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Short communication

Novel pyrazole-5-carboxamide and pyrazole–pyrimidine derivatives: Synthesis and anticancer activity

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ABSTRACT

A series of novel pyrazole-5-carboxamide and pyrazole–pyrimidine derivatives were designed and synthesized. All compounds have been screened for their antiproliferative activity against MGC-803, SGC-7901 and Bcap-37 cell lines *in vitro*. The results revealed that compounds **8a**, **8c** and **8e** exhibited strong inhibitory activity against MGC-803 cell line. The flow cytometric analysis result showed that compound **8e** could inhibit MGC-803 proliferation. Some title compounds were tested against telomerase, and compound **8e** showed the most potent inhibitory activity with IC_{50} value at 1.02 ± 0.08 μM. The docking simulation of compound **8e** was performed to get the probable binding model, among them, LYS 189, LYS 372, LYS 249 and ASP 254 may be the key residues for the telomerase activity.

1. Introduction

Telomerase is active in the early stages of life. In majority of adult somatic cells, it turns to dormancy. However, in cancer cells, telomerase is reactivated to keep the telomere length short in rapidly dividing cells, leading to proliferation. So, telomerase represents one of the promising targets in drug discovery [1–4]. Telomere and telomerase closely related to the occurrence and development of gastric cancer has been reported [5]. In recent publications, one new hypothesis could be utilized to explain the association between telomerase TERT (human telomerase reverse transcriptase) and cancers. The regulation of telomerase TERT predominantly leads to cell proliferation or apoptosis, ultimately resulting in anticancer activity. Unfortunately, these broad-spectrum telomerase TERT inhibitors have been limited by non-specificity and thus non-selective toxicity and dose-limiting efficacy. Therefore, the pursuit of novel telomerase inhibitors with better antitumor effects and more safety profile is still the main issue.

The pyrazole–pyrimidine and their bioisosteres are heterocyclic compounds with important biological functions including antitumor and other activities [6–8]. As is known to all that pyrazole–pyrimidine derivatives PP242, PP30 and PDE5 (Fig. 1) have possessed good kinase selectivity profile used as cyclin-dependent kinase (CDK), ATP-competitive mTORC1/mTORC2 inhibitors [9–16]. It was of our interest to utilize rational chemical approaches to generate and identify novel compounds as potential telomerase inhibitors for cancer therapy. Results of structure–activity relationships (SAR) about these derivatives show that the alkyl of the position 1 or 3 should help the activity, there is some motivations provided in the design idea [17–20]. Furthermore, based on the protein TERT structure of telomerase using LigandFit module, our group has recently reported a novel docking model and we found that ASP 254 was a key residue for activity. Focusing on residue ASP 254 and the volume of the active site of hTERT, we therefore designed some of synthesizable drug-like scaffolds which incorporate the moiety of pyrazole–pyrimidine.

In our recent works [21,22], several pyrazole derivatives were designed, which had potent anticancer activity as potential telomerase inhibitors. Because moiety pyrazole-5-carboxamide is a precursor of title pyrazole–pyrimidine structure (Fig. 2), in order to summarize the SAR, we have also carried out active screening against them. In this study, a series of novel small molecules elaborated around pyrazole-5-carboxamide and pyrazole–pyrimidine scaffolds were designed. We also will built the relative SAR between pyrazole–pyrimidines to their precursors as antitumor agents, which maybe rationalize the experimental

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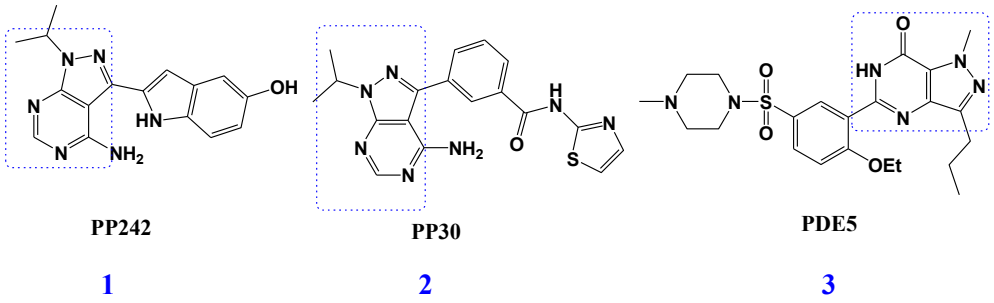

Fig. 1. Pyrazole–pyrimidine scaffold based potential candidates and drugs.

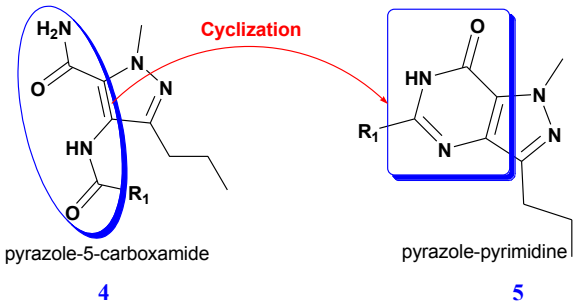

Fig. 2. Design of pyrazole–pyrimidine and its precursor scaffold.

observations and guide us to screen good potency telomerase inhibitors in the further study.

2. Results and discussion

2.1. Chemistry

All derivatives were synthesized by acylation reaction starting from 1-methyl-4-nitro-3-*N*-propylpyrazole-5-carboxamides **7**, which was prepared from 2-pentanone via claisen condensation, hydrazinolytic, cyclization, methylation, hydrolysis, nitration, amidation, reduction according to Scheme 1. Compounds **8a**–**e** were obtained through two steps: First, the substituted carboxylic acids were converted into acyl chlorides with oxalyl chloride at room temperature and excess oxalyl chloride was evaporated, then the acyl chloride was directly reacted with compounds **7** to form amide compounds **8a**–**e**. Compounds **9a**–**e** were obtained from compounds **8a**–**e** via cyclization. The reaction was carried out in the presence of sodium ethoxide, the EtOH used as solvent at reflux condition. All compounds were characterized by means of HR-MS, ^{1}H NMR and ^{13}C NMR spectral analysis.

2.2. Crystal structure analysis

The structures of compounds **8d** and **9a** were determined by X-ray crystallography. Crystal data of **8d**: Colorless crystals, yield, 77%; mp 151–152 °C; $C_{18}H_{24}N_4O_5$, Monoclinic, space group P_12_1/c; a = 14.4205(14), b = 17.7873(16), c = 8.8377(9) (Å); α = 90, β = 100.983(10), γ = 90 (°), V = 2225.4(4) nm^3, T = 293(2) K, Z = 4, Dc = 1.261 g/cm^3, $F(000)$ = 904, Reflections collected/unique = 9519/4373, Data/restraints/parameters = 4373/6/279, Goodness of fit on F^2 = 1.087, Fine, R_1 = 0.0681, $wR(F^2)$ = 0.1722. Crystal data of **9a**: Colorless crystals, yield, 85%; mp 200–201 °C; $C_{17}H_{18}N_4O$, Monoclinic, space group P_12_1/c; a = 16.820(5), b = 5.381(3), c = 18.370(6) (Å); α = 90, β = 112.73(4), γ = 90 (°), V = 1533.4(11) nm^3, T = 293(2) K, Z = 4, Dc = 1.275 g/cm^3, $F(000)$ = 624, Reflections collected/unique = 3274/1312, Data/restraints/parameters = 3274/0/202, Goodness of fit on F^2 = 0.880, Fine, R_1 = 0.0741, $wR(F^2)$ = 0.1818. Their molecular structures were shown in Fig. 3. Crystallographic data (excluding structure factors) for the structures had been deposited with the Cambridge Crystallographic Data Center as supplementary publication No. CCDC-981021 and 1021035.

2.3. In vitro anticancer activity

Recently, we focused on screening lead compounds with specific activity against gastric cancer cells, so gastric cancer cell SGC-7901 and MGC-803 were chosen. In this screening assay studies, all the compounds were evaluated for their cytotoxic activity against SGC-7901, MGC-803 cell lines. In order to examine activity with other cancer cells, Bcap-37 (human breast cancer cell line) was chosen. The results were reported in terms of IC_{50} values (Table 1).

In the initial study of SAR, our title compounds were divided into two series, one was the 5-propyl-1H-pyrazole-3-carboxamide (compounds **8a** ~ **8e**), the other was the pyrazolo[4,3-d]pyrimidin-7(6H)-one (compounds **9a** ~ **9e**). Among them, compound **8** is the precursor of compound **9**. In general, series 5-propyl-1H-pyrazole-3-carboxamide (compounds **8**) leaded to increase in inhibitory activity, among them, compound **8a** was the most potent activity against MGC-803 cell with IC_{50} value of 3.01 ± 0.23 μM, surpassing that of the positive control 5-fluorouracil. Compared with the compounds **8**, compounds **9** generally did not reflect the activity on the tested cells (**9b**, **9d**). Therefore, for this kind of structure moiety, activity of precursor compounds was superior to that cyclization compounds.

The SAR also indicated that all title compounds showed good activity against gastric cancer cells but poor activity against Bcap-37 cells. Scanning from Table 1, it is obvious that compounds **8a**, **8b** and **8c** exhibited the strong inhibitory activity against the MGC-803 cells (with IC_{50s} of 3.44 ± 0.28, 4.57 ± 0.88, 4.24 ± 1.22 μM, respectively) and the values could compare with that of the potent 5-fluorouracil.

2.4. Cell cycle analysis

To understand whether the cell cycle arrest lead to decrease cell proliferation [23], we used flow cytometric analysis to measure the effect of compound **8e** on induction of cell cycle. As shown in Fig. 4, the cells in S phase in the MGC-803 control group accounted for about 30.38%, while after cells treated with compound **8e** MGC-803 for 48 h, the ratio was approximately 42.16%. This showed that the cells were arrested in S phase.