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Synthesis and evaluation of new 2-chloro-4-aminopyrimidine and 2,6-dimethyl-4-aminopyrimidine derivatives as tubulin polymerization inhibitors

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ABSTRACT

Eighteen new 2-chloro-4-aminopyrimidine and 2,6-dimethyl-4-aminopyrimidine derivatives were synthesized and evaluated as tubulin polymerization inhibitor for the treatment of cancer. Among them, compounds **10**, **17**, **20** and **21** exhibited potent antiproliferative activities against five human cancer cell lines. Microtubule dynamics assay showed that compound **17** could effectively inhibit tubulin polymerization. Molecular docking studies were also carried out to understand the binding pattern. Further mechanism studies revealed that **17** could induce G2/M phase arrest, disrupt the organization of the cellular microtubule network and induce cell apoptosis and mitochondrial dysfunction.

Tubulin-microtubule systems play a critical role in cell growth, division, and cytoskeletal organization as well as being implicated in motility, shape, and intracellular transport.[1] These features make microtubules an attractive target for cancer therapy.[2,3] A number of natural compounds, such as paclitaxel, epothilone, vinblastine, combretastatin A-4 (CA-4), dolastatin and colchicine, which interfere the dynamics of tubulin polymerization and depolymerization, have been marketed or is undergoing clinical research.[4] Among them, taxanes and vinca alkaloids have been proved to be effective in the treatment of diverse human cancers.[5] In the last decades, the design and synthesis of structurally diverse tubulin polymerization and depolymerization inhibitors were also flourishing. For instance, MPC-6827 (A)[6–9] and its analogues (B),[10] 4-arylcoumarin analogue (C),[11,12] and Se-aspirin analogue (D)[13] exhibited potent anticancer activities against a broad-spectrum of human cancer cell lines (Fig. 1).

In our study to search for new structural compounds as anticancer agents, we have developed several series of compounds, such as indole-chalcone derivatives, selenium-containing isocombretastatins and phenstatins derivatives, which had been proved to exert their effective antitumor activity through microtubule destabilization in vitro and *in vivo*.[14,15] Inspired by MPC-6827 (which has reached phase II trials for the treatment of recurrent glioblastoma), we expected to search for new more effective anti-tumor lead molecules. Starting from the diversity of molecular structure screening, we decided to make an attempt to synthesize and evaluate the 2-chloro-4-aminopyrimidine and 2,6-dimethyl-4-aminopyrimidine derivatives that have the similar pharmacophores to MPC-6827 but smaller molecular weight.

The synthetic method of new 2,4-dichloropyrimidine derivatives (**3a-3c**) is shown in scheme 1. The reaction of 2,4-dichloropyrimidine with 4-methoxy-*N*-methylaniline or its fluorine, chlorine derivatives (**2a-2c**) in 2-propanol at room temperature provides the target compounds **3a-3c** in a good yields. 4-Fluoro-3-nitroaniline (**4**) was *N*-methylated by reacting with paraformaldehyde in MeONa-MeOH solution and then sodium borohydride to afford 4-methoxy-*N*-methyl-3-nitroaniline (**5**), which reacted with 2,4-dichloropyrimidine to give compound **6**. Compound **10** is synthesized via a four-step procedure. First, the hydroxyl of 2-methoxy-5-nitrophenol (**7**) was protected by MOM, and then hydrogenated in the presence of palladium/C to give intermediate **8**, followed by a methylation of amino group to give compound **9**, finally, **9** reacted with 2,4-dichloropyrimidine to give the target compound **10**.

Scheme 2 listed the synthetic route of compounds containing 4-methoxy-3-bromoalkanoxy or 4-methoxy-3-cyanoselenoalkanoxy group at anilino moiety. The reaction of compound **10** with dibro-

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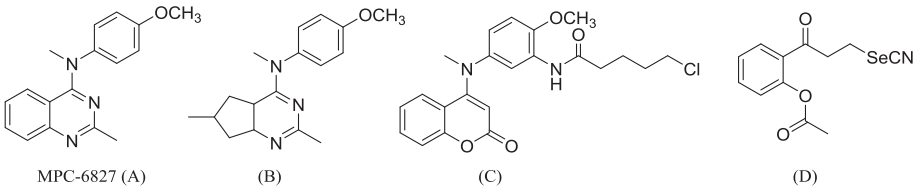
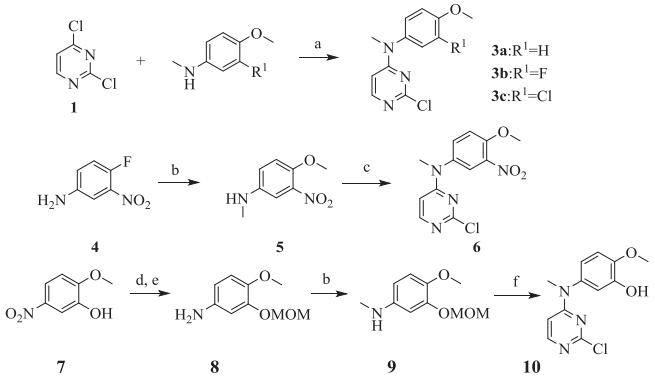


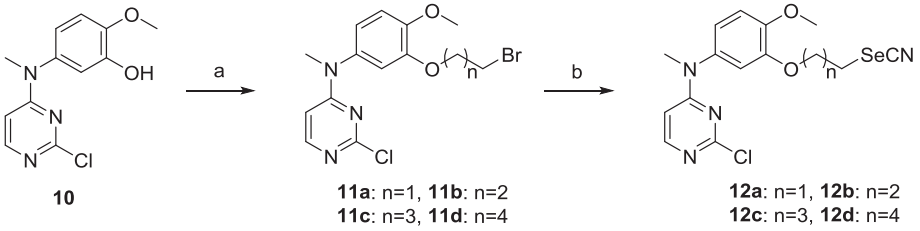
Fig. 1. Some reported tubulin polymerization and depolymerization inhibitors.



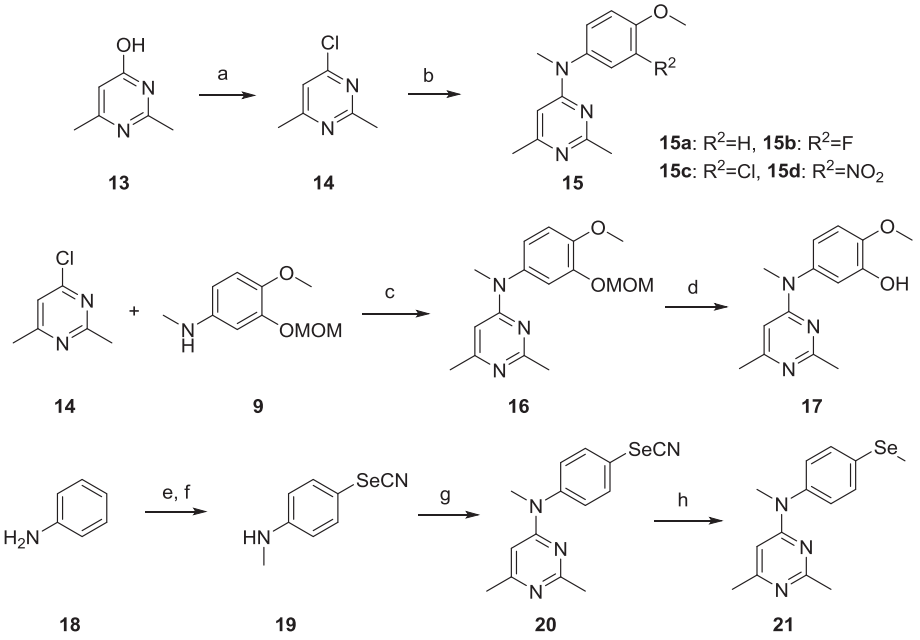
Scheme 1. Reagents and conditions: a) isopropanol, r.t. b) (i) $(CH_2O)_n$, CH_3ONa, MeOH, r.t. (ii) $NaBH_4$, reflux. c) 2,4-dichloropyrimidine, isopropanol, r.t. d) MOMCl, DIPEA, DCM, 0 °C. e) Pd/C, H_2, MeOH, r.t. f) 2,4-dichloropyrimidine, HCl, isopropanol, r.t.

moalkanes in the presence of potassium carbonate gave **11a-11d**, which reacted with potassium cyanide to give compound **12a-12d**.

The synthetic routes to 2,4-dimethylpyrimidine derivatives (**15**, **17**, **20** and **21**) are shown in Scheme 3. The reaction of 2,6-dimethylpyrimidin-4-ol (**13**) with phosphoryl trichloride gave 4-chloro-2,6-dimethylpyrimidine (**14**), which reacted with the corresponding anilines to provide the target compounds **15a**-**15d**. The reaction of compound **14** with **9** afforded compound **16**, followed by a deprotection in the presence of hydrogen chloride in ethyl acetate to afford the target compound **17**. Selenocyanato-containing compound **20** was prepared through the reaction of **14** with 4-selenocyanatoaniline (**19**), which was obtained by nitrogen methylation of aniline followed by the reaction with malononitrile in the presence of selenium dioxide. The methylseleno derivative (**21**) was obtained by the reaction of **20** with sodium borohydride and methyl iodide.



Scheme 2. Reagents and conditions: a) K_2CO_3, $Br(CH_2)_nBr$, CH_3CN, reflux. b) KSeCN, CH_3CN, reflux.



Scheme 3. Reagents and conditions: a) $POCl_3$, toluene, reflux. b) **2** or **5**, isopropanol, r.t. c) isopropanol, r.t. d) HCl, isopropanol, r.t. e) (i) $(CH_2O)_n$, CH_3ONa, MeOH, r.t. (ii) $NaBH_4$, reflux. f) malononitrile, SeO_2, DMSO, r.t. g) **14**, isopropanol, r.t. h) $NaBH_4$, CH_3I, ethanol, r.t.

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