European Journal of Medicinal Chemistry 154 (2018) 267–279

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

Design, synthesis and biological evaluation of N-phenyl-(2,4-dihydroxypyrimidine-5-sulfonamido)benzoyl hydrazide derivatives as thymidylate synthase (TS) inhibitors and as potential antitumor drugs

Xin-yang Li, Jing-wei Liang, Kamara Mohamed O, Ting-jian Zhang, Guo-Qing Lu, Fan-hao Meng*

School of Pharmacy, China Medical University, 77 Puhe Road, North New Area, Shenyang, 110122, China

ARTICLE INFO

Article history:
Received 9 January 2018
Received in revised form
12 April 2018
Accepted 14 May 2018

Keywords:
Thymidylate synthase
Anti-proliferative activity
Apoptosis

ABSTRACT

The Inhibition of cellular nucleotide metabolism to promote apoptosis is a key principle of cancer therapy. Thymidylate synthase (TS) is a key rate-limiting enzyme in the initiation of DNA synthesis in cell. Here, we presented two types of thymidylate synthase inhibitors, and, the key pharmacological properties of these two types of thymidylate synthase inhibitor were extracted and combined to design new compounds with inhibitory activity. Therefore, two series of 42 new compounds with the common biological effect of promoting apoptosis are designed and synthesized by combination principle. Most of the compounds had good anti-proliferative activity on A549, OVCAR-3, SGC7901 and MDA-MB-231 cells. The IC_{50} of compound **10l** on A549 cells was 1.26 μM, which was better than that of pemetrexed (PTX, $IC_{50} = 3.31$ μM), furthermore, the selection index of compound **10l** was higher than PTX. Flow cytometry analysis showed that compound **10l** (the apoptosis rate is 39.4%) could induce A549 cell apoptosis and effectively inhibit tumor cell proliferation. Further western blot analysis showed that compound **10l** could induce intrinsic apoptosis by activating caspase-3, increasing expression of cleaved caspase-3 and reducing the ratio of bcl-2/bax. All of this makes compound **10l** to be a promising compound in future animal tumor models.

1. Introduction

Targeted chemotherapy is an important mean of cancer treatment by inhibiting the growth of cancer cells and promoting apoptosis in order to achieve the purpose of anti-cancer [1]. Essentially, blocking the DNA synthesis of tumor cells can induce apoptosis which can effectively inhibit the proliferation of tumor cells [2,3].

Thymidylate synthase (TS) plays an important role in the synthesis of DNA in living organisms [4]. It is a key enzyme that catalyzes the methylation of dUMP to dTMP, which is necessary for DNA biosynthesis. Since the level of DNA synthesis in tumor cells is significantly higher than that in normal cells [5,6], in the absence of exogenous thymidine, the inhibition of TS activity causes intracellular thymidine loss, resulting in intracellular DNA synthesis that does not proceed normally, followed by defective DNA synthesis and apoptosis of the solution [5,7]. Folic acid structural analogues are a kind of TS inhibitor that well bind to TS and interfere with the normal physiological functions of TS, thus causes inhibition in DNA synthesis [8,9]. The marketed drugs are currently available under branded names such as methotrexate, pemetrexed (Fig. 1) [10], said to contain glutamic acid residue structure, enter the cell by folic acid carrier protein of the cell membrane, and then use folic acid poly-ɣ-glutamate synthase (FPGS) as catalytic enzyme and the poly-ɣ-glutamic acid method to increase intracellular drug concentration, and prolong the retention time of drugs in the cells [11]. However, Costi [4] and other studies have shown that prolonging the retention time of intracellular drugs can stimulate cellular TS protein and FPGS overexpression causes cancer cells become more resistant [12].

Pyrimidine analogs are another TS inhibitors such as 5-fluorouracil (5-Fu) and tegafur. These compounds first converted to fluorouracil deoxynucleotide and then in the presence of 5, 10-mehyltetrahydrofolic acid coenzyme catalytic binding to form TS in vivo. Fluorouracil deoxyribonucleotide C-F bond is very stable,

* Corresponding author.
E-mail address: fhmeng@cmu.edu.cn (F.-h. Meng).

https://doi.org/10.1016/j.ejmech.2018.05.020
0223-5234/

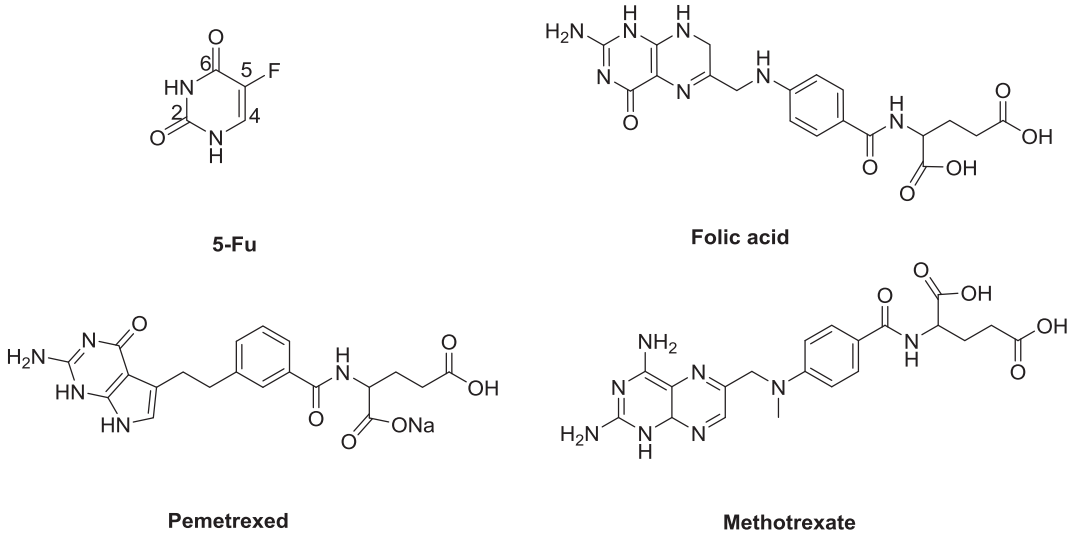

Fig. 1. Chemical structures of selected TS inhibitors.

resulting in the inability to effectively synthesize thymidine, thereby inhibiting the synthesis of DNA, eventually leading to tumor cell death [10]. Although these TS inhibitors have been used clinically as effective anti-tumor drugs, there are some with insurmountable deficiencies such as they do not specifically block TS activity and long-term use of these drugs may also be resisted [13].

In summary, in order to obtain TS inhibitors that can inhibit DNA synthesis and promote tumor cell apoptosis [10,12], this study combines the structural characteristics of folic acid analogues inhibitors and pyrimidine analogues inhibitors, preserving the amino acid structure (P2) (binding to the TS coenzyme site) and the uracil structure (P1) (binding to the thymidine site) [14,15](Fig. 2). In order to obtain the attenuated TS inhibitor and enhance its anticancer effect, C_5-substituted linker amino-benzoic acid structures have been designed and their general structures have also been derived [16]. A total of 42 compounds for the first time have not been reported. These novel TS inhibitors we designed have the following features: (1) they do not contain glutamate components and are therefore not need FPGS. Also they are not prone to accumulate toxic and drug resistance; (2) they are all fat-soluble compounds and do not require the involvement of reduced folate carrier (RFC), which can enter the cell in a passive and diffusive manner, and as well overcomes the resistance caused by the RFC [17,18]. In this article, we describe our preliminary results of the two series of 42 compounds that are synthesized and their biological evaluation of the inhibition of cancer cell proliferation [19].

2. Result and discussion

2.1. Chemistry

The chemical synthesis of *N*-phenyl-(*2,4-dihydroxypyrimidine* -5-sulfonamido)benzoyl hydrazide derivatives **(5a-5g, 6a-6g,** and **7a-7g**) and *N*-benzoyl-(*2,4-dihydroxypyrimidine*-5-sulfonamido) benzoyl hydrazide derivatives (**8h-8n, 9h-9n,** and **10h-10n**) was carried out by synthetic method illustrated in Scheme 1 Preparation of 2,4-dihydroxypyrimidine-5-sulfonylchloride(compound **1**) was done according to the reported method by Pogorelova [20]. In comparison with the previous method this experiment uses a gradient heating method that caused the yield to be increased, and the after-treatment method has been improved to obtain a higher yield. Compound **1** reacted with the corresponding aminobenzoic acid in the presence of pyridine as an acid-binding agent to obtain compound **2**–**4** [21]; which were sequentially reacted with HOBT and EDCI to form an active ester then it was reacted with the corresponding phenyl hydrazine to give the target compounds **(5a-5g, 6a-6g,** and **7a-7g)** or reacted with the corresponding benzoyl hydrazide to give the target compounds **(8h-8n, 9h-9n,** and **10h-10n**). There have been different in the after-treatment method of compounds from the previous after-treatment methods [22], The after-treatment of target compound involved the Hinsberg reaction, and had a specific description in the general methods [23].

2.2. Biological evaluation

2.2.1. MTT assay

Taking pemetrexed (**PTX**) as reference compound, the target compounds (**5a-5g, 6a-6g, 7a-7g**, **8h-8n, 9h-9n,** and **10h-10n**) were evaluated for the anti-proliferative against four cancer cell lines: A549, OVCAR-3, SGC7901 and MDA-MB-231 by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) assay. The results were expressed as IC_{50} values and summarized in Table 1 and the values were the average of at least three independent experiments. As shown in Table 1, more than half of the target compounds showed anti-proliferative activity against A549, OVCAR-3, SGC7901 and MDA-MB-231 cells.

During MTT assay that we found that in the first series, the anti-proliferative activity of compounds from replacement reaction was eliminated when R was replaced by −H; at the same time when R was replaced by 3-CH_3 was unfavorable to the anti-proliferative activity. Therefore, we adjusted the second series, replacing -H with 4-$OCH_2CH_2CH_3$ and replacing 3-CH_3 with 4-CH_3. The result showed R was replaced by 4-$OCH_2CH_2CH_3$, the anti-proliferative activity of compounds was better than -H. However, there had precious few improvements on anti-proliferative activity of compounds when R was replaced by 4-CH_3.

For all targeted compounds, the electron-withdrawing group (-Cl, -F) was superior to the electron-donating group (-CH_3). Simultaneously, R was replaced by -Cl, anti-proliferative activity of compounds was better than replaced by -F. For the first series, when R was replaced by 2-Cl, X was replaced by m-phenyl (**6d**), anti-proliferative activity of compound was better than replaced by p-phenyl (**5d**), and anti-proliferative activity of compound **5d** was