure-activity relations in class 4.

The original research stemmed from attention to renal carbonic anhydrase inhibition, but the crucial finding was the difference between benzenesulfonamide or *p*-chlorbenzenesulfonamide, and 1,3-disulfonamide-6-chlorbenzene. The second sulfonamide group, meta to the first, yielded a chloruretic (saluretic) compound, while the monosulfonamide (Figure 2) showed only the renal effects of carbonic anhydrase inhibition, HCO_3^- and not Cl^- excretion. In the disulfonamide class, addition of a second Cl to the ring yielded dichlorophenamide (Figure 4); because this is sufficiently active against carbonic anhydrase, it is a drug of both classes, i.e. it may be used in glaucoma and as a saluretic.

Ring closure of another of these disulfonamides, chlorodisulfamylaniline, yielded the more active chlorothiazide. Further work yielded hydrochlorothiazide (Figure 4), still more (10 times) active as a chloruretic, but less so as a carbonic anhydrase inhibitor. In hydrochlorothiazide, we see again the fundamental features necessary for activity, the 1,3-disulfamyl relation and the adjacent Cl. Compounds without the halogen have activity, but are very weak (31; and work in the reviewer's laboratory).

Further activity was obtained by continuing in the 3–4 saturated series, and making alkyl substitutions in position 3. The 3-cyclopentylmethyl derivative (cyclopenthiazide, not shown) is about 100 times as active as hydrochlorothiazide. It is interesting, but unexplained, that, although the role of Cl in position 6 is presumably that of electron attraction, CF_3 in this position does not enhance activity. The increasing activity in the series chlorothiazide $\longrightarrow$ hydrochlorothiazide $\longrightarrow$ cyclopenthiazide corresponds with increasing ether/water partition coefficient, although it was recognized that additional (unknown) factors are also at work. There is no relation between pK_a and renal activity (31). The possible relations between lipid solubility, pK_a, drug disposition, and renal activity are well illustrated in a neglected paper, and show that no single or obvious pattern is at work (34).

The advent of chlorthalidone (Figure 4) made it clear that a carbamyl function can substitute effectively for the second (meta) sulfamyl group. The activity of this drug is equal to that of chlorothiazide; its distinction lies in long duration of action. Further departures from the original benzothiadiazine structure are discussed, including such esoterica as replacement of the benzene ring by pyridine and inclusion of boron in the thiadiazine ring (32). It is significant that, even in these types, activity follows only if the fundamental features just described are retained.

The precise nature of the sulfamyl group(s) in these compounds was debated for many years, along with the closely related issue of whether all or part of their pharmacological effect was due to carbonic anhydrase inhibition. Initially, many workers stated or implied that the renal effects were, in some way, due to inhibition of the enzyme (31, 35, 36), and the question is unfortunately still raised (32). It has now been shown clearly that the $R–SO_2NH_2$ group could be substituted, although in all the useful compounds it was free. The free sulfonamide in the 7 position of

hydrochlorothiazide was altered to $-SO_2NHR$, where R was alkyl or acyl, or to $-SO_2-CH_3$. None of these compounds inhibit carbonic anhydrase, yet all were chloruretic (37). Data on 7-acetylhydrochlorothiazide were particularly convincing. This result was not unexpected, since some of the compounds most potent as chloruretics (i.e. cyclopenthiazide) were the weakest as carbonic anhydrase inhibitors and did not alkalinize the urine (31, 33). The minimal requirement for activity thus appears to be the structure shown on the left of Figure 4, although, for maximum activity, the free SO_2NH_2, group and adjacent halogens are necessary. The free SO_2NH_2, however, does not work here through carbonic anhydrase inhibition, since the renal effects of fully effective saluretic doses (of hydrochlorothiazide, for example) are qualitatively different from those of the monosubstituted sulfonamides (Figure 2), whose renal effects are solely due to carbonic anhydrase inhibition (15, 33).

A surprising and significant finding was that a compound of the general type of Figure 4, with both features that usually enhance activity (6-halogen and 3-alkyl), is not only virtually devoid of diuretic action, but blocks the renal effect of other thiazides (i.e. hydrochlorothiazide). This compound, 3,4-dihydro-2-methyl-3-(β-oxopropyl)-7-sulfamyl-6-trifluoromethyl-2H-1,2,4-benzothiadiazine-1,1-dioxide-1-phthalazinylhydrazone (EX 4877), appears to occupy the renal thiazide receptor (37a). The special structural feature may be a ring-substituted hydralazine linked to the 3-alkyl group. It is surprising that these studies have not been pursued, since they could illuminate the nature of this important receptor, which is still entirely unknown. Comparison of the antagonist EX 4877 and the agonists of Figure 4 shows possibilities for making compounds substituted on the 3 position to reveal whether the hydrazine or the additional ring (or other features) are responsible for blockade.

The relation between structure and activity for the antidiuretic action of this type of thiazide drug has received special attention (38). It was found that the same structural criteria that elicit saluresis are responsible for free water retention; this is an interesting but not unexpected finding, in view of theories linking the site of distal sodium reabsorption with that of elaboration of osmotically unobligated water. These and other physiological mechanisms underlying the actions of diuretics have been reviewed (39).

High Ceiling Sulfonamide Saluretics

Figure 4*A* shows a new and separate class of compounds; although derived from thiazide research they have structural similarities with compounds of Figure 4. Chief of these is the meta relation between sulfamyl and carboxy groups on the benzene ring. However, when a series of 5-sulfamylanthranilic acids was synthesized (40), a much higher ceiling of activity was reached. This is clearly shown in the human pharmacology of furosemide (Figure 4*A*), the first and chief drug of the class (41). Dose and potency comparison was made with six of the "thiazides"; furosemide elicited two to three times the sodium excretion at plateau or peak doses of all those drugs. Details of the pharmacology and renal action of furosemide have been well reviewed (33), and physiological differences from the thiazide type have been discussed (39).

With regard to structure-activity relations, it is important to note that the sulfamyl group does appear in all compounds of this class, but that carbonic anhydrase inhibition is relatively low and furosemide does not alkalinize the urine (33). In some experimental compounds the $-SO_2NH_2$ group has one or two N substituents (40) but, since these may be subject to metabolic removal (35), it is not clear whether the sulfamyl group need be intact. Of paramount interest is the finding that furosemide and hydrochlorothiazide do not have a common mode of action (42). This was determined by blocking the renal action of hydrochlorothiazide with the specific antagonist EX 4877 (see above), in which case the effect of furosemide was retained.

Supporting this concept is further work in the structure-activity field. Two departures (at least) are possible from the furosemide type—whose structure does conform to the basic criterion of the thiazides. First, it was possible to move the –NHR groups from ortho (i.e. anthranilic acid type) to meta relative to COOH; second, and most significant, the activating halogen ortho to the sulfonamide group was found unnecessary and, in fact, yielded compounds in the metanilic acid series with low activity (43). This interesting research produced bumetanide (Figure 4*A*), which showed "high ceiling" diuretic activity in animals (43) and man (44) at about one fiftieth the dose of furosemide. The activating groups ortho to the sulfonamide group now appear to be –Y–phenyl where Y = NH, S, or O (43). Of further significance is the fact that these new activating groups yielded inert compounds when substituted for the halogen in the thiazide series (45).

The structure-action relations of the high ceiling types are currently being extended past furosemide and bumetanide to cover dozens of compounds with 2-, 3-, and 4- substituents of 5-sulfamylbenzoic acid. The most recent paper that summarizes work of this group is cited (46). It is now shown that the NH function is not necessary on positions 2 or 3. The most active compounds have a connecting link to the phenyl in the 4 position and a bulky substituent at 3. Figure 4*A* shows the various possibilities. Perhaps the most potent diuretic yet to be described (active in dog at 1 μg/kg) is 4-benzoyl-5-sulfamoyl-3-(3-thenyloxy)benzoic acid. The authors also explain the activity of a series of benzisothiazoles not containing a free $-SO_2-NH_2$, by ring cleavage, which yields the free sulfonamide, meta to –COOH.

A clear criterion for this high ceiling type is the –COOH group, which links it to ethacrynic acid, the other main high ceiling type of seemingly quite different structure. The basic structural criteria for class 4*A* have not been elucidated absolutely. It would be interesting if these did turn out to be similar to ethacrynic acid, since the two types do share, for the most part, a common renal pharmacology (33).

ANTITHYROID COMPOUNDS

The sulfonamides belong to one of three distinct classes of organic compounds that interfere with the synthesis of thyroid hormone (Figure 5). Activity is not actually related to the sulfonamide group, but to the $R–NH_2$ component, which occurs in all antibacterial sulfonamides (Figure 1). None of these sulfonamides or other aromatic amines are in clinical use as antithyroid drugs. However, there are several reasons for inclusion here: historical development, recognition of possible anti-

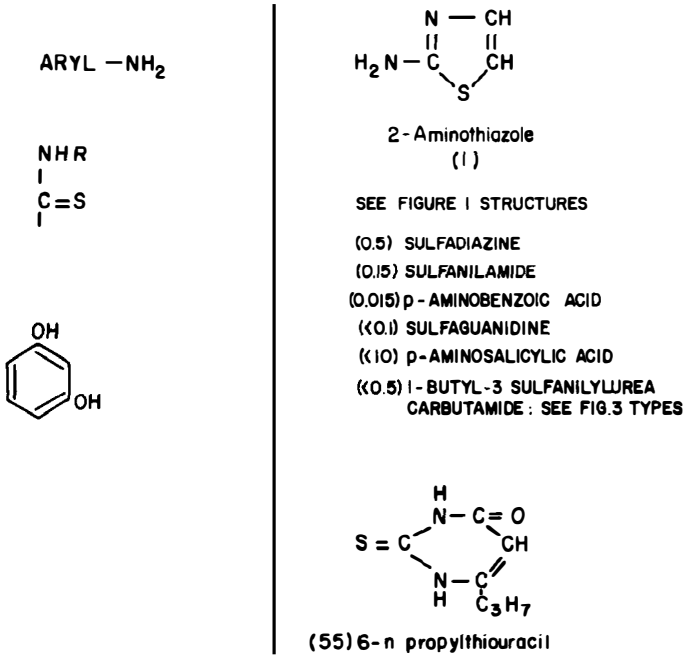


Figure 5 Antithyroid drugs. Relative (to 2-aminothiazole) antithyroid activity based on reduction of I content of rat thyroid. Compiled from (50) and (55).

thyroid activity as an unwanted side effect of drugs of other classes, and the theoretical and possible practical importance of the fact that the sulfonamides (or amines) may work at a site in thyroxine biosynthesis different from that of the thioureas.

The discovery of the antithyroid drugs, like that of the carbonic anhydrase inhibitors and the antidiabetic sulfonylureas, was an accident during a study of the antibacterial sulfonamides. Goiter was observed in rats during work on the effect of sulfaguanidine on intestinal flora (47). This, together with similar chance observations on phenylthiourea during the same year, set off an explosive round of research on physiological mechanisms, chemical syntheses, and structure-action relations of antithyroid drugs. Work of the first few years was summarized by its leading figure (48) and has been brought forward to the present (49). At the end of ten years, almost 1000 compounds had been carefully evaluated (50) but relatively little work of this type has been done since.

It was soon found that goiter was a compensatory change resulting from drug-induced hypothyroidism (48). The possibility of developing drugs for the treatment of hyperthyroidism was immediately conceived, and screening was set up in many pharmaceutical houses. As in all the other classes of drugs discussed, these procedures were rewarded by the discovery of new compounds, hundreds of times as active as those that furnished the lead. However, these were all in the thiourea, rather than in the arylamine series (Figure 5). Nevertheless, enough work was done

to delineate clearly the structural features necessary for activity in the arylamine group.

Drugs were evaluated quantitatively largely by their effect in lowering the iodine content of rat thyroid in vivo (51; reviewed in 50). The activity and structural specificity of sulfanilamide-like compounds were also studied in the synthesis of diiodotyrosine and thyroxine from labeled iodine, in thyroid slices in vitro (52). Extension of quantitative structure-activity relations to man was made possible by external counting of injected ^{131}I in the region of the thyroid, following intravenous injection (53). Syntheses of these varied data make it possible to arrive at intrinsic structural relations unmarred by species peculiarities or factors of metabolic alteration of the drugs.

The simple fact emerges that all aromatic primary amines, $R–NH_2$, are probably active. In many cases such activity is either not observed (as aniline itself) or is weak (as *p*-aminobenzoic acid) in the rat test (50), but at $10^{-3}M$ in vitro all such compounds inhibit organic synthesis of iodide (52, 54). Although in vivo data suggest that the free amino group is necessary (50), several substituted amines (cf acetanilide) were active in vitro (52). Similarly, acetazolamide (Figure 2), which failed to alter thyroid morphology or function in several species studied (15), did lower uptake of radioiodine in thyroid slices (54). The in vitro test also uncovered the activity of certain aromatic hydroxy compounds; however, these studies are marred by lack of dose-response curves (52).

The most active compound of the amine class is 2-aminothiazole (Figure 5), which has about one fiftieth the potency of 6-*n*-propylthiouracil in the rat. However, the startling fact emerges that, in man, 2-aminothiazole is three times as active as 6-*n*-propylthiouracil in the inhibition of iodine uptake to the thyroid (53).

The only compound of this class that has caused hypothyroidism and goiter in man is *p*-aminosalicylic acid (Figure 1). This occurs as a toxic side effect of the chemotherapy of tuberculosis, in which the unusually large dose of 5–10 g per day is given (49, 55). It is clear that, in the structural specificity of amine antithyroid drugs (Figure 5) and antibacterial sulfonamides (Figure 1), the $R–NH_2$ group is shared. Thus all the latter drugs will have the potential for inhibiting thyroid hormone synthesis. As noted, this has not generally been observed in man, although clearly demonstrable in rat (50) and in tissue slices (52, 54). Other primary amines in clinical use include the sulfonylurea, carbutamide, and the inhibitor of adrenal steroid synthesis, amphenone. They are goitrogenic in rat, and inhibit thyroidal ^{131}I uptake in man, but have not caused hypothyroidism or goiter in man (reviewed in 55).

The arylamine class of antithyroid drugs thus fundamentally has nothing to do with the sulfonamide group itself. The association arose out of the chance observation with sulfaguanidine (47) and the fact that many antibacterial sulfonamides, all bearing the arylamine group, were available for investigation. However, it is worth noting that the sulfonamides may have an antithyroid mechanism different from the thioureas or possibly from the anilines themselves. This difference is revealed by the fact that iodide greatly potentiates the inhibitory action of the sulfonamides (i.e. sulfadiazine) but not of the thioureas (55). The sulfonamides may therefore be useful probes in thyroid physiology, as they have been in all the systems reviewed.

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