Review

Folic Acid Antimetabolites (Antifolates): A Brief Review on Synthetic Strategies and Application Opportunities

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Abstract: Antimetabolites of folic acid represent a large group of drugs and drug candidates, including those for cancer chemotherapy. In this current review, the most common methods and approaches are presented for the synthesis of therapeutically significant antimetabolites of folic acid, which are Methotrexate (MTX), Raltitrexed (Tomudex, ZD1694), Pralatrexate, Pemetrexed, TNP-351, and Lometrexol. In addition, the applications or uses of these folic acid antimetabolites are also discussed.

Keywords: antifolates; folic acids; synthesis; applications; drugs

1. Introduction

Antimetabolites, which are antagonists of natural metabolites, belong to a group of highly efficient anticancer drugs. Based on the chemical structure, these groups can be divided into several sub-groups, such as non-natural amino-acids [1] or peptides [2,3], including phospha-analogues [4], analogues of purine and pyrimidine bases, such as competitors in the synthesis of the nucleic acids [5,6], as well as vitamin actions including folic acid [7], hormones [8], coenzymes [9], and other substrates responsible for the normal functioning of cells and tissues of the human body.

The mechanism of antimetabolites action is based on their ability to enter into competitive relationships with structurally similar metabolites of the living, which leads to a lack of the corresponding metabolite and a decrease in the activity of vital biochemical processes in the cell. In order to interfere with the synthesis of the DNA constituents, the most common antimetabolites should be structural analogues of purine and pyrimidine bases/nucleosides, or of folate cofactors [10].

In this current review, we analyze the most common approaches for the synthesis of therapeutically significant antimetabolites of folic acid [11–13], such as Methotrexate (MTX), Raltitrexed (Tomudex, ZD1694), Pralatrexate, Pemetrexed, TNP-351, and Lometrexol.

2. Discussion

2.1. Mechanism of Antifolates Action

Folic acid (**1**) first has to be reduced to THFA (**2**) by dihydrofolate reductase (Figure 1), after which it can attach various one-carbon groups and transfer them to other molecules. In the reaction, once catalyzed by thymidylate synthase, deoxyuridine monophosphate (deoxy-UMP or dUMP) is converted to deoxythymidine monophosphate (deoxy-TMP or dTMP), producing a methylene group from 5,10-methylene-THFA; the latter is oxidized into dihydrofolic acid and must be reduced again to participate in further reactions. Methotrexate (MTX) and other folic acid antagonists with a high affinity for dihydrofolate reductase (K, 0.01–0.2 nmol/L) disrupt the formation of THFA, causing a deficiency of

reduced folates and an accumulation of toxic dihydrofolic acid polyglutamates. At the same time, the transfer reactions of one-carbon groups, which are necessary for the synthesis of purines and dTMP, are inhibited; as a result, the synthesis of nucleic acids and other metabolic processes are disrupted. The toxic action of methotrexate is prevented by calcium folinate (the calcium salt of 5-formyl-THFA), which enters the cell via a reduced folate transporter and is converted into the other THFA derivatives [14] (Scheme 1).
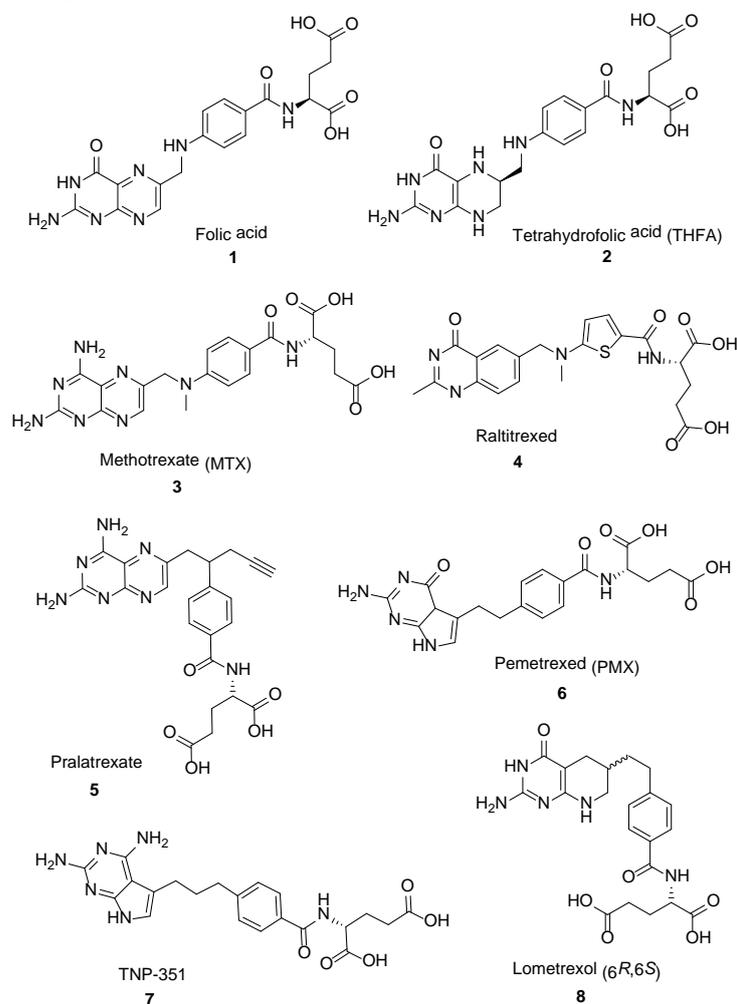
Figure 1. Structure of folic acid (**1**) and tetrahydrofolic acid (THFA) (**2**) and their antimetabolites.

Once it became clear that methotrexate directly inhibits not only dihydrofolate reductase but also the enzymes for the synthesis of purines and thymidylate synthase, the coenzymes of which are reduced folates, a search commenced for folic acid antagonists that selectively inhibit these enzymes. By replacing the N-5, N-8 and N-10 atoms and modifying the side chains of the methotrexate molecule, it was possible to synthesize drugs that retain their inherent ability to form stable polyglutamates inside the cell, but better penetrate the tumor [15], such as the following: raltitrexed, a thymidylate synthase inhibitor; lometrexol, a purine synthesis inhibitor; and pemetrexed, which combines both mechanisms of action [16].

Most folic acid antimetabolites are only partially selective for tumor cells and affect rapidly proliferating normal cells, including bone marrow and gastrointestinal mucosa. Folic acid antagonists act in the S-period and are most active against cells in the logarithmic growth phase [17].
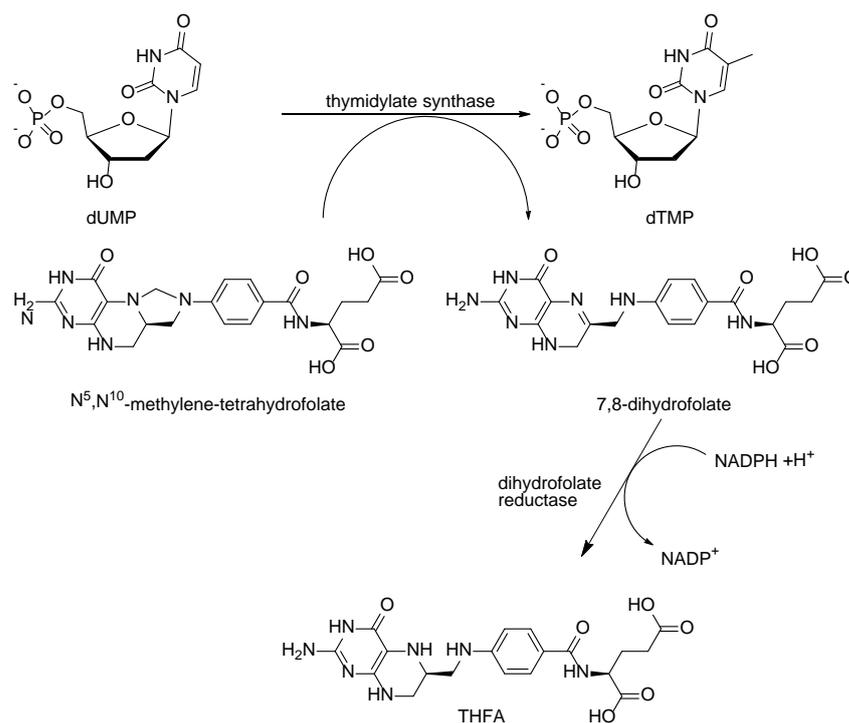
Scheme 1. Main target processes for antifolates in living cells.

2.2. Methotrexate: (S)-2-(4-(((2,4-Diaminopteridin-6-yl)Methyl)(Methyl)Amino)benzamido) Pentanedioic Acid (MTX, Rheumatrex, Amethopterin, Abitrexate, Trexall, Methylaminopterin, Mexate, Metatrexan)

The discovery of the first folic acid antagonist, methotrexate (MTX), with its promising activity for the treatment of a variety of human cancers, prompted the search for other folate analogs [18]. As a structural analogue of folic acid, methotrexate inhibits the activity of the enzyme folate reductase, which prevents the conversion of folic acid into tetrahydrofolic acid, which is involved in cell metabolism and reproduction. Methotrexate is recommended for acute childhood leukemia; chorionepithelioma of the uterus; cancer of the breast, lungs, testicles, and other malignant tumors in adults (in combination with other antiblastoma drugs); and is also used as an immunosuppressive agent.

The most common synthetic strategy for the preparation of MTX **3** involves the post-modification of 3,4-dihydropteridine-2,4-diamines **9,** as depicted in Scheme 2.
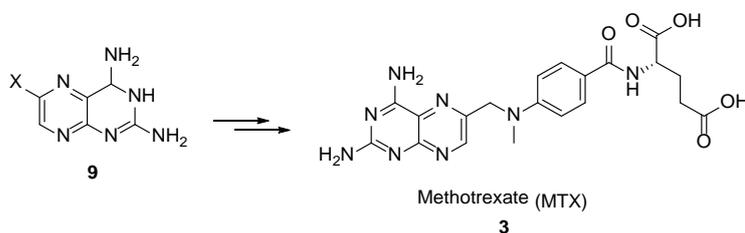
Scheme 2. Synthetic strategy towards MTX by the post-modification of 3,4-dihydropteridine-2,4-diamines **9**.

Thus, MTX was obtained by the reaction of 2,4-diamino-6-bromomethylpteridine hydro-bromide **11** with barium salt dehydrate [19] or Zn^{2+} salt [20] of *p*-(*N*-methyl)-aminobenzoyl-*L*-glutamic acid **10** in 87.5% and 56.1% yields, accordingly (Scheme 3).
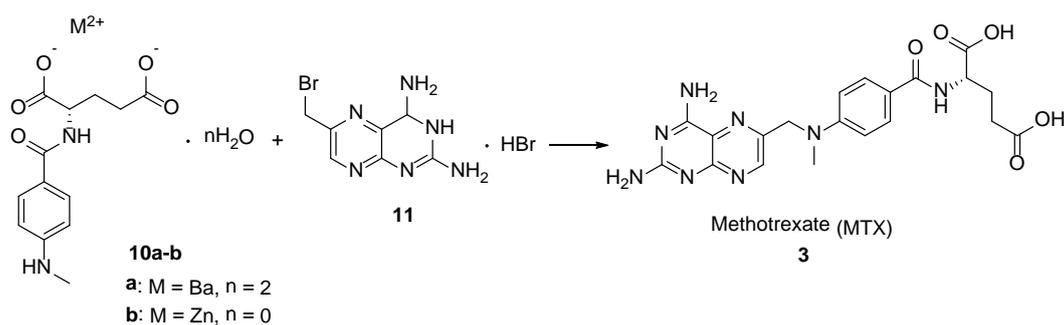
Scheme 3. Synthesis of MTX by the reaction between metal salt of *p*-(*N*-methyl)-aminobenzoyl-*L*-glutamic acid and 2,4-diamino-6-bromomethylpteridine hydrobromide.

The reaction of **11** with the diethyl *p*-(*N*-methyl)-aminobenzoyl-*L*-glutamate **12** followed by basic saponification (Scheme 4) provided lower yields of the target product [21].
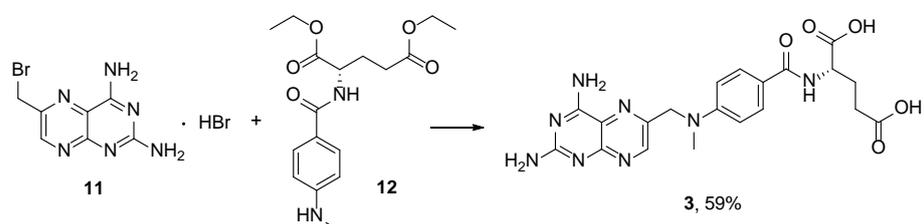
Scheme 4. Synthesis of MTX by the reaction of 2,4-diamino-6-bromomethylpteridine hydrobromide **11** with diethyl *p*-(*N*-methyl)-aminobenzoyl-*L*-glutamate **12**.

Another approach involves the substitution of the azide group in 4-(*N*-methyl-*N′*-(6″-aminopteroil-methyleno)aminobenzoic acid derivative **13** in a reaction with L-glutamic acid **14** in DMSO at room temperature in the presence of tetramethylguanidine (TMG) as the base (Scheme 5) [22]. The reaction resulted in the corresponding desired MTX in a quantitative yield, which is the main advantage of this method.
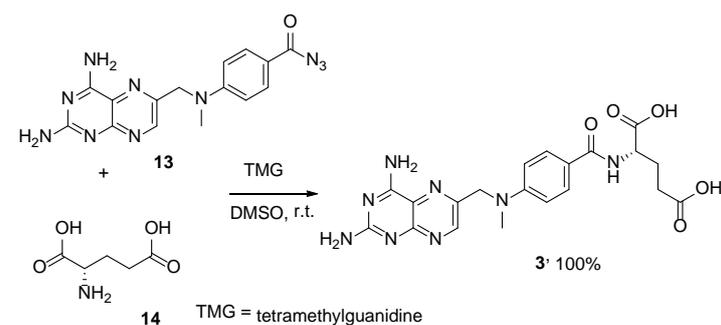
Scheme 5. Quantitative synthesis of MTX by the reaction of 4-(*N*-methyl-*N′*-(6″-aminopteroil-methyleno)aminobenzoic acid derivative **13** with L-glutamic acid **14**.

In addition, MTX was obtained in a 75.7% yield by means of the transformation of its more stable and synthetically available 4-oxoderivative (methopterin hydrate) (**15**) in the presence of pyridine, *p*-toluenesulfonic acid monohydrate and 1,1,1,3,3,3-hexamethyl-disilazane (HMDZ) (Scheme 6) [23].

Along with MTX, its ¹³C-multilabelled forms with ¹³C-enrichment at 2, 7, 9, 4, 7, 8a, 9 and 2, 4a,b positions were synthesized from the di-*tert*-butyl ester of MTX **16** for the NMR study of the mechanisms of drug–enzyme interactions (Scheme 7) [24]. The reaction was carried out by performing 'benzylic' bromination, followed by the substitution of the bromine atom by the di-*t*-butyl *N*-(*p*-methylaminobenzoyl)-*L*-glutamate. The acid treatment of each of the formed methotrexate di-*t*-butyl esters yielded the corresponding

¹³C-enriched methotrexate in 60–90% yields. So far, this is the only method reported for the synthesis of C¹³-MTX.
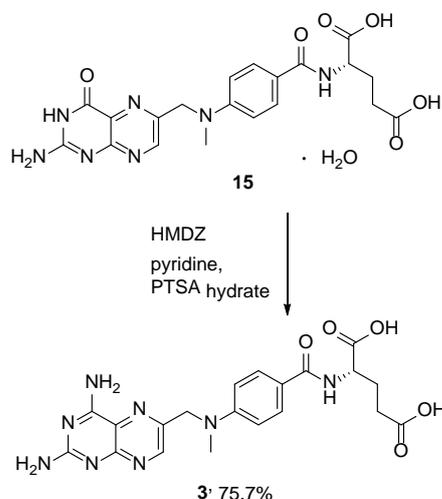
Scheme 6. Synthesis of MTX from 4-oxoderivative of MTX (methopterin hydrate) (**15**).
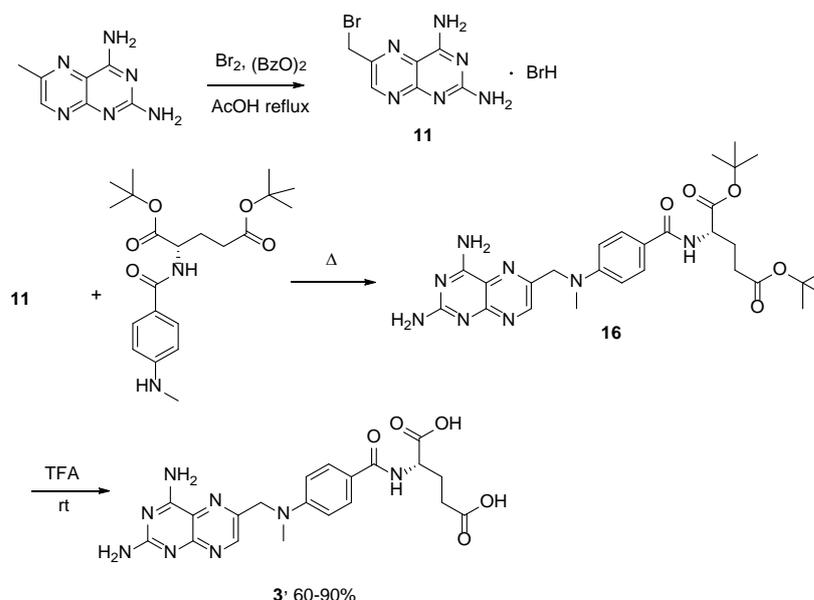
Scheme 7. Multistep synthesis of MTX.

In another method for the synthesis of MTX **3**, the pro-drug of MTX, *N*-(*L*-α-aminoacyl)-derivative of methotrexate **18**, was initially prepared by a reaction between the di-*tert*-butyl ester of MTX **16** and *N*-*tert*-butyloxycarbonyl-*L*-leucine derivative **17**, followed by the acidic deprotection of protective groups [25]. Subsequently, the obtained pro-drug **18** was successfully converted into MTX via the enzymatic cleavage by porcine microsomal leucine aminopeptidase (Scheme 8). Unfortunately, the authors did not provide any yields due to the format of the publication.

Free-form MTX was obtained from the conjugate of the *o*-nitrobenzyl alcohol derivative and MTX **19** during a photolysis experiment in aqueous methanol under UV-light irradiation [26]. This technique was considered by the authors as a possible way to transport the MTX to the cancer cells with the release of MTX free form at up to 50% at a pH level of 7.4 (Scheme 9).
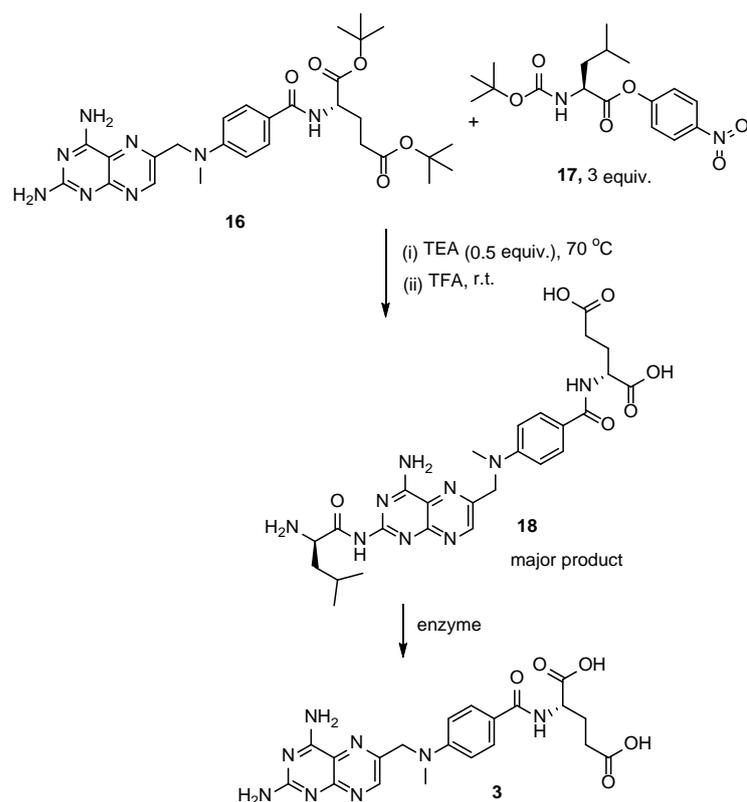
Scheme 8. Three-step synthesis of MTX starting from di-*tert*-butyl ester of MTX **16** and *N-tert*-butyloxycarbonyl-*L*-leucine derivative **17**.
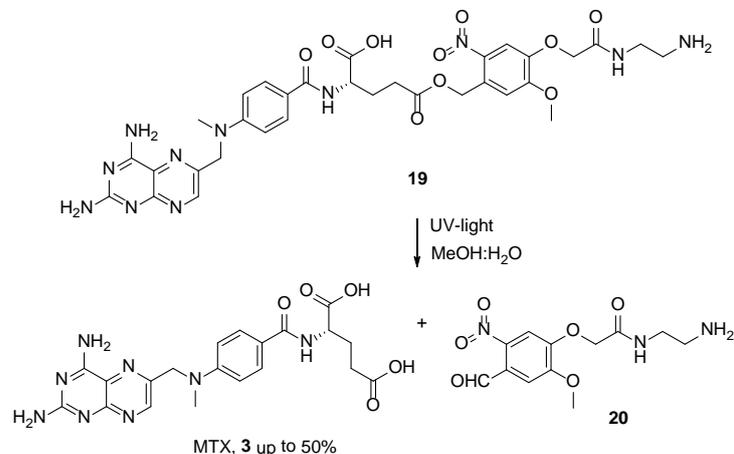
Scheme 9. UV-light promoted synthesis of MTX from **19**. Reproduced with the permission of reference [26]. Copyright © 2011, Elsevier Ltd.

In the literature, there are less common synthetic approaches available that involve the construction of a 3,4-dihydropteridine core starting from aminopyrimidines **21** (Scheme 10).
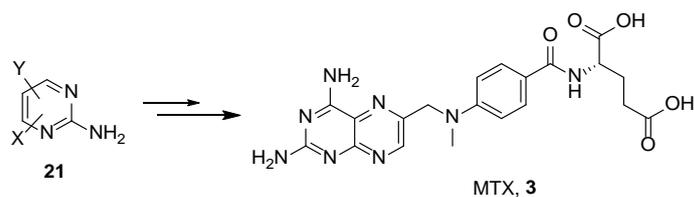
Scheme 10. Synthetic strategy towards MTX by the post-modification of aminopyrimidines **21**.

In this context, MTX was obtained by the tandem multicomponent reaction between Zn^{2+} salt of *N*-(4-*N*-methylaminobenzoil)-*L*-glutamic acid **10b**, 1,1,3-tribromoacetone **23** and 2,2,5,6-tetraaminopyrimidine sulfate **22** under mild conditions (Scheme 11) [27]. This method has a noticeable advantage, such as the possibility to carry out several reactions in one step without the isolation of intermediates during each step.
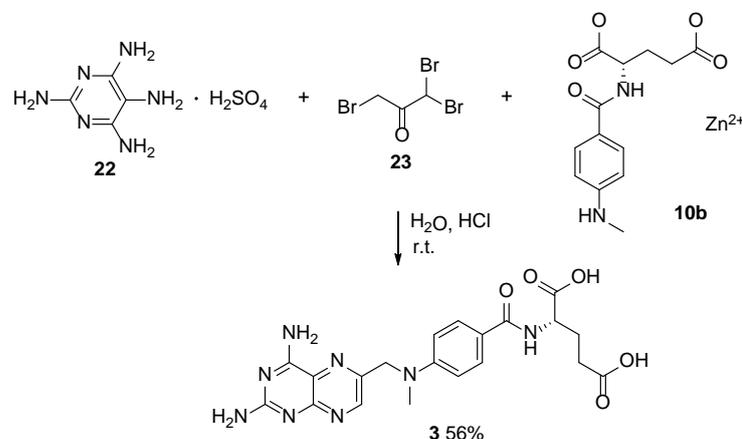
Scheme 11. Multicomponent synthesis of MTX under mild reaction conditions.

In another method, the MTX core was constructed by means of a heterocyclization reaction between commercially available guanidine acetate **24** and easily derived diethyl (4-(((5-amino-6-cyanopyrazin-2-yl)methyl)(methyl)amino)benzoyl)glutamate **25** under heating conditions, followed by basic hydrolysis (Scheme 12) [28].
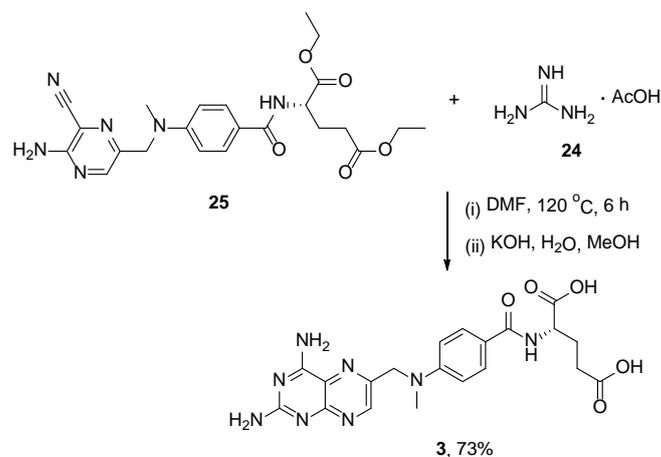
Scheme 12. Synthesis of MTX by the reaction between guanidine acetate **24** and diethyl (4-(((5-amino-6-cyanopyrazin-2-yl)methyl)(methyl)amino)benzoyl)glutamate **25**.

Lastly, the approach for MTX **3** involves a reaction between 2,4,5,6-tetraaminopyrimidine hydrosulphate **22**, 2,3-dibromopropionaldehyde **26**, and *N*-4-(methylamino)benzoyl)-*L*-glutamic acid **12** disodium salt under oxidative conditions (iodine in the presence of KI) (Scheme 13) [29]. In this article, the authors were more concerned about the purity of the obtained compounds than their yields.

2.3. Raltitrexed: (2S)-2-[[5-[Methyl-[(2-Methyl-4-oxo-3H-Quinazolin-6-yl)Methyl]Amino] Thiophene-2-Carbonyl]Amino]Pentanedioic Acid (Tomudex, ZD1694)

Raltitrexed (Tomudex) is a more recent, specific, mixed, and non-competitive inhibitor of thymidylate synthase indicated for use in cancer therapy, especially colorectal cancer [30–32].
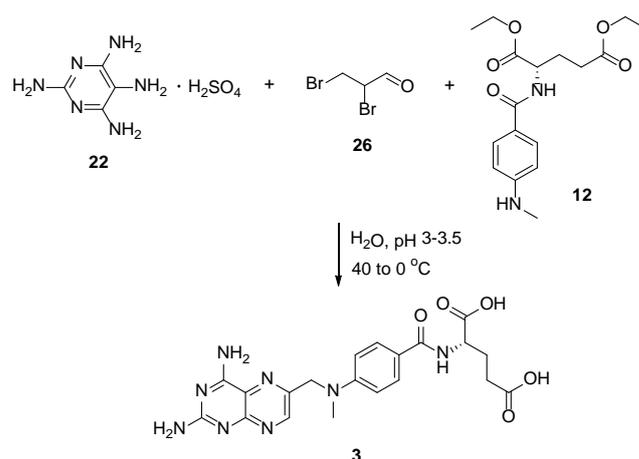
Scheme 13. Synthesis of MTX by the reaction between 2,4,5,6-tetraaminopyrimidine hydrosulphate **22**, 2,3-dibromopropionaldehyde **26**, and *N*-4-(methylamino)benzoyl-*L*-glutamic acid **12** disodium salt under oxidative conditions.

In 1991, Marsham et al. reported the synthesis of a series of C2-methyl-N10-alkylquinazoline-based antifolates, in which the benzene ring was replaced by the heterocycles, i.e., thiophene, thiazole, thiadiazole, pyridine, and pyrimidine (Scheme 14) [33].
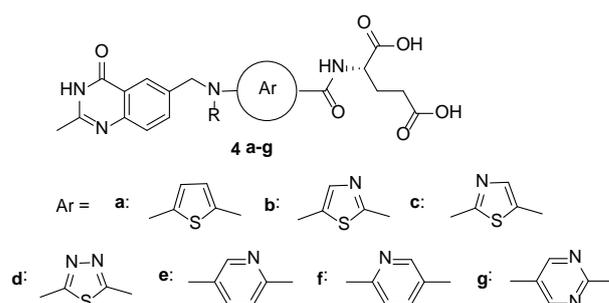
Scheme 14. Structures of a series of C2-methyl-N10-alkylquinazoline-based antifolates. Reproduced with the permission of reference [33]. Copyright © 1991, American Chemical Society.

The thiophene system **4a** and its related thiazole **4b** yielded analogues that were considerably more efficient than the parent benzene series as inhibitors of L1210 cell growth. Although, in general, these heterocycles were somewhat poorer inhibitors of the isolated TS enzyme. Raltitrexed **4a** (R = CH$_3$) was synthesized in a 41% yield starting with the thiophene-2-carboxylic acid, as shown in Scheme 15.
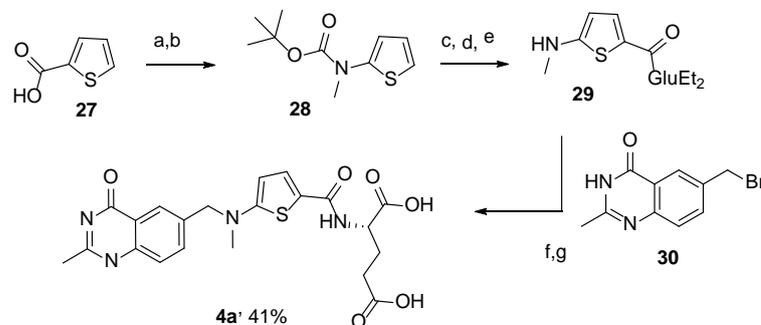
Reagents and conditions: (a) Diphenyl phosphorazidate, Et$_3$N, *t*-BuOH, reflux; (b) sodium hydride, iodomethane, DMF; (c) (i) *n*-BuLi, (ii) CO$_2$; (d) (i) oxalyl chloride, DMF, (ii) diethyl glutamate, Et$_3$N, CH$_2$Cl$_2$; (e) trifluoroacetic acid; (f) 2,6-lutidine, DMF, Ar; (g) 1 N aq. NaOH, Ar.

Scheme 15. Multistep synthesis of Raltitrexed **4a** starting from thiophene-2-carboxylic acid **27**.

Another route to Raltitrexed was reported that started with thiophene-2,5-dicarboxylic acid **31**, which was then converted in four steps to diethyl (5-(methylamino)thiophene-2-carbonyl)-*L*-glutamate **35**. This was followed by an alkylation reaction of the last one with 6-bromomethyl-2-methyl-4-quinazolinone **30** and basic hydrolysis, which resulted in the target product **4a** (Scheme 16) [34].
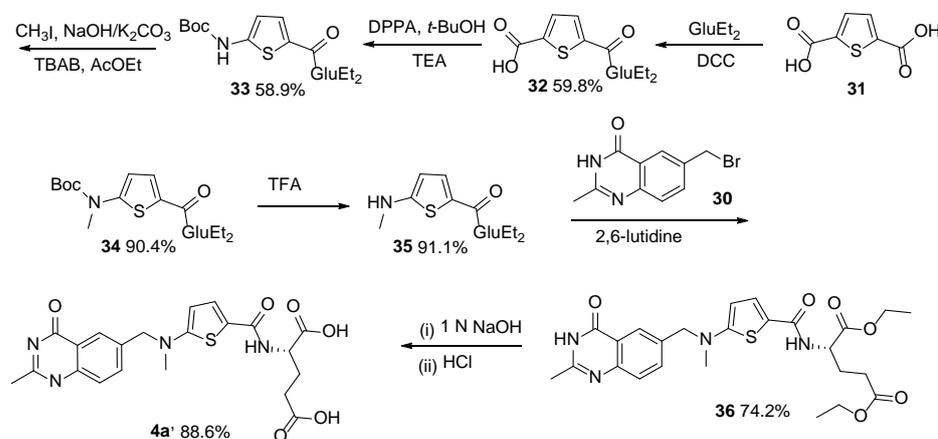
Scheme 16. Multistep synthesis of Raltitrexed starting from thiophene-2,5-dicarboxylic acid **31**.

A similar route to Raltitrexed was reported by Yao et al. starting with 5-nitrothiophene-2-carboxylic acid **37** via the sequence of NaBH$_4$ reduction, alkylation, and saponification (Scheme 17) [35]. The target product was isolated in a lower yield.
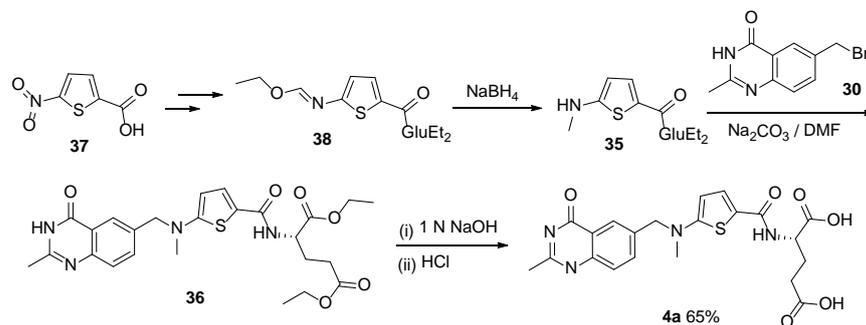
Scheme 17. Multistep synthesis of Raltitrexed starting from 5-nitrothiophene-2-carboxylic acid **37**.

Raltitrexed was also prepared by using the same compound in less reaction steps as reported by Xiqun et al. (Scheme 18) [36].
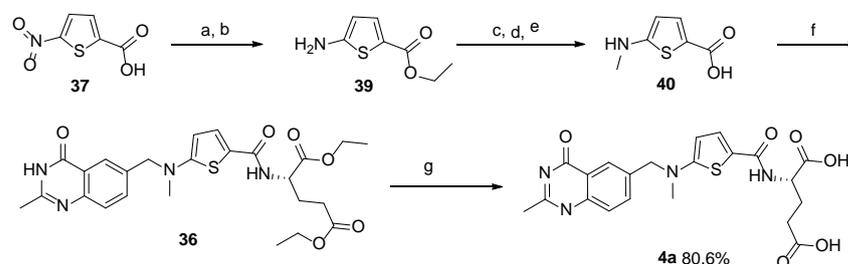
Reagents and conditions: (a) EtOH, H$^+$; (b) H$_2$/Pd/C/NH$_4$COOH; (c) CH(OMe)$_3$; (d) NaBH$_4$; (e) NaOH; (f) (i) SOCl$_2$, (ii) L-GluEt$_2$, (iii) **30**; (g) (i) 1 N NaOH, (ii) HCl.

Scheme 18. Multistep synthesis of Raltitrexed starting from 5-nitrothiophene-2-carboxylic acid **37**.

Moreover, the most recent and—in our opinion—easiest approach was reported in the work of H. Shaojie et al. regarding Raltitrexed, which involves a four-step sequence

using diethyl (5-(*N*-methylacetamido)thiophene-2-carbonyl)-*L*-glutamate **41** as the starting material (Scheme 19) [37].
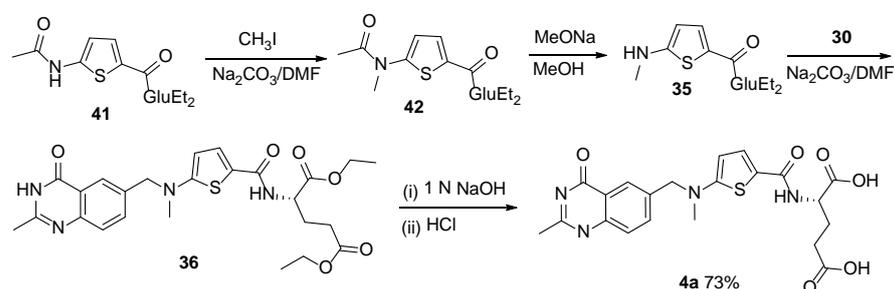
Scheme 19. Synthesis of Raltitrexed by diethyl (5-(*N*-methylacetamido)thiophene-2-carbonyl)-*L*-glutamate **41** as the starting material.

2.4. Pralatrexate (Folotyn):
N-4-[1-(2,4-Diaminopteridin-6-yl)Pent-4-yn-2-yl]Benzoyl-L-Glutamic Acid

Pralatrexate **25** is another folate antagonist and antineoplastic agent with confirmed activity for the treatment of relapsed or refractory peripheral T-cell lymphoma (PTCL). Pralatrexate was approved for medical use in the United States in September 2009, as the first treatment for Peripheral T-cell Lymphoma (PTCL) [38,39], an often-aggressive type of non-Hodgkins lymphoma [40].

Successive alkylation of dimethyl homoterephthalate **43** with propargyl bromide **44** and 2,4-diamino-6-(bromomethy1)pteridine **11** followed by ester saponification at room temperature resulted in 2,4-diamino-4-deoxy-10-carboxy-10-propargy1-10-deazapteroic acid **46**. Subsequently, compound **46** was readily decarboxylated by heating in DMSO at 120 °C to yield diamino-10-propargyl-10-deazapteroic acid **47** as a precursor of Pralatrexate **5**. Additionally, the coupling of **47** with diethyl *L*-glutamate followed by ester hydrolysis, yielded Pralatrexate **5** (Scheme 20) [41].
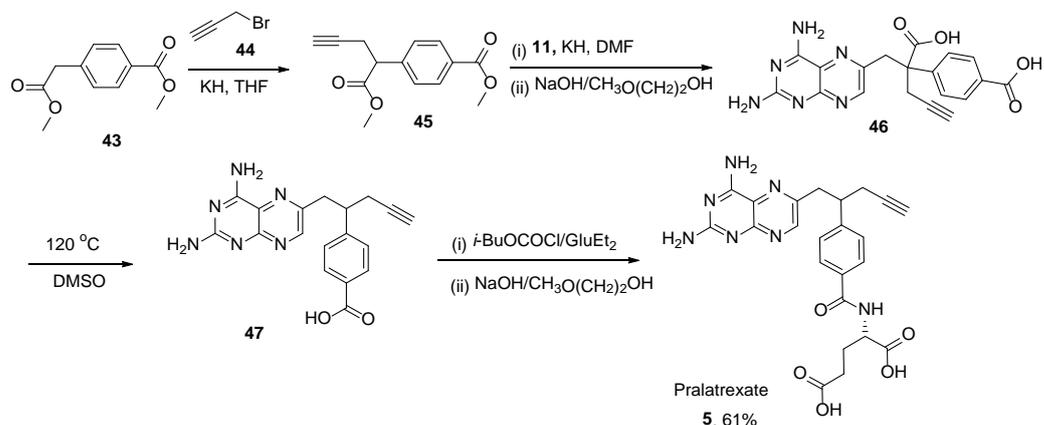
Scheme 20. Synthesis of Pralatrexate starting from the reaction between dimethyl homoterephthalate **43** and propargyl bromide. Reproduced with the permission of reference [41]. Copyright © 1993, American Chemical Society.

After the abovementioned publication, many improved procedures were reported for the preparation of Pralatrexate [42–48]. The synthesis of optically pure diastereomers of Pralatrexate has also been reported [49].

Another approach to producing Pralatrexate was developed by Alla et al. (2013), starting with ethyl 4-formylbenzoate **48**; however, the yield was not specified (Scheme 21) [50].

Scheme 21. Multistep synthesis of Pralatrexate starting from ethyl 4-formylbenzoate **48**.

2.5. Pemetrexed: (S)-2-(4-(2-(2-Amino-4-oxo-4,7-Dihydro-1H-Pyrrolo[2,3-d]Pyrimidin-5-yl)Ethyl)Benzamido)Pentanedioic Acid (PMX, ALIMTA, LY231514, MTA)

Pemetrexed (PMX) **30** is a folate antagonist and antineoplastic agent, used in the treatment of non-small cell lung cancer [51–54] and malignant mesothelioma [55]. The mechanism of action of PMX is based on the inhibition of three enzymes responsible for the purine and pyrimidine synthesis—thymidylate synthase (TS), dihydrofolate reductase (DHFR), and glycinamide ribonucleotide formyltransferase [56]—which prevents the formation of DNA and RNA, which are responsible for the growth of normal and cancer cells.

The first synthetic approach toward PMX was reported starting with *tert*-butyl-4-formylbenzoate **54**. This aliphatic precursor was heterocyclized to the PMX diethyl ester **60** in a few steps, which was converted to PMX **6** by performing hydrolysis (Scheme 22) [57].

Scheme 22. Multistep synthesis of Pemetrexed starting from *tert*-butyl-4-formylbenzoate **54**.

Mitchell-Ryan et al. reported the synthesis of 5-substituted pyrrolo[2,3-*d*]pyrimidine antifolates with one-to-six bridge carbons and a benzoyl ring in the side chain as antitumor agents [58]. The compound with a 4-carbon bridge was the most active analogue and it potentially inhibited the proliferation of the folate receptor (FR) *α*-expressing Chinese hamster ovary and KB human tumor cells. PMX was synthesized from ethyl 4-iodobenzoate **61**, and 1-butene-4-ol **62** using a Heck cross-coupling reaction followed by bromination of the aldehyde at alpha-position. Further heterocyclization with basic hydrolysis and the formation of amide from diethyl-*L*-glutamate resulted in acid derivative **60**. In the final step, PMX **6** was obtained by the basic hydrolysis of the ester groups in a glutamate moiety (Scheme 23).
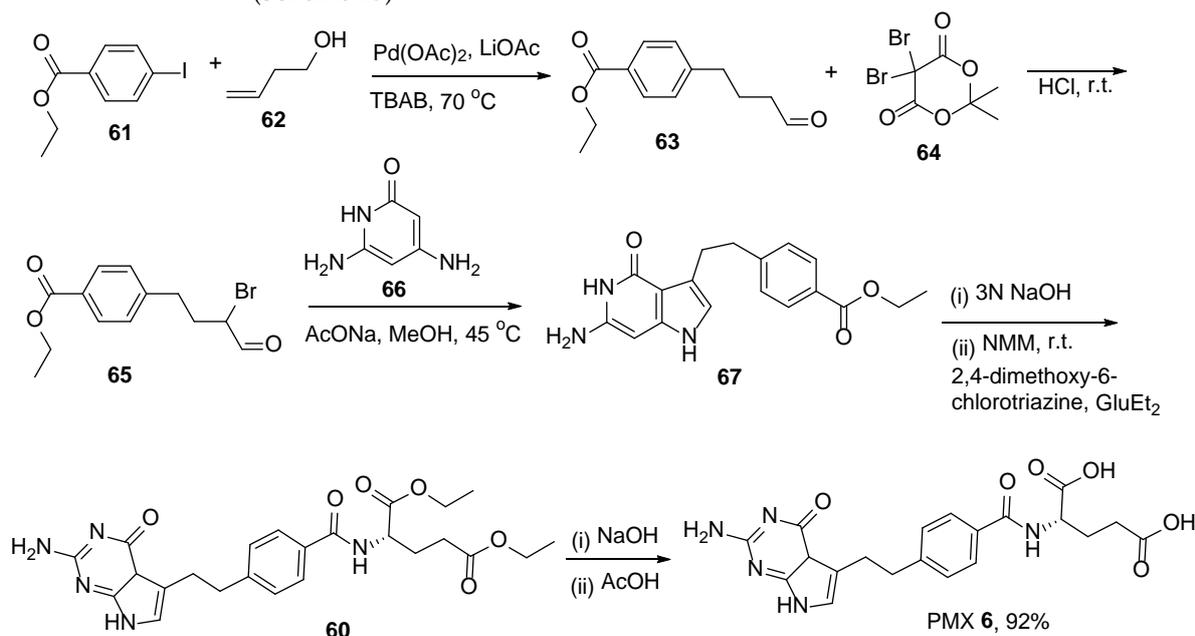
Scheme 23. Synthesis of Pemetrexed starting from ethyl 4-iodobenzoate **61**, and 1-butene-4-ol **62** through Heck cross-coupling reaction. Reproduced with the permission of reference [58]. Copyright © 2013, American Chemical Society.

As an improvement to the abovementioned method, the preparation of lysin salt of PMX was reported [59].

Michalak et al. reported the synthesis of PMX along with its common impurities/side products, starting with 4-[2-(2-amino-4-oxo-4,7-dihydro-1*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoic acid **28** [60].

In the method reported by Tailor et al. for the synthesis of PMX, ethyl-4-(3-oxopropyl)benzoate **67** was used as a starting compound [61–63]. After the Henry reaction with nitromethane, the product was converted to the semi-product with 2,6-diaminopyrimidin-4-ol **69**. The heterocyclization of this semi-product resulted in pyrrolo[2,3-*d*]pyrimidine derivative **70**, which, followed by its functionalization with diethyl-*L*-glutamate and basic hydrolysis, resulted in the desired product **6** in a 92% yield (Scheme 24).

The same research group reported an improved synthesis of PMX, starting from dimethyl (4-ethynylbenzoyl)-*L*-glutamate **73** and *N*-(4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl)pivalamide **71** [64]. The sequence of iodination, reduction, Sonogashira cross-coupling, reduction reactions, and basic hydrolysis in the last step, resulted in the final product, PMX, in a 67% yield (Scheme 25).
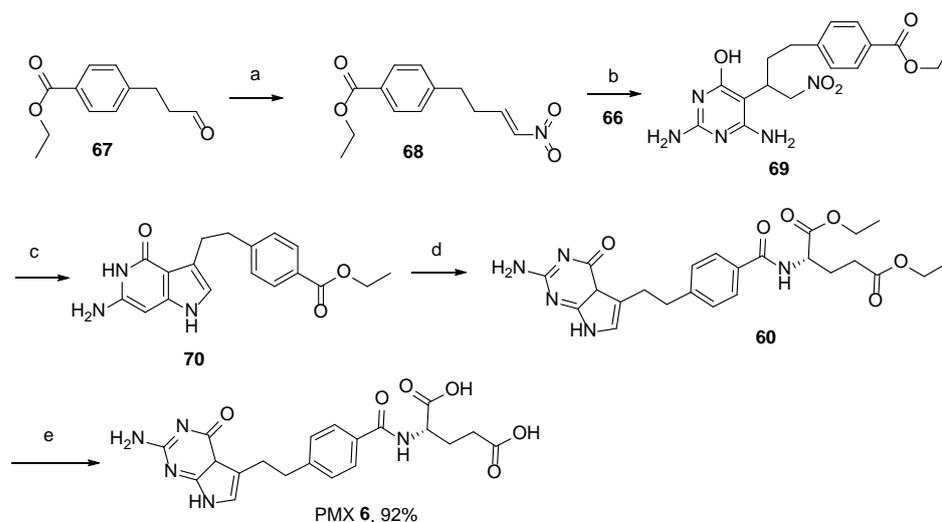
Reagents and conditions: (a) (i) CH_3NO_2/NaOH/EtOH; (ii) CH_3SO_2Cl/Et_3N; (b) EtOAc/H_2O 1:1, 50 °C, 24 h; (c) (i) aq NaOH, rt, 2 h; (ii) add to aq. H_2SO_4 at 0 °C, 3 h; (iii) aq. NaOH to pH 7, rt, 1 h; HOAc, then filter; (d) NMM, 2,4-dimethoxy-6-chlorotriazine, DMF, rt; (e) NaOH, THF/H_2O.

Scheme 24. Synthesis of Pemetrexed starting from ethyl-4-(3-oxopropyl)benzoate **67**.
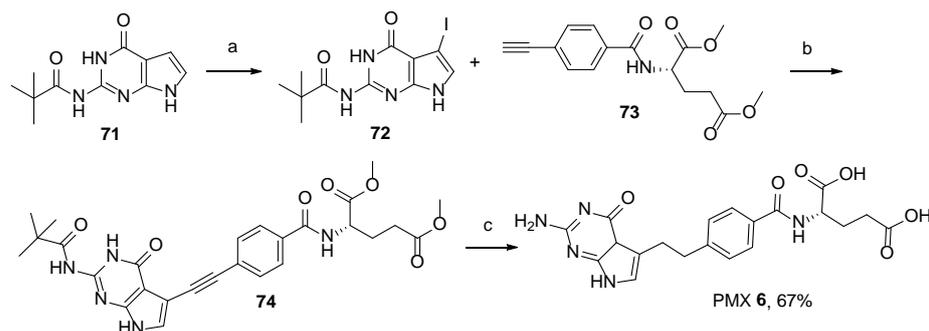
Reagents and conditions: (a) (i) NIS, DMF, rt; (ii) Zn dust, AcOH (b) CuI, TEA, Pd(PPh3)4, rt; (c) (i) H_2 50 psi Pd/C (ii) 1N NaOH, AcOH, 3 d, rt.

Scheme 25. Synthesis of Pemetrexed starting from dimethyl (4-ethynylbenzoyl)-*L*-glutamate **73** and *N*-(4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl)pivalamide **71**.

The same authors also reported the synthesis of PMX starting from methyl (*E*)-3-(but-2-en-1-yl(3,4-dimethoxybenzyl)amino)-3-oxopropanoate [65]. The last one was cyclized to methyl-1-(3,4-dimethoxybenzyl)-2-oxo-4-vinylpyrrolidine-3-carboxylate by the reaction of Mn(III) and Cu(II) acetates. The oxo-group was then converted to the thioxo-group upon treatment with P_2S_5. After the heterocyclization reaction, the obtained 2-amino-7-(3,4-dimethoxybenzyl)-5-vinyl-4*a*,5,6,7-tetrahydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one was subjected to a Heck cross-coupling reaction with diethyl 4-iodobenzoylglutamate. Additionally, the coupling product was identified as one with unexpected double bond migration products in vinyl-bridged pyrrolinopyrimidine to form the ethano-bridged pyrrolopyrimidine. Thus, the authors avoided the reduction of the unsaturated bridge and the subsequent oxidation of the pyrroline ring at the same time. According to the authors, the protection of the N-7 position eliminates the PMX cell growth's inhibitory activity. In addition, deprotection of the N-7 position was finally achieved upon treatment with a H_2SO_4/TFA mixture to facilitate the PMX precursor in a 30% yield, which resulted in the target product after saponification (Scheme 26).

Reagents and conditions: (a) Mn(OAc)$_3$·2H$_2$O, Cu(OAc)$_2$·H$_2$O; (b) P$_2$S$_5$; (c) guanidine; (d) diethyl 4-iodobenzoyl-L-glutamate, Pd(OAc)$_2$; (e) H$_2$SO$_4$/TFA; (f) NaOH.
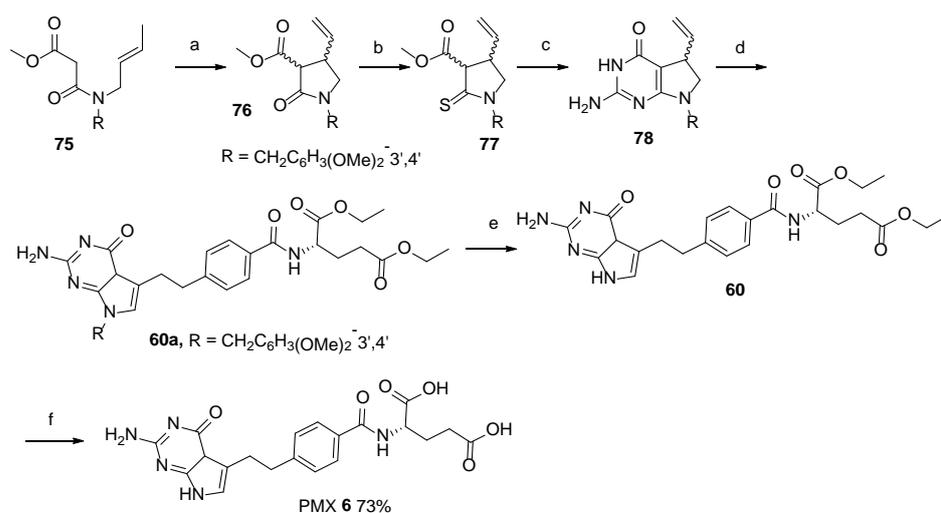
Scheme 26. Synthesis of Pemetrexed starting from methyl (*E*)-3-(but-2-en-1-yl(3,4-dimethoxybenzyl)amino)-3-oxopropanoate **75**.

Finally, very recently, a method for PMX synthesis was developed by means of the reaction of an anomeric amide agent with a secondary amine precursor followed by the deprotection of protective groups (Scheme 27) [66].
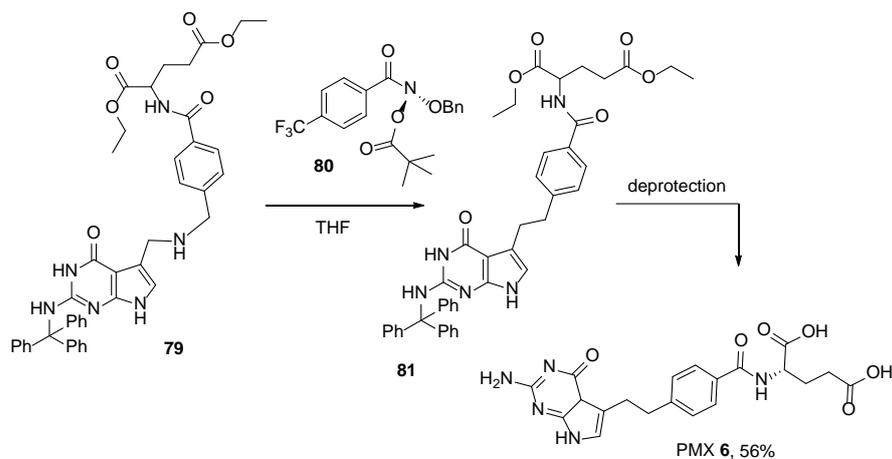
Scheme 27. Synthesis of Pemetrexed by the reaction of anomeric amide agent with secondary amine precursor followed by the deprotection of protective groups.

2.6. TNP-351: (2S)-2-[[4-[3-(2,4-Diamino-7H-Pyrrolo[2,3-d]Pyrimidin-5-yl)Propyl]benzoyl]Amino] Pentanedioic Acid (HY-19095)

TNP-351 is another antifolate from the same family as PMX. As a dihydrofolate reductase (DHFR) inhibitor, TNP-351 has good potential for the treatment of not only leukemia cells but also solid tumor cells, both in vitro and in vivo [67]. The structure of TNP-351 contains three methylene bridges instead of two as in PMX and two amino groups in pyrimidine core.

So far, only two synthetic approaches to TNP-351 **7** have been reported; the first one includes construction of the key intermediary acyclic skeleton, 5-[4-(*tert*-butoxycarbonyl)phenyl]-2-(dicyanomethyl)pentanoate **85**, cyclization with guanidine, followed by reduction to pyrrolo[2,3-*d*]pyrimidine derivatives **87**, and subsequent glutamate coupling and saponification. These antifolates were more growth-inhibitory by approximately one order of

magnitude than methotrexate (MTX) against KB human epidermoid carcinoma cells and A549 human non-small cell lung carcinoma cells with in vitro culture (Scheme 28) [68].

Reagents and conditions: (a) CH₃CH=CHCOOEt, *t*-BuOK, *t*-BuOH-ether; (b) H₂, Pd/C, AcOEt; (c) (i) LDA, THF, (ii) I₂; (d) NCCH₂CN, NaH, DMSO; (e) guanidine hydrochloride, *t*-BuOK, t-BuOH; (f) (i) BH₃-THF, THF, (ii) AcOH-MeOH (iii) RuCl₂(PPh₃)₃, THF; (g) (i) CF₃COOH; (ii) diethyl L-glutamate hidrochloride, DPPA, Et₃N, DMF; (h) (i) NaOH, THF-H₂O, (ii) AcOH.
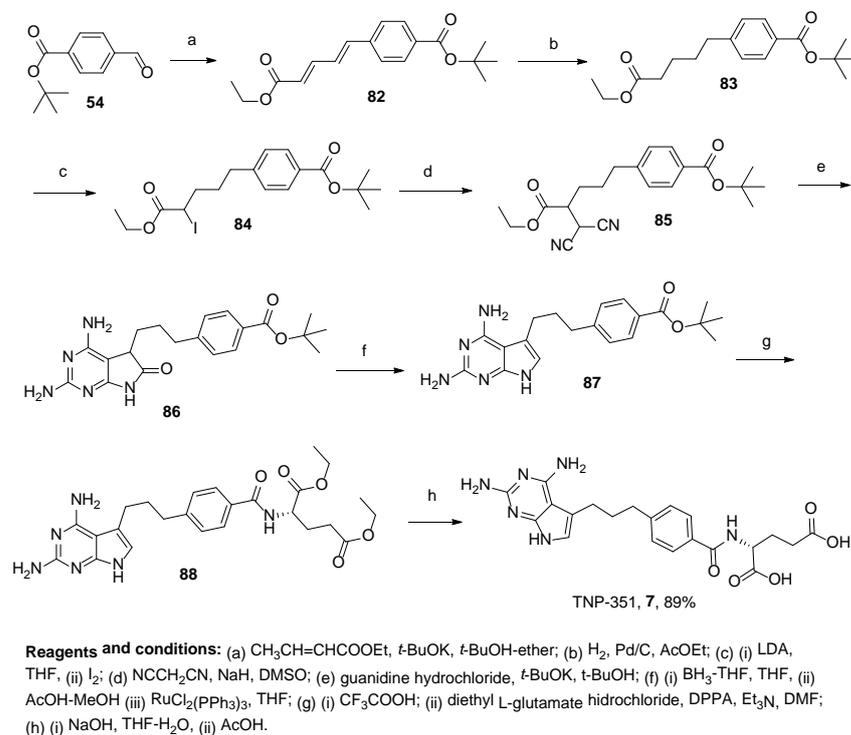
Scheme 28. Multistep synthesis of TNP-351.

The second method belongs to the same article, where the synthesis of TNP-351 has been reported along with PMX synthesis (Scheme 29) [57].
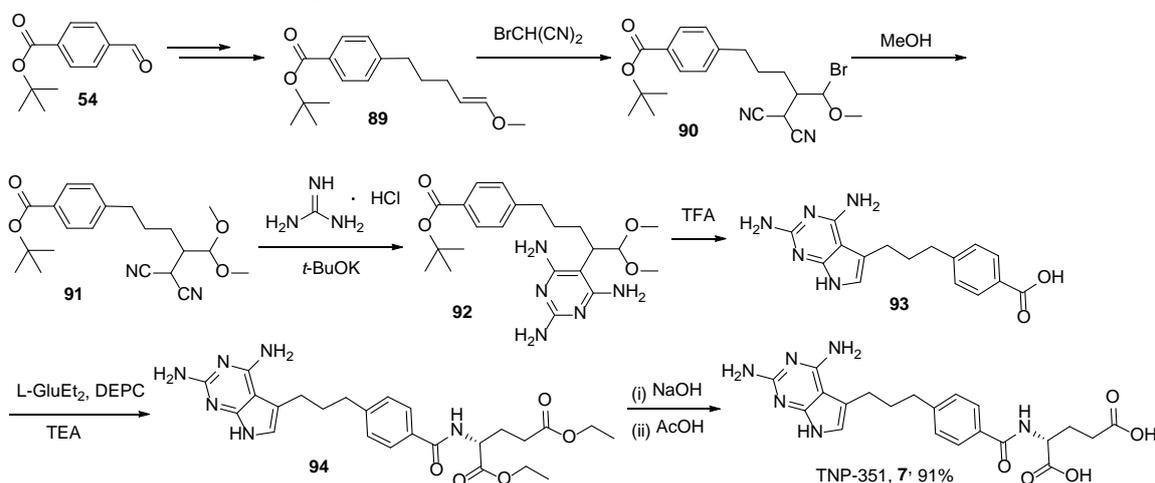
Scheme 29. Multistep synthesis of TNP-351.

2.7. Lometrexol: (2S)-2-[[4-[2-[(6R)-2-Amino-4-oxo-5,6,7,8-Tetrahydro-1H-Pyrido[2,3-d]Pyrimidin-6-yl]Ethyl]Benzoyl]Amino]Pentanedioic Acid (LY 264618, DDATHF-B, Lometrexolum)

Lometrexol (6R)-**8** is a folate analogue antimetabolite with antineoplastic activity [69–71]. As the 6R diastereomer of 5,10-dideazatetrahydrofolate, lometrexol inhibits glycinamide ribonucleotide formyltransferase (GARFT), the enzyme that catalyzes the first step in the de novo purine biosynthetic pathway, thereby inhibiting DNA synthesis, arresting cells in the S phase of the cell cycle, and inhibiting tumor cell proliferation. The agent is active against tumors that are resistant to the folate antagonist methotrexate.

Lometrexol has been used in trials for the treatment of lung cancer, drug/agent toxicity by tissues/organs, as well as for the treatment of unspecified adult solid tumors.

Taylor et al. reported several approaches to Lometrexol. The first of their approaches relates to the synthesis of (mixture of diastereomers) *(6S,6R)*-Lometrexol **8** with a satisfactory yield starting from 5-methyl-2-((4-nitrophenyl)thio)nicotinonitrile **95** (Scheme 30) [72].
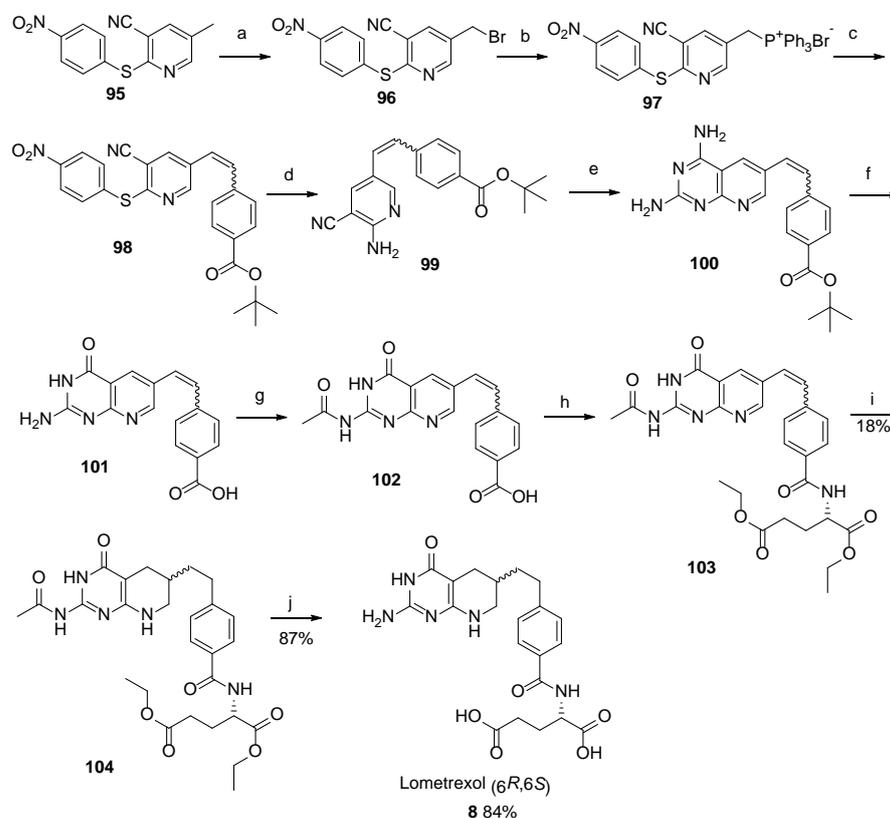
Scheme 30. Synthesis of Lometrexol starting from 5-methyl-2-((4-nitrophenyl)thio)nicotinonitrile **95**.

A key intermediate **109** for the subsequent synthesis of *(6S,6R)*-Lometrexol was also prepared by Taylor et al. via a regiospecific intermolecular inverse electron demand Diels-Alder reaction between fused 1,2,4-triazines, 2-*N*-pivaloyl-7-substituted-6-azapterins, and enamine (Scheme 31) [73].

Reagents and conditions: (a) dry MgSO4, dry THF; (b) Raney Ni, EtOH, rt, 5 d.
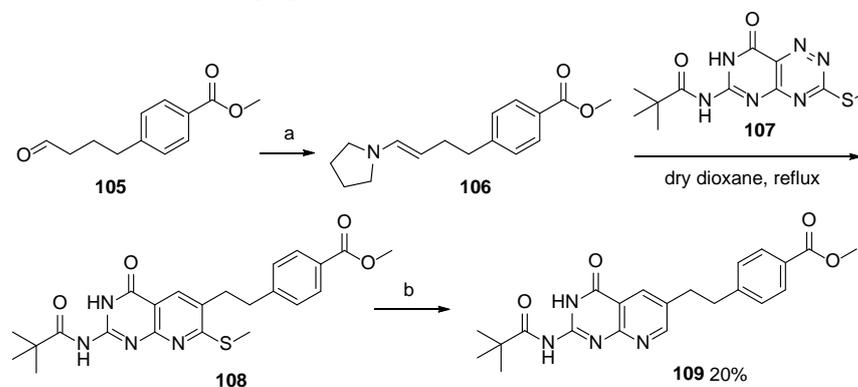
Scheme 31. Synthesis of key intermediate **109**.

In another work, Taylor et al. developed a convenient method for the synthesis of *(6S,6R)*-Lometrexol **8** with good yield via *N*-(6-bromo-4-oxo-3,4-dihydropyrido[2,3-*d*]pyrimidin-2-yl)pivalamide starting with 2,6-diaminopyrimidin-4(3*H*)-one (Scheme 32) [74].
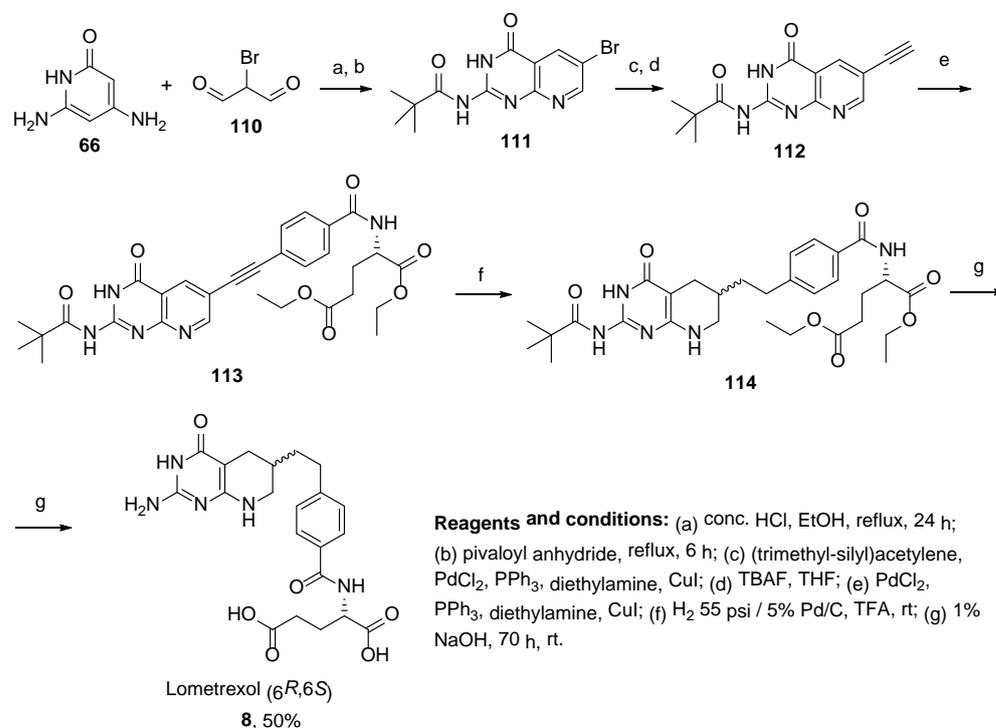
Scheme 32. Synthesis of *(6S,6R)*-Lometrexol **8** via *N*-(6-bromo-4-oxo-3,4-dihydropyrido[2,3-*d*]pyrimidin-2-yl)pivalamide starting from 2,6-diaminopyrimidin-4(3*H*)-one.

Boschelli et al. performed Wittig olefination of 2-acetyl-6-formyl-5-deazapterine to prepare *(6S,6R)*-Lometrexol in three synthetic steps instead of Sonogashira cross-coupling of 2-pyvaloyl-6-formyl-5-deazapterine (Scheme 33) [75].
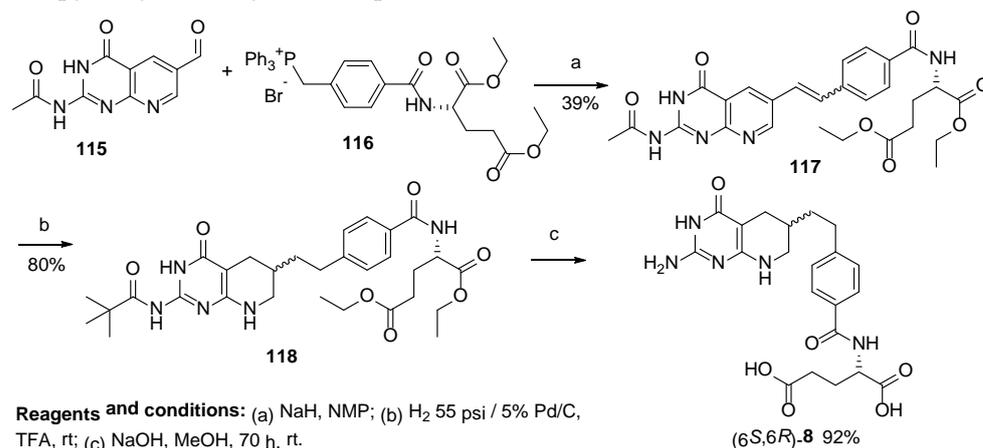
Scheme 33. Synthesis of *(6S,6R)*-Lometrexol **8** by the Wittig olefination of 2-acetyl-6-formyl-5-deazapterine.

Similarly, Wittig olefination was used by Piper et al. for the synthesis of (6*S*,6*R*)-Lometrexol starting from 2,4-diaminopyrido[2,3-*d*]pyrimidine-6-carboxaldehyde **120**, derived from 6-carbonytrile, and [4-(methoxycarbonyl)benzylidene]triphenylphosphorane to yield 9,10-ethenyl precursor **122** [76]. Standard hydrolytic deamination produced 5,10-dideazafolic acid **123**, which was further converted to 5,10-dideazaaminopterin via a coupling reaction with dimethyl *L*-glutamate by using (EtO)$_2$POCN, followed by hydrogenation and ester hydrolysis which led to the final product **8** (Scheme 34).
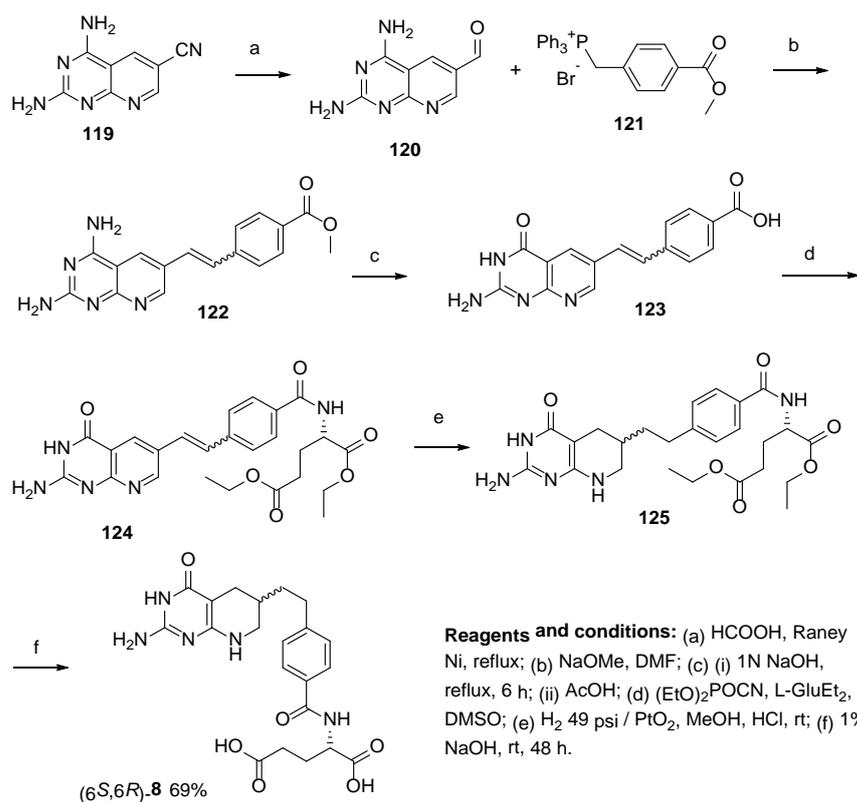
Scheme 34. Synthesis of (6*S*,6*R*)-Lometrexol starting from 2,4-diaminopyrido[2,3-*d*]pyrimidine-6-carboxaldehyde.

Currently, only two synthetic approaches toward diastereometrically pure *6R*-Lometrexol are reported in the literature. In this context, the synthesis of *6R*-Lometrexol was carried out starting from a double deprotected DDAH$_4$Pte–OH **126**, which was obtained with preparative chiral-HPLC in a mixture of diastereomers derived from the route based on the work of Taylor et al. [77]. After the transformation of the benzoic acid residue to a derivative which includes azides, the azide derivative was converted to the final product by the reaction of *L*-glutamic acid in DMSO in the presence of TEA (Scheme 35) [78].

Reagents and conditions: (a) (i) 1 N NaOH; (ii) 1N HCl; (b) (i) ClCO$_2$Et, TEA, DMSO; (ii) NaN$_3$; (c) L-Glu, TEA, DMSO.
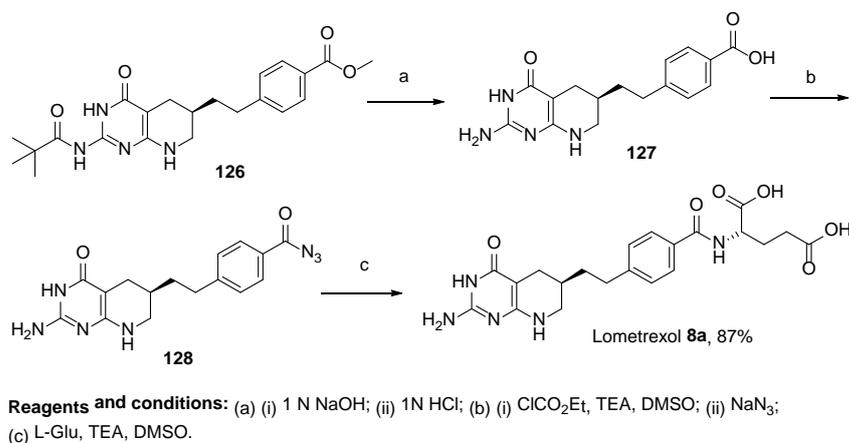
Scheme 35. Synthesis of *6R*-Lometrexol starting from a double deprotected DDAH$_4$Pte–OH **126**.

In another approach, the lipase-catalyzed enantioselective esterification of 2-(4-bromophenethyl) propane-1,3-diol, derived in several steps from 2-(4-bromphenyl)acetic acid, was utilized in the asymmetric synthesis of key (*R*)-2-amino-6-(4-bromophenethyl)-5,6,7,8-tetrahydropyrido

pyrimidin-4(3*H*)-one, which resulted in the target product in two synthetic steps (Scheme 36) [79,80].
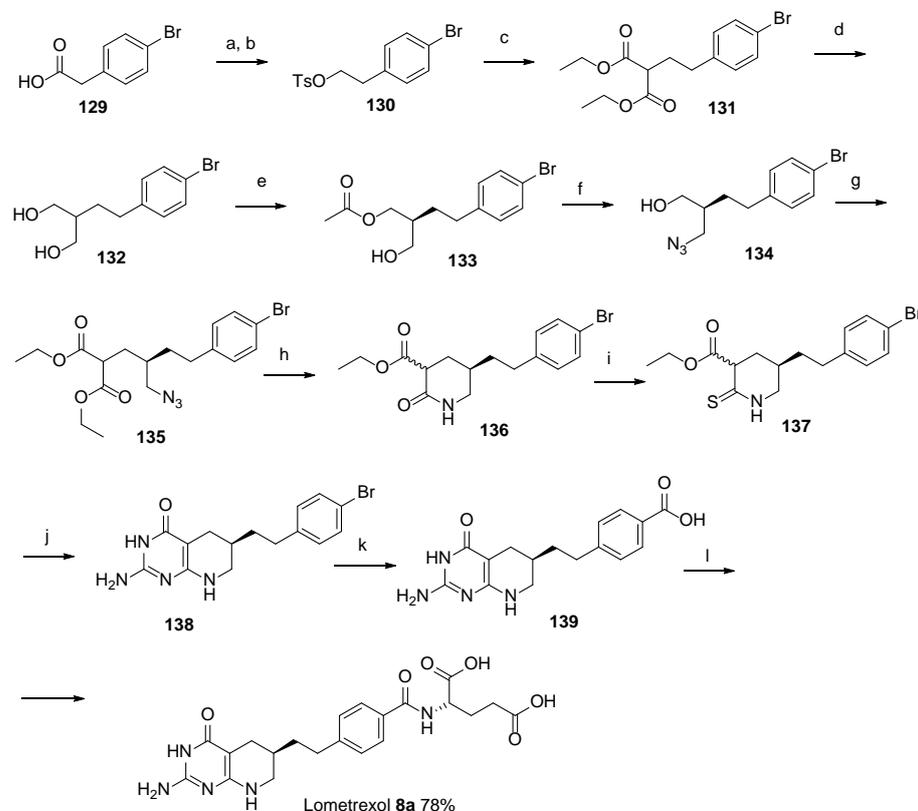
Reagents and conditions: (a) BH$_3$·THF; (b) TsCl, Et3N; (c) NaH, diethyl malonate; (d) LiAlH$_4$, Et$_2$O; (e) PPL-porcine pancreatic lipase (Sigma type II) immobilized on Hyflo Super Cel, MeOAc; (f) (i) MsCl, Et$_3$N; (ii) NaN$_3$' DMF; (iii) HCl, MeOH; (g) (i) TsCl, Et$_3$N; (ii) NaH, diethyl malonate, NaI; (h) Bu$_3$P, THF; (i) P$_2$S$_5$; (j) guanidine; (k) CuCN, NMP, reflux; (l) (i) H$_2$N-L-Glu(OEt)$_2$; (ii) 6M HCl, reflux.

Scheme 36. Multistep synthesis of Lometrexol starting from 2-(4-bromphenyl)acetic acid. Reproduced with the permission of references [79]. Copyright © 1989, Elsevier Ltd.

3. Conclusions and Future Perspectives

In summary, this review represents the analysis of the most up-to-date synthetic approaches for the synthesis of therapeutically significant analogues of folic acid, such as Lometrexol, Methotrexate, Pemetrexed, Pralatrexate, Raltitrexed, and TNP-351. Among the other folic acid analogues exhibiting antimalarial/antiprotozoal [81] and broad-spectrum antimicrobial activity [82–84], the importance and effectiveness of the abovementioned six analogues of folic acid as drugs or drug candidates for the treatment of diseases with a social significance, such as various types of cancers, severe psoriasis, and rheumatoid arthritis, were reported in a large number of original research publications and review articles [18,30–32,40,51–55,67,69–71]. Even though folates were reported to be somehow connected with the severeness of COVID-19 [85–87], several recent studies suggested the effectiveness of antifolates for the therapy of patients with coronavirus SARS-CoV-2 [85,88,89], along with the enhancement of the antiviral efficacy of remdesivir [88], treatment of fungal infections with COVID-19-like symptoms [90], as well as treatment of fungal infections among COVID-19 patients [90].

Most of the synthetic strategies for these important scaffolds presented in research articles and patents are based on similar approaches and have only minor differences from each other. So far, no attention has been paid to methods based on transitional metal (TM)-catalyzation or the TM-free direct C-H-activation/C-H-functionalization of

aza-aromatic rings as the most atom- and step-efficient approaches. We hope that our review will encourage future interest in this research area.

References

1. Ahluwalia, G.S.; Grem, J.L.; Hao, Z.; Cooney, D.A. Metabolism and action of amino acid analog anti-cancer agents. *Pharmacol. Ther.* **1990**, *46*, 243–271. [CrossRef]
2. Xie, M.; Liu, D.; Yang, Y. Anti-cancer peptides: Classification, mechanism of action, reconstruction and modification. *Open Biol.* **2020**, *10*, 200004. [CrossRef]
3. Chiangjong, W.; Chutipongtanate, S.; Hongeng, S. Anticancer peptide: Physicochemical property, functional aspect and trend in clinical application (Review). *Int. J. Oncol.* **2020**, *57*, 678–696. [CrossRef]
4. Mandal, P.K.; Gao, F.; Lu, Z.; Ren, Z.; Ramesh, R.; Birtwistle, J.S.; Kaluarachchi, K.K.; Chen, X.; Bast, R.C., Jr.; Liao, W.S.; et al. Potent and selective phosphopeptide mimetic prodrugs targeted to the Src homology 2 (SH2) domain of signal transducer and activator of transcription 3. *J. Med. Chem.* **2011**, *54*, 3549–3563. [CrossRef] [PubMed]
5. Gut, J. Aza Analogs of Pyrimidine and Purine Bases of Nucleic Acids. *Adv. Heterocycl. Chem.* **1963**, *1*, 189–251. [CrossRef]
6. Gojkovic, Z.; Karlsson, A. Purine and Pyrimidine-Based Analogs and Suicide Gene Therapy. In *Deoxynucleoside Analogs in Cancer Therapy*; Humana Press: Totowa, NJ, USA, 2007; pp. 403–439. [CrossRef]
7. Zelder, F.; Sonnay, M.; Prieto, L. Antivitamins for medicinal applications. *ChemBioChem* **2015**, *16*, 1264–1278. [CrossRef] [PubMed]
8. Howell, A. *The Role of Antihormones*; CRC Press: Boca Raton, FL, USA, 1990.
9. Albert, A. Antimetabolites: Antagonistic analogues of coenzymes and enzyme substrates. In *Selective Toxicity*; Albert, A., Ed.; Springer: Dordrecht, The Netherlands, 1985; pp. 323–378.
10. Lansiaux, A. Les antimétabolites. *Bull. Cancer* **2011**, *98*, 1263–1274. [CrossRef] [PubMed]
11. Petering, H.G. Folic acid antagonists. *Physiol. Rev.* **1952**, *32*, 197–213. [CrossRef]
12. Ellison, R.R. Treating Cancer with Antimetabolites. *Am. J. Nurs.* **1962**, *62*, 79. [CrossRef]
13. Panderi, I.; Koufopantelis, P. Methotrexate, an antimetabolite of folic acid, a brief throwback. *Pharmakeftiki* **2014**, *26*, 45–56.
14. Chu, E.; Drake, J.C.; Boarman, D.; Baram, J.; Allegra, C.J. Mechanism of thymidylate synthase inhibition by methotrexate in human neoplastic cell lines and normal human myeloid progenitor cells. *J. Biol. Chem.* **1990**, *265*, 8470–8478. [CrossRef]
15. Messmann, R.; Allegra, C. Antifolates. In *Cancer Chemotherapy and Biotherapy*; Chabner, B., Longo, D., Eds.; L. Williams & W: Philadelphia, PA, USA, 2001; pp. 139–184.
16. Shih, C.; Chen, V.J.; Gossett, L.S.; Gates, S.B.; MacKellar, W.C.; Habeck, L.L.; Shackelford, K.A.; Mendelsohn, L.G.; Soose, D.J.; Patel, V.F.; et al. Ly231514, a pyrrolo [2,3-d]pyrimidine-based antifolate that inhibits multiple folate-requiring enzymes. *Cancer Res.* **1997**, *57*, 1116–1123. [PubMed]
17. Avendaño, C.; Menéndez, J.C. (Eds.) Antimetabolites. In *Medicinal Chemistry of Anticancer Drugs*; Elsevier: Amsterdam, The Netherlands, 2008; pp. 9–52.
18. Moran, R.G. Folate antimetabolites inhibitory to de novo purine synthesis. In *New Drugs, Concepts and Results in Cancer Chemotherapy. Cancer Treatment and Research*; Muggia, F.M., Ed.; Springer: Boston, MA, USA, 1991; Volume 58, pp. 65–87.
19. Catalucci, E. Process for the Production of Methotrexate. U.S. Patent US4224446A, 23 September 1991.
20. Bin, Y.; Shubin, W.; Zhichao, M.; Quansheng, S.; Yanjiao, X.; Lilian, L. A Kind of Preparation Method of Methotrexate (MTX). U.S. Patent CN109553619A, 2 April 2019.
21. Piper, J.R.; Montgomery, J.A. 6-(Bromomethyl)-2,4-diaminopteridine Hydrobromide. U.S. Patent US4077957A, 11 April 1978.
22. Luo, J.; Smith, M.D.; Lantrip, D.A.; Wang, S.; Fuchs, P.L. Efficient syntheses of pyrofolic acid and pteroyl azide, reagents for the production of carboxyl-differentiated derivatives of folic acid. *J. Am. Chem. Soc.* **1997**, *119*, 10004–10013. [CrossRef]

23. Attoline, E.; Michieletti, M.; Rossi, D.; Allegrini, P. Process for the Preparation of Pteridine Derivatives. U.S. Patent US4767859, 17 November 1988.
24. Cheung, A.H.T.; Smal, M.; Chau, D.D. Synthesis of multi-^{13}C-enriched methotrexate for NMR studies of drug-Enzyme interactions. *Heterocycles* **1987**, *25*, 507–514. [CrossRef]
25. Cheung, A.H.T.; Boadle, D.K.; Tran, T.Q. N-(L-α-aminoacyl) derivatives of methotrexate. *Heterocycles* **1989**, *28*, 751–758. [CrossRef]
26. Choi, S.K.; Verma, M.; Silpe, J.; Moody, R.E.; Tang, K.; Hanson, J.J.; Baker, J.R., Jr. A photochemical approach for controlled drug release in targeted drug delivery. *Bioorg. Med. Chem.* **2012**, *20*, 1281–1290. [CrossRef]
27. Lei, T.; Yi, Z.; Jianta, W.; Gaofeng, Z.; Xing, C. Synthesis Process of Methotrexate. U.S. Patent CN112851676A, 28 May 2021.
28. Wiecko, J. Process for Preparing Methotrexate or an N-Substituted Derivative Thereof and/or a di (lower) Alkyl Ester Thereof and Precursor Therefor. U.S. Patent US4057548A, 30 March 1976.
29. Seeger, D.R.; Cosulich, D.B.; Smith, J.M.; Hultquist, M.E. Analogs of Pteroylglutamic Acid. III. 4-Amino Derivatives. *J. Am. Chem. Soc.* **1949**, *71*, 1753–1758. [CrossRef]
30. Widemann, B.C.; Balis, F.M.; Godwin, K.S.; McCully, C.; Adamson, P.C. The plasma pharmacokinetics and cerebrospinal fluid penetration of the thymidylate synthase inhibitor raltitrexed (Tomudex(TM)) in a nonhuman primate model. *Cancer Chemother. Pharmacol.* **1999**, *44*, 439–443. [CrossRef] [PubMed]
31. Liu, Y.; Wu, W.; Hong, W.; Sun, X.; Wu, J.; Huang, Q. Raltitrexed-based chemotherapy for advanced colorectal cancer. *Clin. Res. Hepatol. Gastroenterol.* **2014**, *38*, 219–225. [CrossRef]
32. Cocconi, G.; Cunningham, D.; Van Cutsem, E.; Francois, E.; Gustavsson, B.; van HazelD Kerr, G.; Possinger, K.; Hietschold, S.M. Open, randomized, multicenter trial of raltitrexed versus fluorouracil plus high-dose leucovorin in patients with advanced colorectal cancer. Tomudex Colorectal Cancer Study Group. *J. Clin. Oncol.* **2016**, *16*, 2943–2952. [CrossRef]
33. Marsham, P.R.; Hughes, L.R.; Jackman, A.L.; Hayter, A.J.; Oldfield, J.; Wardleworth, J.M.; Bishop, J.A.M.; O'Connor, B.M.; Calvert, A.H. Quinazoline Antifolate Thymidylate Synthase Inhibitors: Heterocyclic Benzoyl Ring Modifications. *J. Med. Chem.* **1991**, *34*, 1594–1605. [CrossRef] [PubMed]
34. Cao, S.L.; Wan, R.; Feng, Y.P. New synthesis of thymidylate synthase inhibitor raltitrexed. *Synth. Commun.* **2003**, *33*, 3519–3526. [CrossRef]
35. Yao, T.; Zubing, W.; Shuwang, G.; Jian, W.; Yuzhu, C.; Feng, L.; Dan, X.; Chunxia, Z.; Zhoushan, T. The Formoxyl of 5-((Alkoxy methylene)amino)thienyl-2-formyl Group)-L-glutamic Acid Dialkyl Ester and Preparation Method Thereof. U.S. Patent CN106957296A, 18 July 2017.
36. Xiqun, J.; Wei, W.; Zhoushan, T.; Jun, H.; Jing, W.; Yuzhu, C.; Huaping, W.; Dan, X.; Chunxia, Z.; Chuanjun, W. A kind of Pharmaceutical Composition of Raltitrexed Pharmaceutical Composition and Preparation Method Thereof. U.S. Patent CN107616976A, 23 January 2018.
37. Shaojie, H.; Wei, S.; Zhaobai, Z.; Xu, S. Preparation Method of Raltitrexed. U.S. Patent CN110551114A, 10 December 2019.
38. O'Connor, O.A.; Pro, B.; Pinter-Brown, L.; Bartlett, N.; Popplewell, L.; Coiffier, B.; Lechowicz, M.J.; Savage, K.J.; Shustov, A.R.; Gisselbrecht, C.; et al. Pralatrexate in patients with relapsed or refractory peripheral T-cell lymphoma: Results from the pivotal PROPEL study. *J. Clin. Oncol.* **2011**, *29*, 1182–1189. [CrossRef] [PubMed]
39. Amengual, J.E.; Lichtenstein, R.; Lue, J.; Sawas, A.; Deng, C.; Lichtenstein, E.; Khan, K.; Atkins, L.; Rada, A.; Kim, H.A.; et al. A phase 1 study of romidepsin and pralatrexate reveals marked activity in relapsed and refractory T-cell lymphoma. *Blood* **2018**, *131*, 397–407. [CrossRef] [PubMed]
40. Parker, T.; Barbarotta, L.; Foss, F. Pralatrexate: Treatment of T-cell non-Hodgkin's lymphoma. *Future Oncol.* **2012**, *9*, 21–29. [CrossRef] [PubMed]
41. DeGraw, J.I.; Colwell, W.T.; Piper, J.R.; Sirotnak, F.M. Synthesis and Antitumor Activity of 10-Propargyl-10-deazaaminopterin. *J. Med. Chem.* **1993**, *36*, 2228–2231. [CrossRef]
42. Guimin, Z.; Chengfu, C.; Chuanbing, W. A Kind of Preparation Method of Pralatrexate. U.S. Patent CN108069970, 02 June 2020.
43. Giust, W.; Burton, R.; Gorin, B.; Clayton, J. Process for Preparation of an Antifolate Agent. U.S. Patent WO2013/177713A1, 5 December 2013.
44. O'Connor, O.A.; Sirotnak, F.M. Treatment of T-Cell Lymphoma Using 10-Propargyl-10-Deazaaminopterin. U.S. Patent US2005/267117A1, 1 December 2005.
45. Lahiri, S.; Gupta, N.; Singh, H.K.; Panda, N.; Handa, V.; Abul, A.; Gupta, C.K.; Sanghani, S.; Sonavane, G.M. Process for the Preparation of Pralatrexate. U.S. Patent US9440979B2, 13 September 2016.
46. Lahiri, S.; Gupta, N.; Singh, H.K. Salts of Pralatrexate. U.S. Patent WO2014/20553A1, 6 February 2014.
47. Lahiri, S.; Gupta, N.; Singh, H.K.; Panda, N.; Handa, V.; Abul, A.; Gupta, C.K.; Sanghani, S.; Sonavane, G.M. Improved Process for the Preparation of Pralatrexate. U.S. Patent WO2014016740A2, 30 January 2014.
48. Tiseni, P.S.; Galluzzo, C.; Canavesi, A.; Biljan, T. Processes and Intermediates for Preparing Pralatrexate. U.S. Patent EP2794610B1, 27 June 2013.
49. Pronk, G.J. Optically Pure Diastereomers of 10-Propargyl-10-Deazaaminopterin and Methods of Using Same. U.S. Patent US8835433B2, 4 August 2011.
50. Alla, R.R.V.; Ramarao, C.; Michel, P.T.; Nitlikar, L.H.; Kalam, B.R.; Duduka, R. A Process for Preparing Intermediates of 10-propargyl-10-deazaaminopterin (pralatrexate) Synthesis and the Intermediates Thereof. U.S. Patent WO2013164856A1, 7 November 2013.

51.   Cohen, M.H.; Justice, R.; Pazdur, R. Approval Summary: Pemetrexed in the Initial Treatment of Advanced/Metastatic Non-Small Cell Lung Cancer. *Oncologist* **2009**, *14*, 930–935. [CrossRef] [PubMed]

52.   Rossi, A.; Ricciardi, S.; Maione, P.; de Marinis, F.; Gridelli, C. Pemetrexed in the treatment of advanced non-squamous lung cancer. *Lung Cancer* **2009**, *66*, 141–149. [CrossRef]

53.   Gandhi, L.; Rodríguez-Abreu, D.; Gadgeel, S.; Esteban, E.; Felip, E.; De Angelis, F.; Domine, M.; Clingan, P.; Hochmair, M.J.; Powell, S.F.; et al. Pembrolizumab plus Chemotherapy in Metastatic Non–Small-Cell Lung Cancer. *N. Engl. J. Med.* **2018**, *378*, 2078–2092. [CrossRef] [PubMed]

54.   Azzoli, C.G.; Kris, M.G.; Pfister, D.G. Cisplatin versus carboplatin for patients with metastatic non-small-cell lung cancer–an old rivalry renewed. *J. Natl. Cancer Inst.* **2007**, *99*, 828–829. [CrossRef]

55.   Manegold, C. Pemetrexed (alimta, MTA, multitargeted antifolate, LY231514) for malignant pleural mesothelioma. *Semin. Oncol.* **2003**, *30*, 32–36. [CrossRef] [PubMed]

56.   McLeod, H.L.; Cassidy, J.; Powrie, R.H.; Priest, D.G.; Zorbas, M.A.; Synold, T.W.; Shibata, S.; Spicer, D.; Bissett, D.; Pithavala, Y.K.; et al. Pharmacokinetic and Pharmacodynamic Evaluation of the Glycinamide Ribonucleotide Formyltransferase Inhibitor AG2034l. *Clin. Cancer Res.* **2000**, *6*, 2677–2684. [PubMed]

57.   Miwa, T.; Hitaka, T.; Akimoto, H. A Novel Synthetic Approach to Pyrrolo [2,3-d]pyrimidine Antifolates. *J. Org. Chem.* **1993**, *58*, 1696–1701. [CrossRef]

58.   Mitchell-Ryan, S.; Wang, Y.; Raghavan, S.; Ravindra, M.P.; Hales, E.; Orr, S.; Cherian, C.; Hou, Z.; Matherly, L.H.; Gangjee, A. Discovery of 5-substituted pyrrolo[2,3-d]pyrimidine antifolates as dual-acting inhibitors of glycinamide ribonucleotide formyl-transferase and 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase in de novo purine nucleotide biosynthesis. *J. Med. Chem.* **2013**, *56*, 10016–10032. [CrossRef]

59.   Bonaccorsi, F.; Calvani, F.; Pasqui, F. Process for the Preparation of Pemetrexed and Lysin Salt Thereof. U.S. Patent EP2882753B1, 13 February 2014.

60.   Michalak, O.; Gruza, M.M.; Witkowska, A.; Bujak, I.; Cmoch, P. Synthesis and physicochemical characterization of the impurities of pemetrexed disodium, an anticancer drug. *Molecules* **2015**, *20*, 10004–10031. [CrossRef]

61.   Taylor, E.C.; Liu, B. A simple and concise synthesis of LY231514 (MTA). *Tetrahedron Lett.* **1999**, *40*, 4023–4026. [CrossRef]

62.   Taylor, E.C.; Liu, B. A New and Efficient Synthesis of Pyrrolo[2,3-d]pyrimidine Anticancer Agents: Alimta (LY231514, MTA), Homo-Alimta, TNP-351, and Some Aryl 5-Substituted Pyrrolo[2,3-d]pyrimidines. *J. Org. Chem.* **2003**, *68*, 9938–9947. [CrossRef]

63.   Taylor, E.C.; Liu, B. Process for the Preparation of pyrrolo[2,3-d]pyrimidines. U.S. Patent US6066732 A, 23 May 2000.

64.   Taylor, E.C.; Kuhnt, D.; Shih, C.; Rinzel, S.M.; Grindey, G.B.; Barredo, J.; Jannatipour, M.; Moran, R.G. A Dideazatetrahydrofolate Analogue Lacking a Chiral Center at C-6, N-[4-[2-(2-Amino-3,4-dihydro-4-oxo-7H-pyrrolo[2,3-d]pyrimidin-5yl)ethyl]benzoyl]-l-glutamic Acid is an Inhibitor of Thymidylate Synthase. *J. Med. Chem.* **1992**, *35*, 4450–4454. [CrossRef]

65.   Taylor, E.C.; Liu, B. A novel synthetic route to 7-substituted derivatives of the antitumor agent LY231514 (MTA). *Tetrahedron Lett.* **1999**, *40*, 5291–5294. [CrossRef]

66.   Kennedy, S.H.; Dherange, B.D.; Berger, K.J.; Levin, M.D. Skeletal editing through direct nitrogen deletion of secondary amines. *Nature* **2021**, *593*, 223–227. [CrossRef]

67.   Itoh, F.; Russello, O.; Akimoto, H.; Beardsley, G.P. Novel pyrrolo[2,3-d]pyrimidine antifolate TNP-351: Cytotoxic effect on methotrexate-resistant CCRF-CEM cells and inhibition of transformylases of de novo purine biosynthesis. *Cancer Chemother. Pharmacol.* **1994**, *34*, 273–279. [CrossRef] [PubMed]

68.   Miwa, T.; Hitaka, T.; Akimoto, H.; Nomura, H.J. Novel pyrrolo [2, 3-d] pyrimidine antifolates: Synthesis and antitumor activities. *Med. Chem.* **1991**, *34*, 555–560.

69.   Adams, J.; Elliott, P.J. New agents in cancer clinical trials. *Oncogene* **2000**, *19*, 6687–6692. [CrossRef] [PubMed]

70.   Newell, D.R. Clinical pharmacokinetics of antitumor antifolates. *Semin. Oncol.* **1999**, *26*, 74–81.

71.   Bronder, J.L.; Moran, R.G. Antifolates targeting purine synthesis allow entry of tumor cells into S phase regardless of p53 function. *Cancer Res.* **2002**, *62*, 5236–5241. [PubMed]

72.   Taylor, E.C.; Harrington, P.J.; Fletcher, S.R.; Beardsley, G.P.; Moran, R.G. Synthesis of the Antileukemic Agents 5, 10-Dideazaaminopterin and 5, 10-Dideaza-5, 6, 7, 8-tetrahydroaminopterin. *J. Med. Chem.* **1985**, *28*, 914–921. [CrossRef] [PubMed]

73.   Taylor, E.C.; Harrington, P.M.; Warner, J.C. Diels-Alder reactions of 6-azapterins. An alternative strategy for the synthesis of 5,10-dideaza-5,6,7,8-tetrahydrofolic acid (DDATHF). *Heterocycles* **1988**, *27*, 1925–1928. [CrossRef]

74.   Taylor, E.C.; Wong, G.S.K. Convergent and Efficient Palladium-Effected Synthesis of 5,10-Dideaza-5,6,7,8-tetrahydrofolic Acid (DDATHF). *J. Org. Chem.* **1989**, *54*, 3618–3624. [CrossRef]

75.   Boschelli, D.H.; Webber, S.; Whiteley, J.M.; Oronsky, A.L.; Kerwar, S.S. Synthesis and biological properties of 5,10-dideaza-5,6,7,8-tetrahydrofolic acid. *Arch. Biochem. Biophys.* **1988**, *265*, 43–49. [CrossRef]

76.   Piper, J.R.; McCaleb, G.S.; Montgomery, J.A.; Kisliuk, R.L.; Gaumont, Y.; Thorndike, J.; Sirotnak, F.M. Synthesis and An-tifolate Activity of 5-Methyl-5,10-dideaza Analogues of Aminopterin and Folic Acid and an Alternative Synthesis of 5,10-Dideazatetrahydrofolic Acid, a Potent Inhibitor of Glycinamide Ribonucleotide Formyltransferase. *J. Med. Chem.* **1988**, *31*, 2164–2169. [CrossRef] [PubMed]

77.   Taylor, E.C.; Chaudhari, R.; Lee, K. A simplified and efficient synthesis of 5,10-dideaza-5,6,7,8-tetrahydrofolic acid (DDATHF). *Investig. New Drugs* **1996**, *14*, 281–285. [CrossRef] [PubMed]

78. Tomsho, J.W.; McGuire, J.J.; Coward, J.K. Synthesis of (6R)- and (6S)-5,10-dideazatetrahydrofolate oligo-γ-glutamates: Kinetics of multiple glutamate ligations catalyzed by folylpoly-γ-glutamate synthetase. *Org. Biomol. Chem.* **2005**, *3*, 3388–3398. [CrossRef]

79. Barnett, C.J.; Wilson, T.M. Asymmetric synthesis and absolute configuration of 5,10-dideaza-5,6,7,8-tetrahydropteroic acid and 5,10-dideaza-5,6,7,8-tetrahydrofolic acid (DDATHF). *Tetrahedron Lett.* **1989**, *30*, 6291–6294. [CrossRef]

80. Barnett, C.J.; Wilson, T.M.; Wendel, S.R.; Winningham, M.J.; Deeter, J.B. Asymmetric Synthesis of Lometrexol ((6R)-5,10-Dideaza-5,6,7,8-tetrahydrofolic Acid). *J. Org. Chem.* **1994**, *59*, 7038–7045. [CrossRef]

81. Sirichaiwat, C.; Intaraudom, C.; Kamchonwongpaisan, S.; Vanichtanankul, J.; Thebtaranonth, Y.; Yuthavong, Y. Target Guided Synthesis of 5-Benzyl-2,4-diamonopyrimidines: Their Antimalarial Activities and Binding Affinities to Wild Type and Mutant Dihydrofolate Reductases from Plasmodium falciparum. *J. Med. Chem.* **2004**, *47*, 345–354. [CrossRef] [PubMed]

82. Kimmitt, P.T.; Harwood, C.R.; Barer, M.R. Toxin gene expression by Shiga toxin-producing Escherichia coli: The role of antibiotics and the bacterial SOS response. *Emerg. Infect. Dis.* **2000**, *6*, 458–465. [CrossRef] [PubMed]

83. Heaslet, H.; Harris, M.; Fahnoe, K.; Sarver, R.; Putz, H.; Chang, J.; Subramanyam, C.; Barreiro, G.; Miller, J.R. Structural comparison of chromosomal and exogenous dihydrofolate reductase from Staphylococcus aureus in complex with the potent inhibitor trimethoprim. *Proteins Struct. Funct. Bioinforma.* **2009**, *76*, 706–717. [CrossRef]

84. Brogden, R.N.; Carmine, A.A.; Heel, R.C.; Speight, T.M.; Avery, G.S. Trimethoprim: A Review of its Antibacterial Activity, Pharmacokinetics and Therapeutic Use in Urinary Tract Infections. *Drugs* **1982**, *23*, 405–430. [CrossRef]

85. Topless, R.K.; Green, R.; Morgan, S.L.; Robinson, P.C.; Merriman, T.R.; Gaffo, A.L. Folic acid and methotrexate use and their association with COVID-19 diagnosis and mortality: An analysis from the UK Biobank. *medRxiv* **2022**. [CrossRef]

86. Sheybani, Z.; Dokoohaki, M.H.; Negahdaripour, M.; Dehdashti, M.; Zolghadr, H.; Moghadami, M.; Masoompour, S.M.; Zolghadr, A.R. The Role of Folic Acid in the Management of Respiratory Disease Caused by COVID-19. *ChemRxiv Camb. Camb. Open Engag.* **2020**. [CrossRef]

87. Meisel, E.; Efros, O.; Bleier, J.; Halevi, T.B.; Segal, G.; Rahav, G.; Leibowitz, A.; Grossman, E. Folate Levels in Patients Hospitalized with Coronavirus Disease 2019. *Nutrients* **2021**, *13*, 812. [CrossRef] [PubMed]

88. Stegmann, K.M.; Dickmanns, A.; Gerber, S.; Nikolova, V.; Klemke, L.; Manzini, V.; Schlösser, D.; Bierwirth, C.; Freund, J.; Sitte, M.; et al. The folate antagonist methotrexate diminishes replication of the coronavirus SARS-CoV-2 and enhances the antiviral efficacy of remdesivir in cell culture models. *Virus Res.* **2021**, *302*, 198469. [CrossRef] [PubMed]

89. Zhang, Y.; Guo, R.; Kim, S.H.; Shah, H.; Zhang, S.; Liang, J.H.; Fang, Y.; Gentili, M.; O' Leary, C.N.; Elledge, S.J.; et al. SARS-CoV-2 hijacks folate and one-carbon metabolism for viral replication. *Nat. Commun.* **2021**, *12*, 1676. [CrossRef]

90. Mujwar, S.; Tripathi, A. Repurposing benzbromarone as antifolate to develop novel antifungal therapy for Candida albicans. *J. Mol. Model.* **2022**, *28*, 193. [CrossRef] [PubMed]