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Glycinamide ribonucleotide transformylase (GAR TFase) as a target for cancer therapy

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CONTENTS

Abstract

Cancer of all types is the second leading cause of death in the United States. While there are many different treatment options, antimetabolites inhibit aberrant cell growth by interfering with the formation or utilization of a normal cellular metabolite. Most antimetabolites interfere with the enzymes responsible for DNA synthesis or are incorporated into newly formed DNA, or both. Some antimetabolites are analogues of important cofactors, including the folates. Antifolates have been discovered, synthesized and evaluated that are capable of inhibiting glycinamide ribonucleotide transformylase (GAR TFase), an enzyme within the de novo purine biosynthetic pathway. The establishment and further characterization of GAR TFase as a target for cancer therapy will be reviewed herein.

Introduction

Antifolates were among the first antimetabolites developed and were introduced into clinical use in the late 1940s (aminopterin and methotrexate) (Fig. 1) (1, 2). These antifolates were found to inhibit dihydrofolate reductase (DHFR), which generates tetrahydrofolate (THF) from dihydrofolate and therefore maintains a cellular supply of this important coenzyme. Methotrexate, a nonselective antifolate and cytotoxic agent, suffers from toxicity, ineffectiveness against many types of human cancer and the development of tumor cell resistance (3, 4). Efforts have been focused on developing analogues with greater transport selectivity, lower toxicity and a broader range of activity against human cancers. The three enzymes most studied as targets for antifolate inhibition are DHFR, thymidylate synthase (TS) and, more recently, glycinamide ribonucleotide transformylase (GAR TFase). GAR TFase, a trifunctional protein, catalyzes the fourth step in a series of 11 reactions required for de novo purine biosynthesis. GAR TFase utilizes the cofactor (6*R*)-N^{10}-formyltetrahydrofolate to transfer a formyl group to the primary amine of its substrate, glycinamide ribonucleotide (GAR) (Fig. 2). This one-carbon transfer incorporates the C-8 carbon of purines and is the first of two formyl transfer reactions, with aminoimidazolecarboxamide ribonucleotide transformylase (AICAR TFase) performing the second formyl transfer reaction. It has been shown that many types of transformed cancer cells have an elevated dependence on the de novo purine biosynthetic pathway and an impaired purine salvage pathway, while normal cells can rely solely on the salvage pathway for purines (5, 6). In fact, some sensitive tumor cell lines have been shown to have lost the capacity for purine salvage in the course of genomic deletions responsible for cell transformation (5). This allows such sensitive cancer cells to be targeted selectively over normal cells by inhibitors of de novo purine biosynthesis, including GAR TFase inhibitors.

Two key elements involved in folate and antifolate metabolism are cellular transport and polyglutamation. The reduced folate carrier (RFC) transports reduced folates and many antifolates into cells, while the high-affinity folate-binding protein (FBP) transports primarily folates (7). There are two isoforms of the folate receptor (FR), a class of FBPs that modulate how folates and antifolates are transported. FR-α is highly overexpressed in many epithelial cancers, including ovarian and head and neck cancers, while FR-β is the more common isoform (8). Selectivity for FR-α over FR-β is a desired trait for antifolates that may limit toxicity. FR-α and FR-β exhibit profound differences in their stereospecificities and affinities for folate coenzymes and antifolates (9).

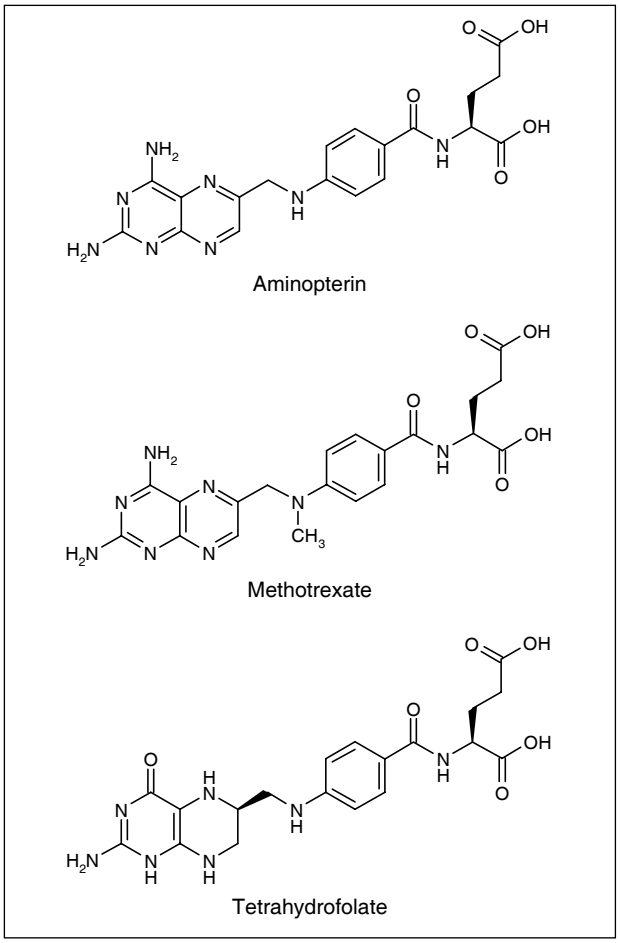


Fig. 1. Antimetabolites and cofactor.

Expression of the folate transport systems varies with cell type and growth conditions. The differential tissue-specific expression of FR isoforms and their elevation in tumors may be important factors that affect the chemotherapeutic potential of antifolates in various tissues.

While the transport of antifolates into the cell is necessary, maintaining a certain level of antifolates within cells is equally important. After entering cells, most folates and many antifolates are modified by the enzyme folylpolyglutamate synthase (FPGS). FPGS attaches additional glutamates (2-9) to the γ-carboxylic acid of folates and antifolates. Polyglutamation of folates and antifolates increases their affinity for the target enzymes and makes them less susceptible to cellular efflux, providing an enhanced and long-lived cellular supply (10). These benefits of polyglutamation, however, are associated with potential limitations. The inability of cells to efflux these polyglutamated antifolates can lead to cumulative toxicity, and many tumor cells experience either an inherent or acquired downregulation of FPGS. This downregulation leads to resistance to classic antifolates that benefit from polyglutamation (11, 12).

Princeton and Lilly inhibitor development

Validation of GAR TFase as a target for cancer therapy came with the discovery that (6*R*)-5,10-dideazatetrahydrofolate (lometrexol, DDATHF) achieves its potent anticancer activity by selective GAR TFase inhibition (Fig. 3) (13). This discovery established GAR TFase and the purine de novo biosynthetic pathway as viable targets for antineoplastic intervention. The original synthesis of racemic lometrexol was reported by Taylor and cowork-

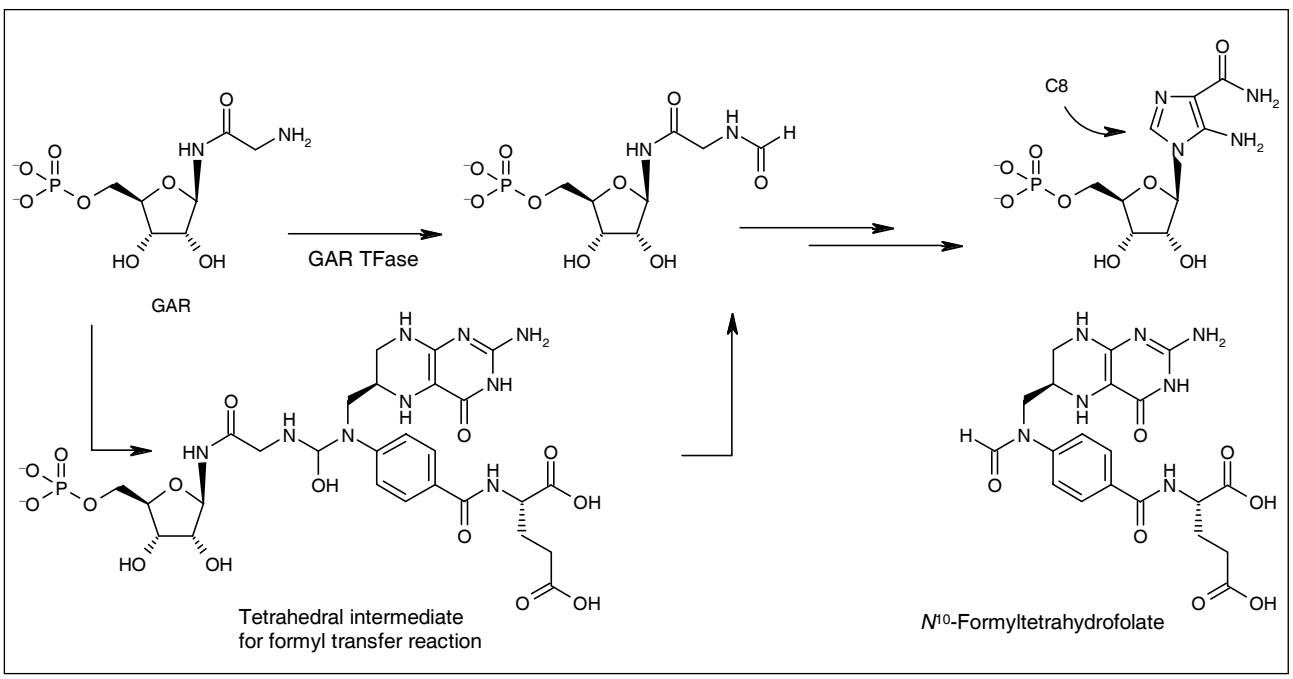


Fig. 2. Reaction catalyzed by glycinamide ribonucleotide transformylase (GAR TFase).

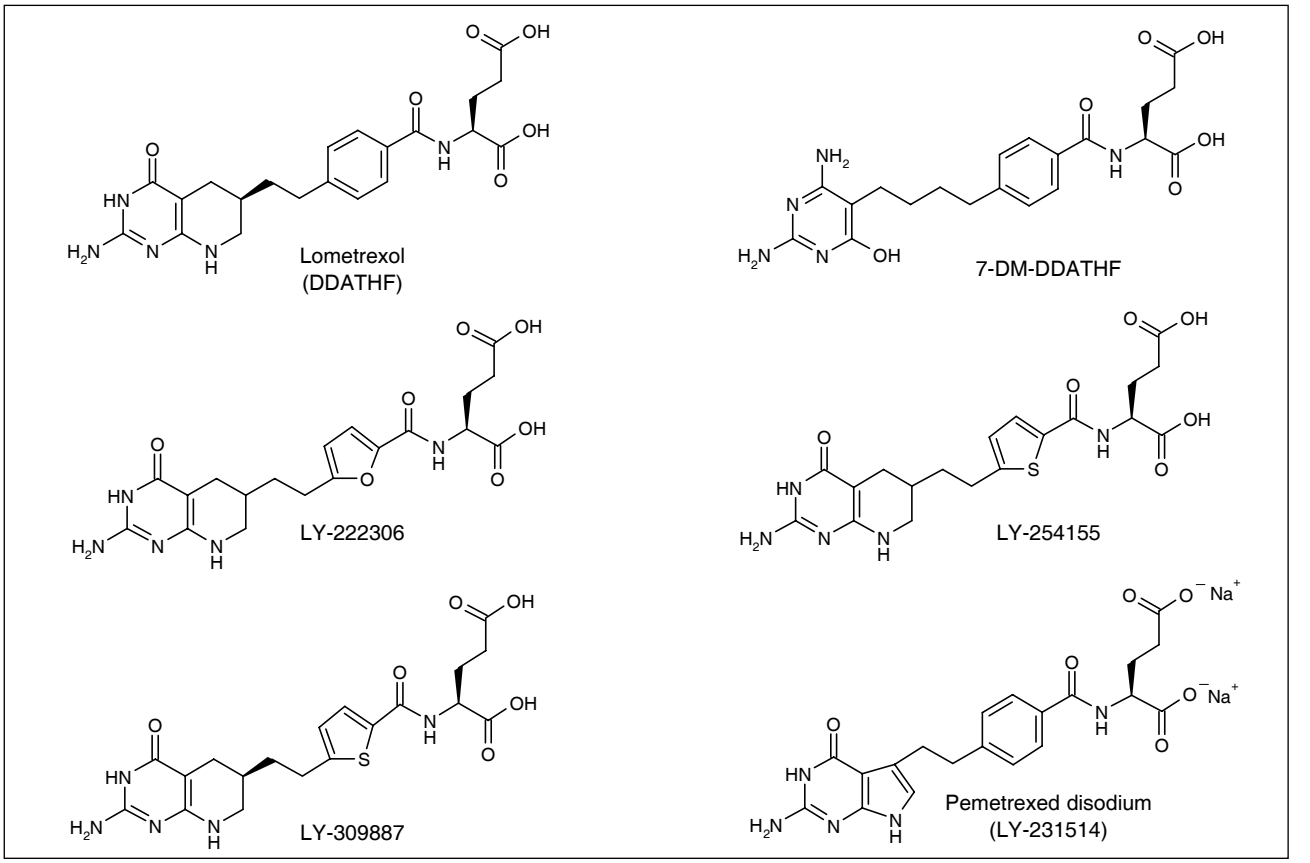


Fig. 3. Princeton/Lilly inhibitors.

ers at Princeton University in 1985 (14). Lometrexol differs from the natural cofactor THF by the placement of carbons at the 5- and 10-positions, precluding it from serving as a cofactor for a one-carbon transfer. In all other aspects, lometrexol is structurally identical to THF. DHFR inhibitors usually possess a 2,4-diamino pattern, while lometrexol is a fully reduced tetrahydrofolate analogue possessing a 2-amino-4-oxopteridine. Lometrexol is a poor inhibitor of both DHFR and TS, but a potent inhibitor of GAR TFase (K_i = 0.006 μM) and a substrate for FPGS (15, 16). The diastereomers were separated and found to be equipotent as inhibitors of cell growth and as substrates for FPGS (16). The (6*R*)-diastereomer, which corresponds to the configuration found in natural tetrahydrofolates, was chosen for further development. Clinical investigations started in 1987, but observations of delayed cumulative toxicity in phase I clinical trials prompted investigations of the effects of folate status on efficacy, metabolism, pharmacokinetics and toxicity (17). Lometrexol's cumulative toxicity is thought to be due in part to its ability to be transported by both the RFC and FBP, resulting in increased cellular levels, as well as its FPGS polyglutamation-inhibiting efflux (18, 19). It was determined that augmentation with folic acid was needed to avert lometrexol toxicity and still allow optimal therapeutic effect (20, 21). In the absence of folic acid supplementation, patients treated with lometrexol in phase I clinical trials developed severe and cumulative myelosuppression and mucositis.

During the development and evaluation of lometrexol, additional analogues were designed under a collaboration between Lilly and Princeton University. Open-chain analogues of lometrexol were synthesized and evaluated for activity (22, 23). These analogues did not possess a stereocenter and were therefore less challenging to synthesize. 7-Desmethylene-DDATHF (7-DM-DDATHF) (Fig. 3) is approximately 8-fold less potent than lometrexol against human leukemia CCRF-CEM cells (IC_{50} = 0.13 μM vs. 0.016 μM) and only 3-fold less active against GAR TFase isolated from murine leukemia L1210 cells. 7-DM-DDATHF is also a substrate for FPGS and its polyglutamated conjugates are more potent inhibitors of GAR TFase than the parent compound. Removal of the annulated tetrahydropyridine ring of lometrexol had minimal effect on binding to GAR TFase.

Unexpected observations of delayed cumulative toxicity with lometrexol led to a search for a second-generation antimetabolite with a more favorable toxicological, biochemical and pharmacological profile. In 1994, Lilly reported a novel class of monoglutamated antifolates that replaced the 1',4'-phenylene of lometrexol with a 2',5'-furan (LY-222306) and a 2',5'-thiophene (LY-254155) (Fig. 3) (24). Both LY-222306 and LY-254155 (mixtures of diastereomers) were found to be potent inhibitors of

CCRF-CEM cell growth (IC_{50} = 27 and 2.3 nM, respectively) and tight binders of human GAR TFase (K_i = 0.77 and 2.1 nM, respectively). The thiophene analogue LY-254155 demonstrated a 6-fold decrease in affinity for membrane FBP compared to lometrexol, indicating that it might display less cumulative toxicity. Polyglutamation of the thiophene analogue is not required to achieve tight-binding inhibition of monofunctional human GAR TFase, possibly allowing it to have increased antitumor activity against solid tumors expressing very low levels of FPGS or tumors resistant due to increased γ-glutamyl hydrolase activity.

Further evaluation of LY-254155 commenced in 1996 after the compound was resolved into its two diastereomers (25, 26). LY-309887 (6*R*-2',5'-thienyl-5,10-dideazatetrahydrofolic acid), like lometrexol, is activated by FPGS, but has a lower first-order rate constant. Polyglutamation of LY-309887 was less extensive and livers of mice accumulated fewer polyglutamates of LY-309887. The affinities of LY-309887 and lometrexol for the isoforms of human FRs were also compared. As mentioned earlier, FR-α is highly overexpressed in many epithelial cancers, including ovarian and head and neck cancers, while FR-β is the more common isoform. Lometrexol has 6-fold higher affinity for FR-α than LY-309887, but the latter has greater selectivity for FR-α over FR-β (10.5 vs. 5.0). In vivo, LY-309887 was more potent than lometrexol at inhibiting tumor growth in the murine C3H mammary tumor model and several tumor xenografts. LY-309887 displayed 9-fold greater inhibitory potency against GAR TFase compared to lometrexol. In preclinical models, LY-309887 appeared to be more active than lometrexol against two pancreatic xenografts and in the human LX-1 lung carcinoma model. Based on these observations and upon completion of preclinical toxicology studies of lometrexol, Lilly discontinued its development in favor of developing LY-309887. In two preliminary reports of phase I studies with LY-309887, all patients received concurrent folic acid but still suffered from cumulative toxicity, suggesting that cumulative toxicity remains a property of LY-309887 (27, 28).

In 1992, Taylor and coworkers reported the synthesis of what would become the FDA-approved drug pemetrexed disodium (*N*-[4-[2-(2-amino-3,4-dihydro-4-oxo-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-L-glutamic acid disodium salt, Alimta, LY-231514) (Fig. 3) (29). Pemetrexed is a structurally novel antifolate that possesses a 6,5-fused pyrrolo[2,3-*d*]pyrimidine nucleus instead of the more common quinazoline or 6,6-fused pteridine structure. Pemetrexed was originally reported to inhibit TS, but it is now recognized that pemetrexed and its polyglutamates also inhibit DHFR and GAR TFase (30). The pentaglutamate of pemetrexed has significantly enhanced inhibitory activity against GAR TFase compared to the monoglutamate (K_i = 65 nM vs. 9300 nM; 144-fold). Pemetrexed is regarded as a multitargeted antifolate (MTA) due to its ability to inhibit multiple folate enzymes. In February 2004, pemetrexed in combination with cisplatin became the first drug to be approved by the U.S. FDA for the treatment of malignant pleural mesothelioma. In August of the same year, it was granted accelerated approval for the second-line treatment of non-small cell lung cancer as a single agent. Pemetrexed was approved in Europe for both indications in September 2004 by the European Medicines Agency (EMEA). At this time, there are dozens of clinical trials ongoing to determine the suitability of pemetrexed as monotherapy and in combination with other chemotherapeutic agents for the treatment of breast, colorectal, bladder, cervical, endometrial and ovarian cancer and sarcoma (31).

Burroughs Wellcome inhibitor development

The former Burroughs Wellcome (now GlaxoSmithKline) also developed inhibitors of GAR TFase in the late 1980s and early 1990s (Fig. 4). 11-Deazahomofolate (**1**) and 11-deaza-10-methylhomofolate (**2**) and their reduced derivatives **3** and **4** showed slight growth inhibition of the bacteria *Streptococcus faecium* and *Lactobacillus casei* and very little activity against GAR TFase isolated from *L. casei* (32). These compounds also did not inhibit DHFR, TS or AICAR TFase. In 1990, Wellcome Research presented an acyclic analogue of 5,6,7,8-tetrahydrofolic acid that inhibited the growth of Detroit 98 cells with an IC_{50} of 110 nM (33). 5-DACTHF (Fig. 4), while it did not inhibit DHFR or TS, did inhibit GAR TFase isolated from hog liver (IC_{50} = 2.6 μM) and showed slight inhibition of AICAR TFase (IC_{50} = 200 μM in L cells). Wellcome Research reported additional 5-DACTHF analogues in 1992 with the modification of the 2-position of the pyrimidine ring, substitution on the benzoyl ring and substitution at N-10 (34). Substitution of carbon, oxygen and sulfur for nitrogen at the 10-position or the addition of fluorine at the 2'- or 3'-position resulted in compounds that were only slightly less potent than 5-DACTHF. Conversely, alterations in the four-carbon chain or in the pyrimidine ring resulted in loss of activity. Lastly, in 1994, Wellcome Research reported the synthesis and activity of thienyl and thiazolyl analogues of 5-DACTHF (35). The thiophene analogue **5** was equal in activity to 5-DACTHF in controlling MCF7 breast cancer cell growth, while thiazole **6** was 9-fold more active than 5-DACTHF and 4 times more active than lometrexol. Thiazole **6** and lometrexol have very similar activity against hog liver GAR TFase.

Agouron/Pfizer inhibitor development

The former Agouron Pharmaceuticals, now Pfizer, disclosed a novel inhibitor of GAR TFase in 1996, AG-2034 (4-[2-(2-amino-4-oxo-4,6,7,8-tetrahydro-3*H*-pyrimidino[5,4-*b*][1,4]thiazin-6-yl)-(*S*)-ethyl]-2,5-thienoyl-L-glutamic acid), that was designed from the x-ray structure of the GAR TFase domain of the human trifunctional enzyme (Fig. 5) (36). Analysis of the GAR TFase active site using the GRID program indicated that the placement of two sulfur atoms should increase affinity for two regions of the folate cofactor binding site. AG-2034 retains substrate activity for RFC and FPGS, while incor-

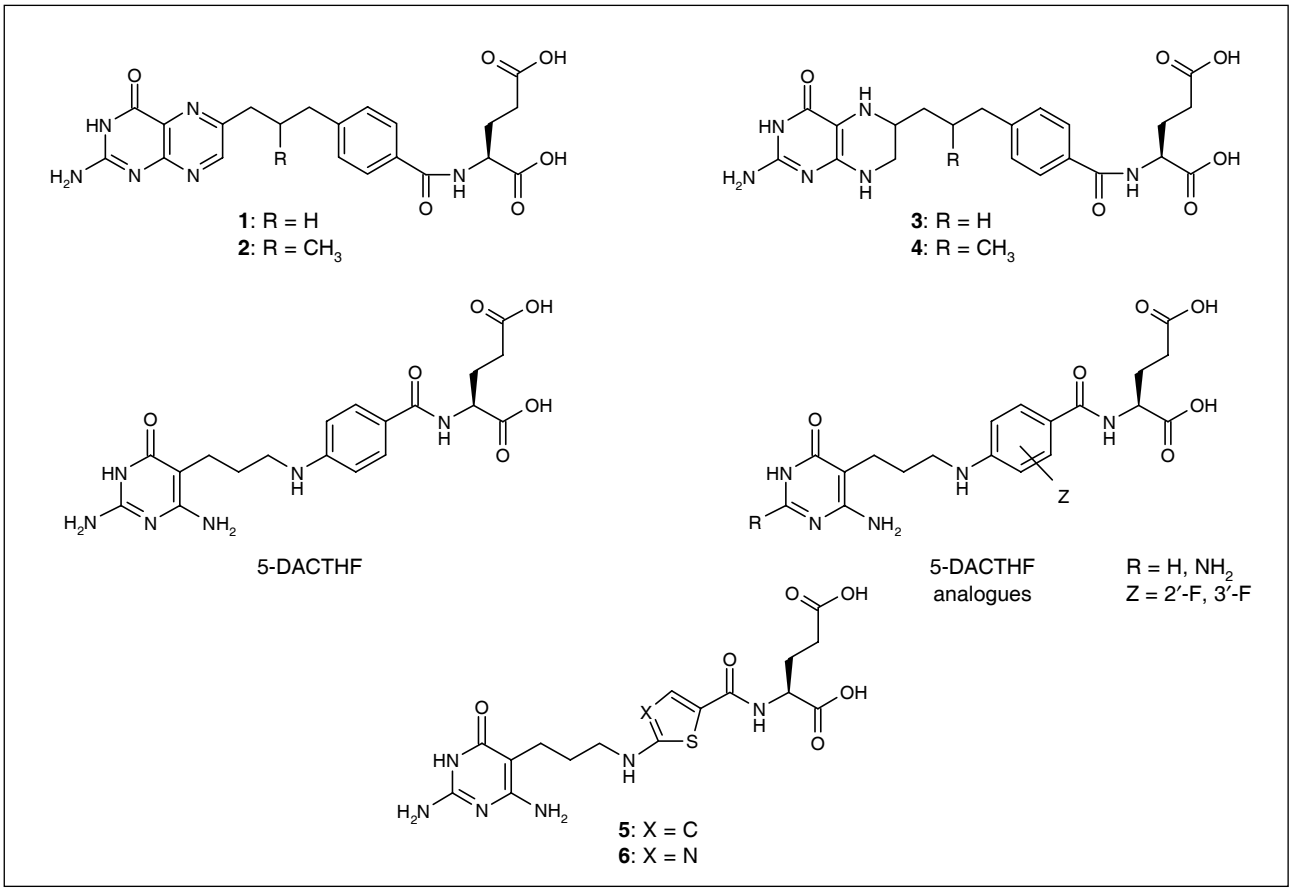


Fig. 4. Burroughs Wellcome inhibitors.

porating two sulfur atoms to maximize binding in the folate cofactor binding site of GAR TFase. It should be noted that AG-2034 differs from LY-309887 by one sulfur substituent within the pteridine ring. AG-2034 inhibits hGAR TFase (K_i = 28 nM) and is a substrate for rat liver FPGS, with similar efficacy as lometrexol, while AG-2034 is a more effective ligand for FBP than lometrexol. AG-2034 inhibited cell growth (IC_{50} = 4.0 and 2.9 nM, respectively, against L1210 and CCRF-CEM cells) and exhibited in vivo antitumor activity against lung carcinomas, lymphosarcomas, mammary and colon adenocarcinomas and melanoma. Like lometrexol, AG-2034 was more toxic to mice fed a folate-deficient diet for 2 weeks before dosing. Phase I clinical studies with AG-2034 were completed without concurrent folic acid treatment (37). AG-2034 displayed much less cumulative myelosuppression than lometrexol, but like lometrexol did result in mucositis and diarrhea. Further study of AG-2034, including with concurrent folic acid administration, is warranted.

Agouron researchers continued their work on x-ray crystal structure-designed analogues, providing 5-thia-2,6-diamino-4(3*H*)-oxopyrimidines (38). Both mono- and bicyclic systems were examined, with the initial conclusions being used to guide further analogue development (Fig. 5). It was concluded that the atoms that link the sulfur at the 5-position of the pyrimidinone to the glutamate portion are flexible and do not optimally fill the available active site space. This led to the synthesis of further compounds to fill this available space. The bicyclic compounds showed increased enzymatic activity over the monocyclic pyrimidinones, and the 2,5-thienyl compounds showed tighter binding than the 1,4-phenylene within the bicyclic series. Addition of a methyl to the 4-position of the thienyl group did not alter the enzyme (GAR TFase) activity of the compound, but this change greatly reduced the compound's affinity for FBP. This decrease in FBP affinity is important because the toxicity of lometrexol is thought to be derived from its transport by both the RFC and FBP.

Agouron/Pfizer's AG-2037 (pelitrexol) is currently in phase I clinical development and varies only slightly from LY-309887 (Fig. 5) (39). The configuration at C-6 is reversed and the thiophene is methylated at the 4-position in AG-2037. AG-2037 is a potent inhibitor of GAR TFase (K_i = 0.5 nM) and exhibits significant antiproliferative effects against tumor cells in vitro and in vivo (40). Several phase I studies have been completed that indicate that AG-2037 is well tolerated and its maximum tolerated dose and schedule for phase II studies have been determined (41, 42). Expected adverse events include mild to moderate myelosuppression, mucositis, diarrhea, anorexia, fatigue and peripheral neuropathy.

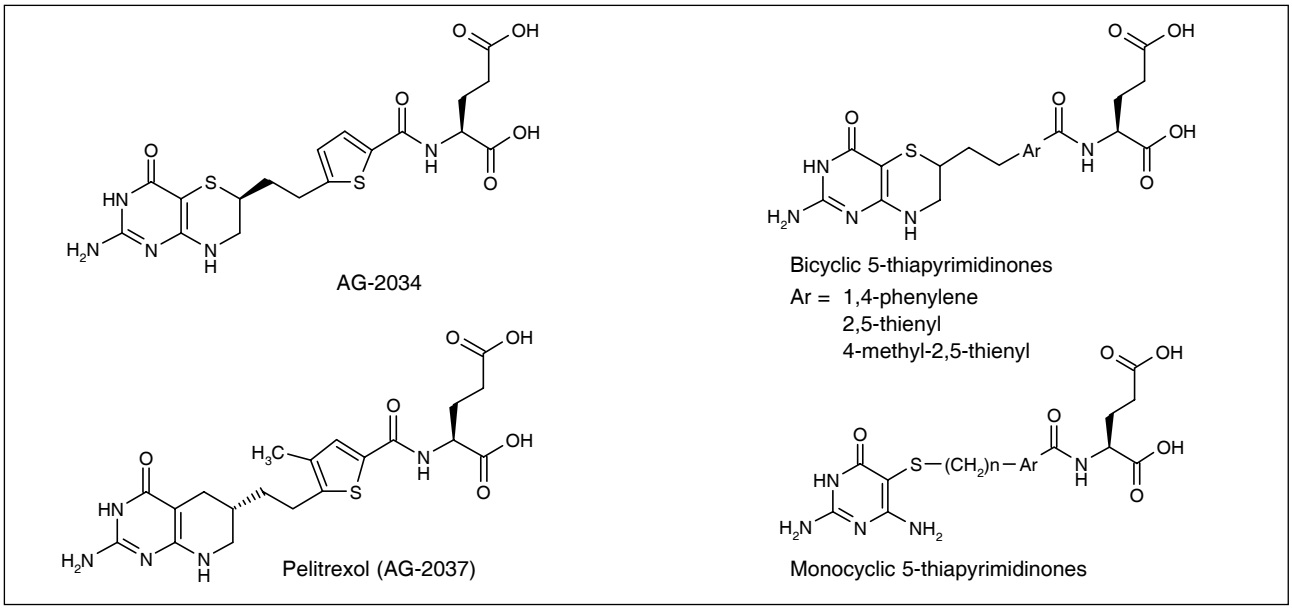


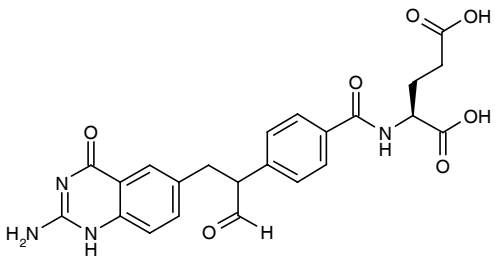
Fig. 5. Agouron/Pfizer inhibitors.

Scripps inhibitor development

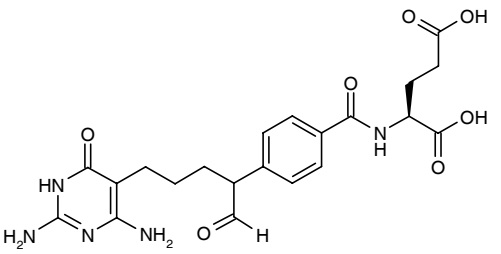
In addition to the pioneering studies of Taylor et al. at Princeton, another major academic contributor to the exploration of GAR TFase as a cancer therapy target is the Boger-Wilson collaboration at The Scripps Research Institute (Fig. 6). Their work started in 1997 with the disclosure of the synthesis and evaluation of 10-formyl-5,8,10-trideazafolic acid (10-formyl-TDAF) as a folate-based inhibitor incapable of transferring the formyl group (43). 10-Formyl-TDAF was designed to take advantage of not only the nontransferable aldehyde, but also the trideazafolate core in which the pterin was replaced with a quinazoline ring system. The use of this substitution was based on previous work that showed 10-formyl-5,8-DDAF constitutes an efficient alternative cofactor for GAR TFase (44). It was designed to form an enzyme-assembled, tight-binding multisubstrate adduct inhibitor (MAI) accessible with the only folate-dependent formyl transfer enzymes GAR TFase and AICAR TFase. 10-Formyl-TDAF exhibited potent inhibition of *pur*N GAR TFase, with a K_i of 0.26 μM, but did not exhibit the expected tight-binding or time-dependent inhibition characteristic of the formation of an enzyme-assembled MAI. 10-Formyl-TDAF was also shown to be sensitive, prone to oxidative deformylation and a poor substrate for the reduced folate carrier. Subsequent x-ray crystallography of 10-formyl-TDAF bound to *Escherichia coli* GAR TFase showed that it bound as the aldehyde gem-diol and not as the designed enzyme-assembled multisubstrate adduct (45). This gem-diol mimics the tetrahedral intermediate of the formyl transfer reaction. This discovery conceptually defined the formyl transfer reaction as one analogous to the classic protease or transaminase reactions, where electrophilic carbonyls have also served as transition-state mimics.

Work continued with the synthesis and evaluation of 10-formyl-5,10-dideaza-acyclic-5,6,7,8-tetrahydrofolic acid (10-formyl-DDACTHF), an acyclic analogue of lometrexol bearing a nontransferable C-10 formyl group and a modified folate core known to be transported by the RFC and to be a substrate for FPGS (46). 10-Formyl-DDACTHF inhibited cell growth (CCRF-CEM IC_{50} = 60 nM) and hGAR TFase (K_i = 14 nM). Unlike 10-formyl-TDAF, 10-formyl-DDACTHF was a substrate for the RFC and FPGS, but like 10-formyl-TDAF, it suffered from instability due to a facile oxidative deformylation. Continued development of such GAR TFase inhibitors was accomplished by combining the 2,4-diaminopyrimidone core of 10-formyl-DDACTHF with a more stable moiety capable of facilitating and stabilizing the formation of a gem-diol, the trifluoromethyl ketone (47). 10-(Trifluoroacetyl)-5,10-dideaza-acyclic-5,6,7,8-tetrahydrofolic acid (10-CF_3CO-DDACTHF) is a potent inhibitor of tumor cell proliferation, with an IC_{50} of 16 nM against CCRF-CEM cells, which represents a 10-fold improvement over lometrexol. 10-CF_3CO-DDACTHF is effectively transported into the cell by the RFC, intracellularly sequestered by polyglutamation, and is stable, displaying no competitive oxidative deacylation. It also specifically inhibits recombinant human GAR TFase (K_i = 15 nM) and is inactive against AICAR TFase, DHFR and TS. All of these properties make this compound a candidate and potential lead for in vivo studies as a chemotherapeutic agent.

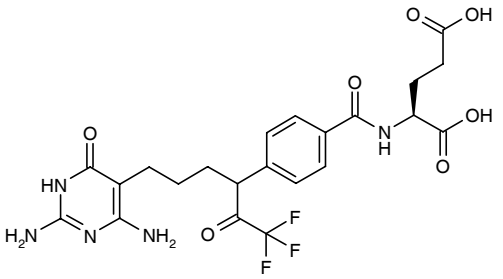
Exploration of analogues containing alternative tetrahedral intermediate mimics continued with the synthesis and evaluation of 10-CH_3SO_2-DDACTHF, 10-CH_3SO_2-5-DACTHF and 10-CH_3S-DDACTHF (48). The sulfone and sulfonamide were chosen to probe their potentially greater stability (no in vivo reduction) and the thiomethyl derivative was synthesized as an intermediate en route to the key analogues. All three compounds exhibited similar



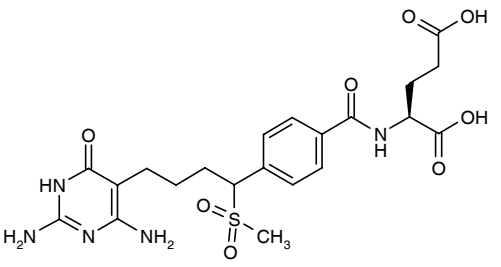
10-Formyl-TDAF



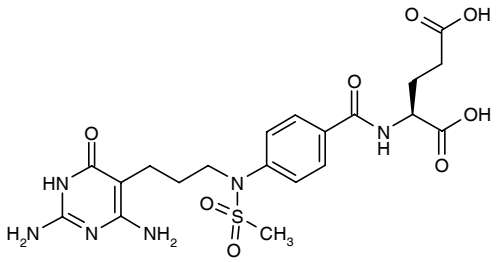
10-Formyl-DDACTHF



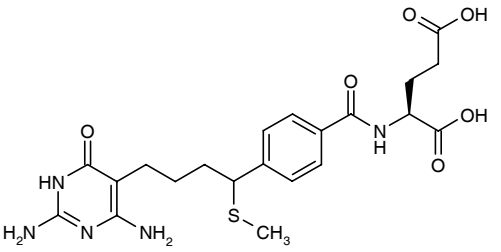
10-CF_3CO-DDACTHF



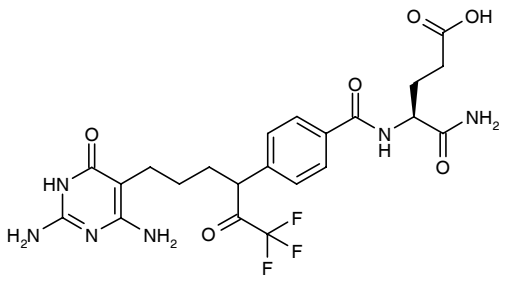
10-CH_3SO_2-DDACTHF



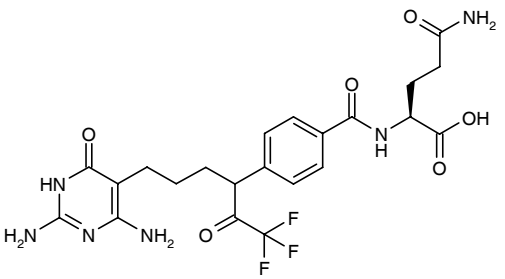
10-CH_3SO_2-5-DACTHF



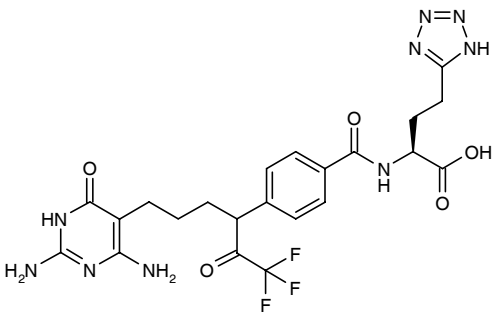
10-CH_3S-DDACTHF



α-$CONH_2$-10-CF_3CO-DDACTHF



γ-$CONH_2$-10-CF_3CO-DDACTHF



γ-Tetrazole-10-CF_3CO-DDACTHF

Fig. 6. Scripps inhibitors.

inhibition of rhGAR TFase (K_i = 230-580 nM), with 10-CH_3SO_2-DDACTHF and 10-CH_3S-DDACTHF being 7 times more potent than the parent unsubstituted DDACTHF. The sulfone and sulfonamide showed moderate cytotoxic activity (IC_{50} = 1 and 2 μM, respectively, against CCRF-CEM cells), whereas 10-CH_3S-DDACTHF proved to be exceptionally potent, exhibiting an IC_{50} of 100 nM (CCRF-CEM). It was also shown to benefit from FPGS polyglutamation and reduced folate carrier transport into the cell. Although 10-CH_3S-DDACTHF does not contain an apparent formyl transfer intermediate mimic, it does incorporate a potential hydrogen bond acceptor and presents a soft hydrophobic substituent for active site binding. Additionally, it bears a C-10 substituent much less prone to epimerization, permitting an independent assessment of the individual C-10 diastereomers. Thus, an asymmetric synthesis of the (10*R*)- and (10*S*)-diastereomers of 10-CH_3S-DDACTHF was conducted, along with several key analogues (49). This work, which defined the unique potency of the thiomethyl substituent, also clarified the importance of the C-10 stereochemistry for this class of inhibitors. Remarkably, both diastereomers are potent and selective inhibitors of rhGAR TFase (K_i = 210 and 180 nM, respectively, for 10*R* and 10*S*) and effective inhibitors of cell growth (IC_{50} = 80 and 50 nM, respectively, against CCRF-CEM cells), which is dependent on intracellular polyglutamation by FPGS but not transport by the RFC. This latter feature suggests a unique opportunity for treating resistant tumor lines that downregulate the RFC.

A further area of examination in these efforts was the glutamic acid portion of 10-CF_3CO-DDACTHF. As stated previously, folates and many antifolates are modified by FPGS after entering cells. FPGS attaches additional glutamates (2-9) to the γ-carboxylic acid of folates and antifolates. Polyglutamation of folates and antifolates increases their affinity for the enzymes and makes them less susceptible to cellular efflux, providing a long-lived cellular supply. However, this long-term enhanced intracellular accumulation of antifolates that results from their polyglutamation contributes to their cumulative toxicity. Therefore, preventing polyglutamation while maintaining potent enzyme binding is potentially a therapeutic asset. γ-$CONH_2$-10-CF_3CO-DDACTHF exhibited potent inhibitory activity against rhGAR TFase (K_i = 56 nM), while α-$CONH_2$-10-CF_3CO-DDACTHF showed decreased affinity (K_i = 4.8 μM) (50). These results are consistent with the x-ray structure of 10-CF_3CO-DDACTHF bound with human GAR TFase, which defined a critical salt bridge between the α-carboxylate and Arg64, while the glutamate γ-carboxylate extends into solvent without making specific interactions with enzyme residues (47). α-$CONH_2$-10-CF_3CO-DDACTHF was inactive in cellular functional assays, whereas γ-$CONH_2$-10-CF_3CO-DDACTHF exhibited purine-sensitive cytotoxic activity (IC_{50} = 300 nM against CCRF-CEM cells). Unlike 10-CF_3CO-DDACTHF, γ-$CONH_2$-10-CF_3CO-DDACTHF is not dependent on FPGS for activity, making it an ideal candidate for in vivo examination. While γ-$CONH_2$-10-CF_3CO-DDACTHF was a potent, nonpolyglutamatable inhibitor of GAR TFase, an even more potent nonpolyglutamatable inhibitor was disclosed in 2006. γ-Tetrazole-10-CF_3CO-DDACTHF exhibits purine-sensitive cytotoxic activity (IC_{50} = 40 nM) and was selective for inhibition of hGAR TFase (K_i = 130 nM) (51). It should be noted that the placement of tetrazoles into glutamic acid moieties has enjoyed much success within another area of antifolate research: TS inhibition (41, 52). As anticipated, the activity of γ-tetrazole-10-CF_3CO-DDACTHF is independent of FPGS activity but benefits from transport by the RFC.

Further development and future success

The knowledge that sensitive tumor cell lines often rely on the de novo biosynthesis of purines since they have an impaired purine salvage pathway, and the recognition that this often results from genetic deletions central to the oncogenic transformation (5, 53), provide a compelling case for the development of selective inhibitors of purine biosynthesis. For example, it is now known that a 9p21 chromosomal deletion that is one of the most prevalent oncogenic transformation steps in many tumors results in the loss of the tumor suppressor genes *CDKN2A* and *CDKN2B* which encode proteins that bind Hdm2, regulating the tumor suppressor p53 and the accompanying loss of the nearby *MTAP* gene. Methythioadenosine phosphorylase (MTAP) is a key purine salvage enzyme whose loss renders the cells completely reliant on de novo synthesis (versus salvage) of purines. Tumors arising from such 9p21 chromosomal deletions are uniquely sensitive to purine biosynthesis inhibitors, whereas normal cells are not. This chromosomal deletion is found in many of the most refractory tumors, including 70% of all gliomas, 50% of all T-cell leukemias, 40% of all lung cancers and 47% of all pancreatic cancers. Genetic typing of a patient or cancer should permit a selection of those that would most favorably respond to a purine biosynthesis inhibitor (54, 55). With this knowledge, it should be possible to more successfully select tumors sensitive to such inhibitors. Central to this pathway is the enzyme GAR TFase, for which selective inhibitors relative to other folate-dependent enzymes have been discovered, making it an especially attractive therapeutic target. Additionally, combining these features with those that are now recognized to avoid the requirements for FPGS polyglutamation or RFC transport many prove especially attractive for the treatment of antifolate-resistant tumors that downregulate FPGS or the RFC.

The success of GAR TFase inhibitors to treat cancer will continue to be dependent not only on their use alone but also in combination with other anticancer agents. Anticancer agents are rarely given alone, but rather as combinations of drugs that act synergistically (56). Most antimetabolites interfere with DNA synthesis, so they are generally combined with drugs that react with DNA. One example of this is the combination of pemetrexed with cis-

platin to treat malignant pleural mesothelioma. Pemetrexed has also been investigated for combined use with gemcitabine, a nucleoside antimetabolite, for the treatment of pancreatic cancer and non-small cell lung cancer (39, 56).

Conclusions

Much effort has been devoted to develop and characterize GAR TFase as a target for cancer therapy by both pharmaceutical companies and academic groups. Although Lilly's pemetrexed is currently the only FDA-approved inhibitor of GAR TFase, it is regarded as a multitargeted antifolate inhibiting many folate-dependent enzymes. Given the compelling case emerging for the clinical use of a selective purine biosynthesis inhibitor, there exists the need and unique opportunity to develop a potent and selective GAR TFase inhibitor. Fortunately, many inhibitor leads have advanced to the stage of definitively characterizing GAR TFase as a cancer therapy target. It will be exciting to witness their in vivo characterization and clinical examination, especially against predictably sensitive tumors.

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