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Perspective

Cancer Chemotherapy: Congener Synthesis

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There are two separate and, in many ways, quite different approaches to the development of new drugs of any kind, including anticancer agents. The difficulty with the first of these, the development of new leads, lies in the inability of those involved in cancer research to identify and define, by whatever means, an exploitable biochemical difference between normal mammalian host cells and invading cancer cells such as exists between mammalian cells and bacterial cells and on which the selective toxicity of antibacterial agents, such as the sulfa drugs, penicillins, and tetracyclines, is dependent. At the same time, few clinically useful agents have resulted from strictly random screening of synthetically prepared compounds (the isolation of useful drugs from complex natural mixtures such as antibiotic beers or plants is obviously another story).

The second approach available to the medicinal chemist, that of congener synthesis to improve the activity of a lead in hand, is the subject of this essay. If one is to attempt to develop a congener that is more useful in the treatment of human cancer than the original lead, one must have goals to attempt to achieve and more specific goals than just simply a "better", or less toxic, agent. These questions must be answered: (1) What are the goals? (2) How can these goals be achieved? (3) How can progress toward these goals be measured? These questions are listed in a logical sequence, but it is helpful to reverse the order of the answers.

First, then, how can progress toward these goals be measured? Obviously, ultimately, by evaluation in humans. But I do not agree with the suggestion that has been made from time to time in the past that congener comparisons can only be meaningfully made in humans, although it may be that more studies of this type should be carried out. I also disagree with another school that, at least in the past, has held that the only valid comparison of analogues is cytotoxicity to cells in culture. Cytotoxicity data can be very important but are not the final basis of selection of a drug for clinical evaluation. Since such a system is one dimensional, host toxicity cannot be related to activity. So, like it or not, we must rely on experimental animal models for congener comparisons. Therefore, the answer to the last question is that we must do most of our measuring of progress by using appropriate animal models. This conclusion, of course, emphasizes the importance of using proper test systems.

If, then, the animal models are accepted, what goals should we try to achieve in these models? In the discussion that follows I have tried to set forth a number of clearly defined goals, the attainment of which I believe should lead to new useful clinical agents. How these goals can be achieved is somewhat more nebulous, but I have attempted to supply examples of approaches that have been taken with varying degrees of success in the animal models and in clinical applications.

First, it would be desirable to obtain *a significantly better cancer cell kill* in a specific test system at host-tolerated doses. This test system should be sensitive to the class of agents in question but not too sensitive; otherwise, meaningful differences in activity cannot be measured.[1] In such a system, or preferably systems, a minimum of a 1-log difference in cell kill[2] at the LD_{10}, either alone or in combination with another agent (or agents), should indicate a new congener with potentially recognizably superior clinical activity. That is to say, if a 1-log greater cell kill in the animal system is predictive of a 1-log greater cell kill at a tolerated dose in man—and there appears to be no data to indicate to the contrary—then the congener should have significantly better clinical activity than the parent now in clinical use. Preferably, this log greater cell kill should be demonstrated against solid tumors and specifically against micrometastases from a solid tumor rather than the primary solid tumor itself, since such activity would indicate its potential utility in surgical adjuvant therapy.[3] The first nitrosourea tested in the L1210 system, *N*-methyl-*N*-nitrosourea (MNU), gave an increase in life span of the leukemic mice to about 60–70%, perhaps a 2- to 3-log cell kill.[4] *N,N'*-Bis(2-chloroethyl)-*N*-nitrosourea (BCNU), about the 30th congener prepared, cured essentially all the leukemic animals at its LD_{10} on its optimal schedule.[5] This result represents an increase in cell kill of about 3 to 4 logs and led to the clinical use of BCNU.[6]

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(2) F. M. Schabel, Jr., D. P. Griswold, Jr., W. R. Laster, Jr., T. H. Corbett, and H. H. Lloyd, *Pharmacol. Ther. Part A*, 1, 411 (1977).
(3) F. M. Schabel, Jr., *Cancer*, **35**, 15 (1975).
(4) H. E. Skipper, F. M. Schabel, Jr., M. W. Trader, and J. R. Thomson, *Cancer Res.*, **21**, 1154 (1961).
(5) T. P. Johnston, G. S. McCaleb, and J. A. Montgomery, *J. Med. Chem.*, **6**, 669 (1963).
(6) S. K. Carter, F. M. Schabel, Jr., L. E. Broder, and T. P. Johnston, *Adv. Cancer Res.*, **16**, 273 (1972).

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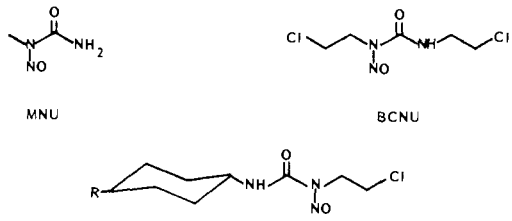
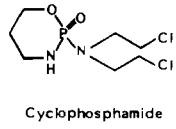


MNU BCNU

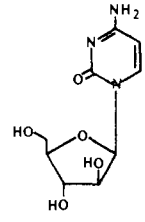
AOCCNU (R = $AcOCH_2$)
CMCCNU (R = $ClCH_2$)
MeCCNU (R = Me)
CCNU (R = H)

Although all nitrosoureas that inhibit the growth of the primary tumor in mice implanted with the Lewis lung carcinoma also increase the life span of the treated animals compared to untreated controls, only two of the compounds studied, *N*-[*trans*-4-[(acetyloxy)methyl]cyclohexyl]-*N'*-(2-chloroethyl)-*N'*-nitrosourea (AOCCNU) and *N*-(2-chloroethyl)-*N'*-[*trans*-(4-chloromethyl)cyclohexyl]-*N*-nitrosourea (CMCCNU), gave good increases in life span with little or no effect on the primary.[1] Since mice implanted with the Lewis lung carcinoma usually die from pulmonary metastases, these compounds appear to be more effective against the metastases than the primary tumor and should be of considerable interest.

Another measure of improved activity, is *a better therapeutic index*. A congener with a significantly greater difference in its LD_{10} and minimum effective dose, defined here as a dose that will kill a minimum of 2 or 3 logs of cells, than the parent should be advantageous, particularly in combination chemotherapy. Schabel and his associates succeeded in curing the plasmacytoma in hamsters by combination chemotherapy with cyclophosphamide (CPA) and 1-β-D-arabinosylcytosine (*ara*-C).[3] They succeeded



Cyclophosphamide

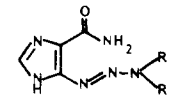


Ara-C

for two reasons: (1) cyclophosphamide, a cycle-nonspecific agent, administered prior to *ara*-C reduces the viable tumor cell burden (cells in cycle and resting), causing the remaining viable cells to go into cycle and become sensitive to *ara*-C; and (2) cyclophosphamide can kill 2 or 3 logs of cells of the plasmacytoma at one-tenth of its LD^{10} and, therefore, this drug could be given in combination with large enough doses of *ara*-C to effect cures. Drugs do differ in their dose–response curves and in their activity at low fractions of the LD_{10}.[7]

A broader spectrum of activity is a clear indication of increased efficacy. If a congener shows real activity against one or more animal tumors that do not respond to the parent, it should become potentially interesting for clinical investigation, since this simply increases the likelihood that it will be active against some form of the human disease. *N*-(2-Chloroethyl)-*N'*-(*trans*-4-methylcyclohexyl)-*N*-nitrosourea (MeCCNU) and several closely related compounds are active against advanced Lewis lung carcinoma and other solid tumors that do not respond to BCNU,[8,9] although there is no general agreement concerning the clinical superiority of MeCCNU to BCNU. Cyclophosphamide is clearly active against a much broader spectrum of animal tumors than the parent nitrogen mustard, and it has proven to be a more useful agent in the treatment of human cancers.[10]

A different factor to be considered is *the lack of cross resistance* (or, in an optimistic vein, collateral sensitivity). If an animal tumor that has become resistant to the parent drug responds to the congener, it should be of interest because of the ease with which neoplasms become resistant to many agents. The evidence today is overwhelming that human cancers that initially respond to drug therapy, but later fail to, have become drug resistant as a result of the overgrowth of mutant resistant cells selected by the treatment employed.[11] In fact, for obvious reasons, the more effective the treatment (short of cure), the faster resistance develops, if the cancer cell population is about 10^6 or greater,[12] which it almost always is by the time of diagnosis. It is tempting to think that the proper use of new agents effective against cells that are biochemically resistant to clinically useful drugs will at least permit much longer extension of remissions and at best contribute to curative chemotherapy. Leukemia L1210 that had become resistant to 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (DTIC) still responded to the bis(2-chloro-



DTIC: R = Me

BID: R = 

ethyl) congener BIC, although the reverse was not true.[13] This, along with a much greater cell kill against leukemia L1210 resulting in a high cure rate, was probably the reason for the interest in evaluating this compound clinically. Unfortunately, the clinical potential of BIC has never been realized for reasons that are not entirely clear,[14] although they could relate to the great instability of this compound. Another example is the activity of 9-β-D-arabinofuranosyladenine (*ara*-A) plus the adenosine deaminase inhibitor 2'-deoxycoformycin against L1210 leukemia resistant to *ara*-C,[15] because these cells are deficient in deoxycytidine kinase, the enzyme that phosphorylates *ara*-C, but contributes only slightly to the phosphorylation of *ara*-A. Although this combination has not yet been used in humans, it seems clearly indicated for the treatment of acute myelogenous leukemias that at first respond but later become resistant to *ara*-C treatment. Such leukemias have been treated with some success with 3-deazauridine, a drug

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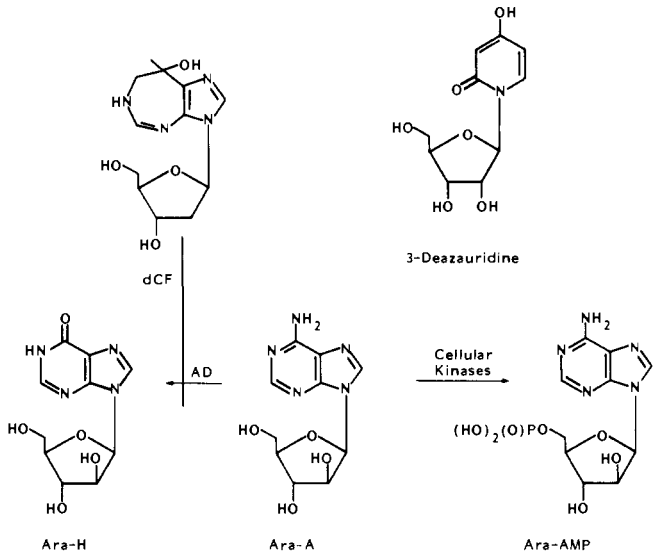
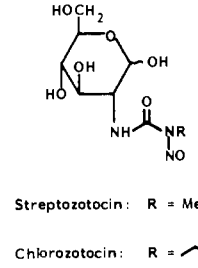
(15) F. M. Schabel in "Nucleoside Analogs", R. T. Walker, E. De-Clercq, and F. Eckstein, Eds., Plenum Press, New York and London, 1979, p 363.

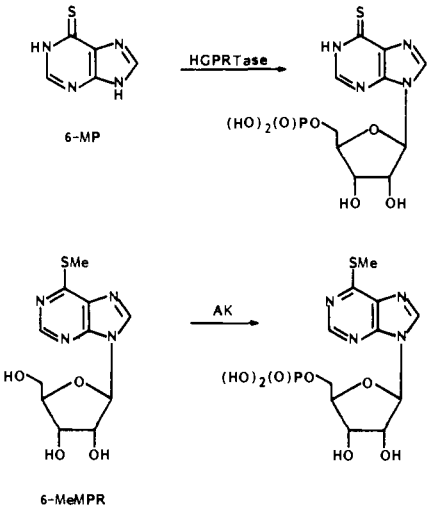


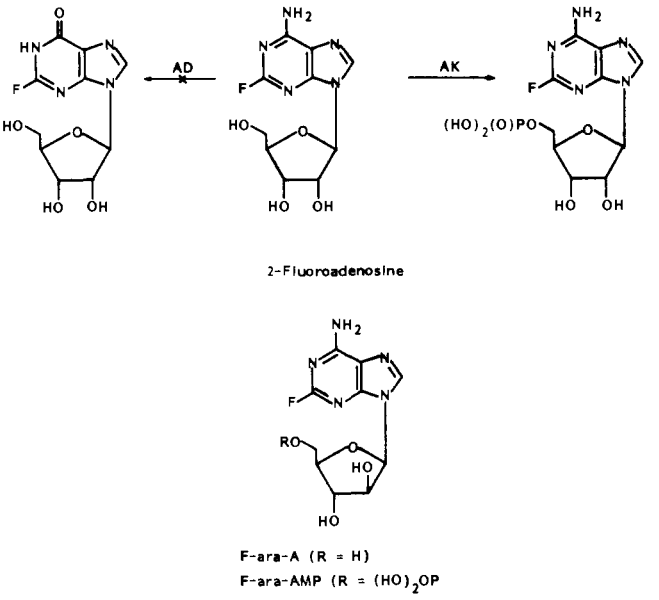
to which leukemia L1210 resistant to *ara*-C has shown collateral sensitivity.[16]

Another goal might be to develop a congener with *a different limiting toxicity to the host*, particularly if the new limiting toxicity is to neither the bone marrow nor the intestinal epithelium. The limiting toxicity of most of the nitrosoureas, such as BCNU and CCNU, is to the bone marrow,[17,18] but this is not true of streptozotocin, a methylnitrosourea antibiotic.[19] This information and the knowledge that all 2-chloroethylnitrosoureas are more active against animal cancer than the corresponding methyl compounds led to the synthesis of chlorozotocin, which proved to be much more active (curative) than streptozotocin in the L1210 system,[20] but no more toxic to the bone marrow of mice.[21] Initial clinical trials indicate that it may be less myelotoxic to humans also.[22-24]



The identification of *congeners that are metabolized differently* is an attractive and, as we now know, an attainable goal. Such a difference underlies the activity of *ara*-A against leukemia L1210 resistant to *ara*-C discussed above. There are a number of other illustrations of this point, one being 6-mercaptopurine and 6-(methylthio)-purine ribonucleoside. 6-Mercaptopurine must be converted by hypoxanthine–guanine phosphoribosyltransferase (HGPRTase) to its ribonucleotide in order to kill cells. On the other hand, 6-(methylthio)purine ribonucleoside is phosphorylated by adenosine kinase (AK) and, therefore, has activity against cell populations that have become resistant to 6-MP because of the overgrowth of cells lacking the transferase.[25] The combination of these two drugs appeared to be synergistic in the treatment of acute myelogenous leukemia in man.[26] A different kind of example is the comparison of 2-fluoroadenosine[27] with adenosine. The placement of the fluorine at position 2 of adenosine reduces drastically its deamination by adenosine deaminase (AD),[28] and, although 2-fluoroadenosine itself





(16) R. W. Brockman, in "Current Chemotherapy", Vol. 1, W. Siegenthaler and R. Luthy, Eds., American Society for Microbiology, Washington, D.C., 1978, p 97.
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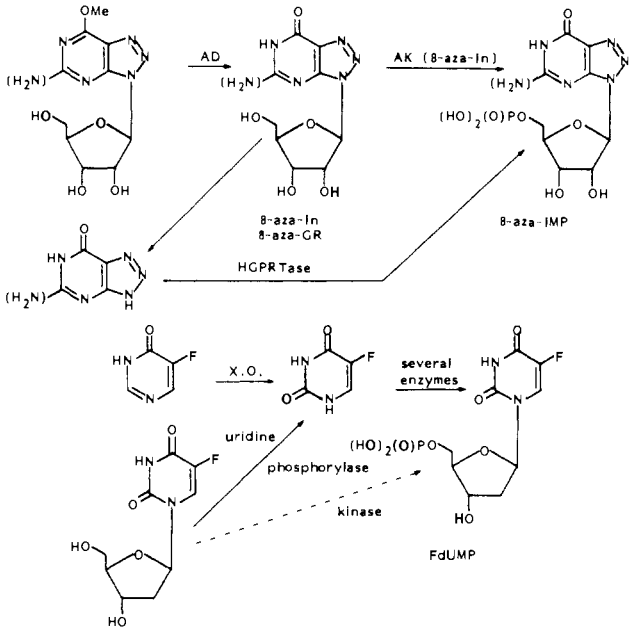
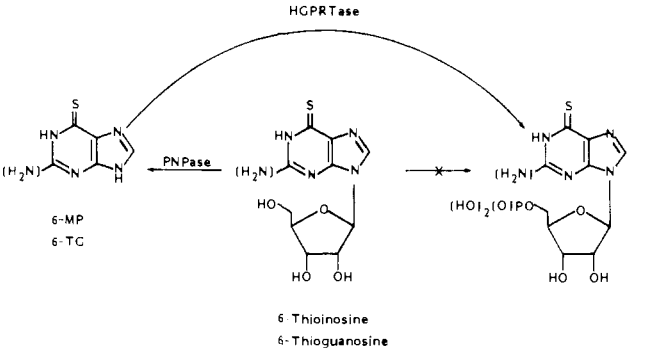
(26) G. P. Bodey, H. S. Brodovsky, A. A. Isassi, M. L. Samuels, and E. J. Freireich, *Cancer Chemother. Rep.*, **52**, 315 (1968).
(27) J. A. Montgomery and K. Hewson, *J. Am. Chem. Soc.*, **79**, 4559 (1957).
(28) O. P. Chilson and J. R. Fisher, *Arch. Biochem. Biphys.*, **102**, 77 (1963).

is too toxic to have any utility as an anticancer agent, this principle was later applied to other nucleosides.[29] For example, the 2-fluoro analogue of *ara*-A was synthesized in the hope that this change would prevent deamination but not phosphorylation. This has proved to be the case: F-*ara*-A is about as active in the L1210 system as *ara*-A given in combination with the deaminase inhibitor 2′-deoxycoformycin.[15,30] Conversion of F-*ara*-A to its 5′-phosphate then gave a water-soluble drug resistant to catabolism, lacking rigid schedule dependency, and highly active in the L1210 system.[15] Catabolic enzymes such as adenosine deaminase can, however, activate other congeneric antimetabolites such as 8-aza-O^6-methylinosine and 8-aza-O^6-methylguanosine, which are converted to 8-aza-

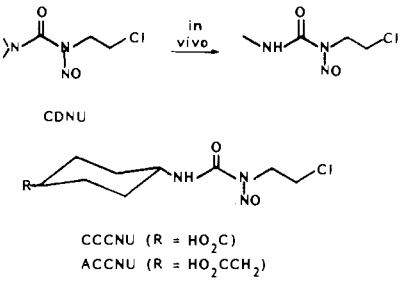


inosine and 8-azaguanosine, both active against leukemia L1210.[31] 5-Fluoropyrimidin-4(1*H*)-one is oxidized by xanthine oxidase to 5-fluorouracil, a conversion that occurs in man,[32] and has demonstrated activity against experimental animal tumors.[33,34]

Not only do we have some guidelines for successful drug modification based on metabolism, we also understand why certain structural modifications of known drugs, such as the ribosylation of 6-mercaptopurine and 8-azaguanine and the deoxyribosylation of 5-fluorouracil, did not result in more active drugs.[35,36] Although these nucleosides can be phosphorylated in cells to their active forms (nucleotides), they are too rapidly cleaved to the corresponding bases for this to occur to any significant extent.



The development of congeners with *more desirable physicochemical properties* but with the same or better activity is a further goal. For example, better stability (either on the shelf or in solution), better solubility in physiologic media, and a more favorable water/lipid solubility ratio (defined by partition coefficient) that might affect drug distribution such as penetration of the blood–brain barrier or of solid tumors are properties that can be designed into certain kinds of agents. Stable nitrosoureas, such as *N*-(2-chloroethyl)-*N*′,*N*′-dimethyl-*N*-nitrosourea (CDNU), that are metabolized in vivo to active forms have



been prepared.[37] The difficulties in formulating MeCCNU for iv administration led to the development of a number of nitrosoureas that are easily soluble at physiological pH. These compounds, CCCNU and ACCNU, contain a carboxyl or carboxymethyl group in the 4 position of the cyclohexane ring in place of the methyl group of MeCCNU.[38] They are quite effective against leukemia L1210 and a number of the solid tumor systems, including the colon tumors developed by Corbett.[10] The conversion of *ara*-A and F-*ara*-A, two very insoluble compounds, to their phosphates (*ara*-AMP and F-*ara*-AMP), which are easily soluble in aqueous media,[38,39] represents the preparation of prodrug congeners (see below).

Some of the goals that are important in the development of congeners with better activity have been set forth, as well as at least one suggestion for measuring progress toward these goals. Some examples which illustrate how these goals were achieved in specific cases have been given. In general, the strategy for the development of a superior congener depends quite a bit on the type of agent and on the specific agent under the general type. For example, the approach to developing better chemically reactive agents such as the nitrogen mustards or the nitrosoureas is quite different from the approach to developing better antimetabolites, be they analogues of *ara*-C, 6-mercaptopurine, 5-fluorouracil, or methotrexate.[40] The activity of a lead compound has been improved in several ways (in

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(36) C. Heidelberger in "Antineoplastic and Immunosuppressive Agents", Part II, A. C. Sartorelli and D. G. Johns, Eds., Springer-Verlag, Berlin, Heidelberg, and New York, 1975, p 193.
(37) R. B. Brundrett, J. W. Cowens, and M. Colvin, *Proc. Am. Assoc. Cancer Res. ASCO*, 17, 102 (1976).
(38) T. P. Johnston, G. S. McCaleb, S. D. Clayton, J. L. Frye, C. A. Krauth, and J. A. Montgomery, *J. Med. Chem.*, **20**, 279 (1977).
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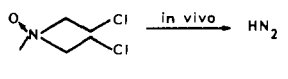
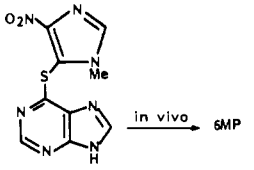
addition to the examples already cited).

(1) By Latentiation. Drug latentiation has been defined as the chemical modification of a biologically active compound to form a new compound which upon in vivo enzymatic attack will liberate the parent compound.[41] Compounds so modified are currently referred to as prodrugs.[42] Examples of latent forms or prodrugs are cyclophosphamide[43] and nitromin[44] (chemically reactive agents) and imuran[45] and *O*-acyl derivatives of nucleosides[46] (antimetabolites).

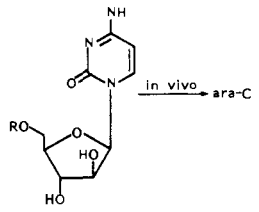


Nitromin

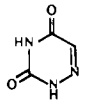


Immuran

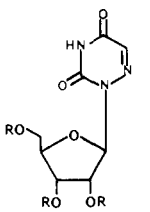
(2) By Altered Drug Absorption and Distribution. One such alteration would be the depot forms of drugs that act by slow release of the active compound, as illustrated by the palmitate (palmo-*ara*-C) or other acyl derivatives



Palmo-ara-C
R = $CH_3(CH_2)_{14}CO$



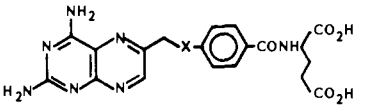
6-Azauracil



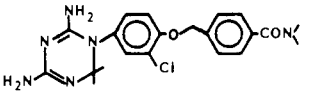
in vivo → 6-Azauridine = R = H
Triacetate: R = Ac

of *ara*-C.[46] This may also be thought of as a special type of latentiation. The triacetate of 6-azauridine is an example of a progression in drug development from 6-azauracil, which was not at all useful, to 6-azauridine, which had better activity and less side effects than the base analogue, but was not absorbed orally, to 6-azauridine triacetate, which had the desirable properties of 6-azauridine and was absorbed from the gut when administered orally.[47] As already mentioned, the partition coefficient of drugs affects distribution also and an alteration in the partition coefficient of drugs such as the nitrosoureas changes their ability, for example, to cross the blood–brain barrier.[48]

(3) By Altered Transport. Methotrexate is an effective agent in the treatment of some human cancers and



Methotrexate: X = N-Me
Aminopterin: X = NH
10-Deazaaminopterin: X = CH_2



Triazinate

its effectiveness, or lack of it, has been related to transport into the target cancer cells.[49,50] It has been established that structural alterations can affect transport favorably. Differential transport may, in fact, be the basis of the greater therapeutic index of methotrexate compared to aminopterin.[51] Further, structural changes can enhance activity against certain types of cancer. For example, 10-deazaaminopterin has a broader spectrum of activity against rodent tumors than methotrexate, and this enhanced activity, which has been related to transport,[52] is the basis of a clinical trial.

(4) By Attachment of Chemically Reactive Groups to the Parent Drug. Although the untimely death of B. R. Baker left his hypothesis for achieving selective cytotoxicity by means of active-site-directed irreversible inhibitors unproven, his work has provided strong support for the concept,[53] and at least one of his diaminodihydrotriazine congeners, triazinate, is being investigated clinically.[54,55]

In summary, then, the validity of congener synthesis is well established, and there are some guidelines to the types of alteration of a drug that can be made in hopes of effecting improvement in the desired activity. One of the biggest stumbling blocks so far has been a lack of mutual understanding between the clinicians and the chemists as to what goals we should be trying to achieve and as to whether or not these goals have been achieved as measured by the best means we have available—animal models.[56] This comment is specifically directed toward the improvement of clinical activity of antitumor agents through congener synthesis.

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