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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

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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,

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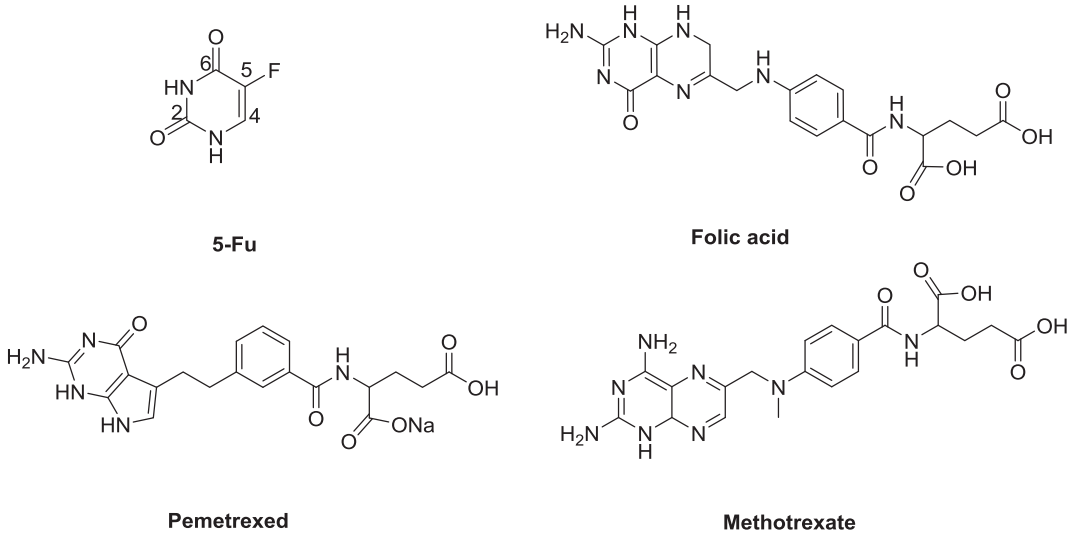


Fig. 1. Chemical structures of selected TS inhibitors.

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

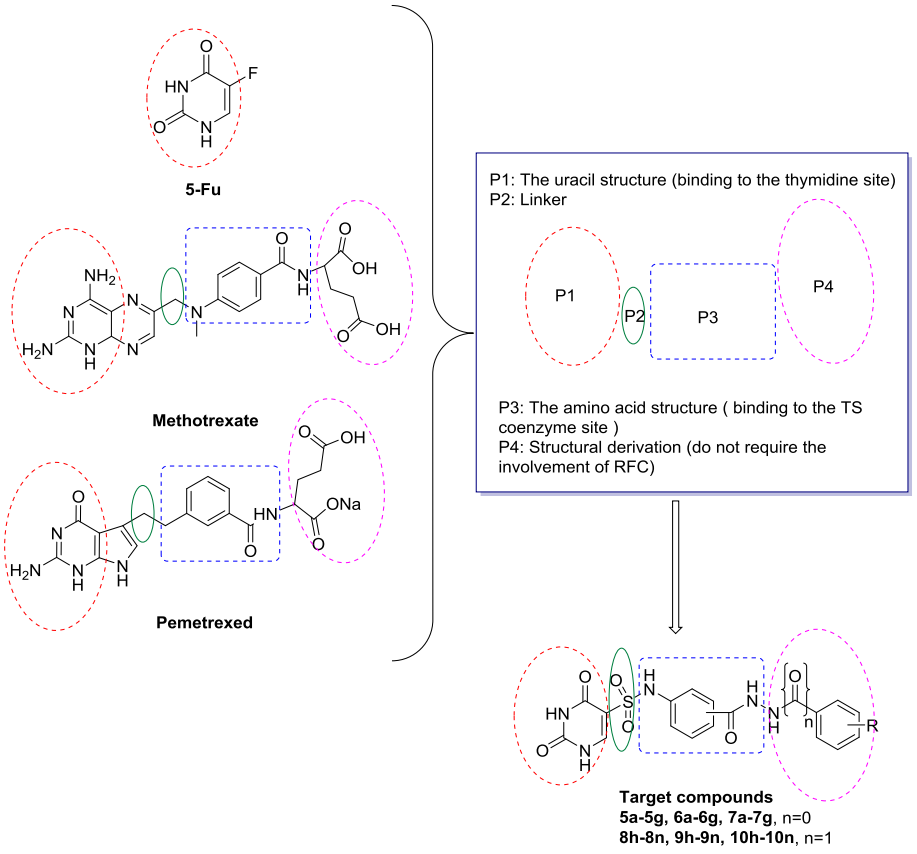
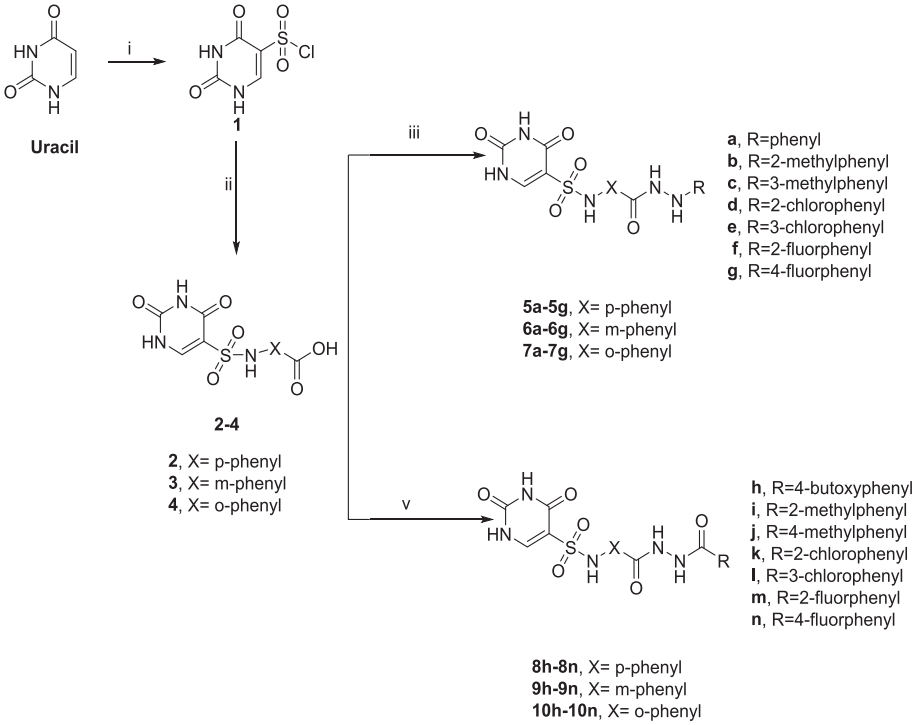


Fig. 2. Design of target compounds as TS inhibitors.



Scheme 1. Reagents and condition: (i) HSO_3Cl, $SOCl_2$, 60–75 °C; (ii) DMF, Pyridine, Corresponding aminobenzoic acid, 25 °C; (iii) HOBT, EDCI, 0 °C, Corresponding phenyl hydrazine; 25 °C; (v) HOBT, EDCI, 0 °C, Corresponding benzoyl hydrazide, 25 °C.

Table 1
IC_{50} values of compounds and PTX against A549, OVCAR-3, SGC7901, and MDA-MB-231 cell lines.

Compounds	IC_{50}[a](μM)			
	A549	OVCAR-3	SGC7901	MDA-MB-231
5a	–	–	–	–
5b	14.46 ± 0.77	>20	>20	>20
5c	>20	>20	>20	>20
5d	3.34 ± 0.24	7.23 ± 0.08	3.63 ± 0.64	4.01 ± 0.23
5e	7.29 ± 0.25	8.41 ± 0.45	11.58 ± 0.81	17.38 ± 1.65
5f	11.74 ± 0.24	>20	16.00 ± 1.43	>20
5g	>20	>20	>20	>20
6a	–	–	–	–
6b	14.22 ± 1.44	>20	>20	>20
6c	>20	>20	>20	>20
6d	1.46 ± 0.09	3.35 ± 0.18	2.27 ± 0.11	2.09 ± 0.05
6e	6.64 ± 0.10	8.04 ± 0.11	11.75 ± 0.09	7.76 ± 0.96
6f	11.14 ± 1.10	17.04 ± 1.61	>20	>20
6g	>20	>20	>20	>20
7a	–	–	–	–
7b	>20	>20	>20	>20
7c	>20	>20	>20	>20
7d	5.48 ± 1.10	14.11 ± 1.16	>20	18.85 ± 1.64
7e	5.29 ± 0.42	5.52 ± 0.42	10.84 ± 0.55	11.84 ± 0.44
7f	9.61 ± 0.17	9.57 ± 0.79	>20	>20
7g	>20	>20	>20	>20
8h	9.01 ± 0.57	12.78 ± 4.18	14.65 ± 0.56	11.90 ± 0.38
8i	15.07 ± 0.62	>20	>20	7.04 ± 1.21
8g	15.93 ± 0.52	>20	>20	>20
8k	1.90 ± 0.11	5.22 ± 0.24	3.24 ± 0.07	13.91 ± 0.59
8l	2.29 ± 0.18	7.71 ± 0.07	8.61 ± 0.10	4.16 ± 0.38
8m	7.61 ± 0.29	16.00 ± 1.43	>20	>20
8n	>20	>20	>20	>20
9h	7.76 ± 0.10	9.20 ± 0.63	7.37 ± 0.24	17.03 ± 3.85
9i	8.01 ± 0.68	>20	>20	18.11 ± 1.50
9g	>20	>20	10.11 ± 0.16	>20
9k	3.74 ± 0.03	7.30 ± 0.19	6.07 ± 0.08	2.36 ± 0.08
9l	6.00 ± 0.04	13.52 ± 2.17	7.31 ± 0.19	3.18 ± 0.25
9m	10.75 ± 0.33	10.81 ± 0.25	>20	>20
9n	>20	>20	>20	>20
10h	4.96 ± 1.17	5.01 ± 0,11	6.89 ± 0.27	6.83 ± 0.31
10i	5.45 ± 0.86	>20	12.97 ± 0.42	5.57 ± 0.47
10j	>20	12.94 ± 0.65	15.46 ± 0.54	>20
10k	5.29 ± 0.133	18.23 ± 0.21	2.41 ± 0.08	8.31 ± 0.56
10l	1.26 ± 0.16	2.08 ± 0.12	1.82 ± 0.08	4.42 ± 1.02
10m	7.15 ± 0.13	15.28 ± 1.55	>20	>20
10n	>20	15.10 ± 0.92	>20	>20
PTX	3.31 ± 0.23	6.90 ± 0.97	9.08 ± 0.52	3.85 ± 0.16

[a] Inhibitory effect was reported as an IC_{50} value (IC_{50} = Mean ± SD). From MTT assay after 24 h of treatment; the values were average from at least 3 independent experiments.

better than replaced by o-phenyl (**7d**); when R was replaced by 3-Cl, X was replaced by o-phenyl (**7e**), anti-proliferative activity of compound was better than replaced by m-phenyl (**6e**), and anti-proliferative activity of compound **6e** was better than replaced by p-phenyl (**5e**). Different from the first series, for the second series, when R was replaced by 2-Cl, X was replaced by p-phenyl (**8k**), anti-proliferative activity of compound was better than replaced by m-phenyl (**9k**), and anti-proliferative activity of compound **9k** was better than replaced by o-phenyl (**10k**); when R was replaced by 3-Cl, X was replaced by o-phenyl (**10l**), anti-proliferative activity of compound was better than replaced by p-phenyl (**8l**), and anti-proliferative activity of compound **8l** was better than replaced by m-phenyl (**9l**). Compared the first series with the second series, we found that anti-proliferative activity of the second series was better than the first series, indicating that the introduction of phenyl hydrazine can enhance the anti-proliferative activity of the targeted compounds, and compound **10l** had the best anti-proliferative activity ($IC_{50} = 1.26 \pm 0.16$ μM) than other compounds.

In short, anti-proliferative activity of the second series of compounds was better than the first series, indicating that the introduction of phenyl hydrazine has enhanced the anti-proliferative activity of these targeted compounds. The electron-withdrawing group (-Cl, -F) was superior to the electron-donating groups (-CH_3, -$OCH_2CH_2CH_3$), and compound **10l** had the best anti-proliferative activity ($IC_{50} = 1.26 \pm 0.16$ μM) than the other compounds.

In conclusion, the results showed that the introduction of formyl groups on aryl groups increased the activity of the target compounds, and the introduction of electron-withdrawing groups was superior to electron-donating groups. Therefore, these two series of for all targeted compounds, the electron-withdrawing group (-Cl, -F) was superior to the electron-donating group (-CH_3). TS inhibitory compounds deserve further antitumor study.

At present, pemetrexed is the first-line drug for the treatment of lung cancer in clinical treatment. The purpose of this study was to obtain a TS inhibitor which is superior to the positive drug (PTX). From the results of MTT assays, Compound **10l** has a better inhibitory effect on lung cancer and the compound **10l** has a higher selection index (SI) than the other three cancers, its SI even higher than PTX, which was shown in Table 2. Therefore, this study mainly focused on A549 cell.

The inhibition rate of compound **10l** on the proliferation of A549 cells was 35.83%, 51.21%, 55.39%, 66.28% and 82.54% at the concentrations of 0.512, 1.28, 3.2, 8 and 20 μM respectively (Fig. 3). The inhibition rate of PTX on the proliferation of A549 cells was 22.93% 49.43%, 56.20%, 61.82% and 64.42%, at the similar concentration to compound 10l above. As shown in Fig. 3, we found that the inhibition rate of compound **10l** was better than PTX at each concentration point, indicating that the compound **10l** have an excellent anti-proliferative effect on A549 cells.

2.2.2. *Effect of compound **10l** on the apoptosis in A549*

TS is over expressed in cancer cells and TS inhibitors can inhibit DNA synthesis and repair; whenever the DNA structure is incomplete can cause cells apoptosis. In this study, the compound **10l** was selected and its mechanism of growth inhibition on A549 cells was evaluated. To determine whether anti-proliferation were associated with apoptosis, A549 cells were treated with different concentrations of compound **10l** (0, 0.5, 1, 1.5, 3 μM) for 24 h, then collected and stained with acridine fluorescein isothiocyanate (FITC)-labeled as Annexin V (Annexin V-FITC) and propidium iodide (PI). Flow cytometry showed that the apoptotic rates are 2.3%, 8.5%, 12.5%, 22.1% and 39.4% (Fig. 4). As the concentration increased, the apoptotic rate obviously increased, of which the early apoptosis rates are 1.2%, 6.9%, 9.8%, 19.6% and 35.5%. It suggested that compound **10l** could inhibit cell proliferation by inducing apoptosis in A549 and the result is significantly different ($P < 0.01$).

2.2.3. *Effect of compound **10l** on the apoptotic protein expression of cleaved caspase-3, bcl-2/bax, and caspase-3 in A549*

According to previous studies that apoptosis can be induced in two major pathways: extrinsic and intrinsic apoptosis signaling [24]. TS inhibitors act on intracellular DNA synthesis and repair

Table 2
Selectivity index of compound 10 l and PTX against HPAEpiC, IOSE80, GES-1, and HTB-125 cell lines.

Compound	SI (Selection index)[a]			
	HPAEpiC	IOSE80	GES-1	HTB-125
10l	16.71	11.29	14.21	5.73
PTX	7.51	3.59	3.22	6.02

[a] SI = CC_{50}/IC_{50}.

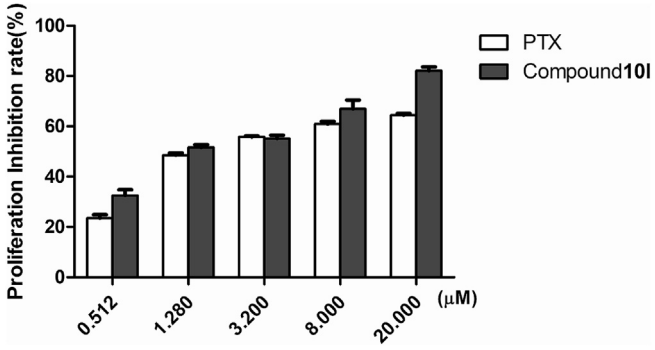


Fig. 3. Comparison of compound **10l** with **PTX** for inhibiting the proliferation of A549 cells.

processes, and are capable of inducing intrinsic apoptosis. An important consequence of intrinsic apoptotic pathway is the mitochondrial dysfunction and cytochrome c release [25]. The bcl-2 family of proteins plays a crucial role in regulating the protein release from mitochondria. This family is divided into two subgroups; pro-apoptotic and anti-apoptotic members. The pro-apoptotic proteins like bax induces cell apoptosis through the mitochondrial membrane permeabilization (MMP), and leads to the release of cytochrome c [26]. Cytochrome c is known to cause the activation of caspase-3 so as to increase the expression of cleaved caspase-3 [27]. The anti-apoptotic proteins like bcl-2, promotes cellular survival and inhibiting the actions of pro-apoptotic proteins. Therefore, when the ratio of bcl-2/bax decreased, the apoptosis increased [28].

To investigate the effect of bax and activation of caspase-3, as well as the expression of bcl-2, A549 cells were treated with different concentrations of (0, 0.5, 1, 1.5, 3 μM) compound **10l** for 24 h and western blot analysis was carried out. It was observed that up-regulation of bax and down-regulation of bcl-2, as well as the active of caspase-3 which in turn caused an increased in cleaved caspase-3 protein levels as shown in Fig. 5, and led the ratio of bcl-2/bax to decrease. However, compound **10l** stimulation could increase the expression of caspase-3, but the expression of caspase-3 had no distinct change when the concentration increased.

Therefore activation of caspase-3, the increase in cleaved caspase-3 and reduction of the ratio of bcl-2/bax by compound **10l** indicated that compound **10l** had the capacity to induce intrinsic apoptosis in A549 cells and the result is significantly different ($P < 0.001$).

3. Conclusions

We designed and synthesized two series of new TS inhibitors in total of 42 compounds and evaluated their activity as anticancer with potential against four human cancer cell lines (A549, OVCAR-3, SGC7901 and MDA-MB-231). More than half of the target compounds showed excellent activity, and the compound **10l** had the more potent inhibitory effect on A549 cells ($IC_{50} = 1.26$ μM) than PTX ($IC_{50} = 3.31$ μM). And the SI of the compound **10l** was higher than PTX. Flow cytometric analysis and Annexin V/propidium iodide (PI) staining revealed that compound **10l** could inhibit the proliferation of A549 cells by induced apoptosis. Further study indicated 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.

In conclusion, the design and synthesis of compound **10l** of TS inhibitors was useful as it showed to activate the intrinsic apoptosis signaling pathway, so as to achieve the purpose of inhibiting A549 cell proliferation and thereby provide a reference for the animal experiments.

4. Materials and methods

4.1. General methods

^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian NMR spectrometers operating at 600 MHz for 1H, and 150 MHz for 13C. All chemical shifts were measured in DMSO-d_6 as solvents. All chemicals were purchased from Sinoreagent Chemical Reagent (Beijing, China) and were used as received, unless stated otherwise. High-resolution mass spectra (HRMS) were measured with an Agilent Accurate-Mass Q-TOF 6530 (Agilent, Santa Clara, CA, USA) in ESI mode. Analytical TLC is performed on silica gel 60 F254 plates (Qingdao Haiyang Chemcal Company, Ltd) and visualized by UV and potassium permanganate staining. Flash column chromatography is performed on gel 60 (40–63 mm) (Qingdao Haiyang Chemcal Company, Ltd). Melting points were determined with an Electro thermal melting point apparatus, are uncorrected.

4.1.1. The synthesis of 2,4-dihydroxypyrimidine-5-sulfonyl chloride (*1*)

2, 4-dihydroxypyrimidine (6 g, 53.57 mmol) and thionylchloride ($SOCl_2$) (10 mL, 89.22 mmol) were added to a 100 mL reaction flask at 25 °C. After chlorosulfonic acid (HSO_3Cl) (16 g, 138.05 mmol) was added at room temperature for 1.5 h. The reaction was stirred at 60 °C for 4 h in an oil bath and then at 75 °C for 7 h. The reaction mixture was added to a mixture of ice and glacial acetic acid (1:1). The precipitate was precipitated and suction filtered, washed repeatedly and dried at 50 °C to give compound **1** as white powder. Yield: 92.30%.

4.1.2. General procedure for the synthesis of compound *2*–*4*

To a solution of 2,4-dihydroxypyrimidine-5-sulfonyl chloride in DMF, corresponding aminbenzoic acid and pyridine were added and the reaction was stirred at 25 °C for 5 h to give compound **2**–**4**.

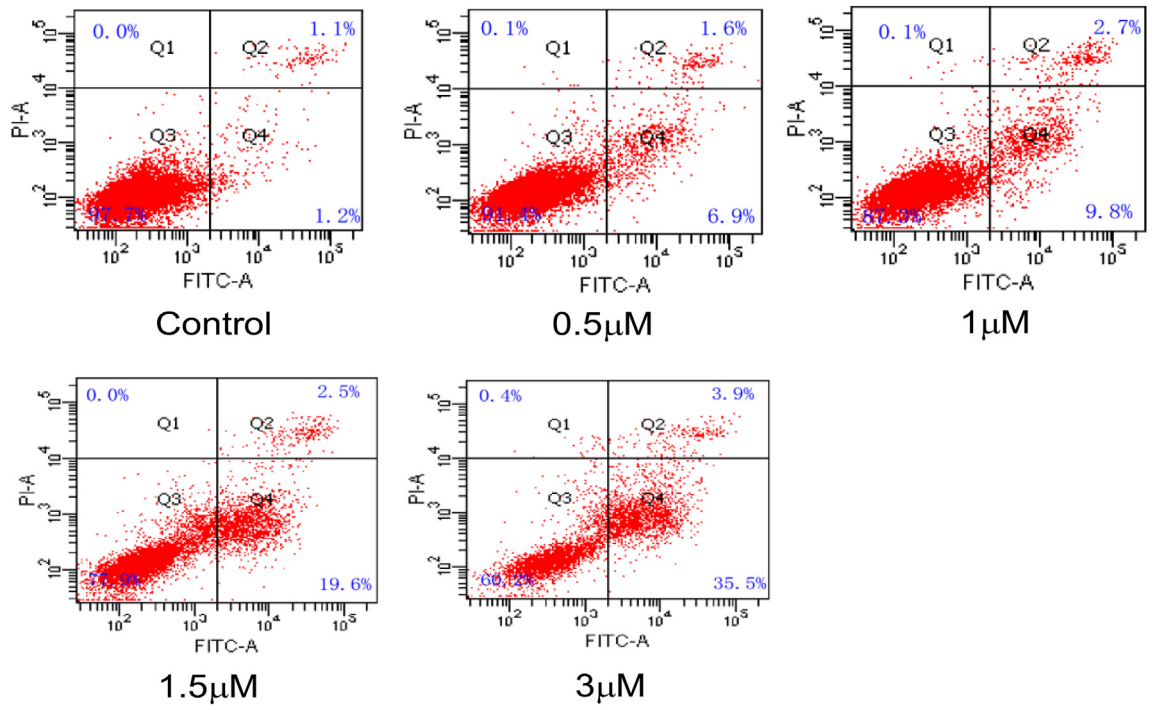
*4.1.2.1. 4-(2,4-dihydroxypyrimidine-5-sulfonamido)benzoic acid (**2**).* To a solution of 2, 4-dihydroxypyrimidine-5-sulfonyl chloride (4 g, 17.65 mmol) in DMF, meth-aminobenzoic acid (3.0 g, 21.86 mmol) and pyridine (0.6 g, 7.58 mmol) were added and the reaction was stirred at 25 °C for 5 h, to give compound **2** as yellow powder, yield 79.96%.

*4.1.2.2. 3-(2,4-dihydroxypyrimidine-5-sulfonamido)benzoic acid (**3**).* To a solution of 2,4-dihydroxypyrimidine-5-sulfonyl chloride (4 g, 17.65 mmol) in DMF, para-aminobenzoic acid (3.0 g, 21.86 mmol) and pyridine (0.6 g, 7.58 mmol) were added and the reaction is stirred at 25 °C for 5 h, to give compound **3** as yellow powder, yield: 80.00%.

*4.1.2.3. 2-(2,4-dihydroxypyrimidine-5-sulfonamido)benzoic acid (**4**).* To a solution of 2,4-dihydroxypyrimidine-5-sulfonyl chloride (4 g, 17.65 mmol) in DMF, ortho-aminobenzoic acid (3.0 g, 21.86 mmol) and pyridine (0.6 g, 7.58 mmol) were added and the reaction was stirred at 25 °C for 5 h, to give **4** as white powder, yield: 79.00%.

4.1.3. General procedure for the synthesis of compounds *5a-5g, 6a-6g,* and *7a-7g*

To a solution of corresponding (2,4-dihydroxypyrimidine-5-sulfonamido)benzoic acid (**2**–**4**) (2.50 g, 7.55 mmol) in DMF (20 mL), then HOBT (1.25 g, 9.68 mmol) and EDCI (1.75 g, 9.68 mmol) were added then the reaction was continued for 1 h at 0 °C. After the reaction was continued for 2 h under at 25 °C,



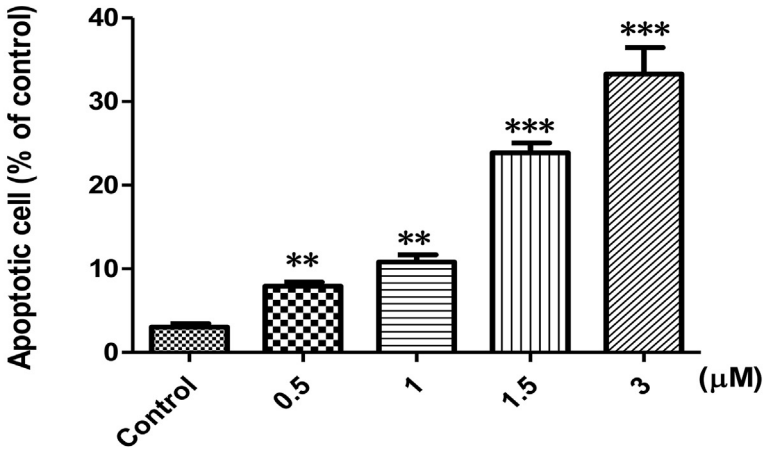


Fig. 4. Compound **10l** induced apoptosis in A549. Effects of compound **10l** on A549 cell death were evidenced by Annexin V-FITC/PI double staining and FACS analysis. Apoptotic cells were Annexin V [+] and PI [−], late apoptotic cells were Annexin V [+] and PI [+], nectotic cells were Annexin V [−] and PI [+] and living cells were Annexin V [−] and PI [−].Three independent experiments were performed, each column represented the mean ± SD of triplicate determinations (*P < 0.05; **P < 0.01 and ***P < 0.001. Compound **10l**-treated group VS control).

corresponding phenylhydrazine (1.25 g, 9.68 mmol) was added to continue the reaction for 5 h. After the reaction was completed, slowly pour the liquid into the water, precipitated the solid, add 10% dilute hydrochloric acid to adjust the solution pH to 2–3, and use suction filter to obtain the solid. The solid was added to a saturated solution of Na_2CO_3, the filtrate was obtained by suction filtered, 10% dilute hydrochloric acid was used to adjust the pH to 2–3, solidify by suction filtered and dried to give **5a-5g**, **6a-6g**, and**d 7a-7 g**.

*4.1.3.1. N′-phenyl-4-(2,4-dihydroxypyrimidine-5-sulfonamido)benzoyl hydrazide (**5a**).* A white solid, yield: 76.00%. Mp: 165–170 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.85 (br. s., 1 H), 11.62 (s, 1 H), 10.58 (s, 1 H), 10.21 (s, 1 H), 8.22 (s, 1 H), 7.81 (d, J = 8.66 Hz, 2 H), 7.19–7.21 (m, 2 H), 7.13 (t, J = 7.91 Hz, 2 H), 6.76 (d, J = 7.72 Hz, 2 H), 6.70 (t, J = 7.34 Hz, 1 H) 10.21 (s, 1 H),; ^{13}C NMR (150 MHz, DMSO-d_6) δ 166.22, 159.00, 150.89, 150.03, 149.33, 141.21, 131.09, 129.17, 128.96, 127.98, 119.02, 118.20, 118.00, 112.76, 111.09. ESI-HRMS calcd. for $C_{17}H_{15}N_5O_5NaS$ $[M + Na]^+$ 424.0692, found: 424.0720.

*4.1.3.2. N′-(2-benzyl)-4-(2,4-dihydroxypyrimidine-5-sulfonamido) benzoyl hydrazide (**5b**).* A white solid, yield: 76.00%. Mp: 172–174 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.85 (br. s., 1 H), 11.62 (s, 1 H), 10.58 (s, 1 H), 10.25 (s, 1 H), 8.22 (s, 1 H), 7.83 (d, J = 8.66 Hz, 2 H), 7.20 (d, J = 8.66 Hz, 2 H), 7.02 (d, J = 7.15 Hz, 1 H), 6.99 (t, J = 7.81 Hz, 1 H), 6.64–6.69 (m, 2 H),2.19 (s, 3 H); ^{13}C NMR (150 MHz, DMSO-d_6) δ166.15, 165.81, 159.02, 154.14, 150.89, 149.32,

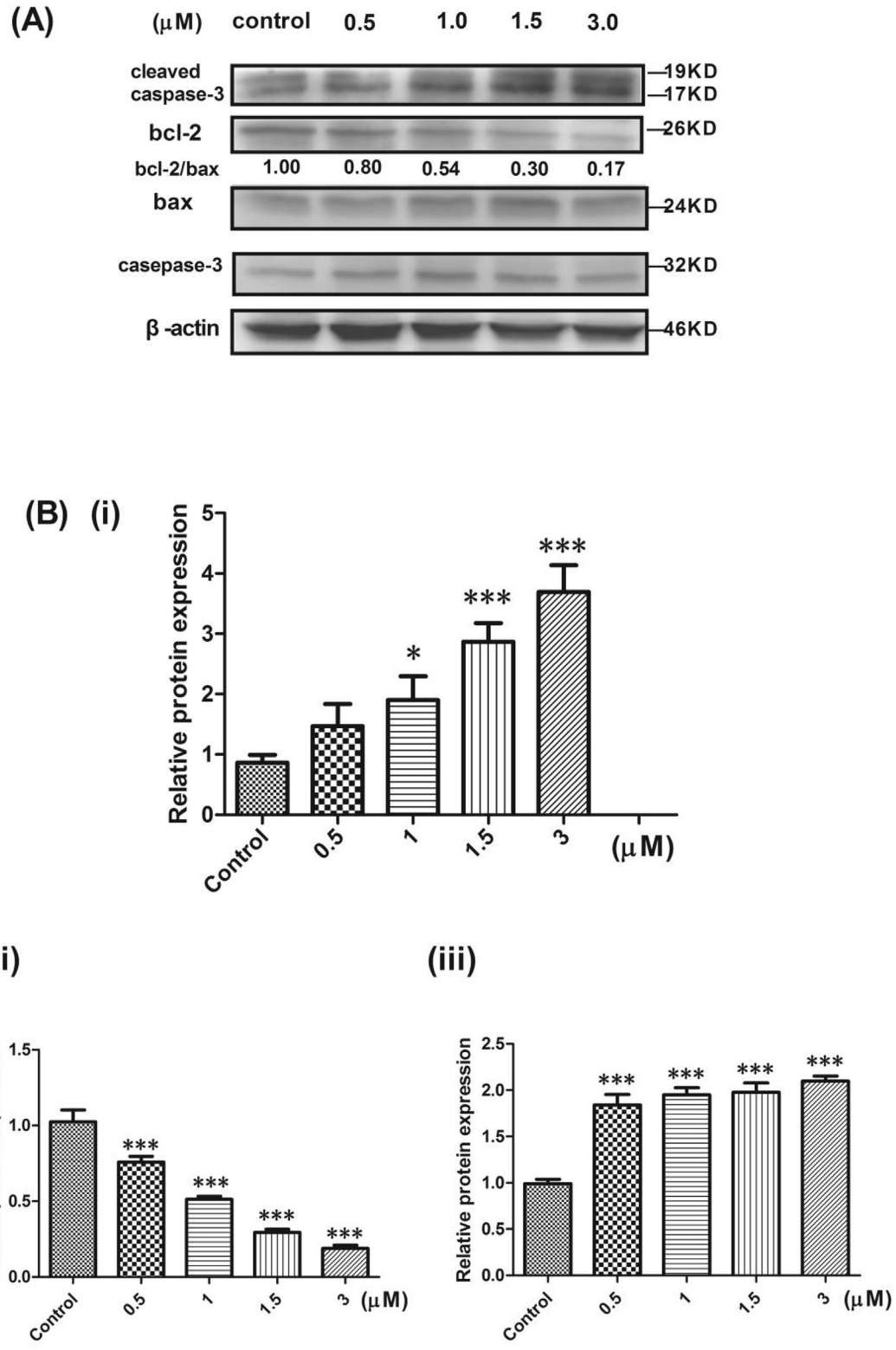


Fig. 5. Effect of compound **10l** on the apoptotic protein expression of cleaved caspase-3, bcl-2/bax and caspase-3 in A549. A549 were incubated for 24 h with indicated compound **10l** after 24 h of serum starvation. (A): The levels of cleaved caspase-3, caspase-3 and bcl-2/bax proteins were analyzed by western blot. (B): (i), (ii) & (iii) Relative protein expression of cleaved caspase-3, bcl-2/bax and caspase-3 in A549 with or without compound **10l.** Each column represented the mean ± SD of triplicate determinations (*P < 0.05; **P < 0.01 and ***P < 0.001. Compound **10l**-treated group VS control).

146.86, 141.23, 130.28, 129.31, 128.76, 126.89, 122.36, 118.24, 111.54, 17.79. ESI-HRMS *m/z* calcd for $C_{18}H_{17}N_5O_5NaS$. [M + Na]+ 438.0827, found: 438.0827.

*4.1.3.3. N'-(3-benzyl)-4-(2,4-dihydroxypyrimidine-5-sulfonamido) benzoyl hydrazide (**5c**).* A light yellow solid, yield: 71.33%. Mp: 173–174 °C.^{1}H NMR (600 MHz, DMSO-d_6) δ 11.80 (br. s., 1 H), 11.62 (s, 1 H), 10.44 (s, 1 H), 10.39 (d, J = 4.52 Hz, 2 H), 8.11 (s, 1 H), 7.95 (s, 1 H), 7.82 (d, J = 8.09 Hz, 2 H), 7.64 (d, J = 1.69 Hz, 1 H), 7.59 (d, J = 7.91 Hz, 1 H), 7.41 (t, J = 7.91 Hz, 1 H), 7.33 (d, J = 7.72 Hz, 2 H),2.38 (s, 3 H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 166.70, 155.35, 152.53, 150.40, 138.22, 129.35, 129.05, 128.76, 123.48, 119.82, 119.74, 113.33, 113.11, 111.97, 110.14, 21.79. ESI-HRMS *m/z* calcd for $C_{18}H_{17}N_5O_5NaS$. [M + Na]+ 424.0692, found: 438.0869.

*4.1.3.4. N'-(2-chlorophenyl)-4-(2,4-dihydroxypyrimidine-5-sulfonamido)benzoyl hydrazide (**5d**).* A white solid, yield: 63.00%. Mp: 163–167 °C.^{1}H NMR (600 MHz, DMSO-d_6) δ 11.50 (br. s., 1 H), 10.38 (s, 1 H), 8.22 (s, 1 H), 8.22 (s, 1 H), 7.82–7.84 (m, 2 H), 7.82–7.84 (m, 2 H), 7.30 (d, J = 7.91 Hz, 1 H), 7.21 (d, J = 8.85 Hz, 2 H), 7.12–7.15 (m, 1 H), 6.81 (d, J = 8.09 Hz, 1 H),6.75 (t, J = 6.87 Hz, 1 H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 166.27, 159.47, 152.54, 151.48, 145.36, 141.79, 129.59, 129.06, 128.27, 127.36, 120.07, 118.16, 117.07, 113.60,110.14. ESI-HRMS calcd for $C_{17}H_{14}ClN_5O_5NaS$. [M + Na]$^+$ 458.0302, found: 458.0244.

*4.1.3.5. N'-(3-chlorophenyl)-4-(2,4-dihydroxypyrimidine-5-sulfonamido) benzoyl hydrazide (**5e**).* A white solid, yield: 76.50%. Mp: 187–190 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.61–11.30 (m, 1 H), 10.23 (s, 1 H), 8.18 (s, 1 H), 7.78 (d, J = 8.66 Hz, 2 H), 7.11–7.19 (m, 3 H), 6.67–6.73 (m, 3 H);^{13}C NMR (150 MHz, DMSO-d_6) δ 166.26, 159.00, 151.66, 150.90, 149.35, 141.38, 133.92,130.90,129.04,127.63,118.45, 118.20, 111.97, 111.36,111.09. ESI-HRMS calcd for $C_{17}H_{14}ClN_5O_5NaS$. [M + Na] + 458.0302, found: 458.0306.

*4.1.3.6. N'-(2-fluorophenyl)-4-(2,4-dihydroxypyrimidine-5-sulfonamido) benzoyl hydrazide (**5f**).* A yellow solid, yield: 80.20%. Mp: 141–144 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.91 (br. s., 1 H), 11.63 (s, 1 H), 10.61 (br. s., 1 H), 10.30 (s, 1 H), 8.23 (s, 1 H), 7.82 (d, J = 8.62 Hz, 2 H), 7.20 (d, J = 8.80 Hz, 2 H), 7.08 (dd, J = 11.65, 7.79 Hz, 1 H), 6.97 (t, J = 7.61 Hz, 1 H), 6.80 (t, J = 8.53 Hz, 1 H), 6.70–6.74 (m, 1 H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 166.26, 159.00, 151.50, 150.86, 149.92, 149.32, 141.33, 137.58, 137.50, 129.04, 127.75, 125.03, 118.22, 114.18, 113.14,111.08. ESI-HRMS calcd for $C_{17}H_{14}FN_5O_5NaS$. [M + Na]$^+$ 442.0597, found: 442.0574.

*4.1.3.7. N'-(4-fluorophenyl)-4-(2,4-dihydroxypyrimidine-5-sulfonamido) benzoyl hydrazide (**5g**).* A yellow solid, yield: 82.00%. Mp: 177–179 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.86 (br. s., 1 H), 11.62 (s, 1 H), 10.59 (s, 1 H), 10.25 (s, 1 H), 8.23 (s, 1 H), 7.81 (d, J = 8.66 Hz, 2 H), 7.28 (d, J = 8.85 Hz, 2 H), 7.20 (d, J = 8.66 Hz, 2 H),6.69–6.76 (m, 2 H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.82, 165.28, 165.28, 159.02, 150.96, 149.48, 141.48, 130.65, 129.15, 127.44, 118.20, 116.08, 115.93,111.02. ESI-HRMS calcd for $C_{17}H_{14}FN_5O_5NaS$. [M + Na]$^+$ 442.0597, found: 442.0611.

*4.1.3.8. N'-phenyl-3-(2,4-dihydroxypyrimidine-5-sulfonamido)benzoyl hydrazide (**6a**).* A yellow solid, yield: 79.00%. Mp: 132–137 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.60 (br. s., 1 H), 10.32 (d, J = 2.82 Hz, 1 H), 8.10 (s, 1 H), 7.86 (d, J = 3.01 Hz, 1 H), 7.61 (d, J = 1.51 Hz, 1 H), 7.34–7.39 (m, 1 H), 7.29 (d, J = 7.91 Hz, 1 H), 7.11–7.20 (m, 2 H), 6.76 (d, J = 7.72 Hz, 2 H),6.71 (t, J = 7.25 Hz, 1 H); ^{13}C NMR (150 MHz, DMSO-d_6) δ169.76, 165.47, 157.99, 149.87, 148.83, 147.94, 137.39, 133.50, 128.60, 128.16, 121.70, 121.42, 118.06, 111.69,110.13. ESI-HRMS calcd for $C_{17}H_{15}N_5O_5NaS$. [M + Na]$^+$ 424.0692, found: 424.0705.

*4.1.3.9. N'-(2-benzyl)-3-(2,4-dihydroxypyrimidine-5-sulfonamido) benzoyl hydrazide (**6b**).* An off yellow solid, yield: 62.34%. Mp: 135–139 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.60 (br. s., 1 H), 10.36 (s, 2 H), 8.10 (s, 1 H), 7.62 (d, J = 1.88 Hz, 1 H), 7.38–7.41 (m, 1 H), 7.30 (d, J = 10.35 Hz, 1 H), 7.25 (d, J = 2.82 Hz, 1 H), 6.98–7.05 (m, 2 H), 6.63–6.71 (m, 2 H),2.20 (s, 3 H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 170.83, 166.44, 159.08, 151.01, 149.09, 147.24, 138.49, 134.59, 130.46, 129.69, 126.92, 122.78, 122.40, 119.22, 119.08, 111.46, 111.17, 17.78. ESI-HRMS calcd for $C_{18}H_{17}N_5O_5NaS$. [M + Na]$^+$ 438.0848, found: 438.0855.

*4.1.3.10. N'-(3-benzyl)-3-(2,4-dihydroxypyrimidine-5-sulfonamido) benzoyl hydrazide (**6c**).* A yellow solid, yield: 73.80%. Mp: 158–160 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.53 (br. s., 1 H), 10.47 (s, 1 H), 10.41 (s, 1 H), 8.11 (s, 1 H), 7.74 (s, 1 H), 7.71 (d, J = 5.84 Hz, 1 H), 7.64 (s, 1 H), 7.59 (d, J = 7.72 Hz, 1 H), 7.38–7.43 (m, 3 H), 7.32 (d, J = 8.09 Hz, 1 H),2.38 (s, 3 H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 167.57, 152.75, 150.18, 149.30, 138.23, 134.42, 129.31, 129.06, 119.85, 118.12, 117.24, 116.25, 114.68, 113.30, 113.27, 112.46, 110.08,21.80. ESI-HRMS calcd for $C_{18}H_{17}N_5O_5NaS$. [M + Na]$^+$ 438.0848, found: 438.0841.

*4.1.3.11. N'-(2-chlorophenyl)-3-(2,4-dihydroxypyrimidine-5-sulfonamido)benzoyl hydrazide (**6d**).* A white powder, yield: 71.33%. Mp: 168–170 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.60 (br. s., 1 H), 10.37 (br. s., 1 H), 10.37 (br. s., 1 H), 8.11 (s, 1 H), 7.65 (s, 1 H), 7.59 (d, J = 7.15 Hz, 1 H), 7.54–7.57 (m, 2 H), 7.52 (t, J = 7.53 Hz, 1 H), 7.45–7.49 (m, 1 H), 7.39 (t, J = 7.81 Hz, 1 H),7.31 (d, J = 8.09 Hz, 1 H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.34, 157.98, 149.66, 147.72, 144.17, 137.37, 133.11, 128.61, 128.54, 127.21, 121.96, 121.60, 118.99, 118.24, 116.69, 112.40,110.09. ESI-HRMS calcd for $C_{17}H_{14}ClN_5O_5NaS$. [M + Na]$^+$ 458.0302, found: 458.0298.

*4.1.3.12. N'-(3-chlorophenyl)-3-(2,4-dihydroxypyrimidine-5-sulfonamido)benzoyl hydrazide (**6e**).* An off white powder, yield: 70.43%. Mp: 170–173 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.41 (br. s., 1 H), 8.23 (s, 1 H), 8.10 (s, 1 H), 7.61 (s, 1 H), 7.57 (d, J = 7.52 Hz, 1 H), 7.35–7.39 (m, 1 H), 7.30 (d, J = 7.70 Hz, 1 H), 7.17 (t, J = 7.89 Hz, 1 H),6.70–6.77 (m, 3 H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 166.55, 159.05, 151.51, 150.93, 149.01, 138.52, 134.25, 133.95, 130.96, 129.75, 122.85, 122.49, 119.09, 118.55, 112.00, 111.33,111.21. ESI-HRMS calcd for $C_{17}H_{14}ClN_5O_5NaS$. [M + Na]$^+$ 458.0302, found: 458.0281.

*4.1.3.13. N'-(2-fluorophenyl)-3-(2,4-dihydroxypyrimidine-5-sulfonamido)benzoyl hydrazide (**6f**).* A yellow solid, yield: 60.86%. Mp: 162–164 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.62 (br. s., 1 H), 10.41 (d, J = 2.20 Hz, 1 H), 8.12 (s, 1 H), 7.81 (s, 1 H), 7.57–7.65 (m, 2 H), 7.40 (t, J = 8.16 Hz, 1 H), 7.26–7.34 (m, 1 H), 7.10 (dd, J = 12.01, 8.16, 1.10 Hz, 1 H), 7.00 (t, J = 7.61 Hz, 1 H), 6.77–6.86 (m, 1 H),6.70–6.77 (m, 1 H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 166.54, 159.04, 151.51, 150.89, 149.93, 148.96, 138.48, 134.33,129.70,125.06, 122.89,122.52,119.11,115.43,115.31,114.11,111.20. ESI-HRMS calcd for $C_{17}H_{14}FN_5O_5NaS$. [M + Na]$^+$ 442.0597, found: 442.0607.

*4.1.3.14. N'-(4-fluorophenyl)-3-(2,4-dihydroxypyrimidine-5-sulfonamido)benzoyl hydrazide (**6g**).* A yellow solid, yield: 80.50%. Mp: 165–169 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.79 (br. s., 1 H), 11.61 (s, 1 H), 10.35 (s, 2 H), 8.10 (s, 1 H), 7.86 (d, J = 3.01 Hz, 1 H), 7.60 (d, J = 1.88 Hz, 1 H), 7.39 (t, J = 8.19 Hz, 1 H), 7.30 (d, J = 7.15 Hz, 1 H), 6.94–7.03 (m, 2 H),6.72–6.82 (m, 2 H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 166.55, 159.03, 150.88, 148.93, 138.45, 134.47, 129.68, 122.82, 122.49, 119.08, 115.75, 115.60, 113.99, 113.94,111.24. ESI-

HRMS calcd for $C_{17}H_{14}FN_5O_5NaS$. $[M + Na]^+$ 442.0597, found: 442.0570.

*4.1.3.15. N′-phenyl-2-(2,4-dihydroxypyrimidine-5-sulfonamido)benzoyl hydrazide (**7a**).* A light brown solid, yield: 74.45%. Mp: 137–142 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.91 (br. s., 1 H), 11.62 (s, 2 H), 10.60 (br. s., 1 H), 8.27 (s, 1 H), 7.92 (d, J = 7.15 Hz, 1 H), 7.52 (d, J = 5.08 Hz, 1 H), 7.44–7.48 (m, 1 H), 7.15–7.21 (m, 3 H), 6.83 (s, 2 H), 6.75 (t, J = 7.25 Hz, 1 H); ^{13}C NMR (150 MHz, DMSO-d_6) δ167.31, 157.70, 149.77, 148.42, 148.32, 137.55, 128.22, 128.11, 122.03, 118.32, 117.98, 116.97, 111.84, 111.71,109.70. ESI-HRMS calcd for $C_{17}H_{15}N_5O_5NaS$. $[M + Na]^+$ 424.0692, found: 424.0768.

*4.1.3.16. N′-(2-benzyl)-2-(2,4-dihydroxypyrimidine-5-sulfonamido) benzoyl hydrazide (**7b**).* A white solid, yield: 76.00%. Mp: 155–160 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.33 (d, J = 2.64 Hz, 1 H), 8.28 (s, 1 H), 7.87 (t, J = 8.38 Hz, 1 H), 7.80 (d, J = 8.85 Hz, 1 H), 7.65 (d, J = 7.72 Hz, 1 H), 7.60 (t, J = 7.62 Hz, 1 H), 6.90 (d, J = 7.15 Hz, 1 H), 6.66–6.73 (m, 1 H), 6.61 (d, J = 7.91 Hz, 1 H), 6.53 (t, J = 7.15 Hz, 1 H),2.07 (s, 3 H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 167.22, 165.71, 157.74, 149.75, 148.21, 145.73, 139.48, 133.56, 132.55, 132.24, 130.57, 129.39, 126.01, 122.04, 121.47, 118.47, 110.66,16.66. ESI-HRMS calcd for $C_{18}H_{17}N_5O_5NaS$. $[M + Na]^+$ 438.0848, found: 438.0845.

*4.1.3.17. N′-(3-benzyl)-2-(2,4-dihydroxypyrimidine-5-sulfonamido) benzoyl hydrazide (**7c**).* A brown solid, yield: 63.13%. Mp: 156–159 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.97 (br. s., 1 H), 11.62 (s, 1 H), 11.05 (s, 1 H), 10.61 (s, 1 H), 8.27 (br. s., 1 H), 7.92 (d, J = 7.70 Hz, 1 H), 7.47–7.57 (m, 1 H), 7.45 (d, J = 8.25 Hz, 1 H), 7.17 (t, J = 7.52 Hz, 1 H), 7.06 (t, J = 7.61 Hz, 1 H), 6.60–6.69 (m, 2 H), 6.57 (d, J = 7.15 Hz, 1 H),2.23 (s, 3 H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 168.38, 158.80, 150.84, 149.57, 149.37, 138.63, 138.42, 133.32, 129.20, 128.92, 123.11, 120.32, 119.07, 118.02, 113.52, 110.80, 110.24, 21.77,. ESI-HRMS calcd for $C_{18}H_{17}N_5O_5NaS$. $[M + Na]^+$ 438.0848, found: 438.0839.

*4.1.3.18. N′-(2-chlorophenyl)-2-(2,4-dihydroxypyrimidine-5-sulfonamido)benzoyl hydrazide (**7d**).* An off white solid, yield: 69.70%. Mp: 163–167 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.48 (s, 1 H), 8.30 (s, 1 H), 7.74–7.78 (m, 1 H), 7.66 (d, J = 8.66 Hz, 1 H), 7.60 (t, J = 7.53 Hz, 1 H), 7.18 (d, J = 7.91 Hz, 1 H), 6.83–6.91 (m, 1 H), 6.78 (d, J = 9.60 Hz, 1 H),6.63 (t, J = 8.38 Hz, 1 H),; ^{13}C NMR (150 MHz, DMSO-d_6) δ 170.16, 166.79, 158.96, 150.69, 149.29, 140.57, 138.50, 134.64, 133.64, 131.65, 128.40, 127.09, 124.21, 124.14, 121.38, 119.55, 110.52. ESI-HRMS calcd for $C_{17}H_{14}ClN_5O_5NaS$. $[M + Na]^+$ 458.0302, found: 458.0297.

*4.1.3.19. N′-(3-chlorophenyl)-2-(2,4-dihydroxypyrimidine-5-sulfonamido)benzoyl hydrazide (**7e**).* An off white solid, yield: 73.03%. Mp: 166–170 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.57 (s, 1 H), 8.46 (s, 1 H), 8.19 (d, J = 7.34 Hz, 1 H), 7.88–7.94 (m, 1 H), 7.80 (d, J = 8.25 Hz, 1 H), 7.61 (t, J = 7.52 Hz, 1 H), 7.21–7.27 (m, 1 H), 6.92 (dd, J = 7.70, 1.10 Hz, 1 H), 6.78 (s, 1 H),6.68 (dd, J = 8.25, 1.65 Hz, 1 H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 168.44, 158.79, 157.35, 155.79, 150.89, 149.46, 145.98, 138.60, 133.37, 128.95, 123.14, 119.08, 118.08, 115.83, 115.68, 114.28,110.72. ESI-HRMS calcd for $C_{17}H_{14}ClN_5O_5NaS$. $[M + Na]^+$ 458.0302, found: 458.0229.

*4.1.3.20. N′-(2-fluorophenyl)-2-(2,4-dihydroxypyrimidine-5-sulfonamido)benzoyl hydrazide (**7f**).* A yellow solid, yield: 75.50%. Mp: 160–164 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.19 (d, J = 6.05 Hz, 1 H), 11.64 (s, 1 H), 10.95 (s, 1 H), 10.78 (s, 1 H), 8.23 (s, 1 H), 7.96 (d, J = 7.89 Hz, 1 H), 7.18 (t, J = 7.98 Hz, 1 H), 7.12 (dd, J = 11.46, 7.61 Hz, 1 H), 7.02–7.07 (m, 1 H), 6.91 (t, J = 9.17 Hz, 1 H),6.75–6.81 (m, 1 H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 168.52, 158.83, 150.74, 149.27, 138.57, 137.01, 136.94, 133.44, 129.09, 125.22, 123.17, 119.03, 118.12, 115.39, 115.27, 114.56, 110.69. ESI-HRMS calcd for $C_{17}H_{14}FN_5O_5NaS$. $[M + Na]^+$ 442.0597, found: 442.0596.

*4.1.3.21. N′-(4-fluorophenyl)-2-(2,4-dihydroxypyrimidine-5-sulfonamido)benzoyl hydrazide (**7g**).* A deep yellow solid, yield: 69.17%. Mp: 162–165 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.91 (br. s., 1 H), 11.62 (s, 1 H), 10.98 (br. s., 1 H), 10.61 (br. s., 1 H), 8.26 (s, 1 H), 7.89 (d, J = 7.72 Hz, 1 H), 7.49–7.56 (m, 1 H), 7.43–7.48 (m, 1 H), 7.17 (t, J = 7.62 Hz, 1 H), 7.03 (t, J = 8.85 Hz, 2 H),6.81–6.87 (m, 2 H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.47, 158.94, 149.35, 147.70, 135.59, 134.02, 133.56, 133.10, 131.32, 128.17, 128.12, 128.10, 127.10, 126.93, 122.41. ESI-HRMS calcd for $C_{17}H_{14}FN_5O_5NaS$. $[M + Na]^+$ 442.0597, found: 442.0542.

*4.1.4. General procedure for the synthesis of compounds **8h-8n, 9h-9n,** and **10h-10n***

To a solution of **2**–**4** (2.50 g, 7.55 mmol) in DMF (20 mL), add HOBT (1.25 g, 9.68 mmol) and EDCI (1.75 g, 9.68 mmol) and allow the reaction to continue for 1 h at 0 °C. After, the reaction was further continued for 2 h at 25 °C, add the corresponding benzoyl hydrazide (1.25 g, 9.68 mmol) and continue the reaction for 5 h. After the reaction has completed, slowly pour the liquid into the water to form precipitate, add 10% dilute hydrochloric acid to adjust the solution pH to 2–3, and use suction filtration to obtain the solid. Furthermore, the solid was added to a saturated solution of Na_2CO_3, the filtrate was obtained by suction filtration, 10% dilute hydrochloric acid was used to adjust the pH to 2–3 and dried to give **8h-8n**, **9h-9n**, and **10h-10n**.

*4.1.4.1. N′-(4-propyl benzoyl)-4-(2,4-dihydroxypyrimidine-5-sulfonamido) benzoyl hydrazide (**8h**).* A white solid, yield: 80.45%. Mp: 200–205 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.90 (br. s., 1 H) 11.62 (s, 1 H), 10.60 (s, 1 H), 10.29 (s, 2 H), 8.22 (br. s., 1 H), 7.89 (d, J = 8.85 Hz, 2 H), 7.82 (d, J = 8.66 Hz, 2 H), 7.21 (d, J = 8.85 Hz, 2 H), 7.02–7.04 (m, 2 H), 4.00–4.03 (m, 2 H), 1.73–1.77 (m, 2 H),0.96–1.01 (m, 3 H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.88, 165.84, 161.93, 159.03, 150.83, 149.31, 141.37, 129.86, 129.17, 127.66, 124.99, 118.27, 114.57, 111.08, 69.64, 22.40, 10.81. ESI-HRMS calcd for $C_{21}H_{21}N_5O_6NaS$. $[M + Na]^+$ 510.1059, found: 510.1081.

*4.1.4.2. N′-(2-methyl benzoyl)-4-(2,4-dihydroxypyrimidine-5-sulfonamido)benzoyl hydrazide (**8i**).* A white solid, yield: 80.65%. Mp: 169–172 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.40 (br. s., 1 H), 10.34 (br. s., 1 H), 8.20 (s, 1 H), 7.80 (d, J = 8.80 Hz, 2 H), 7.74 (s, 1 H), 7.71 (dd, J = 6.14, 2.29 Hz, 1 H), 7.39–7.41 (m, 2 H), 7.18 (d, J = 8.80 Hz, 2 H), 2.37 (s, 3 H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 166.41, 165.88, 160.28, 138.23, 133.09, 132.82, 129.47, 129.06, 129.01, 128.82, 128.52, 127.95, 126.78, 125.01, 120.89, 117.90, 21.42. ESI-HRMS calcd for $C_{19}H_{17}N_5O_6NaS$. $[M + Na]^+$ 466.0797, found: 466.0793.

*4.1.4.3. N′-(4-methyl benzoyl)-4-(24-dihydroxypyrimidine-5-sulfonamido)benzoyl hydrazide (**8j**).* An off yellow solid, yield: 69.16%. Mp: 163–167 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.13 (s, 1 H), 7.82 (d, J = 7.72 Hz, 2 H), 7.73 (d, J = 8.28 Hz, 2 H), 7.31 (d, J = 7.72 Hz, 2 H), 7.11 (d, J = 8.09 Hz, 2 H),2.37 (s, 3 H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 166.22, 166.10, 161.94, 160.47, 144.63, 142.23, 130.32, 129.46, 128.84, 127.96, 125.54, 117.93, 113.10, 106.36, 21.49. ESI-HRMS calcd for $C_{19}H_{17}N_5O_6NaS$. $[M + Na]^+$ 466.0797, found: 466.0888.

*4.1.4.4. N′-(2-chlorobenzoyl)-4-(2,4-dihydroxypyrimidine-5-sulfonamido)benzoyl hydrazide (**8k**).* A white powder, yield: 70.59%. Mp: 178–180 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.51 (br. s., 1 H),

10.38 (d, J = 2.26 Hz, 1 H), 8.22 (s, 1 H), 7.83 (d, J = 8.66 Hz, 2 H), 7.30 (d, J = 6.59 Hz, 1 H), 7.21 (d, J = 8.85 Hz, 2 H), 7.12–7.16 (m, 1 H), 6.81 (d, J = 8.09 Hz, 1 H), 6.73–6.77 (m, 1 H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 166.24, 165.50, 162.78, 160.56, 159.19, 135.17, 131.97, 130.92, 130.37, 129.65, 129.14, 128.98, 129.88, 127.64, 118.98, 118.12, 113.03, 110.64. ESI-HRMS calcd for $C_{18}H_{14}ClN_5O_6NaS$. $[M + Na]^+$ 486.0251, found: 486.0277.

*4.1.4.5. N'-(3-chlorobenzoyl)-4-(2,4-dihydroxypyrimidine-5-sulfonamido)benzoyl hydrazide (**8l**).* An off white solid, yield: 64.28%. Mp: 183–185 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.57 (br. s., 1 H), 10.36 (br. s., 1 H), 8.16 (s, 1 H), 7.94 (t, J = 1.69 Hz, 1 H), 7.87 (d, J = 7.72 Hz, 1 H), 7.76 (d, J = 8.85 Hz, 2 H), 7.67 (d, J = 6.02 Hz, 1 H), 7.54–7.59 (m, 1 H), 7.16 (d, J = 8.66 Hz, 2 H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.92, 165.39, 163.83, 159.38, 151.98, 150.75, 141.70, 130.71, 130.65, 129.44, 129.20, 127.33, 118.28, 116.03, 115.89, 110.48. ESI-HRMS calcd for $C_{18}H_{14}ClN_5O_6NaS$. $[M + Na]^+$ 486.0251, found: 486.0248.

*4.1.4.6. N'-(2-fluorobenzoyl)-4-(2,4-dihydroxypyrimidine-5-sulfonamido)benzoyl hydrazide (**8m**).* A yellow solid, yield: 79.50%. Mp: 193–196 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.21 (br. s., 4 H), 8.14 (br. s., 1 H), 7.73 (d, J = 6.79 Hz, 2 H), 7.68 (br. s., 1 H), 7.57 (br. s., 1 H), 7.32 (br. s., 2 H),7.08 (d, J = 6.05 Hz, 2 H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.52, 163.56, 162.01, 160.57, 158.92, 133.25, 133.20, 130.68, 128.72, 125.00, 123.15, 123.05, 117.92, 116.78, 116.64, 113.06. ESI-HRMS calcd for $C_{18}H_{14}ClN_5O_6NaS$. $[M + Na]^+$ 470.0547, found: 470.0561.

*4.1.4.7. N'-(4-fluorobenzoyl)-4-(2,4-dihydroxypyrimidine-5-sulfonamido)benzoyl hydrazide (**8n**).* A yellow solid, yield: 68.18%. Mp: 192–193 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.54 (br. s., 1 H), 10.42 (br. s., 1 H), 8.23 (s, 1 H), 8.01 (dd, J = 8.80, 5.50 Hz, 2 H), 7.84 (d, J = 8.80 Hz, 2 H), 7.36 (t, J = 8.80 Hz, 2 H), 7.22 (d, J = 8.80 Hz, 2 H); ^{13}C NMR (150 MHz, DMSO-d_6) δ165.90, 164.93, 160.59, 135.01, 133.80, 132.19, 131.07, 128.98, 128.51, 127.73, 126.65, 126.38, 125.00, 117.81. ESI-HRMS calcd for $C_{18}H_{14}ClN_5O_6NaS$. $[M + Na]^+$ 470.0547, found: 470.0544.

*4.1.4.8. N'-(4-propylbenzoyl)-3-(2,4-dihydroxypyrimidine-5-sulfonamido)benzoyl hydrazide (**9h**).* A white solid, yield: 80.12%. Mp: 210–213 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ11.83 (br. s., 1 H), 11.62 (s, 1 H), 10.41 (s, 1 H), 10.38 (s, 1 H), 10.32 (s, 1 H), 8.11 (br. s., 1 H), 7.89 (d, J = 8.85 Hz, 2 H), 7.63 (t, J = 1.69 Hz, 1 H), 7.59 (d, J = 7.91 Hz, 1 H), 7.40 (t, J = 7.91 Hz, 1 H), 7.31 (dd, J = 8.09, 1.51 Hz, 1 H), 7.04 (d, J = 8.85 Hz, 2 H), 3.99–4.02 (m, 2 H), 1.71–1.79 (m, 2 H), 0.97–1.02 (m, 3 H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 166.14, 165.85, 161.98, 159.10, 150.88, 148.94, 138.44, 134.19, 129.79, 124.92, 123.14, 122.83, 119.34, 114.60, 111.26, 69.65, 22.40, 10.79. ESI-HRMS calcd for $C_{21}H_{21}N_5O_6NaS$. $[M + Na]^+$ 510.1059, found: 510.1058.

*4.1.4.9. N'-(2-methylbenzoyl)-3-(2,4-dihydroxypyrimidine-5-sulfonamido)benzoyl hydrazide (**9i**).* A white solid, yield: 69.50%. Mp: 140–142 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.84 (br. s., 1 H), 11.65 (s, 1 H), 10.47 (s, 1 H), 10.42 (s, 2 H), 8.14 (s, 1 H), 7.96 (s, 1 H), 7.84 (d, J = 8.07 Hz, 2 H), 7.65–7.68 (m, 1 H), 7.61 (d, J = 7.70 Hz, 1 H), 7.42 (t, J = 7.89 Hz, 1 H), 7.33 (d, J = 7.89 Hz, 2 H), 2.38 (s, 3 H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 166.27, 159.47, 152.54, 151.48, 145.36, 141.79, 129.63, 129.59, 129.06, 128.27, 127.36, 120.07, 119.07, 118.16, 117.79, 113.60, 113.13, 110.14, 21.62. ESI-HRMS calcd for $C_{19}H_{17}N_5O_6NaS$. $[M + Na]^+$ 466.0797, found: 466.0782.

*4.1.4.10. N'-(4-methylbenzoyl)-3-(2,4-dihydroxypyrimidine-5-sulfonamido)benzoyl hydrazide (**9j**).* A white solid, yield: 79.33%. Mp: 139–143 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.79 (br. s., 1 H), 11.61 (s, 1 H), 10.44 (s, 1 H), 10.37–10.41 (m, 2 H), 8.11 (s, 1 H), 7.82 (d, J = 8.09 Hz, 2 H), 7.63 (s, 1 H), 7.59 (d, J = 7.72 Hz, 1 H), 7.40 (d, J = 15.81 Hz, 1 H), 7.27–7.35 (m, 2 H),2.38 (s, 3 H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 166.15, 166.04, 162.78, 159.07, 150.88, 148.94, 142.36, 138.48, 134.15, 130.18, 129.51, 127.94, 123.10, 122.74, 119.26, 111.24, 21.48. ESI-HRMS calcd for $C_{19}H_{17}N_5O_6NaS$. $[M + Na]^+$ 466.0797, found: 466.0812.

*4.1.4.11. N'-(2-chlorobenzoyl)-3-(2,4-dihydroxypyrimidine-5-sulfonamido)benzoyl hydrazide (**9k**).* An off yellow solid, yield: 74.10%. Mp: 175–180 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.57 (br. s., 1 H), 10.33 (br. s., 1 H), 8.10 (s, 1 H), 7.65 (s, 1 H), 7.54–7.60 (m, 3 H), 7.49–7.54 (m, 1 H), 7.44–7.49 (m, 1 H), 7.38 (t, J = 7.91 Hz, 1 H), 7.30 (d, J = 8.09 Hz, 1 H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 166.17, 165.70, 159.07, 150.82, 148.85, 138.44, 135.07, 133.89, 132.02, 130.92, 130.40, 129.88, 129.71, 127.66, 123.29, 122.89, 119.47, 111.23. ESI-HRMS calcd for $C_{18}H_{14}ClN_5O_6NaS$. $[M + Na]^+$ 486.0251, found: 486.0288.

*4.1.4.12. N'-(3-chlorobenzoyl)-3-(2,4-dihydroxypyrimidine-5-sulfonamido)benzoyl hydrazide (**9l**).* An off yellow solid, yield: 75.40%. Mp: 184–186 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.60 (s, 1 H), 10.17–10.45 (m, 2 H), 8.11 (s, 1 H), 7.58–7.62 (m, 2 H), 7.40 (t, J = 8.00 Hz, 1 H), 7.29–7.33 (m, 1 H), 7.17 (t, J = 8.28 Hz, 1 H), 6.72–6.76 (m, 2 H), 6.71 (d, J = 8.85 Hz, 1 H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.96, 164.89, 159.10, 150.78, 148.84, 138.45, 134.90, 133.98, 133.83, 132.24, 131.09, 129.73, 127.77, 126.68, 123.25, 122.88, 119.46, 111.20. ESI-HRMS calcd for $C_{18}H_{14}ClN_5O_6NaS$. $[M + Na]^+$ 486.0251, found: 486.0234.

*4.1.4.13. N'-(2-fluorobenzoyl)-3-(2,4-dihydroxypyrimidine-5-sulfonamido)benzoyl hydrazide (**9m**).* A yellow solid, yield: 75.65%. Mp: 162–165 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.85 (br. s., 7 H), 11.62 (s, 1 H), 10.57 (s, 1 H), 10.38 (s, 1 H), 10.32 (s, 1 H), 8.11 (s, 1 H), 7.67 (td, J = 7.48, 1.60 Hz, 1 H), 7.65 (s, 1 H), 7.60 (d, J = 7.53 Hz, 2 H), 7.41 (t, J = 7.91 Hz, 1 H), 7.30–7.37 (m, 3 H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 167.68, 165.70, 163.94, 159.07, 150.86, 148.91, 139.77, 138.46, 133.93, 133.57, 130.62, 125.08, 119.40, 119.35, 116.86, 116.72, 111.24, 111.20. ESI-HRMS calcd for $C_{18}H_{14}FN_5O_6NaS$. $[M + Na]^+$ 470.0547, found: 470.0543.

*4.1.4.14. N'-(4-fluorobenzoyl)-3-(2,4-dihydroxypyrimidine-5-sulfonamido)benzoyl hydrazide (**9n**).* A yellow solid, yield: 75.80%. Mp: 172–178 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.56 (br. s., 1 H), 10.50 (br. s., 2 H), 8.11 (s, 1 H), 7.99 (dd, J = 8.75, 5.55 Hz, 2 H), 7.64 (s, 1 H), 7.59 (d, J = 7.91 Hz, 1 H), 7.41 (t, J = 7.91 Hz, 1 H), 7.37 (t, J = 8.85 Hz, 2 H), 7.32 (d, J = 8.09 Hz, 1 H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 166.11, 165.30, 163.88, 159.11, 150.99, 149.07, 138.49, 134.05, 130.67, 130.61, 123.18, 122.78, 119.28, 116.11, 115.96, 111.19. ESI-HRMS calcd for $C_{18}H_{14}FN_5O_6NaS$. $[M + Na]^+$ 470.0547, found: 470.0532.

*4.1.4.15. N'-(4-propylbenzoyl)-2-(2,4-dihydroxypyrimidine-5-sulfonamido)benzoyl hydrazide (**10h**).* A white solid, yield: 75.15%. Mp: 184–186 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.69 (s, 1 H), 8.42 (s, 1 H), 8.21 (d, J = 7.91 Hz, 1 H), 7.98 (d, J = 8.66 Hz, 2 H), 7.88–7.95 (m, 1 H), 7.78 (d, J = 8.09 Hz, 1 H), 7.63 (t, J = 7.43 Hz, 1 H), 7.12 (d, J = 8.66 Hz, 2 H), 4.05 (t, J = 6.59 Hz, 2 H), 1.70–1.83 (m, 2 H), 1.00 (t, J = 7.34 Hz, 3 H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 166.26, 162.70, 159.18, 149.73, 147.80, 135.50, 130.36, 128.08, 126.89, 123.44, 122.45, 114.88, 69.79, 22.40, 10.80. ESI-HRMS calcd for $C_{21}H_{21}N_5O_6NaS$. $[M + Na]^+$ 510.1059, found: 510.1079.

*4.1.4.16. N'-(2-methylbenzoyl)-2-(2,4-dihydroxypyrimidine-5-sulfonamido)benzoyl hydrazide (**10i**).* An off yellow solid, yield:

67.35%. Mp: 153–156 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.87 (s, 1 H), 8.45 (s, 1 H), 8.22 (d, J = 7.89 Hz, 1 H), 7.93 (t, J = 8.34 Hz, 1 H), 7.84 (s, 1 H), 7.78–7.83 (m, 2 H), 7.64 (t, J = 7.52 Hz, 1 H), 7.48–7.51 (m, 2 H),2.42 (s, 3 H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.75, 157.92, 148.54, 146.63, 137.53, 134.48, 132.75, 130.49, 128.04, 127.83, 127.74, 127.53, 127.07, 126.97, 125.83, 124.38, 124.04, 121.36, 20.31. ESI-HRMS calcd for $C_{19}H_{17}N_5O_6NaS$. $[M + Na]^+$ 466.0797, found: 466.0819.

*4.1.4.17. N'-(4-methylbenzoyl)-2-(2,4-dihydroxypyrimidine-5-sulfonamido)benzoyl hydrazide (**10j**).* An off white solid, yield: 69.40%. Mp: 161–163 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.78 (s, 1 H), 8.43 (s, 1 H), 8.21 (d, J = 9.03 Hz, 1 H), 7.91 (d, J = 8.09 Hz, 2 H), 7.85 (d, J = 8.09 Hz, 1 H), 7.78 (d, J = 8.09 Hz, 1 H), 7.62–7.66 (m, 1 H), 7.41 (d, J = 7.91 Hz, 2 H), 2.42 (s, 3 H);^{13}C NMR (150 MHz, DMSO-d_6) δ 166.66, 159.11, 149.65, 147.78, 143.51, 135.55, 129.76, 128.76, 128.35, 128.13, 128.09, 126.91, 122.44, 21.61. ESI-HRMS calcd for $C_{19}H_{17}N_5O_6NaS$. $[M + Na]^+$ 466.0797, found: 466.0861.

*4.1.4.18. N'-(2-chlorobenzoyl)-2-(2,4-dihydroxypyrimidine-5-sulfonamido)benzoyl hydrazide (**10k**).* A white solid, yield: 64.42%. Mp: 165–171 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.93 (s, 1 H), 8.45 (s, 1 H), 8.24 (d, J = 7.91 Hz, 1 H), 7.93 (t, J = 8.38 Hz, 1 H), 7.77–7.81 (m, 2 H), 7.59–7.67 (m, 3 H), 7.53–7.56 (m, 1 H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 170.20, 168.42, 158.85, 150.84, 149.37, 144.84, 138.56, 133.47, 129.61, 129.05, 128.51, 123.23, 120.45, 119.10, 118.26, 117.89, 113.84, 110.72. ESI-HRMS calcd for $C_{18}H_{14}ClN_5O_6NaS$. $[M + Na]^+$ 486.0251, found: 486.0229.

*4.1.4.19. N'-(3-chlorobenzoyl)-2-(2,4-dihydroxypyrimidine-5-sulfonamido)benzoyl hydrazide (**10l**).* A white solid, yield: 63.40%. Mp: 149–154 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.06 (s, 1 H), 8.46 (s, 1 H), 8.22 (d, J = 7.91 Hz, 1 H), 8.05 (s, 1 H), 7.92–7.98 (m, 2 H), 7.75–7.82 (m, 2 H), 7.65 (q, J = 7.22 Hz, 2 H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 168.95, 166.13, 158.97, 150.89, 149.71, 147.81, 138.60, 134.82, 133.56, 130.79, 128.14, 122.75, 121.59, 119.05, 118.04, 150.85, 114.79, 110.60. ESI-HRMS calcd for $C_{18}H_{14}ClN_5O_6NaS$. $[M + Na]^+$ 486.0251, found: 486.1297.

*4.1.4.20. N'-(2-fluorobenzoyl)-2-(2,4-dihydroxypyrimidine-5-sulfonamido)benzoyl hydrazide (**10m**).* An off yellow solid, yield: 65.35%. Mp: 172–175 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.94 (d, J = 5.14 Hz, 1 H), 8.73 (br. s., 1 H), 8.47 (s, 1 H), 8.18 (d, J = 7.34 Hz, 1 H), 8.08 (t, J = 6.97 Hz, 1 H), 7.80 (d, J = 8.25 Hz, 1 H), 7.61 (t, J = 7.43 Hz, 1 H), 7.14 (d, J = 7.15 Hz, 1 H), 6.98 (t, J = 7.52 Hz, 1 H), 6.80 (t, J = 7.34 Hz, 1 H), 6.25 (d, J = 7.89 Hz, 1 H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 170.18, 168.54, 158.83, 150.83, 149.35, 138.60, 133.45, 129.01, 125.25, 123.17, 119.69, 119.65, 119.02, 118.15, 115.42, 115.30, 114.51, 110.75. ESI-HRMS calcd for $C_{18}H_{14}FN_5O_6NaS$. $[M + Na]^+$ 474.0547, found: 470.0530.

*4.1.4.21. N'-(4-fluorobenzoyl)-2-(2,4-dihydroxypyrimidine-5-sulfonamido) benzoyl hydrazide (**10n**).* A brown solid, yield: 62.40%. Mp: 155–159 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.36 (t, J = 8.85 Hz, 2 H) 7.57–7.60 (m, 1 H) 7.75 (d, J = 7.91 Hz, 1 H) 7.85–7.89 (m, 1 H) 8.09 (dd, J = 8.66, 5.65 Hz, 2 H) 8.19 (d, J = 9.22 Hz, 1 H) 8.33 (s, 1 H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 173.66,166.17, 165.65, 164.00, 159.05, 149.74, 147.79, 135.09, 131.14, 131.08, 127.92, 127.74, 126.81, 122.63, 116.01, 115.87. ESI-HRMS calcd for $C_{18}H_{14}FN_5O_6NaS$. $[M + Na]^+$ 470.0547, found: 470.0596.

4.2. HPLC analysis of stability of compound in cell culture medium

The HPLC system consisted of e2695 Seprations Module pump coupled to a 2489 UV/Visible detector (Waters Corp., Milford, MA, USA). Chromatograms was achieved on a Waters BDS C_{18} column (200 mm × 4.6 mm, 5 μm). Detection was performed at a wavelength of 260 nm at 37 °C. The mobile phase consisted of 0.1% phosphoric acid water/methanol (50/50, v/v) at a flow rate of 1.0 mL/min. A10 μL of sample was injected.

4.3. Cell culture and grouping

The human lung adenocarcinoma cell line A549, ovarian cancer cell line OVCAR-3, and gastric carcinoma cell line SGC7901 were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA) and then diluted (1: 10) with Roswell Park Memorial Institute (RPMI) 1640 culture medium (Beijing Thermo Fisher Scientific Company, Beijing, China). These cells were suspended by RPMI 1640 nutrient solution containing 10% heat-inactivated fetal calf serum, 100 μg/mL penicillin and 100 μg/mL streptomycin, these cells were cultured at 37 °C in an incubator with saturated humidity and 5% CO_2 (Memmert Company, Germany). Also, the human breast cancer cell line MBA-MD-231 was purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA), diluted (1:10) with Roswell Park Memorial Institute Leibovitz's L15 culture medium (Beijing Thermo Fisher Scientific Company, Beijing, China), these cells were suspended by L15 nutrient solution containing 10% heat-inactivated fetal calf serum, 100 U/ml penicillin, and 100 μg/mL streptomycin; these cells were cultured at 37 °C in an incubator with saturated humidity and 0% CO_2 (Memmert Company, Germany). These cells were passaged every 2–3 days and cells in the logarithmic growth phase collected for experiments.

4.4. MTT assay

Total cells in the logarithmic growth phase were collected, digested using 0.25% pancreatic enzymes, and then diluted into single-cell suspensions using phosphate-buffered solution (PBS). These cells were seeded into 96-well plates at 5×10^3 cells per well (100 μL per well) and then A549, OVCAR-3 and SGC7901 were cultured at 3 °C in an incubator containing 5% CO_2; MDA-MB-231 cells were cultured at 37 °C in an incubator containing 0% CO_2. After cell complete attachment, 100 μL of different concentrations of compounds (0.512 μM, 1.28 μM, 3.2 μM, 8 μM, 20 μM) were added to the cells after the nutrient solution in each well was discarded, while no compound except 0.1% DMSO was added to cells in the control group; at the same time, each concentration was set up to 5 wells. In addition, 5 wells in each 96-well plate only received 100 μL of nutrient solution as a blank control. The plates were taken out after the cells were incubated with compounds for 24 h, and 20 μL of MTT solution (5 mg/mL) (Wuhan Boster Biotechnology Limited Company, Wuhan, China; item No. AR1156) was added into all the wells. After another time of incubation for 4 h at 37 °C, the nutrient solution in each well was discarded slowly, and 150 μL of DMSO was added to each well. The plates were shaken on a shaking table for 20 min. When the crystals were fully dissolved, the blank wells were used to zero the plate reader. The optical density (OD) of each well was detected at 490 nm. The IC_{50} value was determined as the concentration of the inhibitor that caused 50% inhibition. Using probability unit and weighted regression method to calculate the IC_{50} value of compounds.

4.5. Annexin V/propidium iodide (PI) staining

The density of the A549 cells in the logarithmic growth phase was adjusted to 1×10^6 cells/mL, and then the cell suspension was seeded into a 6-well plate (Becton Dickinson and Company, USA). A total of 1 mL of cell suspension and 1 mL of compound **10 l** at

different concentrations were added into each well with the final compound **10l** concentration in each group as 0 μM, 0.5 μM, 1 μM, 1.5 μM and 3 μM.

After cells were incubated for 24 h at 37 °C, 1 mL of cell suspension per well was collected and centrifuged at 1000 r/min for 10 min at 4 °C, and the supernatant was removed. Then, 1 mL of cold PBS was added to the cell pellet, and was shaken lightly to re-suspend the cells. The cells were centrifuged at 1000 r/min at 4 °C for 10 min, and the supernatant was removed. These steps were repeated twice. The cells were re-suspended in 150 μL of PBS, 10 μL of fluorescein isothiocyanate (FITC)-labeled Annexin V (Annexin V-FITC) (Promega Corporation, USA) and 5 μL of PI (Promega Corporation, USA) were added. The mixture was gently mixed and incubated in the dark at room temperature for 10 min then stained cells were analyzed using flow cytometry (FCM) to test the levels of apoptotic cells (Becton Dickinson and Company, USA; FACS Calibur).

4.6. Western blotting

A549 cells were treated with different concentrations of compound **10l** for 24 h, and collected to establish their respective treatment groups. The cell density is 1×10^7 cells per flask (Becton Dickinson and Company, USA), and the group without compound 10 l is used as the control group. A protein extraction reagent (Beyotime Biotechnology Company, Shanghai, China) was used to extract the proteins, and a BCA kit (Beyotime Biotechnology Company, Shanghai, China) was applied to measure the protein levels.

Extracted proteins were separated from each other using polyacrylamide electrophoresis on a 10% gel (Beyotime Biotechnology Company, Shanghai, China) and transferred to a polyvinylidene fluoride (PVDF) membrane. When the membrane transfer finished, the membranes were placed on a horizontal shaking table and blocked for 1 h with PBS containing either 5% bovine serum albumin (BSA) or dissolved skim milk powder. The sealed PVDF membrane was removed and incubated at 4 °C overnight with the following primary antibodies (100 μl/cm^2): including caspase-3 (19677-1-AP, Proteintech Company, USA), bax (50599-2-lg, Proteintech Company, USA), bcl-2 (12789-1-AP, Proteintech Company, USA), cleaved caspase-3 (25546-1-AP, Proteintech Company, USA) and β-actin (60008-1-lg, Proteintech Company, USA). The secondary antibody was diluted in 5% BSA (Beyotime Biotechnology Company, Shanghai, China) at a density of 100 μl/cm^2 and was added to the membranes, which were then sealed and incubated on a shaking table at room temperature for 1hr. Equal volumes of detection reagents A and B were mixed in a petri dish with no strong light throughout the procedure. The PVDF membrane (Bio-Rad Company, USA) immersed in PBS was placed in ECL working solution for color development, and was scanned using the Bio-Rad Gel Doc 2000 gel imaging system. Image analysis and processing were conducted by Image Lab. Gray values of protein bands were quantitatively analyzed, with the gray value of β-actin band as the reference.

4.7. Statistical analysis

Data were analyzed using GraphPad Prism 5 (GraphPad Software; La Jolla, CA). Normal distributed measurement for two groups was conducted using the *t*-test, and comparisons among multiple groups were determined using one-way analysis of variance (ANOVA). Numeric data were presented as either ratios or percentages, and the chi-square test was applied to compare the two groups. $P < 0.05$ was considered to be statistically significant.

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