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Design, synthesis and biological activity of piperlongumine derivatives as selective anticancer agents

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ABSTRACT

In an effort to expand the structure–activity relationship of the natural anticancer compound piperlongumine, we have prepared sixteen novel piperlongumine derivatives with halogen or morpholine substituents at C2 and alkyl substituents at C7. Most of 2-halogenated piperlongumines showed potent *in vitro* activity against four cancer cells and modest selectivity for lung normal cells. The highly active anticancer compound **11h** exhibited obvious ROS elevation and excellent *in vivo* antitumor potency with suppressed tumor growth by 48.58% at the dose of 2 mg/kg. The results indicated that halogen substituents as electrophilic group at C2 played an important role in increasing cytotoxicity.

1. Introduction

Piperlongumine(PL, Fig. 1) is an alkaloid isolated from *Piper* species and displays anticancer [1–7], anti-diabetic [8], anti-platelet aggregation [9–12], and antifungal activities [13,14]. Recently, Raj et al. demonstrated selectivity of piperlongumine for cancer cells. Compared to the normal cell lines, piperlongumine induced cell death in thirteen cancer lines with an average IC_{50} value lower than 7 μM [15]. Additionally, similar effects were observed *in vivo* in four nude mice models without effect on normal tissue. Further studies revealed a unique mechanism based on reactive oxygen species (ROS). Using a phenotypic screen, they found that two proteins: glutathione-S-transferase-P1 (GSTP1) and carbonyl reductase 1 (CBR1) played a more important role in the cellular response to ROS. This tumor specific effect of piperlongumine on cytotoxicity and ROS shows a new strategy for selective targeting of cancer cells [16–20].

To explore the structure and biological activity relationship of piperlongumine, eighty analogues had been synthesized and screened by Adams et al. [21]. Based on the assays of cellular toxicities, two key pharmacophores: C2–C3 and C7–C8 double bonds had been identified. Both these Michael acceptors were necessary for PL's toxicity to cancer cells. The former was also critical for ROS elevation and glutathione depletion. However, removal of C7–C8 olefin led to a decrease in cytotoxicity, but did not reduce the ROS level. Among all the analogues, PL dimer and trimer displayed more potent activities than the others. SAR indicated that alkynyl substituents can be introduced at position 2 without effect in cytotoxicity. On the contrary, alkyl and aromatic substituents were not tolerated. Although the C7–C8 olefin has been confirmed as one of two key pharmacophores for cellular toxicity with the example of the inactive C7–C8 olefin reduction product, we are unaware of the effect of substituents on the C7–C8 double bond. In order to continue the development of piperlongumine derivatives as selective anticancer agents, we describe herein the synthesis and

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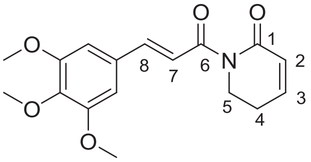


Fig. 1. Structure of piperlongumine (PL).

biological activity of a novel series of piperlongumine derivatives on positions 2 and 7. An active piperlongumine derivative was also assayed for its *in vivo* antitumor activity in aA549 nude mice model.

2. Chemistry

PL and sixteen derivatives were synthesized according to the modified Raj's route [15]. As key reaction intermediates, three dihydropyridin-2(1*H*)-ones **3**, **4** and **6** were prepared in three or four steps from the commercially available piperidin-2-one **1**(Scheme 1). Treatment of **1** with PCl_5 in chloroform yielded 2,2-dichloropiperidin-2-one **2** [22,23]. Dehydrochlorination of **2** at 120 °C in the presence of Li_2CO_3 provided the corresponding 2-chloro-5,6-dihydropyridin-2(1*H*)-one **3** which was readily converted into 2-morpholino-5,6-dihydropyridin-2(1*H*)-one **4**. The third dihydropyridin-2(1*H*)-one **6** was prepared by the same route as **3** in a 63.1% yield.

With three dihydropyridin-2(1*H*)-ones in hand, all target piperlongumine derivatives were synthesized as detailed in Scheme 2. Condensation of 3,4,5-trimethoxy-benzaldehyde **7** with 2-substituted malonic acids in pyridine yielded 2-substituted (*E*)-3-(3,4,5-trimethoxyphenyl)acrylic acids **9a**–**9e**. Treatment of **9a**–**9e** with oxalyl chloride in anhydrous THF under N_2 gave the acyl chlorides **10a**–**10e** which were reacted with the three substituted dihydropyridin-2(1*H*)-ones to afford piperlongumine derivatives **11a** – **11p** using anhydrous THF as solvent and TEA as catalyst.

3. Results and discussion

3.1. In vitro anticancer screening

The first obtained seven piperlongumine derivatives **11a**–**11g** and PL were evaluated for *in vitro* cytotoxicities against four cancer cells (human lung carcinoma A549, human colorectal carcinoma HCT116, human breast carcinoma ZR-75-30, human breast carcinoma MDA-MB-231) and human lung normal cells MRC-5 by MTT assay, respectively. A summary of these results is shown in Table 1. Interestingly, substituents on position 2 of piperlongumine played an important role in antiproliferative activities. For example, compounds **11a** and **11b** without any group at C2 exhibited potent activities. On the other hand, the 2-chloro derivative **11g** also showed potent activities against all four cells. However, compounds **11c**–**11f** with morpholine substituents at C2 were uniformly less active than piperlongumine. In addition, compound **11g** resulted in a nearly two- or three-fold increase in selectivity for three cancer cell lines HCT116, ZR-75-30, MDA-MB-231 over normal cells MRC-5.

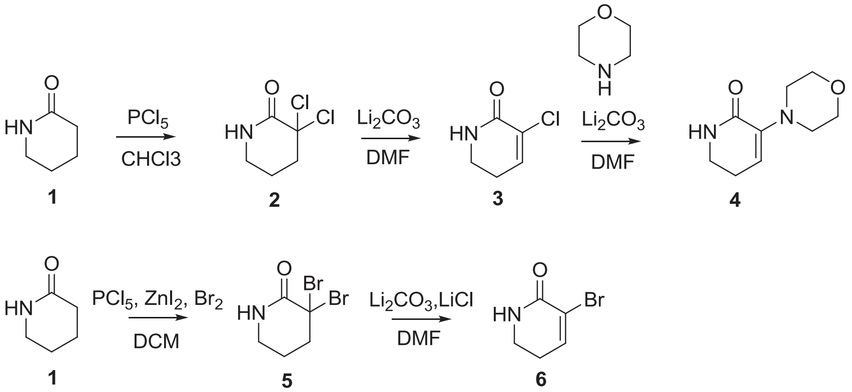
Encouraged by the preliminary design, a series of piperlongumine derivatives with halogen substituent at C2 were synthesized to improve antitumor activity and investigate the SARs. To our delight, most of the 2-halogen substituted piperlongumine derivatives showed potent activities against four cancer cell lines (human lung carcinoma A549, human colorectal carcinoma HCT116, human breast carcinoma MDA-MB-231, human hepatoma Hep3B) and human fetal lung normal cells WI38 (Table 2). Remarkably, both 2-chloro substituted piperlongumines **11h**–**11k** and 2-bromo substituted piperlongumines **11l**–**11p** showed more potent activities than piperlongumine. This fact confirmed that halogen substituents as electrophilic group at C2 increase cytotoxicity. Moreover, selectivity for A549 cells over normal cells was slightly decreased with the length of carbon chain at C7 except for compound **11l**. For example, 7-methyl-2-chloropiperlongumine **11h** with a rate value of 4.98 showed higher selectivity than 7-propyl-2-chloropiperlongumine **11j** in these assays.

3.2. ROS evaluation

Although the natural product piperlongumine, isolated from *Piper* species has been recognized as an antitumor agent, its cellular mechanism has not been defined. Recently a ROS-independent cellular effect has been disclosed as the antitumor mechanism of piperlongumine [15,21]. In order to validate the mechanism of the target compounds, we next determined the effect of piperlongumine derivative **11h** on cellular ROS level in A549 cancer cells by fluorescence microscopy. Treatment with 10 μM of **11h** for 1 h caused an obvious increase in ROS level (Fig. 2). The result indicated that a halogen substituent at C2 of piperlongumine did not affect the ROS elevation.

3.3. In vivo anticancer evaluation

On the basis of the favorable *in vitro* cellular activity and selectivity for normal cells, compound **11h** was selected for further *in vivo* antitumor activity studies on human lung cancer A549



Scheme 1. Synthesis of three key dihydropyridin-2(1*H*)-ones.