N-(Substituted benzyl)-3,5-bis(benzylidene)-4-piperidones: Synthesis and Preliminary Anti-leukemia Activity (I)

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A series of novel *N*-(substituted benzyl)-3,5-bis(benzylidene)-4-piperidones **5a**—**5o** were synthesized with substituted benzylamines as raw materials via a series of Michael addition, Dieckmann condensation, hydrolysis decarboxylation and aldol condensation. The structures were confirmed by ^{1}H NMR, IR, MS techniques and elemental analysis. Assay-based antiproliferative activity study using leukemic cell lines K562 revealed that most of the title compounds have high effectiveness in inhibiting leukemia K562 cells proliferation, among which the compounds **5g** (IC_{50}=7.81 μg•mL^{-1}), **5k** (IC_{50}=6.35 μg•mL^{-1}), **5l** (IC_{50}=7.20 μg•mL^{-1}), and **5o** (IC_{50}=5.79 μg•mL^{-1}) have better inhibition activities than standard 5-fluorouracil (IC_{50}=8.56 μg•mL^{-1}).

Keywords 4-piperidone, synthesis, inhibition, leukemia K562 cell, MTT

Introduction

Chronic myelogenous leukemia (CML) is regarded as a growing public health threat, killing people faster than other diseases. It is caused by clonal expansion of pluripotent hematopoietic stem cells retaining their differentiation potential.[1] Some studies have showed that CML cell line K562 is considerably more resistant to apoptosis than a number of other human hematopoietic cell lines.[2-3] Thus, the development of novel, more effective leukemia drugs for CML K562 is urgently needed.

During last decade different series of compounds have been designed based on the 1,5-diaryl-3-oxo-1,4-pentadienyl pharmacophore,[4-7] which lead to the discovery of a number of potent cytotoxic[8,9] and antitubercular[10] activities. The cytotoxic properties of 3,5-bis(benzylidene)-4-piperidones reported by Dimmock *et al.*,[6,7,11] revealed that molecular modification at the nitrogen atom, *e.g*. *N*-acylation, affects the capacity of transportation of this biologically active molecules via the cellular membrane and therefore resulted in the significantly higher level of anticancer activity. *N*-(4-Substituted benzyl)piperidone derivatives were reported to have certain anti-leukemia activitives in our previous researches.[12-14] In order to find some potent anti-leukemia activity lead compounds, we suggested combination of the 1,5-diaryl-3-oxo-1,4-pentadienyl pharmacophore and *N*-(4-substituted benzyl)piperidones to obtain a series of novel *N*-(4-substituted benzyl)-3,5-bis(benzylidene)-4-piperidones **5a**—**5o**. The structures of all new compounds have been confirmed by ^{1}H NMR, IR, MS techiniques and elemental analysis. The preliminary anti-leukemia activity tests indicate that most of the novel compounds have good effect on the antiproliferative activity against leukemia K562 cells. Compounds **5g** (IC_{50}=7.81 μg•mL^{-1}), **5k** (IC_{50}=6.35 μg•mL^{-1}), **5l** (IC_{50}=7.20 μg•mL^{-1}) and **5o** (IC_{50}=5.79 μg•mL^{-1}) have better inhibition activities than standard 5-fluorouracil (IC_{50}=8.56 μg•mL^{-1}).

Experimental

Materials and apparatus

All the chemical reagents purchased were of analytical grade and used without further purification, except for the toluene, which was dried by refluxing in the presence of sodium and distilled prior to use. ^{1}H NMR spectra were recorded on a Bruker Avance 400 (400 MHz) spectrometer, using $CDCl_3$ or DMSO-d_6 as solvents and tetramethylsilane (TMS) as internal standard. Melting points were determined by an RK1 microscopic melting apparatus (uncorrected). Elemental analysis was performed with a Perkin-Elmer 2400 instrument. MS spectra were recorded on a Trace DSQ mass spectrograph. IR spectra were obtained on a Nicolet 5DX FT-IR spectrophotometer in the region 4000—400 cm^{-1} using KBr discs.

General procedure for the synthesis of *N*-(4-substituted benzyl)-4-piperidone derivatives 4I—4IV

The intermediates of *N*-(4-methylbenzyl)-4-piperidone (**4I**), *N*-(4-methoxybenzyl)-4-piperidone (**4II**), *N*-

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Received October 25, 2010; revised May 4, 2011; accepted June 15, 2011.
Project supported by the Key Laboratory of Rare Earth Functional Materials of Shanghai (No. 07dz22303) and the Key Scientific and Technological Project of Shanghai Science and Technology Commission (No. 09391212100).

(4-bromobenzyl)-4-piperidone (**4III**) and *N*-(4-fluorobenzyl)-4-piperidone (**4IV**) were synthesized in our laboratory according to the literature.[14]

N-(4-Methylbenzyl)-4-piperidone (**4I**): Yellow oil, yield 74%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.32 (s, 3H), 2.46 (t, J=6.0 Hz, 4H), 2.74 (t, J=6.0 Hz, 4H), 3.59 (s, 2H), 7.16 (d, J=8.0 Hz, 2H), 7.27 (d, J=8.0 Hz, 2H); IR (KBr) v: 1718, 1514, 795 cm^{-1}; MS (EI) *m/z*: 203. Anal. calcd for $C_{13}H_{17}NO$: C 76.81, H 8.43, N 6.89; found C 76.68, H 8.38, N 6.93.

N-(4-Methoxybenzyl)-4-piperidone (**4II**): Yellow oil, yield 78%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.37 (t, J=6.0 Hz, 4H), 2.65 (t, J=6.0 Hz, 4H), 3.48 (s, 2H), 3.73 (s, 3H), 6.79 (d, J=8.4 Hz, 2H), 7.18 (d, J=8.4 Hz, 2H); IR (KBr) v: 1716, 1511, 834 cm^{-1}; MS (EI) *m/z*: 219. Anal. calcd for $C_{13}H_{17}NO_2$: C 71.21, H 7.81, N 6.39; found C 71.33, H 7.78, N 6.44.

N-(4-Bromobenzyl)-4-piperidone (**4III**): Yellow oil, yield 68%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.48 (t, J=6.2 Hz, 4H), 2.78 (t, J=6.2 Hz, 4H), 3.60 (s, 2H), 7.36 (d, J=8.1 Hz, 2H), 7.47 (d, J=8.0 Hz, 2H); IR (KBr) v: 1719, 1510, 791 cm^{-1}; MS (EI) *m/z*: 267. Anal. calcd for $C_{12}H_{14}BrNO$: C 53.71, H 5.23, N 5.29; found C 53.75, H 5.26, N 5.22.

N-(4-Fluorobenzyl)-4-piperidone (**4IV**): Yellow oil, yield 71%; ^{1}H NMR ($CDCl_3$) δ: 2.45 (t, J=6.1 Hz, 4H), 2.73 (t, J=6.1 Hz, 4H), 3.58 (s, 2H), 7.02 (t, J=8.7 Hz, 2H), 7.29—7.34 (m, 2H); IR (KBr) v: 1715, 1507, 760 cm^{-1}; MS (EI) *m/z*: 207. Anal. calcd for $C_{12}H_{14}FNO$: C 69.52, H 6.86, N 6.71; found C 69.55, H 6.81, N 6.76.

General procedure for the synthesis of *N*-(4-substituted benzyl)-3,5-bis(benzylidene)-4-piperidone derivatives 5a—5o

To a stirred solution of intermediates **4I**—**4IV** (5 mmol) and the appropriate aldehyde (10 mmol) in 15 mL of absolute ethanol, was added 1 mL of 10% NaOH.[15] The reaction mixture was maintained under room temperature for 0.5—2 h. After this, the separated solid was filtered off and recrystallized from ethanol to obtain title compounds **5a**—**5o** in 68.2%—92.6% yields.

N-(4-Methylbenzyl)-3,5-bis(benzylidene)-4-piperidone (**5a**): Yield 92%, yellow solid, m.p. 168—170 ℃; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.22 (s, 3H), 4.43 (s, 2H), 4.56 (s, 4H), 7.09 (d, J=8.4 Hz, 2H), 7.41 (d, J=7.8 Hz, 2H), 7.46—7.50 (m, 10H), 7.92 (s, 2H); IR (KBr) v: 3055, 2139, 1600, 1569, 1436, 1265, 1181, 990, 818, 763 cm^{-1}; MS (ESI) *m/z*: 379 ([M+H]$^+$). Anal. calcd for $C_{27}H_{25}NO$: C 85.45, H 6.64, N 3.69; found C 85.22, H 6.76, N 3.50.

N-(4-Methylbenzyl)-3,5-bis(4-methylbenzylidene)-4-piperidone (**5b**): Yield 77%, yellow solid, m.p. 170—171 ℃; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.28 (s, 3H), 2.36 (s, 6H), 3.66 (s, 2H), 3.83 (s, 4H), 7.04 (d, J=7.8 Hz, 2H), 7.13 (d, J=7.9 Hz, 2H), 7.17 (d, J=8.0 Hz, 4H), 7.25 (d, J=8.1 Hz, 4H), 7.76 (s, 2H); IR (KBr) v: 2916, 2745, 1670, 1613, 1578, 1558, 1264, 1180, 1072, 813 cm^{-1}; MS (ESI) *m/z*: 408 ([M+H]$^+$). Anal. calcd for $C_{29}H_{29}NO$: C 85.30, H 7.64, N 3.69; found C 85.47, H 7.17, N 3.44.

N-(4-Methylbenzyl)-3,5-bis(4-methoxybenzylidene)-4-piperidone (**5c**): Yield 79%, yellow solid, m.p. 172—174 ℃; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.28 (s, 3H), 3.66 (s, 2H), 3.84 (s, 4H), 3.91 (s, 6H), 7.04 (d, J=7.8 Hz, 2H), 7.13 (d, J=7.9 Hz, 2H), 7.25 (d, J=8.3 Hz, 4H), 7.42 (d, J=8.4 Hz, 4H), 7.80 (s, 2H); IR (KBr) v: 2934, 2835, 1664, 1600, 1510, 1303, 1255, 1171, 1033, 827 cm^{-1}; MS (ESI) *m/z*: 440 ([M+H]$^+$). Anal. calcd for $C_{29}H_{29}NO_3$: C 79.30, H 6.64, N 3.49; found C 79.24, H 6.65, N 3.19.

N-(4-Methoxybenzyl)-3,5-bis(benzylidene)-4-piperidone (**5d**): Yield 71%, yellow solid, m.p. 129—130 ℃; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 3.64 (s, 2H), 3.74 (s, 3H), 3.84 (s, 4H), 6.75 (d, J=8.7 Hz, 2H), 7.15 (d, J=8.6 Hz, 2H), 7.31—7.40 (m, 10H), 7.80 (s, 2H); IR (KBr) v: 2990, 2828, 1671, 1615, 1587, 1508, 1446, 1284, 939, 768 cm^{-1}; MS (ESI) *m/z*: 396 ([M+H]$^+$). Anal. calcd for $C_{27}H_{25}NO_2$: C 82.12, H 6.34, N 3.55; found C 82.00, H 6.37, N 3.54.

N-(4-Methoxybenzyl)-3,5-bis(4-methylbenzylidene)-4-piperidone (**5e**): Yield 89%, yellow solid, m.p. 145—146 ℃; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.36 (s, 6H), 3.68 (s, 2H), 3.78 (s, 3H), 3.90 (s, 4H), 6.90 (d, J=8.7 Hz, 2H), 7.08 (d, J=8.7 Hz, 2H), 7.18 (d, J=8.0 Hz, 4H), 7.25 (d, J=8.0 Hz, 4H), 7.84 (s, 2H); IR (KBr) v: 2996, 2833, 1672, 1615, 1511, 1248, 1180, 1036, 936, 814 cm^{-1}; MS (ESI) *m/z*: 424 ([M+H]$^+$). Anal. calcd for $C_{29}H_{29}NO_2$: C 82.20, H 6.95, N 3.30; found C 82.24, H 6.90, N 3.31.

N-(4-Methylbenzyl)-3,5-bis(4-methoxybenzylidene)-4-piperidone (**5f**): Yield 79%, yellow solid, m.p. 172—174 ℃; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.28 (s, 3H), 3.66 (s, 2H), 3.84 (s, 4H), 3.91 (s, 6H), 7.04 (d, J=7.8 Hz, 2H), 7.13 (d, J=7.9 Hz, 2H), 7.25 (d, J=8.3 Hz, 4H), 7.42 (d, J=8.4 Hz, 4H), 7.80 (s, 2H); IR (KBr) v: 2934, 2835, 1664, 1600, 1510, 1303, 1255, 1171, 1033, 827 cm^{-1}; MS (ESI) *m/z*: 456 ([M+H]$^+$). Anal. calcd for $C_{29}H_{29}NO_3$: C 79.30, H 6.64, N 3.49; found C 79.24, H 6.65, N 3.19.

N-(4-Methoxybenzyl)-3,5-bis(4-chlorobenzylidene)-4-piperidone (**5g**): Yield 90%, yellow solid, m.p. 206—208 ℃; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 3.64 (s, 2H), 3.74 (s, 3H), 3.94 (s, 4H), 6.80 (d, J=8.6 Hz, 2H), 7.10 (d, J=8.7 Hz, 2H), 7.24 (d, J=8.0 Hz, 4H), 7.36 (d, J=8.1 Hz, 4H), 7.78 (s, 2H); IR (KBr) v: 3028, 2980, 1610, 1516, 1491, 1260, 1183, 1094, 1004, 829 cm^{-1}; MS (ESI) *m/z*: 464 ([M + H]$^+$). Anal. calcd for $C_{27}H_{23}Cl_2NO_2$: C 69.80, H 4.98, N 3.03; found C 69.83, H 4.99, N 3.02.

N-(4-Fluorobenzyl)-3,5-bis(benzylidene)-4-piperidone (**5h**): Yield 68%, yellow solid, m.p. 146—148 ℃; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 3.66 (s, 2H), 3.85 (s, 4H), 7.05—7.12 (m, 2H), 7.18—7.21 (m, 2H), 7.33—7.41 (m, 10H), 7.83 (s, 2H); IR (KBr) v: 3128, 2993, 1669, 1615, 1508, 1268, 1222, 1193, 998, 765 cm^{-1};

MS (ESI) *m/z*: 384 ([M + H]$^+$). Anal. calcd for $C_{26}H_{22}FNO$: C 81.30, H 5.64, N 3.53; found C 81.44, H 5.78, N 3.65.

N-(4-Fluorobenzyl)-3,5-bis(4-methylbenzylidene)-4-piperidone (**5i**): Yield 90%, yellow solid, m.p. 165—167 ℃; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.36 (s, 6H), 3.68 (s, 2H), 3.90 (s, 4H), 7.08 (t, J=8.3 Hz, 2H), 7.20 (dd, J=8.7, 5.8 Hz, 2H), 7.22 (d, J=8.0 Hz, 4H), 7.38 (d, J=8.0 Hz, 4H), 7.84 (s, 2H); IR (KBr) *v*: 3031, 2796, 1602, 1507, 1269, 1219, 1181, 1071, 1006, 812 cm^{-1}; MS (ESI) *m/z*: 412 ([M + H]$^+$). Anal. calcd for $C_{28}H_{26}FNO$: C 81.70, H 6.40, N 3.42; found C 81.72, H 6.37, N 3.40.

N-(4-Fluorobenzyl)-3,5-bis(4-methoxybenzylidene)-4-piperidone (**5j**): Yield 92%, yellow solid, m.p. 163—164 ℃; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 3.62 (s, 2H), 3.79 (s, 4H), 3.91 (s, 6H), 7.04 (d, J=7.8 Hz, 2H), 7.12 (d, J=8.2 Hz, 4H), 7.26 (d, J=8.3 Hz, 4H), 7.35 (dd, J=8.0, 5.1 Hz, 2H), 7.80 (s, 2H); IR (KBr) *v*: 2833, 1663, 1597, 1561, 1513, 1258, 1173, 1032, 1011, 828 cm^{-1}; MS (ESI) *m/z*: 444 ([M+H]$^+$). Anal. calcd for $C_{28}H_{26}FNO_3$: C 75.85, H 5.97, N 3.20; found C 75.83, H 5.91, N 3.16.

N-(4-Fluorobenzyl)-3,5-bis(4-chlorobenzylidene)-4-piperidone (5**k**): Yield 88%, yellow solid, m.p. 167—169 ℃; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 3.66 (s, 2H), 3.79 (s, 4H), 7.08—7.15 (m, 2H), 7.25 (d, J=8.3 Hz, 4H), 7.28—7.32 (m, 2H), 7.38 (d, J=8.5 Hz, 4H), 7.74 (s, 2H); IR (KBr) *v*: 2792, 1607, 1508, 1491, 1268, 1222, 1198, 1006, 926, 817 cm^{-1}; MS (ESI) *m/z*: 452 ([M+H]$^+$). Anal. calcd for $C_{26}H_{20}Cl_2FNO$: C 69.05, H 4.47, N 3.10; found C 69.04, H 4.46, N 3.10.

N-(4-Bromobenzyl)-3,5-bis(benzylidene)-4-piperidone (**5l**): Yield 79%, yellow solid, m.p. 132—134 ℃; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 3.68 (s, 2H), 3.85 (s, 4H), 6.87—6.92 (m, 2H), 7.18—7.21 (m, 2H), 7.33—7.41 (m, 10H), 7.88 (s, 2H); IR (KBr) *v*: 3056, 2750, 1665, 1604, 1569, 1267, 1192, 1183, 764, 687 cm^{-1}; MS (ESI) *m/z*: 444 ([M + H]$^+$). Anal. calcd for $C_{26}H_{22}BrNO$: C 70.25, H 4.97, N 3.10; found C 70.28, H 4.99, N 3.15.

N-(4-Bromobenzyl)-3,5-bis(4-methylbenzylidene)-4-piperidone (**5m**): Yield 81%, yellow solid, m.p. 186—187 ℃; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.36 (s, 6H), 3.36 (s, 2H), 3.83 (s, 4H), 7.02 (d, J=7.8 Hz, 2H), 7.10—7.25 (m, 8H), 7.36 (d, J=8.0 Hz, 2H), 7.80 (s, 2H); IR (KBr) *v*: 2817, 2748, 1669, 1609, 1577, 1508, 1264, 1179, 999, 814 cm^{-1}; MS (ESI) *m/z*: 472 ([M+H]$^+$). Anal. calcd for $C_{28}H_{26}BrNO$: C 71.20, H 5.53, N 2.93; found C 71.19, H 5.55, N 2.96.

N-(4-Bromobenzyl)-3,5-bis(4-methoxybenzylidene)-4-piperidone (**5n**): Yield 89%, yellow solid, m.p. 167—170 ℃; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 3.62 (s, 2H), 3.79 (s, 4H), 3.91 (s, 6H), 7.04 (d, J=7.8 Hz, 2H), 7.12 (d, J=8.2 Hz, 4H), 7.26 (d, J=8.3 Hz, 4H), 7.35 (d, J=8.0 Hz, 2H), 7.80 (s, 2H); IR (KBr) *v*: 2833, 1663, 1597, 1561, 513, 258, 1173, 1032, 1011, 828 cm^{-1}; MS (ESI) *m/z*: 504 ([M + H]$^+$). Anal. calcd for $C_{26}H_{20}BrCl_2NO$: C 66.70, H 5.23, N 2.73; found C 66.67, H 5.20, N 2.78.

N-(4-Bromobenzyl)-3,5-bis(4-chlorobenzylidene)-4-piperidone (**5o**): Yield 77%, yellow solid, m.p. 186—188 ℃; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 3.65 (s, 2H), 3.79 (s, 4H), 7.12 (d, J=8.4 Hz, 2H),7.23—7.24 (m, 2H), 7.26—7.37 (m, 8H), 7.74 (s, 2H); IR (KBr) *v*: 2822, 1605, 1489, 1456, 1264, 1264, 1180, 1095, 1003, 817 cm^{-1}; MS (ESI) *m/z*: 512 ([M+H]$^+$). Anal. calcd for $C_{26}H_{20}BrCl_2NO$: C 60.80, H 3.93, N 2.83; found C 60.84, H 3.93, N 2.73.

Inhibiting leukemia K562 cells proliferation test

The growth inhibition activities of the title compounds and 5-fluorouracil (5-FU) against K562 cells were determined by the MTT assay according to the standard bioactivity test procedures of the Zooblast-molecular Biology Laboratory of Shanghai Normal University of China.[16] Specific methods were the same as the previous research.[14] Equation of growth inhibition of cells was shown as follows.

$$\text{Inhibition} = [1 - A(\text{experiment})/A(\text{control})] \times 100\%$$

Results and discussion

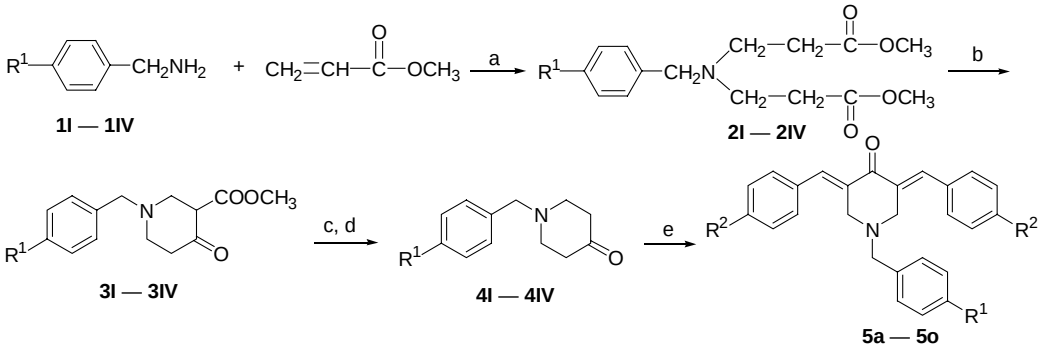
Synthesis of *N*-(substituted benzyl)-3,5-bis(benzylidene)-4-piperidones 5a—5o

A five-step synthetic strategy was adopted for the synthesis of *N*-(substituted benzyl)-3,5-bis(ben-zylidene)-4-piperidones **5a**—**5o**. The general schematic representation describing the routes of syntheses is furnished in Scheme 1. *N*,*N*-Bis[2-(methoxy carbonyl)ethyl] substituted amines **2I**—**2IV** were obtained by the Michael addition of substituted amine and methyl acrylate, in 1∶4 molar ratio. The *N*,*N*-bis[2-(methoxy carbonyl)ethyl]substituted amines **2I** — **2IV** afforded *N*-substituted-4-piperidones **4I**—**4IV** upon reflux with sodium methoxide in the presence of absolute toluene and the fllowed mixture was extracted with 25% HCl, then directly reacted. For the formation of the *N*-(substituted benzyl)-3,5-bis(benzylidene)-4-piperidones **5a**—**5o**, according to the literature,[15] *N*-substituted-4-piperidones **4I**—**4IV** were reacted with appropriate aldehyde, using 0.1 g•mL^{-1} NaOH as catalyst, anhydrous ethyl alcohol as solvent, stirred under room temperature to obtain the title compounds.

Inhibitory effects of compounds 5a—5o on the proliferation of leukemia K562 cells

The growth inhibition activities of the title compounds and 5-fluorouracil (5-FU) against leukemia K562 cells were determined by the MTT assay according to the standard bioactivity test procedures. As indicated in Figure 1, most of our designed compounds exhibited significant inhibition activities against leukemia K562 cells proliferation and had >80% inhibition at

Scheme 1



1I—**4I**: R^1 = Me, **1II**—**4II**: R^1 = OMe, **1III**—**4III**: R^1 = Br, **1IV**—**4IV**: R^1 = F
5a: R^1 = Me, R^2 = H; **5b**: R^1 = Me, R^2 = Me; **5c**: R^1 = Me, R^2 = OMe
5d: R^1 = OMe, R^2 = H; **5e**: R^1 = OMe, R^2 = Me; **5f**: R^1 = OMe, R^2 = OMe
5g: R^1 = OMe, R^2 = Cl; **5h**: R^1 = F, R^2 = H; **5i**: R^1 = F, R^2 = Me
5j: R^1 = F, R^2 = OMe; **5k**: R^1 = F, R^2 = Cl; **5l**: R^1 = Br, R^2 = H
5m: R^1 = Br, R^2 = Me; **5n**: R^1 = Br, R^2 = OMe; **5o**: R^1 = Br, R^2 = Cl

Reagent and conditions: (a) absolute methanol, refluxing; (b) sodium/absolute methanol, toluene, refluxing; (c) 25% HCl, refluxing;(d) 35% sodium hydroxide, r.t.; (e) the appropriate aldehyde, 10% sodium hydroxide, absolute ethanol, r.t.

100 μg/mL, which is higher than that of the anti-leukemia drug of clinical practice 5-FU at the same concentration. As described in Table 1, the title compounds' IC_{50} values are largely influenced by the different substituents on the 1,5-diaryl-3-oxo-1,4-pentadienyl pharmacophore. Electron withdrawing groups on aryl showed excellent inhibition percentage than electron donor groups. Taking **5d**—**5g** for example, when R^1=OCH_3, the inhibition activities of the corresponding analogues decreased in the order R^2=Br (**5g**) >R^2=H (**5d**)>R^2=CH_3 (**5e**)>R^2=OCH_3 (**5f**). On the other hand, the differences of the *N*-(4-substituted benzyl) of piperidine rings also influence the compounds' inhibition percentage, the introduction of 4-bromobenzyl at the *N*-position of piperidine rings showed better inhibitory activities than other substituents. On the basis of above discussions, **5o** is deserved as the most potent compound in this series, having an IC_{50} value of 5.79 μg/mL in suppressing leukemia K562 cells.

Table 1 Growth inhibitory properties IC_{50} values for the compounds **5a**—**5o** and 5-FU at 48 h

Compd.	R^1	R^2	IC_{50}/(μg•mL^{-1})
5a	Me	H	9.17
5b	Me	Me	21.13
5c	Me	OMe	22.52
5d	OMe	H	9.28
5e	OMe	Me	24.41
5f	OMe	OMe	51.58
5g	OMe	Cl	7.81
5h	F	H	8.66
5i	F	Me	32.17
5j	F	OMe	30.20
5k	F	Cl	6.35
5l	Br	H	7.20
5m	Br	Me	17.18
5n	Br	OMe	21.75
5o	Br	Cl	5.79
5-Fu			8.56

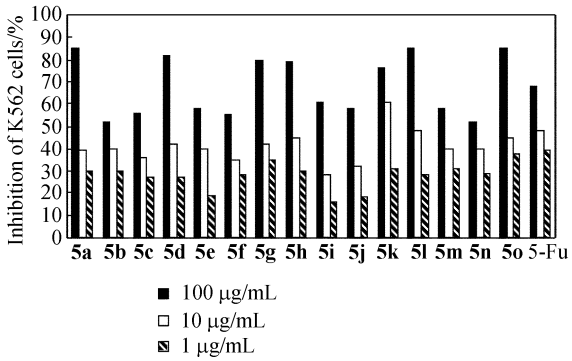


Figure 1 Inhibition of K562 cells proliferation treated with compounds **5a**—**5o**. Cells were seeded in 96-well plates at the density of 6250/cm^2. Cells were treated with the compounds at concentrations of 1, 10, and 100 μg/mL for 48 h. The cell viability was determined by MTT assay.

Conclusions

In summary, we have described a facile approach to prepare *N*-(substituted benzyl)-3,5-bis(benzylidene)-4-piperidones **5a**—**5o**. These compounds were characterized by IR, ^{1}H NMR, MS techniques and elemental analysis. Most of the title compounds have high effectiveness in inhibiting leukemia K562 cells proliferation, among which compounds **5g** (IC_{50}=7.81 μg•mL^{-1}), **5k** (IC_{50}=6.35 μg•mL^{-1}), **5l** (IC_{50}=7.20 μg•mL^{-1}), and **5o** (IC_{50}=5.79 μg•mL^{-1}) have better inhibition activities than standard 5-fluorouracil (IC_{50} = 8.56 μg•mL^{-1}). Further research work will be needed to find piperidine derivatives with more potential anti-leukemia bioactivities.

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(E1010251 Zhao, C.)

