# Inhibitors of de Novo Nucleotide Biosynthesis as Drugs

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**ABSTRACT**

Potent inhibitors of enzymes catalyzing reactions in the de novo pathways for biosynthesis of purine and pyrimidine nucleotides are synthetic or natural-product analogues of pathway intermediates or, more recently, inhibitors rationally designed from a knowledge of the catalytic mechanism. Such inhibitors may be effective drugs against cancer, inflammatory disorders, or various infections. For human cancer, the purine pathway may be a better target for inhibition than the pyrimidine pathway, where toxic side effects are more apparent. Drugs such as methotrexate and 6-mercaptopurine have multiple sites of action, making it difficult to quantitatively predict their effects upon cells. Rational design of inhibitors based upon the X-ray structure of the target enzyme has the prospect of yielding drugs with only one site of action in human cells. Such a drug is VX-497, a potent inhibitor of the purine enzyme, IMP dehydrogenase.

## Introduction

The pathways for de novo biosynthesis of purine and pyrimidine nucleotides produce ATP, GTP, UTP, and CTP, precursors for RNA, and activated metabolites such as UDP-glucose and CDP-choline. Nucleoside diphosphates are converted into dATP, dGTP, dTTP, and dCTP, precursors for DNA. Inhibitors of the enzymes of these pathways may be effective as drugs for treatment of cancer, inflammatory disorders, or various infections. Inhibition of the de novo purine and pyrimidine pathways (Figures 1 and 2) at a particular reaction induces accumulation of intermediates prior to that step and depletion of subsequent intermediates in the pathway, a "metabolic crossover point".[1] The consequent imbalance of deoxynucleotides (dNTPs) may lead to genetic miscoding,[2] and complete depletion of one of the dNTPs would result in arrest of DNA synthesis. Thus, a cell exposed to a nucleotide antagonist may die due to accumulated mutations or DNA strand breaks, usually via apoptosis.[3] The malarial parasite, *Plasmodium falciparum,* can synthesize pyrimidine nucleotides only via the de novo pathway (Figure 1) and is unable to salvage preformed uridine.[4] Potent inhibitors of the de novo pathway may have antimalarial activity with excellent selective toxicity: the parasite dies of a pyrimidine deficiency, while the patient is able to maintain some salvage synthesis of pyrimidine nucleotides from preformed uridine or cytidine in the circulation. This Account is divided into sections for particular enzymes of purine and pyrimidine biosyntheses for which there are potent inhibitors. Numbers for particular reactions of the purine and pyrimidine pathways are derived from Figures 1 and 2.

## P-Rib-PP Synthetase (Rib-5-P → P-Rib-PP)

P-Rib-PP is a substrate for de novo biosynthesis of nucleotides at the first step of the pathway for purines and the fifth step for pyrimidines (Figures 1 and 2). MRPP (Figure 3) is a nucleoside analogue which, following conversion to the 5′-monophosphate (MRPP-MP), is a noncompetitive inhibitor of P-Rib-PP synthetase, with $K_i$ = 190 $\mu$M.[5] MRPP (1 and 10 $\mu$M) inhibited the growth of human HCT 116 colorectal cancer cells. A 4-h exposure to MRPP resulted in significant decreases in nucleotides and P-Rib-PP pools, with maximal decreases after 24 h. The data indicated that MRPP inhibits de novo purine and pyrimidine syntheses and salvage synthesis of nucleotides. ARPP (Figure 3) as the monophosphate derivative, ARPP-MP, is an effective noncompetitive inhibitor of P-Rib-PP synthetase, with $K_i$ = 430 $\mu$M.[6] ARPP-MP accumulates to concentrations approaching 3 mM in human WI-L2 lymphoblasts and acts as an inhibitor of P-Rib-PP synthetase.

## De Novo Purine Biosynthesis

**Amido Phosphoribosyltransferase (APRTase, P-Rib-PP → PRA).** APRTase catalyzes the first committed step of the de novo purine pathway (reaction 1, Figure 1) and is inhibited by AMP, IMP, and GMP.[7] The pentaglutamyl derivative of dihydrofolate and the nonclassical antifolate, piritrexim (Figure 3), are potent inhibitors of APRTase in

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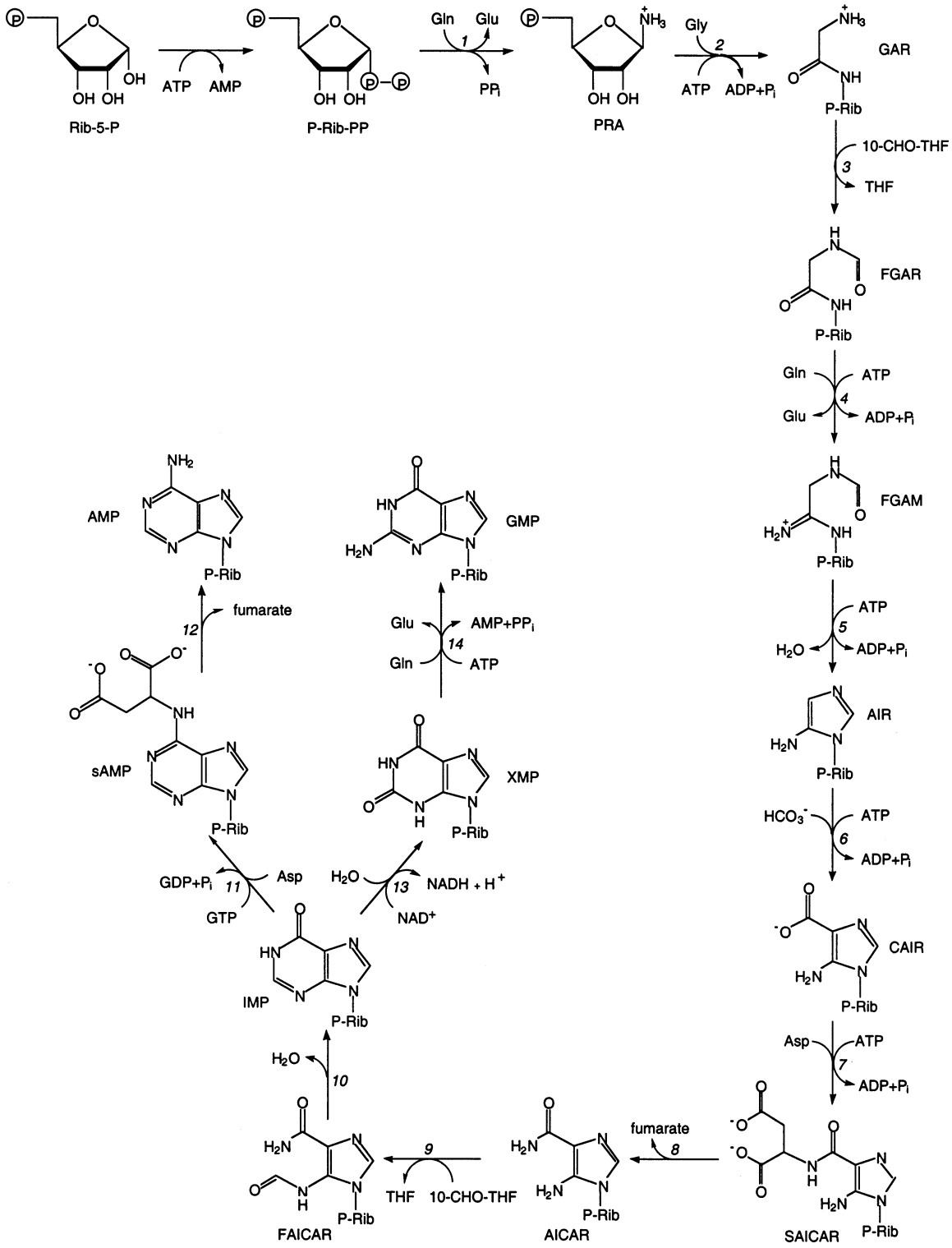


**FIGURE 1.** Pathway for de novo biosynthesis of purine nucleotides. Reaction numbers are indicated above the arrows.

vitro.[7] De novo purine biosynthesis in mouse L1210 leukemia cells exposed to 1 $\mu$M methotrexate is blocked at reactions 1, 3, and 9, presumably due to inhibition of these enzymes by accumulated dihydrofolate polyglutamates.[8] We have shown that APRTase, partially purified from L1210 cells, is subject to noncompetitive (allosteric) inhibition by dihydrofolate pentaglutamate ($K_i$ = 3.1 $\mu$M) and piritrexim ($K_i$ = 6.0 $\mu$M [8]). Specific antifolates could be developed which selectively inhibit APRTase by interaction with this allosteric site. cPRPP is a competitive inhibitor of APRTase from *Escherichia coli*,[9] with $K_i = 116\ \mu$M. cPRPP does not enter growing cells and has been used to probe aspects of the catalytic mechanism.

The thiopurine derivatives 6-mercaptopurine (MP), 6-thioguanine (TG), and 6-methylmercaptopurine riboside (MMPR) have been in clinical use as anticancer drugs for more than 40 years. MP is converted to the nucleoside

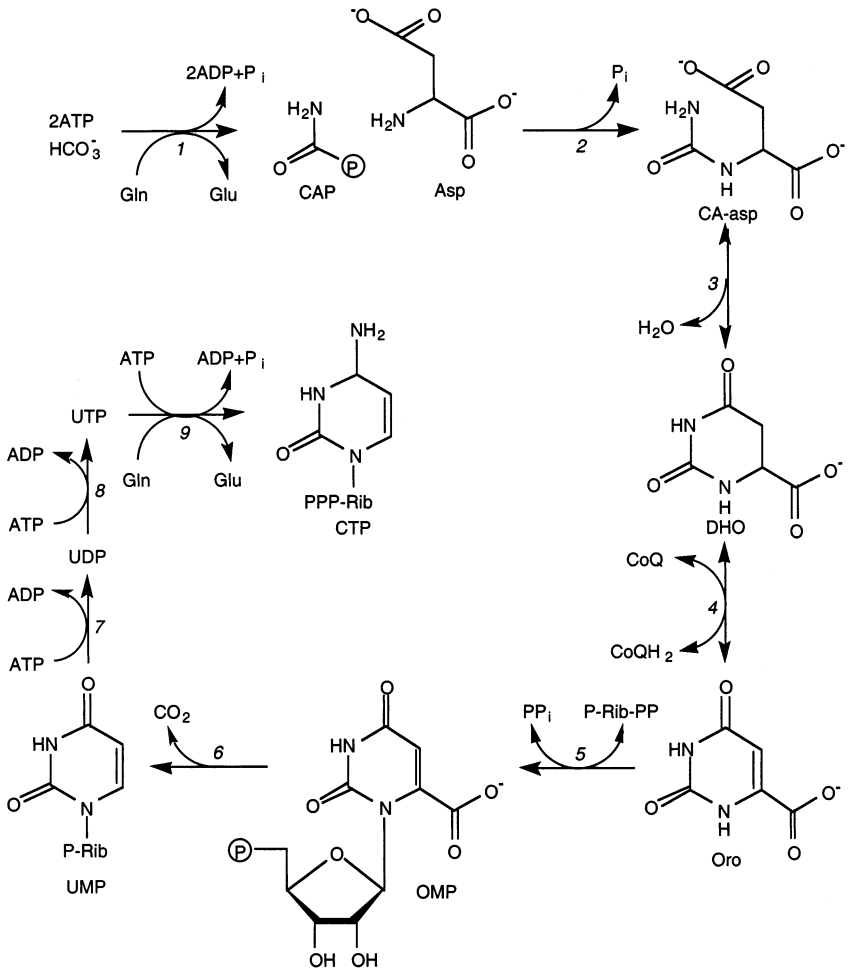


**FIGURE 2.** Pathway for de novo biosynthesis of pyrimidine nucleotides. Reaction numbers are indicated above the arrows.

5′-monophosphate (MPR-MP) and is further metabolized, MP → MPR-MP → thio-XMP → thio-GMP → thio-GDP → thio-GTP, and thioGDP → thio-dGDP → thio-dGTP. MP may also be metabolized via the competing route, MP → MPR-MP → MMPR-MP, with the second reaction catalyzed by thiopurine methyltransferase,[10] which may be expressed at variable levels due to genetic polymorphism. TG is also converted to thio-GTP and thio-dGTP. Misincorporation of MP and TG into nucleic acids is the primary mechanism of cytotoxicity for both drugs. MMPR is converted mostly to MMPR-MP, which inhibits APRTase and the de novo biosynthesis of purine nucleotides in cancer cells. We have determined the apparent $K_i$ values for the three drug monophosphate derivatives as inhibitors of APRTase partially purified from human CCRF−CEM leukemia cells:[11] for MPR-MP, $K_i = 114\ \mu$M; for thio-GMP, $K_i = 6.20\ \mu$M; and for MMPR-MP, $K_i = 3.09\ \mu$M.

**GAR Transformylase (GAR → FGAR).** GAR transformylase catalyzes the transfer of the formyl group from 10-formyl tetrahydrofolate to GAR to produce FGAR and tetrahydrofolate (reaction 3, Figure 1). A number of folate analogues have been identified as inhibitors of GAR transformylase (Figure 4). DDATHF (LY249543) was the first of a new class of inhibitors which affect a folate-dependent enzyme but not dihydrofolate reductase. Lometrexol, the 6(*R*)-diastereomer of DDATHF, lacks the nitrogen atoms at positions 5 and 10 of the pteridine ring and cannot participate in one-carbon-transfer reactions. Polyglutamate derivatives of lometrexol bind to GAR transformylase with an affinity which increases with the length of the polyglutamate chain. The $K_i$ value for interaction of free lometrexol with the human enzyme is 59.7 nM, and the $K_i$ for the pentaglutamate derivative is 5.3 nM.[12] Lometrexol showed anticancer activity in phase I studies but with myelosuppression and mucositis due to accumulation of polyglutamated metabolites. The thiophene (LY254155, $K_i = 2.1\ \mu$M) and furan (LY222306, $K_i = 0.77\ \mu$M) analogues of lometrexol are more potent inhibitors of GAR transformylase. DACTHF is an acyclic analogue of tetrahydrofolate and a specific inhibitor of GAR transformylase ($K_i \approx 0.4\ \mu$M[14]). AG2034 ($K_i = 28$ nM[14,15]) and LY309887 ($K_i = 6.5$ nM) are more potent inhibitors of GAR transformylase than lometrexol.[16] The pentaglutamyl derivative of Alimta, or multitargeted antifolate (MTA, LY231514), is a potent inhibitor of GAR transformylase ($K_i = 65$ nM), AICAR transformylase ($K_i = 265$ nM), thymidylate synthase ($K_i = 1.3$ nM), dihydrofolate reductase ($K_i = 7.2$ nM), and several other enzymes of folate metabolism.[17] The antitumor activity of MTA results from multiple inhibitions of several key folate-requiring enzymes via its polyglutamate derivatives.

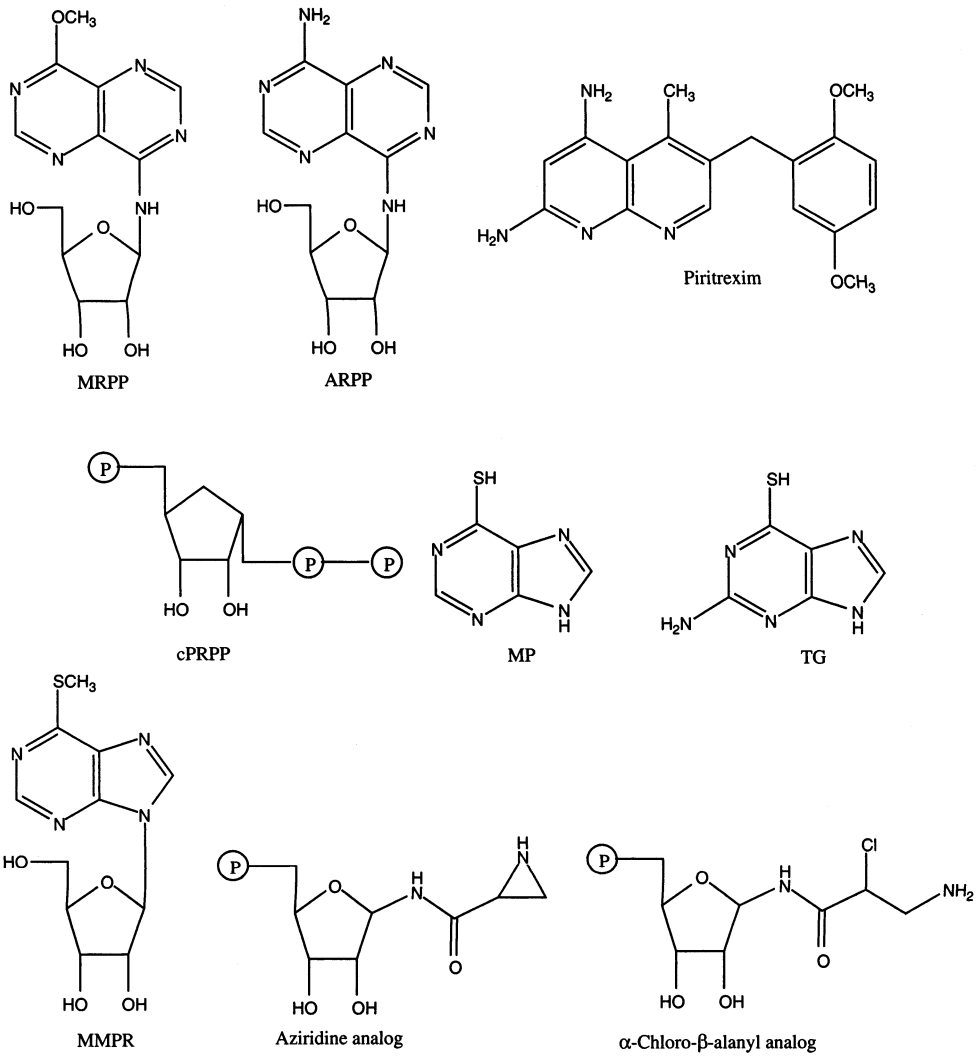


**FIGURE 3.** Inhibitors of P-Rib-PP synthetase, amido phosphoribosyltransferase, and GAR transformylase.

In addition to these antifolates, there are several structural analogues of GAR which inhibit GAR transformylase. The aziridine analogue of GAR (Figure 3) contains a three-membered ring which allows binding to GAR transformylase ($K_i$ = 7.6 $\mu$M) but interferes with catalysis.[18] For the α-chloro-$\beta$-alanyl analogue of GAR, the electronegativity and/or steric bulk of the chloro substituent blocks catalysis, since the $\beta$-alanyl analogue is a relatively good substrate for the transformylase. The binding of the α-chloro-$\beta$-alanyl analogue ($K_i$ = 14 $\mu$M) is comparable to that of GAR ($K_m$ = 10 $\mu$M). These analogues, which inhibit GAR transformylase in vitro, could be prototypes for future development of drugs effective against intact cells.

**AICAR Transformylase (AICAR → FAICAR).** AICAR transformylase catalyzes the transfer of the formyl group from 10-formyl tetrahydrofolate to AICAR to produce FAICAR and tetrahydrofolate (reaction 9, Figure 1). The 6(*S*)-diastereomer of DDATHF is a specific inhibitor of AICAR transformylase.[19] dmAMT is a weak inhibitor of dihydrofololate reductase which inhibits de novo purine and pyrimidine biosyntheses.[20] The tetraglutamyl derivative of dmAMT is a potent inhibitor of human AICAR transformylase ($IC_{50}$ = 0.25 $\mu$M, $K_i$ = 0.09 $\mu$M) and of thymidylate synthase ($IC_{50}$ = 0.53 $\mu$M). For comparison, the $K_i$ value for the tetraglutamyl derivative of methotrexate as an inhibitor of human AICAR transformylase ($K_i$ = 57 nM) is 2500-fold lower than that for free methotrexate ($K_i$ = 140 $\mu$M), supporting the concept that this enzyme has a positively charged region which interacts with the negatively charged tail of polyglutamyl derivatives of folate analogues. A similar pattern is seen for GAR transformylase, where free methotrexate ($K_i$ = 80 $\mu$M) is a less potent inhibitor than the tetraglutamyl derivative ($K_i$ = 2.5 $\mu$M[21]). Methotrexate is also an inhibitor of dihydrofolate reductase ($K_i$ = 5 pM), and the pentaglutamyl derivative inhibits thymidylate synthase ($K_i$ = 47 nM[17]). Agouron Pharmaceuticals Inc. has developed a classical antifolate, AG2009 (Figure 4), which is a specific inhibitor of AICAR transformylase, but there is little published information on this analogue.[22] As stated above, Alimta or multitargeted antifolate (MTA, LY231514, Figure 4) inhibits both purine and thymidylate biosyntheses and dihydrofolate reductase.[17]

**IMP Cyclohydrolase (FAICAR → IMP).** IMP cyclohydrolase catalyzes an intramolecular ring closure (reaction

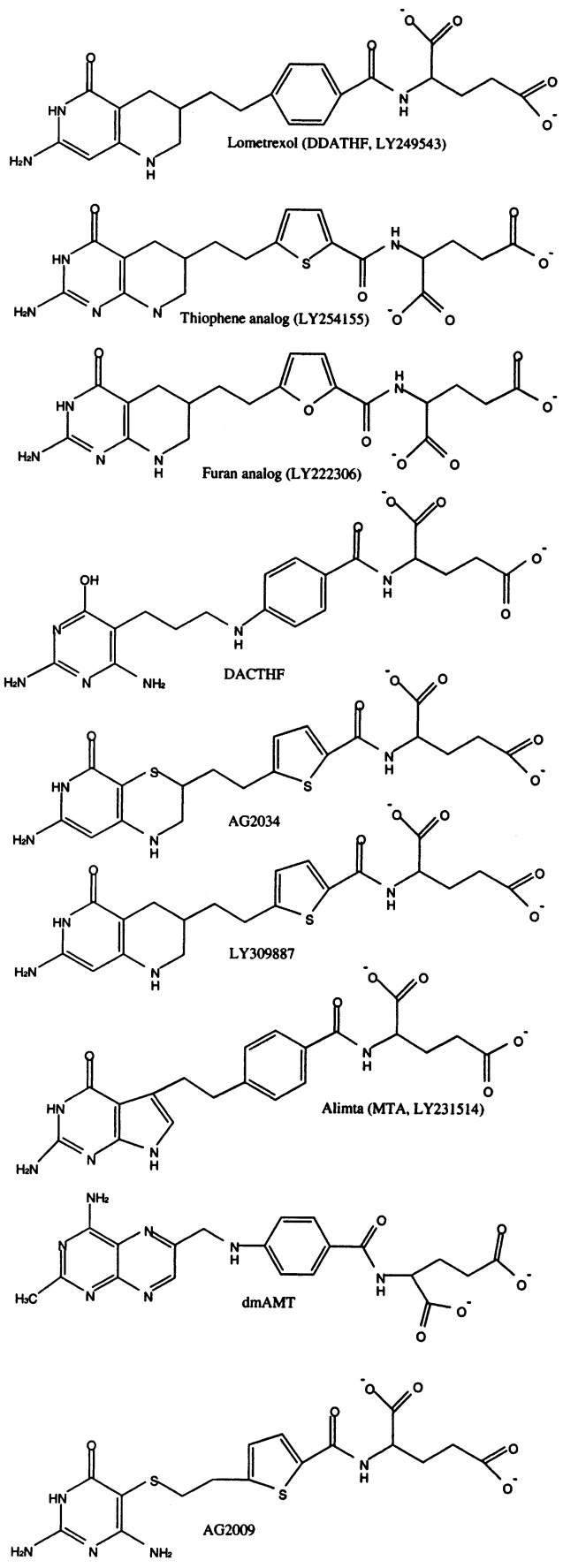


**FIGURE 4.** Antifolates which inhibit GAR and/or AICAR transformylases.

10, Figure 1). We have found that a number of purine nucleoside 5′-monophosphate derivatives (structures not shown) are potent inhibitors of IMP cyclohydrolase: 2-mercaptoinosine 5′-monophosphate ($K_i$ = 0.094 $\mu$M), XMP ($K_i$ = 0.12 $\mu$M), 2-fluoroadenine arabinoside 5′-monophosphate (fludarabine, $K_i$ = 0.16 $\mu$M), 6-mercaptopurine riboside 5′-monophosphate (MPR-MP, $K_i$ = 0.20 $\mu$M), adenosine N1-oxide 5′-monophosphate ($K_i$ = 0.28 $\mu$M), and N6-(carboxymethyl)adenosine 5′-monophosphate ($K_i$ = 1.7 $\mu$M[23]). 2-Fluoro IMP ($K_i$ = 0.19 $\mu$M) and 2-chloro IMP ($K_i$ = 1.9 $\mu$M) are also inhibitors.[24] Szabados et al.[23] proposed that the catalytic mechanism of IMP cyclohydrolase may involve a reaction intermediate with negative charge in the 2-position of the purine ring. Some of the inhibitors listed above have electronegative sulfur, oxygen, fluorine, or chlorine in the 2-position, which may resemble the transition state for the reaction.

**IMP Dehydrogenase (IMP DHase, IMP → XMP).** IMP DHase catalyzes the oxidation of IMP to XMP (reaction 13, Figure 1). Mycophenolate (Figure 5) is a mixed inhibitor of both IMP DHase ($K_i$ = 30 nM[25]) and GMP synthetase ($K_i$ = 80 nM[26]). This natural product has immunosuppressive activity. Mizoribine (bredinin, Figure 5) is an imidazole nucleoside which inhibits IMP DHase and depletes cells of guanine nucleotides.[27] EICAR inhibits the growth of human and mouse leukemia cells and, as the 5′-monophosphate derivative, is a competitive inhibitor with respect to IMP of IMP DHase ($K_i$ = 7 $\mu$M[28]). Ribavirin is a nucleoside analogue (Figure 5) which is phosphorylated to the 5′-monophosphate derivative, a competitive inhibitor with respect to IMP of IMP DHase ($K_i$ = 0.8 $\mu$M[29]). 6-Mercaptopurine riboside, as the 5′-monophosphate (MPR-MP), inhibits amido phosphoribosyltransferase as described above but is also an inhibitor of IMP DHase,[11] presumably competitive with IMP.

Tiazofurin is a structural variant of ribavirin (Figure 5) with a different mechanism of action. This C-nucleoside is phosphorylated to the 5′-monophosphate derivative and then converted to an analogue of NAD. Tiazofurin adenine dinucleotide (TAD) is a potent inhibitor of IMP DHase, binding at the NAD site with $K_i$ = 0.13 $\mu$M.[29] Selenazofurin is an analogue of tiazofurin where selenium replaces sulfur. Similarly, the NAD analogue, SAD, is formed which is a competitive inhibitor with respect to NAD ($K_i$ = 55 nM[30]). Benzamide adenine dinucleotide (BAD) is formed from benzamide riboside in human cells, a competitive inhibitor with respect to NAD of IMP DHase ($K_i$ = 0.12 $\mu$M). Of the three drugs which form TAD, SAD, and BAD in cells, selenazofurin induced the greatest inhibition of IMP DHase in human K562 leukemia cells.[31] VX-497 is a noncompetitive inhibitor of IMP Dhase ($K_i$ = 10 nM[32]), designed from the crystal structure of the enzyme by Vertex Pharmaceuticals Inc. This novel drug selectively inhibits lymphocyte proliferation and acts as an immunosuppressive agent.

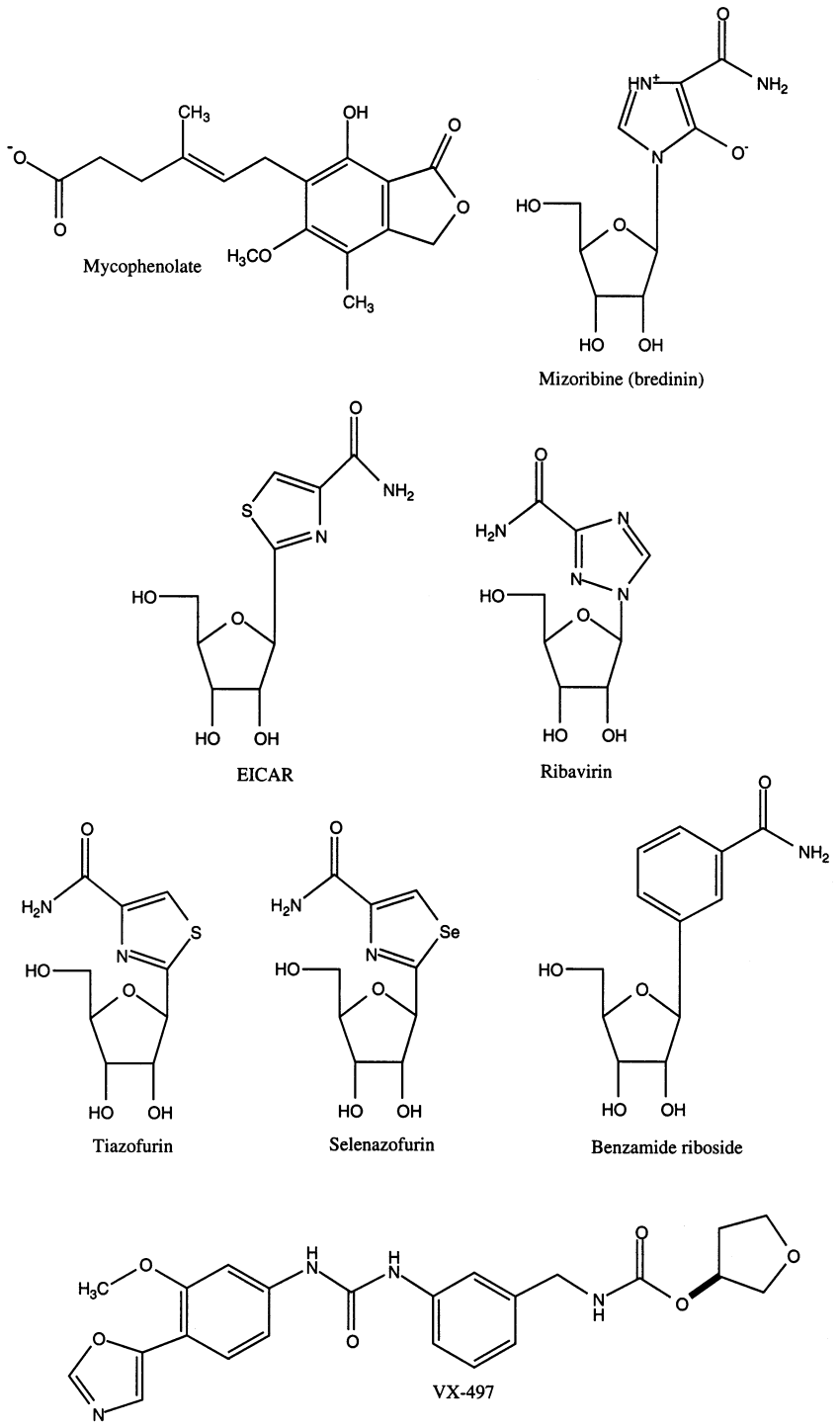


**FIGURE 5.** Inhibitors of IMP dehydrogenase.

## De Novo Pyrimidine Biosynthesis

**Aspartate Transcarbamylase (ATCase, CAP → CA-asp).** The pathway for de novo biosynthesis of pyrimidine nucleotides is shown in Figure 2. ATCase catalyzes the condensation of carbamyl phosphate with aspartate (reaction 2). The best known inhibitor of ATCase is *N*-phosphonacetyl-L-aspartate (PALA, Figure 6), a bisubstrate analogue which resembles both of the substrates, carbamyl phosphate and aspartate.[33] PALA interacts with mouse ATCase with a $K_i$ value of 26 nM [34] and was shown to eradicate solid tumors in mice, but murine leukemias were resistant.[35] Unfortunately, PALA given as a single agent to patients was clinically inactive against leukemias and solid tumors.[36] This inherent resistance of cancer cells to PALA was probably due to high levels of ATCase activity relative to other pyrimidine enzymes normally found in cells,[37] or to salvage of preformed uridine (Urd) found in the circulation (Urd → UMP → UDP → UTP → CTP). In cells which lack carbamyl phosphate phosphatase activity, intracellular PALA may induce "metabolic resistance".[1] Blockade of the ATCase reaction (CAP → CA-asp) by PALA leads to an immediate accumulation of CAP, which may

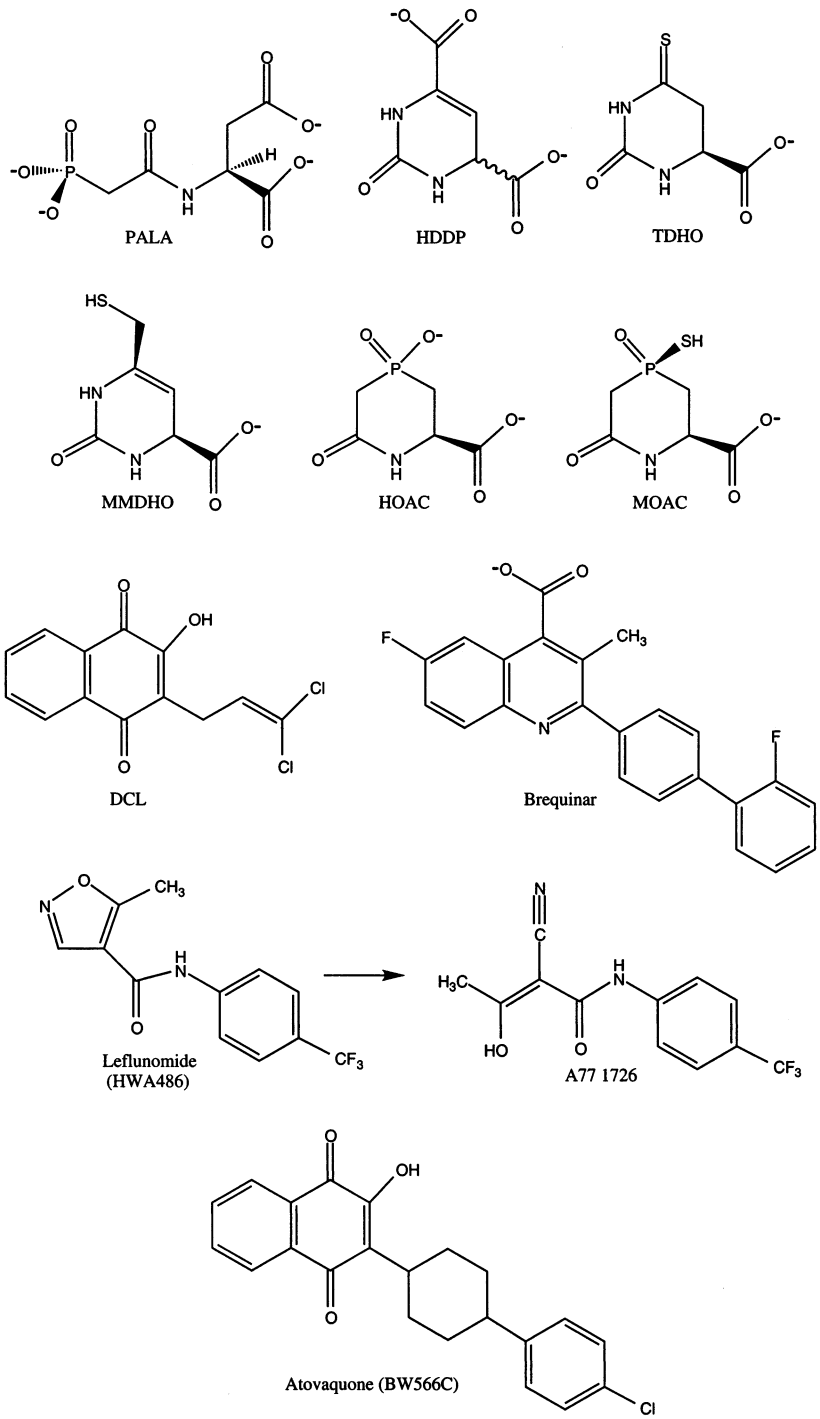


**FIGURE 6.** Inhibitors of aspartate transcarbamylase, dihydroorotase, and dihydroorotate dehydrogenase.

reach a concentration sufficiently high to compete with PALA for binding to ATCase, resulting in restoration of the original flux through the reaction and the pyrimidine pathway. We demonstrated metabolic resistance due to accumulation of CAP in vitro and derived equations which describe this rapid, inherent resistance for an inhibitor–target enzyme interaction in a functioning metabolic pathway.[1]

**Dihydroorotase (DHOase, CA-asp → DHO).** DHOase catalyzes the reversible cyclization of *N*-carbamyl-L-aspartate (CA-asp) to L-dihydroorotate (DHO, reaction 3, Figure 2). The transition state for the reaction is stabilized by formation of an inner-sphere coordination complex with a zinc atom which is tightly bound at the active site.[38] We have designed and synthesized a series of dihydropyrimidine analogues as inhibitors of DHOase[39,40] (Figure 6). HDDP has a $K_i$ for interaction with DHOase of 0.74 $\mu$M, and TDHO has a $K_i$ of 0.85 $\mu$M. Adams et al.[41] synthesized a mercaptomethyl dihydropyrimidine analogue (MMDHO), with $K_i = 0.14$ $\mu$M. Recently, we have synthesized a "second generation" inhibitor, MOAC, which mimics the tetrahedral geometry of the transition state and contains a sulfur atom designed to form a strong coordination bond with the zinc atom at the active site.[42]

Although design of this phosphinothioic acid incorporated multiple structural attributes which individually promote binding to DHOase, the $K_i$ of 2.9 $\mu$M was comparable to the $K_i$ of 4.0 $\mu$M obtained for the parent phosphinic acid (HOAC).

**Dihydroorotate Dehydrogenase (DHO DHase, DHO → Oro).** DHO DHase catalyzes the oxidation of DHO to orotate (Oro) on the outer side of the inner mitochondrial membrane (reaction 4, Figure 2).[43] The pair of electrons abstracted from DHO in this oxidation is transferred to ubiquinone and directly to the electron transport chain also associated with the inner mitochondrial membrane. There are several analogues of ubiquinone which are potent inhibitors. The 1,4-naphthoquinone, lapachol, a natural product isolated from the lapacho tree, is an uncompetitive inhibitor with an apparent dissociation constant for interaction with the DHO DHase−DHO complex from mouse mitochondria of 2.1 $\mu$M.[44] Dichloroallyl lawsone (DCL, Figure 6) is a chemical derivative of lapachol with anticancer activity. DCL is an uncompetitive inhibitor of DHO DHase in isolated mitochondria, with $K_i = 0.27$ nM.[44] Brequinar is a fluorinated carboxyquinoline derivative with anticancer activity which also acts as an analogue of ubiquinone and is a noncompetitive inhibitor of mitochondrial DHO DHase, with an apparent $K_i$ value of 20 nM.[45] Leflunomide has anti-inflammatory and immunoregulatory properties and is converted in vivo to the active metabolite A77 1726, which inhibits DHO DHase as an analogue of ubiquinone ($K_i = 12$ nM[46]) and tyrosine kinases. Elder et al.[47] found with mouse CTLL cells that A77 1726 inhibited pyrimidine biosynthesis, but at higher concentrations of A77 1726, uridine no longer reversed the inhibition of proliferation, attributed to inhibition of protein tyrosine kinases. The higher concentrations of A77 1726 inhibited IL-2-induced tyrosine phosphorylation of the proteins Jak1 and Jak3, and tyrosine phosphorylation of the $\beta$-chain of the IL-2 receptor. Ruckemann et al.[48] proposed that DHO DHase is the prime target of this drug in proliferating human T-lymphocytes. It is apparent that leflunomide and its metabolite, A77 1726, have multiple sites of action dependent upon their concentrations and the type of cell. More recently, Mahajan et al.[49] have designed and synthesized an analogue of A77 1726, called LFM-A13, which is a potent inhibitor of the anti-apoptotic Bruton's tyrosine kinase.

Atovaquone (BW566C) is a 2-hydroxy-1,4-naphthoquinone with potent antimalarial activity. It has an $IC_{50}$ of 1.0 nM against *P. falciparum* in vitro[50] and an $ED_{50}$ of 1.7 nM against Complex III of the malarial electron transport chain. Atovaquone forms a covalent adduct with a protein of molecular weight 11 500 from Complex III of malarial mitochondria,[51] leading to indirect inhibition of DHO DHase with consequent accumulation of CA-asp and DHO and depletion of UTP and CTP.[52] We have found that atovaquone (2.5 $\mu$M, 6 h) reduced the cellular level of dTTP to 28.5%, but dCTP remained virtually unchanged at 96.6%.[53] Clinical trials of atovaquone for treatment of acute *P. falciparum* malaria have shown early resolution of clinical symptoms and clearance of parasitaemia.[50]

**Orotidine-5′-monophosphate Decarboxylase (ODCase, OMP → UMP).** ODCase catalyzes the decarboxylation of OMP to form UMP (reaction 6, Figure 2). There are a number of potent inhibitors of ODCase (Figure 7). 6-Azauracil or 6-azauridine is converted to 6-azauridine-5′-monophosphate within the cell and has a $K_i$ against ODCase from yeast of 0.51 $\mu$M.[54] 6-Azauracil has anticancer activity against a number of experimental tumors.[55] Pyrazofurin is a C-nucleoside readily taken up by mammalian cells and phosphorylated to the 5′-monophosphate derivative (PF-MP), a potent inhibitor of ODCase with a $K_i$ value of 5 nM.[56] Clinical trials have shown that pyrazofurin has anticancer activity limited by toxicity to patients.[57]

Pyrazofurin as the C-nucleoside inhibits malarial OPRTase (Oro → OMP), while the 5′-monophosphate derivative inhibits ODCase[58] (OMP → UMP). Pyrazofurin is an effective antimalarial, retarding the maturation of trophozoites to schizonts; toxicity is not affected by the addition of uracil or uridine to the culture because the parasite lacks the capacity to salvage preformed pyrimidines.[4] The nucleoside-5′-monophosphate derivative of barbiturate (BMP) is an extremely potent inhibitor of yeast ODCase[54] ($K_i = 8.8$ pM) but may not be sufficiently stable to inhibit this enzyme in intact cells. The nucleoside-5′-monophosphate derivatives of allopurinol, oxipurinol, and xanthine,[59] with the ribose linked at the 3- or 9-position of the purine ring (Figure 7), are potent or effective inhibitors of ODCase.

**CTP Synthetase (UTP → CTP).** CTP synthetase catalyzes the amido transfer from glutamine to UTP to form CTP (reaction 9, Figure 2). 3-Deazauridine (Figure 7) is taken up by mammalian cells as the nucleoside and converted to the triphosphate derivative, DAU-TP, a competitive inhibitor of CTP synthetase from mouse L1210 leukemia cells ($K_i = 5.3$ $\mu$M).[60] Cyclopentenyl cytosine (CPEC), as the triphosphate derivative, is a potent competitive inhibitor of CTP synthetase, but the kinetics of the inhibition are complex.[61] CPEC is a carbocyclic analogue of cytosine which is phosphorylated within cells to mono-, di-, and triphosphate derivatives. CPEC-TP induces depletion of CTP and dCTP, with consequent genetic miscoding or arrest of RNA and DNA syntheses. CPEC has shown anticancer activity in a variety of experimental systems, including human colon cancer cell lines[62] and experimental brain tumors in rats.[63]

## Conclusions

Specific inhibition of the de novo purine pathway provides anticancer and immunosuppressive activity. A number of "second generation" antifolates (Figure 4) inhibit GAR and/or AICAR transformylases, effectively blocking the pathway giving useful anticancer activity. Drugs such as mycophenolate, ribavirin, and VX-497 inhibit IMP DHase, leading to immunosuppressive activity, demonstrating the dependence of leukocytes upon guanosine nucleotides.

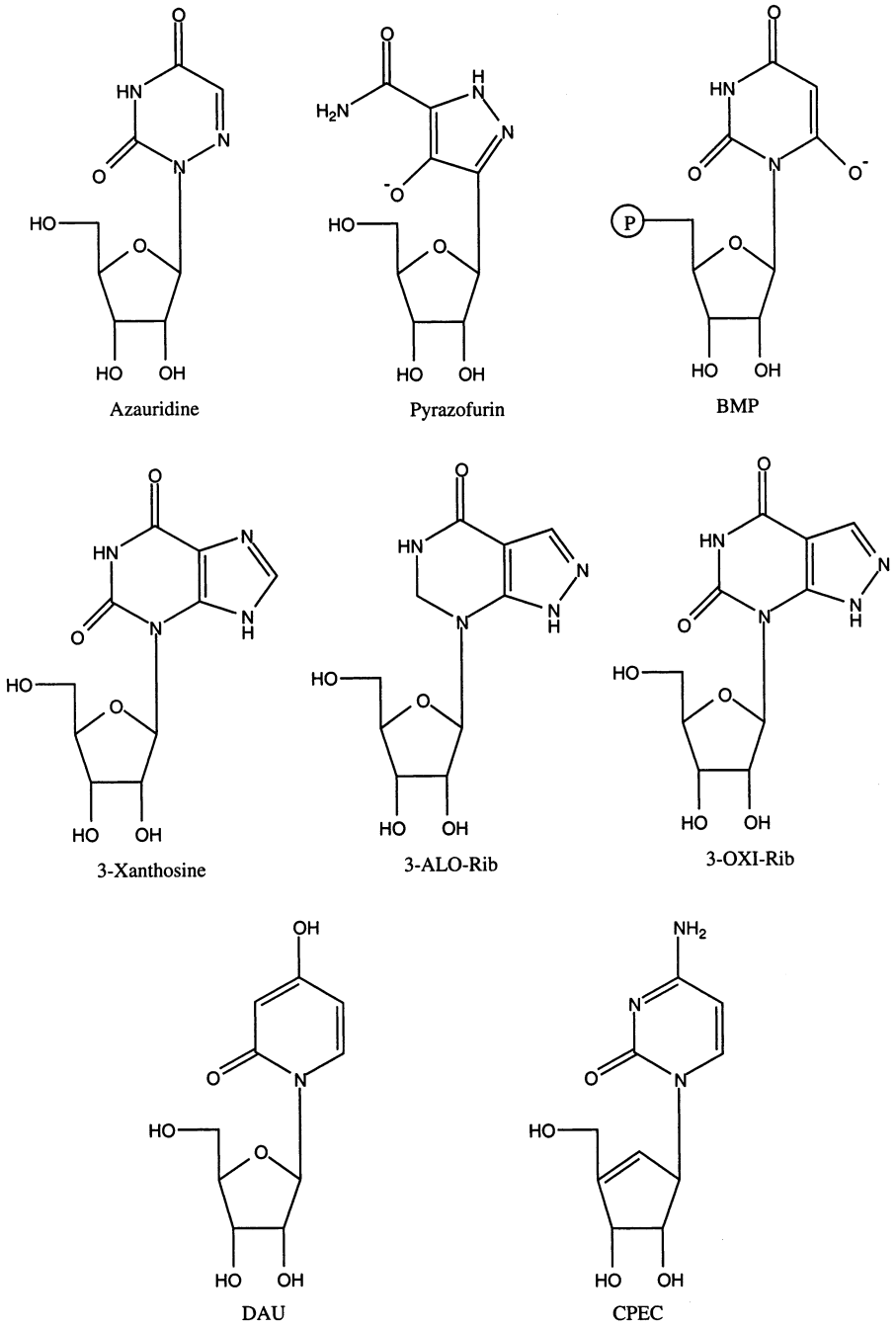


**FIGURE 7.** Inhibitors of OMP decarboxylase and CTP synthetase.

While antifolates such as methotrexate have multiple sites of inhibition in human cells, some "second generation" antifolates may inhibit a single reaction, with potentially fewer side effects. Recently, a new class of C-nucleosidic iminoribitol transition-state analogues, the immucillins, has been developed by Schramm and co-workers.[64,65] The immucillins have a positive charge on the nitrogen of the heterocyclic iminoribitol moiety, resembling the transition state, and have $K_i$ values in the picomolar to nanomolar range. ImmucillinHP and immucillinGP are potent inhibitors of human and malarial hypoxanthine-guanine phosphoribosyltransferases.[64,65] It should be possible to develop homologous inhibitors of amido and orotate phosphoribosyltransferases (APRTase and orotate PRTase).

Potent inhibitors of the initial reactions of the de novo pyrimidine pathway were thought to have potential as anticancer drugs, but this early promise has not been realized. Alternative salvage synthesis of pyrimidine nucleotides combined with the requirement of resting cells for pyrimidine nucleotides in activated intermediates such as UDP-glucose and CDP-choline mean that there is a narrow "therapeutic window" where such inhibitors would have selective toxicity for cancer cells. However, such inhibitors, exemplified by atovaquone, are very useful for treatment of malaria and other parasitic infections (*Pneumocystis carinii* and *Toxoplasma gondii*), such as those seen in AIDS patients. Later in the pathway, inhibitors of CTP synthetase (UTP → CTP, CPEC) and thymidylate

synthase (dUMP → dTMP, tomudex, and FdUMP[66]) are useful anticancer drugs. Inhibitors of dNTP biosynthesis may not affect the availability of activated intermediates such as UDP-glucose and CDP-choline in normal, resting cells.

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