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Research paper

Design, synthesis and biological evaluation of 6-substituted pyrrolo [2,3-*d*]pyrimidines as dual inhibitors of TS and AICARFTase and as potential antitumor agents

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ABSTRACT

A new series of 2-amino-4-oxo-6-substituted pyrrolo[2,3-*d*]pyrimidines, with an isosteric replacement of the side chain amide moiety to a sulfur atom, were designed and synthesized as multitargeted antifolates as well as potential antitumor agents. Starting from previously synthesized 2-amino-4-oxo-pyrrolo[2,3-*d*]pyrimidin-6-yl-acetic acid, a reduction by lithium triethylborohydride and successive mesylation afforded the key mesylate. Nucleophilic substitution by mercaptoacetic or mercaptopropionic acid methyl esters, followed by hydrolysis and condensation with pyridinyl-methylamines provided the nonclassical compounds **1**–**6**, whereas condensation with glutamic acid diethyl ester hydrochloride and saponification afforded the classical analogs **7**–**8**. All target compounds exhibited inhibitory activities toward KB, SW620 and A549 tumor cell lines. The most potent compounds of this series, **7** and **8**, are better inhibitors against A549 cells than methotrexate (MTX) and pemetrexed (PMX). Nucleoside protection assays establish compound **8** a dual inhibitor of thymidylate synthase (TS) and 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase (AICARFTase) targeting both de novo thymidylate and purine nucleotide biosynthesis, which is further verified by the molecular modeling studies. Analogous to PMX, target compound **8** alternates the cell cycle of SW620 cells with S-phase accumulation and induces apoptosis, leading to cell death.

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1. Introduction

In cellular metabolism, folate coenzymes are required in more than twenty interrelated enzymatic reactions, which are necessary to maintain de novo synthesis of the essential building blocks of DNA as well as the synthesis of certain important amino acids [1,2]. Therefore, antifolates, typified by methotrexate (MTX), pemetrexed (PMX), and pralatrexate (PDX) (Fig. 1) remain important chemotherapeutic agents in treating various cancers, microbial infections, inflammatory disorders, and autoimmune diseases [3]. MTX was first synthesized in 1949 and continues to be used for chemotherapy, either alone or in combination with other agents, in the treatment of breast cancer, osteogenic sarcoma, and pediatric acute lymphoblastic leukemia [4]. PDX was identified in the mid-1990s and was approved as a treatment for patients with relapsed or refractory peripheral T-cell lymphoma in 2009 [4–6]. PDX is considered to be more toxic toward cancer cells than normal cells due to its uptake into cells through reduced folate carrier type 1 (RFC-1) [5–7], which is often overproduced in cancer cells to ensure enough folate uptake. Both MTX and PDX are potent inhibitors of dihydrofolate reductase (DHFR), which functions as a catalyst for the reduction of dihydrofolate to tetrahydrofolate [8]. The highly basic 2,4-diaminopyrimidine moiety in the pteridine ring of these compounds is protonated and forms an ionic bond with the active site of the DHFR enzyme and accounts, in part, for their high affinity

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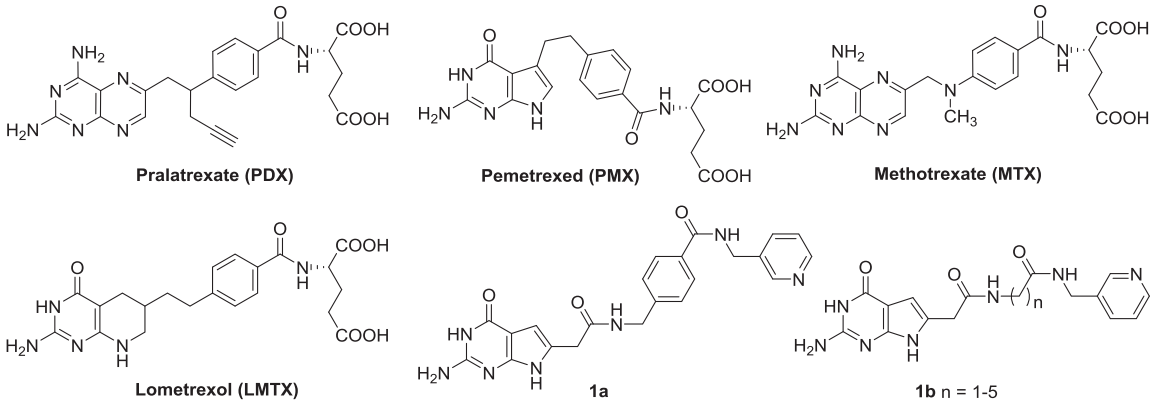

Fig. 1. Structures of classical and nonclassical antifolates.

for DHFR. PMX, originally developed as a thymidylate synthase (TS) inhibitor, is a multitargeted agent with a 6–5 fused pyrrolo [2,3-*d*]pyrimidine nucleus instead of the more common 6-6 fused pteridine or quinazoline ring structure [9]. Preliminary studies revealed that PMX polyglutamates are not only potent inhibitors of TS but also of DHFR and glycinamide ribonucleotide (GAR) formyltransferase (GARFTase) and 5-aminoimidazole-4-carboxamide (AICA) ribonucleotide formyltransferase (AICARFTase) in de novo purine nucleotide biosynthesis [10,11]. PMX has been approved for the treatment of malignant pleural mesothelioma in combination with cisplatin and recently in nonsmall lung cancer in the United States [12–14].

Many types of transformed cancer cells have an elevated dependence on the de novo purine biosynthetic pathway and an impaired purine salvage pathway, while normal cells can rely solely on the salvage pathway for purines [15,16]. This allows such sensitive cancer cells to be targeted selectively over normal cells by inhibitors of de novo purine biosynthesis, including GARFTase and AICARFTase inhibitors [17–20]. Lometrexol (LMTX, Fig. 1), the first GARFTase inhibitor to enter clinical trials, was synthesized in 1985 [21]. LMTX is a poor inhibitor of both DHFR and TS, but a potent inhibitor of GARFTase (Ki = 6 nM) and a substrate for folypoly-γ-glutamate synthetase (FPGS) [22]. Patients treated with LMTX in phase-I clinical trials developed severe and cumulative myelosuppression and mucositis. The cumulative toxicity of LMTX is thought to be due in part to its ability to be transported by both the RFC and folate receptors (FR), resulting in increased cellular levels.

Targeting only one folate-dependent enzyme, as with most currently available antifolates, are frequently associated with drug resistance. Thus, it is of interest to design dual or multitargeted antifolates that could preserve antitumor activity in the event that tumors become resistant as a result of alterations in one or the other targeted enzyme. In our previous studies [23], a series of 2-amino-4-oxo-6-substituted pyrrolo[2,3-*d*]pyrimidines (**1a-b**, Fig. 1) were synthesized and biologically evaluated as potential antifolates targeting both thymidylate and purine nucleotide biosynthesis. These compounds exhibited micromolar to submicromolar antiproliferative potencies against a panel of tumor cell lines including KB, A549 and HepG2. Growth inhibition of compound **1a** toward KB cells resulted in cytotoxicity and G1/G2-phase accumulation, and was partially protected by excess thymidine and adenosine, but was completely reversed in the combination of thymidine and adenosine, indicating both thymidylate and de novo purine nucleotide synthesis as the targeted pathway. The docking studies also showed that **1a** could bind and inhibit both TS and the two folate-dependent purine biosynthetic enzymes (GARFTase and AICARFTase).

As an extension of our research to develop novel multitargeted antifolates as antitumor agents and to optimize the structure for inhibition of folate metabolizing enzymes, in the present study, the straight chain analogs **1b** (Fig. 1) were selected as the lead compounds and the side chain amide moiety was isosterically replaced with a sulfur atom to achieve target compounds **1**–**6** (Fig. 2). This isosteric replacement would decrease somewhat the distance between the pyrrolo[2,3-*d*]pyrimidine nucleus and the side chain pyridine, as well as change the angle between them. The relative position of the nitrogen atom on the side chain pyridine was also considered in the design of these compounds. In addition, the side chain L-glutamate analogs **7**–**8** (Fig. 2), were also synthesized as classical antifolates and were evaluated.

2. Chemistry

Target compounds **1**–**6** were synthesized as shown in Scheme 1. The synthesis started from commercially available ethyl-4-chloroacetoacetate, **9**, which was condensed with 2,4-diamino-6-hydroxypyrimidine, **10**, in sodium acetate-water to give the key intermediate **11** [23]. Ester **11** was first reduced to alcohol **12** by lithium triethylborohydride, and then converted to mesylate **13** under the treatment of methanesulfonyl chloride at 0 °C. Compound **13** was reacted with 2-mercaptoacetic acid methyl ester and 3-mercaptopropanoic acid methyl ester, respectively, followed by saponification to produce carboxylic acids **15a-b**. Final condensation with various pyridinyl-methylamines provided target compounds **1**–**6**. As shown in Scheme 2, target compounds **7**–**8** were successfully obtained by condensation of compounds **15a-b** with glutamic acid diethyl ester hydrochloride followed by hydrolysis. Here compound **8** was selected as an example to elucidate the structures of target compounds. Based on its HRMS (ESI) at m/z 410.1276 $[M-H]^-$, compound **8** had an elemental composition of $C_{16}H_{21}O_6N_5S$. The ^1H NMR and ^{13}C NMR data (experimental section) showed the presence of 21 protons and 16 carbons in **8**. A detailed analysis of ^1H NMR data showed one broad singlet at δ_H 12.31(-COOH), one doublet at δ_H 8.18 (-CONH-), one proton at δ_H 4.19–4.25 (-CH-) and four protons at δ_H 1.70–2.30 (-CH_2-) indicating the successful coupling of glutamic acid. The protons at δ_H 10.85 (7-NH) and 10.17 (3-NH), one broad singlet at δ_H 6.02 (-NH_2) and one singlet at δ_H 5.94 (5-CH) exhibited pyrrolo[2,3-*d*]pyrimidine core structure. The other methylene protons were present at δ_H 2.40–2.73. Consequently, **8** was characterized as (*S*)-2-{3-[2-(2-Amino-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidin-6-yl)- ethylsulfanyl]-propionylamino}-pentanedioic acid.

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