Perspective

Rational Design of Enzyme Inhibitors: Multisubstrate Analogue Inhibitors†

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As medicinal chemists become more and more involved in the "rational"‡ design of enzyme inhibitors as potential drugs, it may be useful to consider a comment made by the late B. R. Baker. Baker, whose pioneering work in inhibitor design provided a conceptual framework upon which much current work is based,[1] boasted wryly that he probably had synthesized more potent enzyme inhibitors than anyone in history, but had never gotten one to the clinic.§ The reasons for this, which will be mentioned below, continue to plague those who work in this field.

The key contribution in Baker's work was the realization that the affinity and specificity made available through binding a substrate mimic to the active site could be greatly enhanced by taking advantage of topographical features near the active site. By a suitable extension from the active site binding moiety into hydrophobic and/or nucleophilic regions on the enzyme surface, Baker found it possible to differentiate among enzymes and, occasionally, isozymes. However, problems with stability, solubility, and/or transport rendered the vast majority of his inhibitors inactive in vivo.

The most important single goal in drug design, whether the target is membrane receptor, enzyme active site, or something else, is increased specificity. For the design of enzyme inhibitors, the subject of this perspective, the initial quest is for *enzyme* specificity. The ultimate goal is isozyme specificity whereby, for example, one might inhibit a critical enzyme in tumor cells but not the isozyme found in normal cells. Despite significant effort, some of which will be described below, the latter goal remains elusive, and even the former is fraught with difficulty.

An example of the difficulty in achieving enzyme specificity is that of the widely used antitumor drug 5-fluorouracil (FU). It was for many years a widely held view that the cytotoxicity of FU was attributable to its anabolism to 2′-deoxy-5-fluorouridine 5′-monophosphate (FdUMP) and subsequent inhibition of thymidylate (dTMP) biosynthesis.[3,4] It is now known that FU is incorporated as its ribonucleotide into RNA[5] and into 5-fluorouridine diphospho sugars,[6] and FdUMP is incorporated into DNA.[7,8] Which of these constitutes the primary cytotoxic event is the subject of enthusiastic debate, and may actually depend on the system under study. In any event, it is clear that FU is a multipotent drug, and that not even the goal of enzyme, let alone isozyme, specificity is achieved.

The three major approaches currently in vogue for the enhancement of specificity in enzyme inhibition are (1) transition-state analogues, (2) suicide or mechanism-based inhibitors, and (3) multisubstrate analogue inhibitors (MAI). There are few good examples of the first class for the obvious reason that transition states represent energy maxima along the reaction coordinate and are intrinsically unstable and very short-lived. Thus, most so-called "transition-state" analogues are more properly "intermediate" analogues. A clear discussion of the criteria that should be met in classifying a compound as a transition-state analogue, along with a description of phosphonamidates as transition-state analogue inhibitors of thermolysin, has been presented by Bartlett.[9] Suicide, or mechanism-based, inhibition requires that the molecule interact with target enzyme in such a way as to initiate the catalytic process. As the reaction proceeds, a latent functional group, usually an electrophile, is unmasked within the active site. Alkylation or acylation of a suitably disposed active-site nucleophile irreversibly inactivates the enzyme.[10-12] In principle, as pointed out by Kalman,[10] such compounds might be expected to be very specific by virtue of the requirements that they be, initially at least, substrates for the enzyme and that the target enzyme have a suitably situated nucleophile to react with the unmasked electrophile. Two problems with this analysis persist. First, it is often the case that several enzymes will process a given substrate to produce various products. It may be that only the target enzyme will be covalently modified; however, many reports consider only the target enzyme, claim success if the putative inhibitor inactivates the target in a time-dependent fashion, and fail to consider whether

† Abbreviations and acronyms used herein include: MAI, multisubstrate analogue inhibitor(s); PALA, phosphonoacetyl-L-asparate; SAM, *S*-adenosylmethionine; SAH, *S*-adenosylhomocysteine; COMT, catechol-*O*-methyltransferase; dc SAM, decarboxylated SAM; Ap_5A, P^1,P^5-diadenosine-5′ pentaphosphate; TS, thymidylate synthase.

‡ "Rational" is placed in quotation marks because, despite tremendous advances in understanding enzyme structure and mechanism of action, there are still more surprises (usually unpleasant) than correct predictions in this business of drug design.

§ Baker would undoubtedly be pleased to know that one of his "nonclassical" antifolates, triazenate (Baker's antifol, NSC 139105) did, in fact, reach clinical trial.[2]

(1) Baker, B. R. *Design of Active Site-Directed Irreversible Enzyme Inhibitors*; Wiley: New York, 1967.
(2) Burkowski, R. M.; La Buglio, A.; McCrucken, J.; Pugh, R. *Cancer Treatment Rep.* **1980**, *64*, 1387.
(3) Moran, R. G.; Spears, C. P.; Heidelberger, C. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *75*, 1456.
(4) Jackman, A. L.; Jones, T. R.; Calvert, A. H. In *Experimental Clinical Progress in Cancer Chemotherapy*; Muggia, F. M., Ed.; Martinus Nijhoff Publishers: Boston, 1985; p 155.
(5) Dolnick, B. J.; Pink, J. J. *J. Biol. Chem.* **1983**, *258*, 13299 and references cited therein.
(6) Weckbecker, G.; Keppler, D. O. R. *Biochem. Pharmacol.* **1984**, *33*, 2291.
(7) Cheng, Y.-C.; Nakayama, K. *Mol. Pharmacol.* **1983**, *23*, 171.
(8) Kufe, D.; Herrick, D.; Gunner, L. *Biochem. Pharmacol.* **1984**, *33*, 2329.
(9) Bartlett, P. A.; Marlowe, C. K. *Biochemistry* **1983**, *22*, 4618 and references cited therein.
(10) Kalman, T. I. *Drug Dev. Res.* **1981**, *1*, 311.
(11) Walsh, C. *Tetrahedron* **1982**, *38*, 871.
(12) Silverman, R. B.; Hoffman, S. J. *Med. Res. Rev.* **1984**, *4*, 415.

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other, related enzymes may also be targets. Second, the partitioning ratios,[11] that is, the ratio of product release to enzyme inactivation events, may be sufficiently high that activated but unreacted inhibitor may be released into the medium. The consequences to a cell of such release are unlikely to be salubrious. Nonetheless, this approach has been productive of interesting chemistry, biochemistry, and pharmacology, and significant advances continue to be made in this area.

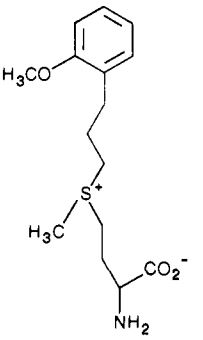
Multisubstrate analogue inhibitors,[13-15] the primary topic of this perspective, offer opportunities for specificity not available to single substrate analogue inhibitors. The combination of two or more substrates required by the target enzyme into a single molecule makes it likely that neither component will be recognized by other enzymes using either substrate, so that a very high order of enzyme specificity may be expected. Since this is a perspective, rather than a review, no attempt will be made to cover all the voluminous literature in this field, but rather to present examples that will illustrate the advantages, drawbacks, and potential of multisubstrate analogue inhibitors.

Essentially, any enzymatic reaction in which two or more substrates (cofactors are considered to be substrates in this context) are simultaneously bound to the enzyme is a candidate for the design of MAI. This includes, but is certainly not limited to, methyl, formyl, and acetyl transferases, dehydrogenases, hydroxylases, kinases, and various synthetic enzymes such as dihydropteroate synthase, ATP:L-methionine *S*-adenosyl transferase, and spermidine synthase.

As the first, and to date only, designed MAI to reach clinical trial,[16] phosphonoacetyl-L-aspartate (PALA, **1**, Figure 1) surely deserves premier treatment as a beacon of hope for the MAI approach. First reported by Collins and Stark in 1971,[17] PALA was designed to inhibit carbamoyl transfer from carbamoyl phosphate to L-aspartate to give *N*-carbamoyl-L-aspartate, the first compound unique to the pyrimidine de novo biosynthetic pathway. PALA binds to L-aspartate carbamoyl transferase some 3 orders of magnitude more tightly than carbamoyl phosphate, the more tightly bound of the two substrates. Based on the observed kinetics of binding, which were competitive versus carbamoyl phosphate and noncompetitive against aspartate, ordered substrate binding with carbamoyl phosphate binding first was proposed. It was further proposed that a major conformational change took place in the enzyme upon interaction with PALA[17] or during the normal transcarbamoylation. It seems likely that conformation changes in enzymes will be frequently required in multisubstrate reactions, especially when ordered binding is observed. This phenomenon is often ignored in the design of MAI. Since conformation changes seem often to be triggered by strong, frequently ionic interactions between substrate and the active site, failure to consider the role of such interactions may account for the relatively weak inhibitory properties of many putative MAI (vide infra).

A great deal of attention has been paid in recent years to the *S*-adenosylmethionine (SAM) dependent methyl transferases. These carry out a large variety of specific heteroatom methylations, ranging from small molecules such as catechols and indoles to macromolecules such as mRNA. Because these reactions are thought to proceed through an S_N2, colinear transition state with the nucleophile attacking the sulfonium-bound methyl group,[19] they have provided the impetus for a number of studies on MAI design and synthesis.

A large number of inhibitors of SAM-dependent methyl transferases have been based upon the finding that *S*-adenosylhomocysteine (SAH) is a potent product inhibitor of most methyl transferase enzymes studied.[19,20] Reports from the Coward group[19] and the Burroughs Wellcome Co.[21] have described MAI approaches to inhibitors of catechol-*O*-methyltransferase (COMT) that delineate both the principles of MAI inhibition and some of the pitfalls that can arise in the design of the inhibitors. Coward[19] demonstrated that sulfonium derivatives such as **2** and **3**



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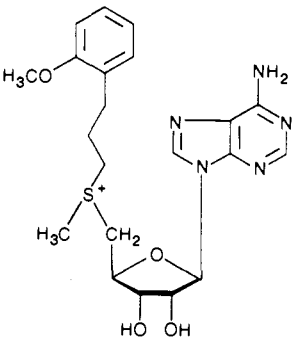

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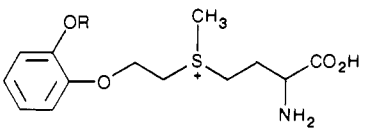
were weak competitive inhibitors of COMT with K_i values in the mM range. The corresponding uncharged thioethers were not inhibitory. It should be noted that neither of these compounds has both the adenosine and methionine functionalities that would be present in the transition state in the methylation reaction. Reasoning that the relatively weak inhibitory potency of the Coward compounds might reside in the substitution of methylene for oxygen in the catechol moiety, Lever and colleagues[21] synthesized some simpler catechol derivatives, such as **4**, and found them to be essentially devoid of inhibitory activity regardless of whether a sulfonium ion or thioether linkage was present.



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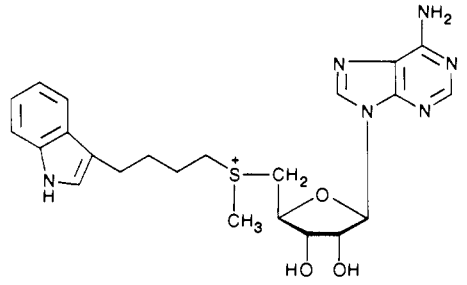
Taken together, these studies imply that the presence of the sulfonium ion may be an important but not sufficient criterion for tight binding, and that the complexity associated with the complete reaction transition state is likely to be required if a truly specific COMT multisubstrate analogue inhibitor is to be developed. It is obvious that in this case, as with many of the others to be discussed herein, the additional complexity remarkably increases the difficulty of synthesis of stable compounds and decreases the probability that the products will be able to penetrate cell membranes in order to reach the target enzyme. Crooks[22] had somewhat better luck in his studies on indole

(13) Wolfenden, R. *Annu. Rev. Biophys.* **1976**, *5*, 271.
(14) Lindquist, R. M. In *Drug Design*; Ariens, S. J., Ed.; Academic: New York, 1975; p 24.
(15) Broom, A. D. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1986**, *45*, 2779.
(16) Muss, H. B.; Slavik, M.; Bundy, B.; Stehman, F. B.; Cressman, W. *J. Clin. Oncol.* **1984**, *7*, 257.
(17) Collins, K. D.; Stark, G. B. *J. Biol. Chem.* **1971**, *246*, 6599.
(18) Collins, K. C.; Stark, G. B. *J. Biol. Chem.* **1969**, *244*, 1869.
(19) Anderson, G. L.; Buzzolotti, D. L.; Coward, J. L. *J. Med. Chem.* **1981**, *24*, 1271 and references cited therein.
(20) Borchardt, R. J. *J. Med. Chem.* **1980**, *23*, 347.
(21) Lever, O. W., Jr.; Hyman, C.; White, H. L. *J. Pharm. Sci.* **1984**, *73*, 1241.

N-methyl transferase. He synthesized a series of compounds including compound **5**, the sulfonium ion con-



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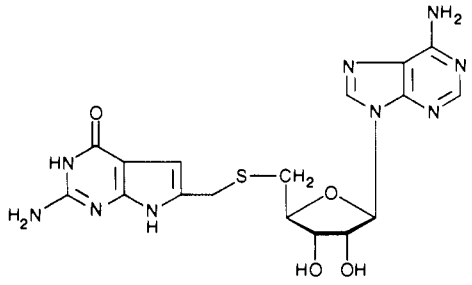
taining both the indole and the adenosine moieties but lacking the amino acid side chain of methionine. This compound turned out to be a reasonable inhibitor of the target enzyme with a K_i of 12 μM, the first bisubstrate analogue to be nearly as potent an inhibitor as the product inhibitor SAH. Importantly, Crooks observed that the compounds in this series were ineffective in inhibition of methylation of 3,4-dihydroxybenzoic acid catalyzed by purified porcine COMT.

From this same laboratory came a recent report[23] of an approach toward a much more ambitious and difficult design problem, inhibition of vaccinia RNA guanine 7-methyl transferase. This is one of a series of enzymes involved in the "capping" reaction essential for viral replication. The chemistry involved in obtaining a good mimic for the transfer of the methyl group of SAM to N-7 of guanine is formidable, indeed. The Crooks group made a start toward this problem by synthesizing compound **6**,



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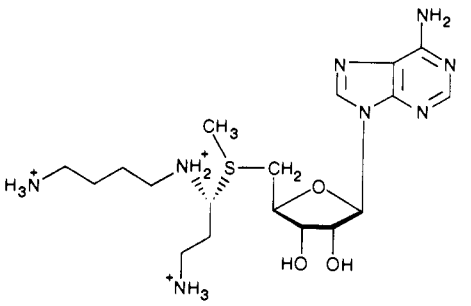
a pyrrolopyrimidine derivative. Despite the alteration in the locus of substitution from the purine-7 to purine-8 position on the pyrrolopyrimidine, compound **6** was a modest inhibitor of the enzyme with a K_i of 9.3×10^{-5} M. This represents a novel approach to understanding more about this complex macromolecular methylating system, and further results in this area are awaited with interest.

Another important set of reactions involves the synthesis of the polyamines spermidine and spermine. The transition state (**7**) in the normal reaction is somewhat different from that of the methyl transferases since in this case, rather than SAM, decarboxylated SAM (dcSAM) is utilized. Attack by putrescine occurs on the aminopropyl side chain at the carbon attached to the sulfonium ion. Based upon this, the design of inhibitor **8** becomes straightforward, although the chemistry involved turned out to be rather challenging. The synthesis of inhibitor **8** was achieved, and it was shown to be a reasonably potent inhibitor ($I_{50} \approx 15$ μM) of spermidine synthase. Oddly, however, the thioether derivative, lacking the methyl group, was a much more potent inhibitor of spermidine



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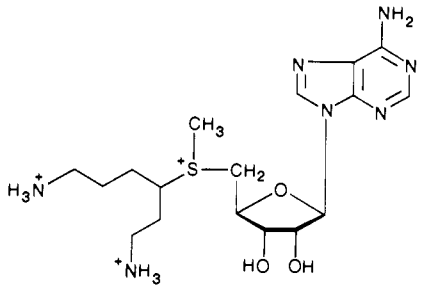

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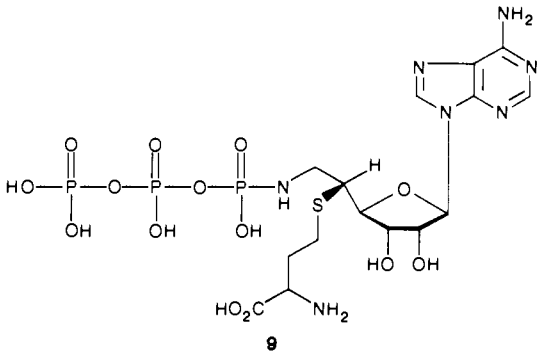
synthase ($I_{50} \approx 0.4$ μM) and was almost completely lacking in inhibitory activity against spermine synthase. Presumably, the cationic sites provided by the amino functions are sufficient to obviate the necessity for a cationic center on the sulfur group in the enhancement of binding to spermidine synthase.[24]

For a number of years, the group of Alexander Hampton has been interested in using multisubstrate analogue inhibitors to try to achieve isozyme specificity as well as enzyme specificity. Quite recently, this group has tackled the problem of methionine adenosyl transferase, comparing the isozyme from rat liver tumor (Novikoff hepatoma) and that from normal rat tissue. Their initial approach[25] led to compounds that contained many important binding sites of the ternary complex that is undoubtedly formed in SAM biosynthesis, but were electronically and sterically somewhat different from the presumed intermediate to be found at the active site of the enzyme. These efforts led to a series of weak inhibitors that bound to the enzyme slightly more tightly than substrate ATP.

However, the second generation of their inhibitors, published quite recently,[26] included the very complex inhibitor **9**, which bears a closer electronic and steric resemblance to the presumed active site complex than prior analogues. Compound **9** turned out to be a rather im-



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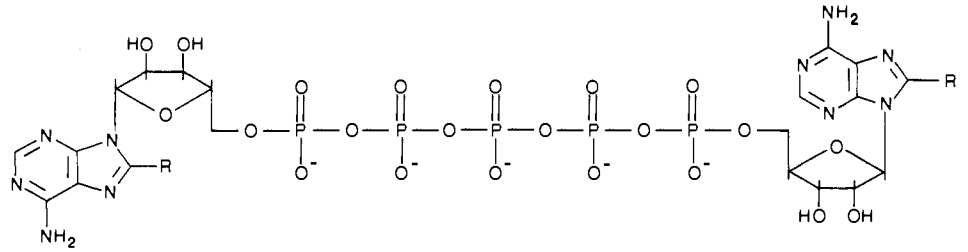
(22) Benghiat, E.; Crooks, P. A. *J. Med. Chem.* **1983**, *26*, 1470.
(23) Benghiat, E.; Crooks, P. A.; Goodwin, R.; Rottman, F. *J. Pharm. Sci.* **1986**, *75*, 142.
(24) Tang, K.-C.; Mariuzza, R.; Coward, J. K. *J. Med. Chem.* **1981**, *24*, 1277.
(25) Kappler, F.; Hai, T. T.; Kotter, R. J.; Hyver, K. J.; Hampton, A. *J. Med. Chem.* **1986**, *29*, 1030.
(26) Vrudhula, V. M.; Kappler, F.; Hampton, A. *J. Med. Chem.* **1987**, *30*, 888.



10a: R = H
b: R = SCH_3

pressive inhibitor of both rat methionine adenosyl transferases, with K_i values of about 0.3 μM versus ATP and K_m/K_i ratios of about 440. No isozyme selectivity was obtained, and of course, these molecules would not be expected to enter cells intact. It was of considerable interest that the 5′*R* isomer was substantially more potent than the 5′*S* isomer; this sheds some light on the probable arrangement of binding functions within the enzyme active site. When X-ray crystallographic and molecular data are not available, this approach provides the medicinal chemist with a tool for probing active-site geometry in a reasonably well defined manner.

The additional problem with the approach of Hampton in targeting this particular enzyme is that, unless isozyme specificity is achievable, it is quite certain that any drug resulting from inhibition of methionine adenosyl transferase will be highly multipotent since SAM participates in such a wide variety of biochemically critical reactions. Thus, this is a case where specificity for a particular enzyme does not mean specificity for a single biochemical pathway.

The story of adenylate kinase inhibition is a very interesting one. Lienhard was the first to develop a multisubstrate inhibitor of the enzyme, which catalyzes phosphate transfer from ATP to AMP, leading to the production of 2 molecules of ADP. Lienhard[27] compared several P^1P^n-diadenosine-5′ polyphosphates and discovered that the pentaphosphate Ap_5A (**10a**) was a potent inhibitor of the enzyme and was competitive against both ATP and AMP. It is obvious that such a pentaphosphate should have all the binding functions to fit into the active site, although it was a bit surprising to find that an extra phosphate residue is required for optimal binding activity. Binding activity fell off very sharply as the number of phosphate residues linking the adenosine moieties was reduced.

The inhibitor is, of course, completely symmetrical about the central phosphate. One might expect, however, that the enzyme would have two discrete binding sites, one for ATP and one for AMP. Were this the case, and if Ap_5A were to bind specifically to both portions of the active site as suggested by the enzyme kinetics, asymmetry might be anticipated in the binding process. Such asymmetry was elegantly demonstrated by Mildred Cohn in 1977,[28] who found that Ap_5A bound to adenylate kinase showed clear asymmetry in the phosphorus NMR spectrum such that five resonances were seen. Furthermore, the addition of magnesium to this complex produced a marked enhancement of the nonequivalence, leading to chemical shift differences of 7 ppm between the 2- and 4-phosphorus nuclei. In a real sense, therefore, one is dealing with a multisubstrate analogue in which magnesium is an important part of the binding process, such that one now sees a quaternary complex analogue rather than a ternary complex analogue. The role of metal ions has infrequently been studied in binding of multisubstrate analogues, and it is an area that might be usefully pursued with state-of-the-art NMR technology.

In 1982, Hampton[29] attempted to use the foregoing information in the design of isozyme-specific inhibitors. Hampton was able to demonstrate selectivity for rat muscle adenylate kinase over rat hepatoma adenylate kinase of greater than 22-fold by substituting both adenosine residues of Ap_5A at the 8-position with ethylthio functions (**10b**). As indicated in his discussion, it is not currently really possible to rationalize the effects of these added substituents on either inhibitory potency or selectivity. This represents a combination of the more classical approach of lead-directed molecular modification with the concept of multisubstrate analogue inhibition. It would be of considerable interest to apply the NMR techniques worked out by Cohn[28] to the kind of system described by Hampton.[29]

The same approach has been very recently applied by Ives' group to the synthesis and evaluation of bisubstrate analogues of deoxynucleoside kinases. In addition to being potent inhibitors of a variety of such kinases, these compounds turned out to be very useful in the resolution of *Lactobacillus* enzymes on affinity media.[30,31]

The relative success of multisubstrate inhibitor approaches to the adenylate kinase system was not very well matched when the same kinds of techniques were applied to the hexokinase system. Hexokinase is the enzyme that transfers phosphate from ATP to the 6-position of hexoses. Dannenberg and Dannenberg synthesized Ap_3glucose and Ap_4glucose and found that these were modest inhibitors of hexokinase with K_i values of 0.43 and 0.37 mM, respectively.[32] The inhibitors were said to be competitive against ATP and uncompetitive against glucose, leading to the conclusion that glucose had to be bound to the enzyme prior to the interaction of the inhibitors. Hampton essentially confirmed this information with yeast and rat hexokinases, demonstrating that inhibitors bound significantly less well than ATP or glucose themselves.[33] The data from these laboratories suggested that, at best, Ap_3glucose and Ap_4glucose were acting as weak ATP antagonists.

A careful reanalysis of the kinetic pattern of inhibition of rat brain hexokinase demonstrated that, while the inhibition versus glucose was indeed uncompetitive, it was

(27) Lienhard, G. E.; Secemski, I. I. *J. Biol. Chem.* **1973**, *248*, 1121.
(28) Rao, B. D. N.; Cohn, M. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 5355.
(29) Kappler, F.; Hai, T. T.; Abo, M.; Hampton, A. *J. Med. Chem.* **1982**, *25*, 1179.
(30) Ikeda, S.; Ives, D. H. *J. Biol. Chem.* **1985**, *260*, 12659.
(31) Ikeda, S.; Chakrabarty, R.; Ives, D. H. *J. Biol. Chem.* **1986**, *261*, 15836.
(32) Dannenberg, P. V.; Dannenberg, K. D. *Biochim. Biophys. Acta* **1977**, *480*, 351.
(33) Hampton, A.; Hai, T. T.; Kapler, F.; Chawla, R. R. *J. Med. Chem.* **1982**, *25*, 801.

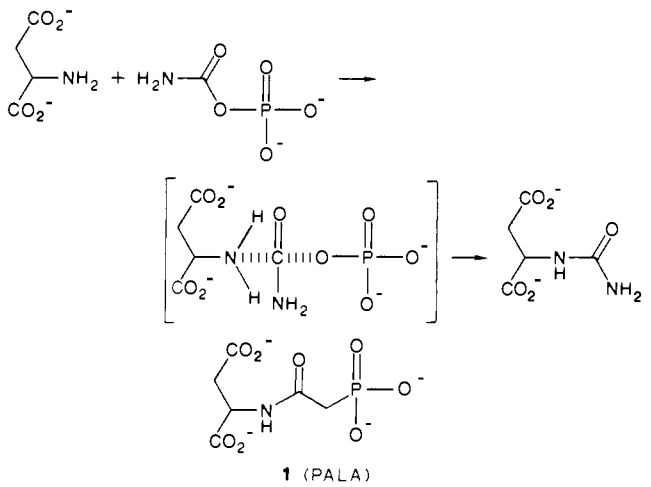
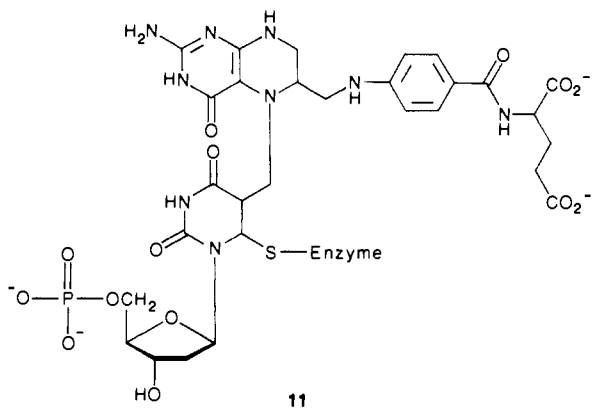


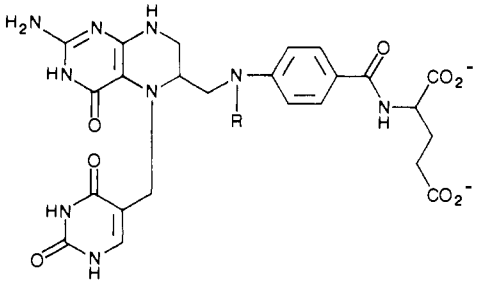
Figure 1.

a mixed inhibitor rather than competitive against ATP. Analysis of the kinetic data led Manning and Wilson to conclude that Ap_3glucose probably binds to an enzyme regulatory site rather than the enzyme active site.[34] This was suggested to have significant evolutionary implications and is of interest from that point of view; it is certainly of little help in the development of effective inhibitors of the enzymes in question.

Several reports have appeared over the last several years describing the synthesis of various amine-acetyl transferase inhibitors.[35-37] These papers describe a rather clever approach to attachment of various amines such as gentamicin[35] or spermidine[36,37] to acetyl-*S*-CoA. Unfortunately, none of these compounds was characterized other than by assumption of its chemical identity and evidence for chromatographic homogeneity. Even granted the substantial complexity of the molecules produced through this methodology, the use of uncharacterized products in order to draw substantial conclusions about enzyme active sites seems unwarranted. These are potentially very interesting compounds, and it is hoped that sufficient amounts of material will be prepared so that they may be fully characterized.

A particularly important carbon methylation reaction is that catalyzed by the enzyme thymidylate synthase (TS) utilizing as substrate 2′-deoxyuridine monophosphate (dUMP) and 5,10-methylenetetrahydrofolate (5,10-CH_2-H_4PteGlu). This enzyme has been studied in great detail in a large number of laboratories, and its basic mechanism of action is quite well understood.[15,38-40] It is a useful target for specific enzyme inhibition because the only role of thymidylate is as a building block for DNA, and its only de novo source is via the TS-catalyzed reaction. The ternary complex involved in the methylation reaction is thought to resemble **11**. This information enabled a number of groups to design approaches to bisubstrate analogue inhibitors of this enzyme that would resemble the nucleotide and folate portions of the ternary complex. To this end, Temple et al.[41] and Charlton and Young[42]

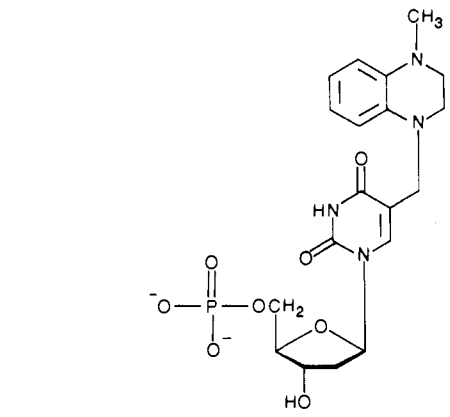




approached the problem beginning with a folate and prepared **12** and **13**, respectively. The latter molecule was synthesized as a chemical model and, upon heating, did indeed form some thymine. Neither of these molecules, however, was an inhibitor of TS, attesting to the importance of the sugar phosphate moiety on the pyrimidine in binding to the enzyme. Mertes and his co-workers[43] used the opposite approach, starting with a preformed 2′-deoxyuridine monophosphate linked through a methylene bridge to a tetrahydroquinoxaline (**13**). This compound turned out to be a modest inhibitor of TS with a K_i of about 0.75 μM. Studies from this laboratory have focused on the use of deazafolates or folate analogues derived from pyrimidines in the construction of **14** and **15** as better mimics of the bisubstrate portion of the ternary complex. These compounds have both turned out to be potent inhibitors of thymidylate synthase with K_i values versus dUMP of 0.06 and 0.03 μM, respectively. The inhibitors bind about 2 orders magnitude more tightly than either the nucleotide or the folate substrate.[44,45]

Neither of these molecules turned out to be a suicide inactivator of the enzyme. That is, the active sulfhydryl group of the enzyme did not attack the thymidine 6-position. This suggests that these compounds may be more closely related to product-substrate analogues, with the pyrimidine portion resembling thymine, than to bisubstrate analogues. Studies involving placing an "electron sink" pendant to the 5-position of the nucleotide are ongoing, and a number of related active site probes have been prepared.[46]

(34) Manning, T. A.; Wilson, J. E. *Biochem. Biophys. Res. Commun.* **1984**, *118*, 90.
(35) Williams, J. W.; Northrop, D. B. *J. Antibiot.* **1979**, *32*, 1147.
(36) Cullis, P. M.; Wolfenden, R.; Cousens, L. S.; Alberts, B. M. *J. Biochem.* **1982**, *257*, 12165.
(37) Erwin, B. G.; Persson, L.; Pegg, A. E. *Biochemistry* **1984**, *23*, 4250.
(38) Benkovic, S. V. *Annu. Rev. Biochem.* **1980**, *49*, 227.
(39) Dannenberg, P. V.; Lockshin, A. *Adv. Enzyme Regul.* **1982**, *20*, 99.
(40) Santi, D. V. *J. Med. Chem.* **1980**, *23*, 103.
(41) Temple, C. L., Jr.; Elliott, R. D.; Rose, J. D.; Montgomery, J. H. In *Chemistry and Biology of Pteridines*; Kisliuk, R. L., Brown, G. M., Eds.; Elsivier/North Holland: Amsterdam, 1979; p 279.
(42) Charlton, P. A.; Young, D. W. *J. Chem. Soc., Chem. Commun.* **1980**, 614.
(43) Park, J. S.; Chang, C. T.-C.; Mertes, M. P. *J. Med. Chem.* **1979**, *22*, 1134.
(44) Srinivasan, A.; Amarnath, V.; Broom, A. D.; Zou, F. C.; Cheng, Y.-C. *J. Med. Chem.* **1984**, *27*, 1710.
(45) Yang, I.-Y.; Slusher, R. M.; Broom, A. D.; Ueda, T.; Cheng, Y.-C. *J. Med. Chem.* **1988**, *31*, 2126.



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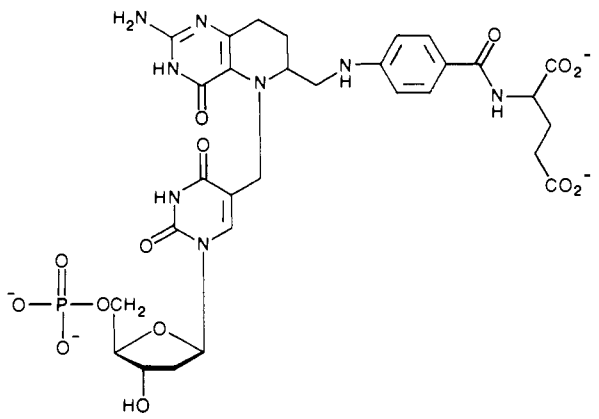

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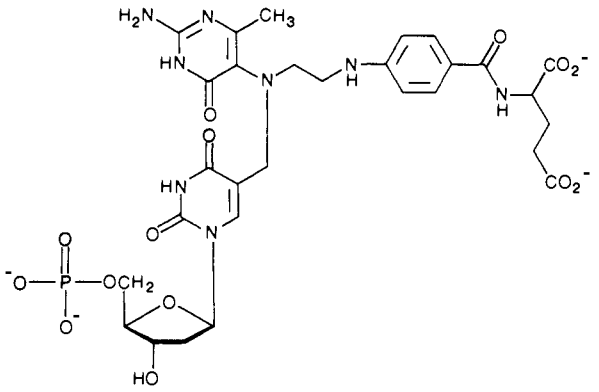
The possibility that inhibition of dopamine β-hydroxylase, a mixed-function oxidase which converts dopamine to norepinephrine, would lead to a potentially clinically useful antihypertensive effect, prompted Kruse and his colleagues at Smith-Kline to design and study a set of multisubstrate inhibitors of dopamine β-hydroxylase.[47] More detailed studies on **16** and a related series of molecules revealed that the *N*-benzyl moiety is a good mimic



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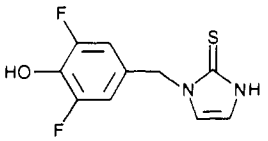

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for the tyramine substrate and the imidazolethione moiety is a good oxygen mimic, binding specifically to Cu^+ in the active site. In fact, this bisubstrate analogue **16** was found to bind 10^5–10^6 times more tightly to the enzyme than substrate. A detailed kinetic analysis supported the view that the interaction between inhibitors and enzyme was that of a multisubstrate analogue. It was both interesting and unusual to note that **16** was found to be orally active as a hypotensive agent in animals. Kruse et al. report[48,49] that a compound closely related to those reported is currently undergoing preclinical evaluation as a hypotensive agent.

Conclusions

The foregoing examples have been selected from a very large literature to illustrate some of the strengths and weaknesses of the multisubstrate approach to the design of enzyme inhibitors. In order for these molecules to be more than intellectual curiosities, they must be able to reach the target enzyme active site in the intact organism. In a very few cases, this has turned out to be possible by the direct administration of the drug, for example, the cases of PALA and the dopamine β-hydroxylase inhibitors designed by the Smith-Kline group. In general, however, targets utilize highly polar compounds that are often transported actively or through facilitated diffusion, as is usually the case with nucleosides and folates. Once the bisubstrate inhibitor is formed, it is no longer recognized by the transport system as a compound worthy of its attention, and is usually not taken up by the cell by a passive diffusion process. These highly polar moleculars, typified by inhibitors of spermidine synthase and thymidylate synthase, will require conversion to prodrugs or incorporation into alternative delivery systems such as liposomes or targeted monoclonal antibodies in order to have potential therapeutic applications. This represents an area of endeavor still in its infancy, and one which must attract the attention of medicinal chemists who are willing to work with pharmaceuticists and pharmacologists in order to prepare compounds of possible clinical utility.

It should also be pointed out that many of the bisubstrate and multisubstrate analogues synthesized to date are so complex that an economically feasible synthesis seems unlikely, even if the transport problem may be overcome. There is value in the synthesis of such complex molecules insofar as they lead to a better understanding of the nature of the target enzyme; however, for practical chemotherapy, the general approach taken by the Smith-Kline group in the design of dopamine β-hydroxylase inhibitors, or of Collins and Stark in the design of PALA, should act as models for future drug design. In other words, once it is determined that multisubstrate analogues behave in the way one wishes for the particular enzyme system, attention should be given to understanding the minimal structural requirements necessary for that specific inhibition in order to design molecules more likely to be of therapeutic value.

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(46) Bayomi, S. M. M.; Brixner, D. I.; Eisa, H.; Broom, A. D. *Nucleosides Nucleotides* **1988**, *7*, 103.
(47) Kruse, L. I.; Kaiser, C.; De Wolf, W. E., Jr.; Frazee, J. S.; Erickson, R. W.; Ezekiel, M.; Ohlstein, E. H.; Ruffalo, R. R., Jr.; Berkowitz, B. A. *J. Med. Chem.* **1986**, *29*, 887.
(48) Kruse, L. I.; Kaiser, C.; We Wolf, W. E., Jr.; Frazee, J. S.; Garvey, E.; Hilbert, E. L.; Faulkner, W. A.; Flaim, K. E.; Sawyer, J. L.; Berkowitz, B. A. *J. Med. Chem.* **1986**, *29*, 2465.
(49) Kruse, L. I.; De Wolf, W. E., Jr.; Chambers, P. A.; Goodhart, B. J. *Biochemistry* **1986**, *25*, 7271.