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Synthesis and potent antileukemic activities of 10-benzyl-9(10H)-acridinones

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ABSTRACT

A novel series of 10-benzyl-9(10H)-acridinones and 1-benzyl-4-piperidones were synthesized and tested for their in vitro antitumor activities against CCRF-CEM cells. Assay-based antiproliferative activity study using CCRF-CEM cell lines revealed that the acridone group and the substitution pattern on the benzene unit had significant effect on cytotoxicity of this series of compounds, among which 10-(3,5-dimethoxy)benzyl-9(10H)-acridinone (**3b**) was found to be the most active compound with IC_{50} at about 0.7 μM. Compound **3b** was also found to have antiproliferative activity against two other human leukemic cell lines K562 and HL60 using the MTT assay. The antitumor effect of **3b** is believed to be due to the induction of apoptosis, which is further confirmed by PI (Propidium iodide) staining and Annexin V-FITC/PI staining assay using flow cytometry analysis.

1. Introduction

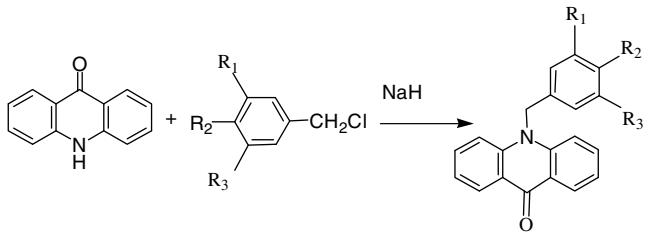
Cancer is now believed to result from unlimited growth of a given cell.[1,2] Cells die in two ways: necrosis and apoptosis. The regulation of apoptosis is crucial for development and sustained health.[3–5] The dysregulation of apoptosis will result in a variety of clinical disorders including cancer. Most chemotherapeutic agents used in cancer therapy kill particular types of tumor cells through apoptosis. Because cells killed by apoptosis do not damage the organism, anticancer drugs, which could kill tumour cells through apoptosis, have been widely studied in medicinal Chemistry.[6–9]

Acridones are naturally occurring alkaloids, which can be considered as aza-analogs of anthrones[10–12] or xanthones.[13] Acridone derivatives show various interesting biological properties,[14–26] which have been studied as antiviral, antiallergy drugs, antitumour agents, etc. Now, many acridone derivatives with potent antitumor activity in vitro/vivo against a range of murine and human tumors have been developed.[19,20,24,25] In our search for potent and selective antitumor agents, a novel series of 10-benzyl-9(10H)-acridinone and 1-benzyl-4-piperidone with substituent(s) at the benzene ring were synthesized for structure-activity relationship (SAR) study. The 10-(3,5-dimethoxy)benzyl-9(10H)-acridinone (**3b**) was found to be the most active compound against human T cell lymphoblast-like cell (CCRF-CEM) growth. We also proved that compound **3b** treatment increases apoptosis in CCRF-CEM cells. In this paper, we describe the synthesis, SAR, and antileukemia effects of 10-benzyl-9(10H)-acridinone and its derivatives in vitro.

2. Results and discussion

2.1. Chemistry

Scheme 1 shows the preparation of the 10-benzyl-9(10H)-acridinone and its derivatives. Starting from the commercially available material acridone **1** and NaH, the *N*-benzylated derivatives of acridone were obtained in high yields by reaction of acridone with the appropriate benzyl chlorides in the presence of catalytic amounts of potassium iodide in *N*,*N*-dimethylformamide. Most benzyl chlorides used in this study were commercially available, while **2c** and **2f** were obtained according to the literature,[27,28]



1 **2a-l** **3a-l**

Scheme 1. Preparation of the 10-benzyl-9(10H)-acridinones derivatives **3a–l**. Reagents and conditions: NaH, DMF, KI, rt, overnight.

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and **2g–2i** were prepared from benzyl alcohols synthesized by the reaction of 3,5-dihydroxybenzylalcohol with the corresponding alkyl bromides.

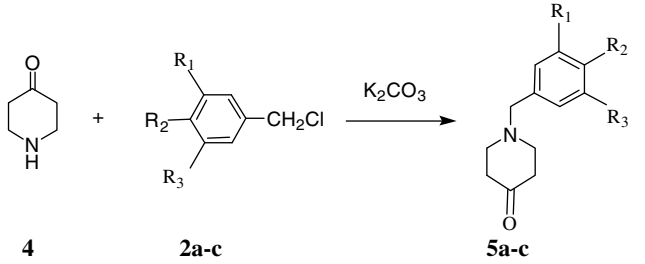
The synthesis of 1-benzyl-4-piperidone and its derivatives **5a–c** were accomplished via the routes illustrated in Scheme 2. The N-alkylation was developed by reaction of the hydrochloric salt of 4-piperidone **4** with substituted benzyl chlorides **2a–c** in dichloromethane in the presence of triethylamine under reflux conditions overnight.[29,30] after workup, products **5a–c** were obtained in moderate yields.

The structures of all compounds were confirmed by NMR and mass spectral data. All the structure features and synthetic yields are listed in Tables 1 and 2.

2.2. In vitro cell growth inhibition assay

The compounds 9(10H)-acridinone **1** and 10-benzyl-9(10H)-acridinone derivatives **3a–l** were initially evaluated for antiproliferative activity against CCRF-CEM leukemia cells. Cell proliferation was determined using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay after 48 h of treatment. As shown in Table 1, the compound **1** showed no cytotoxicity (IC_{50} > 100 μM), while some of the compounds **3** showed minor to good inhibitory effects on CCRF-CEM cell growth. The observed antiproliferative activities were obviously dependent on the substitution pattern of the benzene part. In this series, compound **3a** with no substituents on the benzene ring exhibited moderate inhibitory effects (IC_{50} = 52 μM). Compounds, which only have one methoxy group on either C3 or C4 position (**3d** and **3e**), showed no activity, while those compounds with two or three methoxy groups, such as **3b** and **3c**, showed much stronger inhibition effect. So the amount and position of substituents –OMe did greatly affect the antiproliferative potency. By changing the alkyl chain length of the dialkoxyl substituents on C3 and C5 positions from methyl to butyl, compounds showed decreasing inhibitory effect. Specifically, **3g** which has diethoxy groups was determined to be 9-fold less potent than **3b**, **3h** with dipropoxy substituents was determined to be 56-fold less potent than **3b**, and **3i** with dibutoxy groups was essentially inactive. This comparison clearly demonstrates that the cytotoxicity of 10-(3,5-dialkyloxy)benzyl-9(10H)-acridinone decreases with increasing length and size of the alkyl group.

In contrast to compound **3b**, with the introduction of –OBn to the C3 and C5 positions, **3f** did not display any antiproliferative activity against CCRF-CEM cells. Compounds **3d** with electron-donating methoxy group at 3-position on the benzene ring had no cytotoxicity against CCRF-CEM cells. When the weaker electron-donating group methyl was introduced to the benzene ring, compound **3j** produced high activity (IC_{50}, 8.8 μM). Introduction of electron-withdrawing group at the benzene ring in the case of **3k** (3-fluoro) and **3l** (3-trifluoromethyl) resulted in reduced antiproliferative activity compared with **3j**. Compound **3l** was about 4-fold less potent than **3j**, while **3k** was about 2-fold less potent than **3l**. The results indicated that electron-negativity and steric effect in the benzene ring may change the cytotoxic profile.



4 2a-c 5a-c

Scheme 2. Preparation of the 1-benzyl-4-piperidone derivatives **5a–c**. Reagents and conditions: K_2CO_3, CH_2Cl_2, $N(CH_2CH_3)_3$, reflux, overnight.

Table 1
Chemical data and antiproliferative activity against CCRF-CEM cells of compounds **1** and **3a–l**

	R_1	R_2	R_3	Yield (%)	IC_{50}[a] (μM)
1	—	—	—	—	>100
3a	H	H	H	90	52
3b	OCH_3	H	OCH_3	91	0.7
3c	OCH_3	OCH_3	OCH_3	89	1.5
3d	OCH_3	H	H	91	>100
3e	H	OCH_3	H	90	>100
3f	OBn	H	OBn	87	>100
3g	OCH_2 CH_3	H	OCH_2 CH_3	88	6.7
3h	$O(CH_2)_2CH_3$	H	$O(CH_2)_2CH_3$	85	39.1
3i	$O(CH_2)_3$ CH_3	H	$O(CH_2)_3$ CH_3	95	>100
3j	CH_3	H	H	90	8.8
3k	F	H	H	86	62
3l	CF_3	H	H	85	36

[a] IC_{50} values were determined from MTT proliferation assays after incubation with test compound for 48 h.

Table 2
Chemical data and antiproliferative activity against CCRF-CEM cells of compound **5a–c**

	R_1	R_2	R_3	Yield (%)	IC_{50}[a] (μM)
5a	H	H	H	60	>100
5b	OCH_3	H	OCH_3	65	>100
5c	OCH_3	OCH_3	OCH_3	62	>100

[a] IC_{50} values were determined from MTT proliferation assays after incubation with test compound for 48 h.

Among the twelve compounds, compound **3b** with dimethoxy groups at C3 and C5 positions on the benzene ring had the lowest IC_{50} at about 0.7 μM, which displayed the most potent inhibition activity.

Not only the substitution pattern of the benzene part but also the acridone group played significant influence on the antitumor activity of these classes of compounds. In order to study the role of the acridone group on the 10-benzyl-9(10H)-acridinones, compounds **5a-c** bearing a 4-piperidone group instead of the acridone group were also prepared (Scheme 2). The inhibition of CCRF-CEM leukemia cells growth was also studied (Table 2). Unfortunately, all these three compounds were essentially inactive, indicating that the acridone group is a necessary structure unit in affecting the cytotoxicity of the 10-benzyl-9(10H)-acridinone derivatives. In spite of these factors, the acridone itself was inactive toward CCRF-CEM leukemia cells. These findings, combined with the results above, demonstrated that both the substituted benzyl group and the acridone group affected the cytotoxicity of 10-benzyl-9(10H)-acridinones.

Two other leukemia cells K562 (human chronic myelogenous leukemia cell) and HL60 (human promyelocytic leukemia cell) cells were chosen to further investigate the antiproliferative potential effect of the highly active compounds 10-benzyl-9(10H)-acridinone derivatives **3b** by cellular metabolic activity using the MTT assay. Compound **3b** displayed good cytotoxocities in K562 and HL60 cells with IC_{50} values 11.4 μM and 0.6 μM respectively, suggesting that **3b** has potential antileukemia effect.

2.3. Compound 3b induces apoptosis in CCRF-CEM cells

On the basis of the antiproliferative effect study, compound **3b** was selected for further examinations to determine whether the cytotoxicity is mediated by the induction of apoptosis. Apoptosis

is characterized by morphological and biochemical changes in the nucleus, including chromatin condensation and internucleosomal DNA fragmentation. Flow cytometry is a useful tool to study the cell apoptosis. One of the simple methods is to observe the sub-diploid peak due to the loss of DNA small fragments by propidium iodide (PI) staining. As shown in Figure 1, the sub-Go cell population was observed clearly in a dose-dependent manner after the treatment with **3b** for 24 h. Specifically, cells at sub-Go increased as the concentration of **3b** increased from 0.5 μM to 2.5 μM (see Fig. 1).

In addition, the Annexin-V/PI binding assay was used to further confirm compound **3b** induced apoptosis effect. Annexin-V conjugated with the fluorochrome FITC serves as a marker for apoptotic cells because it has a strong binding affinity to phosphatidylserine (PS), which re-distributes from the inner to the outer layer of the plasma membrane in apoptotic cells.[31–33] As shown in Figure 2, the dual parametric dot plots show four quadrants and among them the lower left quadrant represents the viable cell population (annexin-V negative and PI negative), the upper right represents apoptotic cells undergoing secondary necrosis at the last stage or dead cells (annexin-V and PI double positive), and the lower right represents the early stage apoptotic cell population (annexin-V positive and PI negative). As the concentration of **3b** increased from 1 μM to 5 μM, the annexin-V positive/PI negative cells increased from 9.5% to 27.4%, and then decreased back to 21.7%, whereas the double positive cells increased from 10.0% to 41.6%, suggesting that more and more apoptotic cells progressed from the early stage to the late stage resulting in either death or secondary necrosis under the effect of **3b** at higher concentrations. This confirms again that **3b** induced the cell apoptosis of CCRF-CEM cells.

3. Conclusion

In conclusion, we have designed and synthesized a series of 10-benzyl-9(10H)-acridinones and 1-benzyl-4-piperidones. The preliminary in vitro antileukemia activity test showed that **3b** had significant growth inhibitory effect against CCRF-CEM, K562 and HL60 cells. Structure–activity relationship studies revealed that both the 3,5-dimethoxy substitution and acridone pattern in the 10-benzyl-9(10H)-acridinones played an important role in cellular growth inhibition. Such activity was proved to be associated with the induction of apoptosis by the flow cytometry analysis using PI staining and annexin-V/PI assays. Our results suggest that **3b** may be an attractive lead compound for further development as a chemotherapeutic agent for leukemia therapy. Further investigations of the mechanism of apoptosis induction are under way.

4. Experimental

4.1. Chemistry

General methods: Commercial chemicals were used without further purification unless otherwise indicated. Melting points are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were obtained in $CDCl_3$ at 300 MHz or 500 MHz for ^{1}H NMR and 75 MHz or 125 MHz for ^{13}C NMR with tetramethylsilane as the internal standard, respectively, unless otherwise indicated. Chemical shifts are expressed in ppm relative to TMS (^{1}H, 0.00 ppm) or residual $CDCl_3$ (^{13}C, 77.0 ppm). Splitting patterns are indicated as s, singlet; d, doublet; t, triplet; q, quartet signal; m, multiplet. ESI-MS were obtained with Waters Quattro Premier XE Spectrometer. HRMS were recorded on a QSTAR XL spectrometer and Waters Q-Tof Premier spectrometer. Column chromatography was performed on silica gel under pressure.

4.1.1. General procedure for preparation of 3,5-dialkyloxybenzyl alcohol

3, 5-Dihydroxybenzyl alcohol (700 mg, 5.0 mmol), dried potassium carbonate (1.73 g, 12.5 mmol), 18-crown-6 (264 mg, 1.0 mmol), and the appropriate alkyl bromide (10.0 mmol) in dry

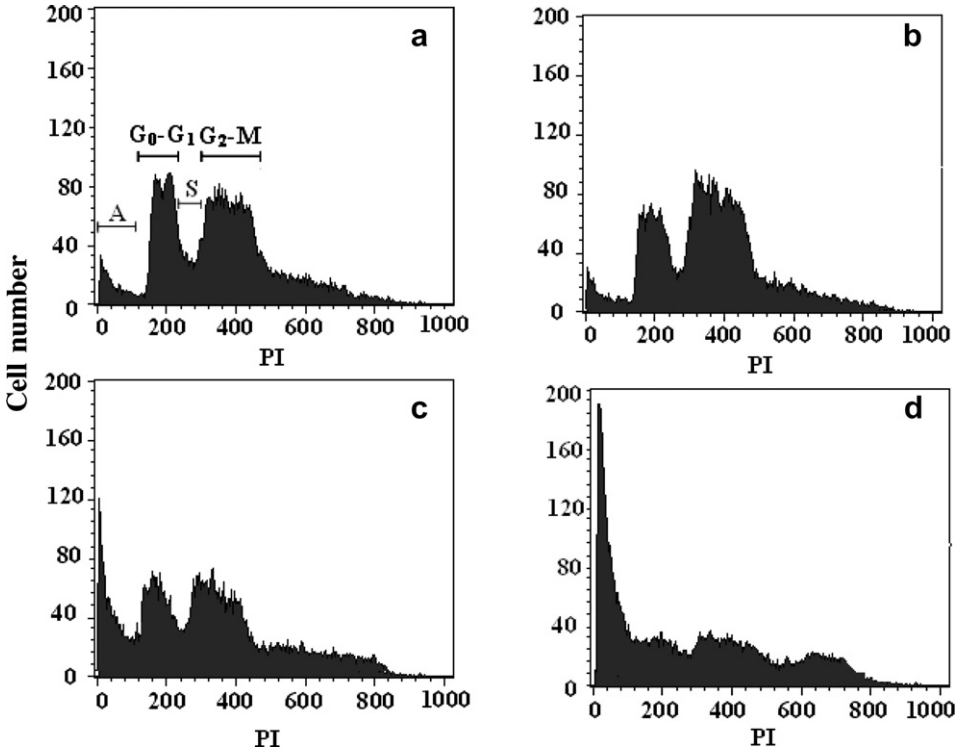


Figure 1. Flow cytometry analysis of cell membrane integrity (PI staining). CCRF-CEM cells were treated with **3b** at different concentrations for 24 h. (a) 0.0 μM, Control; (b) 0.5 μM; (c) 1.0 μM; (d) 2.5 μM. Peak A is the sub-diploid peak.

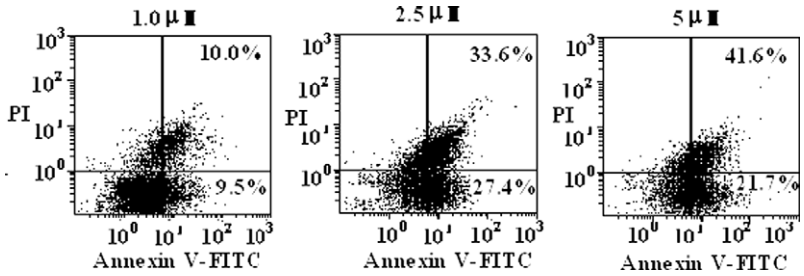


Figure 2. Flow cytometric analysis of phosphatidylserine externalization (annexin V-binding) and cell membrane integrity (PI staining). CCRF-CEM cells were treated with **3b** at 1.0 μM, 2.5 μM and 5 μM for 48 h.

acetone were heated at reflux and stirred vigorously under nitrogen for 48 h. The volatile parts were removed under reduced pressure and the residue was treated with 20 mL CH_2Cl_2 and 20 mL H_2O. The separated water was extracted three times with 20 mL CH_2Cl_2. The combined organic phases were dried with Na_2SO_4 and the products were purified by column chromatography.

4.1.1.1. 3,5-Diethoxybenzyl alcohol.[34] Colorless oil (837 mg), yield 85%, ESI-MS: $[M+H]^+$ 197.

4.1.1.2. 3,5-Dipropoxybenzyl alcohol.[35] Colorless oil (997 mg), yield 89%, ESI-MS: $[M+H]^+$ 225.

4.1.1.3. 3,5-Dibutoxybenzyl alcohol.[36] Colorless oil (1.0 g), yield 80%, ESI-MS: $[M+H]^+$ 253.

4.1.2. General procedure for preparation of 3,5-dialkyloxybenzyl chloride

To a mixture of the appropriate benzyl alcohol (3 mmol) in carbon tetrachloride (10 mL) was added triphenylphosphine (4.0 mmol), and the reaction mixture was heated at reflux under nitrogen overnight. The volatile parts were removed under reduced pressure and the residue was purified by column chromatography.

4.1.2.1. 3,5-Diethoxybenzyl chloride (2g). Colorless crystals (546 mg), yield 85%, mp 48–49 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.40 (t, 6H, ^3J = 7.0 Hz), 4.01 (q, 4H, ^3J = 7.0 Hz), 4.50 (s, 2H), 6.40 (m, 1H), 6.51 (m, 2H).

4.1.2.2. 3,5-Diproxybenzyl chloride (2h). Colorless oil (640 mg), yield 88%; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.03 (t, 6H, ^3J = 7.4 Hz), 1.78 (m, 4H), 3.90 (t, 4H, ^3J = 6.6 Hz), 4.49 (s, 2H), 6.40 (m, 1H), 6.51 (m, 2H).

4.1.2.3. 3,5-Dibutoxybenzyl chloride (2i). Colorless oil (648 mg), yield 80%; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.97 (t, 6H, ^3J = 7.4 Hz), 1.48 (m, 4H), 1.75 (m, 4H), 3.94 (t, 4H, ^3J = 6.5 Hz), 4.50 (s, 2H), 6.40 (m, 1H), 6.51 (m, 2H).

4.1.3. General procedure for preparation of 10-benzyl-9(10H)-acridinones

A mixture of **1** (195 mg, 1 mmol), NaH (48 mg, 1.2 mmol) in dry DMF (10 mL) was stirred vigorously under nitrogen for 1 h at room temperature. The corresponding benzyl chloride **2** (2 mmol) and KI (33.2 mg, 0.2 mmol) were added. The mixture was stirred overnight. Water was slowly added with rapid stirring under ice-water bath. Yellow crystals were obtained after filtration. The crystals were purified by recrystallization from trichloromethane/ethanol.

4.1.3.1. 10-Benzyl-9(10H)-acridinone (3a). Slight yellow crystals (257 mg), yield 90%, mp 180–181 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.61 (s, 2H), 7.20-7.37(m, 9H), 7.62(m, 2H), 8.60 (m, 2H).

4.1.3.2. 10-(3, 5-Dimethoxybenzyl)-9(10H)-acridinone (3b). Yellow crystals (314 mg), yield 91%, mp 229–230 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.72 (s, 6H), 5.51 (s, 2H), 6.35 (m, 2H), 6.39 (m, 1H), 7.26-7.37 (m, 4H), 7.62 (m, 2H), 8.57 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 51.0, 55.4, 99.0, 103.8, 115.2, 121.7, 122.6, 127.7, 134.1, 138.2, 142.6, 161.6, 178.3; HRMS calcd for $C_{22}H_{20}NO_3$ $[M+H]^+$ 346.1443, found 346.1414.

4.1.3.3. 10-(3,4,5-Trimethoxybenzyl)acrid in-9-one (3c). Yellow crystals (334 mg), yield 89%, mp 211–213 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.71 (s, 6H), 3.84 (s, 3H), 5.52 (s, 2H), 6.40 (s, 2H), 7.28-7.39 (m, 4H), 7.65 (m, 2H), 8.59 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 51.3, 56.2, 60.9, 102.4, 115.3, 121.7, 122.6, 127.7, 131.3, 134.1, 137.4, 142.7, 154.0, 178.3; HRMS calcd for $C_{23}H_{22}NO_4$ $[M+H]^+$ 376.1549, found 376.1521.

4.1.3.4. 10-(3-Methoxybenzyl)-9(10H)-acridinone (3d). Slight yellow crystals (287 mg), yield 91%, mp 211–212 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.72 (s, 3H), 5.53 (s, 2H), 6.73–6.84 (m, 3H), 7.23–7.34 (m, 5H), 7.60 (m, 2H), 8.57 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 50.8, 55.2, 111.8, 112.7, 115.2, 117.9, 121.6, 122.5, 127.7, 130.4, 134.1, 137.3, 142.6, 160.3, 178.2; HRMS calcd for $C_{21}H_{18}NO_2$ $[M+H]^+$ 316.1338, found 316.1324.

4.1.3.5. 10-(4-Methoxybenzyl)-9(10H)-acridinone (3e). Slight yellow crystals (284 mg), yield 90%, mp 220–221 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.77 (s, 3H), 5.53 (s, 2H), 6.87 (d, ^3J = 8.7 Hz, 2H), 7.12 (m, 2H), 7.29 (m, 2H), 7.36 (d, ^3J = 8.7 Hz, 2H), 7.62 (m, 2H), 8.58 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 52.2, 57.2, 116.6, 117.1, 123.5, 124.6, 128.8, 129.2, 129.7, 135.9, 144.5, 161.2, 180.1; HRMS calcd for $C_{21}H_{18}NO_2$ $[M+H]^+$ 316.1338, found 316.1337.

4.1.3.6. 10-(3, 5-Dibenzyloxybenzyl)-9(10H)-acridinone (3f). Slight yellow crystals (482 mg), yield 97%, mp 170–171 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.93 (s, 4H), 5.46 (s, 2H), 6.41(m, 2H), 6.55(m, 1H), 7.25–7.31(m, 14H), 7.60(m, 2H), 8.58(m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 52.9, 72.1, 103.2, 106.9, 117.1, 123.5, 124.6, 129.3, 129.7, 129.9, 130.5, 135.9, 138.4, 140.1, 144.5, 162.7, 180.1; HRMS calcd for $C_{34}H_{28}NO_3$ $[M+H]^+$ 498.2069, found 498.2069.

4.1.3.7. 10-(3,5-Diethoxybenzyl)-9(10H)-acridinone (3g). Slight yellow crystals (329 mg), yield 88%, mp 200–201 °C.

^{1}H NMR (500 MHz, $CDCl_3$): δ 1.34 (t, 6H, ^3J = 7.0 Hz), 3.92 (q, 4H, ^3J = 7.0 Hz), 5.49 (s, 2H), 6.32 (m, 2H), 6.37 (m, 1H), 7.29 (m, 2H), 7.36 (m, 2H), 7.63 (m, 2H), 8.58 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.6, 51.1, 63.6, 100.1, 104.3, 115.3, 121.6, 122.6, 127.7, 134.0, 138.1, 142.7, 161.0, 178.3; HRMS calcd for $C_{24}H_{24}NO_3$ $[M+H]^+$ 374.1756, found 374.1747.

4.1.3.8. 10-(3,5-Dipropoxybenzyl)-9(10H)-acridinone (3h). Slight yellow crystals (341 mg), yield 85%, mp 217–218 °C.

^{1}H NMR (500 MHz, $CDCl_3$): δ 0.97 (t, 6H, ^3J = 7.4 Hz), 1.73 (4, 4H), 3.81(t, 4H, ^3J = 6.6 Hz), 5.49 (s, 2H), 6.32 (m, 2H), 6.38 (m, 1H), 7.28 (m, 2H), 7.36 (m, 2H), 7.62 (m, 2H), 8.57 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 10.4, 22.5, 51.2, 69.6, 100.1, 104.2, 115.3, 121.6, 122.6, 127.7, 134.0, 138.0, 142.7, 161.2, 178.3; HRMS calcd for $C_{26}H_{28}NO_3$ $[M+H]^+$ 402.2069, found 402.2059.

4.1.3.9. 10-(3,5-Dibutoxybenzyl)-9(10H)-acridinone (3i). Slight yellow crystals (408 mg), yield 95%, mp 191–192 °C.

^{1}H NMR (500 MHz, $CDCl_3$): δ 0.92 (t, 6H, ^3J = 7.4 Hz), 1.42 (m, 4H), 1.69 (m, 4H), 3.86 (t, 4H, ^3J = 6.5 Hz), 5.50 (s, 2H), 6.32 (m, 2H), 6.38 (m, 1H), 7.30 (m, 2H), 7.37(m, 2H), 7.64 (m, 2H), 8.59 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 13.8, 19.2, 31.2, 51.2, 67.8, 100.1, 104.2, 115.3, 121.6, 122.6, 127.7, 134.0, 138.0, 142.7, 161.2, 178.3; HRMS calcd for $C_{28}H_{32}NO_3$ $[M+H]^+$ 430.2382, found 430.2362.

4.1.3.10. 10-(3-Methylbenzyl)-9(10H)-acridinone(3j). Slight yellow crystals (269 mg), yield 90%, mp 196–198 °C.

^{1}H NMR (500 MHz, $CDCl_3$): δ 2.30(s, 3H), 5.56 (s, 2H), 6.99 (m, 1H), 7.02 (br s, 1H), 7.12 (m, 1H), 7.23 (m, 1H), 7.29 (m, 2H), 7.35 (m, 2H), 7.62(m, 2H), 8.60 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 21.5, 50.9, 115.3, 121.6, 122.6, 122.7, 126.2, 127.8, 128.6, 129.1, 134.0, 135.5, 139.1, 142.7, 178.2; HRMS calcd for $C_{21}H_{18}NO$ $[M+H]^+$ 300.1388, found 300.1373.

4.1.3.11. 10-(3-Fluorobenzyl)-9(10H)-acridinone (3k). Yellow crystals (261 mg), yield 86%, mp 197–198 °C.

^{1}H NMR (500 MHz, $CDCl_3$): δ 5.58 (s, 2H), 6.92 (m, 1H), 7.01 (m, 2H), 7.30–7.34 (m, 5H), 7.63 (m, 2H), 8.60 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 50.4, 112.8, 113.0, 114.8, 114.9, 115.0, 121.3, 121.3, 121.8, 122.7, 128.0, 130.9, 131.0, 134.1, 138.3, 138.4, 142.5, 162.4, 164.5, 178.1; HRMS calcd for $C_{20}H_{15}FNO$ $[M+H]^+$ 304.1138, found 304.1145.

4.1.3.12. 10-(3-Trifluoromethylbenzyl)-9(10H)-acridinone (3l). Yellow crystals (300 mg), yield 85%, mp 200–201 °C.

^{1}H NMR (500 MHz, $CDCl_3$): δ 5.64 (s, 2H), 7.26–7.33 (m, 5H), 7.45 (m, 1H), 7.59 (m, 2H), 7.64 (m, 2H), 8.60 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 50.5, 114.8, 121.9, 122.0, 122.6, 122.6, 122.7, 122.8, 124.8, 124.8, 124.9, 124.9, 125.6, 127.9, 128.9, 129.9, 131.1, 131.5, 131.9, 132.3, 134.2, 136.8, 142.3, 178.1; HRMS calcd for $C_{21}H_{15}F_3NO$ $[M+H]^+$ 354.1106, found 354.1118.

4.1.4. General procedure for the synthesis of 1-benzyl-4-piperidone

To a suspension of 4-piperidone hydrochloride (2 mmol) in CH_2Cl_2 (10 mL) were added the appropriate substituted benzyl chloride (2.2 mmol) and triethylamine (4.4 mmol). The mixture was heated at reflux overnight, diluted with CH_2Cl_2 (20 mL), and then washed with water and brine. The organic layer was separated. The separated water was extracted three times with 20 mL CH_2Cl_2. The combined organic phases dried (Na_2SO_4), and evaporated to give a residue, which was purified by column chromatography on silica gel using EtOAc/petroleum ether/triethylamine (50:50:1, v/v/v) as eluent.

4.1.4.1. 1-Benzyl-4-piperidone (5a). slight yellow oil (227 mg), yield 60%;^{1}H NMR (500 MHz, $CDCl_3$): δ 2.45 (t, 4H, ^3J = 6.0 Hz), 2.75 (t, 4H, ^3J = 6.0 Hz), 3.62(s, 2H), 7.26–7.36 (m, 5H); HRMS calcd for $C_{12}H_{16}NO$ $[M+H]^+$ 190.1232, found 190.1230.

4.1.4.2. 1-(3,5-Dimethoxyl)benzyl-4-piperidone (5b). slight yellow oil (324 mg), yield 65%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.46 (t, 4H, ^3J = 6.1 Hz), 2.75 (t, 4H, ^3J = 6.1 Hz), 3.56(s, 2H), 3.80(s, 6H), 6.38 (m, 1H), 6.54 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 41.3, 53.0, 55.3, 62.0, 99.0, 106.7, 140.7, 160.8, 209.4; HRMS calcd for $C_{14}H_{20}NO_3$ $[M+H]^+$ 250.1443, found 250.1437.

4.1.4.3. 1-(3,4,5-Trimethoxybenzyl)-4-piperidone (5c). Colorless crystals (346 mg), yield 62%, mp 102–103 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.48 (t, 4H, ^3J = 6.1 Hz), 2.76 (t, 4H, ^3J = 6.1 Hz), 3.55(s, 2H), 3.85(s, 3H), 3.88(s, 6H), 6.60 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 41.3, 53.0, 56.1, 60.9, 62.2, 105.5, 134.1, 153.2, 209.3; HRMS calcd for $C_{15}H_{22}NO_4$ $[M+H]^+$ 280.1549, found 280.1549.

4.2. Bioassay

4.2.1. Cell culture

CCRF-CEM leukemia cells (Human T cell lymphoblast-like cell line) and HL60 (human promyelocytic leukemia Cell) were purchased from the Chinese Academy of Sciences Cell Bank. The cells were cultured in RPIM 1640 medium (Cibco), containing 10% fetal bovine serum (FBS) (Hyclone Laboratories Inc.), 100 U/mL penicillin, and 100 μg/mL streptomycin in a 5% CO_2-humidified atmosphere at 37 °C.

4.2.2. Materials

3-(4,5-Dimethyl-thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) and propidium iodide (PI)were purchased from Sigma Chemical Co. Annexin V-FITC Apoptosis Detection Kit I and FITC-conjugated antibody were purchased from Beyotime Institute of Biotechnology.

4.2.3. Cell viability

The cells were suspended at a concentration of 2×10^5 cells/mL and seeded in 96-well microtiter plates. Various concentrations of compound were added to each well in quintuplet followed by incubation for the indicated times. After treatment, the cells were incubated with MTT (5 mg /mL) for 4 h. The formazan precipitate was dissolved in 100 μL DMSO, and the absorbance at 490 nm was measured by a Benchmark microplate reader (Molecular Devices Corporation). IC_{50} values are the concentration at which cell growth was inhibited by 50%.

4.2.4. Flow cytometric analysis of apoptosis

The CCRF-CEM cells were exposed to different concentrations of compound for 24 h at 37 °C, and then detached, and collected. The untreated and treated CCRF-CEM cells were washed twice with phosphate-buffered saline (PBS), fixed with 0.5 mL ice-cold 70% ethanol, and stored at 4 °C for 1–2 h. DNA content was then measured after staining with PI solution (100 μg/mL propidium iodide) for 30 min. Finally, the cells were analyzed with a flow cytometer using an Epics system (Coulter Epics XL) equipped with an argon-ion laser operated at a wavelength of 488 nm. Surface exposure of phosphatidylserine in apoptotic cells was measured by Annexin V-FITC/PI apoptosis detection kit I.

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