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Graphical Abstract

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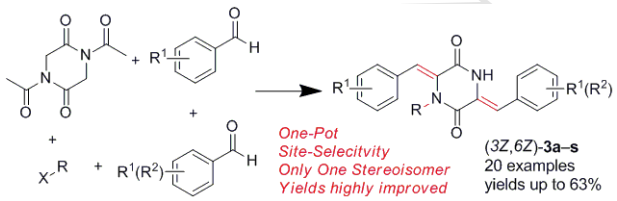

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ABSTRACT

A one-pot multicomponent approach was established for site selective synthesis of novel 1,3,6-trisubstituted 3,6-diunsaturated (3*Z*,6*Z*)-2,5-diketopiperazine derivatives with high stereoselectivity. The computational studies revealed that the steric hindrances between the 2-hydrogen atoms on the aromatic rings and the carbonyl, as well as the steric repulsions between the hydrogen atoms of the CH group in the benzylidene and the CH_2 group in the *N*-alkylative part might be responsible for the *Z*/*E* selectivity. Compound (3*Z*,6*Z*)-**3h** (IC_{50} = 11 nM) has a close activity to the positive compound plinabulin (IC_{50} = 15 nM) against the cancer cell line HL60.

1. Introduction

Natural or synthetic *N*-Monoalkylated 3,6-diunsaturated 2,5-diketopiperazine derivatives (2,5-DKPs) represent a unique kind of bioactive compounds[1] (Figure 1) as they can serve as modulators against multidrug resistance (MDR),[2] inhibitors against plasminogen activator inhibitor-1 (PAI-1),[3] agents against biofouling[4] or cancer,[5] etc. The general protocol for synthesis of the above 2,5-DKPs often consists of three independent steps (Scheme 1) via Aldol condensations of 1,4-diacetyl-2,5-DPK with aromatic aldehydes and the alkylations of halohydrocarbons (such as CH_3I or allyl bromide) on the nitrogen atoms.[3,5a,4,6] However, these operations are complicated and give low total yields of target **3**. Additionally, the purification of intermediate **1** is troublesome due to the enolization of amide group at 4-positon.[7] Therefore, exploring new convenient ways for efficient synthesis of these *N*-monoalkylated 2,5-DKPs with structural and bioactive diversities are necessary.

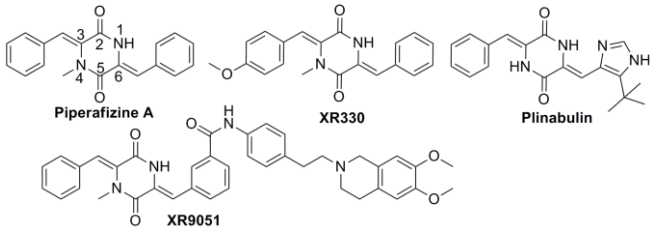

Figure 1. Representative 3,6-Diunsaturated 2,5-Diketopiperazines.

One-pot synthetic strategy has become a powerful tool for rapid access to natural product derivatives in recent years.[8] However, employing this strategy to the synthesis of these *N*-monoalkylated 3,6-diunsaturated 2,5-DKPs might come across difficulties: for instance the methylene groups at 3- or 6-positions on the 2,5-DKP ring would probably compete to react with different aromatic aldehydes, and also with the halohydrocarbons; and the nitrogen and oxygen atoms of the amide could possibly react with halohydrocarbons due to enolization of amide.[9] Theoretically, there will be four possible stereoisomers with two double bonds formed during reaction. Interestingly, only one or two isomers were often observed and isolated from the natural

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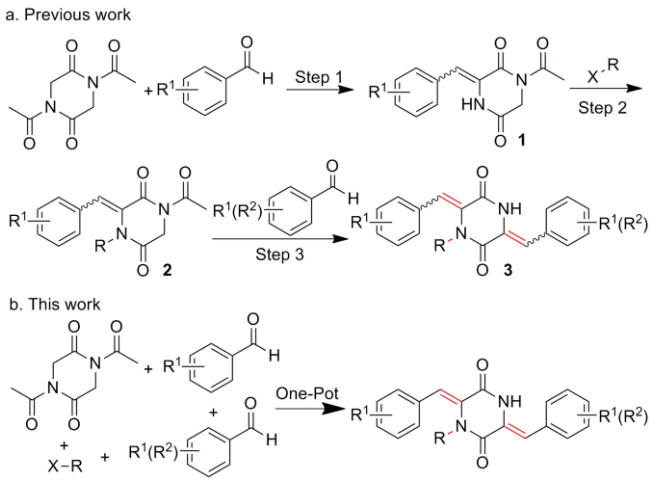
product 2,5-DPKs.[10] This phenomenon inspired us that higher stereoselectivity could be achieved in 2,5-DKPs through one-pot synthesis, possibly, by controlling the reaction temperature [7,9] or changing the order of the starting material addition.

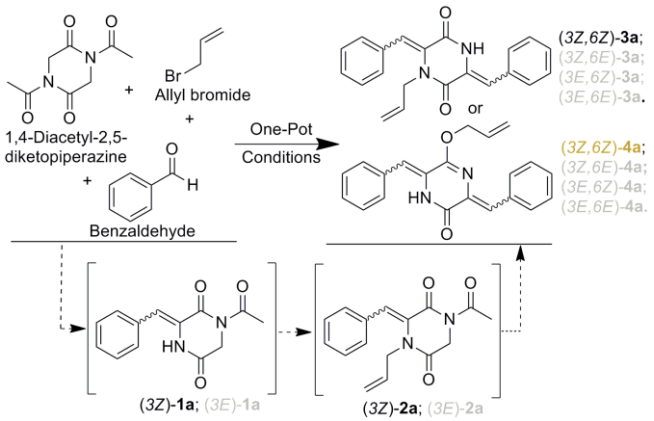


Scheme 1. Routes to *N*-monoalkylated unsaturated 2,5-diketopiperazines.

2. Results and discussion

With the above questions and the previous findings [7,9] in mind, the reaction was carried out by reacting 1,4-diacetyl-2,5-DPK with allyl bromide and benzaldehyde in one pot using NaOH or Cs_2CO_3 as the condensation base. When the molar ratio of allyl bromide to 1,4-diacetyl-2,5-DPK was 1.8:1, the reaction was incomplete and the products were the mixture of intermediates (*3Z*)-**1a** and (*3Z*)-**2a**. No improvement was observed even with the prolonged reaction time (24 h) (Table 1, entries 1 and 2). Therefore, larger amount of the allyl bromide (2.5 equiv.) was used but still no target compound was measured. The products were isolated and identified to be major (*3Z*)-**2a** which was accompanied by an orange compound **4a**, while Cs_2CO_3 had the best efficacy to promote the formation of (*3Z*)-**2a** (entries 3-6). Surprisingly, compound **4a** (identified by X-ray analysis) was a site isomer of the target compound **3a**, that is, the allyl group was alkylated on the oxygen atom, not on the nitrogen atom, similar to the natural products neihumicin, methoxyneihumicin,[11] and nocazines A and B.[12] Then the (*3Z*)-**2a** contained mixture was moved to heat at 95 °C and we were pleased to find that the target compound **3a** was obtained smoothly (entry 7), nevertheless, a small amount of compound **4a** was still observed. Effort was made to improve the yield of the target compound **3a** and achieve a higher *N*-site selectivity. The reaction was performed firstly under a low temperature (- 10 °C, entries 8-10) and we found that Cs_2CO_3 still had the most efficacy to produce compound (*3Z*)-**2a** as the sole product (entry 8). According to these results, the reaction mixture was stirred at - 10 °C using Cs_2CO_3 as the base, until the completion of the 1st Aldol condensation and the alkylation of allyl bromide, and then heated at 95 °C with the target compound **3a** as the only product (entry 11). These results doubtlessly indicated that low temperature could realize the high site selectivity and was beneficial for the *N*-alkylation in this one-pot reaction, thus resulted in higher yield of the target compound. Meanwhile compound **3a** possessed a (*3Z,6Z*)-configuration whereas other stereoisomers of (3Z,6Z)-**3a** were not measured, and this unexpected outcome pushed us to make clear the reasons and a computational study was carried out. Our computational calculations rationalized that the steric

Table 1. Optimization of the reaction conditions[a].



Entry	Base (equiv)	Temp(°C)/ Time(h)	Benzaldehyde (equiv)	Yield(%)[b] **1a**/**2a**/**3a**/**4a**
1[c]	NaOH(3.0)	rt/24	2.0	21/24/0/0
2[c]	Cs_2CO_3(3.0)	rt/24	2.0	33/45/0/0
3[d]	NaOH(3.0)	rt/3	2.0	0/39/0/3
4	Cs_2CO_3(3.0)	rt/4	2.0	0/59/0/5
5	K_2CO_3(3.0)	rt/6	2.0	0/53/0/5
6	DBU(3.0)	rt/4	2.0	0/47/0/7
7	Cs_2CO_3(3.0)	rt/4; 95/3	2.0	0/0/39/4
8	Cs_2CO_3(3.0)	-10/4	2.0	0/77/0/0
9	K_2CO_3(3.0)	-10/16	2.0	0/68/0/0
10	DBU(3.0)	-10/6	2.0	0/49/0/7
11	Cs_2CO_3(3.0)	-10/4; 95/3	2.0	0/0/49/0
12	Cs_2CO_3(3.0)	-20/4; 95/3	2.0	0/0/48/0
13	Cs_2CO_3(2.0)	-10/4; 95/3	2.0	0/0/38/0
14	Cs_2CO_3(2.5)	-10/4; 95/3	2.5	0/0/55/0
15	Cs_2CO_3(2.5)	-10/4; 95/3	3.0	0/0/48/0

[a] Reaction conditions: 1.0 equiv. of 1,4-diacetyl-2,5-DPK (0.25 mmol) was used. The reaction was mixed with 4Å MS in 2 mL dry DMF under a N_2 atmosphere. The reaction was firstly stirred at -10 °C until the completion of the 1st Aldol condensation and the alkylation of the allyl bromide, and then heated at 95 °C for about 4 h.

[b] Isolated yield based on themselves as the products.

[c] 1.8 equiv. of allyl bromide was used.

[d] 2.5 equiv. of allyl bromide was used in entries 3-15.

hindrance between the 2-hydrogen of the aromatic group and the carbonyl might be responsible for the origin of the *E*/*Z* selectivity (2.243 Å for O1···H2 in complex (*3Z,6Z*)-**13A**, while 1.988 Å for O1···H1 in complex (*3E,6Z*)-**13A**, Figure 2, or see the Supporting Information Figure S3), in accordance with Ando S. & co-worker's study.[9] And our further study demonstrated that the steric repulsion between the hydrogen atoms in benzylidene (the CH group) and in the *N*-alkylative part (the CH_2 group) might be another reason (1.952 Å for H2···H5 in complex (*3E,6Z*)-**13A** while 2.304 Å for H1···H5 in complex (*3Z,6Z*)-1**3A**, Figure 2, or see the Supporting Information Figure S3). In addition, the product of halohydrocarbon substituted on 3- or 6-position was also not monitored. We speculated that the contribution of the acetyl group resulted in the condensation of aromatic aldehydes, other than the alkylation of the allyl group,

on the 3- or 6-position more easily.[9] And the optimal reaction conditions were established: using Cs_2CO_3 as the base, the molar ratios of Cs_2CO_3, allyl bromide, and benzaldehyde to 1,4-diacetyl-2,5-DPK were 2.5:1, 2.5:1, and 2.5:1, respectively, and the reaction was firstly stirred at -10 °C until the completion of 1st Aldol condensation and the alkylation of the allyl bromide on (*3Z*)-**1a**, then heated at 95 °C in DMF with the product (*3Z,6Z*)-**3a** 55% of yield (entry 14).

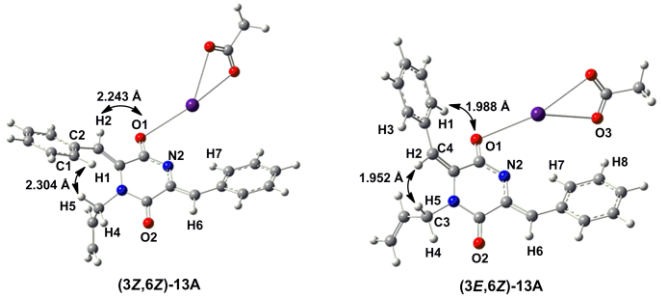
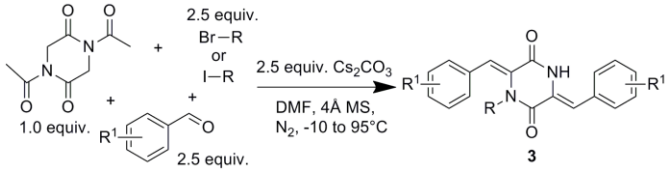


Figure 2. The distances (Å) for the selected atoms in the complex (*3Z,6Z*)-1**3A** (for compound (*3Z,6Z*)-**3a**) and in complex (*3E,6Z*)-**13A** (for compound (*3E,6Z*)-**3a**) calculated by DFT.

A variety of aromatic aldehydes or halohydrocarbons were subjected to the above one-pot method, and the results were summarized in Table 2. Using 3- or 4-substituted aromatic aldehydes, the reactions showed good site- and stereoselectivities to form (*3Z,6Z*)-**3** with yields ranging from 31-64%. However, when used 2-substituted aromatic aldehydes (**3b, 3c, 3j**) as the modifying functions, the products were (*3Z,6Z*)-**3** with trace amount of site isomers (*3Z,6Z*)-**4**, which indicated that 2-substituted aromatic aldehydes had a negligible steric hindrance which influenced the alkylation of halohydrocarbon selectively on the nitrogen or oxygen atom. And the results also showed that distinct *N*-alkylative groups had little influence on the stereo- or siteselectivity in the synthesis of the alkylated 2,5-DKPs. Similarly, different aromatic aldehydes seemed to have little influence on the yields of the derivatives. This one-pot strategy was then used to synthesize the natural product piperafizine A, and the formation of this cytotoxic compound was accomplished easily in 47% yield under the mild conditions.

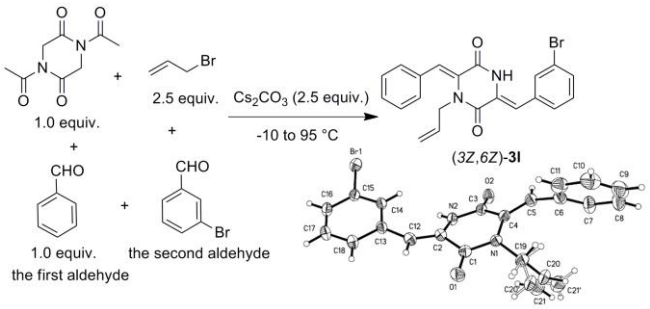
Table 2. Scope of the reaction containing three components.



Compounds	R	R^1	Yield[a](%)
(*3Z,6Z*)-**3a**	Allyl	H	55
(*3Z,6Z*)-**3b**	Allyl	2-Me	53
(*3Z,6Z*)-**3c**	Allyl	2-F	46
(*3Z,6Z*)-**3d**	Allyl	3-F	44
(*3Z,6Z*)-**3e**	Allyl	3-Cl	45
(*3Z,6Z*)-**3f**	Allyl	3-Br	58
(*3Z,6Z*)-**3g**	Allyl	4-CF_3	64
(*3Z,6Z*)-**3h**	Me	3-MeO	56
(*3Z,6Z*)-**3i**	Me	4-MeO	31
Piperafizine A	Me	H	47
(*3Z,6Z*)-**3j**	Bn	2-Cl	45
(*3Z,6Z*)-**3k**	4-CF_3Bn	4-Cl	47

[a] Isolated yield

Table 3. Ratios screening experiments on the synthesis of the compound (*3Z,6Z*)-**3l**[a].



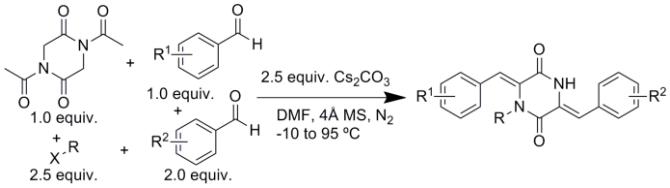
Entry	Benzaldehyde (equiv)	Yield(%)[b] (*3Z,6Z*)-**3l**
1	1.0	39
2	1.5	38
3	2.0	49
4	2.5	36

[a] Reaction conditions: 1,4-diacetyl-2,5-DPK (0.25 mmol), benzaldehyde (0.25 mmol), allyl bromide (0.63 mmol), Cs_2CO_3 (0.63 mmol), and 4Å MS were mixed in 2 mL dry DMF under a N_2 atmosphere. The reaction was firstly stirred at -10 ºC until the completion of the 1st Aldol condensation and the alkylation of the allyl bromide, and then 3-bromobenzaldehyde (1.0 – 2.5 equiv.) was added and the mixture was heated at 95 ºC for about 4 h.

[b] Isolated yield.

The strategy was further employed to the synthesis of derivatives with two different side aromatic groups substituted at 3- and 6-positions. In order to realize the site selectivity of different aromatic aldehydes, the amount of Cs_2CO_3 and allyl bromide were kept unchangeable according to the above optimal conditions, while the addition order of the two different aldehydes was revised by adding the second aldehyde after the first one had completely reacted with 1,4-diacetyl-2,5-DPK. The amount of the first aldehyde was maintained at 1.0 equivalent in avoidance of the formation of the by-products which condensed the same aldehydes at the 3- and 6-positions, and the molar ratios of the second aromatic aldehyde were investigated for high yield of the derivatives. This goal was achieved based on the template synthesis of compound (*3Z,6Z*)-**3l** which used benzaldehyde and 3-bromobenzaldehyde as the first and the second aldehydes, respectively (Table 3).

Table 4. Scope of the reaction containing four components.



Compounds	R	R^1	R^2	Yield(%)[a]
(3Z,6Z)-**3m**	Allyl	2-F	2,3-$(Cl)_2$	43
(3Z,6Z)-**3n**	Allyl	3-F	2,3-$(Cl)_2$	51
(3Z,6Z)-**3o**	Allyl	2-MeO	2,3-$(Cl)_2$	45
(3Z,6Z)-**3p**	Me	H	3-Cl	50
(3Z,6Z)-**3q**	Me	H	3-Br	49
(3Z,6Z)-**3r**	Me	H	2,3-$(Cl)_2$	60
(3Z,6Z)-**3s**	Bn	H	3-Cl	38

(3Z,6Z)-**3t**	4-CF_3Bn	4-Br	3-Me	52	

[c] "-": Not examined.

[a] Isolated yield

Following these changes, another eight 2,5-diketopiperazine derivatives (*3Z,6Z*)-**3m**-**t** were synthesized smoothly with moderate yields (38-60%) (Table 4). Likewise, trace amount of *O*-site isomer (*3Z,6Z*)-**4o** was also observed in the preparation of the known compound (*3Z,6Z*)-**3o**,[5a] which was consistent with the above examples that 2-substituted aromatic aldehydes showed a negligible steric hindrance which influenced the site selectivity of the alkylation.

3. Cytotoxic study

The cytotoxic results were summarized in Table 5. As shown in Table 5, nearly half of these compounds ((*3Z,6Z*)-**3a**–**c**, **3h**, **3j**, **3n**–**q**) displayed strong cytotoxic activities against one to five of the cancer cell lines, and different compounds exhibited distinct inhibitory activities to the cancer cells. From the results, methyl was the best favorable substitutive group for the cytotoxicities of compounds (such as (*3Z,6Z*)-**3h**, (*3Z,6Z*)-**3p**–**r**, and piperafizine A). Allyl was also a better one (such as (*3Z,6Z*)-**3n**, (*3Z,6Z*)-**3a**–**c**) but less effective than the methyl group, however benzyl groups (benzyl, 4-trifluoromethylbeznyl) seemed to be not suitable substitutive functions. The structure — activity relationship was ambiguous because the substitutive phenyl rings or the positions of the substituents on the phenyl rings for the derivatives resulted in inconsistent cytotoxicities. Compared to plinabulin, all the bioactive compounds showed less potency, except compound (*3Z,6Z*)-**3h** (IC_{50} = 11 nM) which has a close activity to plinabulin (IC_{50} = 15 nM)[5e] against the cancer cell line HL60. Nevertheless compared to the compound TSA (trichostatin A), compound (*3Z,6Z*)-**3h** (11 and 104 nM) has stronger cytotoxicities than TSA (42 and 120 nM) against the cancer lines HL60 and K562, respectively.

Table 5. Cytotoxicities of compounds (*3Z,6Z*)-**3a**–**t**, Piperafizine A , plinabulin, and TSA against cancer cell lines[a]

	Cancer Cell Lines: IC_{50} (μM)				
Compound	U937	HL60	DU145	HT29	K562
(*3Z,6Z*)-**3a**	NA[b]	6.9±2.5	NA	NA	2.1±0.3
(*3Z,6Z*)-**3b**	3.1±0.3	1.7±0.0	4.3±1.3	2.6±0.6	NA
(*3Z,6Z*)-**3c**	16.0±7.9	13.4±1.5	10.4±0.1	11.5±1.4	NA
(*3Z,6Z*)-**3d**	NA	NA	NA	NA	NA
(*3Z,6Z*)-**3e**	NA	NA	NA	NA	NA
(*3Z,6Z*)-**3f**	NA	NA	NA	NA	NA
(*3Z,6Z*)-**3g**	NA	NA	NA	NA	NA
(*3Z,6Z*)-**3h**	0.091± 0.006	0.011± 0.001	0.272± 0.047	0.22± 0.05	0.104± 0.012
(*3Z,6Z*)-**3i**	NA	NA	NA	NA	NA
(*3Z,6Z*)-**3j**	20.3±7.4	2.5±0.1	NA	NA	26.1±0.0
(*3Z,6Z*)-**3k**	NA	NA	NA	NA	NA
(*3Z,6Z*)-**3l**	NA	NA	NA	NA	NA
(*3Z,6Z*)-**3m**	NA	NA	NA	NA	NA
(*3Z,6Z*)-**3n**	15.5±1.1	1.5±0.0	NA	NA	NA
(*3Z,6Z*)-**3o**	0.5 ± 0.0	2.0 ± 0.2	-[c]	-	0.9 ± 0.1
(*3Z,6Z*)-**3p**	0.2±0.0	3.2±0.1	0.9±0.2	0.71±0.05	0.28±0.01
(*3Z,6Z*)-**3q**	0.2±0.0	1.2±0.0	1.3±0.7	1.04±0.07	0.38±0.04
(*3Z,6Z*)-**3r**	2.7±0.4	3.1±0.1	4.2±0.1	2.6±0.1	1.2±0.2
(*3Z,6Z*)-**3s**	NA	NA	NA	NA	NA
(*3Z,6Z*)-**3t**	NA	NA	NA	NA	NA
(*3Z,6Z*)-**4a**	NA	NA	NA	NA	NA
Piperafizine A	1.4±0.0	NA	9.4±0.9	6.5±0.9	2.9±0.2
Plinabulin	0.0068± 0.0003	0.015± 0.001	0.0140± 0.0005	0.013± 0.001	0.0063± 0.0007
TSA	0.047± 0.004	0.042± 0.001	0.044± 0.003	0.056± 0.003	0.12± 0.02

[a] Each value represents mean ± SD of three experiments. Plinabulin and TSA (trichostatin A) are used as positive controls.

[b] NA: Not active.

4. Conclusion

In conclusion, a facile and efficient approach for multicomponent one-pot synthesis of *N*-monoalkylated 2,5-DPKs was developed with total yields highly improved (for example the total yield of compound (*3Z,6Z*)-**3o** was improved from 17%[5a] to 45%). This method could achieve three or more site selective modifications on the 2,5-DPK ring, and the synthetic compounds were more preferred to form *Z*-configuration at 3, 6-positions. The compound (*3Z,6Z*)-**3h** might be a new template for further development as anticancer agent.

5. Experimental Section

5.1. General Chemical Methods and Materials

All reactions were carried out with 4Å molecular sieve (4Å MS, activated at 500 °C before to use) in dry solvent under a nitrogen atmosphere. The anhydrous DMF were purchased from Acros Organics and stored under nitrogen. Unless otherwise noted, other chemicals obtained from commercial suppliers were used without further purification. Melting points (m.p.) were determined by using a SGW-X4 melting point instrument (INESA Com. Ltd.) without correction. Analytical thin layer chromatography was performed on Polygram SIL HSGF254 plates. Visualization was accomplished with short wave UV light, or I_2 staining. Flash column chromatography was performed using silica gel (200-300 mesh). The NMR spectra were recorded on a Bruker AC 500 NMR spectrometer with TMS as an internal standard. The data are reported as follows: chemical shift, integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, and m = multiplet), and coupling constant in Hz. HR-ESI-MS data were measured on AQUITY UPLC/Q-TOF mass spectrometer.

*5.2. General procedure for the synthesis of products (3Z,6Z)-**3a**–**k**.*

In a 25 mL two-neck flask saturated with nitrogen, 1,4-diacetyl-2,5-diketopiperazine (50 mg, 0.25 mmol, 1.0 equiv.), aldehydes (0.63 mmol, 2.5 equiv.), halohydrocarbons (0.63 mmol, 2.5 equiv.), Cs_2CO_3 (205 mg, 0.63 mmol, 2.5 equiv.), and 4Å MS (200mg) in 2 mL dry DMF were added. The reaction was firstly stirred at -10 °C until the completion of the 1st Aldol condensation and the alkylation of the halohydrocarbons, and then heated at 95°C for about 4 h (monitored with TLC analysis). The solvent was removed under the reduced pressure, and water (50 mL) and EtOAc (20 mL) were added. The mixture was extracted with EtOAc (20 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 pure product (*3Z,6Z*)-**3** as a slightly yellow oil or solid.

*5.2.1. (3Z,6Z)-4-allyl-3,6-dibenzylidenepiperazine-2,5-dione ((3Z,6Z)-**3a**)*

Following the general procedure, the compound (*3Z,6Z*)-**3a** was obtained in 55% yield as a slightly yellow oil and later changed into solid in about one week. mp = 106 – 108 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.22 (s, 1H), 7.44 (d, *J* = 5.0 Hz, 4H), 7.40 (t, *J* = 5.0 Hz, 2H), 7.36 – 7.34 (m, 2H), 7.32 (d, *J* = 5.0 Hz, 2H), 7.26 (s, 1H), 7.08 (s, 1H), 5.58 – 5.50 (m, 1H), 5.01 (dd, *J* = 10.2, 1.1 Hz, 1H), 4.77 (dd, *J* = 17.1, 1.2 Hz, 1H), 4.28 (d, *J* = 5.0 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 160.3, 159.7, 134.0, 133.1, 131.6, 129.6, 129.1, 128.75, 128.68, 128.4, 126.2, 122.5, 118.5, 118.0, 48.2. NOE correlations between hydrogen atoms in allyl group (δ: 4.28, d, *J* = 5.0 Hz, 2H, CH_2) and in

phenyl group (δ: 7.36 – 7.34, m, 2H, Ph–H), as well as hydrogen atoms in amide (δ 8.22, s, 1H, NH-1) and in phenyl group (δ: 7.44, d, *J* = 5.0 Hz, 2H, Ph–H) were observed. HRMS (ESI): m/z calcd for $C_{21}H_{19}N_2O_2$ $[M+H]^+$ 331.1441, found 331.1445; for $C_{21}H_{18}N_2O_2Na$ $[M+Na]^+$ 353.1260, found 353.1264.

*5.2.2. (3Z,6Z)-5-(allyloxy)-3,6-dibenzylidene-1,6-dihydropyrazin-2(3H)-one ((3Z,6Z)-**4a**)*

Following the general procedure but changing the firstly stirred temperature of the reaction from -10 °C to room temperature, and then the reaction mixture was heated at 95 °C for about 4 h. the compound (*3Z,6Z*)-**4a** accompanied with the compound (*3Z,6Z*)-**3a** was obtained as an orange crystal in 5% yield. mp = 147 – 149 ˚C. ^{1}H NMR (500 MHz, DMSO) δ: 10.14 (s, 1H), 8.10 (d, *J* = 10.0 Hz, 2H), 7.53 (d, *J* = 5.0 Hz, 2H), 7.42 (q, *J* = 10.0 Hz, 4H), 7.36 – 7.30 (m, 2H), 7.13 (s, 1H), 6.57 (s, 1H), 6.20 – 6.12 (m, 1H), 5.51 (d, *J* = 15.0 Hz, 1H), 5.33 (d, *J* = 10.0 Hz, 1H), 4.94 (d, *J* = 5.0 Hz, 2H). ^{13}C NMR (125 MHz, DMSO) δ: 159.4, 153.9, 134.8, 133.2, 132.8, 132.1, 131.3, 129.3, 128.8, 128.7, 128.4, 128.1, 126.0, 123.7, 118.2, 111.3, 67.3. HRMS (ESI): m/z calcd for $C_{21}H_{19}N_2O_2$ $[M+H]^+$ 331.1441, found 331.1441; for $C_{21}H_{18}N_2O_2Na$ $[M+Na]^+$ 353.1260, found 353.1259.

*5.2.3. (3Z,6Z)-4-allyl-3,6-bis(2-methylbenzylidene)piperazine-2,5-dione ((3Z,6Z)-**3b**)*

Following the general procedure, the product (*3Z,6Z*)-**3b** was obtained in 53% yield as a slightly yellow oil and changed into solid in about two weeks. mp = 80 – 83 ˚C. ^{1}H NMR (500 MHz, DMSO) δ: 10.36 (s, 1H), 7.48 (t, *J* = 5.0 Hz, 1H), 7.33 – 7.19 (m, 7H), 7.10 (s, 1H), 6.92 (s, 1H), 5.52 – 5.44 (m, 1H), 4.97 (d, *J* = 10.2 Hz, 1H), 4.55 (d, *J* = 17.1 Hz, 1H), 4.08 (d, *J* = 5.0 Hz, 2H), 2.28 (s, 3H), 2.23 (s, 3H). ^{13}C NMR (125 MHz, DMSO) δ: 159.6, 159.4, 137.0, 136.7, 133.5, 132.2, 132.0, 130.3, 130.1, 129.4, 129.1, 128.7, 128.6, 128.3, 126.8, 126.0, 125.7, 118.9, 117.1, 115.9, 45.8, 19.75, 19.71. HRMS (ESI): m/z calcd for $C_{23}H_{23}N_2O_2$ $[M+H]^+$ 359.1754, found 359.1757; for $C_{23}H_{22}N_2O_2Na$ $[M+Na]^+$ 381.1573, found 381.1577.

*5.2.4.(3Z,6Z)-4-allyl-3,6-bis(2-fluorobenzylidene)piperazine-2,5-dione ((3Z,6Z)-**3c**)*

Following the general procedure, the product (*3Z,6Z*)-**3c** was obtained in 46% yield as a slightly yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.37 (s, 1H), 7.43 (t, *J* = 10.0 Hz, 1H), 7.39 – 7.31 (m, 2H), 7.27 (d, *J* = 10.0 Hz, 1H), 7.23 – 7.17 (m, 2H), 7.15 (s, 1H), 7.13 – 7.10 (m, 1H), 7.06 (s, 1H), 5.58 – 5.47 (m, 1H), 5.02 (d, *J* = 10.2 Hz, 1H), 4.74 (d, *J* = 17.1 Hz, 1H), 4.28 (d, *J* = 5.0 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 160.3 (d, J_{FC} = 250.0 Hz), 160.1 (d, J_{FC} = 247.5 Hz), 159.6, 158.9, 131.3, 131.1, 131.0 (d, J_{FC} = 5.0 Hz), 130.9, 130.3 (d, J_{FC} = 5.0 Hz), 129.9, 127.3, 125.0 (d, J_{FC} = 3.4 Hz), 124.2 (d, J_{FC} = 3.4 Hz), 122.3 (d, J_{FC} = 14.8 Hz), 120.8 (d, J_{FC} = 14.9 Hz), 118.5, 116.7 (d, J_{FC} = 22.0 Hz), 116.0 (d, J_{FC} = 21.3 Hz), 115.1, 111.5, 47.6. HRMS (ESI): m/z calcd for $C_{21}H_{17}F_2N_2O_2$ $[M+H]^+$ 367.1253, found 367.1262; for $C_{21}H_{16}F_2N_2O_2Na$ $[M+Na]^+$ 389.1072, found 389.1081.

*5.2.5. (3Z,6Z)-4-allyl-3,6-bis(3-fluorobenzylidene)piperazine-2,5-dione ((3Z,6Z)-**3d**)*

Following the general procedure, the product (*3Z,6Z*)-**3d** was obtained in 44% yield as a slightly yellow oil and changed into solid in about two days. mp = 110 – 113 ˚C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.47 (s, 1H), 7.43 – 7.35 (m, 2H), 7.21 (d, *J* = 5.0 Hz, 1H), 7.15 (d, *J* = 15.0 Hz, 2H), 7.09 (d, *J* = 10.0 Hz, 1H), 7.06 – 7.00 (m,4H), 5.57 – 5.50 (m, 1H), 5.04 (d, *J* = 10.2 Hz, 1H), 4.79 (d, *J* = 17.1 Hz, 1H), 4.27 (d, *J* = 10.0 Hz, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 163.3 (d, J_{FC} = 246.3 Hz), 162.7 (d, J_{FC} = 247.5 Hz,), 160.1, 159.4, 136.2 (d, J_{FC} = 8.0 Hz), 135.1 (d, J_{FC} = 8.0 Hz), 131.4, 131.2 (d, J_{FC} = 8.4 Hz), 130.3 (d, J_{FC} = 8.3 Hz), 129.1, 126.8 , 125.4 (d, J_{FC} = 2.8 Hz), 124.6 (d, J_{FC} = 2.8 Hz), 121.0, 118.6, 116.9, 116.4 (d, J_{FC} = 22.0 Hz), 116.1 (d, J_{FC} = 8.1 Hz), 116.0 (d, J_{FC} = 8.1 Hz), 115.8 (d, J_{FC} = 22.0 Hz), 48.3. HRMS (ESI): m/z calcd for $C_{21}H_{17}F_2N_2O_2$ $[M+H]^+$ 367.1253, found 367.1266; for $C_{21}H_{16}F_2N_2O_2Na$ $[M+Na]^+$ 389.1072, found 389.1083.

*5.2.6. (3Z,6Z)-4-allyl-3,6-bis(3-chlorobenzylidene)piperazine-2,5-dione ((3Z,6Z)-**3e**)*

Following the general procedure, the product (*3Z,6Z*)-**3e** was obtained in 45% yield as a slightly yellow solid. mp = 132 – 134 ˚C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.62 (s, 1H), 7.44 (s, 1H), 7.38 – 7.34 (m, 3H), 7.31 – 7.28 (m, 3H), 7.20 – 7.18 (m, 1H), 7.10 (s, 1H), 7.01 (s, 1H), 5.57 – 549 (m, 1H), 5.04 (dd, *J* = 10.2, 0.8 Hz, 1H), 4.78 (dd, *J* = 17.1, 0.9 Hz, 1H), 4.26 (d, *J* = 5.0 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 160.2, 159.3, 135.9, 135.4, 134.8, 134.7, 131.3, 130.7, 129.9, 129.4, 129.2, 129.1, 129.0, 128.8, 127.6, 127.0, 126.9, 120.8, 118.6, 116.8, 48.3. HRMS (ESI): m/z calcd for $C_{21}H_{17}Cl_2N_2O_2$ $[M+H]^+$ 399.0662, found 399.0664; for $C_{21}H_{16}Cl_2N_2O_2Na$ $[M+Na]^+$ 321.0481, found 421.0480.

*5.2.7. (3Z,6Z)-4-allyl-3,6-bis(3-bromobenzylidene)piperazine-2,5-dione ((3Z,6Z)-**3f**)*

Following the general procedure, the product (*3Z,6Z*)-**3f** was obtained in 58% yield as a slightly yellow solid. mp = 114 – 116 ˚C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.72 (s, 1H), 7.64 (s, 1H), 7.52 (d, *J* = 10.0 Hz, 1H), 7.45 (d, *J* = 15.0 Hz, 2H), 7.38 (d, *J* = 10.0 Hz, 1H), 7.34 – 7.29 (m, 2H), 7.26 (d, *J* = 10.0 Hz, 1H), 7.10 (s, 1H), 7.03 (s, 1H), 5.59 – 5.52 (m, 1H), 5.07 (d, *J* = 10.2 Hz, 1H), 4.81 (d, *J* = 17.1 Hz, 1H), 4.28 (d, *J* = 10.0 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 160.3, 159.3, 136.1, 135.2, 132.2, 132.0, 131.8, 131.6, 131.3, 130.9, 130.2, 129.2, 128.0, 127.6, 127.0, 123.5, 122.8, 120.6, 118.6, 116.9, 48.3. HRMS (ESI): m/z calcd for $C_{21}H_{17}Br_2N_2O_2$ $[M+H]^+$ 486.9651, found 486.9654; for $C_{21}H_{16}Br_2N_2O_2Na$ $[M+Na]^+$ 508.9471, found 508.9480.

*5.2.8. (3Z,6Z)-4-allyl-3,6-bis(4-(trifluoromethyl)benzylidene)piperazine-2,5-dione ((3Z,6Z)-**3g**)*

Following the general procedure, the product (*3Z,6Z*)-**3g** was obtained in 64% yield as a slightly yellow solid. mp = 130 – 133 ˚C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.64 (s, 1H), 7.71 (d, *J* = 5.0 Hz, 2H), 7.67 (d, *J* = 10.0 Hz, 2H), 7.57 (d, *J* = 5.0 Hz, 2H), 7.42 (d, *J* = 5.0 Hz, 2H), 7.17 (s, 1H), 7.10 (s, 1H), 5.56 – 5.48 (m, 1H), 5.04 (dd, *J* = 10.2, 0.6 Hz, 1H), 4.75 (dd, *J* = 17.1, 0.7 Hz, 1H), 4.24 (d, *J* = 5.0 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 160.0, 159.2, 137.7, 136.6, 131.1, 130.9 (dd, J_{FC} = 32.7, 11.3 Hz), 129.8, 129.7, 129.3, 127.3, 126.4 (d, J_{FC} = 3.6 Hz), 125.7 (d, J_{FC} = 3.7 Hz), 124.0 (d, J_{FC} = 271.0 Hz), 120.6, 118.8, 116.7, 48.4. HRMS (ESI): m/z calcd for $C_{23}H_{17}F_6N_2O_2$ $[M+H]^+$ 467.1189, found 467.1197; for $C_{23}H_{16}F_6N_2O_2Na$ $[M+Na]^+$ 489.1008, found 489.1015.

*5.2.9. (3Z,6Z)-3,6-bis(3-methoxybenzylidene)-4-methylpiperazine-2,5-dione ((3Z,6Z)-**3h**)*

Following the general procedure, the product (*3Z,6Z*)-**3h** was obtained in 56% yield as a slightly yellow solid. mp = 141 – 143 ˚C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.30 (s, 1H), 7.35 (t, *J* = 10.0 Hz, 1H), 7.29 (t, *J* = 10.0 Hz, 1H), 7.23 (s, 1H), 7.02 (s, 1H), 7.01 (d, *J* = 5.0 Hz, 1H), 6.92 (s, 1H), 6.89 – 6.85 (m, 3H), 6.81 (s, 1H), 3.81 (s, 6H), 3.01 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$)

δ: 160.4, 159.52, 159.46, 135.4, 134.4, 130.6, 129.5, 126.2, 122.1, 121.0, 120.7, 117.3, 115.0, 114.5, 114.4, 114.3, 55.49, 55.47, 36.8. HRMS (ESI): m/z calcd for $C_{21}H_{21}N_2O_4$ $[M+H]^+$ 365.1496, found 365.1505; for $C_{21}H_{20}N_2O_4Na$ $[M+Na]^+$ 387.1315, found 387.1322.

*5.2.10. (3Z,6Z)-3,6-bis(4-methoxybenzylidene)-4-methylpiperazine-2,5-dione ((3Z,6Z)-**3i**)*

Following the general procedure, the product (*3Z,6Z*)-**3i** was obtained in 31% yield as a slightly yellow solid. mp = 148 – 150 ˚C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.16 (s, 1H), 7.38 (d, *J* = 10.0 Hz, 2H), 7.23 (d, *J* = 10.0 Hz, 2H), 7.20 (s, 1H), 6.99 (s, 1H), 6.95 (d, *J* = 10.0 Hz, 2H), 6.91 (d, *J* = 5.0 Hz, 2H), 3.83 (s, 3H), 3.82 (s, 3H), 3.02 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 160.2, 160.1, 160.0, 131.3, 130.3, 129.4, 126.2, 125.5, 124.8, 121.1, 117.3, 115.0, 114.0, 55.6, 55.5, 36.8. HRMS (ESI): m/z calcd for $C_{21}H_{21}N_2O_4$ $[M+H]^+$ 365.1496, found 365.1506; for $C_{21}H_{20}N_2O_4Na$ $[M+Na]^+$ 387.1315, found 387.1323.

*5.2.11. (3Z,6Z)-4-benzyl-3,6-bis(2-chlorobenzylidene)piperazine-2,5-dione ((3Z,6Z)-**3j**)*

Following the general procedure, the product (*3Z,6Z*)-**3j** was obtained in 45% yield as a slightly yellow solid. mp = 170 – 172 ˚C. ^{1}H NMR (500 MHz, DMSO) δ: 10.80 (s, 1H), 7.69 (d, *J* = 5.0 Hz, 1H), 7.53 (t, *J* = 5.0, 2H), 7.46 (d, *J* = 5.0 Hz, 1H), 7.43 – 7.35 (m, 4H), 7.21 (dd, *J* = 4.9, 1.5 Hz, 3H), 7.02 (s, 1H), 6.96 (s, 1H), 6.75 (t, *J* = 5.0 Hz, 2H), 4.70 (s, 2H). ^{13}C NMR (125 MHz, DMSO) δ: 159.4, 159.3, 135.7, 133.31, 133.26, 132.4, 131.5, 131.3, 130.6, 130.4, 130.0, 129.6, 129.3, 128.4, 128.1, 127.9, 127.44, 127.38, 127.0, 126.8, 116.5, 113.5, 47.3. NOE correlations between hydrogen atoms in benzyl group (δ: 4.7, s, 2H, CH_2) and in phenyl group (δ: 6.75, t, *J* = 5.0 Hz, 1H, Ph–H), as well as hydrogen atoms in amide (δ: 10.80, s, 1H, NH-1) and in phenyl group (δ 7.69, d, *J* = 5.0 Hz, 1H, Ph–H) were observed. HRMS (ESI): m/z calcd for $C_{25}H_{19}Cl_2N_2O_2$ $[M+H]^+$ 449.0818, found 449.0823; for $C_{25}H_{18}Cl_2N_2O_2Na$ $[M+Na]^+$ 471.0638, found 471.0604.

*5.2.12. (3Z,6Z)-3,6-bis(4-chlorobenzylidene)-4-(4-(trifluoromethyl)benzyl)piperazine-2,5-dione ((3Z,6Z)-**3k**)*

Following the general procedure, the product (*3Z,6Z*)-**3k** was obtained in 47% yield as a slightly yellow solid. mp = 190 – 192 ˚C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.52 (s, 1H), 7.46 (d, *J* = 10.0 Hz, 2H), 7.41 – 7.35 (m, 6H), 7.22 (d, *J* = 10.0 Hz, 2H), 7.12 (s, 1H), 7.03 (s, 1H), 7.00 (d, *J* = 10.0 Hz, 2H), 4.86 (s, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 159.9, 159.8, 140.0, 135.4, 135.1, 132.0, 131.3, 130.9, 130.2, 129.8, 129.1, 127.8, 126.1, 125.8, 125.7, 121.3, 117.6, 49.0. HRMS (ESI): m/z calcd for $C_{26}H_{18}Cl_2F_3N_2O_2$ $[M+H]^+$ 517.0692, found 517.0702; for $C_{26}H_{17}Cl_2F_3N_2O_2Na$ $[M+Na]^+$ 539.0511, found 539.0517.

5.2.13. Piperafizine A

Following the general procedure, the natural product **piperafizine A** was obtained in 47% yield as a slightly yellow solid. mp = 174 – 177 ˚C (lit: 181 – 182 ˚C).[1] ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.06 (s, 1H), 7.47 – 7.41 (m, 4H), 7.39 (d, *J* = 5.0 Hz, 2H), 7.36 – 7.32 (m, 2H), 7.30 (s, 2H), 7.29 (s, 1H), 7.08 (s, 1H), 3.01 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 159.7, 159.6, 134.1, 133.1, 130.5, 129.7, 129.1, 128.8, 128.7, 128.5, 126.1, 121.3, 117.4, 37.0. HRMS (ESI): m/z calcd for $C_{19}H_{17}N_2O_2$ $[M+H]^+$ 305.1285, found 305.1281; for $C_{19}H_{16}N_2O_2Na$ $[M+Na]^+$ 327.1104, found 327.1100.

*5.3 General procedure for the synthesis of products (3Z,6Z)-**3l**–**t***

In a 25 mL two-neck flask saturated with nitrogen, 1,4-diacetyl-2,5-diketopiperazine (50 mg, 0.25 mmol, 1.0 equiv.), the first aldehydes (0.25 mmol, 1.0 equiv.), halohydrocarbons (0.63 mmol, 2.5 equiv.), Cs_2CO_3 (205 mg, 0.63 mmol, 2.5 equiv.), and 4Å MS (200mg) in 2 mL dry DMF were added. The reaction was stirred at -10 ℃ until the completion of the 1st Aldol condensation and the alkylation of the halohydrocarbons, 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 solvent was removed under the reduced pressure, and water (50 mL) and EtOAc (20 mL) were added. The mixture was extracted with EtOAc (20 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 pure product (*3Z,6Z*)-**3** as a slightly yellow solid.

*5.3.1. (3Z,6Z)-4-allyl-3-benzylidene-6-(3-bromobenzylidene)piperazine-2,5-dione ((3Z,6Z)-**3l**)*

Following the general procedure, the product (*3Z,6Z*)-**3l** was obtained in 49% yield as a slightly yellow solid. mp = 87 – 90 ˚C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.67 (s, 1H), 7.62 (s, 1H), 7.44 – 7.39 (m, 3H), 7.37 – 7.35 (m, 2H), 7.32 – 7.27 (m, 3H), 7.17 (s, 1H), 6.99 (s, 1H), 5.57 – 5.49 (m, 1H), 5.01 (d, *J* = 10.0 Hz, 1H), 4.76 (dd, *J* = 17.1, 1.0 Hz, 1H), 4.27 (t, *J* = 10.0 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 160.7, 159.5, 135.3, 133.9, 131.8, 131.6, 131.5, 130.9, 129.6, 129.2, 128.7, 128.2, 127.5, 127.2, 123.6, 122.9, 118.5, 116.4, 48.2. HRMS (ESI): m/z calcd for $C_{21}H_{18}BrN_2O_2$ $[M+H]^+$ 409.0546, found 409.0553; for $C_{21}H_{17}BrN_2O_2Na$ $[M+Na]^+$ 431.0366, found 431.0367.

*5.3.2. (3Z,6Z)-4-allyl-6-(2,3-dichlorobenzylidene)-3-(2-fluorobenzylidene)piperazine-2,5-dione ((3Z,6Z)-**3m**)*

Following the general procedure, the product (*3Z,6Z*)-**3m** was obtained in 43% yield as a slightly yellow solid. mp = 67 – 68 ˚C. ^{1}H NMR (500 MHz, DMSO) δ: 10.77 (s, 1H), 7.60 (dd, *J* = 15.0, 5.0 Hz, 2H), 7.48 – 7.40 (m, 3H), 7.31 – 7.27 (m, 2H), 7.02 (s, 1H), 6.89 (s, 1H), 5.55 – 5.51 (m, 1H), 4.97 (dd, *J* = 10.2, 1.0 Hz, 1H), 4.57 (dd, *J* = 17.1, 1.2 Hz, 1H), 4.18 (d, *J* = 10.0 Hz, 2H). ^{13}C NMR (125 MHz, DMSO) δ: 159.5 ($J_{C\text{-}F}$ = 246.3 Hz), 159.4, 158.7, 134.2, 132.0, 131.9, 131.2, 131.1, 131.0, 130.6, 130.0, 129.3, 128.8, 128.3, 124.4, 121.8 ($J_{C\text{-}F}$ = 13.8 Hz), 117.3, 115.6 ($J_{C\text{-}F}$ = 21.3 Hz), 113.1, 112.6, 46.3. NOE correlations between hydrogen atoms in allyl group (δ: 4.18, d, *J* = 10.0 Hz, 2H, CH_2) and in phenyl group (δ: 7.48 – 7.40, m, 1H, Ph–H), as well as hydrogen atoms in amide (δ: 10.77, s, 1H, NH-1) and in phenyl group (δ: 7.60, dd, *J* = 15.0, 5.0 Hz, 1H, Ph–H) were observed. HRMS (ESI): m/z calcd for $C_{21}H_{16}Cl_2FN_2O_2$ $[M+H]^+$ 417.0567, found 417.0575; for $C_{21}H_{15}Cl_2FN_2O_2Na$ $[M+Na]^+$ 439.0387, found 439.0388.

*5.3.3. (3Z,6Z)-4-allyl-6-(2,3-dichlorobenzylidene)-3-(3-fluorobenzylidene)piperazine-2,5-dione ((3Z,6Z)-**3n**)*

Following the general procedure, the product (*3Z,6Z*)-**3n** was obtained in 51% yield as a slightly yellow solid. mp = 152 – 155 ˚C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.71 (s, 1H), 7.43 (d, *J* = 10.0 Hz, 1H), 7.40 – 7.36 (m, 1H), 7.34 (d, *J* = 5.0 Hz, 1H), 7.25 (t, *J* = 10.0 Hz, 1H), 7.12 (s, 1H), 7.09 – 7.04 (m, 2H), 7.03 (s, 1H), 7.01 (d, *J* = 10.0 Hz, 1H), 5.57 – 5.49 (m, 1H), 5.04 (dd, *J* = 10.2, 0.6 Hz, 1H), 4.77 (dd, *J* = 17.1, 0.8 Hz, 1H), 4.28 (d, *J* = 5.0 Hz, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 162.7 ($J_{C\text{-}F}$ = 246.3 Hz), 160.1, 158.9, 136.1 ($J_{C\text{-}F}$ = 8.8 Hz), 134.5, 133.8, 132.9, 131.3, 130.6, 130.3 ($J_{C\text{-}F}$ = 8.8 Hz), 128.8, 127.91, 127.89, 125.35, 125.33, 121.1, 118.7, 116.3 ($J_{C\text{-}F}$ = 21.3 Hz), 116.1 ($J_{C\text{-}F}$ = 20.0 Hz), 115.0, 48.2. HRMS (ESI): m/z calcd for $C_{21}H_{16}Cl_2FN_2O_2$

$[M+H]^+$ 417.0567, found 417.0575; for $C_{21}H_{15}Cl_2FN_2O_2Na$ $[M+Na]^+$ 439.0387, found 439.0391.

*5.3.4. (3Z,6Z)-4-allyl-6-(2,3-dichlorobenzylidene)-3-(2-methoxybenzylidene)piperazine-2,5-dione ((3Z,6Z)-**3o**)*

Following the general procedure, the product (*3Z,6Z*)-**3o** was obtained in 45% yield as a slightly yellow solid. mp = 139 – 141 °C (lit: 134 – 136 °C).[2] ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.97 (s, 1H), 7.46 (d, *J* = 5.0 Hz, 1H), 7.37 (t, *J* = 10.0 Hz, 2H), 7.29 (m, 7.31 – 7.27, 2H), 7.22 (d, *J* = 10.0 Hz, 1H), 7.11 (s, 1H), 6.99 (t, *J* = 10.0 Hz, 1H), 6.93 (d, *J* = 5.0 Hz, 1H), 5.62 – 5.49 (m, 1H), 5.01 (d, *J* = 10.2 Hz, 1H), 4.76 (d, *J* = 17.1 Hz, 1H), 4.26 (d, *J* = 5.0 Hz, 2H), 3.86 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 160.0, 158.5, 157.4, 134.6, 133.7, 132.8, 131.3, 130.8, 130.6, 130.5, 128.0, 127.9, 127.4, 122.7, 120.3, 119.1, 118.4, 113.6, 110.7, 55.54, 47.49. HRMS (ESI): m/z calcd for $C_{22}H_{19}Cl_2N_2O_3$ $[M+H]^+$ 429.0767, found 429.0769; for $C_{22}H_{19}Cl_2N_2O_3Na$ $[M+Na]^+$ 451.0587, found 451.0586.

*5.3.5. (3Z,6Z)-3-benzylidene-6-(3-chlorobenzylidene)-4-methylpiperazine-2,5-dione ((3Z,6Z)-**3p**)*

Following the general procedure, the product (*3Z,6Z*)-**3p** was obtained in 50% yield as a slightly yellow solid. mp = 137 – 139 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.51 (s, 1H), 7.43 (s, 1H), 7.41 – 7.34 (m, 4H), 7.32 – 7.28 (m, 4H), 7.23 (s, 1H), 6.99 (s, 1H), 3.00 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 159.9, 159.4, 135.5, 135.0, 134.0, 130.7, 130.3, 129.6, 129.0, 128.83, 128.76, 128.5, 127.0, 126.9, 121.7, 115.9, 37.0. HRMS (ESI): m/z calcd for $C_{19}H_{16}ClN_2O_2$ $[M+H]^+$ 339.0895, found 339.0895; for $C_{19}H_{15}ClN_2O_2Na$ $[M+Na]^+$ 361.0714, found 361.0716.

*5.3.6. (3Z,6Z)-3-benzylidene-6-(3-bromobenzylidene)-4-methylpiperazine-2,5-dione((3Z,6Z)-**3q**)*

Following the general procedure, the product (*3Z,6Z*)-**3q** was obtained in 49% yield as a slightly yellow solid. mp = 159 – 161 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.73 (s, 1H), 7.60 (s, 1H), 7.46 (d, *J* = 10.0 Hz, 1H), 7.41 – 7.37 (m, 2H), 7.35 (d, *J* = 5.0 Hz, 2H), 7.33 (d, *J* = 5.0 Hz, 1H), 7.29 (d, *J* = 10.0 Hz, 3H), 7.01 (s, 1H), 3.00 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 160.2, 159.4, 136.5, 135.2, 134.0, 131.9, 131.7, 131.0, 130.3, 129.7, 128.9, 128.6, 127.5, 126.9, 121.8, 116.2, 37.0. HRMS (ESI): m/z calcd for $C_{19}H_{16}BrN_2O_2$ $[M+H]^+$ 383.0390, found 383.0393; for $C_{19}H_{15}BrN_2O_2Na$ $[M+Na]^+$ 405.0209, found 405.0213.

*5.3.7. (3Z,6Z)-3-benzylidene-6-(2,3-dichlorobenzylidene)-4-methylpiperazine-2,5-dione (3Z,6Z)-**3r**)*

Following the general procedure, the product (*3Z,6Z*)-**3r** was obtained in 60% yield as a slightly yellow solid. mp = 189 – 190 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.60 (s, 1H), 7.46 (d, *J* = 10.0 Hz, 1H), 7.43 – 7.39 (m, 2H), 7.38 – 7.35 (m, 2H), 7.29 (t, *J* = 10.0 Hz, 3H), 7.16 (s, 1H), 7.12 (s, 1H), 3.01 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 159.7, 158.9, 134.5, 134.0, 133.9, 132.9, 130.6, 130.1, 129.6, 128.8, 128.5, 127.93, 127.86, 121.7, 114.1, 37.0. HRMS (ESI): m/z calcd for $C_{19}H_{15}Cl_2N_2O_2$ $[M+H]^+$ 373.0505, found 373.0507.

*5.3.8. (3Z,6Z)-4-benzyl-3-benzylidene-6-(3-chlorobenzylidene)piperazine-2,5-dione (3Z,6Z)-**3s**)*

Following the general procedure, the product (*3Z,6Z*)-**3s** was obtained in 38% yield as a slightly yellow solid. mp = 121 – 123 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.75 (s, 1H), 7.46 – 7.37 (m, 4H), 7.33 – 7.29 (m, 4H), 7.24 (d, *J* = 5.0 Hz, 1H), 7.19 – 7.18 (m, 3H), 7.14 (s, 1H), 7.02 (s, 1H), 6.90 – 6.88 (m, 2H), 4.84 (s, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 160.7, 159.8, 136.2, 135.3, 134.9, 133.8, 130.6, 129.7, 129.3, 128.9, 128.8, 128.74, 128.65, 128.5, 127.8, 127.7, 127.12, 127.06, 123.0, 116.7, 49.1. HRMS (ESI): m/z calcd for $C_{25}H_{20}ClN_2O_2$ $[M+H]^+$ 415.1208, found 415.1209; for $C_{25}H_{19}ClN_2O_2Na$ $[M+Na]^+$ 437.1027, found 437.1032.

*5.3.9. (3Z,6Z)-3-(4-bromobenzylidene)-6-(3-methylbenzylidene)-4-(4-(trifluoromethyl)benzyl)piperazine-2,5-dione ((3Z,6Z)-**3t**)*

Following the general procedure, the product (*3Z,6Z*)-**3t** was obtained in 52% yield as a slightly yellow solid. mp = 176 – 179 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.11 (s, 1H), 7.52 (d, *J* = 5.0 Hz, 2H), 7.46 (d, *J* = 5.0 Hz, 2H), 7.34 (t, *J* = 10.0 Hz, 1H), 7.22 (s, 2H), 7.17 (d, *J* = 10.0 Hz, 2H), 7.14 (d, *J* = 10.0 Hz, 2H), 7.08 (s, 1H), 7.01 (d, *J* = 5.0 Hz, 2H), 4.87 (s, 2H), 2.38 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 160.0, 159.6, 140.0, 139.6, 132.7, 132.6, 132.0, 131.1, 130.2, 129.6, 129.32, 129.31, 127.8, 125.8, 125.75, 125.72, 125.5, 123.5, 121.0, 119.2, 49.0, 21.7. HRMS (ESI): m/z calcd for $C_{27}H_{21}BrF_3N_2O_2$ $[M+H]^+$ 541.0733, found 541.0741; for $C_{27}H_{20}BrF_3N_2O_2Na$ $[M+Na]^+$ 563.0552, found 563.0558.

5.4. Cytotoxic experiments[5a]

Cell lines, U937, HL60, DU145, HT29, and K562 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 ℃. 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).

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Supplementary Material

Supplementary data (^{1}H, ^{13}C NMR spectra for all compound, NOESY NMR spectra for compounds (*3Z,6Z*)-**3a**, (*3Z,6Z*)-**3j**, and (*3Z,6Z*)-**3m**, X-ray analysis data for compounds (*3Z,6Z*)-**3l** and (*3Z,6Z*)-**4a**, and computational calculations) associated with this article can be found in the online version, at http://xxxxxxxxxxxxxxxxxx

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