

REVIEW ARTICLE

FROM BENCH TO BEDSIDE: A THOROUGH EXPLORATION OF FOLATE ANTAGONISTS IN MEDICINE

Smriti Dewangan¹, Anamika Singh^{2*}

¹*Amity School of Pharmacy, Amity University, Raipur, Chattisgarh, India.*

²*Indukaka Ipcowala College of Pharmacy, The Charutar Vidyha Mandal (CVM) University, Gujarat, India.*

Corresponding Author: Anamika Singh, Orcid ID- 0000-0001-7673-894X, Email ID: anamikarajni@gmail.com

Received: 05-07-2025 Accepted: 22-08-2025, Published: 22-07-2026

ABSTRACT

Folate antagonists have considerable potentials in medical therapeutics especially occasions in oncology and antimicrobial therapy. Herein, this exhaustive review looks into their actions at molecular levels, into clinical applications as well as assesses the currently emerging state of use. It starts with describing the activity of folate in DNA synthesis and cell division which forms the basis to comprehend the mechanism of disruption of these processes by folate antagonists. The talk is related to the main antifolate drugs, such as methotrexate, aminopterin, and trimethoprim, whose mechanism of action reflects an impact on an enzyme facilitating folate metabolism, namely dihydrofolate reductase (DHFR). The review in oncology outlines their use as chemotherapeutic agents, including mechanisms of action, dose selection, and related toxicities, and focuses in particular on the trade-offs between therapeutic effect and hematologic and gastrointestinal toxicities. With regards to antimicrobial therapy, the review deals with the application of trimethoprim on bacterial DHFR used as a treatment of infection. Future views are concluded in the paper on the development of new agents, innovative ones and development of a drug delivery, as well as the strategy of overcoming resistance. The given review has two different purposes: it can be helpful to the researchers and to the clinicians in order to gain deeper insight into the use of the folate antagonists and their modernization in contemporary medicine.

KEYWORDS: *Folate antagonists, Antifolate drugs, Methotrexate, Cancer chemotherapy, antimicrobial therapy, Drug development, Translational medicine*

INTRODUCTION

Cancer The term cancer describes a complicated set of diseases dealing with uncontrolled development and dispersion of rogue cells. These cells may infiltrate and destroy the tissues and organs around causing interference with the normal body functions. Cancer may appear in pretty much any tissue or organ in the body and may be either benign (non-cancerous) or malignant (cancerous) [1]. Antimetabolites are also a powerful type of antitumor remedy that are antagonistic with regards to natural metabolites. The aforementioned chemicals can be categorised into multiple subgroups based on their chemical structures [2]. They may consist of peptides including phosphate-analogues and non-natural amino acids. Analogues of purine and pyrimidine bases, which compete with one another in the production of nucleic acids can also be included in this category [3]. The group includes chemicals that replicate the effects of vitamins, folic acid, hormones, coenzymes and other substances necessary for the proper operation of the human body's cells and tissues. In order to understand the mechanism of therapeutic application of antifolates, it is necessary to investigate the main compound of their metabolic process: folic acid. This is a metabolite in B-vitamin family, which is a group of small water-soluble molecules used as cofactors in enzymatic reactions in

different metabolism roles [4]. In particular, the words folic acid or folate is used frequently when speaking of a tetrahydrofolate molecule (THF). THF is made up of a pteridine fragment, a p-aminobenzoic acid (PABA) and an L-glutamate residue (Figure 1). Nonetheless, due to its use as a one-carbon donor it is possible that its structure could undergo minor changes based on the carbon transfer reactions in which it is involved and this would include more than 150 molecules under this broad superstructure name [5].

Folate antagonists are substances that interfere with the normal function of folate, a vital B-vitamin that is essential for many bodily processes. Vitamin B9 is called folate and has a very significant role in reproduction, synthesis, and repairing of the DNA, and cell division. This is especially during high cell growth and development e.g. during pregnancy when the intake of folate is very crucial [6]. Folate antagonists act by binding or its counteractive chemicals acting at folate enzyme and inhibit the metabolism of folate therefore interrupting DNA and RNA production. This impairment on nucleic acid synthesis impacts on quickly dividing cells such as cancer cells and this makes folate antagonists useful in chemotherapy of cancer. Nevertheless, they may not be applied exclusively in treating cancer cases because they can also be utilized in treatment of some

autoimmune conditions and inflammatory diseases [7].

The most present form of folate in plasma is in the form of monoglutamate in humans and Penta polyglutamate derivative having five glutamate residues in cells. It is important to point out the fact that polyglutamated forms are better extrapolated towards most folate-dependent enzymes than folate derivatives in monoglutamate. Their closely related inhibitors too also show this enhanced affinity. In regard to its purpose, the folic acid metabolism plays an important role in the adequate performance of all the living cells. Folate is an active participant in many reactions that are which play an important part in a variety of biosynthetic processes, including the proper synthesis of proteins and nucleic acids in different species. On the one hand, it functions in the production of formylated transfer RNA, which is a necessary step in the protein procession, and amino acids such as glutamate,

histidine, glycine, serine, and methionine. Second, by the aforementioned one-carbon transfer processes, it epigenetically regulates the occurrence of gene expression and aids in the synthesis of both purine and pyrimidine nitrogenous bases. Folic acid supplementation is particularly important during pregnancy and in terms of regeneration because of these essential functions. The significance of chemicals with antagonistic effects, such as antifolates, is underscored by the critical role that metabolic processes play in cell survival. Usually, these metabolites fall into one of two categories. Classical antifolates are first group and have structural similarity to folic acid. Such compounds offset several enzymes hence hindering or rather blocking the metabolic process of folic acid. These include the second group of antifolates which affect the biosynthesis of folic acid referred to as non-classical antifolates. These molecules differ structurally with folate (as shown in Figure 1) [8].

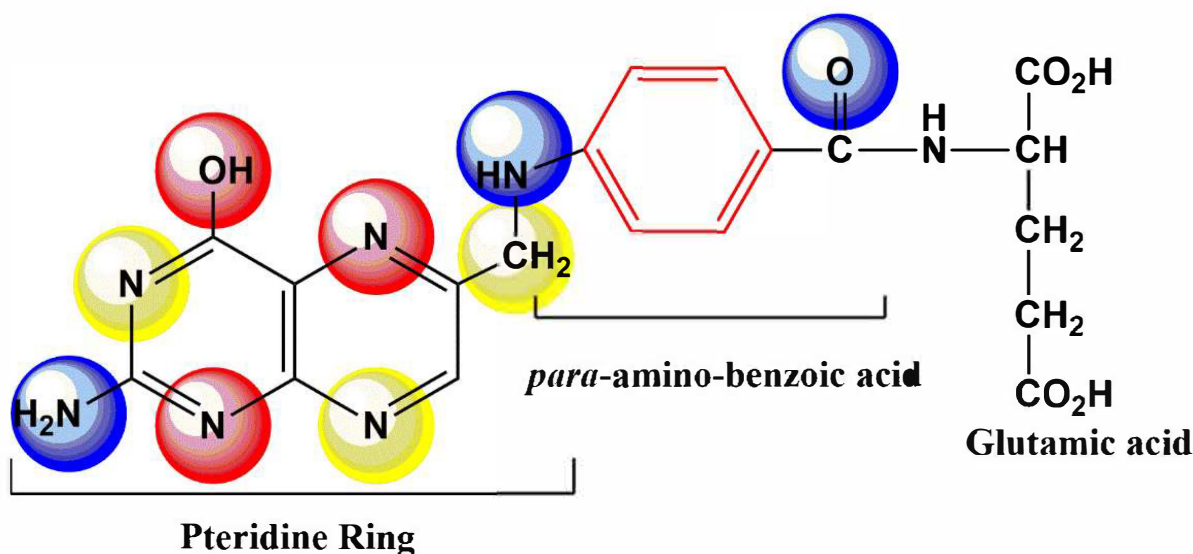


Figure 1: Folic acid structure

This review is a review of clinical application of antifolate, antibacterials, antimalarials, anticancer and immunomodulating agents. This is with reference to how they work, their activity spectra, indications, adverse reactions, and newly developing resistances that have stimulated the search of additional options. We also expose the current, biomedical research on the potential application with a spotlight cast on the novel fields of inquiry [9,10].

FOLATE ANTAGONIST

Folic acid antagonists, are cytotoxic medications that are utilised as immune-suppressors, antimicrobials, anti-inflammatory, and antineoplastics. Despite the development of numerous folate antagonists, and some of these being in clinical trial, methotrexate (MTX) represents the antifolate with the longest history and with broadest application [11]. Another crucial medication is MTX, which is used in chemotherapy cycles to treat patients with choriocarcinoma, osteosarcoma, and acute lymphoblastic leukaemia (ALL). In addition to treating patients with non-cancerous conditions such rheumatoid arthritis, psoriasis, and graft-versus-host disease, it is an important chemical in the treatment of patients with lymphoma, breast, bladder, and head and neck cancer [12]. One of the first contemporary anticancer drugs were the antimetabolites, or antifolates, which are the oldest in their class. The first documented remissions in juvenile

leukaemia were caused by 2,4-diamino-pteroylglutamate (4-amino-folic acid; aminopterin; AMT), the first therapeutically effective antifolate, which was initially described in 1947 [13]. Due to its toxicity, AMT was soon further displaced by its 10-methyl analogue, methotrexate (MTX); now, with one small exception, it is the only antifolate-type anticancer medication used in clinical settings. Many efforts were made to create antifolates that are more therapeutically specific or have a wider tumour range because of the safety and effectiveness of MTX [14]. Initially, the building was based on the growing understanding of the routes and determinants of MTX's mechanism of action that depend on folate. These factors include transport, MTX metabolism to poly - gamma - glutamate (Glu(n)) metabolites, and tight-binding of its target (the folate-dependent enzyme dihydrofolate reductase (DHFR)) [15]. Both the so-called classical analogues, which use the same cellular transport systems as MTX and are metabolised to Glu(n), and the so-called nonclassical (i.e., lipophilic) analogues, which do not use cellular transport systems and are not metabolised to Glu(n), are the other antifolate DHFR inhibitors that emerged from these early studies.

Even though some of these analogs have successfully been taken through clinical trial, none of them has been seen to be better than MTX. A critical analysis of the specifics of MTX's cytotoxicity and selectivity showed that blocking both

dTMP synthesis and de novo purine synthesis, which resulted from DHFR inhibition and thioguanine nucleotide production, inhibited DNA synthesis and, ultimately, cell death; blocking other processes that require folate did not appear to be a prerequisite for these cells to die. Later, it was shown that different cell lines had varying degrees of participation from suppression of dTMP/purine production in cell death [16-18]. These data were tempting to suppose that targeting one of these pathways, in isolation, could (at least in some instances) be of therapeutic advantage as compared to the dual targeting that is caused by MTX. Thus, two new kinds of antifolate enzyme inhibitors were created in both structure-based design studies and rational design studies: direct inhibitors of thymidylate synthase (TMPS) and direct inhibitors of one or both of the two folate-dependent enzymes of de novo purine biosynthesis. Composition of each of the classes consisted of classical and nonclassical members. Following preclinical trials, some of them have been shifted to clinical trials [19-20]. Only a single new TMPS inhibitor has to date undergone a successful clinical trial and been approved into practice; this agent is Tomudex (D1694, raltitrexed) which was approved as a drug of routine use only in Europe and solely in cases of colon cancer. This is nevertheless a new step in the right direction of antifolates, as MTX was equally and notoriously ineffective in colon cancer; therefore, Tomudex is a widening of the tumor spectrum of antifolates. Progress is still being made as

far as antifolate development is concerned. Given that there is a wealth of research on the mechanism of action of antifolates, some of its key points have been covered. There have been reports of other, more recent antifolates that can inhibit different folate metabolism targets, enhance delivery to a single pathway, or act on several folate metabolism pathways. Those new analogues have different preclinical and clinical developmental stages [21].

HISTORY AND PRESENT SCENARIO OF FOLATE ANTAGONIST

Antifolates Folate antagonists (also known as antifolates) are drugs that inhibit folate-dependent enzymes important in DNA synthesis and cell division. In 1940s, the discovery of folic acid and its importance in nucleotide synthesis contributed the first antifolate, aminopterin, as a treatment agent against childhood leukemia. Soon after, when methotrexate (MTX) appeared, it became the foundation of antifolate treatment, it entered wide application in the field of oncology, in the treatment of autoimmune conditions, such as rheumatoid arthritis, and in psoriasis. In succeeding decades newer antifolates were synthesized with enhanced selectivity and less toxic side effects. Raltitrexed and pemetrexed bind both thymidylate synthase and various enzymes that depend on folates, and they are effective against colorectal and lung cancer. Pralatrexate approved in 2009 to treat peripheral T-cell lymphoma is characterized by increased uptake in

malignant cells. The current situation indicates a paradigm transition to the targeted delivery, minimized systemic toxicity, and resistance. Some of the strategies are liposomal MTX, folate-receptor targeting agents such as vintafolide and combination therapies using immune checkpoint inhibitors [22]. Artificial intelligence is currently used in the design of new antifolates

features enhanced pharmacodynamics. Nevertheless, there are still problems like resistance and narrow therapeutic indexes that, nevertheless, do not eliminate the indispensability of antifolates in contemporary medicine, some of which are still based on the optimization of their effect with the help of advanced delivery systems and personalized treatment. All this is stated in Table 1.

Table 1: Evolution and Present Scenario of Folate Antagonists (upto 2025) [23]

Period	Milestone / Development	Drugs / Concepts Introduced	Clinical / Research Impact
1940s	Discovery of folic acid and its role in cell proliferation	Folic acid	Identified as essential for nucleotide synthesis; linked to cancer cell growth
1948–1951	Synthesis of first	Aminopterin, Methotrexate (MTX)	MTX used to treat pediatric leukemia; one of the first successful chemotherapies
1950s–1970s	Clinical expansion of MTX use	Methotrexate	Widely adopted for cancers (leukemias, lymphomas), RA, psoriasis
1980s–1990s	Second-generation with improved selectivity	Trimetrexate, Edatrexate, Lometrexol	Developed to reduce toxicity and overcome resistance; limited clinical success
1990s–2000s	Development of multitargeted folates	Raltitrexed, Pemetrexed	Inhibit TS, DHFR, GARFT; effective in colorectal, lung, and mesothelioma
2009	FDA approval of Pralatrexate for relapsed/refractory peripheral T -cell lymphoma (PTCL)	Pralatrexate	First antifolate approved specifically for PTCL; high - affinity uptake in cancer cells

2010–2015	Focus on targeted delivery and toxicity reduction	Liposomal MTX, nanoparticle formulations	Enhanced drug delivery to tumor cells; reduced systemic toxicity
2015–2020	Folate receptor-targeting strategies	Vintafolide (EC145, folate–drug conjugates)	Selective targeting of folate receptor- α on cancer cells (especially ovarian and breast)
2020–2023	Integration with immunotherapy and emerging resistance management	Antifolate + checkpoint inhibitors (experimental)	Investigated for synergistic effects and immune activation in cancer
2023–2025	Advancements in antifolate design using AI and computational drug repurposing	AI-based novel antifolates; combination with epigenetic drugs	Predictive modeling for efficacy and resistance; new structures for clinical trials
Ongoing (2025)	Overcoming resistance via metabolic reprogramming and folate receptor inhibitors	DHFR-resistant MTX analogs, antifolate prodrugs	Focus on novel antifolates that bypass common resistance mechanisms; cancer metabolism focus

Mechanism of action of Folate antagonists

Mechanism of antifolates functioning depends on the target enzyme. Although the number and name of these enzymes indulged in folate metabolism are legion, some specific ones have to be mentioned because they form the most significant role by playing historical role in use of folate as therapeutic agents. Figure 2 shows these main enzymes, the pathways in which they are involved, as well as some of the chemicals that are the inhibitor of these enzymes [24].

Dihydrofolate reductase: Folate antagonists act in principle to inhibit the production of folic acid, a necessary compound of the tissues. They have a high affinity to dihydrofolate reductase (DHFR) and hence efficiently block transformation of dihydrofolic acid into tetrahydro folic acid along with NADPH utilization. The typical DHFR inhibitors are classical, which conform to a competitive inhibition pattern toward DHF, with the exception of folinate, which can be considered competitive antagonist of NADPH and noncompetitive antagonist of DHF [16].

But each of them contributes to the death of the cells due to the loss of THF pool [25].

Dihydropteroate Synthase: Dihydropteroate synthase (DHPS) is an enzyme which is pivotal in folate biosynthetic pathway. It plays a role in the production of dihydropteroate which is an intermediate in the synthesis of tetrahydrofolate (THF) a vital co-factor in a number of cellular processes, such as DNA synthesis and repair. DHPS is involved in the reaction of p-aminobenzoic acid (PABA) and 6-hydroxymethyl-7,8 -dehydration pyrophosphate (DHPP) to give dihydropteroate. This is an important reaction in the de novo synthesis of folate in microorganisms and in selected plants. Special interest should be paid to the fact that DHPS is also a target of some of the antibiotics, like sulfonamides which are competitive inhibitors of PABA, which interferes with dihydropteroate synthesis which in turn inhibits bacterial folate synthesis [26]. Inhibition of DHPS by the antibiotics has been exploited in the area of medicine especially to treat bacterial diseases. These antibiotics kill bacteria by interfering with their growth as well as replication by interfering with folate production. It is critical to mention that the human cells lack the ability to synthesize folate de novo and obtain its sources through the diet, which is why DHPS is a promising target of a potential antibacterial agent that will not severely affect human cells [27].

Thymidylate Synthase: Folate pathway is linked with pyrimidine synthesis via thymidylate synthase (TS) to recover the depleted dihydrofolate (DHF) in the cycle. Besides, in this process, 2-deoxyuridine-5-monophosphate (dUMP) is methylated in the presence of enzyme TS using N5, N10-Methylene THF, and the outcome is that 2-deoxythymidine-5-monophosphate (dTMP) is formed [8]. The reaction that is catalyzed by the overall response of this enzyme is shown as Figure 3.

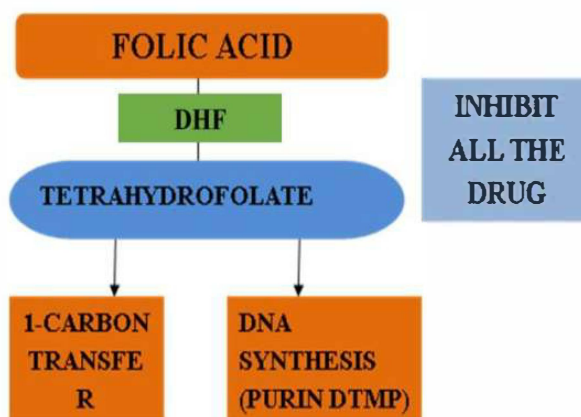


Figure 2: Mechanism of action

Another mechanism of synthesizing dTMP is to block the production of the purines and thymidines. Antifolates kill cells by hindering. The mechanism of action of folate antagonists or analogs is thought to be that of a cytotoxic agent with effects upon one or more steps in several biosynthetic pathways triggered by the cofactors folates. The possibilities of these analogs may be realized by many mechanisms, inhibition of the folate coenzyme formation, competition with the folates concerning the cellular absorption, inhibition of reactions catalyzed by the

folate coenzymes. Clinically significant antineoplastic folate analogs however mainly act by blocking dihydrofolate reductase (DHFR) [28-30].

Molecular basis of antifolate resistance

Folates play a key role in one-carbon metabolism, which is essential for thymidylate, purine production, and ultimately DNA replication. Nea Folates's logical design Since it directly contributes to one-carbon metabolism, which is essential for the manufacture of purines, thymidylate, and ultimately DNA replication, the antifolate methotrexate has been logically created. Nearly 60 years ago, the folate-dependent enzyme dihydrofolate reductase (DHFR) was severely inhibited by methotrexate, a logically developed antifolate that temporarily relieved acute leukaemia in children. The new antifolates raltitrexed and pemetrexed, which target glycineamide ribonucleotide transformylase (GARTF) and thymidylate synthase (TS), have recently been introduced to treat malignant pleural mesothelioma and colorectal cancer. The reduced folate carrier (RFC) is primarily responsible for delivering the divalent anion (anti)folates into the cell. A folylpoly-gamma-glutamate synthetase (FPGS) analyses the polyglutamy of the (anti)folates. Polyglutamylation is a crucial component of antifolate cytotoxicity since the intracellular concentration of antifolates influences its pharmacologic impact [31]. But owing to the phenomenon

of anticancer drug resistance, curative dealing with cancer through the use of drugs has become a major challenge. Action mechanism of folic acid in figure 2A vast number of antifolate- resistance mechanisms have been identified (predominantly) in the pre-clinical and medical studies; it is commonly linked to quantitative and/or qualitative changes to influx and/or efflux transporters of (anti)folates and to folate- dependent enzymes. These include down-regulation of the RFC and/or inactivating mutations, as well as additional modifications to the target enzymes DHFR, TS, and FPGS. Furthermore, it has recently been shown that the ATP-dependent (anti)folate efflux transporters, which have a high capacity and low affinity, belong to the ATP-binding cassette (ABC) superfamily, as well as the multidrug resistance proteins (MRP/ABCC) and the breast cancer resistance protein (BCRP/ABCG2). This transportation exercise is besides their already existing facility to extrude several cytotoxic agents. Therefore, the overexpressed MRPs and/or BCRP actively extrude antifolates thereby causing antifolate resistance. All drug folate antagonist properties and utilities in mention in Table 2, Additionally, down-regulation of MRPs and /or BCRP also leads to down-regulation of folate efflux causing an increase in the intracellular folate store causing an increase in the resistance of antifolate. The spectrum of molecular mechanisms of antifolate resistance in preclinical tumour cell

cultures, in vitro and in vivo, and cancer patients is reviewed and discussed in this chapter. New strategies for overcoming antifolate resistance that are currently emerging are described. Finally, there is evidence that the identification and characterisation of the molecular mechanisms of antifolate-resistance can be helpful in the future rationally-based development of novel antifolates and strategies that could theoretically overcome the drug-resistance phenomenon that occurred just 60 years ago in order to produce temporary remissions in childhood acute leukaemia by potently inhibiting the folate-dependent enzyme dihydrofolate reductase (DHFR). More recently, colorectal cancer and malignant pleural mesothelioma have been treated with novel antifolates called raltitrexed and pemetrexed, which work against thymidylate synthase (TS) and glycineamide ribonucleotide transformylase (GARTF) [32-38].

Drugs used as Folate Antagonist

Methotrexate

Amethopterin or methotrexate is an immune-system suppressant and a chemotherapy agent. It is also applied to cure cancer, autoimmune, and ectopic

pregnancies. Some of the types of cancers that it is used in are breast cancer, leukemia, lung cancer, lymphoma, gestational trophoblastic disease and osteosarcoma. Psoriasis, rheumatoid arthritis and Crohn disease are among types of autoimmune diseases in which it is administered. It can be administrable orally or via injection. Some of the common side effects are nausea, fatigue, fever, susceptibility to infections, low white blood cell levels, and destruction of skin in the mouth cavity. Other side effects can also be liver disease, lung disease, lymphoma, and skin rash severe. Side effects to people under long-term treatment should be checked regularly. During breastfeeding it is not safe [39]. Decreased doses can be required in people with kidney issues. It works by preventing the body to utilize folic acid. Methotrexate has been produced in 1947 and was initially employed in treating cancer since it was much less toxic than the available therapies of the time. The design is presented in Figure 3. It made the initial cures of a metastatic cancer in 1956. The World Health Organization is listed in the List of Essential Medicines. Methotrexate can be taken in generic form. In 2022, it became the 132d most frequent prescription drug in the United States, comprising over 4 million written prescriptions.

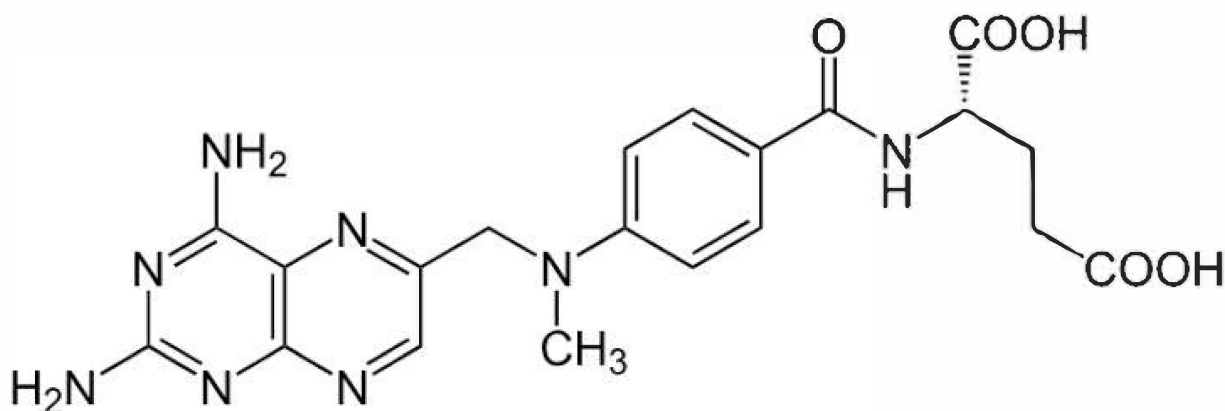


Figure 3: Chemical Structure of Methotrexate

Raltitrexed

Raltitrexed is a TYMS-selective and direct inhibitor. Similar to the cofactor of the THF, it is unable to be integrated within a DNA molecule, and due to the accumulation of dUMP in the cells, raltitrexed resistance is not observed. It can conveniently be administered at 3 week intervals, since it has a long duration of TYMS inhibition. In most countries (not the United States) raltitrexed is adopted in the treatment of advanced colorectal cancer, and is used primarily when the patient is unable to tolerate 5-fluorouracil (5-FU). Figure 4 Raltitrexed (cis-Benzilpidine; a thymidylate synthase inhibitor) has similar agent used in the treatment of colorectal cancer. Even though raltitrexed showed similar activity

as 5-FU/LV against advanced colorectal cancer, there were several raltitrexed-related deaths because of combined gastrointestinal and hematologic toxicity. With all the phase III MCRC trials combined, the rate of mortality with raltitrexed (1.6%-4%) was higher than with the 5-FU (1.2%-2.8%) despite a greatly reduced all-cause serious toxicity rate with raltitrexed. This has been seen despite the significantly lower rate of all-cause serious toxicity with raltitrexed though has been attributed to the use of raltitrexed as rescue therapy after a toxic event or in the presence of renal impairment. The management of patients undergoing raltitrexed requires patient education, renal functional monitoring and supportive care [40].

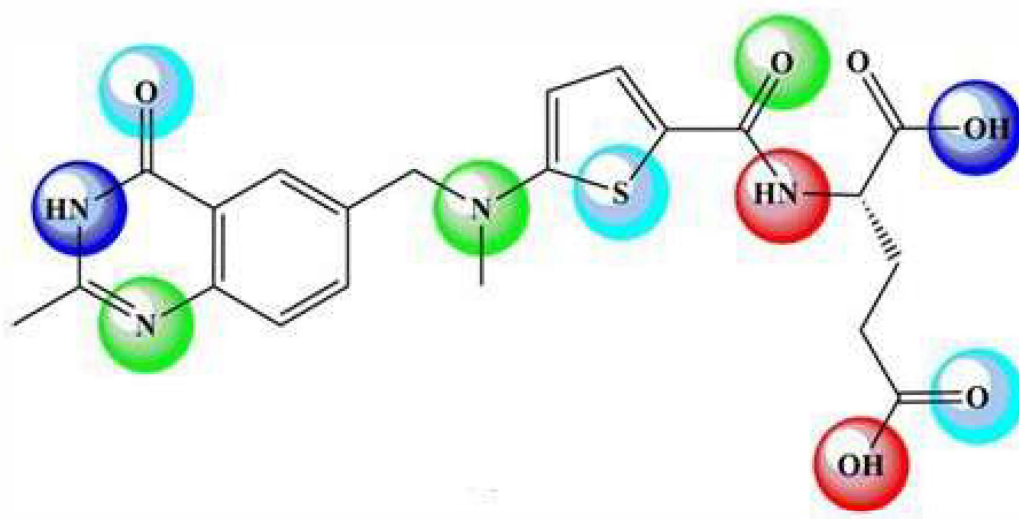


Figure 4: Chemical Structure of Raltitrexed

Pralatrexate

Pralatrexate (PDX; 100144¹⁹-propargyl 10-deazaaminopterin) is a more recently developed antifolate, which was designed rationally to be a more efficient binder of the RFC, which translates to a higher level of cellular internalization. PDX showed partial activity in a phase II study of lymphoma against peripheral T-cell lymphoma (TCL). The multicenter confirmatory phase II PROPEL (Pralatrexate in Relapsed or Refractory Peripheral T-cell Lymphoma) trial is a related study that has been conducted. As a result, PDX has been licensed in the US for use in the relapsed or refractory context of TCL. Twelve patients (11 percent) in the PROPEL trial obtained a full response, whereas the treatment response among the 109 evaluable patients was estimated to be 29 percent.⁷⁹ The median response time was 10.1 months. More than 70 percent of patients had mucosal inflammation that was generally mild to moderate. Clinical profile of structural representation of

pralatrexate is as shown in Figure 5. Haematological toxicity includes severe anaemia in 16 out of 100 participants and severe thrombocytopenia in 2 out of 3 patients. It was observed that 5 percent of the cases had febrile neutropenia. B12 and folic acid supplementation should be given to patients in order to prevent dangerous toxicity. PDX was also logically developed with high affinity toward the RFC and since it is internalized more in the cells, it had a greater antitumor relative to MTX. The structural distinction of The foundation of PDX and MTX is the substitution of the propargyl group at carbon 10 for the methyl group in MTX. This prerequisite of superior efficacy of PDX in vitro rests on the fact that it binds at a higher rate with the RFC, although there is no information on whether this is sufficient to supersede the MTX resistant. A strong inhibitor that binds to and deactivates DHFR, PDX has a high affinity for the protein. This depletes intracellular reduced folate pools and stops the production of DNA [41-42].

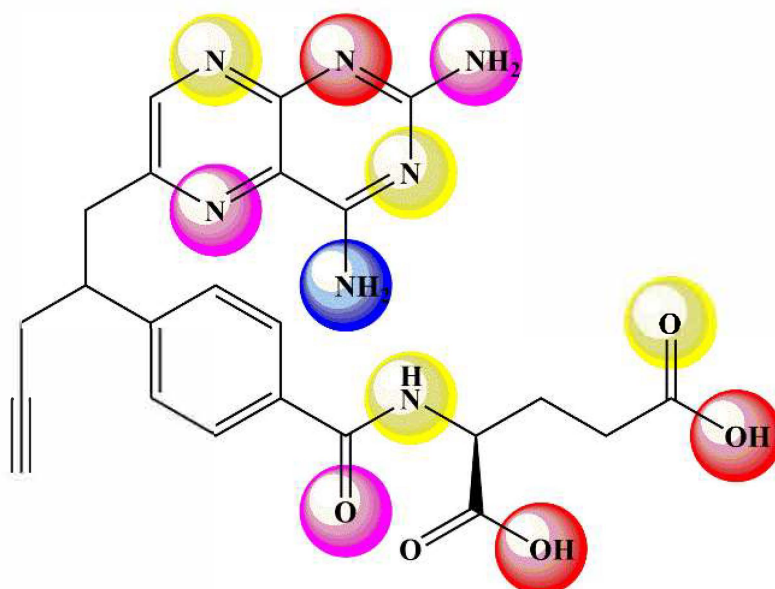


Figure 5: Chemical Structure of Pralatrexate

Lometrexol

Lometrexol (LMTX) is highly selective and effective GARFT inhibitor, having a wide range of antitumor activity. GARFT promotes the synthesis of purines by reacting 10 146 formyltetrahydrofolate (10 146 FTHF) to THF. With its inhibition, the intracellular purine levels are depleted.

LMTX is uptaken by the RFC and has wide-reaching polyglutamation, and slow removal of polyglutamates [43]. There will be severe cumulative myelosuppression and mucositis without the supply of folic acid. LMTX is currently not accepted as an anticancer agent. Lometrexol structure is presented in Figure 6.

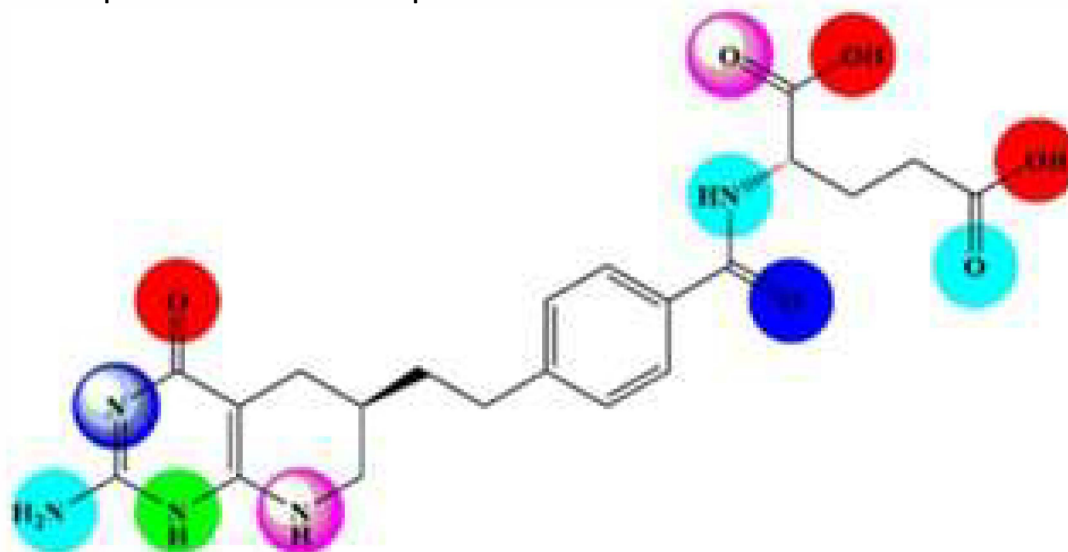


Figure 6: Chemical Structure of Lometrexol

Edatrexate

Edatrexate (EDX) is an old antifolate that has a superior intracellular polyglutamation, compared to MTX. EDX polyglutamates, on the contrary, have a very high affinity towards DHFR with a relatively low affinity towards TYMS. EDX has saturable, nonlinear Michaelis

Menten pharmacokinetics and lovely and only a maximum of 55 percent of EDX is excreted renally the parent unchanged entity. Since EDX was connected to toxic dermatitis, severe stomatitis, and even death, clinical development was stopped [44]. Table 2 presents a comparison of key antifolate drugs. Lometrexol is structure is presented in Figure 7.

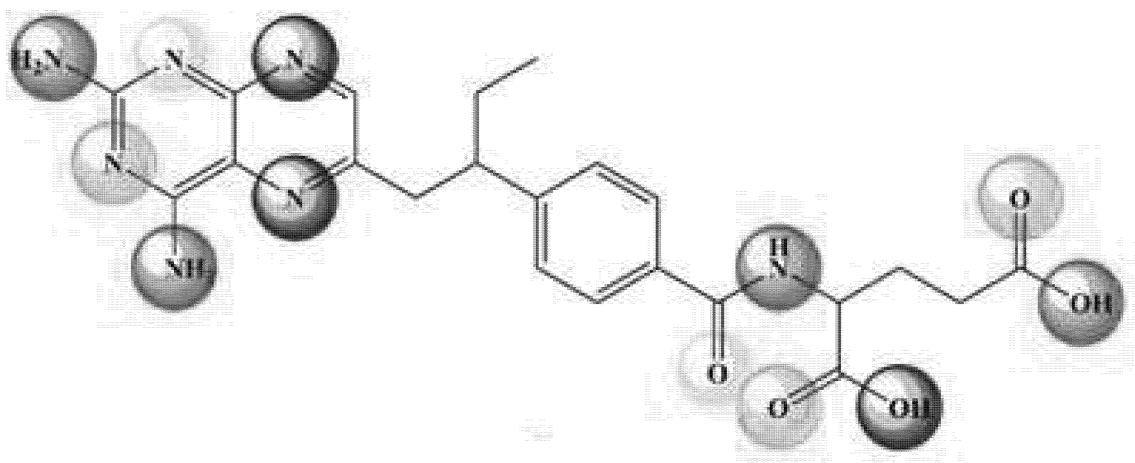


Figure 6: Chemical Structure of Lometrexol

Table 2- Comparison of Key Antifolate Drugs [45-50]

Drug Name	Mechanism of Action	Properties	Clinical Uses	Why/When Preferred Over MTX?	Key Liabilities & Mitigation
Methotrexate (MTX)	Inhibits DHFR → ↓ THF → inhibits dTMP and purine synthesis → DNA replication arrest	Classical antifolate; polyglutamated for retention; good oral & parenteral bioavailability	ALL, osteosarcoma, lymphoma, breast, bladder, head and neck cancers; RA, psoriasis, GVHD	Broadest evidence base; low cost; mature protocols with TDM and leucovorin rescue	Renal clearance → ensure hydration & urine alkalinization; monitor plasma MTX; manage DDIs

Raltitrexed (Tomudex)	Direct TS inhibitor → blocks dTMP synthesis → DNA synthesis arrest	Nonclassical antifolate; long half-life; not a DHFR inhibitor; IV only	Colorectal cancer (esp. MTX/5-FU intolerance); trials in other tumours	When DHFR-targeting not feasible (e.g., DHFR resistance/intolerance)	Long t _{1/2} → prolonged toxicity if renal/hepatic dysfunction; avoid in organ impairment; careful DDI review
Pralatrexate	Inhibits DHFR; high affinity for RFC-1; polyglutamated	Designed for improved uptake and selectivity for tumor cells; rapid intracellular retention	Peripheral T-cell lymphoma; trials for other cancers	In RFC-high tumour's; MTX resistance from poor uptake	Severe mucositis and myelosuppression → folate & B12 premedication; monitor CBC
Lometrexol	Inhibits GARFT → blocks de novo purine synthesis	Classical antifolate; potent purine synthesis inhibitor; requires folinic acid rescue	Investigational in lung/colon cancer; combination with leucovorin	When intensified purine blockade is desired in controlled trial settings	Profound myelosuppression → leucovorin rescue required; close CBC monitoring
Edatrexate	Inhibits DHFR & TS → dual blockade of purine and pyrimidine synthesis	MTX analogue; improved uptake; reduced toxicity in animals	NSCLC, head and neck cancer; phase II/III trials	When dual pathway inhibition is hypothesized to improve response	Added toxicity from dual target inhibition; careful dose titration; monitor organ function

DRUG RESISTANCE AND TOXICITY

Although folate antagonists are work clinically, a serious therapeutic restriction is represented by the growth of drug resistance. For the purpose to reduce the medications' affinity, the mechanisms of resistance can include point mutations on target enzymes like thymidylate synthase (TS) and dihydrofolate reductase (DHFR)

as well as gene amplification. Moreover, the change in membrane transport proteins, including the lowered expression or loss of functionality of reduced folate carrier (RFC1), disables intracellular drug to be uptake. Lower intracellular levels of antifolates are also caused by improved efflux via ATP-binding cassette (ABC) transporters, particularly P-glycoprotein (P-gp) and multidrug resistance-associated

proteins (MRPs). Further, enhanced production of folylpolyglutamate synthetase (FPGS) or glutamate may be protective in malignant cells in a paradoxical way, because folate retention is strengthened [46-48]. Folate antagonists produce toxicity that is mostly dose dependent with special observation in high rates of cell division in normal tissues such as the bone marrow, gastrointestinal tract and hair follicles. Side effects are so typical like myelosuppression, mucositis, hepatotoxicity, nephrotoxicity, and gastrointestinal disorders. Use of high-dose

methotrexate requires the use of leucovorin (folinic acid), known as the methotrexate rescue, to avert life-threatening toxicity by restoring depleted folate pools of normal cells. Therapeutic drug monitoring (TDM) and renal functional evaluation should be important when high-dose antifolate therapy is undergoing as to be safe and effective. The overcoming of resistance and reducing the degree of toxicity are important goals of the next generation of antifolate based therapies [49-50]. Table 3 describes the comparative assessment of folate antagonists based on toxicity, bioavailability, and selectivity

Table 3: Comparative Assessment of Folate Antagonists Based on Toxicity, Bioavailability, and Selectivity [51]

Drug Name	Toxicity Profile	Bioavailability & Pharmacokinetics	Selectivity / Target Specificity
Methotrexate (MTX)	Hematologic, GI, hepatic, renal toxicity; well-characterized; manageable with leucovorin rescue and hydration/alkalinization	Good oral and parenteral bioavailability; TDM widely available; polyglutamation ensures intracellular retention	Broad inhibition via DHFR → ↓ THF → blocks both purine & pyrimidine synthesis
Raltitrexed (Tomudex)	Myelosuppression, hepatotoxicity; risk of prolonged toxicity due to long half-life; no standard rescue	IV only; long half-life (~200h); accumulation risk in renal/hepatic dysfunction	Direct TS inhibitor; narrow target → useful when DHFR resistance or intolerance exists
Pralatrexate	Severe mucositis, myelosuppression; requires folate/B12 supplementation	IV; rapid uptake via RFC-1; efficient intracellular polyglutamation	DHFR inhibitor with enhanced tumor selectivity via RFC-1 transport

Lometrexol	Profound myelosuppression; toxicity requires folinic acid rescue	IV; moderate half-life; less clinical data on systemic bioavailability	GARFT inhibitor → selective blockade of purine synthesis
Edatrexate	Toxicity from dual DHFR & TS inhibition; may exceed MTX toxicity in some regimens	IV; improved uptake over MTX in models; not widely adopted clinically	Dual blockade of purine & pyrimidine synthesis (DHFR + TS)

FUTURE DIRECTIONS

Latest developments within the area of folate antagonist research has been aimed at attempts to increase the specificity and decrease the systemic toxicity of the drugs, as well as thwarting mechanisms of resistance. A prospective intervention includes the formation of targeted delivery vectors where antifolates are able to enter a tumor with much better accuracy like in liposomal formulations, polymeric nanoparticles and antibody drugs conjugate (ADCs) in order to enhance both therapeutic index and minimize the off-target effects. There has also been an emphasis of using pharmacogenomic profiling in predicting the patient-specific responses to methotrexate and pemetrexed, thus facilitating more personalized dosing regimens in terms of genetic polymorphism of the transporters (e.g. RFC1), enzymes metabolizing (e.g. MTHFR), and target enzymes (e.g. DHFR, TS). Moreover, logical drug combos like folate blockers with immunological checkpoint inhibitors or other PC blockers are tried out in preclinical and primary phases of experiments to enhance the

anticancer activity. Advanced novel antifolates which were found to be of enhanced bioavailability when taken orally and decrease the dependency on polyglutamate and specific selective binding to TS or DHFR are also in the development stage. In the future, one would also foresee additional optimization of the therapeutic use of folate antagonists through multi-omics integration, tumor microenvironment modulation, and machine learning-guided drug discovery to initiate a new age of precision antifolate therapy, not only in the oncology fields.

CONCLUSION

Surprisingly little progress has been made in replacing or supplementing MTX as a clinical standard, despite extensive research being conducted even in molecular modelling, synthesis, preclinical testing, and clinical trials. This is only partially because it has so much clinical experience with MTX and because its toxicity is so well-understood and generally manageable that it is a medication that is comfortable for clinical use. As a result, a novel antifolate that

supplements or replaces MTX must exhibit one of the two qualities listed below: be significantly better in MTX-sensitive tumours or work well in MTX-refractory tumours with inadequately effective treatments. Since the majority of antifolates are novel and must pass a number of rigorous clinical trials in order to be licensed, it may be that this is why none of them have been used in routine medicine. They could anticipate that either a more effective schedule or a responsive tumour type would be discovered as the better new antifolates, such as Tomudex, are put through progressive clinical development as single agents and in combination chemotherapy. Additionally, using more advanced molecular characterisation of response determinants (such microarrays), it would be possible to identify patient cohorts for whom a pharmacogenomic profile could allow for customised treatment with one of these new antifolates. Furthermore, the knowledge that a patient's folate status affects toxicity and that oral folic acid supplementation can be utilised to lower toxicity—especially delayed toxicity—will increase the therapeutic index of some antifolates on its own. When taken as a whole, these new findings may make it possible to employ at least some of the more recent drugs that target DHFR, TMPS, or GARFT for at least specialised clinical usage. Additional targets in the folate pathway presenting the possibility of less indiscriminate treatment have yet to be found. FPGS is a possible target since a fortuitous mutant misses FPGS expression

altogether and has lethal phenotype. It is possible to identify other targets in folate metabolism. Gene knock-out technology can also be one such method of determining these targets because this type of technology will enable the determination of those genes whose functioning cannot be neglected. Instead of that, non-essential genes are of less interest with regard to chemotherapy. The above discussion reveals that given the occurrence and, hopefully, the identification of such targets in the future, the instrumentation to urgently bring the drug to trial so that it can be understood that it is a viable chemotherapeutic target will be available.

REFERENCES

1. International Cancer Genome Consortium, Hudson, T. J., Anderson, W., Artez, A., Barker, A. D., Bell, C., Bernabé, R. R., et al. (2010). International network of cancer genome projects. *Nature*, 464(7291), 993–998.
2. Weinstein, J. N., Collisson, E. A., Mills, G. B., Shaw, K. R., Ozenberger, B. A., Ellrott, K., ... & Stuart, J. M. (2013). The cancer genome atlas pan-cancer analysis project. *Nature genetics*, 45(10), 1113-1120.
3. Zhang, J., Baran, J., Cros, A., Guberman, J. M., Haider, S., Hsu, J., ... & Kasprzyk, A. (2011). International Cancer Genome

- Consortium Data Portal—a one-stop shop for cancer genomics data. *Database*, 2011, bar026.
4. Parsons, D. W., Jones, S., Zhang, X., Lin, J. C. H., Leary, R. J., Angenendt, P., ... & Kinzler, K. W. (2008). An integrated genomic analysis of human glioblastoma multiforme. *science*, 321(5897), 1807-1812.
 5. Chan, E. S., & Cronstein, B. N. (2010). Methotrexate—how does it really work?. *Nature Reviews Rheumatology*, 6(3), 175-178.
 6. Chande, N., Wang, Y., MacDonald, J. K., & McDonald, J. W. (2014). Methotrexate for induction of remission in ulcerative colitis. *Cochrane Database of Systematic Reviews*, (8).
 7. Saeed, A., Jabeen, A., Mushtaq, I., & Murtaza, I. (2024). Cancer: Insight and strategies to reduce the burden. In N. Rezaei (Ed.), *Pathophysiology of cancer: An interdisciplinary approach* (Vol. 22). Interdisciplinary Cancer Research. Springer, Cham.
 8. Piñeros, M., Mery, L., Soerjomataram, I., Bray, F., & Steliarova-Foucher, E. (2021). Scaling up the surveillance of childhood cancer: a global roadmap. *JNCI: Journal of the National Cancer Institute*, 113(1), 9-15.
 9. de Martel, C., Geores, D., Bray, F., Ferlay, J., & Clifford, G. M. (2020). Global burden of cancer attributable to infections in 2018: a worldwide incidence analysis. *The Lancet global health*, 8(2), e180-e190.
 10. World Health Organization. (2023). *Assessing national capacity for the prevention and control of noncommunicable diseases: report of the 2021 global survey*. <https://www.who.int/publications/i/item/978924007>
 11. Alam, A., Geddes, A. J., & North, A. C. T. (1983). Methods and mechanisms for the development of MTX resistance in human lymphoid cells. *International Journal of Cancer*, 31(1), 45-50.
 12. Haber, M., Reed, C., Kavallaris, M., Norris, M. D., & Stewart, B. W. (1989). Resistance to drugs associated with the multidrug resistance phenotype following selection with high-concentration methotrexate. *JNCI: Journal of the National Cancer Institute*, 81(16), 1250-1254.
 13. Bech Hansen, N. T., Till, J. E., & Ling, V. (1976). Pleiotropic phenotype of colchicine resistant CHO cells: cross resistance and collateral sensitivity. *Journal of cellular physiology*, 88(1), 23-31.
 14. Beck, W. T. (1983). Vinca Alkaloid-Resistant Phenotype in Cultured Human Leukemic Lymphoblasts¹. *Cancer Treatment Reports*, 67(10), 875.

15. Fahey, J. B., & DiMaggio, C. (2007). High-dose methotrexate and primary central nervous system lymphoma. *Journal of Neuroscience Nursing*, 39(2), 83-88.
16. Zhu, J. J., Gerstner, E. R., Engler, D. A., Mrugala, M. M., Nugent, W., Nierenberg, K., ... & Batchelor, T. T. (2009). High-dose methotrexate for elderly patients with primary CNS lymphoma. *Neuro-oncology*, 11(2), 211-215.
17. Gong, F., Meng, Q., Liu, C., & Zhao, Y. (2019). Efficacy and association analysis of high dose methotrexate in the treatment of children with acute lymphoblastic leukemia. *Oncology letters*, 17(5), 4423-4428.
18. De Wilde, V., Dierickx, D., Schroyens, W., Van Den Neste, E., Bonnet, C., André, M., ... & van Hoof, A. (2016). BHS guidelines for primary central nervous system lymphoma V. De. *Belgian Journal of Hematology*, 7, 69-78.
19. Bergner, N., Monsef, I., Illerhaus, G., Engert, A., & Skoetz, N. (2012). Role of chemotherapy additional to high dose methotrexate for primary central nervous system lymphoma (PCNSL). *Cochrane Database of Systematic Reviews*, (11).
20. Singh, A., Chaturvedi, P., & Tiwari, A. (2022). Synthesis characterization pharmacological activity of some novel Schiff base. *International Journal of Creative Research Thoughts*, 10(1), a944–a956.
21. Kim, D. J., Venkataraman, A., Jain, P. C., Wiesler, E. P., DeBlasio, M., Klein, J., ... & Iwasaki, A. (2020). Vitamin B12 and folic acid alleviate symptoms of nutritional deficiency by antagonizing aryl hydrocarbon receptor. *Proceedings of the National Academy of Sciences*, 117(27), 15837-15845.
22. Nogueira, E., Sárria, M. P., Azoia, N. G., Antunes, E., Loureiro, A., Guimarães, D., ... & Cavaco-Paulo, A. (2018). Internalization of methotrexate conjugates by folate receptor- α . *Biochemistry*, 57(49), 6780-6786.
23. Yadav, P., Patel, P., Shukla, R., Detholia, K., & Singh, A. (2023). Development and validation of stability indicating RP-HPLC method for determination of aspirin and pantoprazole sodium in synthetic mixture stability indicating HPLC method. *International Journal of Pharmaceutical Chemistry and Analysis*, 10(1), 31–38.
24. Van Der Heijden, J. W., Oerlemans, R., Dijkmans, B. A., Qi, H., Laken, C. J. V. D., Lems, W. F., ... & Jansen, G. (2009). Folate receptor β as a potential delivery route for novel folate antagonists to macrophages in the synovial tissue of rheumatoid

- arthritis patients. *Arthritis & rheumatism*, 60(1), 12-21.
25. Lopez Olivo, M. A., Siddhanamatha, H. R., Shea, B., Tugwell, P., Wells, G. A., & Suarez Almazor, M. E. (2014). Methotrexate for treating rheumatoid arthritis. *Cochrane Database of Systematic Reviews*, (6).
 26. Blits, M., Jansen, G., Assaraf, Y. G., van de Wiel, M. A., Lems, W. F., Nurmohamed, M. T., ... & Verweij, C. L. (2013). Methotrexate normalizes up regulated folate pathway genes in rheumatoid arthritis. *Arthritis & Rheumatism*, 65(11), 2791-2802.
 27. Bhaskar, L. V. K. S., Murthy, J., & Babu, G. V. (2011). Polymorphisms in genes involved in folate metabolism and orofacial clefts. *Archives of oral biology*, 56(8), 723-737.
 28. Koźmiński, P., Halik, P. K., Chesori, R., & Gniazdowska, E. (2020). Overview of dual-acting drug methotrexate in different neurological diseases, autoimmune pathologies and cancers. *International journal of molecular sciences*, 21(10), 3483.
 29. Singh, A., & Chaturvedi, P. (2021). Review on Schiff bases. *World Journal of Pharmaceutical Sciences*, 9(8), 133–140.
 30. Bock, K. W. (2023). Aryl hydrocarbon receptor (AHR): Towards understanding intestinal microbial ligands including vitamin B12 and folic acid as natural antagonists. *Biochemical Pharmacology*, 214, 115658.
 31. Czeizel, A. E., Dudás, I., Vereczkey, A., & Bánhidy, F. (2013). Folate deficiency and folic acid supplementation: the prevention of neural-tube defects and congenital heart defects. *Nutrients*, 5(11), 4760-4775.
 32. Bhunia, S. K., Maity, A. R., Nandi, S., Stepensky, D., & Jelinek, R. (2016). Imaging cancer cells expressing the folate receptor with carbon dots produced from folic acid. *ChemBioChem*, 17(7), 614-619.
 33. Ramakrishna, J. K., Gowda, P. S. K. R., Rahamanulla, A., Nagaraju, P. K., Ramesh, B., & Chandramouli, M. (2025). The Impact of Novel Purine, Pyrimidine and Folate Analogues on Cancer Treatment: A Review. *Journal of Young Pharmacists*, 17(1), 26-35.
 34. Yang, T. Y., Chang, G. C., Hsu, S. L., Huang, Y. R., Chiu, L. Y., & Sheu, G. T. (2013). Effect of folic acid and vitamin B12 on pemetrexed antifolate chemotherapy in nutrient lung cancer cells. *BioMed research international*, 2013(1), 389046.
 35. Rossignol, D. A., & Frye, R. E. (2021). Cerebral folate deficiency, folate receptor alpha autoantibodies and leucovorin (folinic acid) treatment in autism spectrum disorders: a

- systematic review and meta-analysis. *Journal of personalized medicine*, 11(11), 1141.
36. Ho, P. I., Ashline, D., Dhitavat, S., Ortiz, D., Collins, S. C., Shea, T. B., & Rogers, E. (2003). Folate deprivation induces neurodegeneration: roles of oxidative stress and increased homocysteine. *Neurobiology of disease*, 14(1), 32-42.
 37. Sahakyan, V., Duellen, R., Tam, W. L., Roberts, S. J., Grosemans, H., Berckmans, P., ... & Sampaolesi, M. (2018). Folic acid exposure rescues spina bifida aperta phenotypes in human induced pluripotent stem cell model. *Scientific reports*, 8(1), 2942.
 38. Shafiq, A., Shah, H., Khan, A., Khan, S., Akram, F., Shafique, M., ... & Madni, A. (2025). Folate receptor-mediated targeting strategies for potential management & treatment of rheumatoid arthritis. *Journal of Drug Delivery Science and Technology*, 106916.
 39. Jacobson, D. L., Crider, K. S., DeMarrais, P., Brummel, S., Zhang, M., Pfeiffer, C. M., ... & Lockman, S. (2024). Dolutegravir-Versus Efavirenz-Based Treatment in Pregnancy: Impact on Red Blood Cell Folate Concentrations in Pregnant Women and Their Infants. *The Journal of infectious diseases*, 230(5), 1224-1234.
 40. Finnell, R. H., & Zhu, H. (2024). Periconceptional maternal folate supplementation impacts a diverse range of congenital malformations. *Pediatric Research*, 95(4), 880-882.
 41. Li, X., Li, S., Mu, D., Liu, Z., Li, Y., Lin, Y., ... & Zhu, J. (2013). The association between periconceptional folic acid supplementation and congenital heart defects: a case-control study in China. *Preventive medicine*, 56(6), 385-389.
 42. Mölzer C, Wilson HM, Kuffova L, Forrester JV. A Role for Folate in Microbiome Linked Control of Autoimmunity. *Journal of Immunology Research*. 2021;2021(1):9998200.
 43. Singh, A., Rajput, A., Kureshi, G., Carpenter, G., & Vashi, J. (2023). An RP-HPLC method performance and validation for azelnidipine measurment and metoprolol succinate within a synthetic mixture. *Pharmacophore*, 14(3-2023), 1-6.
 44. Sadaka, C., Ellsworth, E., Hansen, P. R., Ewin, R., Damborg, P., & Watts, J. L. (2018). Review on abyssomicins: Inhibitors of the chorismate pathway and folate biosynthesis. *Molecules*, 23(6), 1371.
 45. Xu, M., Wu, S., Wang, Y., Zhao, Y., Wang, X., Wei, C., ... & Hu, C. (2022). Association between high-dose methotrexate-induced toxicity

- and polymorphisms within methotrexate pathway genes in acute lymphoblastic leukemia. *Frontiers in Pharmacology*, 13, 1003812.
46. Hanoodi, M., & Mittal, M. (2026). Methotrexate. In StatPearls. StatPearls Publishing, Treasure Island (FL). Available from: <https://www.ncbi.nlm.nih.gov/books/NBK556114/>
 47. Stamp, L. K., & Roberts, R. L. (2011). Effect of genetic polymorphisms in the folate pathway on methotrexate therapy in rheumatic diseases. *Pharmacogenomics*, 12(10), 1449-1463.
 48. Van der Heijden, J. W., Assaraf, Y. G., Gerards, A. H., Oerlemans, R., Lems, W. F., Scheper, R. J., ... & Jansen, G. (2014). Methotrexate analogues display enhanced inhibition of TNF- α production in whole blood from RA patients. *Scandinavian journal of rheumatology*, 43(1), 9-16.
 49. Wang, W., Zhou, H., & Liu, L. (2018). Side effects of methotrexate therapy for rheumatoid arthritis: A systematic review. *European journal of medicinal chemistry*, 158, 502-516.
 50. Kakkadath, M., Naidu, D., Kanthlal, S. K., & Sharun, K. (2025). Combating Methotrexate Resistance in Cancer Treatment: A Review on Navigating Pathways and Enhancing Its Efficacy With Fat Soluble Vitamins. *Scientifica*, 2025(1), 8259470.

