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Preliminary communication

# One-pot synthesis and antiproliferative activity of novel 2,4-diaminopyrimidine derivatives bearing piperidine and piperazine moieties

Wei-Feng Ma [b, 1], Hai-Kui Yang [a, 1], Meng-Jin Hu [a], Qian Li [a], Tian-Zhu Ma [a], Zhong-Zhen Zhou [a], Rui-Yuan Liu [a], Wen-Wei You [a, *], Pei-Liang Zhao [a, *]

[a] Department of Chemistry, School of Pharmaceutical Science, Southern Medical University, Guangzhou 510515, PR China
[b] Department of Microbiology, School of Public Health and Tropical Medicine, Southern Medical University, Guangzhou 510515, PR China

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ABSTRACT

A series of novel 2,4-diaminopyrimidines containing piperidine and piperazine moieties were synthesized *via* an efficient one-pot methodology. The bioassay tests demonstrated that compounds **27** and **28** displayed much stronger antitumor activities against four human cancer cell lines (HepG2, A549, MDA-MB-231 and MCF-7) than positive control fluorouracil. Particularly, compound **28** showed a two-fold improvement compared to fluorouracil in inhibiting MDA-MB-231 and A549 cell proliferation with $IC_{50}$ values of 7.46 and 12.78 μM, respectively. Further flow-activated cell sorting analysis revealed that the most promising compound **28** displayed a significant effect on $G_2$/M cell-cycle arrest in a dose-dependent manner in MDA-MB-231 cells.



## 1. Introduction

Pyrimidines are an important class of heterocyclic structures found in many synthetic and natural occurring products with a remarkable spectrum of biological activities [1–6]. Among the existing large numbers of structurally diverse pyrimidine derivatives, 2,4-diaminopyrimidines have attracted considerable attention due to their important chemopreventive and chemotherapeutic effects on cancer [7–15]. For example, as shown in Fig. 1, NU6027 (**1**), originally identified as a cyclin-dependent kinase 2 inhibitor [16,17], more recently has emerged as a potential antitumor agent inhibiting ataxia telangiectasia mutated and Rad3-related kinase (ATR) [18]. On the other hand, literature survey has revealed that the structural modification of 2,4-diaminopyrimidines by introduction of nitrogen-containing piperidine and piperazine heterocycles, can further enhance the antitumor activity of candidate compounds. For example, the piperidine-pyrimidine analog R547 (**2**), shown in Fig. 1, was identified as a selective adenosine triphosphate-competitive cyclin-dependent kinase inhibitor and is currently being tested in phase I clinical trials [19,20]. While the piperazine-pyrimidine compound XL228 (**3**), a multitargeted protein kinase inhibitor, has been proved effective in patients with solid tumors or hematologic malignancies in phase I clinical trials [11,21,22]. More interestingly, Font et al. documented that the length of amino side chain in pyrimidine 2- and 4-position of compound **4** had an important effect on its antiproliferative activity [23], which indicated that the flexibility of the amino group might be a vital factor for the activity of compound **4**.

Based on the above considerations, and in a continuation of our interest in the synthesis of nitrogen-containing heterocycles with anticancer activity [24–26], we developed an idea that introducing 4-amino piperidine and N-methylpiperazine moiety into the C2 or C4 -position of the pyrimidine scaffold with phenylalkylamine as a flexible linkage might result in new 2,4-diaminopyrimidine derivatives with high cytotoxic activity (Fig. 2). In this study, we described the one-pot synthesis and screening results, and structure-activity relationships of these compounds **8**–**31**.

* Corresponding authors.
*E-mail addresses:* youww@smu.edu.cn (W.-W. You), plzhao@smu.edu.cn (P.-L. Zhao).
[1] These authors contributed equally to this work.

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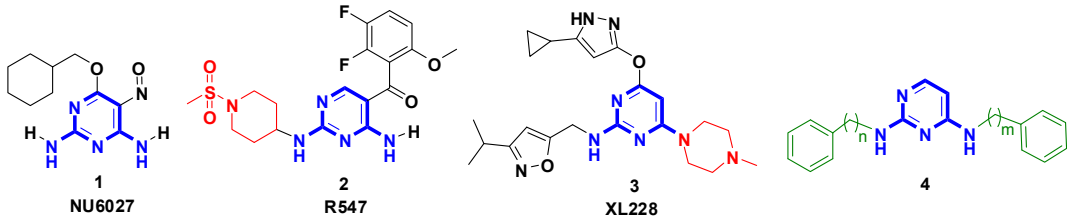



**Fig. 1.** Reported 2,4-diaminopyrimidines with antitumor activity.

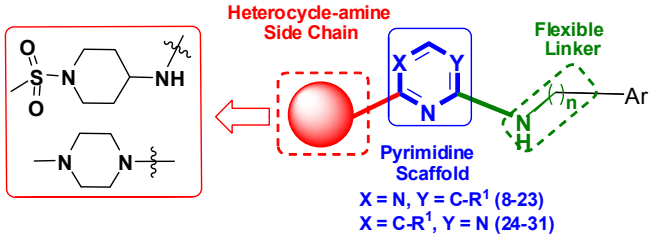



**Fig. 2.** Design strategy of the title compounds **8**–**31**.

## 2. Chemistry

Owing to their important biological roles in chemopreventive action and the broad range of pharmacological properties, many methods have been developed for synthesis of 2,4-diaminopyrimidine derivatives [14,27–30]. All of the methodology involved two steps from the substituted 2,4-dichloropyrimidine as starting material. The halopyrimidines were firstly converted to 4-aminopyrimidine by a nucleophilic substitution reaction at C-4, and following purification, the C-2 chlorine was displaced by various amines in the second step. Although, many efforts were made to synthesize new 2,4-diaminopyrimidine derivatives, no publications described the one-pot reaction between the substituted 2,4-dichloropyrimidine and amine. Herein, Scheme 1 shows the newly developed an efficient one-pot methodology for synthesis of 2,4-diaminopyrimidine derivatives bearing piperidine and piperazine moieties **8**–**31**. The reliable model procedure involved the treatment of 5-substituted 2,4-diaminopyrimidine (**5**) with 1.0 equiv of amine (**6**) and 2.0 equiv of *N,N*-diisopropylethylamine (DIPEA) as base in absolute 2-methoxyethanol at room temperature for about 12 h under $N_2$ flow. The resulting mixture reacted with 1.1 equiv of various another amine (**7**) at 90 °C for 12–18 h to successfully afford the desirable target compounds **8**–**31** in moderate to good isolated yields (58–84%).

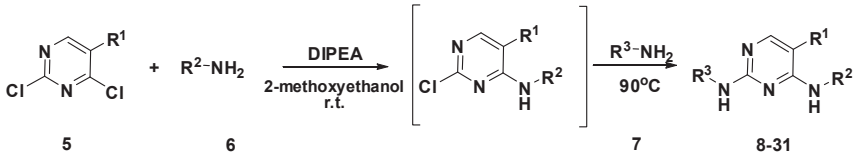
The structures of the prepared 2,4-diaminopyrimidine derivatives **8**–**31** were characterized by $^1$H NMR, $^{13}$C NMR, ESI-MS and elemental analysis and the results are shown in the Experimental section. In addition, the representative compound **25** was further confirmed by single-crystal X-ray diffraction. As shown in Fig. 3, the crystal structure showed that **25** had a C-shape, while the six-membered piperidine ring adopts a chair conformation.

## 3. Pharmacology results and discussion

The *in vitro* antitumor activities of the synthesized compounds **8**–**31** against four human cancer cell lines, including HepG2 (human hepatoma cells), A549 (human alveolar epithelial cells), MDA-MB-231 (human breast cancer cells) and MCF-7 (human mammary adenocarcinoma cells), were assayed by MTT method [31]. Fluorouracil which is one of the most effective anticancer agents was used as the reference drug and the results expressed as $IC_{50}$ (μM) were summarized in Table 1. Here, the $IC_{50}$ value represents the concentration of one compound resulting in a 50% inhibition in cell growth after a 48 h incubation, and is the average of three independent experiments.

For the convenience of structure-activity relationship analysis, compounds **8**–**20**, **21**–**23**, **24**–**28** and **29**–**31** were defined as C2-piperidine, C2-piperazine, C4-piperidine and C4-piperazine pyrimidine derivatives, respectively. As indicated in Table 1 and Fig. 4, unfortunately, the results indicated that all C2- and C4-piperazine pyrimidine derivatives (**21**–**23**, **29**–**31**) were ineffective ($IC_{50}$ >200 μM). Remarkably, most of the C2- and C4-piperidine pyrimidines (**8**–**20**, **24**–**28**) displayed substantial antiproliferative activities. For example, the $IC_{50}$ values of compounds **27** and **28** ranged from 7.46 to 32.76 μM against all of the tested cell lines. The $IC_{50}$ values of compound **16** were 11.73, 32.41 and 46.80 μM against MDA-MB-231, MCF-7 and A549 cells, respectively. In most cases, C4-piperidine pyrimidine derivatives displayed much higher antitumor activities against HepG2, A549 and MCF-7 than C2-piperidine pyrimidine derivatives, for example, compounds **9** and **25** ($n = 0$, $R^1 = H$, $R^2 = 3\text{-MeOC}_6H_4$), **10** and **28** ($n = 1$, $R^1 = H$, $R^2 = 4\text{-MeOC}_6H_4CH_2$).

Further analysis on the structure–activity relationship investigated the effects of several substituents on the pyrimidine ring. Among the series of C2-piperidine pyrimidine derivatives, as showed in Table 1, the antitumor activities were influenced by the C5-substituents on the pyrimidine ring, and overall, hydrogen substitution (**8**–**11**) resulted in higher activities against all of the tested cell lines than those of the corresponding compounds with chloro substitution (**12**–**20**). However, compound **16** is an exception, which exhibited broad-spectrum antiproliferative activities against most of the tested cell lines. Nevertheless, within the series of C4-piperidine pyrimidine derivatives, most compounds displayed potent antitumor activities. Particularly, two potent compounds **27** and **28** exhibited significantly higher activity than fluorouracil against all of the tested cell lines.




**Scheme 1.** One-pot synthesis of compounds **8**–**31**.