Design, Synthesis and Anticancer Activity of Novel 3,6-Diunsaturated 2,5-Diketopiperazines

Xiaolin Li , Tianrong Xun , Huayan Xu , Xiaoyan Pang , Bin Yang , Junfeng Wang , Xuefeng Zhou , Xiuping Lin , Suiyi Tan * , Yonghong Liu * , Shengrong Liao *

Posted Date: 27 April 2023

doi: 10.20944/preprints202304.1053.v1

Keywords: 2,5-diketopiperazine derivative; intermolecular bond; liposolubility; electron property; anticancer

Article

Design, Synthesis and Anticancer Activity of Novel 3,6-Diunsaturated 2,5-Diketopiperazines

Xiaolin Li [1,5,†], Tianrong Xun [3,†], Huayan Xu [2], Xiaoyan Pang [1], Bin Yang [1,5], Junfeng Wang [1,5], Xuefeng Zhou [1,5], Xiuping Liu [1], Suiyi Tan [4,*], Yonghong Liu [1,2,5,*] and Shengrong Liao [1,5,*]

[1] CAS Key Laboratory of Tropical Marine Bio-Resources and Ecology, Guangdong Key Laboratory of Marine Materia Medica, Research Center for Marine Microbes, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, China
[2] Wuya College of Innovation, Shenyang Pharmaceutical University, Shenyang 110016, China
[3] Department of Pharmacy, Southern Medical University, Shenzhen 518100, China.
[4] NMPA Key Laboratory for Research and Evaluation of Drug Metabolism, Guangdong Provincial Key Laboratory of New Drug Screening, School of Pharmaceutical Sciences, Southern Medical University, Guangzhou 510515, China
[5] University of Chinese Academy of Sciences, Beijing 100049, China
* Correspondence: suiyitan@smu.edu.cn (S.T.), yonghongliu@scsio.ac.cn (Y.L.), srliao@scsio.ac.cn (S.L.)
† These authors contributed equally to this work.

Abstract: Based on the marine natural products piperafizine B, X334 and our previously reported compound **4m**, fourteen novel 3,6-diunsaturated 2,5-diketopiperazine (2,5-DPK) derivatives (**1**-**2**, **4**-**6**, **8**-**16**), together with two known ones (**3** and **7**), were designed and synthesized as anticancer agents against cell lines A549 and Hela. The MTT assay results showed that the derivatives **6**, **8**-**12** and **15** had moderate to good anticancer capacities with the IC_{50} values ranging from 0.7 to 8.9 μM. Among them, compound **11** with naphthalen-1-ylmethylene and 2-methoxybenzylidene functions at the 3- and 6-positions of 2,5-DPK ring, respectively, displayed good inhibitory activities to both cancer cells A549 (IC_{50} = 1.2 μM) and Hela (IC_{50} = 0.7 μM). It could also induce apoptosis and obviously block the cell cycle progression at G2/M phase in both cells at 1.0 μM. The electron withdrawing functions might not be favorable for the derivatives with high anticancer activities. Additionally, compared to piperafizine B and X334, these semi-N-alkylated derivatives have high liposolubilities (> 1.0 mg•mL^{-1}). Compound **11** can be further developed aiming to the discovery of novel anticancer candidate.

Keywords: 2,5-diketopiperazine derivative; intermolecular bond; liposolubility; electron property; anticancer

1. Introduction

2,5-diketopiperazine (2,5-DPK) is the smallest cyclic peptide and a useful scaffold frequently found in numerus structurally diverse natural product [1-3]. 2,5-Diketopiperazine derivatives (2,5-DPKs) have higher stability than their linear counterparts against enzymolysis [4], and are often conformational rigidity and able to interact with various biological targets [5] thus giving rise to a broad range of biological activities [3, 6], such as antivirus [7], anticancer [8-12], antifouling [13, 14], antioxidation [15], anti PAI-1 [16, 17], and so on. As a result, 2,5-DPK has been becoming an attractive and privileged scaffold for the discovery of highly active pharmaceutical agents. One class of 2,5-DPKs which have unsaturated C-C double bonds at the 3- and/or 6-positions of the 2,5-DPK ring, such as the marine natural products XR334 [16], piperafizine B [18], phenylahistin [19] and its synthetic derivative plinabulin [20], possessing two pairs of hydrogen bond donor and acceptor (Figure 1), show markedly different anticancer properties. The 2,5-DPKs furnished with phenyl rings at the 3- and 6-positions, like piperafizine B and XR334, will assemble in line and/or net frameworks due to the formation of the intermolecular hydrogen bond as well as the π-π stacking interactions [3, 11] (Figure 2). These compounds have very poor liposolubilities and weak anticancer activities and their further biological investigations are then prevented [17]. However, those 2,5-DPKs, such as phenylahistin and plinabulin, with an imidazole (or 2-pyridyl) group as a side component will have

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 27 April 2023 doi:10.20944/preprints202304.1053.v1

intramolecular hydrogen bond formed preferentially between the amide hydrogen of the 2,5-DPK ring and the nitrogen atom of the side imidazole moiety [21] (Figure 1), and the tendency for the formation of the intermolecular hydrogen bonds is largely weakened. Their liposolubilities and anticancer activities are greatly increased [12]. The lipophilicities of this kind of compounds can also be improved by the introduction of protective groups, similar to the semi-N-methylation of piperafizine B to A or X334 to X330, on the amide nitrogen atom of 2,5-DPK ring. Previous studies demonstrated that these semi-N-protected 2,5-DPKs had improved liposolubilities and could cross the cell membrane easily along with enhanced stable capacities away from the degradation caused by enzymes [4]. Furthermore, the enhanced lipophilicity of 2,5-DPKs was beneficial to their anticancer activity [22].

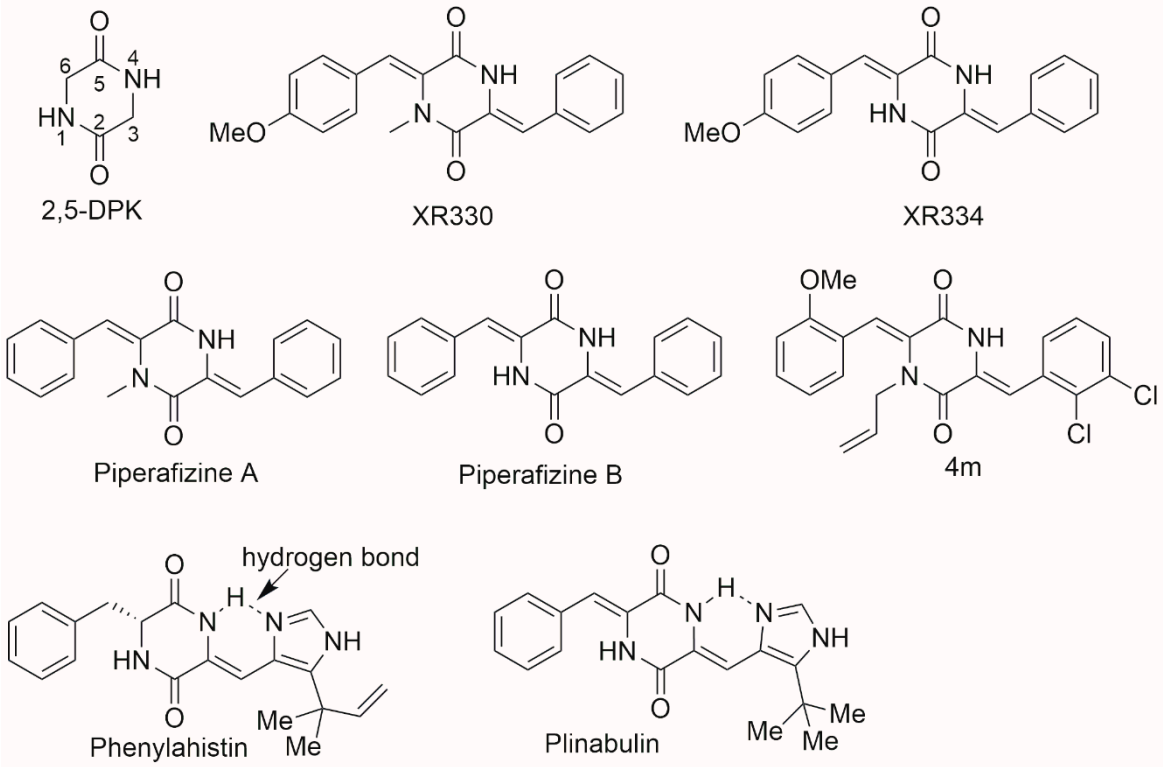


Figure 1. Structure of 2,5-DPK and its representative 2,5-DPKs with unsaturated C-C double bonds.

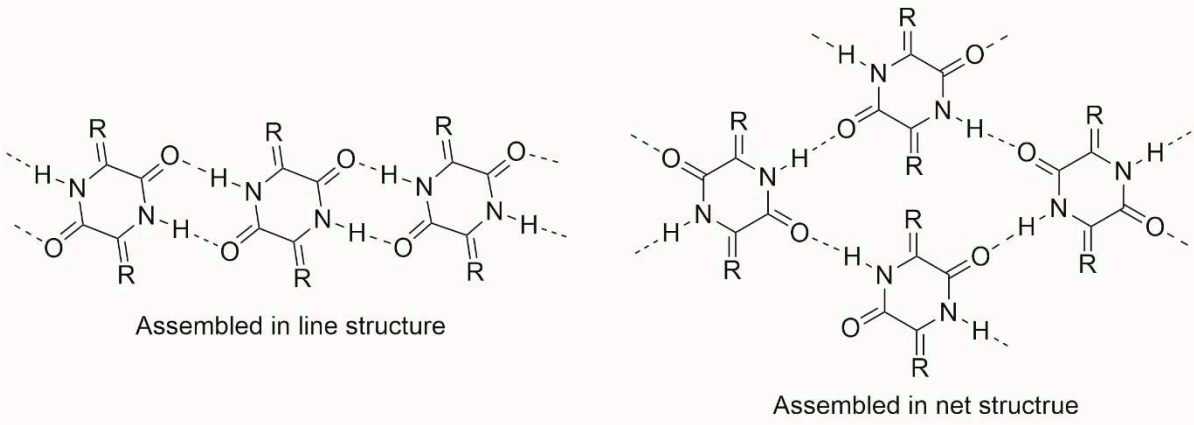
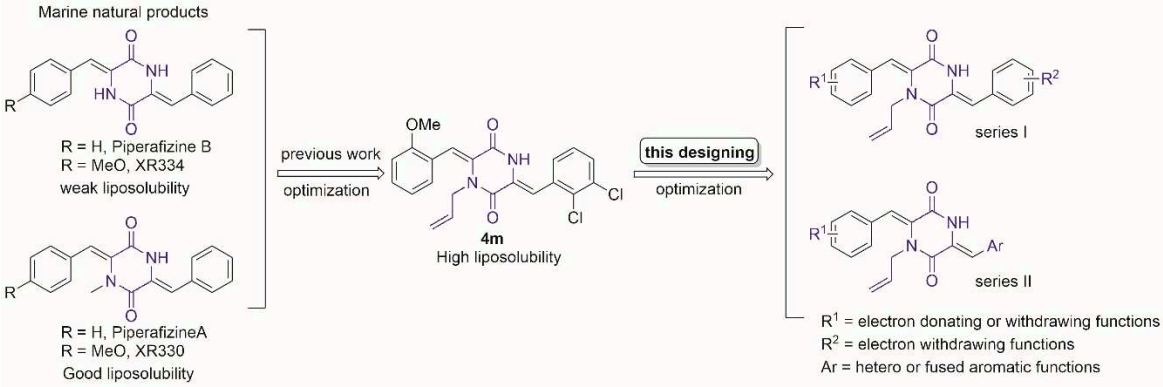


Figure 2. 2,5-DPKs with unsaturated C-C double bonds assembled in line or net structure.

In our previous study, one compound **4m** showed good anticancer activities against several cell lines including A549 and Hela [11]. An allyl group was introduced to the 1-nitrogen atom of the 2,5-DPK ring to interrupt the formation of the intermolecular hydrogen bonds. Compared to the methyl group, this function with a little longer side chain could also disturb the π-π stacking interactions

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 27 April 2023 doi:10.20944/preprints202304.1053.v1

between the layers of the frameworks, which then contributing to the good lipophilicities of derivatives. On the other hand, the biological results showed that the 2-methoxy group on the phenyl ring at the 6-position of the 2,5-DPK ring played critical role in improving the anticancer activity, whereas the impacts of its two chlorine atoms and some other strong electron withdrawing groups (e.g., NO_2, CF_3, CN) on the anticancer activities of derivatives are not clearly investigated, as electron properties often exhibit significant (positive or negative) impact on derivative's bioactivity [23, 24]. Similarly, the size or skeleton of the substituent will also have different influences on the anticancer activities of derivatives [5, 25]. Therefore, based on the marine natural products piperafizine B and AR334, in this context, a small library of novel 2,5-DPKs were designed as following (Scheme 1): 1) similar to compound **4m**, the allyl group on the 1-nitrogen atom was retained, 2) the electron withdrawing groups such as NO_2, CN, CF_3, Br, Cl, F on the phenyl group (series I), and the electron donating group MeO on benzene or naphthlene were introduced, 3) a few electron-rich (e.g., furan and thiophen), electron-deficient (e.g., pyridyl) heteroaromatic scaffolds or fused naphthyl, quinolyl functions (series II) were employed. Thus fourteen novel 2,5-DPKs (**1**-**2**, **4**-**6**, **8**-**16**), along with two known ones (**3** and **7**), were designed and synthesized, and their anticancer activities against two cell lines A549 and Hela were evaluated aiming to the discovery of highly active anticancer agents.



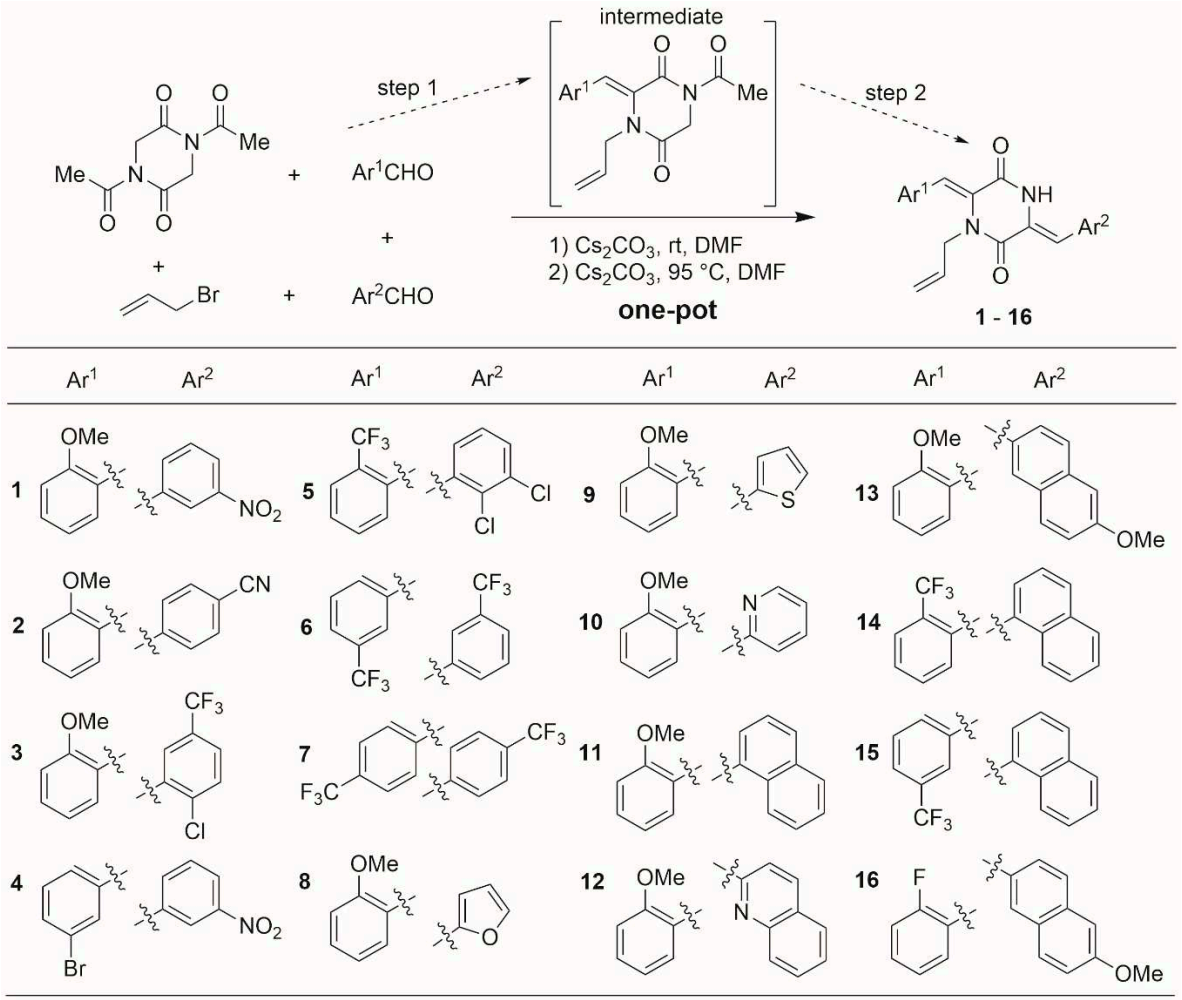
Scheme 1. Designing of the 2,5-DPKs in this study.

2. Results and Discussion

2.1. Synthesis of compound

By following our previous reported procedure [26], the compounds were synthesized using a one-pot operation (Scheme 2). For the synthesis of compounds **1**-**5**, **8**-**16**, after the completion of the reaction of 2,5-diketopiperazine with aldehyde and allyl bromide under base condition at room temperature, the second aldehyde was added directly and the reaction was subsequently heated until the disappearance of the intermediate. The syntheses of compounds **6** and **7** were similar to the above procedure whereas the aldehydes were added just for once in the first step. The solubility test demonstrated that all the compounds could be soluble in common organic solvents such as dimethyl sulfone (DMSO), N,N-dimethylformamide (DMF), ethyl acetate (EtOAc), dichloromethane (DCM), chloroform and acetone with the solubilities > 1.0 $mg \bullet mL^{-1}$ at room temperature.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 27 April 2023 doi:10.20944/preprints202304.1053.v1



Scheme 2. Synthesis of derivatives **1**-**16**.

2.2. Biological evaluation of derivatives

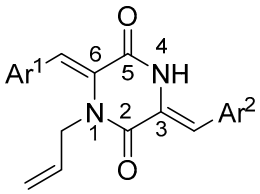
2.2.1. Inhibiting proliferation of cancer cells

First of all, we screened the antiproliferative effects of all compounds against cancer cell lines A549 and Hela at a preliminary concentration of 10 μM. Those with the inhibition > 50% were selected for the further detailed evaluations and their IC_{50} values were subsequently calculated and obtained (Table 1). It was found that compounds **8**-**11** showed moderate to good inhibitory capacities against cell line A549 (IC_{50} = 1.2 - 7.3 μM), while compounds **6**, **8**-**12**, **15** showed moderate to good activities against cell line Hela (IC_{50} = 0.7 - 8.9 μM). It seemed that the active compounds had a little stronger anticancer activity against Hela than A549. In comparison to compound **4m**, the antiproliferative activities of the active compounds were not obviously improved except compound **11**, which had a little stronger activity (IC_{50} = 1.2 μM) than compound **4m** (IC_{50} = 1.6 μM) against cell line A549, and around 3-fold activity (IC_{50} = 0.7 μM) stronger than compound **4m** (IC_{50} = 2.4 μM) against cell line Hela. The results showed that the compounds with electron withdrawing groups, such as 3-NO_2 (**1**), 4-CN (**2**) or 2-Cl-5-CF_3 (**3**), on the phenyl group at the 3-position of 2,5-DPK ring had weak activities (IC_{50} > 10.0 μM). Similar to our previous study, replacing 2-MeO with 3-Br (**4**), 2-CF_3 (**5**), 3-CF_3 (**6**) or 4-CF_3 (**7**) on the phenyl group at the 6-position of 2,5-DPK ring, only compound **6** had moderate inhibitory activity against Hela cells (IC_{50} = 8.9 μM). Of note is that these compounds also contain electron withdrawing groups including 3-NO_2 (**4**), 2,3-Cl (**5**), 3-CF_3 (**6**) or 4-CF_3 (**7**) on the phenyl group at the 3-position of 2,5-DPK ring. These results imply that electron withdrawing groups might be not suitable functions. Delightedly, using electron-rich heteroaromatic cycles such as furan (**8**) and

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 27 April 2023 doi:10.20944/preprints202304.1053.v1

thiophene (**9**) or electron-deficient pyridine (**10**) as the substituents on the 3-position of 2,5-DPK ring, compounds had moderate to good activities against both cell lines A549 (IC_{50} = 3.7 - 7.3 μM) and Hela (IC_{50} = 4.7 - 5.9 μM). Changing the substituent of 2-naphthalene (**11**) into 2-quinoline (**12**) or 6-methoxy-3-naphthalene (**13**), compound **12** displayed only moderate activity against Hela cells (IC_{50} = 6.2 μM), while compound **13** showed no obvious activities to both of the cancer cells. Surprisingly, compound **14**, by taking place of the 2-MeO with 2-CF_3 on the phenyl group at 6-position of 2,5-DPK ring, could also suppress the growth of cell line Hela with good activity (IC_{50} = 3.9 μM). However, moving of the 2-CF_3 from 2-position to 3-position (**15**) or replacing 2-MeO with 2-F (**16**), the activities of the compounds were all loss. Collectively, in terms of the above results, although compound **14** with a 2-CF_3 moiety shows good activity, other examples (compounds **1**-**7**) indicate that the electron withdrawing functions on phenyl groups at both sites (3- and 6-positions) of the 2,5-DPK ring might be not favorable. Besides the vital role of 2-MeO group, the substitutive motifs with suitable size or skeleton at the 6-position of the 2,5-DPK ring, might be another critical factor for the 2,5-DPKs with high anticancer activities. It should yet mention that multiple factors, like the skeleton, size, type and/or position of the substituent, might give combined impacts on the anticancer properties of these derivatives.

Table 1. IC_{50} values of compounds against cancer cell lines A549 and Hela.



Compound	IC_{50}/μM [a]	
	A549	Hela
4m	1.6 ± 0.2	2.4 ± 0.2
1	>10	>10
2	>10	>10
3	>10	>10
4	>10	>10
5	>10	>10
6	>10	8.9 ± 0.7
7	>10	>10
8	7.3 ± 1.2	5.9 ± 1.6
9	3.7 ± 0.4	4.8 ± 1.4
10	5.6 ± 1.0	4.7 ± 2.8
11	1.2 ± 0.1	0.7 ± 0.2
12	>10	6.2 ± 0.4
13	>10	>10
14	>10	3.9 ± 0.2
15	>10	>10
16	>10	>10
Paclitaxol	0.004 ± 0.2	0.004 ± 0.8

[a] All results were presented as mean ± standard deviation (SD) from three parallel experiments.

The results also showed that compound **11** could inhibit the growth of both cell lines in dose-dependent manners after treatment for 48 h, and this compound had a little stronger inhibitory activity against Hela than A549 (Figure 3).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 27 April 2023 doi:10.20944/preprints202304.1053.v1

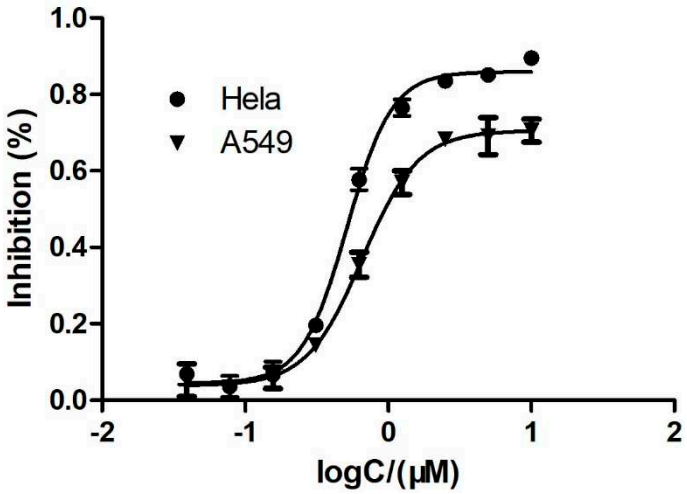
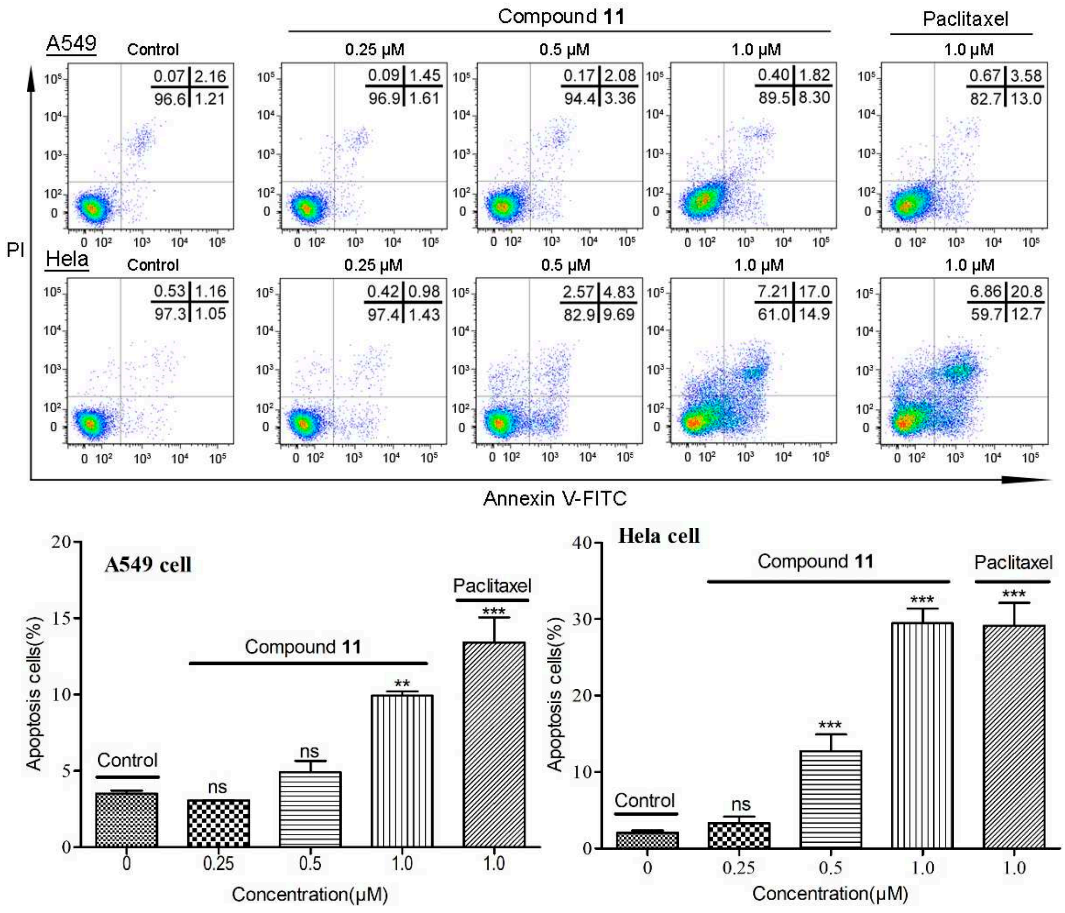


Figure 3. Relationships between the inhibition and the concentration of compound **11**. All results were presented as mean ± standard deviation (SD) from three parallel experiments.

2.2.2. Apoptosis in cancer cell lines induced by compound 11

The apoptosis in both cell lines induced by compound **11** was investigated by using flow cytometry with Annexin V-FITC and PI dual staining. As shown in Figure 4, Compound **11** could obviously induce apoptosis in both cells at 1 μM after the 48 h treatment [27]. The dose-dependent effects of compound **11** inducing apoptosis in both cells were observed, especially for Hela cells, indicating that Hela cells were more sensitive to this compound, which is consistent with the MTT results that compound **11** had stronger inhibitory ability against Hela than A549.



Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 27 April 2023 doi:10.20944/preprints202304.1053.v1

Figure 4. Compound **11** induced apoptosis in cancer cells A549 and Hela. All results were presented as mean ± standard deviation (SD). "ns" means no statistical differences, ** $p < 0.01$, *** $p < 0.001$, student t-test.

2.2.3. Cell cycle arrest induced by compound 11.

The cell cycle arrest experiments were also preformed to investigate which phase of the cell cycle progression was blocked by the compound **11**. As shown in Figure 5, the results showed that both cancer cells could be blocked at G2/M phase with the percentages of 85.87 (A549) and 90.37% (Hela), respectively, at 1.0 μM after the treatment of **11** for 24 h. The results were similar to the cells blocked by paclitaxel, indicating our derivative may have the same biological target as paclitaxel, while not maintain the stability of the microtubule, but more probably similar to plinabulin, inhibit tubulin polymerization and perturb the stability of microtubule, thus resulting in the apoptosis of cancer cells.

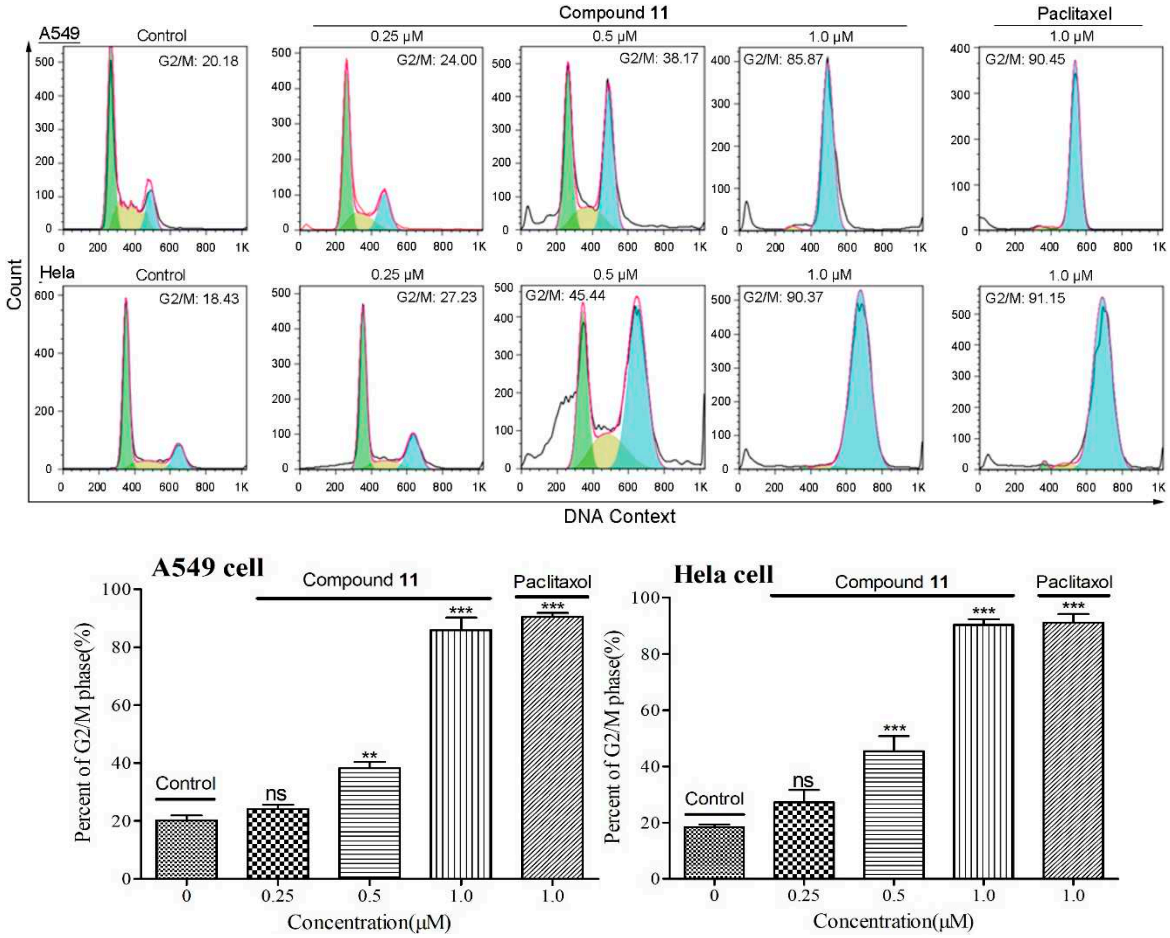


Figure 5. Compound **11** significantly induced G2/M phase arrest in both cancer cell lines compared to control. All results were presented as mean ± standard deviation (SD). "ns" means no statistical differences, ** $p < 0.01$, *** $p < 0.001$, student t-test.

3. Materials and Methods

3.1. Chemicals

Solvents and chemicals are commercially available in analytical grade and used without further purification. Analytical thin layer chromatography (TLC) analyses were performed on HSGF254 plates which were visualized using UV light (254 nm) or I_2 staining. Flash column chromatography was performed using silica gel (200–300 mesh). Melting points (m.p.) were determined by using a SGW-X4 melting point instrument without correction. Mass spectrometry data were collected with a Bruker amaZon SL instrument for low-resolution or a Bruker maXis/QTOF instrument for high-

 doi:10.20944/preprints202304.1053.v1

resolution both using ESI ionization. The NMR spectra were recorded on Bruker AC 500 or 700 NMR spectrometer with TMS as an internal standard. The residual solvent peaks were used for the chemical shifts as an internal reference (ppm): ^{1}H ($CDCl_3$: δ 7.26); ^{13}C ($CDCl_3$: δ 77.0).

3.1.1. Synthesis of compounds

General procedure for the synthesis of products 1-16

Into a 4 mL vial, 1,4-diacetyl-2,5-diketopiperazine (50 mg, 0.25 mmol, 1.0 equiv.) and the first aldehydes (0.25 mmol, 1.0 equiv.) dissolved in 2 mL dry DMF were added, followed with allyl bromide (54 μL, 0.63 mmol, 2.5 equiv.) and Cs_2CO_3 (205 mg, 0.63 mmol, 2.5 equiv.). The reaction was stirred at room temperature until the completion of the 1st Aldol condensation and the alkylation of allyl bromide, and then the second aldehydes (0.5 mmol, 2.0 equiv.) was added and the mixture was heated at 95 °C for about 4 h. The reaction mixture was added into water (15 mL) and extracted with EtOAc (5 mL × 3). The organic layer was dried over Na_2SO_4, filtered, and removed. The residues were purified by flash column chromatography on silica to afford the target products **1**-**5** and **8**-**16**. The procedure for the synthesis of compounds **6** and **7** is similar to the above synthetic method, however, the aldehydes (3.0 equiv.) were added just for once only in the first step. Compound **3** [28] and **7** [26] are known and their analytic data are identical to the reported ones.

1-allyl-6-(2-methoxybenzylidene)-3-(3-nitrobenzylidene)piperazine-2,5-dione (1)

Following the general procedure, the product **1** was obtained in 51% yield as a slightly yellow solid. mp = 138 – 141 ˚C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.99 (s, 1H), 8.46 (s, 1H), 8.01 (d, *J* = 8.2 Hz, 1H), 7.77 (d, *J* = 7.7 Hz, 1H), 7.55 (t, *J* = 8.0 Hz, 1H), 7.36 (t, *J* = 8.4 Hz, 1H), 7.18 (d, *J* = 7.0 Hz, 1H), 7.06 (s, 1H), 6.98 (t, *J* = 7.4 Hz, 1H), 6.92 (d, *J* = 8.5 Hz, 1H), 6.90 (s, 1H), 5.54 – 5.46 (m, 1H), 4.98 (d, *J* = 11.1 Hz, 1H), 4.72 (d, *J* = 17.1 Hz, 1H), 4.19 (d, *J* = 5.9 Hz, 2H), 3.84 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 161.4, 158.8, 157.2, 148.4, 135.0, 134.8, 131.2, 130.7, 130.3, 129.8, 128.1, 127.8, 123.7, 122.7, 122.3, 120.2, 118.8, 118.1, 114.9, 110.6, 55.4, 47.3 ppm. HRMS (ESI): m/z calcd for $C_{22}H_{20}N_3O_5$ $[M+H]^+$ 406.1397, found 406.1390; for $C_{21}H_{19}N_3O_5Na$ $[M+Na]^+$ 428.1217, found 428.1211.

4-((Z)-(4-allyl-5-((Z)-2-methoxybenzylidene)-3,6-dioxopiperazin-2-ylidene)methyl)benzonitrile (2)

Following the general procedure, the product **2** was obtained in 46% yield as a slightly yellow solid. mp = 175 – 178 ˚C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.34 (s, 1H), 7.73 (d, *J* = 8.3 Hz, 2H), 7.56 (d, *J* = 8.2 Hz, 2H), 7.44 – 7.34 (m, 1H), 7.33 (s, 1H), 7.21 (d, *J* = 6.8 Hz, 1H), 7.01 (d, *J* = 7.9 Hz, 1H), 6.99 (t, *J* = 7.4 Hz, 1H), 6.94 (d, *J* = 8.3 Hz, 1H), 5.54 (ddt, *J* = 16.3, 10.2, 6.0 Hz, 1H), 5.02 (dd, *J* = 10.2, 1.1 Hz, 1H), 4.77 (dd, *J* = 17.1, 1.2 Hz, 1H), 4.25 (d, *J* = 6.0 Hz, 2H), 3.87 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 160.3, 158.6, 157.4, 137.8, 132.9, 131.2, 130.9, 130.4, 129.3, 128.1, 127.8, 122.5, 120.3, 119.5, 118.4, 118.3, 114.6, 112.0, 110.7, 55.5, 47.5 ppm. HRMS (ESI): m/z calcd for $C_{23}H_{20}N_3O_3$ $[M+H]^+$ 386.1499, found 386.1498.

(3Z,6Z)-1-allyl-6-(3-bromobenzylidene)-3-(3-nitrobenzylidene)piperazine-2,5-dione (4)

Following the general procedure, the product **4** was obtained in 63% yield as a slightly yellow solid. mp = 182 – 185 ˚C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.61 (s, 1H), 8.48 (s, 1H), 8.09 (d, *J* = 8.1 Hz, 1H), 7.75 (d, *J* = 7.7 Hz, 1H), 7.61 (t, *J* = 8.0 Hz, 1H), 7.51 (d, *J* = 8.0 Hz, 1H), 7.41 (s, 1H), 7.30 (t, *J* = 7.8 Hz, 1H), 7.21 (d, *J* = 7.7 Hz, 1H), 7.10 (s, 1H), 6.81 (s, 1H), 5.56 – 5.48 (m, 1H), 5.05 (d, *J* = 10.2 Hz, 1H), 4.78 (d, *J* = 17.1 Hz, 1H), 4.23 (d, *J* = 5.8 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 160.7, 158.9, 148.6, 135.7, 135.1, 134.7, 132.0, 131.0, 130.1, 130.0, 128.8, 127.80, 127.78, 123.5, 123.0, 122.6, 120.5, 118.5, 115.6, 48.1 ppm. HRMS (ESI): m/z calcd for $C_{21}H_{17}BrN_3O_4$ $[M+H]^+$ 454.0397, found 454.0400;

1-allyl-3-((Z)-2,3-dichlorobenzylidene)-6-((Z)-2-(trifluoromethyl)benzylidene)piperazine-2,5-dione (5)

Following the general procedure, the product **5** was obtained in 21% yield as a orange solid. mp = 109 – 111 ˚C. ^{1}H NMR (700 MHz, $CDCl_3$) δ 8.94 (s, 1H), 7.74 (d, *J* = 7.9 Hz, 1H), 7.57 (t, *J* = 7.6 Hz,

1H), 7.48 (t, *J* = 7.7 Hz, 1H), 7.42 – 7.37 (m, 2H), 7.35 (d, *J* = 7.6 Hz, 1H), 7.26 (t, *J* = 7.9 Hz, 1H), 7.24 (s, 1H), 7.13 (s, 1H), 5.56 – 5.46 (m, 1H), 5.00 (dd, *J* = 10.3, 1.1 Hz, 1H), 4.76 (dd, *J* = 17.1, 1.1 Hz, 1H), 4.09 (d, *J* = 7.0 Hz, 2H).^{13}C NMR (176 MHz, $CDCl_3$) δ 159.2, 158.4, 134.1, 133.3, 132.6, 132.5, 131.6, 130.9, 130.4 (d, J_{C-F} = 2.9 Hz), 129.8, 128.8, 128.6 (q, J_{C-F} = 30.3 Hz), 127.8, 127.6, 127.3, 126.3 (d, J_{C-F} = 4.6 Hz), 123.7 (q, J_{C-F} = 273.9 Hz), 117.9, 117.7, 114.8, 48.07 ppm. HRMS (ESI): m/z calcd for $C_{22}H_{16}Cl_2F_3N_2O_2$ $[M+H]^+$ 467.0538, found 467.0535.

1-allyl-3,6-bis((Z)-3-(trifluoromethyl)benzylidene)piperazine-2,5-dione (6)

Following the general procedure, the product **6** was obtained in 39% yield as a white solid. mp = 154 – 155 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.97 (s, 1H), 7.71 (s, 1H), 7.63 (s, 2H), 7.60 – 7.51 (m, 4H), 7.47 (d, *J* = 7.2 Hz, 1H), 7.10 (d, *J* = 7.0 Hz, 2H), 5.55 – 5.44 (m, 1H), 5.03 (d, *J* = 10.2 Hz, 1H), 4.72 (d, *J* = 17.0 Hz, 1H), 4.23 (s, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 160.0, 159.0, 134.7, 133.7, 132.3, 131.9, 131.7 (q, J_{C-F} = 37.2 Hz), 131.1 (q, J_{C-F} = 32.6 Hz), 131.0, 129.8, 129.3, 129.0, 126.9, 126.0 (d, J_{C-F} = 3.6 Hz), 125.6 (d, *J* = 3.7 Hz), 125.4 (d, *J* = 3.5 Hz), 125.3 (d, *J* = 3.5 Hz), 123.7 (q, J_{C-F} = 272.7 Hz), 123.7 (q, J_{C-F} = 272.6 Hz). 120.3, 118.4, 116.8, 48.0 ppm. HRMS (ESI): m/z calcd for $C_{23}H_{17}F_6N_2O_2$ $[M+H]^+$ 467.1189, found 467.1199.

1-allyl-3-(furan-2-ylmethylene)-6-(2-methoxybenzylidene)piperazine-2,5-dione (8)

Following the general procedure, the product **8** was obtained in 65% yield as a slightly yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.14 (s, 1H), 7.57 (s, 1H), 7.34 (s, 1H), 7.32 (d, *J* = 8.4 Hz, 1H), 7.18 (d, *J* = 7.3 Hz, 1H), 6.95 (t, *J* = 7.4 Hz, 1H), 6.90 (d, *J* = 8.3 Hz, 1H), 6.78 (s, 1H), 6.57 (d, *J* = 3.4 Hz, 1H), 6.51 (dd, *J* = 3.4, 1.8 Hz, 1H), 5.56 – 5.49 (m, 1H), 4.98 (d, *J* = 11.2 Hz, 1H), 4.74 (d, *J* = 17.1 Hz, 1H), 4.23 (d, *J* = 5.9 Hz, 2H), 3.83 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.5, 158.9, 157.3, 150.8, 143.9, 131.4, 130.4, 130.3, 128.3, 123.7, 123.0, 120.1, 118.1, 117.9, 114.2, 112.3, 110.5, 103.4, 55.4, 47.4 ppm.. HRMS (ESI): m/z calcd for $C_{20}H_{19}N_2O_4$ $[M+H]^+$ 351.1339, found 351.1346.

1-allyl-6-(2-methoxybenzylidene)-3-(thiophen-2-ylmethylene)piperazine-2,5-dione (9)

Following the general procedure, the product **9** was obtained in 46% yield as a slightly yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.06 (s, 1H), 7.45 (d, *J* = 5.1 Hz, 1H), 7.35 – 7.32 (m, 2H), 7.28 (d, *J* = 3.6 Hz, 1H), 7.20 (d, *J* = 5.0 Hz, 2H), 7.13 (dd, *J* = 5.1, 3.7 Hz, 1H), 6.96 (t, *J* = 7.5 Hz, 1H), 6.91 (d, *J* = 8.2 Hz, 1H), 5.59 – 5.48 (m, 1H), 4.99 (d, *J* = 11.3 Hz, 1H), 4.75 (d, *J* = 17.1 Hz, 1H), 4.22 (d, *J* = 6.0 Hz, 2H), 3.84 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.9, 159.1, 157.3, 135.8, 131.4, 130.5, 130.3, 129.7, 128.3, 128.1, 127.6, 124.1, 122.7, 120.2, 118.4, 118.0, 110.6, 110.4, 55.4, 47.3 ppm. HRMS (ESI): m/z calcd for $C_{20}H_{19}N_2O_3S$ $[M+H]^+$ 367.1111, found 367.1114.

(3Z,6Z)-1-allyl-6-(2-methoxybenzylidene)-3-(pyridin-2-ylmethylene)piperazine-2,5-dione (10)

Following the general procedure, the product **10** was obtained in 38% yield as a slightly yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.66 (s, 1H), 8.63 (s, 1H), 7.71 (t, *J* = 7.7 Hz, 1H), 7.38 (s, 1H), 7.33 (t, *J* = 7.9 Hz, 2H), 7.19 (d, *J* = 7.3 Hz, 2H), 6.96 (t, *J* = 7.4 Hz, 1H), 6.90 (d, *J* = 8.3 Hz, 1H), 6.79 (s, 1H), 5.58 – 5.50 (m, 1H), 4.99 (d, *J* = 10.2 Hz, 1H), 4.75 (d, *J* = 17.1 Hz, 1H), 4.26 (d, *J* = 5.6 Hz, 2H), 3.84 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.5, 158.8, 157.2, 154.9, 148.4, 136.9, 131.4, 130.9, 130.35, 130.32, 128.6, 125.9, 123.2, 122.0, 120.1, 118.2, 117.9, 110.6, 109.8, 55.4, 47.4 ppm. HRMS (ESI): m/z calcd for $C_{21}H_{20}N_3O_3$ $[M+H]^+$ 362.1499, found 362.1507.

1-allyl-6-(2-methoxybenzylidene)-3-(naphthalen-1-ylmethylene)piperazine-2,5-dione (11)

Following the general procedure, the product **11** was obtained in 58% yield as a slightly yellow solid. mp = 80 – 82 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.02 – 7.98 (m, 2H), 7.90 – 7.86 (m, 2H), 7.56 – 7.50 (m, 5H), 7.36 (t, *J* = 8.4 Hz, 1H), 7.32 (s, 1H), 7.25 (d, *J* = 8.3 Hz, 1H), 7.00 (t, *J* = 7.3 Hz, 1H), 6.93 (d, *J* = 8.3 Hz, 1H), 5.63 – 5.55 (m, 1H), 5.03 (d, *J* = 11.2 Hz, 1H), 4.80 (d, *J* = 15.9 Hz, 1H), 4.31 (d, *J* = 6.0 Hz, 2H), 3.87 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.8, 158.9, 157.3, 133.8, 131.5, 131.4, 130.5, 130.4,

 doi:10.20944/preprints202304.1053.v1

129.8, 129.3, 128.7, 128.2, 127.6, 126.9, 126.6, 126.0, 125.5, 124.5, 122.9, 120.2, 118.5, 118.1, 115.3, 110.6, 55.4, 47.4 ppm. HRMS (ESI): m/z calcd for $C_{26}H_{23}N_2O_3$ $[M+H]^+$ 411.1703, found 411.1696.

1-allyl-6-(2-methoxybenzylidene)-3-(quinolin-2-ylmethylene)piperazine-2,5-dione (12)

Following the general procedure, the product **12** was obtained in 56% yield as a slightly yellow solid. mp = 170 – 173 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 13.23 (s, 1H), 8.18 – 8.14 (m, 2H), 7.78 (d, *J* = 8.1 Hz, 1H), 7.74 (t, *J* = 7.1 Hz, 1H), 7.54 (t, *J* = 7.5 Hz, 1H), 7.43 (t, *J* = 5.1 Hz, 2H), 7.34 (t, *J* = 7.8 Hz, 1H), 7.22 (d, *J* = 7.3 Hz, 1H), 6.97 (t, *J* = 7.4 Hz, 1H), 6.92 (d, *J* = 10.4 Hz, 2H), 5.61 – 5.53 (m, 1H), 5.01 (d, *J* = 11.2 Hz, 1H), 4.79 (d, *J* = 18.2 Hz, 1H), 4.30 (d, *J* = 5.9 Hz, 2H), 3.86 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.6, 158.6, 157.3, 155.1, 146.8, 136.8, 132.2, 131.4, 130.39, 130.37, 130.2, 128.8, 128.6, 127.5, 127.0, 126.7, 123.8, 123.2, 120.2, 118.4, 117.9, 110.6, 109.3, 55.4, 47.5 ppm. HRMS (ESI): m/z calcd for $C_{25}H_{22}N_3O_3$ $[M+H]^+$ 412.1656, found 412.1660.

(Z)-1-allyl-6-((Z)-2-methoxybenzylidene)-3-((6-methoxynaphthalen-2-yl)methylene)piperazine-2,5-dione (13)

Following the general procedure, the product **13** was obtained in 45% yield as a slightly yellow solid. mp = 156 – 158 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.24 (s, 1H), 7.86 (s, 1H), 7.77 (dd, *J* = 15.0, 8.7 Hz, 2H), 7.48 (dd, *J* = 8.5, 1.5 Hz, 1H), 7.40 – 7.33 (m, 1H), 7.32 (s, 1H), 7.23 (d, *J* = 7.0 Hz, 1H), 7.21 – 7.16 (m, 2H), 7.12 (d, *J* = 2.3 Hz, 1H), 6.98 (t, *J* = 7.4 Hz, 1H), 6.92 (d, *J* = 8.3 Hz, 1H), 5.57 (ddt, *J* = 16.3, 10.3, 6.0 Hz, 1H), 5.01 (dd, *J* = 10.2, 1.1 Hz, 1H), 4.78 (dd, *J* = 17.1, 1.2 Hz, 1H), 4.26 (d, *J* = 6.0 Hz, 2H), 3.93 (s, 3H), 3.86 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 160.2, 159.4, 158.6, 157.4, 134.4, 131.5, 130.5, 130.4, 129.7, 128.8, 128.5, 128.2, 127.9, 127.9, 126.5, 125.6, 122.9, 120.2, 119.8, 118.3, 118.0, 117.7, 110.6, 105.8, 55.5, 55.4, 47.4 ppm. HRMS (ESI): m/z calcd for $C_{27}H_{25}N_2O_4$ $[M+H]^+$ 441.1809, found 441.1815.

(Z)-1-allyl-3-(naphthalen-1-ylmethylene)-6-((Z)-2-(trifluoromethyl)benzylidene)piperazine-2,5-dione (14)

Following the general procedure, the product **14** was obtained in 30% yield as a slightly yellow solid. mp = 98 – 101 °C. ^{1}H NMR (700 MHz, $CDCl_3$) δ 8.52 (s, 1H), 8.04 – 7.94 (m, 1H), 7.83 (t, *J* = 7.0 Hz, 2H), 7.74 (d, *J* = 7.9 Hz, 1H), 7.59 (s, 1H), 7.58 – 7.55 (m, 2H), 7.55 – 7.50 (m, 3H), 7.48 (t, *J* = 7.7 Hz, 1H), 7.38 (d, *J* = 7.6 Hz, 1H), 7.33 (s, 1H), 5.57 (ddd, *J* = 22.7, 10.6, 5.6 Hz, 1H), 5.04 (dd, *J* = 10.3, 0.9 Hz, 1H), 4.81 (dd, *J* = 17.1, 1.0 Hz, 1H), 4.15 (s, 2H).^{13}C NMR (176 MHz, $CDCl_3$) δ 158.9, 158.8, 133.8, 133.0, 131.6, 131.5, 131.1, 130.6, 130.12, 129.6, 129.4, 128.7 (q, J_{C-F} = 30.6 Hz), 128.7, 127.0, 126.9, 126.6, 126.4 (d, J_{C-F}= 3.1 Hz), 125.5, 124.5, 123.8 (q, J_{C-F} = 273.4 Hz), 121.5, 117.8, 117.3, 116.4, 48.1 ppm. HRMS (ESI): m/z calcd for $C_{26}H_{20}F_3N_2O_2$ $[M+H]^+$ 449.1471, found 449.1480.

(Z)-1-allyl-3-(naphthalen-1-ylmethylene)-6-((Z)-3-(trifluoromethyl)benzylidene)piperazine-2,5-dione (15)

Following the general procedure, the product **15** was obtained in 37% yield as a slightly yellow solid. mp = 111 – 113 °C. ^{1}H NMR (700 MHz, $CDCl_3$) δ 8.32 (d, *J* = 19.6 Hz, 1H), 8.02 – 7.97 (m, 1H), 7.87 (dd, *J* = 11.7, 5.6 Hz, 2H), 7.62 (t, *J* = 7.6 Hz, 1H), 7.60 (d, *J* = 9.1 Hz, 1H), 7.56 (dd, *J* = 6.6, 1.3 Hz, 2H), 7.55 (dd, *J* = 5.3, 2.3 Hz, 2H), 7.54 (s, 1H), 7.52 (t, *J* = 6.3 Hz, 1H), 7.50 (t, *J* = 6.0 Hz, 1H), 7.15 (s, 1H), 5.54 (ddt, *J* = 16.1, 10.3, 5.8 Hz, 1H), 5.05 (dd, *J* = 10.2, 1.1 Hz, 1H), 4.75 (dd, *J* = 17.1, 1.1 Hz, 1H), 4.28 (d, *J* = 5.8 Hz, 2H).^{13}C NMR (176 MHz, $CDCl_3$) δ 159.2, 159.0, 135.0, 133.8, 132.3, 131.5, 131.1, 130.9 (q, J_{C-F} = 32.8 Hz), 129.6, 129.4, 129.3, 128.9, 128.6, 127.1, 126.9, 126.6, 126.2, 126.0 (d, J_{C-F} = 2.4 Hz), 125.4, 125.2 (d, J_{C-F} = 2.4 Hz), 124.5, 123.5 (q, J_{C-F} = 272.6 Hz), 119.6, 118.2, 116.6, 48.0 ppm. HRMS (ESI): m/z calcd for $C_{26}H_{20}F_3N_2O_2$ $[M+H]^+$ 449.1471, found 449.1475.

(Z)-1-allyl-6-((Z)-2-fluorobenzylidene)-3-((6-methoxynaphthalen-2-yl)methylene)piperazine-2,5-dione (16)

Following the general procedure, the product **16** was obtained in 67% yield as a bright yellow solid. mp = 152 – 155 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.71 (s, 1H), 7.87 (s, 1H), 7.77 (d, *J* = 8.5 Hz, 1H),

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 27 April 2023 doi:10.20944/preprints202304.1053.v1

7.74 (d, J = 9.0 Hz, 1H), 7.48 (dd, J = 8.5, 1.5 Hz, 1H), 7.36 (td, J = 7.3, 1.5 Hz, 1H), 7.26 – 7.22 (m, 1H), 7.21 (s, 1H), 7.19 – 7.16 (m, 1H), 7.16 – 7.13 (m, 1H), 7.11 (t, J = 9.2 Hz, 1H), 7.07 (d, J = 9.3 Hz, 2H), 5.53 (ddt, J = 16.2, 10.3, 5.9 Hz, 1H), 5.01 (dd, J = 10.2, 1.0 Hz, 1H), 4.74 (dd, J = 17.1, 1.1 Hz, 1H), 4.28 (d, J = 5.9 Hz, 2H), 3.88 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 156.0 (d, J_{C-F} = 251.1 Hz), 159.7, 159.3, 158.6, 134.4, 131.2, 130.7, 130.7, 126.0, 129.7, 128.7, 128.0, 128.0, 127.8, 126.5, 125.2, 123.9 (d, J_{C-F} = 3.3 Hz), 122.2 (d, J_{C-F} = 14.7 Hz), 119.7, 118.6, 118.2, 115.7 (d, J_{C-F} = 21.3 Hz), 114.4, 105.7, 55.3, 47.2. HRMS (ESI): m/z calcd for $C_{26}H_{22}FN_2O_3$ $[M+H]^+$ 429.1609 found 429.1601.

3.1.2. liposolubility tests

In a 1.5 mL vial each containing 1.0 mg of the synthetic compound, the solvent (DMAO, DMF, AcOEt, DCM, chloroform or acetone) was then added dropwise and the mixture was stirred simultaneously at the room temperature until the complete dissolubility of the compound. The liposolubilites for all compounds were estimated and calculated on the basis of the volume (less than 1.0 mL each) of the above solvents used.

3.2. Biological evaluations

3.2.1. Cytotoxicity bioassay

Cell viability was analyzed by 3-(4,5)-dimethylthiahiazo (-z-y1)-3,5-di-phenytetrazoliu mromide (MTT) assay as previously described [29]. In brief, cells were seeded in a 96-well plate at a density of 3 × 10^3 per well treated with compounds (0.039, 0.078, 0.156, 0.312, 0.625, 1.25, 2.5, 5, 10 μM) in an incubator (5% CO_2, 37℃) for 48 h. The OD_{570} values were measured using an ELISA Reader. The experiment was independently repeated for three times.

3.2.2. Apoptosis and cell cycle assay

A549 and Hela cells were seeded and incubated in the six-well plate at a density of 2.0 × 10^5 cells per well treated with DMSO (0.1 %, v/v), paclitaxel (1.0 μM) or compound **11** (0.25, 0.5, 1.0 μM), respectively, for 48 h. The cells were then collected and stained with both annexin V-FITC and PI solutions, by following the manufacturer's protocol (BMS500FI-300, Thermo Fisher Scientific,Waltham, MA, USA). Apoptotic rates and cell cycle distribution of both cells were examined and analyzed by a flow cytometer (BD Fac SCanto II, USA). Three parallel experiments were performed for each concentration.

4. Conclusion

Sixteen 2,5-DPKs with different functions substituted at the 3,6-positions of the 2,5-DPK ring were designed, synthesized and investigated as anticancer agents against cell lines A549 and Hela. 2-MeO on the phenyl group at the 6-position and the suitable size or skeleton substituents at the 3-postion on 2,5-DPK ring might give combined impacts on the anticancer activities of derivatives. The one-pot method for the synthesis of these derivatives is simple and operationally convenient, which is also a guarantee of providing enough amounts of the active derivatives for the further druggability assessments. The biological evaluation disclosed that the activities of most derivatives were not obviously improved except compound **11**, which is a promising skeleton for the further development to explore high anticancer agents, and therefore our ongoing studies are now mainly focusing on this compound.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org. Figure S1-S28: the NMR spectra for all the derivatives.

Author Contributions: Conceptualization and design, S.T., S.L. and Y.L.; chemical synthesis & data collection, X.L., H.X and B.Y.; biological evaluation and data analysis, T.X., X.P. and X.L. writing—original draft preparation, X.L. and T.X.; writing—review and editing, S.T. and S.L.; project administration, J.W., X.Z. and S.L.;

supervision, S.L. and Y.L.; funding acquisition, S.T., S.L. and Y.L.. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Finance Science and Technology Project of Hainan Province (ZDKJ202018), the National Natural Science Foundation of China (82073762, 82072276), the Guangdong Basic and Applied Basic Research Foundation (2021B1515120046, 2021A1515011523).

Informed Consent Statement: Not applicable.

Acknowledgments: We are grateful to Z. Xiao and X. Zheng (for NMR); A. Sun, Y. Zhang, and X. Ma (for MS) at SCSIO for analytic data recording.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Huang, R. M.; Yi, X. X.; Zhou, Y.; Su, X.; Peng, Y.; Gao, C. H., An update on 2,5-diketopiperazines from marine organisms. *Mar. Drugs* **2014,** *12*, 6213-6235.
2. Song, Z.; Hou, Y.; Yang, Q.; Li, X.; Wu, S., Structures and Biological Activities of Diketopiperazines from Marine Organisms: A Review. *Mar. Drugs* **2021,** *19*, 403.
3. Borthwick, A. D., 2,5-Diketopiperazines: Synthesis, Reactions, Medicinal Chemistry, and Bioactive Natural Products. *Chem. Rev.* **2012,** *112*, 3641-3716.
4. Huber, E. M., Epipolythiodioxopiperazine-Based Natural Products: Building Blocks, Biosynthesis and Biological Activities. *Chembiochem* **2022,** *23*, e202200341.
5. Borgman, P.; Lopez, R. D.; Lane, A. L., The expanding spectrum of diketopiperazine natural product biosynthetic pathways containing cyclodipeptide synthases. *Org. Biomol. Chem.* **2019,** *17*, 2305-2314.
6. Mishra, A. K.; Choi, J.; Choi, S. J.; Baek, K. H., Cyclodipeptides: An Overview of Their Biosynthesis and Biological Activity. *Molecules* **2017,** *22*, 1796.
7. Winyakul, C.; Phutdhawong, W.; Tamdee, P.; Sirirak, J.; Taechowisan, T.; Phutdhawong, W. S., 2,5-Diketopiperazine Derivatives as Potential Anti-Influenza (H5N2) Agents: Synthesis, Biological Evaluation, and Molecular Docking Study. *Molecules* **2022,** *27*, 4200.
8. Fu, Z. Y.; Hou, Y. W.; Ji, C. P.; Ma, M. X.; Tian, Z. H.; Deng, M. Y.; Zhong, L. L.; Chu, Y. Y.; Li, W. B., Design, synthesis and biological evaluation of anti-pancreatic cancer activity of plinabulin derivatives based on the co-crystal structure. *Bioorgan. Med. Chem.* **2018,** *26*, 2061-2072.
9. Ding, Z. P.; Ma, M. X.; Zhong, C. J.; Wang, S. X.; Fu, Z. Y.; Hou, Y. W.; Liu, Y. Q.; Zhong, L. L.; Chu, Y. Y.; Li, F.; Song, C.; Wang, Y. X.; Yang, J. L.; Li, W. B., Development of novel phenoxy-diketopiperazine-type plinabulin derivatives as potent antimicrotubule agents based on the co-crystal structure. *Bioorgan. Med. Chem.* **2020,** *28*, 115186.
10. Ganesher, A.; Chaturvedi, P.; Karkara, B. B.; Chatterjee, I.; Datta, D.; Panda, G., One pot synthesis of N-monoalkylated plinabulin derivatives via multicomponent protocol and their application as anticancer agents. *J. Mol. Struct.* **2021,** *1229*, 129830.
11. Liao, S.; Qin, X.; Li, D.; Tu, Z.; Li, J.; Zhou, X.; Wang, J.; Yang, B.; Lin, X.; Liu, J.; Yang, X.; Liu, Y., Design and synthesis of novel soluble 2,5-diketopiperazine derivatives as potential anticancer agents. *Euro. J. Med. Chem.* **2014,** *83*, 236-244.
12. Yamazaki, Y.; Tanaka, K.; Nicholson, B.; Deyanat-Yazdi, G.; Potts, B.; Yoshida, T.; Oda, A.; Kitagawa, T.; Orikasa, S.; Kiso, Y.; Yasui, H.; Akamatsu, M.; Chinen, T.; Usui, T.; Shinozaki, Y.; Yakushiji, F.; Miller, B. R.; Neuteboom, S.; Palladino, M.; Kanoh, K.; Lloyd, G. K.; Hayashi, Y., Synthesis and structure-activity relationship study of antimicrotubule agents phenylahistin derivatives with a didehydropiperazine-2,5-dione structure. *J. Med. Chem.* **2012,** *55*, 1056-71.
13. Labriere, C.; Cervin, G.; Pavia, H.; Hansen, J. H.; Svenson, J., Structure-Activity Relationship Probing of the Natural Marine Antifoulant Barettin. *Mar. Biotechnol.* **2021,** *23*, 904-916.
14. Sjogren, M.; Johnson, A. L.; Hedner, E.; Dahlstrom, M.; Goransson, U.; Shirani, H.; Bergman, J.; Jonsson, P. R.; Bohlin, L., Antifouling activity of synthesized peptide analogs of the sponge metabolite barettin. *Peptides* **2006,** *27*, 2058-2064.
15. Labriere, C.; Andersen, J. H.; Albrigtsen, M.; Hansen, J. H.; Svenson, J., Heterocyclic cellular lipid peroxidation inhibitors inspired by the marine antioxidant barettin. *Bioorgan. Chem.* **2019,** *84*, 106-114.
16. Bryans, J.; Charlton, P.; Chicarelli-Robinson, I.; Collins, M.; Faint, R.; Latham, C.; Shaw, I.; Trew, S., Inhibition of plasminogen activator inhibitor-1 activity by two diketopiperazines, XR330 and XR334 produced by Streptomyces sp. *J. Antibio.* **1996,** *49*, 1014-1021.
17. Wang, S. M.; Golec, J.; Miller, W.; Milutinovic, S.; Folkes, A.; Williams, S.; Brooks, T.; Hardman, K.; Charlton, P.; Wren, S.; Spencer, J., Novel inhibitors of plasminogen activator inhibitor-1: Development of new templates from diketopiperazines. *Bioorgan. Med. Chem. Lett.* **2002,** *12*, 2367-2370.

18. Kamei, H.; Oka, M.; Hamagishi, Y.; Tomita, K.; Konishi, M.; Oki, T., Piperafizines A and B, potentiators of cytotoxicity of vincristine. *J. Antibio.* **1990,** *43*, 1018-1020.
19. Kanoh, K.; Kohno, S.; Asari, T.; Harada, T.; Katada, J.; Muramatsu, M.; Kawashima, H.; Sekiya, H.; Uno, I., (-)-phenylahistin: A new mammalian cell cycle inhibitor produced by Aspergillus ustus. *Bioorgan. Med. Chem. Lett* **1997,** *7*, 2847-2852.
20. Nicholson, B.; Lloyd, G. K.; Miller, B. R.; Palladino, M. A.; Kiso, Y.; Hayashi, Y.; Neuteboom, S. T. C., NPI-2358 is a tubulin-depolymerizing agent: in-vitro evidence for activity as a tumor vascular-disrupting agent. *Anti-Cancer Drug.* **2006,** *17*, 25-31.
21. Ma, M. X.; Ding, Z. P.; Wang, S. X.; Ma, L. L.; Wang, Y. X.; Zhong, L. L.; Li, Z. P.; Yang, J. L.; Li, W. B., Polymorphs, co-crystal structure and pharmacodynamics study of MBRI-001, a deuterium-substituted plinabulin derivative as a tubulin polymerization inhibitor. *Bioorgan. Med. Chem.* **2019,** *27*, 1836-1844.
22. Chen, X.; Chen, X. Y.; Steimbach, R. R.; Wu, T.; Li, H. M.; Dan, W. J.; Shi, P. D.; Cao, C. Y.; Li, D.; Miller, A. K.; Qiu, Z. X.; Gao, J. M.; Zhu, Y., Novel 2, 5-diketopiperazine derivatives as potent selective histone deacetylase 6 inhibitors: Rational design, synthesis and antiproliferative activity. *Euro. J. Med. Chem.* **2020,** *187*, 111950.
23. Guo, H. Y.; Chen, Z. A.; Shen, Q. K.; Quan, Z. S., Application of triazoles in the structural modification of natural products. *J. Enzym. Inhib. Med. Chem.* **2021,** *36*, 1115-1144.
24. Guo, L. H.; Zhang, H. R.; Tian, M.; Tian, Z. Z.; Xu, Y. J.; Yang, Y. L.; Peng, H. W.; Liu, P.; Liu, Z., Electronic effects on reactivity and anticancer activity by half-sandwich N,N-chelated iridium(III) complexes. *New. J. Chem.* **2018,** *42*, 16183-16192.
25. Labriere, C.; Kondori, N.; Caous, J. S.; Boomgaren, M.; Sandholm, K.; Ekdahl, K. N.; Hansen, J. H.; Svenson, J., Development and evaluation of cationic amphiphilic antimicrobial 2,5-diketopiperazines. *J. Pept. Sci.* **2018,** *24*, e3090.
26. Liao, S. R.; Du, L. J.; Qin, X. C.; Xu, L.; Wang, J. F.; Zhou, X. F.; Tu, Z. C.; Li, J.; Liu, Y. H., Site selective synthesis of cytotoxic 1,3,6-trisubstituted 3,6-diunsaturated (3Z,6Z)-2,5-diketopiperazines via a one-pot multicomponent method. *Tetrahedron* **2016,** *72*, 1051-1057.
27. Zhang, C. C.; Li, C. G.; Wang, Y. F.; Xu, L. H.; He, X. H.; Zeng, Q. Z.; Zeng, C. Y.; Mai, F. Y.; Hu, B.; Ouyang, D. Y., Chemotherapeutic paclitaxel and cisplatin differentially induce pyroptosis in A549 lung cancer cells via caspase-3/GSDME activation. *Apoptosis* **2019,** *24*, 312-325.
28. Liao, S. R.; Xu, Y.; Tang, Y.; Wang, J. F.; Zhou, X. F.; Xu, L.; Liu, Y. H., Design, synthesis and biological evaluation of soluble 2,5-diketopiperazines derivatives as potential antifouling agents. *Rsc. Adv.* **2015,** *5*, 51020-51026.
29. Cai, J.; Wang, X. N.; Gan, X.; Zhou, Q.; Luo, X. W.; Yang, B.; Liu, Y. H.; Ratnasekera, D.; Zhou, X. F., New Chlorinated Metabolites and Antiproliferative Polyketone from the Mangrove Sediments-Derived Fungus Mollisia sp. SCSIO41409. *Mar. Drugs* **2023,** *21*, 32.