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Perspective

The Renewed Potential for Folate Antagonists in Contemporary Cancer Chemotherapy

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Introduction

Cancer chemotherapy is entering a new era of research based, in large part, on the study of oncogenes and their protein products. Hopefully, these studies will reveal new directions in the search for rational means to detect, treat, and prevent cancer.[1] Regrettably, the exploitation of new insights and technology has historically been slow to impact directly on patient care. Although antioncogene/antiprotein research will no doubt contribute to revolutionizing our understanding, this crest in the cycle of periodic advances cannot reasonably be expected to effect patient care or cancer mortality in a meaningful way during the next 5–10 years.

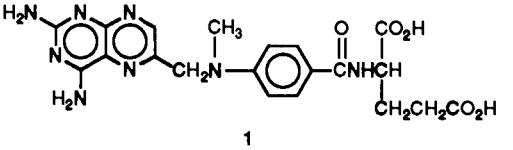
It will be necessary, then, to bridge the gap preceding the introduction of the new generation of cancer therapeutic agents. There are, of course, immediate roles for relatively new adjuvants like colony-stimulating factors,[2] immunomodulators, immunotoxins/immunoconjugates,[3] and monoclonals, though some of these will be appropriate only for less common malignancies. Some application will also be made of modern molecular genetics in cancer diagnostics. However, increases in survival or improvement in the quality of life for most cancer patients during the next decade will likely be accomplished in large part by second or even third-generation traditional cytotoxics. With this in mind, it is important that we look critically and insightfully at our current drugs and ask what rational extensions can be made to improve chemotherapy over the near term.

The antifolate area[4a,b] has been the recipient of just such a reexamination. No other traditional area of cancer chemotherapy has generated more interest or enthusiasm for renewed potential. This enthusiasm is sparked by new strategies for circumventing some forms of resistance and toxicity and by the recent recognition of enzymatic targets where intervention with inhibitors has just recently been shown to be of therapeutic value.

In this perspective, we briefly review five relatively new antifolates in some phase of clinical development.[4c] We hope to identify the enlightened strategies and approaches which have rejuvenated this area while speculating on their more far-reaching implications for future drug discovery.

Inhibitors of Dihydrofolate Reductase: Old Target, New Strategies

Methotrexate (MTX, 1), an inhibitor of dihydrofolate reductase (DHFR), has an accepted place in cancer chemotherapy, both as a single agent and in combination regimens. It continues to be used widely and effectively as the treatment of choice for choriocarcinoma and in acute lymphocytic leukemia. It is included in a variety of combination regimens to treat diffuse lymphomas, osteogenic sarcoma, and head and neck, lung, cervical, ovarian, and bladder carcinomas.



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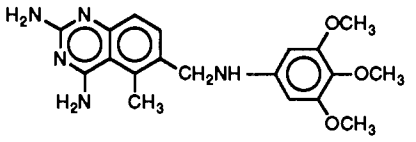
Like most cytotoxic antineoplastics, however, the therapeutic value of MTX is not without clinical liabilities.[5] These shortcomings include marrow-related toxicities, the common development of acquired resistance, and inherent resistance (limited tumor spectrum). Although medicinal chemists have searched for a "better MTX" for the last 30 years, their efforts have been largely unsuccessful. However, the extensive study some of the older DHFR inhibitors have received has not been without benefit. New findings relevant to drug pharmacology, drug resistance, schedule dependency, and tumor selectivity have emerged from this work and this information has led, as we will describe below, to the clinical development of both modified DHFR inhibitors and potent inhibitors of new folate targets.

Nonclassical. The 1960s saw the emergence of a unique class of DHFR inhibitors. These quinazoline and pyrimidine analogues of folic acid are called nonclassical or lipophilic because they lack the glutamate residue found in classical DHFR inhibitors like MTX. The overriding consideration in the design of these agents was their potential to circumvent the resistance due to (1) impaired transport associated with classical antifolates or (2) reduced intracellular drug concentrations due to altered or low levels of folylpolyglutamate synthetase (FPGS). This early attempt to design rationally new DHFR inhibitors for the circumvention of MTX resistance is one of the first mechanistically motivated strategies to impact the antifolate area.[6] Metoprine (DDMP), a first generation lipid-soluble DHFR inhibitor, demonstrated clinical antitumor activity but was plagued by nonfolate-related toxicity. These side effects were eventually attributed to the drugs long half-life and DDMP's potent inhibition of histamine-*N*-methyltransferase.

Two second-generation nonclassical DHFR inhibitors have now been taken to clinical trial. Both trimetrexate



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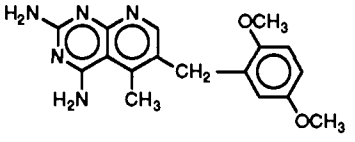

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(**2**, CI-898; 5-methyl-6-[[(3,4,5-trimethoxyphenyl)amino]-methyl]-2,4-quinazolinediamine)[7] and piritrexim (**3**, BW-301U; 2,4-diamino-6-(2,5-dimethoxybenzyl)-5-methylpyrido[2,3-*d*]pyrimidine)[8] are nonclassical DHFR inhibitors which do not require the MTX reduced folate carrier system (active transport) to enter cells.[9] In addition, they are not substrates for folylpolyglutamate synthetase and, as such, do not depend on polyglutamation to achieve intracellular retention, tumor selectivity, or high affinity for their target enzyme, DHFR. Their means of cellular retention is not known.

In preclinical models[4,7b] trimetrexate has a tumor spectrum superior to that of MTX with activity against P388 and L1210 leukemias, B16 melanoma, CD8F1 mammary carcinoma, colon adenocarcinomas 26, 36, 38, and M5076 sarcoma. It is inactive against the Lewis lung carcinoma and the MX-1, CX-1, and LX-1 human tumor xenografts in nude mice, as in MTX. The biochemical pharmacology of trimetrexate has been reviewed.[4,11] Studies have demonstrated that in vitro in tumor cell lines resistant to MTX due to defective drug transport, trimetrexate retains activity. Some MTX-resistant tumors are collaterally sensitive to trimetrexate. Trimetrexate penetrates cells rapidly. Its transport is independent of the reduced folate carrier system, yet not entirely attributable to passive diffusion.[4,10,12]

Recent work by Klohs and others has shown that unlike MTX, trimetrexate is vulnerable to multidrug resistance (MDR).[12,13] MDR, whether acquired or intrinsic, has been shown to be a clinically relevant form of resistance wherein drug is effluxed out of cells. Several mechanisms for the efflux have been advanced. Klohs,[14] Beck,[15] Ramu,[16,17] and many others have reported on a variety of agents which somehow modulate drug trafficking to inhibit drug efflux and permit drug retention. In this way, these agents potentiate the activity of antifolates and many other anticancer drugs made ineffective by MDR and so restore some level of drug sensitivity to tumor cells.

Klohs has completely reversed the effects of MDR and achieved the complete restoration of full activity for trimetrexate in several MDR cell lines with reserpine (**4**).[18]

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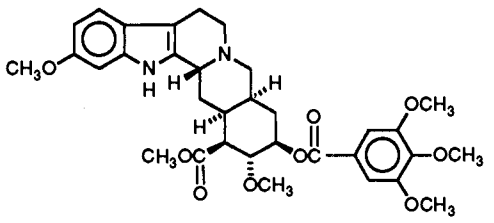

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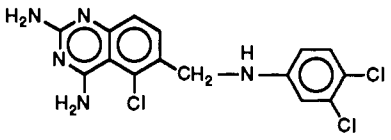
In addition, these workers have identified several classes of lipophilic, nonclassical DHFR inhibitors, including quinazolines related to trimetrexate, which are not subject to MDR.[19] Specifically, 5, a chlorinated analogue of



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trimetrexate completely overcomes MDR in vivo in a P388 leukemia MDR (adriamycin resistant) model, giving activity identical with that seen in the adriamycin-sensitive tumor.

It is intriguing to speculate on the significance of such findings and the contributions they could make to understanding MDR and to the discovery of superior antifolates. For example, what structural relationship might there be between those agents which potentiate drug activity in MDR tumors and drugs which either have the inherent ability to overcome MDR or an inherent susceptibility to it? How could this be relevant to the rational design of new antifolates which overcome MDR?

At this point, it might be reasonably concluded that we have indeed achieved two significant advances needed in an improved anticancer antifolate. For example, the nonclassical compound 5 is not subject either to active transport related resistance or to MDR. It is problematic, therefore, to explain why this compound is inactive in a variety of colon tumor xenografts.[20] Colon tumors, notorious for their inherent resistance to chemotherapy, have been shown by Klohs to express intrinsically the MDR phenotype.

It is now clear that the ability of a drug to overcome transport-related resistance and MDR is a necessary yet insufficient criterion for activity in colon carcinomas and possibly other refractory malignancies. Extensive studies by Klohs and co-workers specifically have addressed the inadequacy of lipophilic antifolates which overcome these forms of resistance but which nevertheless are inactive in colon tumor xenografts. They have demonstrated in vitro that pharmacokinetics may play a key role in their failure.[18] Although none of these drugs bind to P-glycoprotein, the membrane-associated protein responsible for MDR drug efflux, the intracellular concentration of lipophilic compounds are not maintained at therapeutically adequate levels in drug free media. This is indicative of passive diffusion of the drug out of the cell. A primary challenge in the design of new nonclassical agents, therefore, must be either the ability to achieve adequate intracellular drug concentrations through some retentive process (such as intracellular metabolism) or to design exceptionally potent agents without increased toxic liabilities.

In clinical trials of trimetrexate, responses have been observed in pretreated patients who had been unsuccessfully treated with other agents. Responses have been reported in patients with colon, head and neck, breast, and nonsmall cell lung cancer. Relatively mild hematologic toxicity was observed, with rapid recovery, and no cumulative dose effects were noted.[21]

Clinical trials with trimetrexate have been equivocal, however.[22] In Phase 1 trials some activity was observed in patients with lung cancer and colon cancer. Yet, in some Phase 2 trials only minimal responses in nonsmall cell lung cancer were seen.[23] In one Phase 2 trial, however, the response rate for nonsmall cell lung cancer approached 21% in 55 previously untreated patients.[24] This response rate could be exciting since only a few drugs have significant antitumor effects (≥15%) in nonsmall cell lung cancer when used as single agents. The Phase 2 results become more meaningful if one considers evidence suggesting that the scheduling in the initial studies may have been less than optimal for a nonclassical antifolate (see below). It may be possible to achieve greater therapeutic gains with an alternative schedule, such as one employing continuous infusion.

For example, clinical experience with piritrexim suggests that schedules with prolonged drug exposure time may be optimal for nonclassical antifolates.[25] Since nonclassical antifols cannot benefit from enhanced cellular retention associated with polyglutamation, it seems logical that this schedule might help to overcome the effects of passive efflux of lipid-soluble compounds from cells. Indeed, this observation may prove critical for deriving maximal efficacy with nonclassical antifols. The generality of this approach should be tested in prolonged low-dose scheduling of trimetrexate in clinical trials.

Piritrexim (3), like trimetrexate, is a nonclassical, lipophilic DHFR inhibitor which does not require active transport and which overcomes MTX-transport resistance.[8b,26] Like trimetrexate, this drug is also subject to MDR.[12] Unlike, metoprine, Wellcome's earlier lipophilic antifolate, it is a relatively weak inhibitor of histamine *N*-methyltransferase and diamine oxidase. Inhibition of these enzymes had been linked to the central nervous system (CNS) toxicity which plagued metoprine in the clinic. More importantly, piritrexim has an improved

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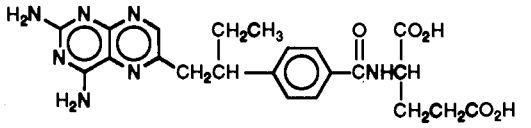
pharmacokinetic profile with a greatly reduced plasma half-life compared to metroprine.

In preclinical in vivo tumor models, the drug has significant antitumor activity in Walker 256 carcinoma, P388 leukemia, sarcoma 180, and Ehrlich ascites carcinoma, but only marginal activity in L1210 leukemia and B16 melanoma.[27]

The possible reduction in the side-effect profile of piritrexim coupled with potential advantages it might have against some tumor cells resistant to classical antifols made piritrexim an attractive candidate for clinical trials. Like trimetrexate, it has demonstrated clinical anticancer activity.[28] In Phase 1 clinical trials with drug administered intravenously the major toxicities were hematologic, skin rash, oral mucositis, and phlebitis. No CNS toxicity was observed. In early Phase 2 trials the drug had only minimal activity on patients with advanced (Stage III) nonsmall cell lung cancer. In subsequent trials, it was reported to have activity against malignant melanomas, lung cancer, colon cancer, sarcoma, and head and neck cancer.

Classical. Another interesting DHFR inhibitor currently in clinical trials, *N*-[4-[1-[(2,4-diamino-6-pteridinyl)methyl]propyl]benzoyl]-L-glutamic acid (10-ethyl-10-deazaaminopterin, 10-EdAM, **6**), is a classical glutamate-



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containing analogue of aminopterin.[4,29] In preclinical models, 10-EdAM has been shown to be superior to MTX, including activity in tumor xenografts where MTX is inactive. It should be noted that evaluation of each of the resolved C-10 diastereomers of 10-EdAM in vivo against L1210 leukemia in mice showed no significant difference in efficacy for the two isomers.[30] The isomers were essentially comparable with respect to biochemistry and transport as well.

10-EdAM is particularly interesting because it is among the few anticancer agents which exploits differences between tumor cells and normal cells to achieve greater selectivity and, hence, less toxicity.[31] Specifically, it has been demonstrated to have enhanced uptake, retention, and polyglutamate formation in tumor cells with concomitant rapid clearance from normal cells.[31b] Comparative studies have shown that EdAM is only a slightly better inhibitor of DHFR than MTX, yet it is considerably more effective in in vivo models as an antitumor agent.[31c] This has been attributed to its differential active transport and enhanced polyglutamation in tumor cells. That membrane transport is a key determinant of selective cytotoxicity had been demonstrated previously in the aminopterin series where it was shown preclinically that N-10 alkyl analogues are transported less efficiently into normal proliferative tissues, like gut, than into tumor tissue.

In a randomized Phase 2 study comparing MTX and 10-EdAM in patients with advanced head and neck squamous cell cancer, the activity of the two compounds was essentially equivalent.[32] Among a total of 25 evaluable patients, 10-EdAM gave a response rate of 27% (3/11 patients) and MTX gave a 21% (3/14 patients) response rate. With respect to side effects, the dose-limiting toxicities were stomatitis and bone marrow suppression.

Hair loss and cutaneous toxicity were more severe in patients treated with 10-EdAM than for those receiving MTX. In another study in nonsmall cell lung cancer (NSCLC) with previously untreated patients with Stage III or IV disease, 6 of 19 (32%) experienced a major objective response.[33] In this case mucositis was the most common toxic side effect. Myelosuppression was minimal.

Recently, preclinical combination chemotherapy studies of 10-EdAM with 5-FU or alkylating agents against advanced metastatic disease in murine tumor models have been reported.[34a] The combination of 10-EdAM and cyclophosphamide was the most active combination with curative responses and therapeutic synergy against three tumor models (E0771 mammary adenocarcinoma, T241 fibrosarcoma, and advanced L1210 leukemia).

In clinical combination chemotherapy study,[34b] a combination regimen employing 10-EdAM/mitomycin/vinblastine was evaluated in patients with advanced NSCLC who had not received prior chemotherapy. This study produced a 60% response rate. Overall, clinical data thus far indicates that 10-EdAM warrants continued advanced clinical evaluation, particularly in comparison to standard agents currently used for NSCLC treatment.

A consideration of the respective advantages offered by the lipophilic antifols and those of classical ones like 10-EdAM poses some dilemmas for rational drug discovery. For example, one might ask if it is necessary to sacrifice the tumor selectivity achieved by a glutamate-bearing drug

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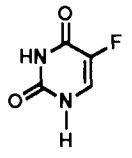
like 10-EdAM in order to overcome transport-related resistance. Is it possible (or even desirable) to design a folate antagonist that does not require active transport but which, once inside the cell, can be sequestered or trapped through a cellular chemical modification akin to polyglutamation. Rosowsky[35] pioneered this kind of strategy in attempting to circumvent the transport defect of MTX-resistant tumor cells by designing lipophilic glutamate esters of MTX where cellular uptake was achieved by passive transport.

Inhibitors of Thymidylate Synthase: *N*-10-Propargyl-5,8-dideazafolic Acid (CB 3717) and Analogues

Known structure–activity relationships among quinazoline analogues of folic acid suggested that 2-amino-4-hydroxyquinazolines rather than 2,4-diamino analogues had greater affinity for thymidylate synthase (TS) than DHFR and that alkylation of N-10 enhanced TS inhibition.[36] Until recently, however, there were no known folic acid analogues with *selectivity* for inhibitory activity against TS. Jackson and Niethammer reported that the effective target of MTX changes from DHFR to TS in cells resistant to MTX due to overproduction of DHFR.[37] In these resistant cells, TS became rate-limiting for growth in the presence of MTX. TS has been considered a viable biochemical target for chemotherapeutic intervention for many years.[38c] 5-Fluorouracil (5-FU, **7**) for example, is



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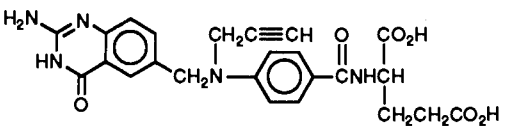
alleged to be a suicide inhibitor of TS and it has useful clinical activity as a single agent and in combination chemotherapy.[38,39] 5-FU treatment has several drawbacks including (1) toxic side effects possibly resulting from its misincorporation into RNA, (2) resistance often develops due to reductions in the cellular levels of kinases required for metabolic activation, and (3) as a single agent it has a narrow spectrum of activity. A folic acid analogue with inhibitory activity against TS could have potential therapeutic advantages in that it might be less toxic and would not require a kinase.

With these considerations in mind, Jones studied the effects of N-10 substitution on quinazoline analogues of folic acid. He found that the N-10 substituent was an important determinant of TS inhibitory activity.[40] Overall, it was clearly the propargyl group which imparted the greatest TS activity to the quinazolines. The structure–activity relationships further revealed that large polar or charged substituents on N-10 were unfavorable for TS activity.

N-[4-[[(2-amino-3,4-dihydro-4-oxo-6-quinazolinyl)-methyl]-2-propynylamino]benzoyl]-L-glutamic acid (N^{10}-propargyl-5,8-dideazafolic acid, CB 3717, **8**), a selective inhibitor of TS, was synthesized by Jones and coworkers and first reported in 1981.[41] CB 3717 is a tight-binding, selective inhibitor with a K_i of 4.5 nM and a 10-fold greater affinity for TS than DHFR. Intracellular polyglutamation of **8** serves two important functions. First, it transforms the parent drug from a form which readily diffuses out of cells to the tetra- and pentaglutamates which are retained well and are themselves extremely potent inhibitors of TS.[42] This has been observed with some, but not all, C-2 modified analogues (see below) as well.[43] Second, compared to the parent **8**, the polyglutamates are more selective for inhibition of TS than DHFR. Although the in vitro and in vivo activity of this compound as well as its biochemistry have been reviewed elsewhere,[4] several findings should be noted here.



8

First, in murine models **8** is only weakly active against P388 leukemia, a standard in vivo preclinical test model.[45] The preclinical evaluation of TS inhibitors in murine tumor models is complicated by the high circulating thymidine levels in rodents which might obscure the effects of TS inhibition.[44] Second, in vitro, **8** is active against MTX-resistant tumor cells which overproduce DHFR.[45] In addition, **8** does not utilize the MTX carrier for transport, though this is not true for some analogues (see below).[46]

In Phase 1 clinical trials, **8** had modest activity against breast, ovarian, and liver cancer.[42b,47] However, dose-

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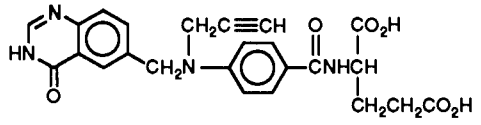
limiting renal and hepatic toxicity with associated malaise were severe and life threatening. Since the renal toxicity was attributed to precipitation of the drug in the kidneys, the design of less toxic analogues focused on increasing solubility at physiological pH. Toward that end, C-2 substituted analogues were evaluated.[48] Interestingly, the 2-desamino analogue **9** (*N*-[4-[[(3,4-dihydro-4-oxo-6-



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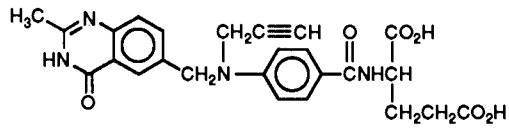
quinazolinyl)methyl]-2-propynylamino]benzoyl]-L-glutamic acid, CB 3804) was 8-fold less potent than **8** as a TS inhibitor, but 10-fold more potent than **8** against L1210 cells in culture. The desamino analogue retained selectivity for TS (K_i = 26 nM) with only weak inhibition of DHFR (K_i = 2.5 μM). Most importantly, the compound is cleared rapidly and no renal or hepatic toxicities were observed in mice after the iv administration of 500 mg/kg. **9** causes significant toxicity at 100 mg/kg. Presumably, the reduced toxicity and superior antitumor activity can both be attributed to its greater solubility at physiological pH.

A series of 2-desamino-2-substituted analogues of **8** were evaluated with respect to TS inhibition, folylpolyglutamate synthase (FPGS) substrate activity, cytotoxicity, plasma clearance, and hepatic/renal toxicity.[43,49] Surprisingly, TS inhibition was generally tolerant of increasing steric bulk at the 2-position, whereas cytotoxicity was not. Heteroatom substituents at the 2-position were well-tolerated. All of the analogues were more water soluble than **8** and all were devoid of liver and renal toxicity in mice. 2-Desamino-2-methyl analogue (*N*-[4-[[(3,4-dihydro-2-



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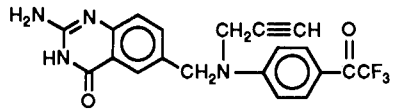
methyl-4-oxo-6-quinazolinyl)methyl]-2-propynylamino-[benzoyl]-L-glutamic acid, CB 3819) is noteworthy among the series of C-alkylated analogues. **10** was 30 times more cytotoxic than CB 3717 despite the fact that it had only half the affinity for TS.

That the 2-desamino compound and 2-substituted analogues retain significant activity is an interesting finding suggesting that the 2-position of DHFR inhibitors or other antifolates may also be vulnerable to modifications without significant loss of activity. This opens the door to new approaches to solubilizing folic acid analogues such as quinazolines where poor solubility could impede clinical development.

A series of selective, nonclassical quinazoline TS inhibitors have also been reported recently.[50a,b] These 10-propargyl-5,8-dideazafolic acid derivatives were investigated for structure–activity relationships emanating from variations in the phenyl substituent at the 4-position which bears a glutamate in the CB 3717 series. In the 2-amino quinazoline series, potent inhibition of TS was achieved with several substituents with 4-(trifluoroacetyl) analogue **11** (2-amino-6-[[propynyl[4-(trifluoroacetyl)phenyl]-



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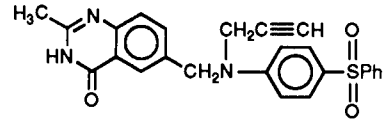
amino]methyl]-4(3*H*)-quinazolin-4-one) being optimal. As with the classical TS inhibitors, the 2-desamino analogues exhibited diminished, yet significant TS inhibitory activity relative to their 2-amino counterparts. Many of the analogues of both the 2-amino and 2-desamino series also overcame MDR in vitro in a P388 adriamycin-resistant leukemia cell line. Unfortunately, most of the analogues were very insoluble and had poor dose potency with respect to cytotoxicity.

More recently, Jones also reported on lipophilic TS inhibitors designed with the benefit of the high-resolution X-ray structure of the *Escherichia coli* TS.[50c]

In these studies, optimal activity was achieved with diphenyl sulfone analogue **12** (4-[*N*-[(3,4-dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-(prop-2-ynyl-amino]diphenyl phenyl sulfone). This compound has a K_i of 27 nM for human TS and an IC_{50} value of 1.0 μM for L1210 cells. In addition, the log *P* for **12** is relatively high at 2.9

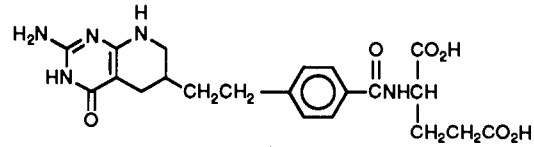


12

Thus reinvestigation of this target has revealed both substantial potential for clinical efficacy and new areas for further exploration.[42c] We await the selection and clinical development of an analogue of **8** in the future.

5,10-Dideaza-5,6,7,8-tetrahydrofolic Acid (DDATHF)

5,10-Dideaza-5,6,7,8-tetrahydrofolic acid (**13**, DDATHF, *N*-[4-[2-(2-amino-3,4,5,6,7,8-hexahydro-4-oxopyrido[2,3-*d*]pyrimidin-6-yl)ethyl]benzoyl]-L-glutamic acid)[51] is the



13

most recently discovered folic acid cofactor analogue, whose site of action is unique among the traditional targets

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(51) DDATHF refers to **12** as a mixture of diastereomers at C-6.

for inhibitors of folate metabolism. DDATHF is a potent, selective inhibitor of de novo purine synthesis through the direct inhibition of glycinamide ribonucleotide (GAR) transformylase.[4,52] It has insignificant inhibitory activity with respect to DHFR and TS, but it is an excellent substrate for FPGS. Biological equivalence with respect to GAR transformylase inhibition and in vitro antitumor activity has been established for **13** as its diastereomeric mixture and for each of the two diastereomers individually.[53] The diastereomers do differ, however, in their transport rates (uptake), metabolism, and in their spectrum of in vivo antitumor activity. The mixture of diastereomers has been antitumor activity against a broad spectrum of preclinical in vivo murine solid tumors, many of which are insensitive to MTX.[54] **13** has activity against X-5563 myeloma, AC 755 adenocarcinoma, 6C3HED lymphosarcoma, colon 26 carcinoma, B-16 melanoma, and Lewis and Madison lung carcinomas. Interestingly, when the individual diastereomers were evaluated spearately against these tumors, it was found that one of the isomers was only active in 6C3HED lymphosarcoma while the other isomer was active in all of the tumors in this panel.[58] This difference might be attributable to differences in transport and metabolism for the two isomers. Specifically, it has been shown that the isomers differ significantly in their degree of conversion to polyglutamate forms and in their distribution among various polyglutamate chain lengths.

New analog synthesis has focused on the 5-deaza-5,6,7,8-tetrahydrofolic acid analogue with N-10 substituents.[55] In general, reintroduction of the nitrogen at the 10-position does not have a deleterious effect on GAR transformylase inhibitory activity, in vitro cytotoxicity, or FPGS substrate activity. This suggests that substitution of carbon for nitrogen at position 5 of DDATHF is the critical change necessary for changing the locus of action from DHFR to GAR transformylase.

The N-10 unsubstituted, 10-formyl, 10-acetyl, and 10-methyl analogues all exhibit potent in vitro antitumor activity. In particular, the N-10 unsubstituted analogue 5-DATHF was even more potent than the parent DDATHF in inhibiting the growth of leukemia cells in culture. It has comparable in vivo antitumor activity against 6C3HED lymphosarcoma. The diastereomers of this analogue were evaluated and found to be equipotent in vitro. 5-DATHF is also superior to the parent as a substrate for FPGS. Modifications of the phenyl ring of the parent compound DDATHF have also been reported to produce compounds with antitumor activity against murine solid tumors in mice.[56]

In in vitro combinations studies, synergy between lipophilic DHFR inhibitors **2** and metoprine with DDATHF has been reported.[57] The in vivo demonstration of the effect has not yet been reported.

Here then we have a new folate pathway target which also seems to have excellent potential for clinical utility.

Conclusion

This brief review considers the current status of antifolates as anticancer agents. Recent developments in this area have directed attention to new considerations. Classical and nonclassical antifolates each offer their own advantages and liabilities relevant to selectivity, tumor spectrum, resistance, and toxicity. Similar considerations arise with regard to inhibitors of new enzymatic targets such as TS or GAR when compared with the more tried and true, but wanting, DHFR inhibitors. The data emerging from new structure–activity relationships for traditional molecules, nonclassical structural variants, and new enzymatic targets suggests that these areas may offer considerable potential for improved therapeutic efficacy for patients. Investigators in the field must now turn their attention to the exploration in depth of these compelling questions in an effort to convert hypothesis into viable patient care.

Registry No. Folic acid, 59-30-3.

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