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Table of Contents Graphic and Synopsis

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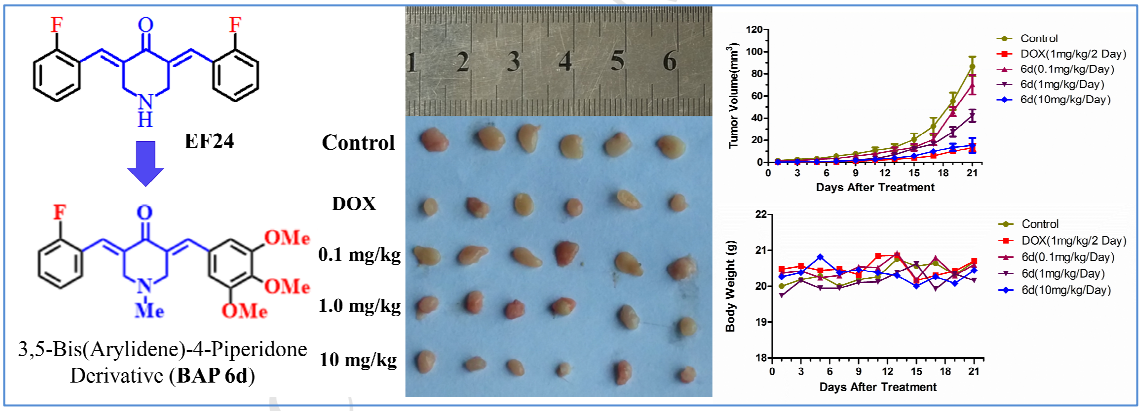
Thirty-five novel dissymmetric 3,5-bis(arylidene)-4-piperidone derivatives (**BAPs**) were generated and evaluated as potential antitumor agents *in vitro* and *in vivo*.



Novel dissymmetric 3,5-bis(arylidene)-4-piperidones as potential antitumor agents with biological evaluation *in vitro* and *in vivo*

Ning Li[a,1], Wen-Yu Xin[a,1], Bin-Rong Yao[a,1], Chun-Hua Wang[a], Wei Cong[a], Feng Zhao[a], Hong-Juan Li[a], Yun Hou[a], Qing-Guo Meng[b,*] , Gui-Ge Hou[a,*]

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Abstract: Thirty-five novel dissymmetric 3,5-bis(arylidene)-4-piperidone derivatives (BAPs, **6a-h**, **7a-h**, **8a-g**, **9a-g**, **10a-e**) were synthesized and evaluated the cytotoxicity. BAPs **6d**, **7h**, **8g**, **9g** demonstrated the most potentially inhibitory activities against HepG2 and THP-1 but lower cytotoxicity toward LO2. *In vitro*, **6d**, **7h**, **8g**, **9g** can effectively up-regulate BAX expression, down-regulate Bcl-2 expression in HepG2 cell. They could reasonably bind to the active site of Bcl-2 protein proved by molecular docking modes. The most active BAP **6d** induced HepG2 cells apoptosis in a dose-dependent manner by flow cytometrey. The cellular uptake of HepG2 cells showed **6d** mainly accumulated into the nuclei by confocal laser scanning microscopy (CLSM). *In vivo*, **6d** suppressed the growth of HepG2 xenografts in nude mice and relatively nontoxic to mice. These results suggest that **6d** could be therapeutically beneficial as potential therapeutic agent for the early clinical treatment of liver cancers.

Keywords: 3,5-bis(arylidene)-4-piperidone; cytotoxicity; cellular uptake; molecular docking; tumor xenograft

1. Introduction

Malignant tumor seriously hazard to human health, and its incidence has increased in recent years [1, 2]. Targeted oncology drugs may be most promising strategy of the treatment for malignant tumors [3, 4]. Curcumin (Fig. 1), due to its antiproliferative and antiangiogenic properties, as well as minimal toxicity [5, 6], has attracted great attention as a possible novel anticancer agent. Unfortunately, its low bioavailability hinders further clinical application [7]. In order to improve these defects, many improved curcumin analogues have been reported [8]. Therein, (*3E,5E*)-3,5-bis(arylidene)-4-piperidone (BAP) derivatives, have displayed good antitumor activities because of their double *α,β*-unsaturated ketone structural characteristics, which have a greater preference or exclusive affinity for bio-thiols in contrast to amino and hydroxy groups resulting a greater chemosensitivity to tumors rather than with normal cells [9-13]. In addition, two of *α,β*-unsaturated keto groups enable sequential attacks of cellular thiols to display the better activities [14].

3,5-Bis(2-fluorobenzylidene)-4-piperidone (EF24, Fig. 1) is a potent antitumor agent that inhibits tumor growth and metastasis by inhibiting NF-*κ*B dependent signaling pathways [15]. EF24 can result in a decrease in Akt and MDM2 and an increase in the expression of p53 by up-regulation of PTEN, thereby inducing G2/M arrest and apoptosis in human ovarian cancer cells [16]. Also, EF24 can disrupt normal mitosis, interphase microtubule organization and stabilize cellular microtubule [17]. In addition, EF24 exhibites a more potent cytotoxic effect than curcumin, directly inhibits the IKK*β* kinase activity [18]. Another curcuminoid 4-(3,5-bis(2-chlorobenzylidene-4-oxo-piperidine-1-yl)-4-oxo-2-butenoic acid) (CLEFMA) with the better anti-proliferative activity in H441 cells than EF24 was reported [11]. Furthermore, nitrogen-containing heterocyclic dienones, such as 4-piperidone, displayed higher inhibitory properties toward human carcinoma cell lines compared with their homocyclic dienone analogs (such as cyclohexanone) [19-21].

Recently, we have reported many novel symmetric BAPs as antitumor agents or multidrug resistance (MDR) reverting agents [9, 13, 20-24]. However, all the previous BAPs are symmetric compounds, and dissymmetric BAPs were rarely documented. Only some

allylated mono-carbonyl analogs of curcumin (MACs) acts as potent anti-inflammatory agents against LPS-induced acute lung injury (ALI) in rats, such as (*3E,5E*)-3-(3-allyl-4-hydroxybenzylidene)-1-cyclopropyl-5-(4-(4-methylpiperazin-1-yl)benzylidene)piperidin-4-one (MAC 7a) [25]. Our interests lie in incorporation of different substituent groups on both sides of BAPs, and find the desired and improved antitumor activities in contrast to symmetric derivatives. In this study, some novel dissymmetric BAPs as potential antitumor agents were synthesized and evaluated *in vivo* and *in vitro*.

Fig. 1

2. Results and Discussion

2.1. Design and synthesis of dissymmetric BAPs

The titled compounds were synthesized by following a step synthesis protocol of Claisen-Schmidt condensation. As shown in (Fig. 2A), 2-fluorobenzaldehyde (**1**), *m*-nitrobenzaldehyde (**2**), and *N*-methyl-4-piperidinone (**3**) in 1:1:1 ratio interact under the catalysis of aqueous sodium hydroxide to generate three components, which can be purified on silica gel by column using petroleum ether/EtOAc as the eluent. According to the different polarity, they can outflow in turn, symmetric ((3*E*,5*E*)-3,5-bis(2-fluorobenzylidene)-1-methylpiperidin-4-one (**4**), dissymmetric (3*E*,5*E*)-3-(2-fluorobenzylidene)-5-(3-nitrobenzylidene)-1-methylpiperidin-4-one (**6g**), and symmetric (3*E*,5*E*)-3,5-bis(3-nitrobenzylidene)-1-methylpiperidin-4-one) (**5**) [21,22]. BAP **6g** as the principal product of them were prepared with a 48% yield. In this synthetic strategy, the reaction of Claisen-Schmidt condensation can be catalyzed by dry HCl, aqueous sodium hydroxide, or other base [26]. Considering the environment friendly and operability in laboratory, 10% aqueous sodium hydroxide was selected to catalyze the reaction as shown in (Fig. 2A). Similarly, other series of BAPs were synthesized (Fig. 2B) and described in experiment part, and the yields of BAPs can reach ca. 41%~52%.

Fig. 2

2.2. Structural characterization of BAPs

The structures of BAPs were fully characterized by NMR, FTIR spectroscopy, and elemental analysis. The ^{1}H NMR signals of -C=CH- in the *α,β*-unsaturated ketone split into two groups of evident unimodal signals, chemical shifts 7.99~7.70 ppm. Coincidentally, chemical shifts of -CH_2 groups in central piperidone ring also are divided into two groups in the range of 3.85~3.57 ppm. These proved the different electronic environment in two sides of BAPs and proved the different structure characteristic in BAPs. From the FTIR spectra, the characteristic banded at around 1660-1694 cm^{-1} are attributed to -C=O group in piperidone. The strong baned at around 1620~1600 cm^{-1} are attributed to –C=C group in *α,β*-unsaturated ketone. Additionly, ^{13}C NMR, element analysis further confirmed the correctness of their structures.

2.3. Single-Crystal Structure of 8a

The crystallographic structure of compound **8a** was detected using *X*-ray diffraction. The ORTEP diagram is presented in Fig. 2C. **8a** crystallizes in the Monoclinic space group *P2(1)/c*. Single-crystal structure showed the $\theta_{C2\text{-}C6\text{-}C7\text{-}C12}$ torsion angle value is 20.60(28)° and the $\theta_{C5\text{-}C13\text{-}C14\text{-}C19}$ torsion angle value is -36.42(22)°. The data confirmed that two benzene ring on both sides of piperidone in **8a** adopt the *E* stereochemistry of the olefinic double bonds and the *E, E* isomer [9].

2.4. Cytotoxicity in vitro

Some reports shows BAPs with electron-withdrawing groups demonstrate remarkable cytotoxicity, while compounds with the presence of electron-rich groups display poor inhibitory activity against human neoplastic cell lines [10-13]. Whereas, curcumin analog (*3E,5E*)-3,5-bis(3,4,5-trimethoxybenzylidene)-1-methylpiperidin-4-one (L49H37) with fertile electron-donating substitutes exhibits more potent inhibitory effects than curcumin against pancreatic stellate cells (PSCs) cell cycle [27]. Inspired by EF24, L49H37 and their interesting biological activity, series **6** (**6a-h**) were prepared with 2-fluorobenzaldehyde and other aromatic aldehydes with *N*-methyl-4-piperidinone by Claisen-Schmidt condensation interaction (Fig. 2B). These aromatic aldehydes contain great electron-donating substitutes

(-OMe, -CMe_3) or electron-withdrawing substitutes (-CF_3, -CN, and -NO_2) as shown in Fig. 2B. Initial screening showed that a majority of series **6** (**6a-h**) exhibit effective inhibitory activities against four malignant cells, especially against HepG2 and THP-1 cell lines (Table 1). Including 2-fluorobenzylidene group, **6c** and **6d** with electron-donating 3,5-dimethoxy and 3,4,5-triethoxy groups led to more potent inhibitory activities against HepG2 and THP-1 at IC_{50} values about 1.24 μM (**6c**, HepG2), 1.17 μM (**6c**, THP-1), 0.67 μM (**6d**, HepG2), 0.64 μM (**6d**, THP-1). Similarly, **6g** and **6h** with electron-withdrawing substitutes (-CN and -NO_2), displayed more potent inhibitory activities, which are shown in Table 1.

In order to examine the substituent effect of dissymmetric BAPs, especially strong electron-donating 3,5-dimethoxy-substituted BAPs (**7a-h**), 3,4,5-triethoxy-substituted BAPs (**8a-h**) and strong electron-withdrawing nitro-substituted BAP derivatives (**9a-g**), cyano-substituted BAPs (**10a-e**) were synthesized (Fig. 2B). For series **7** and **8**, BAPs **7f**, **7g**, **8f** and **8g** with strong electron-withdrawing -NO_2, -CN groups and **7h** with 3,4,5-triethoxy group displayed desired inhibitory activities against HepG2 and THP-1. Their IC_{50} values against HepG2 and THP-1 cells are below 2.0 μM (Table 1). For series **9** and **10**, only **9g** with electron-withdrawing trifluoromethyl group showed accredited inhibitory activities against HepG2 and THP-1 (1.04 μM and 0.97 μM, respectively, Table 1).

In order to study their biocompatibility of BAPs, non-malignant LO2 cell line was selected to evaluate their cytotoxicity. As shown in Table 1, all IC_{50} values toward LO2 cell line exceed 10 μM except for **9c** and **9f**. Most inportantly, the cytotoxicity of BAPs **6d**, **6h**, **7g**, **7h**, **8g**, **9g** were all only in the range of 11.00~18.60 μM, which were distinctly lower than that of DOX (6.93 μM). Unfortunately, the cytotoxicity of **6d**, **6h**, **7g**, **7h**, **8g**, **9g** are higher than Curcumin (25.67 μM).

Table 1

2.5. BAPs 6d, 7h, 8g, 9g indicated dose-dependent inhibitory effect

The BAPs **6d**, **7h**, **8g**, **9g** (Fig. 3A) were selected for further biological characterization. All survival rates induced by different concentrations of **6d**, **7h**, **8g**, **9g** after 24 h treatment in experimental cell lines presented S-curves (Fig. 3B). For LO2, all survival rate curves were

relatively flat, and this indicated that survival effect of **6d**, **7h**, **8g**, **9g** was short of dose-dependent. For four human tumor cell lines, survival effect of **6d**, **7h**, **8g**, **9g** was clearly dose-dependent. In the concentrations of 0~2.0 μM, the dose-dependent inhibitory effects are more highlighted, which indicated that they are more sensitive to human tumor cell lines than normal cell line.

Fig. 3

2.6. BAPs 6d, 7h, 8g, 9g can regulate the expression level of BAX and Bcl-2 in HepG2

Yadav reported CLEFMA inhibited growth of lung cancer xenografts through inhibiting anti-apoptotic markers (cellular inhibitor of apoptosis protein-1(cIAP1), Bcl-xL, Bcl-2, and survivin) and up-regulated the pro-apoptotic BAX and BID [28]. Subramaniam reported EF24 inhibited the expression of antiapoptotic genes Bcl-2 and Bcl-xL protein but it had no detectable effect on the expression levels of apoptosis-promoting total BAX protein, which resulting in decreased Bcl-2 to BAX and Bcl-xL to BAX protein ratios [29]. In accordance with these previous observations, the ratio of BAX/β-actin and BCL-2/β-actin of BAPs **6d**, **7h**, **8g** and **9g** expression level was shown in Fig. 3C. The results showed they all can up-regulate the expression level of BAX, and down-regulate the expression level of Bcl-2 compared with the negative control. The effect of BAPs **6d**, **7h**, **8g** and **9g** on the Bcl-2 and BAX expression pattern, in order, is **6d, 7h, 8g**, and**9g**, which is basically identical to their inhibitory effects on HepG2 (Table 1). Collectively, introduction of 3,4,5-triethoxy phenyl group on the basis of BAPs can effectively up-regulate the expression level of BAX and down-regulate the expression level of Bcl-2 in HepG2.

2.7. The molecular docking study for Bcl-2 protein

Bcl-2 protein is key regulators of the mitochondrial apoptosis pathway and an attractive new anti-cancer target. The ratio of Bcl-2 to BAX dictates a cell's susceptibility to an apoptotic stimulus [30, 31]. The results showed that **6d**, **7h**, **8g** and **9g** down-regulate the ratio of Bcl-2 to BAX. Compounds might block the heterodimerization between the Bcl-2 and BAX through hydrophobic interactions with Bcl-2. So theoretical modeling calculations using

Surflex-Dock program in Sybyl 2.0 was performed to elucidate the mode of Bcl-2 inhibition by these compounds using in silico docking simulations. The results showed in Fig. 3D. There is a narrow and long groove on the surface of the anti-apoptotic Bcl-2 protein. Its bottom is bound by α5, and the side is bound by α2, α3, α4, and α8. The active site comprises three main areas: the P1 and P2 are two big and deep pockets, and the L1 is a narrow and shallow channel linking P1 and P2. The 3,4,5-triethoxy phenyl group of **6d**, **7h**, **8g** inserts into P1 hydrophobic pocket, and could reasonably bind to P1 pocket compared to the 4-CF_3 phenyl group of **9g**. The 2-F phenyl group of **6d** binds α4 active site. The 3,5-bis(arylidene)-4-piperidone core structure of **7h**, **8g**, **9g** bind to L1 active site. The 3,5-dimethoxy, 4-CN, 3-NO_2 phenyl group of **7h**, **8g**, **9g**, respectively, could bind to P2 pocket. Above all, the simulation results are basically identical to their cytotoxic activity (Table 1).

Fig. 4

2.8. BAP 6d induces HepG2 cells apoptosis

To further investigate the underlying mechanism of decreased cell proliferation observed in the MTT assay, the HepG2 apoptosis mediated by **6d** was determined by flow cytometry and confocal microscop. As shown in Fig 4A, the results indicated that HepG2 cells showed a dose-dependent apoptosis after **6d** treatment for 24 h.

2.9. The investigation of cellular uptake in HepG2 cell for BAP 6d

Cellular uptake of BAP **6d** was detected by confocal laser scanning microscopy (CLSM), as their fluorescence can allow the visualization inside the cells. The effect of representative BAP **6d** on the cellular uptake of HepG2 cells in different time was shown in Fig. 4B. It can be found that **6d** mainly accumulate in the cytoplasma, only a small amount can be observed in the nuclei after treatment for 1 h, 2 h and 3 h. While, the distribution of **6d** was exactly opposite after 4 h treatment, and with the distribution changed, cell morphology disrupted, which may be attribute to the cytotoxic activity of **6d**.

2.10. BAP 6d inhibits tumor growth in vivo

HepG2-bearing mice were used to evaluate the in vivo anticancer effect of **6d**. The tumor volume and body weight was measured every 2 days. At the end of the experiments, all tumors were excised and photographed (Fig. 5A). The results showed that **6d** (10 mg/kg) significantly inhibited the tumor growth, and the inhibitory effect of **6d** was similar to DOX. The tumor growth curves and the body weight curves of all groups were shown in Fig. 5B and Fig. 5C. In general, the tumors in control group grew continuously during the experimental period, whereas the tumor growth in the **6d** (10 mg/kg)-treated and DOX (1 mg/kg)-treated mice was suppressed significantly. However, there was no apparent change in body weight in the animals (Fig. 5C). Therefore, **6d** is a potential therapeutic agent for early treatment of liver cancers.

Fig. 5

3. Conclusions

In this study, thirty-five dissymmetric BAPs were synthesized by Claisen-Schmidt condensation interaction. BAPs **6d**, **7h**, **8g**, **9g** possess optimistic and preferential inhibition activity against experimental malignant cells with relative safe cytotoxicity *in vitro*. Structure-activity relationship analysis showed that dissymmetric BAPs containing two of electron-donating 3,5-dimethoxy, 3,4,5-triethoxy groups or electron-withdrawing 2-F, 4-CN, 3-NO_2, 4-CF_3 groups demonstrated the most potent inhibitory activities against HepG2 and THP-1 and lower cytotoxicity toward LO2 cell lines. Potential BAPs **6d**, **7h**, **8g**, **9g** indicated dose-dependent inhibitory effect in survival effect, and they can effectively promote cell apoptosis through up-regulating BAX expression and down-regulating Bcl-2 expression by the western blot. Molecular docking modes showed **6d**, **7h**, **8g**, **9g** could reasonably bind to the active site of Bcl-2 protein. BAP **6d** induced HepG2 cells apoptosis a dose-dependent manner. The cellular uptake of HepG2 cells from CLSM proveed BAP **6d** mainly accumulate into the nuclei after 4 h. BAP **6d** suppressed the growth of HepG2 xenografts without no apparent body weight changes. Taken together, **6d** is a potential therapeutic agent for early treatment of liver cancers. The data presented in this work can be the guideline for constructing dissymmetric BAPs with both preferential anticancer activity and relative safe

cytotoxicity.

4. Materials and Methods

4.1. Chemistry

4.1.1. General

N-Methyl-4-piperidinone and all aromatic aldehydes were purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China) and were used as obtained without further purification. Infrared (IR) spectra were obtained in the 400-4000 cm^{-1} range using a Perkin-Elmer Frontier Mid-IR FTIR Spectrometer. ^{1}H NMR data were collected using a Bruker Avance 300 or 400 MHz. ^{13}C NMR data were collected at 100 MHz on a Bruker Avance 400 MHz spectrometer. Chemical shifts were reported in δ relative to TMS. Elemental analyses were performed on a Perkin–Elmer Model 240c analyzer.

4.1.2. General synthesis of compounds 6a-h

2-Fluorobenzaldehyde (0.62 g, 0.005 mol), *N*-methyl-4-piperidone (0.57 g, 0.005 mol), and aromatic aldehyde (0.005 mol) were dissolved in 5 mL of methanol. After 2.0 mL 10% NaOH solution added, the mixture was stirring for 2 h at ambient temperature (monitored by TLC). Then removal of solvent through pouring process, the residues were purified on silica gel by column using petroleum ether/EtOAc (3:1, v/v) as the eluent to afford light yellow powders **6a-h**.

4.1.2.1. *(3E,5E)-3-(2-fluorobenzylidene)-5-(4-methoxybenzylidene)-1-methylpiperidin-4-one (**6a**):* Light yellow powder; yield: 47%; mp: 127-129℃; IR (cm^{-1}): 2940(m), 2779(m), 1669(s), 1603(s), 1573(s), 1507(s), 1454(s), 1302(s), 1259(s), 1165(s), 1023(s), 922(s), 826(s), 759(s). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (s, 1H, -C=CH), 7.82 (s, 1H, -C=CH), 7.39 (d, *J* = 8.6 Hz, 2H, $-C_6H_4$), 7.35 (d, *J* = 6.1 Hz, 1H, $-C_6H_4$), 7.30 (d, *J* = 7.1 Hz, 1H, $-C_6H_4$), 7.19 (t, *J* = 7.4 Hz, 1H, $-C_6H_4$), 7.13 (t, *J* = 9.2 Hz, 1H, $-C_6H_4$), 6.97 (d, *J* = 8.6 Hz, 2H, $-C_6H_4$), 3.86 (s, 3H, $-OCH_3$), 3.83 (s, 2H, $-CH_2$), 3.67 (s, 2H, $-CH_2$), 2.47 (s, 3H, $-NCH_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 186.06, 160.77 (d, J = 251.6 Hz), 150.09, 148.75, 137.42, 134.56, 130.80, 130.72, 129.08, 128.01, 123.93, 123.88, 123.16 (d, *J* = 13.8 Hz), 115.88 (d, *J* = 22.0 Hz), 112.38 (d, *J*

= 293.7 Hz), 57.21, 56.50, 55.91, 45.42. Elemental analysis (%) calcd. for $C_{21}H_{20}FNO_2$ (337.38): C 74.76, H 5.97, N 4.15; Found: C 74.80, H 5.94, N 4.13.

4.1.2.2. *(3E,5E)-3-(2-fluorobenzylidene)-5-(3,4-dimethoxybenzylidene)-1-methylpiperidin-4-one (**6b**):* Light yellow powder; yield: 52%; mp: 116-118℃; IR (cm^{-1}): 2934(m), 2782(m), 1668(s), 1608(s), 1508(s), 1446(s), 1255(s),1146(s), 1021(s), 922(s), 810(s), 764(s). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (s, 1H, -C=CH), 7.80 (s, 1H, -C=CH), 7.36 (dd, *J* = 12.8, 6.5 Hz, 1H, -C_6H_4), 7.32 – 7.27 (m, 1H, -C_6H_4), 7.19 (t, *J* = 7.5 Hz, 1H, -C_6H_4), 7.16 – 7.10 (m, 1H, -C_6H_4), 7.02 (d, *J* = 8.2 Hz, 1H, -C_6H_3), 6.98 – 6.90 (m, 2H, -C6H_3), 3.94 (s, 3H, -OCH_3), 3.92 (s, 3H, -OCH_3), 3.83 (s, 2H, -CH_2), 3.66 (s, 2H, -CH_2), 2.46 (s, 3H, -NCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 186.06, 160.77 (d, *J* = 251.6 Hz), 160.45, 137.33, 134.52, 132.48, 132.40, 130.79, 130.74, 130.71, 129.07, 127.72, 123.90 (d, *J* = 3.6 Hz), 123.17 (d, *J* = 13.8 Hz), 115.88 (d, *J* = 21.9 Hz), 114.13, 57.21, 56.49, 56.47, 55.34, 45.42. Elemental analysis (%) calcd. for $C_{22}H_{22}FNO_3$ (367.41): C 71.92, H 6.04, N 3.81; Found: C 71.87, H 6.07, N 3.83.

4.1.2.3. *(3E,5E)-3-(2-fluorobenzylidene)-5-(3,5-dimethoxybenzylidene)-1-methylpiperidin-4-one (**6c**):* Light yellow powder; yield: 46%; mp: 93-95℃; IR (cm^{-1}): 2937(m), 2763(m), 1694(s), 1591(s), 1454(s), 1332(s), 1266(s),1152(s), 1098(s), 1059(s), 932(s), 832(s), 751(s). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90 (s, 1H, -C=CH), 7.76 (s, 1H, -C=CH), 7.43 – 7.29 (m, 2H, -C_6H_4), 7.23 – 7.11 (m, 2H, -C_6H_4), 6.56 (s, 1H, -C_6H_3), 6.55 (s, 1H, -C_6H_3), 6.50 (t, *J* = 2.2 Hz, 1H, -C_6H_3), 3.85 (d, *J* = 7.7 Hz, 6H, -OCH_3), 3.78 (s, 2H, -CH_2), 3.65 (s, 2H, -CH_2), 2.45 (s, 3H, -NCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 186.38, 160.83 (d, *J* =252.5Hz), 160.62, 136.86, 134.74, 133.29, 130.87, 130.78, 130.69, 129.45 (d, *J* = 3.8 Hz), 129.03 (d, *J* = 3.9 Hz), 123.92 (d, *J* = 3.6 Hz), 123.10 (d, *J* = 13.7 Hz), 115.84 (d, *J* = 21.9 Hz), 108.36, 101.02, 57.09, 56.75, 55.38, 45.63. Elemental analysis (%) calcd. for $C_{22}H_{22}FNO_3$ (367.41): C 71.92, H 6.04, N 3.81; Found: C 71.88, H 6.06, N 3.85.

4.1.2.4. *(3E,5E)-3-(2-fluorobenzylidene)-5-(3,4,5-trimethoxybenzylidene)-1-methylpiperidin-4-one (**6d**):* Light yellow powder; yield: 45%; mp: 103-105℃; IR (cm^{-1}): 2940(m), 2848(m), 2788(m), 1671(s), 1619(s), 1581(s), 1505(s), 1484(s), 1451(s), 1417(s), 1386(m), 1326 (s), 1295(m), 1267(s), 1232(s), 1178(s), 1152(s), 1123(s), 1058(s), 1002(s), 974(s), 926(s), 842(s), 827 (m), 791(s), 772(s), 668(m). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (s, 1H, -C=CH), 7.76 (s,

1H, -C=CH), 7.42 – 7.36 (m, 2H, -C_6H_4), 7.13 (t, *J* = 8.3 Hz, 2H, -C_6H_4), 6.64 (s, 2H, -C_6H_2), 3.90 (s, 9H, -OCH_3), 3.80 (s, 2H, -CH_2), 3.75 (s, 2H, -CH_2), 2.49 (s, 3H, -NCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 186.56, 162.87 (d, *J* = 250.8 Hz), 153.09, 139.22, 136.67, 135.15, 132.76 (d, *J* = 1.4 Hz), 132.34, 132.28, 132.20, 131.40, 131.36 130.65, 115.70 (d, *J* = 21.7 Hz), 107.94, 60.91, 56.98, 56.88, 56.23, 45.70. Elemental analysis (%) calcd. for $C_{23}H_{24}FNO_4$ (397.44): C, 69.51; H, 6.09; N, 3.52; Found: C 69.46, H 6.05, N 3.62.

4.1.2.5. *(3E,5E)-3-(2-fluorobenzylidene)-5-(4-tert-butylbenzylidene)-1-methylpiperidin-4-one (**6e**):* Light yellow powder; Yield: 48%; mp: 128~130℃. IR (cm^{-1}): 2963(s), 2793(m), 1675(m), 1611(s), 1575(s), 1479(s), 1269(s), 1173(s), 1096(s), 991(s), 922(s), 830(s), 765(s). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (s, 1H, -C=CH), 7.82 (s, 1H, -C=CH), 7.45 (d, *J* = 7.1 Hz, 2H, -C_6H_4), 7.37 (d, *J* = 7.3 Hz, 3H, -C_6H_4), 7.29 (m, 1H, -C_6H_4), 7.22-7.09 (m, 2H, -C_6H_4), 3.80 (s, 2H, -CH_2), 3.63 (s, 2H, -CH_2), 2.44 (s, 3H, -NCH_3), 1.35 (s, 9H, -CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 186.45, 160.81 (d, *J* = 251.6 Hz,), 152.55, 136.91, 134.96, 132.28 (d, *J* = 18.3 Hz), 130.73, 130.71, 130.63, 130.46, 128.83 (d, *J* = 3.7 Hz), 125.56, 123.86 (d, *J* = 3.7 Hz), 123.27 (d, *J* = 13.9 Hz), 115.86 (d, *J* = 22.0 Hz), 57.36, 56.77, 45.72, 34.81, 31.14. Elemental analysis (%) calcd. for $C_{24}H_{26}FNO$ (363.46): C 79.31, H 7.21, N 3.85; Found: C 79.26, H 7.23, N 3.82.

4.1.2.6. *(3E,5E)-3-(2-fluorobenzylidene)-5-(4-(trifluoromethyl)benzylidene)-1-methylpiperidin-4-one (**6f**):* Light yellow powder; yield: 42%; mp: 103-105℃; IR (cm^{-1}): 2937(m), 2779(m), 1739(s), 1670(s), 1612(s), 1488(m), 1452(s), 1323(s), 1237(s), 1163(s), 1111(s), 983(s), 926(s), 826(s). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.92 (s, 1H, -C=CH), 7.84 (s, 1H, -C=CH), 7.70 (d, *J* = 8.1 Hz, 2H, -C_6H_4), 7.51 (d, *J* = 8.1 Hz, 2H, -C_6H_4), 7.43 – 7.29 (m, 2H, -C_6H_4), 7.25 – 7.11 (m, 2H, -C_6H_4), 3.76 (s, 2H, -CH_2), 3.67 (s, 2H, -CH_2), 2.46 (s, 3H, -NCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 186.16, 160.85 (d, *J* = 251.9 Hz), 138.65, 134.77, 134.50, 131.01, 130.92, 130.70, 130.67, 130.35, 130.29, 129.58 (d, *J* = 3.8 Hz), 125.44 (q, *J* = 3.7 Hz), 123.94 (d, *J* = 3.7 Hz), 123.02 (d, *J* = 13.7 Hz), 115.92 (d, *J* = 21.9 Hz), 56.98, 56.81, 45.70. Elemental analysis (%) calcd. for $C_{21}H_{17}F_4NO$ (375.35): C 67.20, H 4.56, N 3.73; Found: C 67.28, H 4.51, N 3.70.

4.1.2.7. *(3E,5E)-3-(2-fluorobenzylidene)-5-(3-nitrobenzylidene)-1-methylpiperidin-4-one (**6g**):*

Light yellow powder; Yield: 48%; mp:128-130℃; IR(cm^{-1}): 2927(m), 2731(m), 1675(s), 1617(s), 1523(s), 1449(s), 1347(s), 1265(s), 1173(s), 1100(s), 1054(s), 983(s), 919(s), 809(s), 762(s). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (d, *J* = 7.2 Hz, 2H, -C_6H_4), 7.97 (s, 1H, -C=CH), 7.87 (s, 1H, -C=CH), 7.76 (d, *J* = 7.8 Hz, 1H, -C_6H_4), 7.68 (dd, *J* = 10.3, 6.2 Hz, 1H, -C_6H_4), 7.43 (dt, *J* = 7.2, 3.7 Hz, 1H, -C_6H_4), 7.36 (t, *J* = 6.9 Hz, 1H, -C_6H_4), 7.26 (t, *J* = 7.4 Hz, 1H, -C_6H_4), 7.22 – 7.15 (m, 1H, -C_6H_4), 3.82 (s, 2H, -CH_2), 3.72 (s, 2H, -CH_2), 2.51 (s, 3H, -NCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 185.95, 160.87 (d, *J* = 252.0 Hz), 148.27, 136.02, 136.00 (d, *J* =141.4 Hz), 134.33, 133.54, 131.11, 131.03, 130.69 (d, *J* = 2.5 Hz), 129.80 (d, *J* = 3.8 Hz), 129.63, 124.35, 123.98 (d, *J* = 3.7 Hz), 123.46, 122.93 (d, *J* = 13.7 Hz), 115.95 (d, *J* = 22.0 Hz), 56.82, 56.76, 45.75. Elemental analysis (%) calcd. for $C_{20}H_{17}FN_2O_3$ (352.35): C 68.17, H 4.86, N 7.95; Found: C 68.10, H 4.90, N 7.98.

4.1.2.8. *(3E,5E)-3-(2-fluorobenzylidene)-5-(4-cyanobenzylidene)-1-methylpiperidin-4-one (**6h**):* Light yellow powder; Yield: 41%; mp:120-122℃; IR (cm^{-1}): 2942(m), 2773(m), 2224(s), 1675(s), 1614(s), 1587(s), 1501(m), 1485(s), 1454(m), 1329(m), 1290(s), 1268(s), 1225(s),1169(s), 1101(s), 1060 (s), 983(s), 924(s), 826(s), 760(s). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (s, 1H, -C=CH), 7.78 (s, 1H, -C=CH), 7.72 (d, *J* = 8.1 Hz, 2H, -C_6H_4), 7.48 (d, *J* = 8.1 Hz, 2H, -C_6H_4), 7.39 (dd, *J* = 12.9, 6.6 Hz, 1H, -C_6H_4), 7.31 (t, *J* = 7.4 Hz, 1H, -C_6H_4), 7.21 (t, *J* = 7.4 Hz, 1H, -C_6H_4), 7.15 (t, *J* = 9.2 Hz, 1H, -C_6H_4), 3.73 (s, 2H, -CH_2), 3.66 (s, 2H, -CH_2), 2.45 (s, 3H, -NCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 185.99, 160.85 (d, *J* = 252.0 Hz), 139.67, 135.55, 134.38, 134.06, 132.21, 131.04 (d, *J* =8.08 Hz), 130.66 (d, *J* = 2.5 Hz), 130.52, 129.78 (d, *J* = 3.8 Hz), 123.96 (d, *J* = 3.7 Hz), 122.94 (d, *J* = 13.7 Hz), 118.41, 115.94 (d, *J* = 22.0 Hz), 112.21, 56.95 56.75, 45.68. Elemental analysis (%) calcd. for $C_{21}H_{17}FN_2O$ (332.37): C 75.89, H 5.16, N 8.43; Found: C 75.87, H 5.09, N 8.44.

4.1.3. General synthesis of compounds 7a-h

3,5-dimethoxybenzaldehyde (0.83 g, 0.005 mol), *N*-methyl-4-piperidone (0.57 g, 0.005 mol), and aromatic aldehyde (0.005 mol) were dissolved in 5 mL of methanol. After 2.0 mL 10% NaOH solution added, the mixture was stirring for 2~4 h at ambient temperature (monitored by TLC). Then removal of solvent through pouring process, the residues were purified on silica gel by column using petroleum ether/EtOAc (4:1, v/v) as the eluent to afford light

yellow powders **7a-h**.

4.1.3.1. *(3E,5E)-3-(3,5-dimethoxybenzylidene)-5-(4-fluorobenzylidene)-1-methylpiperidin-4-one (**7a**):* Light yellow powder; Yield: 46%; mp: 93-95℃; IR (cm^{-1}): 2942(m), 2839(m), 1664(s), 1588(s), 1507(s), 1455(s), 1426(s), 1325(m), 1292(m), 1269(s), 1226(m), 1205(s), 1153(s), 1059(s), 1014(m), 989(m), 919(s), 831(s), 791(m), 679(m). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (s, 1H, -C=CH), 7.74 (s, 1H, -C=CH), 7.39 (dd, *J* = 8.0, 5.7 Hz, 2H, $-C_6H_4$), 7.13 (t, *J* = 8.5 Hz, 2H, $-C_6H_4$), 6.54 (s, 2H, $-C_6H_3$), 6.50 (s, 1H, $-C_6H_3$), 3.83 (s, 6H, $-OCH_3$), 3.76 (s, 2H, $-CH_2$), 3.74 (s, 2H, $-CH_2$), 2.47 (s, 3H, $-NCH_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 186.71, 162.88 (d, *J* =251.5 Hz), 160.66, 136.96, 136.38, 135.17, 133.45, 132.81 (d, *J* = 1.5 Hz), 132.26 (d, *J* = 8.3 Hz), 131.37 (d, *J* = 3.4 Hz), 115.80, 115.58, 108.33, 100.99, 56.99, 56.94, 55.39, 45.78. Elemental analysis (%) calcd. for $C_{22}H_{22}FNO_3$ (367.41): C 71.92, H 6.04, N 3.81; Found: C 71.90, H 5.99, N 3.83.

4.1.3.2. *(3E,5E)-3-(3,5-dimethoxybenzylidene)-5-(4-chlorobenzylidene)-1-methylpiperidin-4-one (**7b**):* Pale yellow oil; Yield: 49%. IR (cm^{-1}): 2937(m), 2835(m), 2755(m), 1678(s), 1615(s), 1589(s), 1492(s), 1456(s), 1422(s), 1386(m), 1351(m), 1332(m), 1271(s), 1206(s), 1183(s), 1154(s), 1128(m), 1092(m), 1060(s), 1011(s), 976(s), 919(s), 824 (s), 783(s), 738(m), 682(m), 669(m).^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, 2H, -C=CH), 7.41 (d, *J* = 8.1 Hz, 2H, $-C_6H_4$), 7.33 (d, *J* = 8.1 Hz, 2H, $-C_6H_4$), 6.54 (s, 2H, $-C_6H_3$), 6.50 (s, 1H, $-C_6H_3$), 3.83 (s, 6H, $-OCH_3$), 3.76 (s, 2H, $-CH_2$), 3.73 (s, 2H, $-CH_2$), 2.47 (s, 3H, $-NCH_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 186.64, 160.67, 136.91, 136.53, 135.00, 134.92, 133.65, 133.56, 133.38, 131.51, 128.81, 108.35, 101.03, 56.97, 56.94, 55.39, 45.78. Elemental analysis (%) calcd. for $C_{22}H_{22}ClNO_3$ (383.87): C 68.83, H 5.78, N 3.65; Found: C 68.85, H 5.77, N 3.63.

4.1.3.3. *(3E,5E)-3-(3,5-dimethoxybenzylidene)-5-(4-bromobenzylidene)-1-methylpiperidin-4-one (**7c**):* Light yellow powder; Yield: 43%; mp:120-122℃; IR(cm^{-1}): 2938(m), 2834(m), 2755(m), 1676(s), 1614(s), 1587(s), 1489(s), 1449(s), 1420(s), 1385(m), 1351(m), 1333(s), 1314(m), 1270(s), 1252(m), 1206(s), 1183(s), 1153(s), 1100(m), 1060(s), 1008(s), 989(s), 978(s), 920(s), 871(s), 826(s), 818(s), 783(s), 728(m), 683(s), 662(s). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (d, 2H, -C=CH), 7.56 (d, *J* = 8.0 Hz, 2H, $-C_6H_4$), 7.27 (d, *J* = 8.0 Hz, 2H, $-C_6H_4$), 6.54 (s, 2H, $-C_6H_3$), 6.50 (s, 1H, $-C_6H_3$), 3.84 (s, 6H, $-OCH_3$), 3.76 (s, 2H, $-CH_2$), 3.72

(s, 2H, -CH_2), 2.47 (s, 3H, -NCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 186.65, 160.63, 136.88, 136.60, 135.01, 134.05, 133.62, 133.34, 131.78, 131.75, 123.36, 108.34, 100.98, 56.99, 56.97, 55.41, 45.84. Elemental analysis (%) calcd. for $C_{22}H_{22}BrNO_3$ (428.32): C 61.69, H 5.18, N 3.27; Found: C 61.66, H 5.17, N 3.23.

4.1.3.4. *(3E,5E)-3-(2,4-dichlorobenzylidene)-5-(3,5-dimethoxybenzylidene)-1-methylpiperidin-4-one (**7d**):* Light yellow powder; Yield: 44%; mp:156-158℃; IR (cm^{-1}): 2838(m), 2765(m), 1672(s), 1619(s), 1592(s), 1452(s), 1425(s), 1382(m), 1335(s), 1275(s), 1252(m), 1205(s), 1187(m), 1102(s), 1045(m), 1003(s), 989(s), 923(s), 860(s), 829(m), 786(s), 689(m), 678(s). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (s, 1H, -C=CH), 7.77 (s, 1H, -C=CH), 7.49 (s, 1H, -C_6H_3), 7.30 (d, *J* = 8.0 Hz, 1H, -C_6H_3), 7.18(d, *J* =8.0Hz, -C_6H_3), 6.54 (s, 2H, -C_6H_3), 6.50 (s, 1H, -C_6H_3), 3.84 (s, 6H, -OCH_3), 3.77 (s, 2H, -CH_2), 3.57(s, 2H, -CH_2), 2.42 (s, 3H, -NCH_3).^{13}C NMR (100 MHz, $CDCl_3$) δ 186.22, 160.64, 137.25, 136.81, 135.86, 135.15, 134.88, 133.11, 132.91, 132.13, 130.97, 129.83, 126.82, 108.39, 101.06, 57.13, 56.43, 55.42, 45.68. Elemental analysis (%) calcd. for $C_{22}H_{21}Cl_2NO_3$ (418.31): C 63.17, H 5.06, N 3.35; Found: C 63.11, H 5.07, N 3.33.

4.1.3.5. *(3E,5E)-3-(3,4-dichlorobenzylidene)-5-(3,5-dimethoxybenzylidene)-1-methylpiperidin-4-one (**7e**):* Light yellow powder; Yield: 46%; mp:109-111℃; IR (cm^{-1}): 2935(m), 2839(m), 2781(m), 1667(s), 1611(s), 1595(s), 1453(s), 1420(s), 1382(m), 1355(s), 1277(s), 1255(m), 1209(s), 1183(m), 1108(s), 1062(m), 1026(s), 977(s), 933(s), 859(s), 822(m), 756(s), 671 (s). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (s, 1H, -C=CH), 7.67 (s, 1H, -C=CH), 7.50 (d, *J* = 8.3 Hz, 1H, -C_6H_3), 7.47 (s, 1H, -C_6H_3), 7.22 (d, *J* = 7.9 Hz, 1H, -C_6H_3), 6.53 (s, 2H, -C_6H_3), 6.50 (s, 1H, -C_6H_3), 3.83 (s, 6H, -OCH_3), 3.76 (s, 2H, -CH_2), 3.71 (s, 2H, -CH_2), 2.47 (s, 3H, -NCH_3).^{13}C NMR (100 MHz, $CDCl_3$) δ 186.42, 160.64, 136.88, 136.77, 135.15, 134.52, 133.49, 133.16, 133.05, 132.79, 131.67, 130.54, 129.33, 108.38, 101.04, 56.92, 56.78, 55.41, 45.8. Elemental analysis (%) calcd. for $C_{22}H_{21}Cl_2NO_3$ (418.31): C 63.17, H 5.06, N 3.35; Found: C 63.11, H 5.07, N 3.33.

4.1.3.6. *(3E,5E)-3-(3,5-dimethoxybenzylidene)-5-(3-nitrobenzylidene)-1-methylpiperidin-4-one (**7f**):* Light yellow powder; Yield: 47%; mp:195-197℃; IR (cm^{-1}): 3086(m), 3013(m), 2942(m), 2842(m), 2780(m), 1688(s), 1584(s), 1522(s), 1454(s), 1427(m), 1343(m), 1305(m),

1270(s), 1202(s), 1150(s), 1107(s), 1064(s), 982(m), 916(s), 852(m), 803(m), 745(m), 674(s), 653(m), 595(m). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (d, *J* = 7.2 Hz, 2H, -C_6H_4), 7.82 (s, 1H, -C=CH), 7.77 (s, 1H, -C=CH), 7.71 (d, *J* = 8.0 Hz, 1H, -C_6H_4), 7.62 (t, *J* = 8.0 Hz, 1H, -C_6H_4), 6.54 (s, 2H, -C_6H_3), 6.51 (s, 1H, -C_6H_3), 3.84 (s, 6H, -OCH_3), 3.82 (s, 2H, -CH_2), 3.79 (s, 2H, -CH_2), 2.49 (s, 3H, -NCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 186.16, 160.71, 148.32, 137.42, 136.75, 136.64, 135.88, 135.20, 133.32, 132.78, 129.61, 124.34, 123.42, 108.41, 101.21, 56.81, 56.55, 55.42, 45.62. Elemental analysis (%) calcd. for $C_{22}H_{22}N_2O_5$ (394.42): C 66.99, H 5.62, N 7.10; Found: C 66.88, H 5.59, N 6.96.

4.1.3.7. *(3E,5E)-3-(3,5-dimethoxybenzylidene)-5-(4-cyanobenzylidene)-1-methylpiperidin-4-one (**7g**):* Light yellow powder; Yield: 41%; mp:130-132℃; IR (cm^{-1}): 2940(m), 2842(m), 2781(m), 2225(s), 1740(s), 1668(s), 1605(s), 1582(s), 1503(s), 1453(s), 1425(s), 1388(m), 1321(s), 1270(s), 1203(m), 1182(s), 1161(s), 1099(s), 1065(s), 919(s), 845(s), 812(s), 782(s). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (s, 2H, -C=CH), 7.71 (d, *J* = 8.1 Hz, 2H, -C_6H_4), 7.47 (d, *J* = 8.1 Hz, 2H, -C_6H_4), 6.53 (s, 2H, -C_6H_3), 6.50 (s, 1H, -C_6H_3), 3.83 (s, 6H, -OCH_3), 3.77 (s, 2H, -CH_2), 3.71 (s, 2H, -CH_2), 2.46 (s, 3H, -NCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 186.39, 160.67, 139.69, 137.19, 136.67, 135.67, 133.71, 133.03, 132.22, 130.51, 118.45, 112.13, 108.42, 101.12, 56.95, 56.83, 55.42, 45.80. Elemental analysis (%) calcd. for $C_{23}H_{22}N_2O_3$ (374.43): C 73.78, H 5.92, N 7.48; Found: C 73.81, H 5.97, N 7.43.

4.1.3.8. *(3E,5E)-3-(3,4,5-trimethoxybenzylidene)-5-(3,5-dimethoxybenzylidene)-1-methylpiperidin-4-one (**7h**):* Pale yellow oil; Yield: 43%. IR(cm^{-1}): 2938(m), 2836(m), 1674(s), 1588(s), 1504(s), 1450(s), 1414(m), 1387(m), 1326(s), 1265(m), 1241(s), 1199(s), 1148(s), 1123(s), 1060(s), 993(s), 931(s), 916(s), 829(s), 792(m), 739(m), 684(s). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (d, 2H, -C=CH), 6.61 (s, 2H, -C_6H_2), 6.51 (s, 2H, -C_6H_3), 6.46 (s, 1H, -C_6H_3), 3.88 (s, 3H, -OCH_3), 3.88 (s, 6H, -OCH_3), 3.80 (s, 6H, -OCH_3), 3.76 (s, 2H, -CH_2), 3.75 (s, 2H, -CH_2), 2.45 (s, 3H, -NCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 186.62, 160.60, 153.01, 139.01, 136.92, 136.64, 136.32, 133.43, 132.38, 130.66, 108.31, 107.79, 100.86, 60.89, 57.00, 56.86, 56.15, 55.36, 45.67. Elemental analysis (%) calcd. for $C_{25}H_{29}NO_6$ (439.50): C 68.32, H 6.65, N 3.19; Found: C 68.31, H 6.70, N 6.54.

4.1.4. General synthesis of compounds 8a-g

3,4,5-trimethoxybenzaldehyde (0.98 g, 0.005 mol), *N*-methyl-4-piperidone (0.57 g, 0.005 mol), and aromatic aldehyde (0.005 mol) were dissolved in 5 mL of methanol. After 2.0 mL 10% NaOH solution added, the mixture was stirring for 3~6 h at ambient temperature (monitored by TLC). Then removal of solvent through pouring process, the residues were purified on silica gel by column using petroleum ether/EtOAc (4:1, v/v) as the eluent to afford light yellow powders **8a-g**.

4.1.4.1. *(3E,5E)-3-(3,4,5-trimethoxybenzylidene)-5-(4-fluorobenzylidene)-1-methylpiperidin-4-one (**8a**):* Light yellow powder; Yield: 44%; mp: 151-153℃; IR (cm^{-1}): 2939(m), 2844(m), 2777(m), 1669(s), 1617(s), 1603(s), 1578(s), 1504(s), 1450(s), 1416(s), 1386(m), 1326(s), 1269(s), 1239(s), 1182(s), 1155(s), 1122(s), 1062(s), 1015(s), 995(m), 972(m), 952(m), 928 (s), 852 (m), 835 (s), 787(s), 716(m), 673(m). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (s, 1H, -C=CH), 7.78 (s, 1H, -C=CH), 7.39 (dd, *J* = 8.1, 5.6 Hz, 2H, $-C_6H_4$), 7.13 (t, *J* = 8.5 Hz, 2H, $-C_6H_4$), 6.63 (s, 2H, $-C_6H_2$), 3.91 (s, 3H, $-OCH_3$), 3.90 (s, 6H, $-OCH_3$), 3.84 (s, 2H, $-CH_2$), 3.79 (s, 2H, $-CH_2$), 2.50 (s, 3H, $-NCH_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 186.17, 164.19, 161.70, 153.09, 139.18, 137.35, 135.81, 132.36, 132.28, 131.18 (d, *J* = 3.2 Hz), 130.48, 115.79 (d, *J* = 21.7 Hz), 107.80, 60.96, 56.67, 56.59, 56.21, 45.29. Elemental analysis (%) calcd. for $C_{23}H_{24}FNO_4$ (397.44): C 69.51, H 6.09, N 3.52; Found: C 69.53, H 6.07, N 3.53.

4.1.4.2. *(3E,5E)-3-(3,4,5-trimethoxybenzylidene)-5-(4-chlorobenzylidene)-1-methylpiperidin-4-one (**8b**):* Light yellow powder; Yield: 47%; mp:154-156℃; IR (cm^{-1}): 2938(m), 2842(m), 2773(m), 1669(s), 1615(s), 1578(s), 1506(s), 1490(s), 1467(m), 1449(s), 1416(s), 1385(s), 1325(s), 1266(s), 1241(s), 1182(s), 1158(s), 1124(s), 1094(s), 1061(s), 1011(m), 995(m), 973(s), 952(s), 927(s), 835(s), 813(s), 792 (m), 698(m), 661(s). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (d, 2H, -C=CH), 7.41 (d, *J* = 8.2 Hz, 2H, $-C_6H_4$), 7.33 (d, *J* = 8.2 Hz, 2H, $-C_6H_4$), 6.62 (s, 2H, $-C_6H_2$), 3.91 (s, 3H, $-OCH_3$), 3.90 (s, 6H, $-OCH_3$), 3.84 (s, 2H, $-CH_2$), 3.79 (s, 2H, $-CH_2$), 2.49 (s, 3H, $-NCH_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 186.08, 153.09, 139.23, 137.51, 135.59, 135.18, 133.44, 131.54, 131.45, 130.43, 128.89, 128.00, 107.83, 60.96, 56.66, 56.56, 56.22, 45.29. Elemental analysis (%) calcd. for $C_{23}H_{24}ClNO_4$ (413.89): C 66.74, H 5.84, N 3.38; Found: C 66.67, H 5.88, N 3.33.

4.1.4.3. *(3E,5E)-3-(3,4,5-trimethoxybenzylidene)-5-(4-bromobenzylidene)-1-methylpiperidin-*

*4-one (**8c**):* Light yellow powder; Yield: 50%; mp: 153-155℃; IR(cm^{-1}): 2938(m), 2842(m), 1668(s), 1615(s), 1578(s), 1506(s), 1487(s), 1449(s), 1416(s), 1384(s), 1324(s), 1267(s), 1241(s), 1179(s), 1158(s), 1124(s), 1073(s), 1061(s), 995(s), 973(s), 927(s), 837(s), 812(s), 781(s). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (s, 1H, -C=CH), 7.74 (s, 1H, -C=CH), 7.57 (d, *J* = 8.2 Hz, 2H, -C_6H_4), 7.27 (d, *J* = 8.5 Hz, 2H, -C_6H_4), 6.64 (s, 2H, -C_6H_2), 3.91 (s, 3H, -OCH_3), 3.90 (s, 6H, -OCH_3), 3.80 (s, 2H, -CH_2), 3.73 (s, 2H, -CH_2), 2.48 (s, 3H, -NCH_3).^{13}C NMR (100 MHz, $CDCl_3$) δ 186.51, 153.07, 139.13, 136.91, 135.01, 134.05, 133.57, 132.22, 131.79, 131.73, 130.60, 123.35, 107.84, 60.95, 56.99, 56.87, 56.21, 45.74. Elemental analysis (%) calcd. for $C_{23}H_{24}BrNO_4$ (458.34): C 60.27, H 5.28, N 3.06; Found: C 60.21, H 5.22, N 3.08.

4.1.4.4. *(3E,5E)-3-(2,4-dichlorobenzylidene)-5-(3,4,5-trimethoxybenzylidene)-1-methylpiperidin-4-one (**8d**):* Light yellow powder; Yield: 50%; mp: 172-174℃; IR (cm^{-1}): 2926(m), 2849(m), 2787(m), 1669(s), 1615(s), 1578(s), 1505(s), 1466(m), 1416(s), 1381(s), 1337(s), 1319(s), 1268(s), 1251(m), 1178(s), 1123(s), 1104(m), 1052(s), 995(s), 972(m), 923(s), 872(s), 835(m), 768(m), 734(m), 655(m). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (s, 1H, -C=CH), 7.78 (s, 1H, -C=CH), 7.49 (s, 1H, -C_6H_3), 7.30 (d, *J* = 9.3 Hz, 1H, -C_6H_3), 7.18 (d, *J* = 8.3 Hz, 1H, -C_6H_3), 6.63 (s, 2H, -C_6H_2), 3.91 (s, 3H, -OCH_3), 3.90 (s, 6H, -OCH_3), 3.80 (s, 2H, -CH_2), 3.59 (s, 2H, -CH_2), 2.44 (s, 3H, -NCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 186.05, 153.10, 139.35, 137.50, 135.81, 135.13, 134.91, 132.22, 132.06, 132.02, 130.93, 130.53, 129.82, 126.82, 108.04, 60.91, 57.14, 56.28, 56.24, 45.50. Elemental analysis (%) calcd. for $C_{23}H_{23}Cl_2NO_4$ (448.34): C 61.62, H 5.17, N 3.12; Found: C 61.67, H 5.18, N 3.13.

4.1.4.5. *(3E,5E)-3-(3,4,5-trimethoxybenzylidene)-5-(3,4-dichlorobenzylidene)-1-methylpiperidin-4-one (**8e**):* Light yellow powder; Yield: 52%; mp: 159-161℃; IR (cm^{-1}): 2936(m), 2840(m), 2779(m), 1666(s), 1610(s), 1577(s), 1505(s), 1470(m), 1417(s), 1384(s), 1327(s), 1273(s), 1246(m), 1208(m), 1184(s), 1128(s), 1063(s), 996(s), 975(m), 931(s), 872(s), 869(m), 782(m), 759(m), 661(m). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (s, 1H, -C=CH), 7.68 (s, 1H, -C=CH), 7.50 (d, *J* = 8.2 Hz, 1H, -C_6H_3), 7.47 (s, 1H, -C_6H_3), 7.23 (d, *J* = 8.0 Hz, 1H, -C_6H_3), 6.64 (s, 2H, -C_6H_2), 3.91 (s, 3H, -OCH_3), 3.91 (s, 6H, -OCH_3), 3.80 (s, 2H, -CH_2), 3.72 (s, 2H, -CH_2), 2.49 (s, 3H, -NCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 186.28, 153.08, 139.23, 137.21, 135.16, 134.47, 133.49, 133.05, 132.80, 132.03, 131.65, 130.55, 130.50, 129.33, 107.88,

60.96, 56.95, 56.67, 56.22, 45.73. Elemental analysis (%) calcd. for $C_{23}H_{23}Cl_2NO_4$ (448.34): C 61.62, H 5.17, N 3.12; Found: C 61.64, H 5.16, N 3.16.

4.1.4.6. *(3E,5E)-3-(3,4,5-trimethoxybenzylidene)-5-(3-nitrobenzylidene)-1-methylpiperidin-4-one (**8f**):* Pale yellow oil; Yield: 47%. IR (cm^{-1}): 2941(m), 2839(m), 1674(s), 1612(s), 1579(s), 1525(s), 1505(s), 1454(s), 1420(s), 1389(m), 1347(s), 1289(m), 1265(s), 1182(s), 1123(s), 1058(s), 1003(s), 927(m), 896(m), 822(s), 778(m), 741(s), 715(m), 675(s). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (d, *J* = 7.2 Hz, 2H, -C_6H_4), 7.76 (s, 1H, -C=CH), 7.73 (s, 1H, -C=CH), 7.68 (d, *J* = 7.6 Hz, 1H, -C_6H_4), 7.59 (t, *J* = 8.1 Hz, 1H, -C_6H_4), 6.61 (s, 2H, -C_6H_2), 3.88 (s, 9H, -OCH_3), 3.79 (s, 2H, -CH_2), 3.74 (s,2H, -CH_2), 2.46 (s, 3H, -NCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 186.12, 153.05, 148.20, 139.25, 137.38, 136.73, 135.92, 135.40, 133.06, 131.91, 130.38, 129.62, 124.31, 123.35, 107.90, 60.90, 56.92, 56.50, 56.18, 45.70. Elemental analysis (%) calcd. for $C_{23}H_{24}N_2O_6$ (424.45): C 65.08, H 5.70, N 6.60; Found: C 65.11, H 5.72, N 6.54.

4.1.4.7. *(3E,5E)-3-(3,4,5-trimethoxybenzylidene)-5-(4-cyanobenzylidene)-1-methylpiperidin-4-one (**8g**):* Light yellow powder; Yield: 41%; mp:167-169℃; IR (cm^{-1}): 2940(m), 2838(m), 2226(s), 1674(s), 1578(s), 1503(s), 1452(s), 1416(s), 1326(s), 1268(m), 1242(s), 1184(s), 1158(s), 1123(m), 1063(s), 996(s), 928(s), 833(s), 623(m). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, 2H, -C=CH), 7.72 (d, *J* = 8.0 Hz, 2H, -C_6H_4), 7.48 (d, *J* = 8.0 Hz, 2H, -C_6H_4), 6.64 (s, 2H, -C_6H_2), 3.92 (s, 3H, -OCH_3), 3.91 (s, 6H, -OCH_3), 3.81 (s, 2H, -CH_2), 3.73 (s, 2H, -CH_2), 2.48 (s, 3H, -NCH_3).^{13}C NMR (100 MHz, $CDCl_3$) δ 186.22, 153.13, 139.74, 139.48, 137.45, 135.69, 133.62, 132.20, 131.93, 130.46, 130.40, 118.40, 112.16, 108.09, 60.92, 56.98, 56.70, 56.26, 45.68. Elemental analysis (%) calcd. for $C_{24}H_{24}N_2O_4$ (404.46): C 71.27, H 5.98, N 6.93; Found: C 71.21, H 5.92, N 6.94.

4.1.5. General synthesis of compounds 9a-g

3-nitrobenzaldehyde (0.76 g, 0.005 mol), *N*-methyl-4-piperidone (0.57 g, 0.005 mol), and aromatic aldehyde (0.005 mol) were dissolved in 5 mL of methanol. After 2.0 mL 10% NaOH solution added, the mixture was stirring for 4~8 h at ambient temperature (monitored by TLC). Then removal of solvent through pouring process, the residues were purified on silica gel by column using petroleum ether/EtOAc (4:1, v/v) as the eluent to afford light yellow powders

9a-g.

4.1.5.1. *(3E,5E)-3-(3-nitrobenzylidene)-5-(4-fluorobenzylidene)-1-methylpiperidin-4-one (**9a**):* Light yellow powder; Yield: 49%; mp:172-174℃; IR (cm^{-1}) 2848(m), 2766(m), 1674(s), 1613(s), 1598(m), 1583(s), 1526(s), 1505(s), 1455(m), 1350(s), 1286(s), 1271(s), 1222(s), 1179(s), 1159(s), 1132(m), 1107(s), 1058(s), 1009(m), 990(s), 922(s), 893 (m), 852(m), 820 (s), 808(s), 789(s), 741(s),725(s), 692(s), 671(s). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (d, *J* = 7.2 Hz, 2H, -C_6H_4), 7.78 (d, 2H, -C=CH), 7.69 (d, *J* = 7.7 Hz, 1H, -C_6H_4), 7.60 (t, *J* = 8.2 Hz, 1H, -C_6H_4), 7.38 (dd, *J* = 8.2, 5.6 Hz, 2H, -C_6H_4), 7.12 (t, *J* = 8.5 Hz, 2H, -C_6H_4), 3.76 (s, 4H, -CH_2), 2.49 (s, 3H, -NCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 186.20, 163.02 (d, *J* = 251.4 Hz), 148.27, 136.75, 135.99, 135.98, 135.35, 133.17, 132.44 (d, *J* = 8.4 Hz), 132.26 (d, *J* = 1.3 Hz), 131.10 (d, *J* = 3.3 Hz), 129.63, 124.32, 123.43, 115.83 (d, *J* = 21.7 Hz), 56.94, 56.60, 45.88. Elemental analysis (%) calcd. for $C_{20}H_{17}FN_2O_3$ (352.36): C 68.17, H 4.86, N 7.95; Found: C 68.23, H 4.39, N 7.98.

4.1.5.2. *(3E,5E)-3-(3-nitrobenzylidene)-5-(4-chlorobenzylidene)-1-methylpiperidin-4-one (**9b**):* Light yellow powder; Yield: 49%; mp:193-195℃; IR (cm^{-1}): 2945(m), 2845(m), 2764(m), 1673(s), 1614(s), 1586(s), 1523(s), 1489(s), 1455(s), 1409(s), 1349(s), 1284(s), 1270(s), 1171(s), 1130(m), 1089(s), 1055(s), 1012(s), 989(s), 937(m), 919(s), 891(s), 839(m), 823(s), 806(s), 775(s), 740(s), 729(s), 712(s), 670(s), 651(s). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25(d, *J* = 7.2 Hz, 2H, -C_6H_4), 7.83 (s, 1H, -C=CH), 7.79 (s, 1H, -C=CH), 7.73 (d, *J* = 8.0 Hz, 1H, -C_6H_4), 7.64 (m, 1H, -C_6H_4), 7.44 (d, *J* = 8.0 Hz, 2H, -C_6H_4), 7.36 (d, *J* = 8.0 Hz, 2H, -C_6H_4),7.28(s, 2H, -C_6H_4), 3.78 (s, 4H, -CH_2), 2.47 (s, 3H, -NCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 186.18, 148.29, 136.72, 135.99, 135.75, 135.32, 133.37, 133.30, 133.04, 131.63, 131.17, 129.64, 128.92, 124.32, 123.47, 56.95, 56.63, 45.89. Elemental analysis (%) calcd. for $C_{20}H_{17}ClN_2O_3$ (368.81): C 65.13, H 4.65, N 7.60; Found: C 65.23, H 4.69, N 7.68.

4.1.5.3. *(3E,5E)-3-(3-nitrobenzylidene)-5-(4-bromobenzylidene)-1-methylpiperidin-4-one (**9c**):* Light yellow powder; Yield: 46%; mp: 200-202℃; IR (cm^{-1}): 2941(m), 2840(m), 2767(m), 1680(s), 1618(s), 1590(m), 1521(s), 1484(m), 1343(s), 1268(s), 1175(s), 1071(m), 991(s), 916(s), 808(s), 739(m), 671(s). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (d, *J* = 7.2 Hz, 2H, -C_6H_4), 7.81 (s, 1H, -C=CH), 7.76 (s, 1H, -C=CH), 7.71 (d, *J* = 7.6 Hz, 1H, -C_6H_4), 7.63 (t, *J*

= 8.1 Hz, 1H, -C_6H_4), 7.58 (d, *J* = 8.0 Hz, 2H, -C_6H_4), 7.28 (d, *J* = 8.0 Hz, 2H, -C_6H_4), 3.77 (s, 2H, -CH_2), 3.75 (s, 2H, -CH_2), 2.49 (s, 3H, -NCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 186.16, 148.33, 136.74, 135.92, 135.74, 135.34, 133.82, 133.29, 133.19, 131.87, 131.78, 129.62, 124.31, 123.66, 123.44, 56.91, 56.61, 45.82. Elemental analysis (%) calcd. for $C_{20}H_{17}BrN_2O_3$ (413.26): C 58.13, H 4.15, N 6.78; Found: C 58.23, H 4.09, N 6.76.

4.1.5.4. *(3E,5E)-3-(2,4-dichlorobenzylidene)-5-(3-nitrobenzylidene)-1-methylpiperidin-4-one* ***(9d)****:* Light yellow powder; Yield: 46%; mp:193-195℃; IR(cm^{-1}): 2945(m), 2858(m), 2788(m), 1679(s), 1614(s), 1587(m), 1520(s), 1462(s), 1348(s), 1294(m), 1272(s), 1183(s), 1107(s), 1047(m), 989(m), 923(s), 847(m), 822(s), 742(m), 672(s), 641(m), 562(s). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (d, *J* = 7.2 Hz, 2H, -C_6H_3), 7.99 (s, 1H, -C=CH), 7.90 (s, 1H, -C=CH), 7.71 (d, *J* = 8.0 Hz, 1H, -C_6H_4), 7.65 (t, *J* = 8.0 Hz, 1H, -C_6H_4), 7.51 (s, 1H, -C_6H_4), 7.33(d, *J* = 8.0 Hz, 1H, -C_6H_3), 7.19(d, *J* = 8.0 Hz, 1H, -C_6H_3),3.90 (s, 2H, -CH_2), 3.73 (s, 2H, -CH_2), 2.50 (s, 3H, -NCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 185.77, 148.32, 136.66, 135.98, 135.92, 135.42, 135.12, 134.50, 133.88, 132.77, 131.92, 130.93, 129.91, 129.64, 126.89, 124.33, 123.52, 56.79, 56.37, 45.68. Elemental analysis (%) calcd. for $C_{20}H_{16}Cl_2N_2O_3$ (403.26): C 59.57, H 4.00, N 6.95; Found: C 59.23, H 4.09, N 6.96.

4.1.5.5. *(3E,5E)-3-(3,4-dichlorobenzylidene)-5-(3-nitrobenzylidene)-1-methylpiperidin-4-one* ***(9e)****:* Light yellow powder; Yield: 46%; mp: 167-169℃; IR (cm^{-1}): 2949(m), 2857(m), 2795(m), 1678(s), 1617(s), 1594(s), 1531(s), 1473(s), 1404(m), 1347(s), 1312(m), 1274(s), 1183(s), 1153(m), 1129(s), 1101(s), 1059(s), 1029(s), 1003(s), 992(s), 927(s), 884(s), 822(s), 805(s), 779(s), 745(s), 729(s), 688(s). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (d, *J* = 7.2 Hz, 2H, -C_6H_4), 7.79 (s, 1H, -C=CH), 7.68 (m, 2H, -C=CH, -C_6H_4), 7.61 (t, *J* = 7.5 Hz, 1H, -C_6H_4), 7.51 – 7.45 (m, 2H, -C_6H_3), 7.21 (d, *J* = 7.8 Hz, 1H, -C_6H_3), 3.77 (s, 2H, -CH_2), 3.74 (s, 2H, -CH_2), 2.49 (s, 3H, -NCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 185.93, 148.29, 136.58, 135.98, 135.05, 134.86, 134.29, 133.98, 133.66, 133.38, 132.91, 131.75, 130.63, 129.68, 129.38, 124.36, 123.56, 56.69, 56.55, 45.82. Elemental analysis (%) calcd. for $C_{20}H_{16}Cl_2N_2O_3$ (403.26): C 59.57, H 4.00, N 6.95; Found: C 59.23, H 4.09, N 6.86.

4.1.5.6. *(3E,5E)-3-(3-bromobenzylidene)-5-(3-nitrobenzylidene)-1-methylpiperidin-4-one* ***(9f)****:* Light yellow powder; Yield: 46%; mp:159-161℃; IR(cm^{-1}): 2926m), 2853(m), 2785(m),

1671(s), 1614(s), 1588(s), 1530(s), 1462(m), 1348(s), 1289(s), 1183(s), 1110(s), 1001(m), 993(m), 921(s), 862(m), 758(s), 738(m), 668(s). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (d, *J* = 7.2 Hz, 2H, -C_6H_4), 7.82 (s, 1H, -C=CH), 7.75 (s, 1H, -C=CH), 7.71 (d, *J* = 8.0 Hz, 1H, -C_6H_4), 7.63 (t, *J* = 8.0 Hz, 1H, -C_6H_4), 7.53 (s, 2H, -C_6H_4), 7.33 (d, *J* = 4.0 Hz, 2H, -C_6H_4), 3.79 (s, 4H, -CH_2), 2.50 (s, 3H, -NCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 185.99, 148.33, 136.96, 136.65, 135.91, 135.45, 135.10, 133.58, 132.88, 132.08, 130.10, 129.64, 128.77, 124.35, 123.49, 122.70, 56.61, 56.54, 45.67. Elemental analysis (%) calcd. for $C_{20}H_{17}BrN_2O_3$ (413.26): C 58.13, H 4.15, N 6.78; Found: C 58.23, H 4.09, N 6.76.

4.1.5.7. *(3E,5E)-3-(3-nitrobenzylidene)-5-(4-(trifluoromethyl)benzylidene)-1-methylpiperidin-4-one (**9g**):* Light yellow powder; Yield: 47%; mp:186-188℃; IR(cm^{-1}): 2948(m), 2850(m), 2785(m), 1682(s), 1619(s), 1594(m), 1522(s), 1465(m), 1413(m), 1324(s), 1270(s), 1168(s), 1107(s), 1069(s), 1009(m), 990(m), 917(s), 831(s), 733(m), 706(m), 671(s) .^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (d, *J* = 7.2 Hz, 2H, -C_6H_4), 7.83 (d, 2H, -C=CH), 7.73 (s, 1H, -C_6H_4), 7.70 (d, *J* = 8.0 Hz, 2H, -C_6H_4), 7.65 (t, *J* = 12.4 Hz, 1H, -C_6H_4), 7.50 (d, *J* = 8.0Hz, 2H, -C_6H_4), 3.79 (s, 4H, -CH_2), 2.50 (s, 3H, -NCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 185.99, 148.34, 138.38, 136.59, 135.92, 135.24, 134.99, 134.33, 133.78, 130.31, 129.67, 125.52 (q, *J* = 3.7 Hz,), 124.35, 123.56, 56.68, 56.57, 45.68. Elemental analysis (%) calcd. for $C_{21}H_{17}F_3N_2O_3$ (402.37): C 62.69, H 4.26, N 6.96; Found: C 62.68, H 4.29, N 6.96.

4.1.6. General synthesis of compounds 10a-e

4-cyanobenzaldehyde (0.66 g, 0.005 mol), *N*-methyl-4-piperidone (0.57 g, 0.005 mol), and aromatic aldehyde (0.005 mol) were dissolved in 5 mL of methanol. After 2.0 mL 10% NaOH solution added, the mixture was stirring for 4 h at ambient temperature (monitored by TLC). Then removal of solvent through pouring process, the residues were purified on silica gel by column using petroleum ether/EtOAc (5:1, v/v) as the eluent to afford light yellow powders **10a-e**.

4.1.6.1. *(3E,5E)-3-(4-fluorobenzylidene)-5-(4-cyanobenzylidene)-1-methylpiperidin-4-one (**10a**):* Light yellow powder; Yield: 44%; mp:157-159℃; IR (cm^{-1}): 2946(m), 2845(m), 2221(s), 1677(s), 1618(s), 1601(s), 1505(m), 1412(s), 1291(m), 1272(s), 1181(s), 1157(s), 1104(m), 1060(s), 985(s), 922(s), 836(s), 790(s), 653(m). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79

(s, 1H, -C=CH), 7.76 (s, 1H, -C=CH), 7.72 (d, *J* = 8.1 Hz, 2H, -C_6H_4), 7.48 (d, *J* = 8.1 Hz, 2H, -C_6H_4), 7.40 (dd, *J* = 8.1, 5.6 Hz, 2H, -C_6H_4), 7.14 (t, *J* = 8.5 Hz, 2H, -C_6H_4), 3.76 (s, 2H, -CH_2), 3.72 (s, 2H, -CH_2), 2.48 (s, 3H, -NCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 186.27, 163.02 (d, *J* = 251.4 Hz), 139.67, 135.99, 135.59, 133.71, 132.43 (d, *J* = 8.4 Hz), 132.28 (d, *J* = 1.8 Hz), 132.23, 131.10 (d, *J* = 3.3 Hz), 130.51, 118.46, 115.83 (d, *J* = 21.7 Hz), 112.16, 56.97, 56.78, 45.88. Elemental analysis (%) calcd. for $C_{21}H_{17}FN_2O$ (332.37): C 75.89, H 5.16, N 8.43; Found: C 75.81, H 5.20, N 8.51.

4.1.6.2. *(3E,5E)-3-(4-chlorobenzylidene)-5-(4-cyanobenzylidene)-1-methylpiperidin-4-one* ***(10b)****:* Light yellow powder; Yield: 42%; mp: 158-160℃; IR (cm^{-1}): 2933(m), 2851(m), 2226(s), 1672(s), 1614(s), 1580(s), 1490(s), 1456(m), 1408(s), 1331(m), 1272(s), 1170(s), 1087(s), 1009(s), 984(s), 922(m), 830(s), 776(s), 688(s). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (s, 2H, -C=CH), 7.72 (d, *J* = 8.1 Hz, 2H, -C_6H_4), 7.48 (d, *J* = 8.1 Hz, 2H, -C_6H_4), 7.41 (d, *J* = 8.3 Hz, 2H, -C_6H_4), 7.34 (d, *J* = 8.3 Hz, 2H, -C_6H_4), 3.75 (s,2H, -CH_2), 3.72 (s, 2H, -CH_2), 2.48 (s, 3H, -NCH_3).^{13}C NMR (100 MHz, $CDCl_3$) δ 186.22, 139.61, 135.74, 135.52, 135.33, 133.85, 133.35, 133.03, 132.24, 131.62, 130.52, 128.91, 118.44, 112.20, 56.94, 56.78, 45.86. Elemental analysis (%) calcd. for $C_{21}H_{17}ClN_2O$ (348.83): C 72.31, H 4.91, N 8.03; Found: C 72. 34, H 4.89, N 8.08

4.1.6.3. *(3E,5E)-3-(4-bromobenzylidene)-5-(4-cyanobenzylidene)-1-methylpiperidin-4-one* ***(10c)****:* Light yellow powder; Yield: 42%; mp:165-167℃; IR (cm^{-1}): 2938(m), 2773(m), 2231(s), 1673(s), 1611(s), 1581(s), 1501(m), 1486(s), 1457(m), 1403(m), 1330(m), 1270(s), 1168(s), 1130(s), 1089(s), 1005(s), 982(s), 925(s), 827(s), 775(s), 699(m). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (s, 1H, -C=CH), 7.74 (s, 1H, -C=CH), 7.72 (d, *J* = 8.1 Hz, 2H, -C_6H_4), 7.57 (d, *J* = 8.1 Hz, 2H, -C_6H_4), 7.48 (d, *J* = 8.0 Hz, 2H, -C_6H_4), 7.27 (d, *J* =8.0 Hz, 2H, -C_6H_4), 3.73 (s, 2H, -CH_2), 3.72 (s, 2H, -CH_2), 2.48 (s, 3H, -NCH_3).^{13}C NMR (100 MHz, $CDCl_3$) δ 186.20, 139.60, 135.77, 135.51, 133.88, 133.78, 133.15, 132.23, 131.87, 131.80, 130.52, 123.69, 118.43, 112.21, 56.92, 56.77, 45.84. Elemental analysis (%) calcd. for $C_{21}H_{17}BrN_2O$ (393.28): C 64.13, H 4.36, N 7.12; Found: C 64.23, H 4.39, N 7.08.

4.1.6.4. *(3E,5E)-3-(2,4-dichlorobenzylidene)-5-(4-cyanobenzylidene)-1-methylpiperidin-4-on e* ***(10d)****:* Light yellow powder; Yield: 45%; mp:140-142℃; IR (cm^{-1}): 2943(m), 2854(m),

2224(s), 1741(s), 1676(s), 1611(s), 1582(s), 1501(m), 1463(s), 1412(m), 1381(m), 1335(s), 1275(s), 1237(m), 1183(s), 1104(s), 1052(s), 988(s), 921(s), 826(s), 784(s), 730(m). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (s, 1H, -C=CH), 7.78 (s, 1H, -C=CH), 7.72 (d, *J* = 8.1 Hz, 2H, -C_6H_4), 7.48 (d, *J* = 8.4 Hz, 3H, -C_6H_4), 7.30 (dd, *J* = 12.0, 4.9 Hz, 1H, -C_6H_3), 7.18 (d, *J* = 8.3 Hz, 1H, -C_6H_3), 3.72 (s, 2H, -CH_2), 3.59 (s, 2H, -CH_2), 2.43 (s, 3H, -NCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 185.83, 139.52, 135.93, 135.43, 135.27, 134.50, 134.44, 132.83, 132.25, 131.87, 130.93, 130.56, 129.91, 126.90, 118.41, 112.30, 56.95, 56.38, 45.71. Elemental analysis (%) calcd. for $C_{21}H_{16}Cl_2N_2O$ (383.27): C 65.81, H 4.21, N 7.31; Found: C 65.84, H 4.20, N 7.30.

4.1.6.5. *(3E,5E)-3-(4-(trifluoromethyl)benzylidene)-5-(4-cyanobenzylidene)-1-methylpiperidin-4-one (**10e**):* Light yellow powder; Yield: 45%; mp: 181-183℃; IR (cm^{-1}): 2942(m), 2848(m), 2224(s), 1680(s), 1612(s), 1590(s), 1504(m), 1465(m), 1413(s), 1323(s), 1272(s), 1162(s), 1107(s), 1068(s), 1014(s), 987(s), 918(s), 839(s), 779(s), 675(s). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (s, 1H, -C=CH), 7.78 (s, 1H, -C=CH), 7.72 (dd, *J* = 12.2, 8.3 Hz, 4H, -C_6H_4), 7.52 – 7.47 (m, 4H, -C_6H_4), 3.76 (s, 2H, -CH_2), 3.74 (s, 2H, -CH_2), 2.48 (s, 3H, -NCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 186.17, 139.50, 138.38, 135.36, 135.12, 134.48, 134.20, 132.26, 130.88, 130.55, 130.33, 125.51 (q, *J* = 3.7 Hz), 118.41, 112.32, 56.83, 56.80, 45.84. Elemental analysis (%) calcd. for $C_{22}H_{17}F_3N_2O$ (382.38): C 69.10, H 4.48, N 7.33; Found: C 69.14, H 4.42, N 7.31.

4.2. Single-Crystal Structure Determination of 8a

Suitable single crystal of **8a** was prepared by recrystallization via solvent evaporation in methanol solution under the ambient condition. It measured at 153 K on a Bruker phi and omega scans-based diffractometer (Cu Kα radiation, λ = 1.54178 Å) using a SMART and SAINT programs. The structure was solved by direct methods and refined on F^2 by full-matrix least-squares methods with SHELXTL version 6.1. Crystal data of **8a**: $C_{23}H_{24}FNO_4$, *M* = 397.43, Monoclinic, space group *P2(1)/c*, yellow block, *a* = 31.9452(8) Å, *b* = 8.2631(2) Å, *c* = 7.5532(2) Å, β = 91.379(2)°, *V* = 1993.21(9) Å^3, Z = 4, *D*c = 1.324 g·cm^{-3}, μ(Cu-Kα) = 0.797 mm^{-1}, *T* = 153(2) K. 7094 unique reflections [R_{int} = 0.0215]. Final R_1 [with $I > 2\sigma(I)$] = 0.0439, wR_2 (all data) = 0.1096. CCDC 1567525 (**8a**) contains the

supplementary crystallographic data for this paper. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44)1223-336-033; e-mail: *deposit@ccdc.cam.ac.uk*).

4.3. In vitro cytotoxicity testing with MTT method

All compounds were screened against human neoplastic cell lines such as human liver hepatocellular carcinoma cell line (HepG2), human cervical carcinoma cells (HeLa), human chronic myelogenous leukemia cell line (K562), human acute mononuclear granulocyte leukemia (THP-1), human normal heptical cell line (LO2) using modified MTT assay (Dojindo Laboratories, Tokyo, Japan). The THP-1, HeLa, K562, HepG2 and LO_2 cell line, was maintained at 37 °C in a humidified 5% CO_2 and 95% air atmosphere. The HepG2 cell lines were culture in DMEM containing 10% fetal bovine serum. Other cell lines were grown in 1640 and supplemented with 10% fetal bovine serum. The tested compounds and positive control (Doxorubicin, DOX) were initially dissolved in dimethylsulfoxide (DMSO), and then the solutions were diluted 1:1000 in DMEM medium or 1640 medium, which final concentration of DMSO was always 0.1% (v/v). Controls were prepared with 0.1% DMSO (v/v) only.

The cells were seeded in a 96-well plate in 200 μL medium per well at a density of 1×10^4 cells/well for 24 h. The cells were treated with serial concentrations of compound and incubated for 24 h. Cells with only culture media were used as control. After the media removed, 20 μL of MTT (5 mg/mL) was added then the plates were incubated for 4 h at 37 ℃ in cell culture incubator. The MTT containing media were removed and then 150 μL of DMSO was added to dissolve the dark-blue formazan crystals. The optical density (OD) was measured by a multi-well plate reader (TECAN, Männedorf, Switzerland) at 570 nm. The results are expressed as a decrease in the cell viability (%) in comparison to untreated controls. The concentration of each compound was examined in triplicate. The concentrations of the compounds were 10, 8, 5, 3, 2, 1, 0.5, 0.1, 0.05 and 0.01 μg/mL. Doxorubicin (DOX) was used as a positive control. The concentrations of DOX used were 1.5, 1.2, 1.0, 0.8, 0.5, 0.3, 0.1, 0.05 and 0.01 μg/mL.

4.4. Western Blot

The HepG2 cell line treated for 3 h by compounds **6d**, **7h**, **8g**, **9g**, respectively, in 50 μM concentration were washed twice with PBS, and then lysed in ice-cord modified RIPA buffer containing PMSF (RIPA : PMSF = 100:1; the ultimate density of PMSF is 1 mM). The lysate was stand for 30 minutes on the ice, and then centrifuged for 15 min at 12000 rpm/min at 4 ℃ and the supernatant collected. The protein was boiling at 95 ℃ at 5 min with loading buffer (the volune ratio of protein and the loading buffer is 4:1). Each 50 μg protein of cell lysates was separated by 10% SDS-PAGE gel electrophiresis. The proteins were then transferred onto polyvinylidene fluorides. The membrane was probed with antibody and anti-β-actin. The Western blot was visualized using an enhanced chemiluminescence (ECL) detection kit according to the manufacturer instruction.

4.5. Molecular docking study

The molecular docking of compounds **6d**, **7h**, **8g**, **9g**, against Bcl-2 protein was performed using SYBYLX 2.0 software (Tripos, Certara Inc., St. Louis, MO, USA) in DELL7500 workstation. The structures were assigned with Gasteiger-Hückel charges. Other parameters that are not mentioned were set at default values.

4.6. Apoptosis assay

HepG2 cells was plated at a density of 1×10^5 cells/well in 24-well plates. After treatment with **6d** (0.4, 0.6 and 0.8 μM) and DMSO for 24 h, HepG2 cells treated with **6d** was harvested, washed twice with pre-chilled PBS and suspended in 1X binding buffer at a concentration of 1×10^6 cells/mL. One hundred microliters of such solution (1×10^5 cells) was mixed with 5μL of Annexin V-FITC and 5μL of Propidium Iodide (BD Biosciences, San Jose, CA, USA) according to the manufacturer's instruction. The mixed solution was gently vortexed and incubated in the dark at room temperature (25℃) for 15 min. Four hundreds microliters of 1X binding buffer were then added to each tube and cell apoptosis analysis was performed by flow cytometry (BD FACS Calibur) within 1 h.

4.7. Cellular uptake studies in HepG2 cells using CLSM

HepG2 cells were seeded in 6-well plates at a density of 2×10^5 cells/well in 2.0 mL of culture medium and cultivated for 24 h, and then incubated with **6d** for 1 h, 2 h, 3 h, 4 h, respectively. The concentration of **6d** is 10 mg/mL. Then, the culture medium was removed

and the cells were washed with PBS for three times. Whereafter, 4% (w/v) paraformaldehyde was utilized to fix the cells for 1 h at room temperature and 100 mL (10 mg/mL) Hoechst 33342 was applied to stain the nuclei for 15 min. Following this, the glass cover slips containing cells were mounted onto slides and then observed under confocal laser scanning microscopy (CLSM, TCS SPE, Leica, Germany).

4.8. In vivo antitumor efficacy

The antitumor effect was investigated on the seven-week-old female Balb/c nude mice (n = 30) weighing 19 to 21 g (Shanghai Laboratory Animal Center). Briefly, 0.2 mL HepG2 cells (1×10^7 /mL) were inoculated subcutaneously into the alar left of each mouse. At the third day after the inoculation, the HepG2-bearing mice were randomly divided into five groups (n=6/group) and administrated the according drug or solvent. For the treatment groups, **6d** was dissolved in saline containing 1% DMSO, and DOX was dissolved in saline. **6d** administered by daily i.p. injection of (10 mg/kg/d, 1.0 mg/kg/d and 0.1 mg/kg/d) for 20 days, DOX (1.0 mg/kg/2d) administered by every two days injection of 1mg/kg for 20 days. While the control group was administrated saline as negative control. The body weight was recorded starting from the day of treatment, and tumor volumes were also calculated at the same time points using the following equation: tumor volume = length × (width)2 × π/6. Animal experiments were reviewed and approved by the Binzhou Medical University Experimental Animal Committee.

4.9. Statistical analysis

The molecular docking was performed using SYBYLX 2.0 software (Tripos, Certara Inc., St. Louis, MO, USA) in DELL7500 workstation. All data were analyzed by one-way ANOVA followed by Dunnett's test (*P<0.05, **P<0.01, ***P<0.001). All values reported in this study are the means of three replicates.

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Figure 1. The structures of curcumin and some BAPs.

Figure 2. (A) Synthetic strategy of dissymmetric compound **6g**. (B) Synthetic strategy and structures of five series of novel BAPs. (C) The ORTEP figure of **8a** (Displacement ellipsoids with 40% probability).

Figure 3. (A) The structures of **6d**, **7h**, **8g**, **9g**. (B) Dose-dependent inhibitory effect of compounds **6d**, **7h**, **8g**, **9g** and DOX on experimental cell lines after 24 h, respectively. The curves were obtained under logarithm concentration. (C) The effect of **6d**, **7h**, **8g**, **9g** on the expression level of resistance protein BAX and Bcl-2 detected by western blot. Protein was from HepG2 cell line treated for 3 h by **6d**, **7h**, **8g**, **9g**, respectively, in 50 μM concentration. The data are representative of three independent experiments. *P<0.05, **P<0.01, ***P<0.001 vs the negative control (one-way ANOVA followed by Dunnett's test). (D) The molecular docking modes of compound **6d**, **7h**, **8g**, **9g** in the active site of Bcl-2 protein.

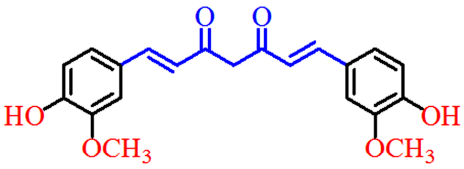
Figure 4. (A) HepG2 cell lines were incubated with **6d** (0.4, 0.6 or 0.8 μmol/L) for 24 h and analyzed for apoptosis by Annexin V-FITC staining. **6d** treatment increased number of apoptotic cells in a dose-dependent manner compared with the untreated control. (B) The cellular uptake of HepG2 cells in different time (1 h, 2 h, 3 h, 4 h) observed by CLSM. Scale bar: 50 μm.

Figure 5. 6d inhibits HCC tumor growth *in vivo*. (A) HepG2 cells were injected to the flanks of nude mice were allowed to develop for 3 days. Subsequently, **6d** (10 mg/kg, 1.0 mg/kg and 0.1 mg/kg) was injected daily i.p. for up to 20 days. On day 21, tumors were excised and subjected to further analyses. Representative tumors of each group were showed. (B) Tumor size were measured every two days. (C) Mice body weight were measured every two days. There was a significant reduction in relative tumor volume from **6d** (10 mg/kg)-treated and DOX (1 mg/kg)-treated animals when compared with untreated controls.

Table 1. Cytotoxicity of BAPs, Curcumin and DOX.

Table 1. Cytotoxicity of BAPs, Curcumin and DOX.

Compds	$IC_{50}(\mu M)$					Compds	$IC_{50}(\mu M)$				
	HepG2	Hela	K562	THP-1	LO2		HepG2	Hela	K562	THP-1	LO2
6a	12.25±0.63	23.45±3.05	18.37±0.91	15.42±2.51	22.10±0.17	**8d**	8.80±0.32	＞20	＞20	5.02±0.17	13.38±0.83
6b	8.76±0.72	14.35±1.36	17.98±1.39	11.19±0.89	10.50±0.11	**8e**	6.96±0.06	13.50±0.76	16.15±0.52	5.33±0.28	18.37±0.13
6c	1.24±0.32	5.45±0.26	6.32±0.27	1.17±0.19	11.92±0.10	**8f**	1.89±0.11	5.54±0.32	4.20±0.11	0.89±0.08	13.57±0.26
6d	0.67±0.20	3.22±0.21	4.34±0.32	0.64±0.23	12.05±0.04	**8g**	1.14±0.17	3.42±0.13	4.29±0.40	0.82±0.04	18.60±0.42
6e	9.12±0.56	15.54±1.26	18.21±1.98	13.41±0.79	25.80±0.96	**9a**	9.34±0.12	6.25±0.05	11.25±0.32	7.16±0.25	17.60±0.13
6f	4.14±0.17	6.56±0.13	6.34±0.11	5.93±0.35	18.62±0.15	**9b**	8.21±0.14	＞20	＞20	7.87±0.43	＞20
6g	1.08±0.12	2.34±0.12	3.89±0.04	1.01±0.12	13.45±0.07	**9c**	8.80±0.45	11.90±0.23	＞20	7.69±0.15	9.85±0.13
6h	0.59±0.14	1.34±0.09	2.67±0.17	0.81±0.06	11.00±0.42	**9d**	4.80±0.04	8.90±0.28	12.41±0.12	3.56±0.21	13.98±0.09
7a	6.14±0.08	10.23±0.17	11.25±0.26	5.04±0.46	21.89±0.57	**9e**	5.96±0.05	9.12±0.20	15.54±0.34	5.02±0.27	14.83±0.13
7b	5.95±0.03	11.23±0.12	13.32±0.78	5.86±0.78	18.90±0.14	**9f**	4.89±0.07	11.12±0.32	12.20±1.05	4.03±0.14	8.07±0.26
7c	4.84±0.08	5.90±0.23	7.42±0.06	4.27±0.12	21.26±0.98	**9g**	1.04±0.10	3.56±0.21	4.78±0.20	0.97±0.16	12.43±0.21
7d	6.25±0.12	7.76±0.20	11.90±0.12	6.07±0.21	18.75±0.89	**10a**	6.14±0.08	10.23±0.17	11.25±0.26	5.03±0.46	20.89±0.57
7e	3.84±0.25	6.90±0.34	9.49±0.27	3.26±0.11	10.61±0.57	**10b**	5.95±0.03	11.23±0.12	13.32±0.78	3.18±0.78	16.20±0.14
7f	1.84±0.08	2.56±0.13	4.42±0.42	1.77±0.19	10.78±1.12	**10c**	4.84±0.08	5.90±0.23	7.42±0.06	4.40±0.12	10.50±0.98
7g	0.88±0.07	3.42±0.47	4.65±0.13	0.94±0.05	14.00±0.43	**10d**	6.25±0.12	7.76±0.20	11.90±0.12	1.91±0.21	10.75±0.89
7h	0.89±0.02	2.30±0.25	3.47±0.27	0.72±0.07	14.56±0.67	**10e**	0.98±0.04	3.21±0.16	4.01±0.12	1.43±0.12	17.20±0.21
8a	7.34±0.17	7.25±0.03	10.25±0.03	6.13±0.35	10.73±0.27	Curcumin	13.78±0.31	31.89±0.27	23.12±0.14	22.54±0.34	25.67±0.23
8b	8.25±0.21	＞20	＞20	7.20±0.23	14.00±0.83	DOX	0.68±0.12	2.23±0.17	1.58±0.10	0.67±0.09	6.93±0.32
8c	6.80±0.14	11.90±0.23	12.41±0.01	5.33±0.22	15. 40±1.13						



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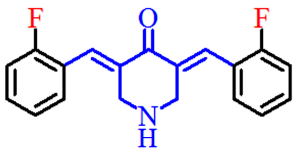

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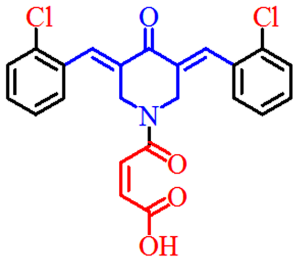

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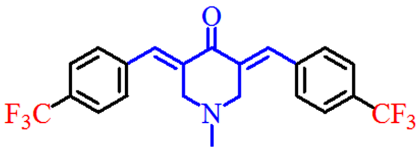

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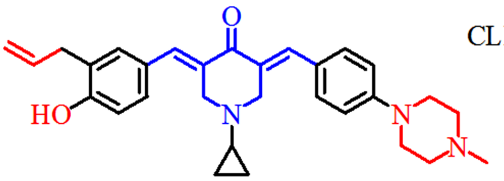
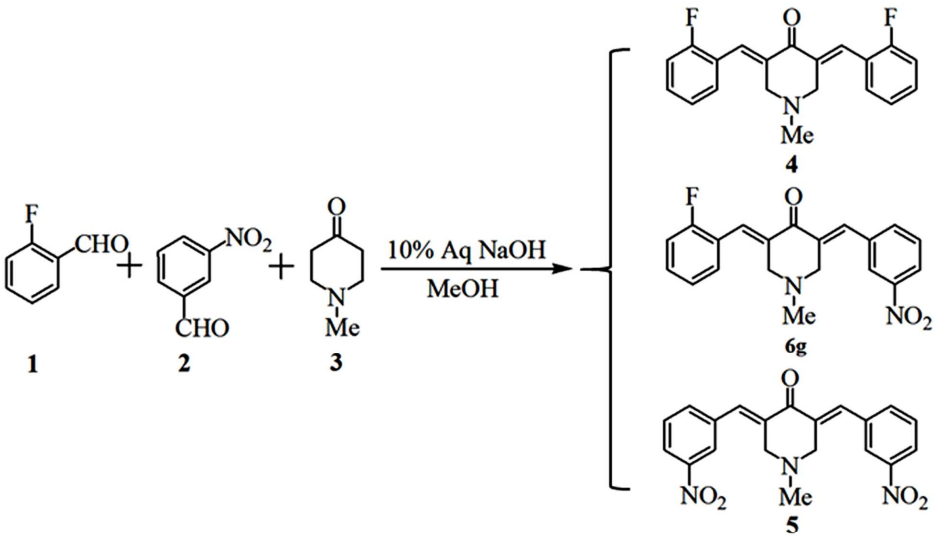


MAC 7a

A



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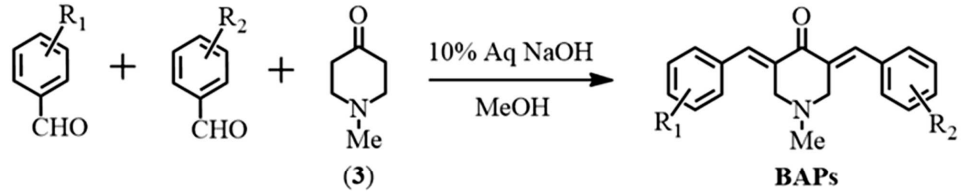

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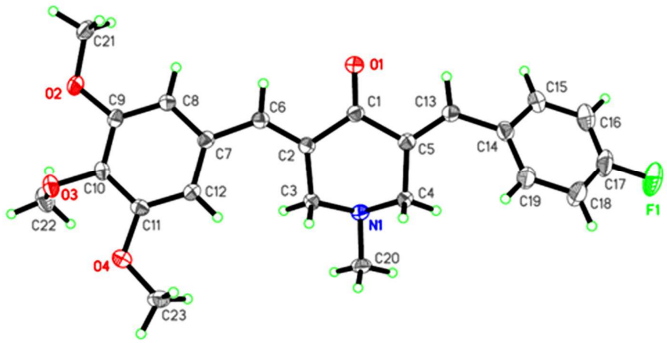
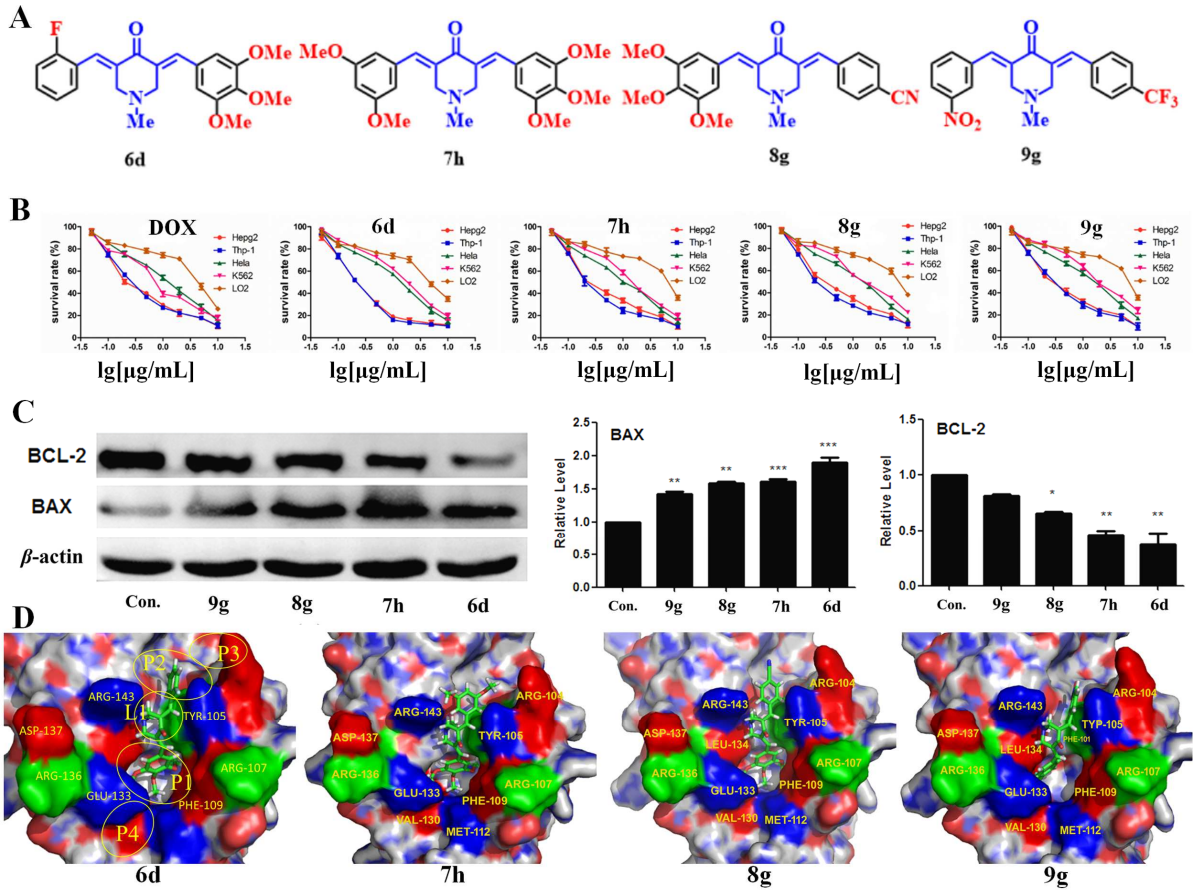
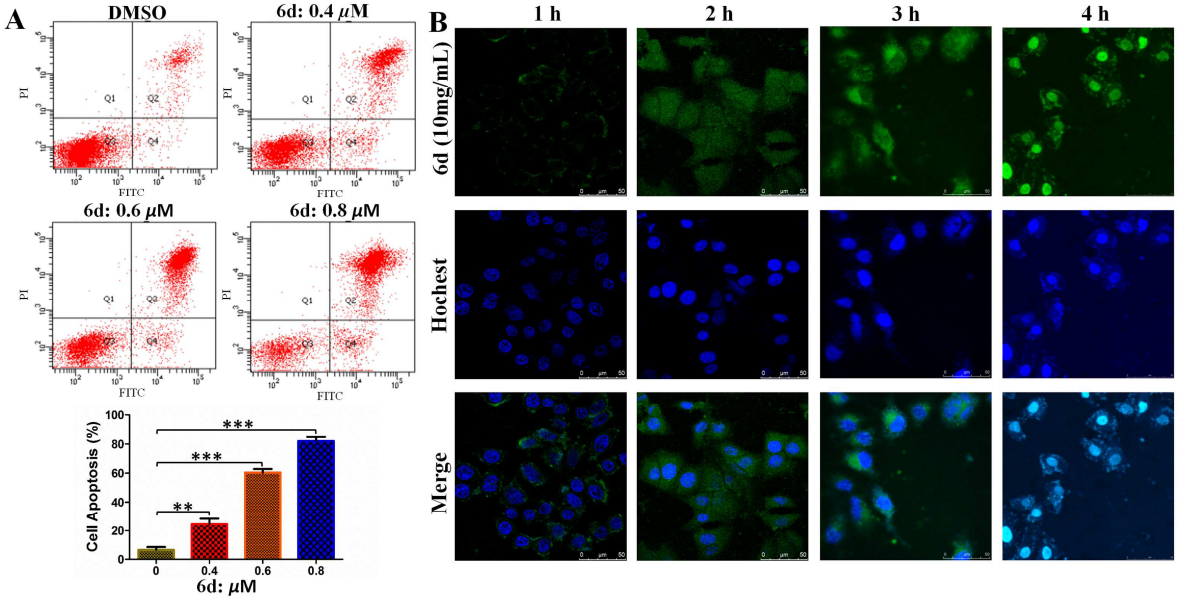
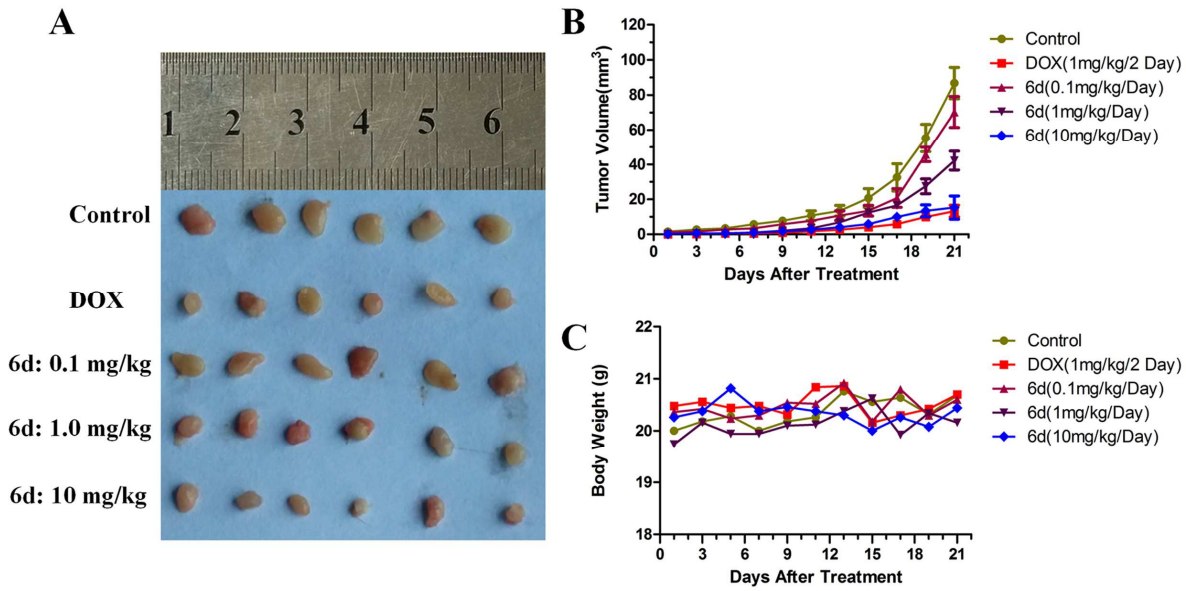
3,4-OMe	**(6b)**	4-Cl	**(7b)**	4-Cl	**(8b)**	4-Cl	**(9b)**	4-Cl	**(10b)**
3,5-OMe	**(6c)**	4-Br	**(7c)**	4-Br	**(8c)**	4-Br	**(9c)**	4-Br	**(10c)**
3,4,5-OMe	**(6d)**	2,4-Cl	**(7d)**	2,4-Cl	**(8d)**	2,4-Cl	**(9d)**	2,4-Cl	**(10d)**
4-CMe_3	**(6e)**	3,4-Cl	**(7e)**	3,4-Cl	**(8e)**	3,4-Cl	**(9e)**	4-CF_3	**(10e)**
4-CF_3	**(6f)**	3-NO_2	**(7f)**	3-NO_2	**(8f)**	3-Br	**(9f)**		
3-NO_2	**(6g)**	4-CN	**(7g)**	4-CN	**(8g)**	4-CF_3	**(9g)**		
4-CN	**(6h)**	3,4,5-OMe	**(7h)**						

C









Highlights

1. Thirty-five novel dissymmetric 3,5-bis(arylidene)-4-piperidone derivatives (**6a-h, 7a-h, 8a-g, 9a-g, 10a-e**) were generated and characterized.
2. **6d, 7h, 8g, 9g** demonstrated the most potential inhibitory activities against HepG2 and THP-1 and lower cytotoxicity toward LO2.
3. **6d, 7h, 8g, 9g** indicated dose-dependent inhibitory effect in survival effect.
4. **6d, 7h, 8g, 9g** can effectively promote cell apoptosis through up-regulating BAX expression and down-regulating Bcl-2 expression *in vitro*.
5. Tumor xenograft experiments *in vivo* of **6d** suppress the growth of HepG2 xenografts in nude mice.