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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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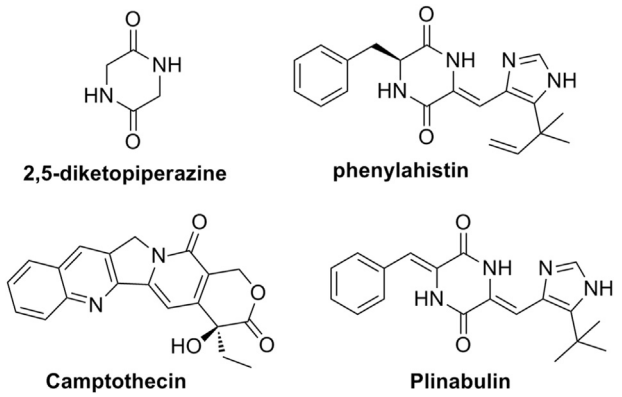


Fig. 1. The structures of Camptothecin, 2,5-diketopiperazine and its derivatives.

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

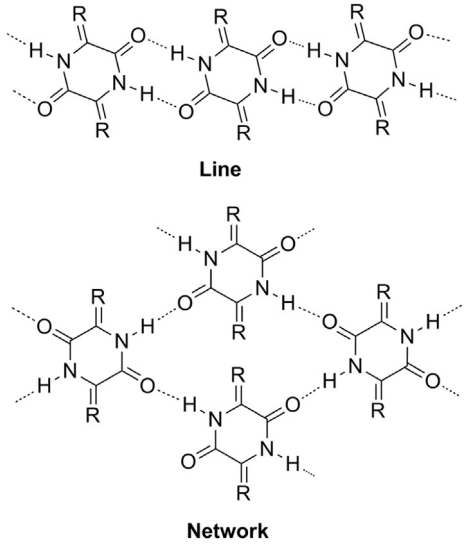


Fig. 2. Schematic view of H-bonding patterns for 2,5-diketopiperazine derivatives.

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.

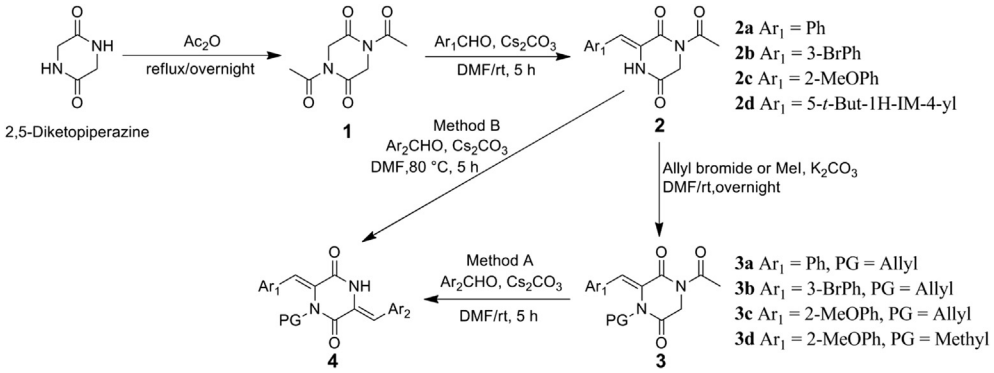
2.2. Biological activity

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



4a Ar_1 = Ph, Ar_2 = 3,4-ClPh, PG = Allyl
4b Ar_1 = Ph, Ar_2 = 3,4,5-MeOPh, PG = Allyl
4c Ar_1 = 3-BrPh, Ar_2 = 3-BrPh, PG = Allyl
4d Ar_1 = 3-BrPh, Ar_2 = 3,4,5-MeOPh, PG = Allyl
4e Ar_1 = 2-MeOPh, Ar_2 = 3-BrPh, PG = Allyl
4f Ar_1 = 2-MeOPh, Ar_2 = 3-ClPh, PG = Allyl
4g Ar_1 = 2-MeOPh, Ar_2 = 3-FPh, PG = Allyl
4h Ar_1 = 2-MeOPh, Ar_2 = 3-MePh, PG = Allyl
4i Ar_1 = 2-MeOPh, Ar_2 = 2-ClPh, PG = Allyl
4j Ar_1 = 2-MeOPh, Ar_2 = 2-FPh, PG = Allyl
4k Ar_1 = 2-MeOPh, Ar_2 = 2-CF_3Ph, PG = Allyl
4l Ar_1 = 2-MeOPh, Ar_2 = 2-MeOPh, PG = Allyl
4m Ar_1 = 2-MeOPh, Ar_2 = 2,3-ClPh, PG = Allyl
4n Ar_1 = 2-MeOPh, Ar_2 = 3,4-ClPh, PG = Allyl
4o Ar_1 = 2-MeOPh, Ar_2 = 5-Br-2-FPh, PG = Allyl
4p Ar_1 = 2-MeOPh, Ar_2 = 3-Br-4-FPh, PG = Allyl
4q Ar_1 = 2-MeOPh, Ar_2 = 5-F-2-MePh, PG = Allyl
4r Ar_1 = 2-MeOPh, Ar_2 = 4-MePh, PG = Allyl
4s Ar_1 = 2-MeOPh, Ar_2 = 2-MeOPh, PG = Methyl
4t Ar_1 = 2-MeOPh, Ar_2 = 2-ClPh, PG = Methyl
4u Ar_1 = 2-MeOPh, Ar_2 = 2,3-ClPh, PG = Methyl
4v Ar_1 = 2-MeOPh, Ar_2 = 2-MeOPh, PG = H
4w Ar_1 = 2-MeOPh, Ar_2 = 2,3-ClPh, PG = H
4x Ar_1 = 5-*t*-But-1*H*-IM-4-yl, Ar_2 = 2-MeOPh, PG = H

Scheme 1. Synthesis of the 2,5-diketopiperazine derivatives.

growth, whereas **4s** had moderate activity. The unprotected compounds **4v** and **4w** had no activity, while **4x** and plinabulin had high anticancer activity (Table 2). These results were similar to those for their suppressing the growth of other six cancer cell lines. Combining the results in Tables 1 and 2, we found that almost half of the derivatives had high and broad-spectrum activity against these ten cancer cell lines, and all active derivatives seemed to be more effective against cell line U937, especially the allyl-protected compound **4m** with IC_{50} value of 0.5 μM.

The IC_{50} values of compound **4m** for ten cancer cell lines were compared as shown in Fig. 3. It was found that **4m** had high inhibitory activity to cell lines U937 (IC_{50} = 0.5 μM) and K562

Table 1
The IC_{50} values (μM) of derivatives against cancer cell lines.

Compd.	Cell lines[a]					
	BGC-823	Hela	Huh-7	MCF-7	H1975	A549
4a	NA[b]	NA	NA	NA	NA	NA
4b	NA	NA	NA	NA	NA	NA
4c	NA	NA	NA	NA	NA	NA
4d	NA	NA	NA	NA	NA	NA
4e	5.9 ± 0.6	5.4 ± 0.3	2.3 ± 0.7	5.4 ± 0.2	NA	5.4 ± 0.2
4f	4.6 ± 0.7	5.5 ± 0.9	8.9 ± 0.9	5.2 ± 0.1	5.1 ± 0.1	5.2 ± 0.1
4g	5.6 ± 0.1	9.1 ± 0.3	27.2 ± 9.5	13.7 ± 1.7	25.6 ± 5.2	19.7 ± 6.0
4h	20.4 ± 0.1	19.3 ± 2.0	21.4 ± 0.7	20.7 ± 0.3	32.8 ± 5.4	22.7 ± 1.1
4i	4.6 ± 0.1	5.1 ± 0.8	6.4 ± 0.7	4.7 ± 0.3	9.2 ± 0.5	5.3 ± 0.3
4j	18.9 ± 3.5	45 ± 12.9	31.3 ± 0.5	23.0 ± 4.0	25.1 ± 3.9	NA
4k	5.1 ± 0.2	4.6 ± 0.2	9.5 ± 1.7	6.5 ± 0.9	14.5 ± 4.1	7.0 ± 0.6
4l	13.4 ± 2.4	6.9 ± 1.2	21.2 ± 2.9	41.1 ± 8.0	21.5 ± 2.8	22.4 ± 3.7
4m	2.2 ± 0.9	1.6 ± 0.2	1.2 ± 0.2	2.1 ± 0.6	4.5 ± 0.5	2.3 ± 0.2
4n	NA	NA	NA	NA	NA	NA
4o	NA	34.6 ± 4	NA	28.1 ± 5.0	NA	NA
4p	NA	NA	NA	NA	NA	NA
4q	1.6 ± 0.8	2.9 ± 0.7	5.6 ± 0.5	3.1 ± 0.7	7.3 ± 0.5	4.9 ± 0.1
4r	NA	NA	NA	NA	NA	NA
4s	NA	NA	NA	9.0 ± 6.0	16.9 ± 9.0	7.0 ± 3.0
4t	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1
4u	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1
4v[c]	NA	NA	NA	NA	NA	NA
4w[c]	NA	NA	NA	NA	NA	NA
4x	0.09 ± 0.04	0.08 ± 0.02	0.09 ± 0.02	0.10 ± 0.01	0.10 ± 0.02	0.09 ± 0.02
Plinabulin[d]	0.009 ± 0.005	0.010 ± 0.001	0.031 ± 0.006	0.010 ± 0.002	0.011 ± 0.001	0.010 ± 0.002
TSA[d]	0.080 ± 0.007	0.11 ± 0.02	0.10 ± 0.01	0.060 ± 0.001	0.21 ± 0.02	0.050 ± 0.001

[a] Each value represents mean ± SD of three experiments.
[b] NA means no activity.
[c] These results might not be accurate because of the compounds' poor solubility.
[d] Plinabulin and TSA (trichostatin A) are used as positive controls.

Table 2
The IC_{50} values (μM) of derivatives against four leukemic cell lines.

Compd.	Cell lines[a]			
	HL60	K562	MOLT-4	U937
4a	NA[b]	NA	NA	NA
4b	NA	NA	NA	NA
4c	NA	NA	NA	NA
4d	NA	NA	NA	NA
4e	5.2 ± 0.5	2.4 ± 0.1	5.0 ± 0.1	1.1 ± 0.0
4f	4.5 ± 0.3	2.7 ± 0.4	4.9 ± 0.1	1.2 ± 0.3
4g	8.89 ± 1.0	4.1 ± 0.2	6.5 ± 0.9	2.5 ± 0.2
4h	16.7 ± 2.1	8.8 ± 0.9	16.0 ± 1.9	5.1 ± 0.3
4i	4.2 ± 0.9	2.5 ± 0.1	4.4 ± 0.8	0.7 ± 0.1
4j	18.5 ± 1.7	5.6 ± 1.2	20.0 ± 0.5	5.5 ± 0.8
4k	4.6 ± 0.4	2.7 ± 0.4	4.4 ± 0.6	1.5 ± 0.1
4l	15.7 ± 3.0	7.4 ± 0.8	13.1 ± 0.5	5.1 ± 0.3
4m	2.0 ± 0.2	0.9 ± 0.1	1.2 ± 0.1	0.5 ± 0.0
4n	NA	NA	NA	NA
4o	17.4 ± 1.7	5.1 ± 0.1	12.9 ± 1.8	4.7 ± 0.3
4p	NA	NA	NA	NA
4q	2.6 ± 0.7	1.8 ± 0.3	3.8 ± 0.8	1.0 ± 0.0
4r	NA	NA	NA	NA
4s	16.2 ± 6.4	2.2 ± 0.2	–[c]	1.50 ± 0.08
4t	0.20 ± 0.02	0.11 ± 0.01	–	0.10 ± 0.01
4u	0.20 ± 0.02	0.11 ± 0.01	–	0.10 ± 0.01
4v[d]	NA	NA	–	NA
4w[d]	NA	NA	–	NA
4x	0.07 ± 0.02	0.060 ± 0.001	–	0.050 ± 0.003
Plinabulin[e]	0.010 ± 0.001	0.0080 ± 0.0006	–	0.0060 ± 0.0004
TSA[e]	0.08 ± 0.01	0.41 ± 0.01	0.030 ± 0.002	0.10 ± 0.01

[a] Each value represents mean ± SD of three experiments.
[b] NA means no activity.
[c] "–" means that the date are not obtained.
[d] These results might not be accurate because of the compounds' poor solubility.
[e] Plinabulin and TSA (trichostatin A) are used as positive controls.

(IC_{50} = 0.9 μM), without obvious activity difference to other cell lines. The relationship for the inhibitory activity versus the concentrations (0.01, 0.03, 0.1, 0.3, 1.0, 5.0, and 20.0 μM) of compound **4m** against cell lines U937, K562, and H1975 are shown in Fig. 4. Compound **4m** exhibited good concentration-dependent activity on these three cell lines, and almost completely suppressed the cell growth when its concentration was higher than 20 μM.

The viability assays of cancer cell lines U937, K562 and H1975 treated with compound **4m** at different concentrations (5 or 10 μM) for 24 h or 48 h were also carried out, and the results are summarized as shown in Table 3. No obvious change was found for the growth of cell lines K562 and H1975 treated with 5.0 or 10.0 μM of **4m** for 24 h, while the cell growth was significantly suppressed after 48 h incubation. In comparison, the growth of cell line U937 was significantly suppressed even after 24 h incubation with **4m** at 5.0 μM concentration. These results are consistent with the IC_{50} values as shown in Tables 1 and 2

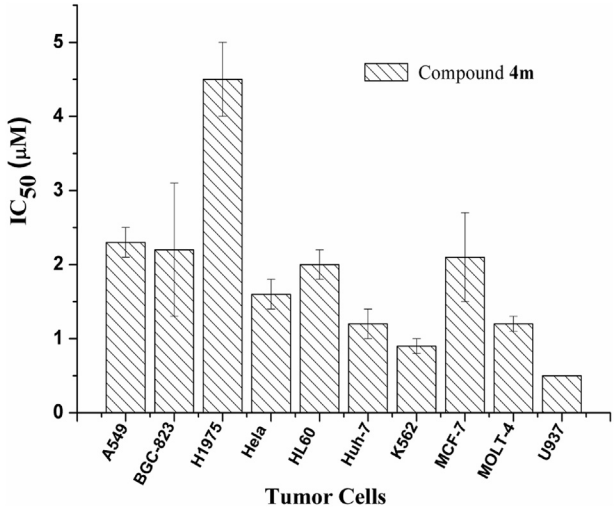


Fig. 3. A comparison for the effect of compound **4m** to ten cancer cell lines.

3. Conclusion

A novel series of mono-protected 2,5-diketopiperazine derivatives were synthesized and showed good solubility, and the non-protected 2,5-diketopiperazine derivatives with imidazole group as the substituent also showed good solubility. Our CCK8 assay results demonstrated that most of the active derivatives had high and broad-spectrum anticancer activity. Among the allyl-protected derivatives, compound **4m** might become a promising lead compound for further development for anticancer agents. Further studies for the effects of different protective groups on the anticancer activity of 2,5-diketopiperazine derivatives for their quantitative structure–activity relationship are currently in progress.

4. Experimental

4.1. Materials and synthetic methods

Melting points (m.p.) were determined by using a SRSO-ptiMelt automated melting point instrument without correction. NMR spectra were recorded with Bruker Avance spectrometers operating at 500 MHz for ^1H, and at 125 MHz for ^{13}C by using TMS as internal standard. IR spectra were recorded on a Shimadzu IR Affinity-1 fourier transform infrared spectrometer. Mass spectrometry data were collected with an HRMS-TOF instrument or a low-resolution MS instrument by using ESI ionization. Silica gel (200–300 mesh) and TLC plates (25 × 10 × 0.04 cm) from Qingdao Mar. Chem. Ind. Co. Ltd were used for chromatography. All solvents were analytical grade. DMF was dried in CaH_2 and distilled. Glycine anhydride was purchased from Alfa Aesar, trichostatin A (TSA, ≥98%) from Sigma Aldrich, and plinabulin (>98%) and others from Aladdin. All chemicals were used without further purification.

4.1.1. Synthesis of intermediate 1,4-diacetylpiperazine-2,5-dione (*1*)

Glycine anhydride (500 mg, 2.5 mmol) and acetic anhydride (20 mL) were heated under reflux overnight, and the excess acetic anhydride were removed under reduce pressure. The residue was purified by using silica gel chromatography and intermediate **1** was obtained as a white solid with yield of 97%. ^1H NMR (500 MHz, $CDCl_3$) δ = 4.56 (s, 4H, CH_2), 2.54 (s, 6H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 170.65, 165.83, 47.04, 26.58 ppm; MS (ESI) *m/z* (%) = 197.2 (100) $[M-H]^-$.

4.1.2. General procedure for synthesis of intermediates

1-Acetyl-3-benzylidenepiperazine-2,5-dione (**2a**),
1-Acetyl-3-(3-bromobenzylidene)piperazine-2,5-dione (**2b**),
1-Acetyl-3-(2-methoxybenzylidene)piperazine-2,5-dione (**2c**), and
1-Acetyl-3-((5-(tert-butyl)-1H-imidazole-4-yl)methylene) piperazine-2,5-dione (**2d**).

Cs_2CO_3 (1.5 equiv., 4.9 g) was added into the solution containing intermediate **1** (1.5 equiv., 3.0 g) and aromatic aldehyde (1.0 equiv., 10 mmol) in dry DMF (20 mL), and the mixture was stirred at room temperature for about 5 h. After the reaction was completed, the mixture was poured into crashed ice and the solid was filtered, washed with water for three times and dried. The target intermediates were obtained as white solids.

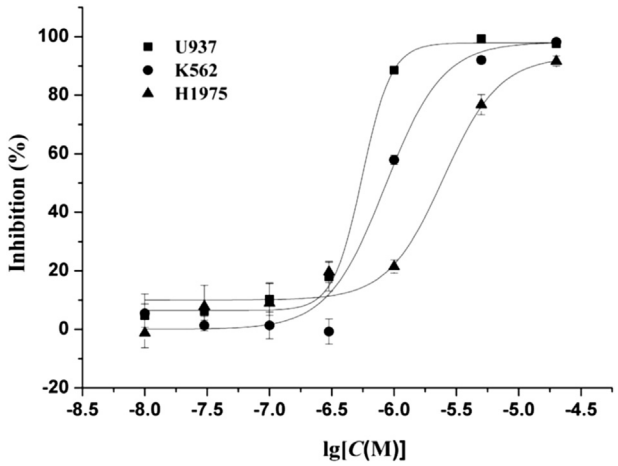


Fig. 4. The suppression of cancer cell line growth with varying concentrations (0.01, 0.03, 0.1, 0.3, 1.0, 5.0, and 20.0 μM) of compound **4m**.

*4.1.2.1. 1-Acetyl-3-benzylidenepiperazine-2,5-dione (**2a**).* Yield: 87%. ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.99 (s, 1H, N–H), 7.46 (t, J = 10.0 Hz, 2H, Ar–H), 7.40–7.38 (m, 3H, Ar–H), 7.18 (s, 1H, =C–H), 4.51 (s, 2H, CH_2), 2.66 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 172.47, 162.71, 159.95, 132.49, 129.56, 129.38, 128.52, 125.66, 119.93, 46.11, 27.19; MS (ESI) *m/z* (%) = 245.1 (5) $[M+H]^+$, 267.1 (4) $[M+Na]^+$, 243.3 (40) $[M-H]^-$, 201.3 (5).

*4.1.2.2. 1-Acetyl-3-(3-bromobenzylidene)piperazine-2,5-dione (**2b**).* Yield: 88%. ^{1}H NMR (500 MHz, $CDCl_3$) δ = 8.02 (s, 1H, N–H), 7.52 (d, J = 5.0 Hz, 2H, Ar–H), 7.36–7.31 (m, 2H, Ar–H), 7.09 (s, 1H, =C–H), 4.52 (s, 2H, CH_2), 2.66 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 172.40, 162.82, 159.59, 134.54, 132.32, 131.41, 130.99, 126.98, 126.62, 123.66, 118.1, 46.1, 27.2 ppm; MS (ESI) *m/z* (%) = 321.2 (100), 323.2 (98) $[M-H]^-$.

*4.1.2.3. 1-Acetyl-3-(2-methoxybenzylidene)piperazine-2,5-dione (**2c**).* Yield: 69%. ^{1}H NMR (500 MHz, $CDCl_3$) δ = 8.48 (s, 1H, N–H), 7.38 (t, J = 10.0 Hz, 1H, Ar–H), 7.30 (d, J = 10.0 Hz, 1H, Ar–H), 7.16 (s, 1H, =C–H), 7.03 (t, J = 10.0 Hz, 1H, Ar–H), 6.99 (d, J = 5.0 Hz, 1H, Ar–H), 4.46 (s, 2H, CH_2), 3.93 (s, 3H, OCH_3), 2.64 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 172.59, 162.50, 160.28, 156.29, 131.10, 131.05, 125.63, 121.67, 121.56, 117.25, 111.99, 55.97, 46.11, 27.13 ppm; MS (ESI) *m/z* (%) = 273.2 (70) $[M-H]^-$, 231.2 (35).

*4.1.2.4. 1-Acetyl-3-((5-(tert-butyl)-1H-imidazole-4-yl)methylene) piperazine-2,5-dione (**2d**).* Yield: 42%. ^{1}H NMR (500 MHz, $CDCl_3$) δ = 12.13 (s, 1H, N–H), 9.17 (s, 1H, N–H) 7.57 (s, 1H, Ar–H), 7.18 (s, 1H, =C–H), 4.47 (s, 2H, CH_2), 2.65 (s, 3H, CH_3), 1.46 (s, 9H, $3CH_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 172.81, 162.45, 160.53, 140.82, 132.31, 131.58, 123.90, 108.83, 46.46, 31.83, 30.70, 27.33 ppm; MS (ESI) *m/z* (%) = 313.3 (80) $[M+Na]^+$, 603.1 (50) [2 M+Na]$^+$, 289.4 (100) $[M-H]^-$.

4.1.3. General procedure for synthesis of intermediates

1-Acetyl-4-allyl-3-benzylidenepiperazine-2,5-dione (**3a**),

1-Acetyl-4-allyl-3-(3-bromobenzylidene)piperazine-2,5-dione (3**b**),

1-Acetyl-4-allyl-3-(2-methoxybenzylidene)piperazine-2,5-dione (**3c**), and

1-Acetyl-3-(2-methoxybenzylidene)-4-methylpiperazine-2,5-dione (**3d**).

K_2CO_3 (2 equiv., 1.38 g) was added into the solution containing intermediate **2** (1 equiv., 5.0 mmol) and allyl bromide (1.2 equiv., 0.5 mL) or CH_3I (1.2 equiv., 0.37 mL) in dry DMF (20 mL), and the mixture was stirred at room temperature overnight. After the reaction was completed, the mixture was poured into crashed ice and the solid was filtered, washed with water for three times and dried. The target intermediates were obtained as white solids.

*4.1.3.1. 1-Acetyl-4-allyl-3-benzylidenepiperazine-2,5-dione (**3a**).* Yield: 45%. ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.42–7.37 (m, 3H, Ar–H), 7.34 (d, J = 10.0 Hz, 2H, Ar–H), 7.29 (s, 1H, =C–H), 5.54–5.47 (m, 1H, =C–H), 5.01 (d, J = 10.0 Hz, 1H, =CH–H), 4.72 (d, J = 15.0 Hz, 1H, =CH–H), 4.52 (s, 2H, CH_2), 4.09 (d, J = 5.0 Hz, 2H, CH_2), 2.61 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 171.29, 164.80, 164.35, 132.63, 131.02, 129.67, 129.57, 129.26, 128.71, 126.64, 118.84, 46.35, 45.22, 26.61 ppm; MS (ESI) *m/z* (%) = 285.2 (10) $[M+H]^+$, 307.1 (10) $[M+Na]^+$, 241.3 (5).

*4.1.3.2. 1-Acetyl-4-allyl-3-(3-bromobenzylidene)piperazine-2,5-dione (3**b**).* Yield: 28%. ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.57 (d, J = 5.0 Hz, 1H, Ar–H), 7.53 (s, 1H, Ar–H), 7.36–7.31 (m, 2H, Ar–H), 7.30 (s, 1H, =C–H), 5.61–5.53 (m, 1H, =C–H), 5.10 (d, J = 10.0 Hz, 1H, =CH–H), 4.81 (d, J = 20.0 Hz, 1H, =CH–H), 4.58 (s, 2H, CH_2), 4.14 (d, J = 5.0 Hz, 2H, CH_2), 2.68 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 171.25, 164.68, 163.91, 134.82, 132.58, 132.24, 130.89, 130.49, 130.23, 127.99, 124.56, 122.87, 119.08, 46.63, 45.25, 26.70; MS (ESI) *m/z* (%) = 263.3 (15), 365.1 (14) $[M+H]^+$, 385.1 (15), 387.1 (15) $[M+Na]^+$.

*4.1.3.3. 1-Acetyl-4-allyl-3-(2-methoxybenzylidene)piperazine-2,5-dione (**3c**).* Yield: 44%. ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.44 (s, 1H, =C–H), 7.38 (t, J = 10.0 Hz, 1H, Ar–H), 7.23 (d, J = 10.0 Hz, 1H, Ar–H), 6.99 (t, J = 10.0 Hz, 1H, Ar–H), 6.93 (d, J = 10.0 Hz, 1H, Ar–H), 5.55–5.47 (m, 1H, =C–H), 5.01 (d, J = 10.0 Hz, 1H, =CH–H), 4.73 (d, J = 15.0 Hz, 1H, =CH–H), 4.51 (s, 2H, CH_2), 4.02 (d, J = 5.0 Hz, 2H, CH_2), 3.85 (s, 3H, OCH_3) 2.63 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 171.44, 164.61, 164.43, 157.52, 131.30, 131.05, 130.08, 129.34, 122.45, 121.69, 120.41, 118.64, 110.81, 55.57, 45.77, 45.23, 26.62 ppm; MS (ESI) *m/z* (%) = 313.2 (45), 315.2 (14) $[M+H]^+$, 337.2 (50) $[M+Na]^+$.

*4.1.3.4. 1-Acetyl-3-(2-methoxybenzylidene)-4-methylpiperazine-2,5-dione (**3d**).* Yellow solid, yield: 60.4%. ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.44 (s, 1H, =C–H), 7.36 (t, J = 10 Hz, 1H, Ar–H), 7.19 (d, J = 10 Hz, 1H, Ar–H), 6.98 (t, J = 10 Hz, 1H, Ar–H), 6.92 (d, J = 5 Hz, 1H, Ar–H), 4.52 (s, 2H, CH_2), 3.86 (s, 3H, OCH_3), 2.83 (s, 3H, N–CH_3),

Table 3
The viability of cancer cell lines treated with compound **4m**.

Compd. **4m**						
Cells	U937		K562		H1975	
Treatment	24 h	48 h	24 h	48 h	24 h	48 h
5 μM	7.0 ± 4.1[a]	0.9 ± 0.3	104.4 ± 0.5	24.2 ± 0.7	107.4 ± 1.2	42.7 ± 1.4
10 μM	8.7 ± 3.2	1.3 ± 0.2	103.2 ± 4.6	14.9 ± 0.4	98.9 ± 3.0	42.6 ± 0.8

[a] Each value represents mean ± SD of three experiments.

2.63 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 171.58, 164.71, 163.81, 157.48, 131.79, 131.05, 130.21, 121.73, 121. 42, 120.36, 110.66, 55.57, 45.30, 33.76, 26.71 ppm; MS (ESI) *m/z* (%) = 289.1 (100) $[M+H]^+$, 311.1 (50) $[M+Na]^+$, 599.0 (50) $[2\ M+Na]^+$.

*4.1.4. General procedure for synthesis of the target 2,5-diketopiperazine derivatives (**4**)*

Method A: Cs_2CO_3 (1.5 equiv.) was added into the solution containing intermediate **3** (1.0 equiv., 50.0 mg) and aromatic aldehyde (1.2 equiv.) in dry DMF (1.5 mL), and the mixture was stirred at room temperature for about 5 h. After the reaction was completed, the mixture was slowly poured into water and extracted with AcOEt for three times, and the organic layer was dried, filtered, and purified with silica gel column to afford the target compound.

Method B: Cs_2CO_3 (1.5 equiv.) was added into the solution containing intermediate **2** (1.0 equiv., 50.0 mg) and aromatic aldehyde (1.2 equiv.) in dry DMF (1.5 mL), and the mixture was stirred at 80 °C for about 5 h. The purification of the target compound was described as follows: compounds **4v** and **4w** were recrystallized in hot DMF, and compound **4x** was purified according to a previously reported procedure by using a semi-preparative HPLC column (ODS-AQ C_{18}, 250 × 10 mm, d.s-5 μM, 12 nm), and eluted with a mixture of solvent MeOH:H_2O = 60:40 in 0.1% aqueous TFA over 27 min at a flow rate of 2 mL min^{-1}. The desired fraction was collected, concentrated through evaporation, and then lyophilized to give the target compound.

*4.1.4.1. 1-Allyl-6-benzylidene-3-(3,4-dichlorobenzylidene)piperazine-2,5-dione (**4a**).* According to method A, compound **4a** was synthesized as a yellow solid with a yield of 46.3%. m.p. 155–157 °C. ^1H NMR (500 MHz, $CDCl_3$) δ = 7.96 (s, 1H, N–H), 7.52 (t, *J* = 5.0 Hz, 1H, Ar–H), 7.41 (t, *J* = 5.0 Hz, 1H, Ar–H), 7.37 (d, *J* = 5.0 Hz, 1H, Ar–H), 7.31 (d, *J* = 10.0 Hz, 1H, Ar–H), 7.29 (s, 1H, Ar–H, 1H, =C–H), 6.94 (s, 1H, =C–H), 5.58–5.50 (m, 1H, =C–H), 5.03 (d, *J* = 10.0 Hz, 1H, =CH–H), 4.77 (d, *J* = 15.0 Hz, 1H, =CH–H), 4.27 (d, *J* = 5.0 Hz, 2H, =CH–H); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 160.14, 159.03, 133.83, 133.75, 132.97, 131.37, 131.29, 130.34, 129.45, 129.06, 128.56, 127.92, 127.67, 127.32, 122.97, 118.41, 114.76, 48.09 ppm; FT-IR (neat): 3179, 1682, 1620, 1474, 1380, 1354, 1265 cm^{-1}; HRMS (ESI) (*m/z*) $[M+H]^+$ for ($C_{21}H_{17}N_2O_2$) calculated 399.0662 found 399.0662.

*4.1.4.2. 1-Allyl-6-benzylidene-3-(3,4,5-trimethoxybenzylidene)piperazine-2,5-dione (**4b**).* According to method A, compound **4b** was synthesized as a yellow solid with a yield of 62.3%. m.p. 151–153 °C. ^1H NMR (500 MHz, $CDCl_3$) δ = 7.99 (s, 1H, N–H), 7.41 (t, *J* = 10.0 Hz, 2H, Ar–H), 7.36 (d, *J* = 5.0 Hz, 1H, Ar–H), 7.32 (d, *J* = 5.0 Hz, 2H, Ar–H), 7.29 (s, 1H, =C–H), 7.01 (s, 1H, =C–H), 6.62 (s, 2H, Ar–H), 5.58–5.51 (m, 1H, =C–H), 5.02 (d, *J* = 10.0 Hz, 1H, =CH–H), 4.77 (d, *J* = 15.0 Hz, 1H, =CH–H), 4.28 (d, *J* = 5.0 Hz, 2H, CH_2), 3.89 (s, 9H, $3OCH_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 160.02, 159.51, 153.95, 138.64, 133.86, 131.43, 129.44, 128.94, 128.52, 128.32, 128.24, 125.84, 122.35, 118.27, 117.90, 105.64, 60.98, 56.31, 48.03 ppm; FT-IR (neat): 3210, 1682, 1624, 1581, 1504, 1454, 1373, 1323, 1246 cm^{-1}; HRMS (ESI) (*m/z*) $[M+H]^+$ for ($C_{24}H_{25}N_2O_5$) calculated 421.1758 found 421.1763.

*4.1.4.3. 1-Allyl-3,6-bis(3-bromobenzylidene)piperazine-2,5-dione (**4c**).* According to method A, compound **4c** was synthesized as a yellow solid with a yield of 56.7%. m.p. 141–143 °C. ^1H NMR (500 MHz, $CDCl_3$) δ = 8.53 (s, 1H, N–H), 7.62 (s, 1H, Ar–H), 7.51 (t, *J* = 10.0 Hz, 2H, Ar–H), 7.47 (d, *J* = 5.0 Hz, 1H, Ar–H), 7.38 (d, *J* = 5.0 Hz, 1H, Ar–H), 7.35–7.31 (m, 2H, Ar–H), 7.28 (t, *J* = 10.0 Hz, 1H, Ar–H), 7.17 (s, 1H, =C–H), 7.07 (s, 1H), 5.60–5.53 (m, 1H, =C–H), 5.08 (d, *J* = 10.0 Hz, 1H, =CH–H), 4.82 (d, *J* = 20.0 Hz, 1H, =CH–H), 4.29 (d, *J* = 10.0 Hz, 2H, CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 159.63, 158.98, 135.94, 134.90, 132.06, 131.88, 131.36, 131.10, 130.90, 129.98, 129.01, 127.86, 127.03, 126.97, 126.71, 122.61, 120.50, 118.43, 116.32,116.26, 48.17 ppm; FT-IR (neat): 3179, 1682, 1620, 1589, 1558, 1474, 1373, 1246 cm^{-1}; HRMS (ESI) (*m/z*) $[M+H]^+$ for ($C_{21}H_{17}Br_2N_2O_2$) calculated 486.9651 found 486.9652.

*4.1.4.4. 1-Allyl-6-(3-bromobenzylidene)-3-(3,4,5-trimethoxybenzylidene)piperazine-2,5-dione (**4d**).* According to method A, compound **4d** was synthesized as a yellow solid with a yield of 68.3%. m.p. 163–165 °C. ^1H NMR (500 MHz, $CDCl_3$) δ = 8.25 (s, 1H, N–H), 7.56 (d, *J* = 5.0 Hz, 1H, Ar–H), 7.54 (s, 1H, Ar–H), 7.37–7.32 (m, 2H, Ar–H, 1H, =C–H), 7.10 (s, 1H, =C–H), 6.69 (s, 2H, Ar–H), 5.66–5.58 (m, 1H, =C–H), 5.13 (d, *J* = 10.0 Hz, 1H, =CH–H), 4.87 (d, *J* = 15.0 Hz, 1H, =CH–H), 4.34 (d, *J* = 5.0 Hz, 2H, CH_2), 3.961 (s, 3H, OCH_3), 3.957 (s, 6H, $2CHO_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 159.52, 159.33, 153.84, 138.61, 136.04, 132.05, 131.74, 131.22, 129.91, 129.28, 128.12, 127.84, 125.50, 122.55, 120.02, 118.39, 118.25, 105.64, 60.91, 56.22, 48.08 ppm; FT-IR (neat): 3224, 1682, 1620, 1582, 1504, 1454, 1373, 1319, 1246 cm^{-1}; HRMS (ESI) (*m/z*) $[M+H]^+$ for ($C_{24}H_{24}BrN_2O_5$) calculated 499.0863 found 499.0873.

*4.1.4.5. 1-Allyl-3-(3-bromobenzylidene)-6-(2-methoxybenzylidene)piperazine-2,5-dione (**4e**).* According to method A, compound **4e** was synthesized as a yellow solid with a yield of 52.3%. m.p. 167–169 °C. ^1H NMR (500 MHz, $CDCl_3$) δ = 8.01 (s, 1H, N–H), 7.56 (s, 1H, Ar–H), 7.47 (d, *J* = 5.0 Hz, 1H, Ar–H), 7.37–7.30 (m, 3H, Ar–H; 1H, =C–H), 7.21 (d, *J* = 5.0 Hz, 1H, Ar–H), 6.98 (d, *J* = 5.0 Hz, 1H, Ar–H), 6.97 (s, 1H, =C–H), 6.92 (d, *J* = 5.0 Hz, 1H, Ar–H), 5.58–5.50 (m, 1H, =C–H), 5.01 (d, *J* = 10.0 Hz, 1H, =CH–H), 4.76 (d, *J* = 15.0 Hz, 1H, =CH–H), 4.23 (d, *J* = 5.0 Hz, 2H, CH_2), 3.86 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 160.20, 158.85, 157.35, 135.16, 131.60, 131.39, 131.32, 130.74, 130.65, 130.36, 128.09, 127.12, 127.03, 123.41, 122.70, 120.24, 118.85, 118.16, 115.38, 110.65, 55.47, 47.41 ppm; FT-IR (neat): 3186, 1682, 1620, 1558, 1485, 1458, 1381, 1353, 1246 cm^{-1}; HRMS (ESI) (*m/z*) $[M+H]^+$ for ($C_{22}H_{20}BrN_2O_3$) calculated 439.0652 found 439.0651.

*4.1.4.6. 1-Allyl-3-(3-chlorobenzylidene)-6-(2-methoxybenzylidene)piperazine-2,5-dione (**4f**).* According to method A, compound **4f** was synthesized as a yellow solid with a yield of 62.4%. m.p. 143–145 °C. ^1H NMR (500 MHz, $CDCl_3$) δ = 8.10 (s, 1H, N–H), 7.41 (s, 1H, Ar–H), 7.38 (t, *J* = 10.0 Hz, 2H, Ar–H), 7.33–7.31 (m, 2H, Ar–H; 1H, =C–H), 7.21 (d, *J* = 5.0 Hz, 1H, Ar–H), 6.98 (t, *J* = 10.0 Hz, 1H, Ar–H; 1H, =C–H), 6.92 (d, *J* = 10.0 Hz, 1H, Ar–H), 5.58–5.51 (m, 1H, =C–H), 5.01 (d, *J* = 10.0 Hz, 1H, =CH–H), 4.76 (d, *J* = 15.0 Hz, 1H, =CH–H), 4.23 (d, *J* = 5.0 Hz, 2H, CH_2), 3.86 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 160.18, 158.84, 157.37, 135.37, 134.83, 131.32, 130.69, 130.60, 130.38, 128.78, 128. 51, 128.08, 127.07, 126.54, 122.69, 120.26, 118.92, 118.22, 115.50, 110.67, 55.49, 47.45 ppm; FT-IR (neat): 3232, 1682, 1620, 1578, 1489, 1462, 1381, 1354, 1246 cm^{-1}; HRMS (ESI) (*m/z*) $[M+H]^+$ for ($C_{22}H_{20}ClN_2O_3$) calculated 395.1157 found 395.1163.

*4.1.4.7. 1-Allyl-3-(3-fluorobenzylidene)-6-(2-methoxybenzylidene)piperazine-2,5-dione (**4g**).* According to method A, compound **4g** was synthesized as a yellow solid with a yield of 54.2%. m.p. 108–110 °C. ^1H NMR (500 MHz, $CDCl_3$) δ = 8.56 (s, 1H, N–H), 7.42 (t, *J* = 5.0 Hz, 1H, Ar–H), 7.37 (t, *J* = 10.0 Hz, 1H, Ar–H), 7.29 (s, 1H, =C–H), 7.24 (t, *J* = 10.0 Hz, 2H, Ar–H), 7.17 (d, *J* = 10.0 Hz, 1H, Ar–H), 7.05–6.98 (m, 2H, Ar–H; 1H, =C–H), 6.94 (d, *J* = 10.0 Hz, 1H, Ar–H), 5.59–5.52 (m, 1H, =C–H), 5.01 (d, *J* = 10.0 Hz, 1H, =CH–H), 4.77 (d, *J* = 15.0 Hz, 1H, =CH–H), 4.24 (d, *J* = 5.0 Hz, 2H, CH_2), 3.87 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 163.00 (d, J_{FC} = 246.3 Hz), 160.26, 158.93, 157.28, 135.10 (d, J_{FC} = 7.5 Hz), 131.30,

130.77 (d, J_{FC} = 8.8 Hz), 130.54, 130.30, 128.09, 126.91, 124.26, 122.69, 120.17, 118.61, 118.03, 115.77, 115.60 (d, J_{FC} = 3.8 Hz), 115.42 (d, J_{FC} = 2.5 Hz), 110.60, 55.39, 47.32 ppm; FT-IR (neat): 3197, 1682, 1620, 1581, 1489, 1381, 1350, 1242 cm^{-1}; HRMS (ESI) (*m*/*z*) $[M+H]^+$ for ($C_{22}H_{20}FN_2O_3$) calculated 379.1452 found 379.1455.

*4.1.4.8. 1-Allyl-6-(2-methoxybenzylidene)-3-(3-methylbenzylidene) piperazine-2,5-dione (**4h**).* According to method A, compound **4h** was synthesized as a yellow solid with a yield of 50.7%. m.p. 146–148 °C. ^1H NMR (500 MHz, $CDCl_3$) δ = 8.05 (s, 1H, N–H), 7.36–7.31 (m, 3H, Ar–H), 7.22 (d, *J* = 10.0 Hz, 2H, Ar–H; 1H, =C–H), 7.16 (d, *J* = 10.0 Hz, 1H, Ar–H), 7.04 (s, 1H, =C–H), 6.98 (t, *J* = 10.0 Hz, 1H, Ar–H), 5.59–5.51 (m, 1H, =C–H), 5.00 (d, *J* = 10.0 Hz, 1H, =CH–H), 4.76 (d, *J* = 20.0 Hz, 1H, =CH–H), 4.23 (d, *J* = 5.0 Hz, 2H, CH_2), 3.85 (s, 3H, OCH_3), 2.38 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 160.01, 159.21, 157.35, 139.18, 132.97, 131.47, 130.52, 130.38, 129.59, 129.27, 129.02, 128.38, 126.00, 125.55, 122.87, 120.22, 118.29, 118.03, 117.40, 110.63, 55.46, 47.36, 21.44 ppm; FT-IR (neat): 3201, 1682, 1620, 1578, 1489, 1458, 1381, 1350, 1246 cm^{-1}; HRMS (ESI) (*m*/*z*) $[M+H]^+$ for ($C_{23}H_{23}N_2O_3$) calculated 375.1703 found 375.1709.

*4.1.4.9. 1-Allyl-3-(2-chlorobenzylidene)-6-(2-methoxybenzylidene) piperazine-2,5-dione (**4i**).* According to method A, compound **4i** was synthesized as a yellow solid with a yield of 57.9%. m.p. 167–169 °C. ^1H NMR (500 MHz, $CDCl_3$) δ = 7.86 (s, 1H, N–H), 7.49 (d, *J* = 5.0 Hz, 1H, Ar–H), 7.44 (d, *J* = 5.0 Hz, 1H, Ar–H), 7.37–7.30 (m, 3H, Ar–H; 1H, =C–H), 7.22 (d, *J* = 5.0 Hz, 1H, Ar–H), 7.15 (s, 1H, =C–H), 6.98 (t, *J* = 10.0 Hz, 1H, Ar–H), 6.92 (d, *J* = 10.0 Hz, 1H, Ar–H), 5.60–5.52 (m, 1H, =C–H), 5.01 (d, *J* = 10.0 Hz, 1H, =CH–H), 4.76 (d, *J* = 20.0 Hz, 1H, =CH–H), 4.26 (d, *J* = 5.0 Hz, 2H, CH_2), 3.86 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.97, 158.65, 157.37, 134.47, 131.46, 131.37, 130.64, 130.58, 130.42, 129.97, 129.12, 128.02, 127.44, 127.39, 122.78, 120.23, 118.82, 118.25, 114.01, 110.64, 55.49, 47.40 ppm; FT-IR (neat): 3183, 1682, 1620, 1489, 1381, 1354, 1246 cm^{-1}; HRMS (ESI) (*m*/*z*) $[M+H]^+$ for ($C_{22}H_{20}ClN_2O_3$) calculated 395.1157 found 395.1165.

*4.1.4.10. 1-Allyl-3-(2-fluorobenzylidene)-6-(2-methoxybenzylidene) piperazine-2,5-dione (**4j**).* According to method A, compound **4j** was synthesized as a yellow solid with a yield of 51.3%. m.p. 126–128 °C. ^1H NMR (500 MHz, $CDCl_3$) δ = 7.99 (s, 1H, N–H), 7.43 (t, *J* = 10.0 Hz, 1H, Ar–H), 7.38–7.33 (m, 2H, Ar–H; 1H, =C–H), 7.22 (t, *J* = 5.0 Hz, 2H, Ar–H), 7.16 (t, *J* = 10.0 Hz, 1H, Ar–H), 7.04 (s, 1H, =C–H), 6.98 (t, *J* = 10.0 Hz, 1H, Ar–H), 6.92 (d, *J* = 10.0 Hz, 1H, Ar–H), 5.59–5.52 (m, 1H, =C–H), 5.01 (d, *J* = 10.0 Hz, 1H, =CH–H), 4.76 (d, *J* = 15.0 Hz, 1H, =CH–H), 4.24 (d, *J* = 5.0 Hz, 2H, CH_2), 3.86 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 160.00, 159.89 (d, J_{FC} = 247.5 Hz), 158.82, 157.35, 131.38, 130.66, 130.59, 130.38, 129.95 (d, J_{FC} = 2.5 Hz), 128.13, 127.49, 124.82 (d, J_{FC} = 2.5 Hz), 122.79, 120.82 (d, J_{FC} = 13.8 Hz), 120.23, 118.74, 118.14, 116.48 (d, J_{FC} = 22.5 Hz), 110.65, 110.37, 55.47, 47.43 ppm; FT-IR (neat): 3190, 1682, 1620, 1578, 1489, 1454, 1381, 1354, 1246 cm^{-1}; HRMS (ESI) (*m*/*z*) $[M+H]^+$ for ($C_{22}H_{20}FN_2O_3$) calculated 379.1452 found 379.1454.

*4.1.4.11. 1-Allyl-6-(2-methoxybenzylidene)-3-(2-(trifluoromethyl) benzylidene)piperazine-2,5-dione (**4k**).* According to method A, compound **4k** was synthesized as a yellow solid with a yield of 57.1%. m.p. 134–136 °C. ^1H NMR (500 MHz, $CDCl_3$) δ = 7.93 (s, 1H, N–H), 7.77 (d, *J* = 10.0 Hz, 1H, Ar–H), 7.62 (d, *J* = 10.0 Hz, 1H, Ar–H), 7.51–7.46 (m, 2H, Ar–H), 7.36 (t, *J* = 10.0 Hz, 1H, Ar–H), 7.29 (s, 1H, =C–H), 7.22 (t, *J* = 10.0 Hz, 1H, Ar–H; 1H, =C–H), 6.99 (t, *J* = 10.0 Hz, 1H, Ar–H), 6.92 (d, *J* = 10.0 Hz, 1H, Ar–H), 5.59–5.51 (m, 1H, =C–H), 5.01 (d, *J* = 10.0 Hz, 1H, =CH–H), 4.75 (d, *J* = 15.0 Hz, 1H, =CH–H), 4.26 (d, *J* = 10.0 Hz, 2H, CH_2), 3.86 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 160.03, 158.42, 157.33, 132.43, 131.44, 131.31, 130.62, 130.40, 129.64, 129.37, 128.60, 127.99, 127.92, 126.91 (q, J_{FC} = 10.0, 5.0 Hz), 123.64 (d, J_{FC} = 272.5 Hz), 122.77, 120.22, 118.88, 118.20, 113.45, 110.63, 55.45, 47.33 ppm; FT-IR (neat): 3156, 1686, 1620, 1578, 1485, 1381, 1367, 1312, 1254 cm^{-1}; HRMS (ESI) (*m*/*z*) $[M+H]^+$ for ($C_{23}H_{20}N_2O_3$) calculated 429.1421 found 429.1427.

*4.1.4.12. 1-Allyl-3,6-bis(2-methoxybenzylidene)piperazine-2,5-dione (**4l**).* According to method A, compound **4l** was synthesized as a yellow solid with a yield of 74.4%. m.p. 124–126 °C. ^1H NMR (500 MHz, $CDCl_3$) δ = 8.50 (s, 1H, N–H), 7.36–7.32 (m, 3H, Ar–H), 7.31 (s, 1H, =C–H), 7.20 (d, *J* = 5.0 Hz, 1H, Ar–H), 7.06 (s, 1H, =C–H), 7.02 (t, *J* = 10.0 Hz, 1H, Ar–H), 7.00–6.95 (m, 2H, Ar–H), 6.90 (d, *J* = 5.0 Hz, 1H, Ar–H), 5.60–5.52 (m, 1H, =C–H), 4.99 (d, *J* = 10.0 Hz, 1H, =CH–H), 4.76 (d, *J* = 20.0 Hz, 1H, =CH–H), 4.24 (d, *J* = 5.0 Hz, 2H, CH_2), 3.94 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 159.70, 159.44, 157.31, 156.31, 131.60, 130.93, 130.35, 128.63, 126.18, 123.02, 122.27, 121.41, 120.14, 117.89, 117.84, 114.30, 111.89, 110.58, 55.94, 55.42, 47.35 ppm; FT-IR (neat): 3271, 1682, 1624, 1597, 1489, 1462, 1378, 1358, 1246 cm^{-1}; HRMS (ESI) (*m*/*z*) $[M+H]^+$ for ($C_{23}H_{23}N_2O_4$) calculated 391.1652 found 391.1650.

*4.1.4.13. 1-Allyl-3-(2,3-dichlorobenzylidene)-6-(2-methoxybenzylidene)piperazine-2,5-dione (**4m**).* According to method A, compound **4m** was synthesized as a yellow solid with a yield of 58.2%. m.p. 134–136 °C. ^1H NMR (500 MHz, $CDCl_3$) δ = 8.21 (s, 1H, N–H), 7.44 (d, *J* = 5.0 Hz, 1H, Ar–H), 7.36 (t, *J* = 10.0 Hz, 2H, Ar–H), 7.29–7.26 (m, 1H, Ar–H; 1H, =C–H), 7.21 (d, *J* = 5.0 Hz, 1H, Ar–H), 7.11 (s, 1H, =C–H), 6.99 (d, *J* = 10.0 Hz, 1H, Ar–H), 6.92 (d, *J* = 5.0 Hz, 1H, Ar–H), 5.58–5.51 (m, 1H, =C–H), 5.01 (d, *J* = 10.0 Hz, 1H, =CH–H), 4.76 (d, *J* = 15.0 Hz, 1H, =CH–H), 4.25 (d, *J* = 5.0 Hz, 2H, CH_2), 3.86 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 160.08, 158.48, 157.34, 134.42, 133.68, 132.69, 131.28, 130.68, 130.44, 130.39, 127.96, 127.87, 127.76, 127.45, 122.70, 120.24, 119.01, 118.28, 113.68, 110.65, 55.49, 47.41 ppm; FT-IR (neat): 3179, 1682, 1620, 1489, 1462, 1381, 1358, 1246 cm^{-1}; HRMS (ESI) (*m*/*z*) $[M+H]^+$ for ($C_{22}H_{19}Cl_2N_2O_3$) calculated 429.0767 found 429.0775.

*4.1.4.14. 1-Allyl-3-(3,4-dichlorobenzylidene)-6-(2-methoxybenzylidene)piperazine-2,5-dione (**4n**).* According to method A, compound **4n** was synthesized as a yellow solid with a yield of 64.2%. m.p. 166–168 °C. ^1H NMR (500 MHz, $CDCl_3$) δ = 8.17 (s, 1H, N–H), 7.53 (s, 1H, Ar–H), 7.51 (d, *J* = 10.0 Hz, 1H, Ar–H), 7.36 (t, *J* = 10.0 Hz, 1H, Ar–H), 7.31 (s, 1H, =C–H), 7.26 (d, *J* = 5.0 Hz, 1H, Ar–H), 7.21 (d, *J* = 5.0 Hz, 1H, Ar–H), 6.98 (t, *J* = 10.0 Hz, 1H, Ar–H), 6.93 (s, 1H, =C–H), 6.92 (d, *J* = 5.0 Hz, 1H, Ar–H), 5.58–5.50 (m, 1H, =C–H), 5.01 (d, *J* = 10.0 Hz, 1H, =CH–H), 4.76 (d, *J* = 15.0 Hz, 1H, =CH–H), 4.23 (d, *J* = 5.0 Hz, 2H, CH_2), 3.86 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 160.22, 158.73, 157.39, 133.64, 133.06, 132.73, 131.25, 130.75, 130.39, 130.33, 127.98, 127.72, 127.35, 122.61, 120.27, 119.13, 118.29, 114.33, 110.67, 55.50, 47.46 ppm; FT-IR (neat): 3167, 1678, 1620, 1578, 1458, 1381, 1358, 1250 cm^{-1}; HRMS (ESI) (*m*/*z*) $[M+H]^+$ for ($C_{22}H_{19}Cl_2N_2O_3$) calculated 429.0767 found 429.0774.

*4.1.4.15. 1-Allyl-3-(5-bromo-2-fluorobenzylidene)-6-(2-methoxybenzylidene)piperazine-2,5-dione (**4o**).* According to method A, compound **4o** was synthesized as a yellow solid with a yield of 67.2%. m.p. 158–161 °C. ^1H NMR (500 MHz, $CDCl_3$) δ = 8.38 (s, 1H, N–H), 7.58 (d, *J* = 5.0 Hz, 1H, Ar–H), 7.41–7.39 (m, 1H, Ar–H), 7.36 (d, *J* = 10.0 Hz, 1H, Ar–H), 7.27 (s, 1H, =C–H), 7.22 (d, *J* = 5.0 Hz, 1H, Ar–H), 7.21 (d, *J* = 5.0 Hz, 1H, Ar–H), 7.04 (t, *J* = 10.0 Hz, 1H, Ar–H), 6.99 (t, *J* = 10.0 Hz, 1H, Ar–H), 6.94 (s, 1H, =C–H), 6.92 (d, *J* = 10.0 Hz, 1H, Ar–H), 5.59–5.51 (m, 1H, =C–H), 5.02 (d, *J* = 10.0 Hz, 1H, =CH–H), 4.76 (d, *J* = 15.0 Hz, 1H, =CH–H), 4.24 (d, *J* = 5.0 Hz, 2H, CH_2), 3.87 (s, 3H, OCH_3); ^{13}C NMR (125 MHz,

$CDCl_3$) δ = 160.17, 158.99 (d, J_{FC} = 248.8 Hz), 158.50, 157.37, 133.22 (d, J_{FC} = 8.8 Hz), 132.52 (d, J_{FC} = 3.8 Hz), 131.28, 130.70, 130.39, 128.47, 127.93, 123.04 (d, J_{FC} = 16.3 Hz), 122.69, 120.27, 119.18, 118.24, 118.12 (d, J_{FC} = 23.8 Hz), 117.27 (d, J_{FC} = 3.8 Hz), 110.68, 108.69, 55.49, 47.46 ppm; FT-IR (neat): 3179, 1682, 1620, 1578, 1485, 1462, 1381, 1358, 1242 cm^{-1}; HRMS (ESI) (*m*/*z*) $[M+H]^+$ for ($C_{22}H_{19}BrFN_2O_3$) calculated 457.0558 found 457.0564.

*4.1.4.16. 1-Allyl-3-(3-bromo-4-fluorobenzylidene)-6-(2-methoxybenzylidene)piperazine-2,5-dione (**4p**).* According to method A, compound **4p** was synthesized as a yellow solid with a yield of 67.4%. m.p. 172–174 °C. ^1H NMR (500 MHz, $CDCl_3$) δ = 7.91 (s, 1H, N–H), 7.62 (d, *J* = 5.0 Hz, 1H, Ar–H), 7.38–7.34 (m, 2H, Ar–H; 1H, =C–H), 7.22–7.18 (m, 2H, Ar–H), 6.98 (t, *J* = 10.0 Hz, 1H, Ar–H), 6.94 (s, 1H, =C–H), 6.92 (d, *J* = 10.0 Hz, 1H, Ar–H), 5.58–5.50 (m, 1H, =C–H), 5.01 (d, *J* = 10.0 Hz, 1H, =CH–H), 4.77 (d, *J* = 20.0 Hz, 1H, =CH–H), 4.23 (d, *J* = 5.0 Hz, 2H, CH_2), 3.86 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 160.21, 158.84 (d, J_{FC} = 248.8 Hz), 158.80, 157.39, 133.61, 131.30, 130.73, 130.67 (d, J_{FC} = 3.8 Hz), 130.37, 129.23 (d, J_{FC} = 7.5 Hz), 128.04, 126.99, 122.64, 120.27, 119.01, 118.25, 117.46, 117.28, 114.45, 110.68, 55.50, 47.45 ppm; FT-IR (neat): 3190, 1682, 1620, 1578, 1492, 1462, 1381, 1354, 1246 cm^{-1}; HRMS (ESI) (*m*/*z*) $[M+H]^+$ for ($C_{22}H_{19}BrFN_2O_3$) calculated 457.0558 found 457.0557.

*4.1.4.17. 1-Allyl-3-(5-fluoro-2-methylbenzylidene)-6-(2-methoxybenzylidene)piperazine-2,5-dione (**4q**).* According to method A, compound **4q** was synthesized as a yellow solid with a yield of 55.2%. m.p. 205–207 °C. ^1H NMR (500 MHz, $CDCl_3$) δ = 7.83 (s, 1H, N–H), 7.36 (d, *J* = 5.0 Hz, 1H, Ar–H), 7.33 (s, 1H, =C–H), 7.25–7.21 (m, 2H, Ar–H), 7.04 (d, *J* = 10.0 Hz, 1H, Ar–H), 7.02 (s, 1H, =C–H), 6.98 (t, *J* = 10.0 Hz, 2H, Ar–H), 6.92 (d, *J* = 10.0 Hz, 1H, Ar–H), 5.59–5.51 (m, 1H, =C–H), 5.01 (d, *J* = 10.0 Hz, 1H, =CH–H), 4.77 (d, *J* = 20.0 Hz, 1H, =CH–H), 4.25 (d, *J* = 5.0 Hz, 2H, CH_2), 3.86 (s, 3H, OCH_3), 2.29 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 161.08 (d, J_{FC} = 245.0 Hz), 159.93, 158.74, 157.34, 133.24, 133.15 (t, J_{FC} = 3.8 Hz), 132.32 (d, J_{FC} = 8.8 Hz), 131.36, 130.57, 130.37, 128.04, 127.21, 128.81, 120.20, 118.74, 118.12, 115.52 (d, J_{FC} = 21.3 Hz), 115.08, 115.50 (d, J_{FC} = 21.3 Hz), 110.64, 55.45, 47.38, 19.23 ppm; FT-IR (neat): 3182, 1682, 1620, 1582, 1489, 1458, 1381, 1354, 1246 cm^{-1}; HRMS (ESI) (*m*/*z*) $[M+H]^+$ for ($C_{23}H_{22}FN_2O_3$) calculated 393.1609 found 393.1616.

*4.1.4.18. 1-Allyl-6-(2-methoxybenzylidene)-3-(4-methylbenzylidene) piperazine-2,5-dione (**4r**).* According to method A, compound **4r** was synthesized as a yellow solid with a yield of 46.7%. m.p. 97–99 °C. ^1H NMR (500 MHz, $CDCl_3$) δ = 8.00 (s, 1H, N–H), 7.36–7.32 (m, 3H, Ar–H, 1H, =C–H), 7.24 (d, *J* = 5.0 Hz, 2H, Ar–H), 7.22 (d, *J* = 10.0 Hz, 1H, Ar–H), 7.04 (s, 1H, =C–H), 6.97 (t, *J* = 10.0 Hz, 1H, Ar–H), 6.91 (d, *J* = 5.0 Hz, 1H, Ar–H), 5.59–5.51 (m, 1H, =C–H), 5.00 (d, *J* = 10.0 Hz, 1H, =CH–H), 4.77 (d, *J* = 20.0 Hz, 1H, =CH–H), 4.23 (d, *J* = 5.0 Hz, 2H, CH_2), 3.85 (s, 3H, OCH_3), 2.38 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 160.07, 159.33, 157.34, 138.98, 131.48, 130.50, 130.37, 130.08, 130.05, 128.47, 128.40, 125.53, 122.86, 120.20, 118.21, 118.01, 117.44, 110.61, 55.45, 47.35, 21.33 ppm; FT-IR (neat): 3217, 1682, 1620, 1578, 1489, 1458, 1377, 1354, 1246 cm^{-1}; HRMS (ESI) (*m*/*z*) $[M+H]^+$ for ($C_{23}H_{23}N_2O_3$) calculated 375.1703 found 375.1695.

*4.1.4.19. 3,6-Bis(2-methoxybenzylidene)-1-methylpiperazine-2,5-dione (**4s**).* According to method A, compound **4s** was synthesized as a yellow solid with a yield of 65.6%. m.p. 115–117 °C. ^1H NMR (500 MHz, $CDCl_3$) δ = 8.53 (s, 1H, N–H), 7.37–7.32 (m, 3H, Ar–H), 7.30 (s, 1H, =C–H), 7.14 (d, *J* = 10 Hz, 1H, Ar–H), 7.05 (s, 1H, =C–H), 7.03 (t, *J* = 10 Hz, 1H, Ar–H), 6.99 (d, *J* = 10 Hz, 1H, Ar–H), 6.96 (t, *J* = 10 Hz, 1H, Ar–H), 6.90 (d, *J* = 10 Hz, 1H, Ar–H), 3.95 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 2.97 (s, 3H, NCH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 159.47, 158.98, 157.29, 156.33, 130.96, 130.51, 130.37, 130.04, 126.03, 123.17, 122.28, 121.45, 120.04, 116.87, 113.92, 111.91, 110.39, 56.96, 55.42, 35.95 ppm; FT-IR (neat): 3251, 2943, 2835, 1678, 1620, 1489, 1338, 1242 cm^{-1}; HRMS (ESI) (*m*/*z*) $[M+H]^+$ for ($C_{21}H_{21}N_2O_4$) calculated 365.1496 found 395.1491.

*4.1.4.20. 3-(2-chlorobenzylidene)-6-(2-methoxybenzylidene)-1-methylpiperazine-2,5-dione (**4t**).* According to method A, compound **4t** was synthesized as a yellow solid with a yield of 42.6%. m.p. 185–187 °C. ^1H NMR (500 MHz, $CDCl_3$) δ = 7.93 (s, 1H, N–H), 7.49 (d, *J* = 10 Hz, 1H, Ar–H), 7.43 (d, *J* = 10 Hz, 1H, Ar–H), 7.36–7.31 (m, 4H, Ar–H, =C–H), 7.16 (d, *J* = 5.0 Hz, 1H, Ar–H), 7.13 (s, 1H, =C–H), 6.98 (t, *J* = 10 Hz, 1H, Ar–H), 6.92 (d, *J* = 10 Hz, 1H, Ar–H), 3.87 (s, 3H, OCH_3), 2.99 (s, 3H, NCH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 159.19, 158.65, 157.31, 134.44, 131.47, 130.54, 130.52, 130.35, 130.29, 129.94, 129.15, 127.39, 127.28, 122.90, 120.10, 117.77, 113.61, 110.45, 55.45, 36.00 ppm; FT-IR (neat): 3175, 3005, 2936, 1681, 1620, 1431, 1381, 1342, 1253 cm^{-1}; HRMS (ESI) (*m*/*z*) $[M+H]^+$ for ($C_{20}H_{18}ClN_2O_3$) calculated 369.1000 found 369.1005.

*4.1.4.21. 3-(2,3-dichlorobenzylidene)-6-(2-methoxybenzylidene)-1-methylpiperazine-2,5-dione (**4u**).* According to method A, compound **4u** was synthesized as a yellow solid with a yield of 39.0%. m.p. 176–178 °C. ^1H NMR (500 MHz, $CDCl_3$) δ = 7.89 (s, 1H, N–H), 7.48 (d, *J* = 10 Hz, 1H, Ar–H), 7.36 (t, 2H, *J* = 5.0 Hz, Ar–H), 7.32 (s, 1H, =C–H), 7.29 (t, *J* = 10 Hz, 1H, Ar–H), 7.16 (d, *J* = 10 Hz, 1H, Ar–H), 7.10 (s, 1H, =C–H), 6.98 (t, *J* = 10 Hz, 1H, Ar–H), 6.92 (d, *J* = 10 Hz, 1H, Ar–H), 3.87 (s, 3H, OCH_3), 2.99 (s, 3H, NCH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 159.17, 157.33, 134.60, 133.68, 132.75, 130.55, 130.52, 130.40, 130.18, 127.86, 127.78, 127.28, 122.80, 120.13, 118.13, 113.18, 110.48, 55.48, 36.06 ppm; FT-IR (neat): 3178, 3005, 2943, 1682, 1624, 1435, 1373, 1346, 1250 cm^{-1}; HRMS (ESI) (*m*/*z*) $[M+H]^+$ for ($C_{20}H_{17}Cl_2N_2O_3$) calculated 403.0611 found 403.0605.

*4.1.4.22. 3,6-Bis(2-methoxybenzylidene)piperazine-2,5-dione (**4v**).* According to method B, compound **4v** was synthesized as a yellow solid with a yield of 20.1%. m.p. 279–281 °C. ^1H NMR (500 MHz, $DMSOd_6$) δ = 9.99 (s, 1H, N–H), 7.48 (d, *J* = 10.0 Hz, 1H, Ar–H), 7.35 (t, *J* = 10.0 Hz, 1H, Ar–H), 7.07 (d, *J* = 10.0 Hz, 1H, Ar–H), 7.01 (t, *J* = 10.0 Hz, 1H, Ar–H), 6.84 (s, 1H, =C–H), 3.85 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 157.39, 156.84, 129.99, 126.28, 121.72, 120.61, 117.33, 111.42, 110.81, 55.56 ppm; FT-IR (neat): 3209, 1674, 1628, 1597, 1492, 1462, 1396, 1354, 1246 cm^{-1}; HRMS (ESI) (*m*/*z*) $[M+H]^+$ for ($C_{20}H_{19}N_2O_4$) calculated 351.1339 found 351.1344.

*4.1.4.23. (2,3-dichlorobenzylidene)-6-(2-methoxybenzylidene)piperazine-2,5-dione (**4w**).* According to method B, compound **4w** was synthesized as a yellow solid with a yield of 32.8%. m.p. >300 °C. ^1H NMR (500 MHz, $CDCl_3$) δ = 7.60 (d, *J* = 10.0 Hz, 1H, Ar–H), 7.53 (d, *J* = 5.0 Hz, 1H, Ar–H), 7.48 (d, *J* = 5.0 Hz, 1H, Ar–H), 7.40 (t, *J* = 10.0 Hz, 1H, Ar–H), 7.36 (t, *J* = 10.0 Hz, 1H, Ar–H), 7.07 (d, *J* = 5.0 Hz, 1H, Ar–H), 7.01 (t, *J* = 5.0 Hz, 1H, Ar–H), 6.86 (s, 1H, =CH–H), 6.74 (s, 1H, =CH–H), 3.85 (s, 3H, OCH_3); Attempt to gather ^{13}C NMR was failure; FT-IR (neat): 3190, 1678, 1632, 1574, 1492, 1466, 1400, 1354, 1250 cm^{-1}; Attempt to obtain HRMS (ESI) was failure because of its poor solubility.

*4.1.4.24. 3-((5-(tert-butyl)-1H-imidazole-4-yl)methylene)-6-(2-methoxybenzylidene)piperazine-2,5-dione (**4x**).* According to method B, compound **4x** was synthesized as a yellow solid with a yield of 48%. m.p. 264–267 °C. ^1H NMR (500 MHz, $CDCl_3$) δ = 11.79 (s, 1H, N–H), 9.90 (s, 1H, N–H), 8.17 (s, 1H, Ar–H), 7.42 (d, *J* = 5.0 Hz, 1H, Ar–H), 7.31 (t, *J* = 10.0 Hz, 1H, Ar–H), 7.03 (d, *J* = 10.0 Hz, 1H, Ar–H), 6.96 (t, *J* = 10.0 Hz, 1H, Ar–H), 6.79 (s, 1H, =C–H), 6.72 (s,

1H, =C–H), 3.82 (s, 3H, OCH_3), 1.33 (s, 9H, $3CH_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 158.45, 158.16, 156.83, 139.91, 134.17, 130.08, 129.95, 126.29, 121.75, 120.65, 119.26, 111.46, 110.69, 110.26, 99.55, 55.57, 31.81, 30.27 ppm; FT-IR (neat): 3178, 2954, 1666, 1624, 1412, 1369, 1342, 1246 cm^{-1}; HRMS (ESI) (m/z) $[M+H]^+$ for ($C_{20}H_{23}N_4O_3$) calculated 367.1765 found 367.1757.

4.2. Cell culture and cell viability assay

Cell lines, Hela, HL60, MCF-7, BGC-823, A549, Huh-7, K562, U937, H1975, and Molt-4 were purchased from Shanghai Cell Bank, Chinese Academy of Sciences. Cells were routinely grown and maintained in mediums RPMI or DMEM with 10% FBS and 1% penicillin/streptomycin. All cell lines were incubated in a Thermo/Forma Scientific CO_2 Water Jacketed Incubator with 5% CO_2 in air at 37 °C. Cell viability assay was determined by using the CCK8 (DOjinDo, Japan) assay. Cells were seeded at a density of 400–800 cells/well in 384 well plates and treated with varying concentrations of compounds or solution as control. After 72 h incubation, CCK8 reagent was added, and absorbance was measured at 450 nm using Envision 2104 multi-label Reader (Perkin Elmer, USA). Dose response curves were plotted to determine the IC_{50} values using Prism 5.0 (GraphPad Software Inc., USA).

Conflict of interest

We declare that we have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.06.030.

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