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Original article

Design and synthesis of novel 1,2,3-triazole-pyrimidine hybrids as potential anticancer agents

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ABSTRACT

A series of novel 1,2,3-triazole-pyrimidine hybrids were designed, synthesized and evaluated for their anticancer activity against four selected cancer cell lines (MGC-803, EC-109, MCF-7 and B16-F10). Most of the synthesized compounds exhibited moderate to good activity against all the cancer cell lines selected. Compound **17** showed the most excellent anticancer activity with single-digit micromolar IC_{50} values ranging from 1.42 to 6.52 μM. Further mechanism studies revealed that compound **17** could obviously inhibit the proliferation of EC-109 cancer cells by inducing apoptosis and arresting the cell cycle at G2/M phase.

1. Introduction

As one of the leading causes of death globally, cancer causes a great burden to both single human lives and the society as a whole. Although there have been progresses in the development of prevention and treatment of cancer, the successful treatment of cancer remains a challenge. Therefore, there is still an urgent need to search for some newer and safer anticancer agents that have broader spectrum of cytotoxicity to tumor cells [1,2]. Molecular hybridization which covalently combines two or more drug pharmacophores into a single molecule is an effective tool to design highly active novel entities [3,4]. In addition, the hybrids may also minimize the unwanted side effects and allow for synergic action [5].

The multi-functionalized pyrimidinones scaffold represents a class of heterocyclic compounds with significant pharmacological efficiency, including anti-viral [6,7], anti-HIV [8–10], anti-bacterial [11], especially anticancer [12–18]. For example, Compound (**1**), a benzimidazole–pyrimidine conjugates as potent antitumor agents, exhibited more potent cytotoxic activities than 5-fluorouracil against cervical carcinoma KB cells [19]. In addition, NSC23766 (**2**) is the first-generation small-molecule inhibitor of Rac GTPase targeting Rac activation by GEF with the ability to inhibit cell proliferation, anchorage-independent growth and invasion against human prostate cancer PC-3 cells [20]. Hoff et al. reported that thienopyrimidine (**3**) was identified as a novel and proprietary small molecule scaffold for potential antitumor agents as EGFR inhibitor [21] (Fig. 1).

On the other hand, 1,2,3-triazole has been a fruitful source of inspiration for medicinal chemists for many years. Due to their synthetic accessibility by click chemistry as well as their diverse inhibitory activities, including anti-fungal, anti-bacterial, anti-allergic, anti-inflammatory and others [22–29], we paid a lot of attention to that. Recent research on 1,2,3-triazoles became more appealing and promising for the design of anticancer agents. For example, M.J. Miller group reported that compound (**4**) exhibited an IC_{50} of 46 nM against MCF-7 cancer cell line [30]. Compound (**5**), a 1, 2, 3-triazol-dithiocarbamate-urea hybrid, showed IC_{50} values of 1.62 and 1.86 μM against MGC-803 and MCF-7 cell line, respectively [31]. Carboxyamidotriazole (**6**) [32], a 1,2,3-triazole-containing anticancer agent, is now available in the market (Fig. 2).

The study of new hybrid systems in which 1,2,3-triazole and pyrimidine are combined comprises an unexplored field of research. We have previously reported some 1,2,3-triazole-dithiocarbamate hybrids with good anticancer activity [33]. These findings have encouraged us to investigate the potential synergistic effect of 1,2,3-triazole and pyrimidine scaffolds. Herein, for the first time, we report the hybridization of these two pharmacophores

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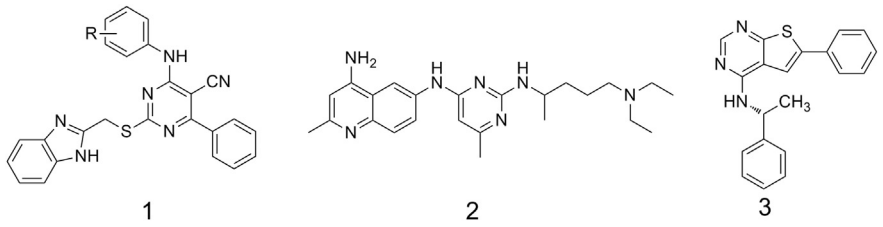

Fig. 1. Pyrimidine derivatives with anticancer activity.

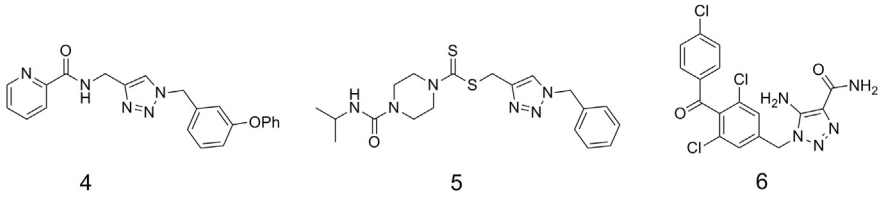

Fig. 2. 1,2,3-triazole derivatives with anticancer activity.

and their anticancer ability against the four selected tumor cell lines.

2. Results and discussion

2.1. Chemistry

The general route for the synthesis of the target 1,2,3-triazole-pyrimidine hybrids was depicted in Scheme 1. The 6-aryl-5-cyano-2-thiouracils **10a**–**f** were prepared via prolonged heating of aldehydes**7a**–**f**, ethylcyanoacetate**8**, and thiourea**9** in ethanol, in the presence of potassium carbonate [34]. A mixture of the appropriate 2-mercapto-dihydroyrimidine derivatives **10a**–**f**, the propargyl bromide, and anhydrous potassium carbonate was refluxed in dry dioxane. Upon completion, phosphorous oxychloride was added to yield the target derivatives **11a**–**f**. These highly activated intermediates were then reacted with different aryl amines to obtain compounds **13a**–**e**. The compounds **12a**–**i** were prepared via click reaction of compound **11a**–**f** with appropriately substituted benzyl azides. The substituted benzyl azides were readily synthesized from the corresponding halides and sodium azide following literature procedures [35,36]. Target compounds **14**–**40** were synthesized in moderate to high yield using the same reaction condition as **13a**–**e**.

All the synthesized compounds were fully characterized by ^{1}H, ^{13}C NMR and high resolution mass spectra as described for compound **18** (Fig. 3). In the 1H NMR spectra of **18**, the NH proton resonated at δ 10.06 ppm as singlet. We have identified compound **18** from 1D NMR (^{1}H NMR, ^{13}C NMR and DEPT135) and 2D NMR (HSQC, COSY and HMBC). The numbers of the hydrogens and carbons corresponding to **18** were showed below (Fig. 3). The protons attached to S-CH_2, Ar-CH_2 and triazole-H occurred at δ 4.41 (s, 2H), 5.61 (s, 2H) and 7.73 (s, 1H), respectively. The carbons attached to S-CH_2, Ar-CH_2 and triazole-H occurred at δ 25.66, 51.04 and 124.17, respectively. In addition, some direct C–H correlations were observed, confirming that the signals of the aryl chain carbons appeared at 122.69–131.81 ppm and the aryl photons appeared at 7.12–7.89. The presence of a molecular ion peak at $m/z = 566.0699$ ($[M+Na]^+$) in the mass spectrum (calcd. 566.0697) further confirmed the structure of **18**. For all the spectra of compound **18**, please refer to the Supporting information.

2.2. Evaluation of biological activity

2.2.1. Anticancer activity

All synthesized compounds were evaluated for their anticancer activity against four cancer cell lines, MGC-803 (human gastric cancer cell line), MCF-7 (human breast cancer cell line), B16-F10 (mouse melanoma cell line), and EC-109 (human esophageal cancer cell line) using MTT assay method and compared with the well-known anticancer drug 5-fluorouracil [37].

The anti-proliferative results of preliminary evaluation against the MGC-803, EC-109, B16-F10 and MCF-7 cancerous cell lines for the candidate compounds were shown in Table 1. The replacement of the alkyne substituent by the 1,2,3-triazole scaffolds resulted in a powerful improvement of activity for all the compounds (**14**–**18**), compared with the corresponding pyrimidine-analogs (**13a**–**e**). Especially, compound **17** showed excellent inhibitory effect against EC109 with an IC_{50} value of **1.42** μM (>90-fold and 7-fold more potent than 13**d** and 5-Fu, respectively). This result suggests that 1,2,3-triazole moiety may play an important role in determining activity. In order to complete an SAR study, a series of 1,2,3-triazole-pyrimidine hybrids were prepared and evaluated for their anti-proliferative activity (Table 2).

The SAR studies analysis, as listed on Table 2 showed that the majority of the synthesized compounds showed moderate to good cytotoxic activities against EC-109, MCF-7, and MGC-803. Among them, it was observed that compounds (**14**, **17**, **19**, and **24**) have an excellent anticancer activity with single-digit micromolar IC_{50} values against all the assayed cell lines. On the other hand, the electronic effect and the position of substituent on the aryl amine and benzyl groups had a remarkable effect on their cytotoxic activity. Compounds **14**–**24** were more cytotoxic than **25** against all the assayed cell lines, which means that the substitution on the aryl amine was important for the *in vitro* anticancer activity. Compounds **14**, **19** and **24** with electron-donating groups on the arylamine group have more potent inhibitory effect (7.96, 9.67 and 9.74 μM, respectively) against EC-109 than compounds (**15**, **16**, **18**, and **20**–**23**) (IC_{50} > 15 μM) with electron- withdrawing groups. Compared with compounds (**16**, **19**, and **22**) at the 2-substitution on the arylamine group, compounds (**17**, **14** and **23**) at the 3,4-substitution performed a relatively weak inhibitory effect against MCF-7 and MGC-803. The 4-substitution on the arylamine group (**17**) was more effective against MCF-7 and MGC-803 than those