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Synthesis and biological evaluation of 2,4-diaminopyrimidines as selective Aurora A kinase inhibitors

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ABSTRACT

The Aurora kinases are a family of serine/threonine kinases that interact with components of the mitotic apparatus and serve as potential therapeutic targets in oncology. Here we synthesized 15 2,4-diaminopyrimidines and evaluated their biological activities, including antiproliferation, inhibition against Aurora kinases and cell cycle effects. These compounds generally exhibited more potent cytotoxicity against tumor cell lines compared with the VX-680 control, especially compound **11c**, which showed the highest cytotoxicities, with IC_{50} values of 0.5–4.0 μM. Compound **11c** had more than 35-fold more selectivity for Aurora A over Aurora B, and molecular docking analysis indicated that compound **11c** form better interaction with Aurora A both from the perspective of structure and energy. Furthermore, compound **11c** induced G2/M cell cycle arrest in HeLa cells. This series of compounds has the potential for further development as selective Aurora A inhibitors for anticancer activity.

1. Introduction

The Aurora kinase family is a subfamily of serine/threonine kinases that is essential for the regulation of centrosome maturation, mitotic spindle formation, chromosome segregation and cytokinesis during mitosis [1,2]. The family includes three kinases designated as Aurora A, B, and C, which are very closely related in the kinase domain sequence. However, these kinases have quite different and nonoverlapping functions during mitosis [3]. Aurora A regulates the cell cycle and is associated with late S phase and entry into the M phase. It associates with the spindle poles and is involved in both centrosomal and acentrosomal spindle assembly [4,5]. Aurora B localizes to the centromeres in prometaphase, and then relocates to the spindle midzone at anaphase. It has functions associated with histone phosphorylation and chromatin condensation in prophase, chromosome alignment and segregation, regulation of a mitotic checkpoint at metaphase and a role in cytokinesis [6]. Aurora C has similar functions as Aurora B [7].

The expressions of Aurora A and Aurora B are elevated in a variety of human cancers and are associated with poor prognosis [8]. The potential roles of Aurora kinases in regulating cell mitosis and tumorigenesis make them attractive targets for anticancer therapy [9]. Many Aurora kinase inhibitors have been developed and introduced into clinical trials, including VX-680/MK-0457, ZM447439, Hesperadin, MLN8054, MLN8237, and AZD1152 (Fig. 1) [10–12].

ZM447439 [13], Hesperadin [14] and VX-680/MK-0457 [15] were the first generation of Aurora kinase inhibitors. These three small molecule chemical inhibitors occupy the ATP-binding site in Aurora kinases to inhibit catalytic activity. Unlike pan-Aurora kinase inhibitors, MLN8054 and MLN8237 are ATP-competitive and reversible Aurora A selective inhibitors, and are approximately 40-fold and 200-fold more sensitive towards Aurora A, respectively, compared with Aurora B [12]. MLN8237 is more potent than MLN8054 and causes less benzodiazepine-like side effects based on structure modulation by the addition of a methoxy group to either end of the MLN8054 molecule [16,17]. AZD1152 is an Aurora B selective inhibitor that showed 1000-fold selectivity for Aurora B over Aurora A and a panel of 50 additional kinases in enzymatic assays

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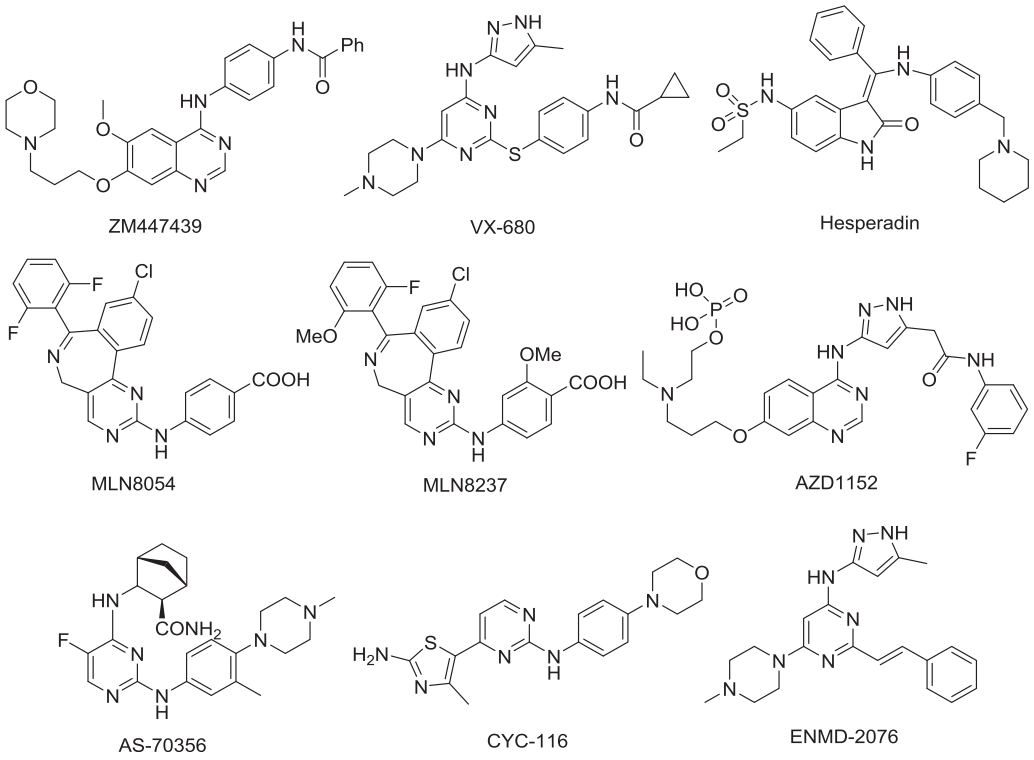

Fig. 1. Aurora kinase inhibitors.

[18–21]. It thus still remains uncertain how exactly aurora A and B pan- or monospecific inhibitors induce tumor cell death and which type of inhibitor will be preferable from a therapeutic viewpoint. Recently, the research of novel selective Aurora inhibitors has become a new trend, and a lot of new active compounds have been developed [22–24].

Pyrimidine is the important pharmacology core in many Aurora inhibitors, such as VX-680, ENMD-2076, CYC-116 and ENMD-2076 [12]. To identify additional effective Aurora inhibitors, we designed a series of 2,4-diaminopyrimidine compounds, our modeling studies suggested that the pyrimidine core as well as the secondary aromatic amine of the compounds form hydrogen bonds with the hinge region of the kinase domain and show selectively inhibition to Aurora A over Aurora B. Introduction of cyclopentyl amine on the C-4 in pyrimidine can adopt a binding mode similar to VX-680 [25]. Furthermore, the differences of F, Cl, Br and NO_2 at 5-C of pyrimidine was to investigate the effects of the electron-withdrawing on anti-proliferation and inhibition of Aurora kinase. Herein, we reported the synthesis, and evaluated their anti-proliferation activities, inhibition of Aurora kinase and effects on the cell cycle.

2. Results and discussion

2.1. Chemistry

The general synthetic routes for intermediate anilines **4a**–**b** and **7a**–**b** are illustrated in Schemes 1 and 2, respectively. Treatment of *p*-aminobenzoic acid **1a** with ditertbutyl dicarbonate ($(BOC)_2O$) afforded 4-Boc-amino-benzoic acid **2a**, and then condensation of compound **2a** with *N*-methyl-4-amino-piperidine generated **3a** under condensing agent tri(dimethylamino)benzotriazol-1-yloxyphos phonium hexafluorophosphate (BOP) in the presence of *N,N*-diisopropylethylamine (DIPEA). Finally, removal of the protecting group provided aniline **4a** in the dichloromethane solution of trifluoroacetic acid [26]. To obtain compound **4b**, 3-methoxy-4-amino-benzoic acid (**1b**), as the raw material, the reaction process was similar to preparing **4a**. Another intermediate aniline **6a**–**b** was prepared by substituted reaction of 4-chloro-1-nitrobenzene with morpholine or 4-methyl-piperazine, and the nitro was reduced by catalytic hydrogen under the catalysis of 10% Pd/C [27].

Our approach to the preparation of 2,4-diaminopyrimidines based on the double SN_2 displacement of pyrimidine is shown in Scheme 3. The displacement of the 4-chloro group of 2,4-dichloro-5-substituted pyrimidine by cyclopentyl amine, cyclopropyl amine, and n-propylamine provided **8a**–**d**, **9** and **10**, which has already been widely reported in the literature [28]. Treatment of **8a**–**d**, **9** and **10** with the different anilines **4a,b** or **7a,b** in isopropanol in the presence of hydrochloride at 80 °C gave the target compounds **11a**–**d**, **12b,c** and **13**–**19** [29]. The target compound **20** was synthesized by the nitro at C-5 of **16** and reduced with hydrogen gas under the catalysis of 10% Pd/C. The newly synthesized compounds were characterized by physicochemical and spectral means, and both analytical and spectral data of all the compounds were in full agreement with the proposed structures.

2.2. Biological activity

2.2.1. Cytotoxicities of compounds *11*–*20*

The *in vitro* cytotoxicities of target compounds **11**–**20** were evaluated in a panel of four human tumor cell lines (cervical carcinoma HeLa, lung carcinoma A-549, human colorectal adenocarcinoma HCT-8 and hepatic carcinoma Hep-G2 cells), with VX-680 as a reference compound. The screening procedure was based on the standard MTT method [30], and the results are summarized in Table 1.

All the target compounds showed better or equivalent