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Original article

Design and synthesis of novel soluble 2,5-diketopiperazine derivatives as potential anticancer agents

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ABSTRACT

Non-protected 2,5-diketopiperazine derivatives have poor solubility thus with negative impact on their bioavailability. In the present study, twenty-one novel soluble mono-protected, and three non-protected 2,5-diketopiperazine derivatives were designed and synthesized. Their anticancer activity to ten cell lines were evaluated by using CCK8 assay, and the results showed that about half of the mono-protected derivatives had broad-spectrum anticancer activity. Among allyl-protected derivatives, compound **4m** had strong activity to all the cell lines (IC_{50} = 0.5–4.5 μM), especially to the cancer cell lines U937 (IC_{50} = 0.5 μM) and K562 (IC_{50} = 0.9 μM). Compound **4m** could become a lead compound for further development for anticancer agents.

1. Introduction

Cancer is one of the most serious diseases in the world. Despite huge effort has been made by researchers during past several decades, the search of effective clinical approaches for the treatment of cancer is still a tough challenge. Apart from surgery, immunotherapy, and radiotherapy, chemotherapy using anticancer agents is another useful option for the cancer treatment [1]. For a long time, the development of novel anticancer agents is a highly active research field, and much effort has been focused on natural products because of their fewer side effects [2]. However, solubility is one major problem for some active natural or synthetic compounds in the early stage study of these compounds [3,4]. One classic instance is about the natural product Camptothecin (CPT, Fig. 1), whose low solubility limits its broad use as cancer therapeutic agent, and some optimized derivatives have been synthesized for its improvement in the following years [5–8].

2,5-Diketopiperazine (Fig. 1) is an important scaffold in many natural products which have a variety of biological activities [9], while most have complicated chemical structures. Therefore, many 2,5-diketopiperazine derivatives with simple structures have been synthesized based on 2,5-diketopiperazine scaffold and show good activities [10,11]. One significant example is plinabulin (NPI-2358/KPU-2, Fig. 1), which has been derived from natural phenylahistin (Fig. 1) and first developed as a vascular disrupting agent (VDA) [12], and is now under phase II clinical trials as an anticancer drug [13]. Some recent studies have shown that plinabulin is also a potent anti-microtubule agent with colchicine-like tubulin depolymerization activity [14]. A few of its derivatives modified at the aromatic moieties have also been synthesized with good efficacy [14,15]. Another study has shown that 2,5-diketopiperazine derivatives have weak activity (100 μg mL^{-1}) in inhibiting the nauplii movement without causing their death [16]. But for most non-protected derivatives, their solubility is poor [17], presumably due to the combination of intermolecular hydrogen bonds and π–π stacking interactions from lines or networks of 2,5-diketopiperazine templates (Fig. 2) [9,18], thus with negative influence on their solubility and purification. A useful solution to this problem is to interrupt the formation of hydrogen bonds and disturb the π–π stacking interactions by introducing protective groups to replace one or two of the amide hydrogen atoms, resulting in a non-planar structure of 2,5-diketopiperazine

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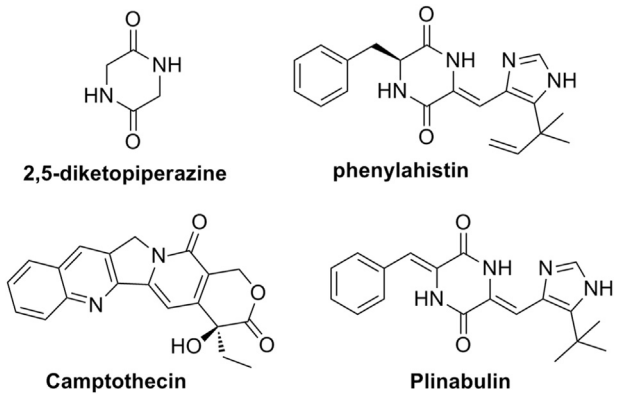

Fig. 1. The structures of Camptothecin, 2,5-diketopiperazine and its derivatives.

template [19,20]. Interestingly, some previous articles [21–23] have shown that garlic derivatives, e.g. S-allylcysteine [24,25] with an allyl-protection on the sulfur atom, can well suppress the growth of a broad spectrum of tumors, and their allyl groups play important roles for their inhibitory activities [26]. Therefore, in the present study, a novel series of allyl-protected 2,5-diketopiperazine derivatives were designed and synthesized, and their anticancer activities against ten cell lines were evaluated by using CCK8 assay [27]. Besides, several methyl-protected or non-protected derivatives were also synthesized for comparative studies.

2. Results and discussion

2.1. Chemistry

The synthesis of the target 2,5-diketopiperazine derivatives is shown in Scheme 1. The purchased compound 2,5-diketopiperazine was heated under reflux in acetic anhydride overnight to yield product **1** [28], which was treated with aromatic aldehydes (Ar_1CHO) and Cs_2CO_3 in dry DMF at room temperature to afford the intermediate **2** [15]. Compound **2** was then protected with allyl or methyl group to give compound **3**. The final target compound **4** was obtained from aromatic aldehydes (Ar_2CHO) by reacting either with intermediate **3** under the same condition as

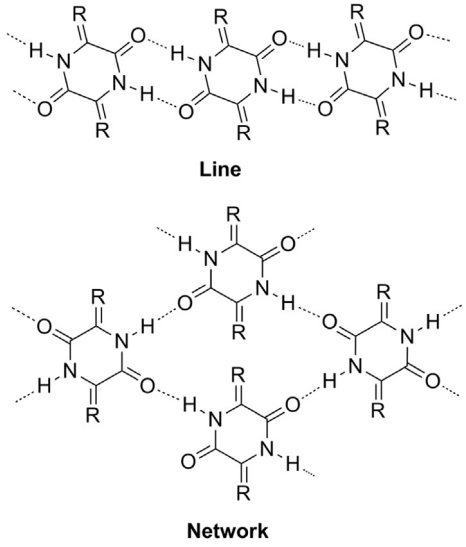

Fig. 2. Schematic view of H-bonding patterns for 2,5-diketopiperazine derivatives.

that for the synthesis of intermediate **2**, or with intermediate **2** in the presence of Cs_2CO_3 at 80 °C. All the protected compounds can be easily dissolved in normal solvents, such as ethyl acetate (AcOEt), dichloromethane (DCM), chloroform, methanol (MeOH), ethanol (EtOH), *N*,*N*-dimethylmethanamide (DMF), dimethylsulfoxide (DMSO), etc. The non-protected compound **4v** and **4w** had poor solubility in all above solvents, while compound **4x** and plinabulin could be dissolved in DMF, DMSO, a mixture of MeOH with DCM or AcOEt.

2.2. Biological activity

We first investigated the anticancer activity of the allyl-protected 2,5-diketopiperazine derivatives (**4a**–**r**) to six cancer cell lines (BGC-823, Hela, Huh-7, MCF-7, H1975, A549), and their IC_{50} values are listed in Table 1. It was found that their inhibitory activities to these cancer cell lines were different. When Ar_1 was the phenyl (Ph) or 3-bromo phenyl (3-BrPh) group, the derivatives (**4a**, **4b**, **4c**, and **4d**, Table 1) had no activities, indicating that Ph and 3-BrPh were not good substitutive groups for their biological activity. However, when Ar_1 was 2-MeOPh, most of the derivatives had high activity against the cell lines. Therefore, we continued our further synthesis of derivatives with 2-MeOPh as Ar_1, and with other phenyl groups as Ar_2 in the following studies. As shown in Table 1, when Ar_2 was 3-BrPh (**4e**), 3-ClPh (**4f**), 2-ClPh (**4i**), 2-CF_3Ph (**4k**), or 5-F-2-MePh (**4q**), the derivatives had high activity, except compound **4j** with 2-FPh as Ar_2. Among all these derivatives, compound **4m** with 2,3-ClPh as Ar_2 showed the highest inhibitory activity to all cancer cell lines. In comparison, when its chlorine atom at ortho-position was moved to para-position, compound **4n** lost its activity to all these cell lines. To our surprise, compound **4l** with 2-MeOPh as Ar_2 had high activity only against cell line Hela, while **4g** with 3-FPh as Ar_2 had high activity only against cell lines BGC-823 and Hela. On the other hand, compound **4h** with 3-MePh and compound **4o** with 5-Br-2-FPh as Ar_2, both had low activity, while compound **4p** with 3-Br-4-FPh and **4r** with 4-MePh as Ar_2, had no activity at all.

The methyl-protected compounds **4t** and **4u** showed slightly higher anticancer activity than their corresponding allyl-protected compounds **4i** and **4m**. The methyl-protected compound **4s** had low or no anticancer activity, just like its corresponding allyl-protected compound **4l**. For unprotected compounds, **4v** and **4w** had no activity, while **4x** and the control compound plinabulin had higher anticancer activity even than the protected derivatives. This might indicate that imidazole was beneficial to the solubility of **4x** and plinabulin, resulting in their improved anticancer activity, similar to those reported previously [15].

Based on the above results, their structure–activity relationships were still not very clear, because the aromatic groups, the positions for the substituents, and the protective groups of the derivatives had somehow different impacts on their bioactivities. However, some of the allyl-protected derivatives, especially **4m**, should be worthwhile for further study as lead compounds in searching for strong and broad-spectrum anticancer agents.

With the above findings, we next studied compound **4m**, along with the other twenty-three compounds against four leukemic cell lines HL60, K562, MOLT-4, and U937, which are related to acute or chronic malignant diseases, and the results are shown in Table 2. It was found that the allyl-protected derivatives **4a**, **4b**, **4c**, **4d**, **4n**, **4p**, and **4r** had no activity to these cell lines, while compound **4m** and compounds **4e**, **4f**, **4g**, **4i**, **4k**, and **4q**, had high activity to these cell lines, and in comparison, **4h**, **4j**, **4l**, and **4o** showed high activity against cell lines K562 and U937 only. The methyl-protected compounds **4t** and **4u** had high activity in suppressing the cancer cell