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Design, synthesis, anticancer activity and cytotoxicity of novel 4-piperidone/cyclohexanone derivatives

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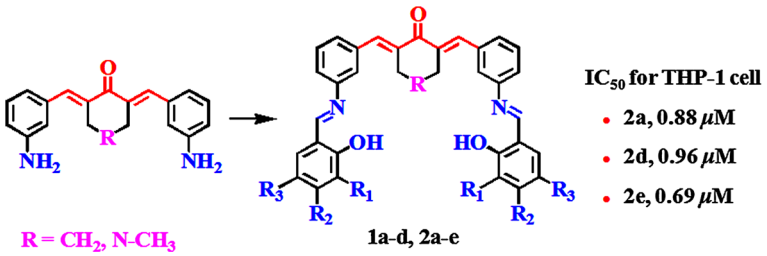
Abstract Design and synthesis of two series of novel double Schiff-base substituted 4-piperidone/cyclohexanone derivatives, like curcumin analogues, series **1** (**1a–d**) and **2** (**2a–e**) were generated and characterized by ^{1}H NMR, ^{13}C NMR, IR, and elemental analysis. The anticancer activities against human carcinoma cell lines HePG2, HeLa, K562, THP-1, and their cytotoxicities for LO2 cell lines were subsequently evaluated by the MTT method. The results show that series **2** (**2a–e**) have the better anticancer activity than series **1** (**1a–d**) in spite of the bigger cytotoxicity of series **2**. Structure analysis shows that the *N*-methyl-4-piperidinone group and the introduction of hydroxy is helpful for improving their anticancer activity, especially **2a**, **2d** and **2e**, and their IC_{50} values against THP-1 cells can reach 0.69–0.96 μM.

Graphical Abstract



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Introduction

Curcumin (Diferuloylmethane, Fig. 1), a member of the curcuminoid family, is the yellow-coloured phenolic pigment of the rhizome of Curcuma longa or turmeric from the Zingiberaceae family. Since antiquity, Curcuma longa or turmeric has been used to improve colour and flavor of food products [1, 2]. More important, curcumin, the active principle of turmeric [3, 4], has been reported to possess anti-inflammatory, anticoagulant, antibacterial, antifungal, anticarcinogenic, antitumor, anti-HIV, anti-mutagenic, antiproliferative, antioxidant, anti-protozoal, and anti-venom activities [5–10]. Curcumin and its analogues containing the pharmacophore of 1,5-diaryl-3-oxo-1,4-pentadienyl, is thought to interact at the primary binding site and bio-thiols from susceptible neoplasms. The pharmacophore of methoxyphenol groups align at an auxiliary site and also influence their bio-activities. However, curcumin and some analogues have many major problems in clinical applications, such as poor aqueous solubility, relatively low bioavailability, intense staining colour and so on [11]. In order to improve these defects, many improved curcumin

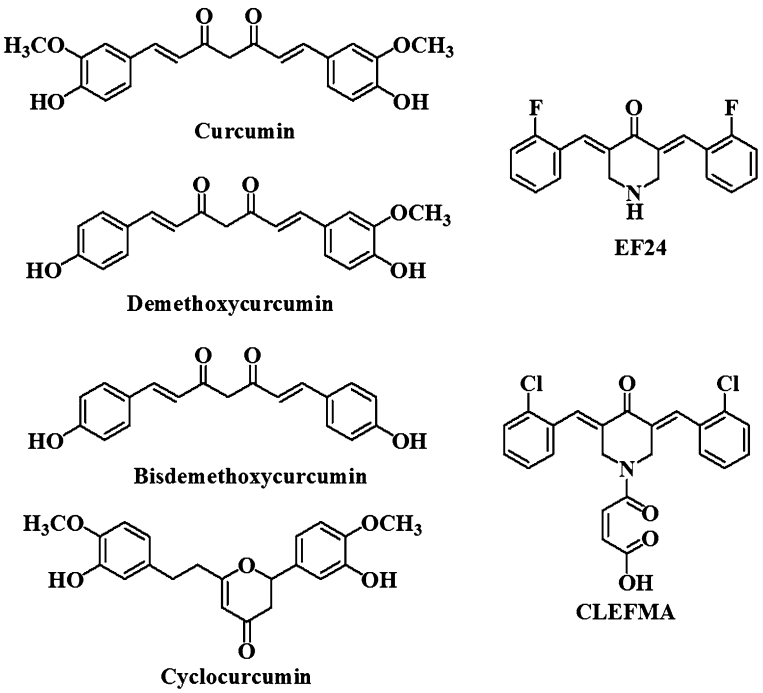


Fig. 1 Structures of curcumin and some curcumin analogues

analogues have been reported, such as demethoxycurcumin, bisdemethoxycurcumin, cyclocurcumin, and so on [12–19].

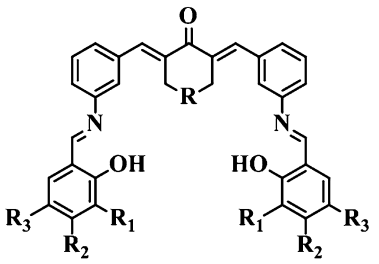
In recent years, some curcumin analogues 3,5-bis(arylidene)-4-piperidone derivatives were synthesized and evaluated for bioactivity. They incorporate two α,β-unsaturated ketos and β-aminoketones structures. These possess 1,5-diphenyl-penta-1,4-dien-3-ones pharmacophore, which could combine with sensitive parts of the tumor cells and induce apoptosis or autophagy [20]. The nitrogen-containing heterocyclic dienones, such as 4-piperidone, can display higher inhibitory properties toward human carcinoma cell lines compared with their homocyclic dienone analogs [21]. Another advantage is that these analogues have lower toxicity toward normal cells. In other words, these compounds can be easier to attack malignant cells rather than normal cells. 3,5-Bis(2-fluorobenzylidene)-4-piperidone (EF24, Fig. 1) with excellent anti-proliferative activity was first reported by Adams [22]. Another derivative 4-[3,5-bis(2-chlorobenzylidene-4-oxo-piperidine-1-yl)-4-oxo-2-butenoic acid] (CLEFMA, Fig. 1) with the better anti-proliferative activity in H441 cells than EF24 was reported by Awasthi [23]. Recently, our group has also reported some novel *N*-substituted-3,5-bis(arylidene)-4-piperidone derivatives as anticancer agents with fluorescent properties [24]. The results show an interesting relationship between cytotoxic activities and fluorescent properties. In addition, some bispyridyl-substituted piperidone derivatives [25, 26] and their pharmaceutical co-crystals with coformers were reported recently with interesting luminescent properties [27] and the better anticancer activities against human neoplastic cell lines and lower cytotoxicities, which showed their improved anticancer activities may be dependent on the synergistic effect between pharmaceutical ingredients and gallic acid [28]. However, the anticancer activities and the water solubility of these derivatives need to be further improved. The structure-property relationship between structures and anticancer activities also needs to continue refinement. In addition, Schiff-base compounds with active –C=N group can be seen as a good donor site or an active ligand, which exhibits a range of biological activities, including antifungal, antiradical, antimalarial, antiviral, anticancer, antibacterial, antiinflammatory, and antipyretic properties [29–32].

In this study, intermediate (2*E*,6*E*)-2,6-bis(3-aminobenzylidene)cyclohexanone (**3**) and (3*E*,5*E*)-3,5-bis(3-aminobenzylidene)-1-methylpiperidin-4-one (**4**) were synthesized via the Claisen-Schmidt condensation reaction and the reduction reaction of stannous chloride. Next, some aromatic aldehydes with phenol substitution groups were selected to synthesize two series of target compounds **1a–d** and **2a–e** (Fig. 2). Their anticancer activities against human HePG2, HeLa, K562, THP-1 cell lines, and their cytotoxicities for LO2 cell lines were evaluated by the MTT method.

Experimental section

Materials and methods

Cyclohexanone, *N*-methyl-4-piperidinone, *m*-nitrobenzaldehyde, tetrabutyl ammonium bromide (TBAB), stannous chloride, and all aromatic aldehydes were



R = CH_2
1a: R_1=H, R_2=H, R_3=H
1b: R_1=OH, R_2=H, R_3=H
1c: R_1=H, R_2=OH, R_3=H
1d: R_1=OH, R_2=OH, R_3=H

R = NCH_3
2a: R_1=H, R_2=H, R_3=H
2b: R_1=OH, R_2=H, R_3=H
2c: R_1=H, R_2=OH, R_3=H
2d: R_1=H, R_2=H, R_3=OH
2e: R_1=OH, R_2=OH, R_3=H

Fig. 2 Structures of double Schiff-base substituted 4-piperidone/cyclohexanone derivatives

purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China) and were used as obtained without further purification. ^{1}H NMR data were collected using a Bruker Avance 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. Infrared (IR) spectra were obtained in the 400–4000 cm^{-1} range using a Perkin-Elmer Frontier Mid-IR FTIR Spectrometer.

Preparation of 3 and 4

*(2E,6E)-2,6-bis(3-aminobenzylidene)cyclohexanone (**3**)*

Cyclohexanone (0.98 g, 0.01 mol) and *m*-nitrobenzaldehyde (3.32 g, 0.022 mol) was mixed with 40 mL glacial acetic acid, stirred on a magnetic stirrer at room temperature for about 30 min, during which time a clear solution was obtained. Dry hydrogen chloride gas was bubbled into this mixture for about 2 h, at this time a large amount of precipitation appeared. Then, the hydrogen chloride gas device was removed, and the mixture continued stirring at room temperature for 24 h (monitored by TLC). The precipitate was collected and dried under the vacuum to obtain crude intermediates (2*E*,6*E*)-2,6-bis(3-nitrobenzylidene)cyclohexanone, which were untreated and directly used in the following reaction. The crude intermediates dissolved in a solution of trichloromethane (10 mL), water (20 mL), and methanol (16 mL) with TBAB (0.32 g, 0.001 mol). In addition, stannous chloride (6.75 g, 0.03 mol) dissolved in concentrated hydrochloric acid (12 mL), was slowly added to the reaction system. The mixture was stirred at 40 °C for 8 h. After cooled to temperature, the mixture was adjusted to pH 12 by 40 % sodium hydroxide. The precipitate was collected and dried under vacuum to get yellow powder **3**. Yield: 57 %; mp: 70–72 °C. IR (cm^{-1}): 3408(m), 3299(m), 2985(m), 2872(m), 1647(m), 1592(s), 1549(w), 1486(w), 1456(w), 1381(m), 1315(m), 1262(s), 1162(s), 1135(w), 1062(w), 986(m), 881(m), 783(m). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.71 (s, 2H), 7.22 (t, J = 8.0 Hz, 2H), 6.89 (d, J = 8.0 Hz, 2H), 6.78 (s, 2H), 6.69 (d, J = 8.0 Hz, 2H), 3.73 (s, 4H),

2.92 (t, $J = 8.0$ Hz, 4H), 1.85–1.75 (m, 2H). Elemental analysis (%) calcd. for $C_{20}H_{20}N_2O$ (304.38): C 78.92, H 6.67, N 9.20; Found: C 78.87, H 6.70, N 9.23.

*(3E,5E)-3,5-bis(3-aminobenzylidene)-1-methylpiperidin-4-one (**4**)*

N-Methyl-4-piperidinone (1.13 g, 0.01 mol) and *m*-nitrobenzaldehyde (3.32 g, 0.022 mol) was mixed with 40 mL glacial acetic acid. Dry hydrogen chloride gas was bubbled into this mixture for about 2 h, at this time a large amount of precipitation appeared. Then, hydrogen chloride gas device was removed, and the mixture continued stirring at room temperature for 24 h (monitored by TLC). The precipitate was collected and dried under vacuum to obtain crude intermediates (3*E*,5*E*)-1-methyl-3,5-bis(3-nitrobenzylidene)piperidin-4-one, which were untreated and directly used in the following reaction. Then, the crude intermediates were dissolved in a solution of water (20 mL) and methanol (16 mL) with TBAB (0.32 g, 0.001 mol). Stannous chloride (6.75 g, 0.03 mol) was dissolved in concentrated hydrochloric acid (12 mL), was slowly added to the reaction system. The mixture was stirred at 40 °C for 8 h. After cooled to temperature, the precipitate was collected and washed with 5 % sodium hydroxide solution, and dried under vacuum to get yellow powder **4**. Yield: 52 %; mp: 101–103 °C. IR (cm^{-1}): 3355(w), 3347(w), 3222(w), 1670(m), 1600(s), 1583(s), 1452(w), 1316(w), 1259(s), 1171(s), 1095(w), 1051(w), 991(w), 915(w), 862(m), 780(s), 688(s). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (s, 2H), 7.31–7.17 (m, 2H), 6.81 (d, $J = 7.7$ Hz, 2H), 6.78–6.66 (m, 4H), 3.76 (d, 8H), 2.47 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 187.00, 146.45, 136.76, 136.27, 132.97, 129.38, 120.71, 116.68, 115.85, 57.07, 45.75. Elemental analysis (%) calcd. for $C_{20}H_{21}N_3O$ (319.40): C 75.21, H 6.63, N 13.16; Found: C 75.27, H 6.60, N 13.09.

Preparation of 1a–d and 2a–e

3 (0.30 g, 0.001 mol) or **4** (0.32 g, 0.001 mol) and aromatic aldehyde (0.002 mol) were dissolved in methanol (5 mL). A drop of formic acid was added into the mixture. The mixture was stirred for 3–8 h at ambient temperature (monitored by TLC). The precipitate was collected and recrystallized by methanol to afford **1a–d** and **2a–e**.

1a: Light yellow powder; Yield: 72 %; mp: 133–135 °C. IR (cm^{-1}): 1667(w), 1619(s), 1596(m), 1568(s), 1282(s), 1169(s), 1149(s), 1031(w), 982(w), 820(m), 804(s), 794(m), 747(s), 734(m), 695(m), 684(m), 535(m). ^{1}H NMR (400 MHz, $CDCl_3$): δ 13.13 (bs, 2H), 8.66 (s, 2H), 7.84 (s, 2H), 7.50–7.20(m, 12H), 7.07 (d, $J = 8.0$ Hz, 2H), 6.98 (t, $J = 8.0$ HZ, 2H), 2.99 (t, $J = 4.0$ Hz, 4H), 1.90–1.80 (m, 2H). ^{13}C NMR (100 MHz, DMSO): δ 188.91, 164.19, 160.24, 148.45, 136.95, 136.59, 135.52, 133.47, 132.65, 129.65, 128.57, 123.01, 121.96, 119.29, 119.20, 116.61, 27.85, 22.33. Elemental analysis (%) calcd. for $C_{34}H_{28}N_2O_3$ (512.59): C 79.67, H 5.51, N 5.46; Found: C 79.78, H 5.45, N 5.42.

1b: Light yellow powder; Yield: 72 %; mp: 182–184 °C. IR (cm^{-1}): 1670(m), 1625(w), 1610(w), 1576(m), 1461(m), 1356(m), 1272(m), 1210(s), 1170(s), 1144(s), 1031(w), 992(w), 886(w), 863(m), 784(s), 724(s), 684(s), 540(m),

504(m). ^{1}H NMR (400 MHz, DMSO): δ 13.65 (bs, 2H), 8.64 (s, 2H), 7.84 (s, 2H), 7.50 (t, J = 8.0 Hz, 2H), 7.45–7.35 (m, 4H), 7.30 (s, 2H), 7.10 (d, J = 8.0 Hz, 2H), 7.02 (d, J = 8.0 Hz, 2H), 6.88 (t, J = 8.0 Hz, 2H), 5.80 (bs, 2H), 2.99 (t, J = 4.0 Hz, 4H), 1.89–1.80 (m, 2H). ^{13}C NMR (100 MHz, DMSO): δ 188.93, 164.60, 149.27, 148.22, 145.61, 136.97, 136.61, 135.32, 129.67, 128.54, 122.93, 121.89, 121.19, 119.36, 119.09, 118.83, 27.86, 22.34. Elemental analysis (%) calcd. for $C_{34}H_{28}N_2O_5$ (544.59): C 74.98, H 5.18, N 5.14; Found: C 75.06, H 5.13, N 5.10.

1c: Light yellow powder; Yield: 74 %; mp: 55–57 °C. IR (cm^{-1}): 3196(br), 1667(m), 1616(s), 1597(s), 1574(s), 1488(w), 1356(m), 1212(s), 1169(s), 1119(m), 978(m), 842(m), 796(s), 689(s), 627(m), 538(m), 511(w). ^{1}H NMR (400 MHz, DMSO): δ 13.35 (bs, 2H), 10.37 (s, 2H), 8.85 (s, 2H), 7.67 (s, 2H), 7.55–7.35 (m, 10H), 6.44 (d, J = 8.0 Hz, 2H), 6.32 (s, 2H), 3.00–2.90 (m, 4H), 1.89–1.80 (m, 2H). ^{13}C NMR (100 MHz, DMSO): δ 190.79, 163.29, 163.00, 162.68, 148.39, 136.85, 136.54, 135.42, 134.60, 132.65, 129.60, 122.73, 112.00, 108.59, 107.98, 102.34, 27.86, 22.37. Elemental analysis (%) calcd. for $C_{34}H_{28}N_2O_5$ (544.59): C 74.98, H 5.18, N 5.14; Found: C 75.07, H 5.14, N 5.11.

1d: Light yellow powder; Yield: 76 %; mp: 210–212 °C. IR (cm^{-1}): 3055(br), 1668(m), 1617(s), 1577(m), 1513(w), 1280(m), 1211(w), 1149(s), 1078(m), 991(m), 884(w), 778(m), 680(s), 541(m), 482(w), 438(w). ^{1}H NMR (400 MHz, DMSO): δ 13.51 (s, 2H), 9.77 (s, 2H), 8.83 (s, 2H), 8.52 (s, 2H), 7.68 (s, 2H) 7.55–7.35 (m, 8H), 6.98 (d, J = 8.0 Hz, 2H), 6.45 (d, J = 8.0 Hz, 2H), 2.96 (t, J = 4.0 Hz, 4H), 1.80–1.70 (m, 2H). ^{13}C NMR (100 MHz, DMSO): δ 188.95, 163.80, 151.36, 150.48, 148.14, 136.89, 136.58, 135.41, 132.38, 129.62, 127.89, 124.24, 122.71, 121.61, 112.32, 108.36, 107.85, 27.87, 13.49. Elemental analysis (%) calcd. for $C_{34}H_{28}N_2O_7$ (576.59): C 70.82, H 4.89, N 4.86; Found: C 70.75, H 4.92, N 4.90.

2a: Light yellow powder; Yield: 69 %; mp: 114–116 °C. IR (cm^{-1}): 1675(w), 1615(m), 1563(m), 1278(m), 1182(w), 1152(w), 1102(w), 1058(w), 974(w), 922(w), 881(w), 800(s), 743(m), 691(m), 539(w), 424(w). ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.95 (s, 2H), 8.50–8.35 (m, 2H), 7.74–7.58 (m, 2H), 7.40–7.00 (m, 12H), 6.95–6.70 (m, 4H), 3.70–3.50 (m, 4H), 2.40–2.20 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 186.62, 163.47, 161.08, 148.94, 136.49, 135.80, 133.73, 133.48, 132.46, 129.63, 128.58, 122.99, 121.58, 119.22, 119.05, 117.29, 56.98, 45.89. Elemental analysis (%) calcd. for $C_{34}H_{29}N_3O_3$ (527.61): C 77.40, H 5.54, N 7.96; Found: C 77.49, H 5.51, N 7.92.

2b: Light yellow powder; Yield: 80 %; mp: 140–142 °C. IR (cm^{-1}): 3253(br), 1672(m), 1614(s), 1573(s), 1460(m), 1363(m), 1273(s), 1209(s), 1183(m), 1028(w), 939(w), 850(w), 789(m), 734(s), 684(s), 540(w). ^{1}H NMR (400 MHz, DMSO): δ 13.03 (s, 2H), 9.26 (s, 2H), 8.97 (s, 2H), 7.69 (s, 2H), 7.62–7.50 (m, 4H), 7.50–7.40 (m, 4H), 7.13 (d, J = 8.0 Hz, 2H), 6.98 (d, J = 8.0 Hz, 2H), 6.81 (t, J = 8.0 Hz, 2H), 3.82 (s, 4H), 2.42 (s, 3H). ^{13}C NMR (100 MHz, DMSO): δ 186.27, 164.75, 149.25, 148.42, 145.63, 135.85, 134.47, 134.04, 129.86, 128.46, 123.32, 122.85, 122.25, 119.38, 119.14, 118.87, 56.17, 45.15. Elemental analysis (%) calcd. for $C_{34}H_{29}N_3O_5$ (559.61): C 72.97, H 5.22, N 7.51; Found: C 72.91, H 5.25, N 7.53.

2c: Light yellow powder; Yield: 72 %; mp: 65–67 °C. IR (cm^{-1}): 3144(br), 1672(w), 1607(s), 1568(s), 1509(w), 1455(w), 1329(w), 1281(w), 1208(s), 1176(m), 1127(m), 977(m), 850(m), 788(s), 691(s), 651(w), 538(w). ^{1}H NMR (400 MHz,

DMSO): δ 13.38 (s, 2H), 10.50 (s, 2H), 8.84 (s, 2H), 7.66 (s, 2H), 7.53 (t, $J = 8$ Hz, 2H), 7.46 (d, $J = 8$ Hz, 4H), 7.38 (t, $J = 8$ Hz, 4H), 6.43 (d, $J = 8$ Hz, 2H), 6.31 (s, 2H), 3.78 (s, 4H), 2.40 (s, 3H). ^{13}C NMR (100 MHz, DMSO): δ 186.38, 163.44, 162.95, 162.66, 148.70, 135.83, 134.56, 134.46, 134.12, 129.78, 127.77, 123.03, 122.09, 112.06, 107.99, 102.36, 56.26, 45.23. Elemental analysis (%) calcd. for $C_{34}H_{29}N_3O_5$ (559.61): C 72.97, H 5.22, N 7.51; Found: C 72.92, H 5.23, N 7.56.

2d: Light yellow powder; Yield: 78 %; mp: 152–154 °C. IR (cm^{-1}): IR: 3173(br), 1674(w), 1615(s), 1568(s), 1485(s), 1433(w), 1373(w), 1349(w), 1292(s), 1217(s), 1148(s), 874(m), 824(m), 787(s), 727(w), 681(s), 642(w), 615(w), 530(m). ^{1}H NMR (400 MHz, DMSO): δ 12.10 (s, 2H), 8.89 (s, 2H), 7.68 (s, 2H), 7.55 (t, $J = 8.0$ Hz, 2H), 7.50 (s, 2H), 7.43 (t, $J = 7.6$ Hz, 4H), 7.07 (d, $J = 4.0$ Hz, 2H), 6.90 (dd, $J = 7.6$ Hz, 2H), 6.82 (d, $J = 7.8$ Hz, 2H), 3.79 (s, 4H), 2.41 (s, 3H). ^{13}C NMR (100 MHz, DMSO): δ 186.46, 163.90, 153.05, 149.67, 149.03, 135.85, 134.35, 134.23, 129.79, 128.30, 123.19, 122.35, 121.35, 119.30, 117.23, 116.85, 56.28, 45.25. Elemental analysis (%) calcd. for $C_{34}H_{29}N_3O_5$ (559.61): C 72.97, H 5.22, N 7.51; Found: C 72.90, H 5.25, N 7.54.

2e: Light yellow powder; Yield: 72 %; mp: 160–162 °C. IR (cm^{-1}): 3061(br), 1672(m), 1613(m), 1578(m), 1516(w), 1452(w), 1276(w), 1207(m), 1169(m), 1082(m), 1057(w), 979(m), 935(w), 875(w), 771(w), 681(w), 606(w), 592(w), 573(m), 564(m), 553(m), 538(w), 532(w). ^{1}H NMR (400 MHz, DMSO): δ 13.47 (s, 2H), 9.79 (s, 2H), 8.83 (s, 2H), 8.54 (s, 2H), 7.68 (s, 2H), 7.54 (t, $J = 8.0$ Hz, 2H), 7.48 (s, 2H), 7.45–7.35 (m, 4H), 6.98 (d, $J = 7.8$ Hz, 2H), 6.46 (d, $J = 7.8$ Hz, 2H), 3.82 (s, 4H), 2.43 (s, 3H). ^{13}C NMR (100 MHz, DMSO): δ 193.04, 163.97, 151.30, 150.54, 148.33, 135.81, 132.39, 129.83, 127.83, 124.26, 123.04, 121.98, 112.33, 107.89, 56.17, 45.14. Elemental analysis (%) calcd. for $C_{34}H_{29}N_3O_7$ (591.61): C 69.03, H 4.94, N 7.10; Found: C 69.10, H 4.90, N 7.05.

Anticancer testing with MTT method

Compounds **1a–d** and **2a–e** were screened against human neoplastic cell lines such as human cervical carcinoma cells (HeLa), human liver hepatocellular carcinoma cell line (HePG2), human chronic myelogenous leukemia cell line (K562), human acute mononuclear granulocyte leukemia (THP-1), and human normal heptical cell line (LO2) (Table 1) using modified the MTT assay (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide, MTT, Dojindo Laboratories, Tokyo, Japan). 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 was removed, 20 μL of MTT (5 mg/mL) was added, then the plates were incubated for 4 h at 37 °C in a 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, and the IC_{50} values are expressed in Table 1. The concentrations of the compounds were 10, 8, 5,

Table 1 Solubility, anticancer activity, and cytotoxicity of **1a–d**, **2a–e** and DOX

Compds.	Solubility (mg/mL)		IC_{50}^{a} (μM)				
	In water	In PBS	Hepg2	Hela	K562	THP-1	LO2
1a	0.14 ± 0.04	0.26 ± 0.09	3.25 ± 0.03	9.90 ± 0.31	3.38 ± 0.01	1.47 ± 0.06	8.16 ± 0.15
1b	0.78 ± 0.12	2.12 ± 0.31	4.41 ± 0.05	6.99 ± 0.09	3.2 ± 0.10	2.83 ± 0.08	6.94 ± 0.17
1c	0.14 ± 0.02	0.36 ± 0.09	>10	>10	2.58 ± 0.09	2.63 ± 0.13	9.39 ± 0.12
1d	0.04 ± 0.02	0.15 ± 0.08	6.66 ± 0.04	>10	2.40 ± 0.08	4.30 ± 0.09	8.80 ± 0.11
2a	0.16 ± 0.05	0.24 ± 0.03	2.59 ± 0.10	6.34 ± 0.15	2.76 ± 0.07	0.88 ± 0.09	4.56 ± 0.13
2b	0.27 ± 0.10	0.42 ± 0.16	1.32 ± 0.11	2.45 ± 0.09	>10	1.41 ± 0.07	4.81 ± 0.11
2c	0.42 ± 0.10	1.23 ± 0.18	2.54 ± 0.07	2.14 ± 0.04	3.44 ± 0.05	1.04 ± 0.05	4.72 ± 0.07
2d	0.72 ± 0.11	2.20 ± 0.36	1.04 ± 0.05	1.98 ± 0.06	2.36 ± 0.13	0.96 ± 0.03	3.02 ± 0.15
2e	0.60 ± 0.08	1.66 ± 0.21	0.92 ± 0.05	1.50 ± 0.05	1.26 ± 0.06	0.69 ± 0.01	2.32 ± 0.09
DOX	9.8 ± 0.65	13.3 ± 1.06	0.57 ± 0.07	2.02 ± 0.02	1.42 ± 0.04	0.3 ± 0.02	6.93 ± 0.32

[a] IC_{50} is the concentration of compounds required to inhibit the growth of the cells by 50 %

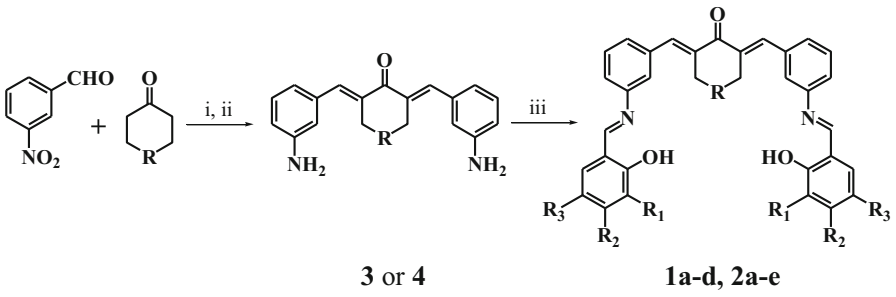
3, 2, 1, 0.5, 0.1, 0.05 and 0.01 μg/mL. Doxorubicin (DOX) was used as a positive control. The concentrations of DOX used were 1.5, 1.2, 1.0, 0.8, 0.5, 0.3, 0.1, 0.05, and 0.01 μg/mL.

Results and discussion

Structural analysis

As described in the experimental section, the intermediate (2*E*,6*E*)-2,6-bis(3-nitrobenzylidene)cyclohexanone and (3*E*,5*E*)-1-methyl-3,5-bis(3-nitrobenzylidene)piperidin-4-one were obtained via a Claisen-Schmidt condensation reaction from *m*-nitrobenzaldehyde with cyclohexanone or *N*-methyl-4-piperidinone. They were untreated and directly used to synthesize intermediate **3** and **4** by the reduction reaction of stannous chloride (Scheme 1). Then, some aromatic aldehydes with phenol substitution groups were introduced into a -NH_2 fragment of **3** or **4** to generate a serie of target compounds **1a–d** and **2a–e** (Fig. 2) through a Schiff-base condensation reaction (Scheme 1), from which target compounds can be obtained from precipitate in high yield. Their structures were confirmed by ^{1}H NMR, ^{13}C NMR, IR and elemental analysis.

According to the ^{1}H NMR spectra, the chemical shift 3.73 ppm in **3** and 3.76 ppm in **4** are attributed to the amino groups, respectively. After Schiff-base condensation reaction, two of them disappear, while single bands in the range of 8.50–8.97 ppm appear and are attributed to the protons of –CH=N– groups. In target compounds, the chemical shift of *ortho*-hydroxyl proton is in the range of 12.10–13.65 ppm. The single band of –C=CH– in the α,β-unsaturated ketone is about 8.97–8.50 ppm, which proves the existence of 1,5-diphenylpenta-1,4-dien-3-ones pharmacophore. In addition, the target compounds of **1a–d** have the chemical shift of methylene of that around ca. 2.96 and ca. 1.85 ppm, while compounds **2a–e** show the chemical shifts of *N*-methyl and methylene around δ ca. 3.78 and ca. 2.40 ppm. From the FTIR spectra, the characteristic band at around 1667–1675 cm^{-1} are attributed to –C=O group in cyclohexanone or piperidone.



Scheme 1 Synthesis of **1a–d** and **2a–e**. The reaction conditions as follows: *i* dry HCl, HAc, *ii* $SnCl_2$/HCl, CH_3OH/H_2O, *iii* aromatic aldehyde, formic acid, CH_3OH

The strong bands at around 1619–1607 cm^{-1} are attributed to –C=C bonds of α,β-unsaturated ketone and –C=N bonds of Schiff-base groups. Additionally, ^{13}C NMR and element analysis further confirm the correctness of their structures.

The solubility of the synthesized compounds **1a–d** and **2a–e** was evaluated by dispersing the samples in different solvents at room temperature. They can be soluble in many organic solvents, such as methanol, alcohol, chloroform, DMSO, and DMF. In water, their solubility is very small. Only **1b**, **2d**, and **2e** can reach 0.6–0.78 mg/mL (Table 1). Fortunately, all of them exhibit slightly improved solubility in a 20 mM PBS buffer system (pH 7.4), and **1b**, **2c**, **2d**, and **2e** can reach 2.12, 1.23, 2.20, and 1.66 mg/mL (Table 1), respectively. Compared with **1a**, the solubility of **1b** in the PBS buffer system is obviously improved because of the joining of the adjacent hydroxyl group in **1b**. Compared with **2a**, the solubilities of **2b–e** are also obviously improved in water and the PBS buffer system, which may be caused by additional phenol substitution groups of **2b–e**.

Anticancer activity and cytotoxicity

The anticancer activities against human carcinoma cell lines HePG2, HeLa, K562, THP-1, and their cytotoxicities toward LO2 cell lines were evaluated by the MTT method. Doxorubicin (DOX) was selected as positive control. The results of the biological activity evaluation can be found in Table 1. The results suggest that about 80 % of the IC_{50} values of the compounds against HeLa, HePG2, K562, and THP-1 are lower than 5 μM.

For series **1**, their IC_{50} values against Hepg2 and Hela cells are larger, which can exceed 5.0 μM, except **1a** and **1b** against Hepg2. But for K562, THP-1, the anticancer activity of series **1** is significantly better and their IC_{50} values are lower than 5.0 μM, especially for **1a**, its IC_{50} value is 1.47 μM. More importantly, cytotoxicity of series **1** is very small. Their IC_{50} values toward the LO2 cell line are in the range of 6.94–9.39 μM, which has cytotoxicity lower than that of DOX (6.93 μM), except **1b**.

For series **2**, 87.5 % of the compounds are more active than the analogs in series **1** against HeLa, HePG2, K562, and THP-1 (Table 1). For example, for HeLa, HePG2, and THP-1, all of the compounds in series **2** have the lower IC_{50} values than that of series **1** with the same aryl substitution pattern, respectively. Only for K562, the anticancer activities of **2b** and **2c** weaken compared with **1b** and **1c**, respectively. In addition, 85 % of the compounds in series **2** show lower IC_{50} values (<3 μM) against experimental human carcinoma cell lines. kWhat is more, target compounds **2a**, **2c–e** against THP-1 cells are the best compared with other experimental human carcinoma cell lines. Especially, the IC_{50} values of compounds **2a**, **2d**, and **2e** against THP-1 cells can reach 0.69–0.96 μM. Compounds **2b** and **2c** against THP-1 cells have small IC_{50} values (1.41 and 1.04 μM) compared with **1b** and **1c**. For Hepg2, compound **2e** displays the best anticancer activity with the IC_{50} value 0.92 μM, while **2b** and **2d** also have the lower IC_{50} value (1.32 μM for **2b** and 1.04 μM for **2d**). For Hela and K562, the IC_{50} values can be below 2.0 μM for **2d**, **2e** toward Hela and **2e** toward K562, respectively. According to these analyses, compounds **2d** and **2e** display the best anticancer activities against HeLa, HePG2,

K562, and THP-1. For the LO2 cell line, their cytotoxicity of series **2** is higher than that of DOX and series **1** with the same aryl substitution pattern, respectively. Their IC_{50} values are in the range of 2.32–4.81 μM. This shows that the compounds in series **2** have great cytotoxicity.

In general, series **2** have better anticancer activity than series **1** against experimental human carcinoma cell lines. However, the cytotoxicity of series **2** is slightly greater than that of series **1** and DOX. Structure analysis shows that series **1** are 2,6-bis(3-benzylidene)cyclohexanone derivatives, while series **2** are 3,5-bis(3-benzylidene)-1-methylpiperidin-4-one derivatives; their anticancer activity and cytotoxicity are very different in spite of the same phenol substitution groups. The results show the *N*-methyl-4-piperidinone group is more beneficial for anticancer activity than the cyclohexanone group [21]. In addition, the introduction of second or third hydroxy through Schiff-base condensation reaction is helpful for improving their anticancer activity for 4-piperidinone derivatives, especially **2d** and **2e**.

Conclusions

In this study, some aromatic aldehydes with phenol substitution groups were introduced into the -NH_2 fragment of **3** or **4** to generate two series of novel double Schiff-base substituted 4-piperidone/cyclohexanone derivatives (**1a–d**, **2a–e**). Their structures were characterized by ^{1}H NMR, ^{13}C NMR, IR, and elemental analysis. The anticancer activities against human carcinoma cell lines HePG2, HeLa, K562, THP-1, and their cytotoxicities for LO2 cell lines were subsequently evaluated by the MTT method. The results show that **2a–e** have better anticancer activity than **1a–d** against experimental cell lines. But the cytotoxicities of **2a–e** are slightly greater than that of **1a–d** and DOX. Structure analysis shows that *N*-methyl-4-piperidinone group and the introduction of hydroxy is helpful for improving their anticancer activity, especially **2d** and **2e**.

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