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Short communication

Berbamine derivatives: A novel class of compounds for anti-leukemia activity

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ABSTRACT

Our previous studies showed that the natural compound berbamine, from Chinese herb *Berberis amurensis*, selectively induces apoptosis of imatinib (IM)-resistant-Bcr/Abl-expressing leukemia cells from the K562 cell line and CML patients. Here, a series of new berbamine derivatives were obtained by synthesis. In this series, high to very high activity *in vitro* has been found. Compounds **2e**, **2g**, **3f**, **3k**, **3q** and **3u** exhibited consistent high anti-tumor activity for imatinib-resistant K562 leukemia cells. Their IC_{50} values at 48 h were 0.36–0.55 μM, whereas berbamine IC_{50} value was 8.9 μM. Cell cycle analysis results showed that compound **3h** could reduce G0/G1 cells. In particular, these compounds displayed potent inhibition of the cytoplasm-to-nucleus translocation of NF-κB p65 which plays a critical role in the survival of leukemia stem cells. These results suggest that berbamine could be a good starting point for the development of novel lead compounds in the fight against leukemia.

1. Introduction

Berbamine is a well-known bisbenzylisoquinoline alkaloid isolated from traditional Chinese herbal medicines such as *Berberis amurensis* (Fig. 1). Berbamine has been demonstrated to possess a number of interesting and potent biological activities [1–8]. Gleevec (also called Imatinib or STI571), which is an inhibitor of the bcr/abl tyrosine kinase [9], has been a remarkable success for the treatment of chronic myelogenous leukemia (CML). However, a significant proportion of patients chronically treated with Gleevec develop resistance [10]. Thus, it is necessary to identify novel inhibitors that are active against Gleevec-resistant mutants of bcr/abl oncoprotein.

In our previous report, we found that berbamine can selectively induce cell death of both Gleevec sensitive- and resistant-Ph^+ chronic myeloid leukemia (CML) cells [11]. Moreover, we found that berbamine can selectively induce caspase-3-dependent apoptosis of leukemia NB4 cells via the survivin-mediated pathway, suggesting that berbanine may be a novel agent for the treatment of leukemia [12]. However, its IC_{50} value for various leukemia cells was only modest (from 4 to 8.9 μM) [11,12]. Some synthetic berbamine derivatives for anti-tumor activity have been reported, such as *O*-(4-ethoxybutyl)-berbamine (EBB). EBB displays various activities such as inhibition of hepatoma [13], inhibition of the trypsin-activated $Ca^{2+} + Mg^{2+}$-ATPase [14]. E6, another berbamine derivative, has also shown potent biological activity [15,16]. These previous studies prompted us to develop novel berbamine derivatives designed to treat leukemia. Interestingly, although berbamine derivatives display various anticancer activities, there are currently no publications that systematically demonstrated the structure–activity relationship of this class of compounds for anticancer activity.

In order to discover novel anti-leukemia berbamine derivatives in this study we focus specifically on compounds that are active against Gleevec-resistant leukemia cells. We describe in this report the results of berbamine derivatives synthesis as well as their anti-leukemia activity for imatinib-resistant K562 leukemia cells.

2. Results and discussions

2.1. Chemistry

The phenolic hydroxyl group of berbamine could be etherified, esterified or sulfonylated facilely. The synthesis of a series of berbamine derivatives are illustrated in Scheme 1. Berbamine derivatives **2a–g** were synthesized according to Williamson

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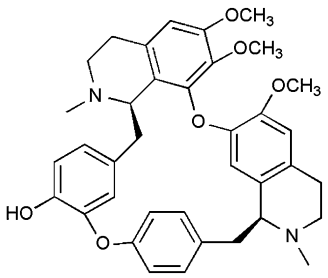


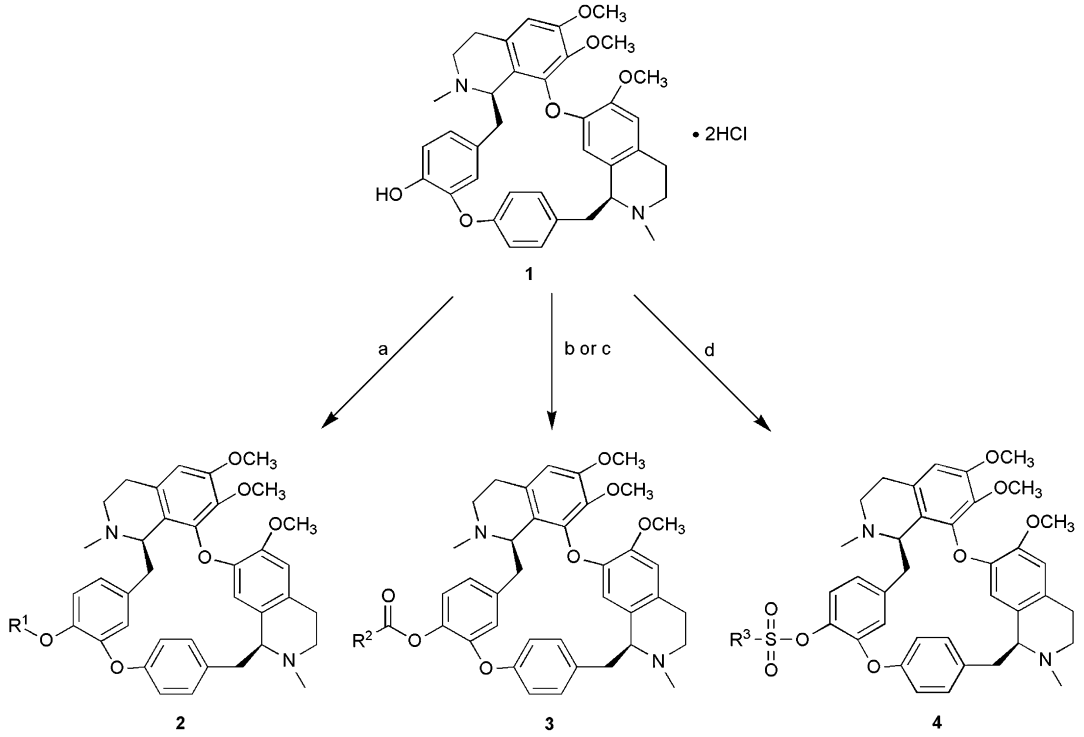
Fig. 1. The structure of berbamine.

reaction. Etherified derivatives (route a) were synthesized starting from berbamine dihydrochloride **1**, which was dissolved in DMF, the solution was cooled to 0 °C. NaH was added to the solution and the solution was stirred for 1 h at 0 °C. Then an alkyl halide such as benzyl bromide was added dropwise and the solution was stirred for 1 h at 0 °C. The solution was allowed to warm to room temperature and was stirred for 1–8 h to afford products **2a**–**g**. Esterfied derivatives (route b) were synthesized starting with the treatment of berbamine dihydrochloride **1** with Et_3N at 0 °C in CH_2Cl_2 for 1 h. Then an acyl chloride was added dropwise and the solution was stirred for 1 h at 0 °C. The solution was allowed to warm to room temperature to afford products **3a**–**t**. An additional esterfied derivative, compound **3u**, was synthesized (route c) via reaction of berbamine with 1*H*-indole-2-carboxylic acid in the presence of *N*,*N*′-dicyclohexylcarbodiimide (DCC) in CH_2Cl_2 at room temperature. Finally sulfonylated derivatives were formed (route d) by first neutralizing berbamine dihydrochloride **1** with Et_3N. A sulfonyl chloride was then added dropwise to the solution. The mixture was stirred at 0 °C for 1 h to afford products **4a** and **4b**. The yields of berbamine derivatives **2**–**4** are listed in Table 1. The products were characterized by ESI-MS and NMR spectra.

2.2. Biological activities

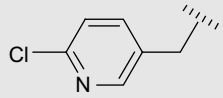
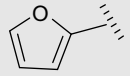
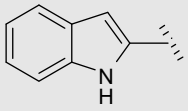
All compounds were evaluated for their cytotoxic activity *in vitro* against human imatinib-resistant K562 leukemia cell line. The results are summarized in Table 1.

As shown in Table 1, most compounds exhibited inhibitions on the growth of imatinib-resistant K562 leukemia cells. Preliminary structure–activity relationship of this series of compounds for cytotoxicity against imatinib-resistant K562 leukemia cell line was investigated. From the IC_{50} data of these compounds, we find that etherification, esterification or sulfonylation of berbamine could improve its cytotoxicity against imatinib-resistant K562 leukemia cell line significantly. In general, when the R^1, R^2 or R^3 group contained an aromatic ring, the activity was better than when these substituents were aliphatic chain. For example, **2a** ($R^1 = CH_3CH_2$, $IC_{50} = 3.14$ μM) compared to **2c** ($R^1 = PhCH_2$, $IC_{50} = 0.80$ μM), **3a** ($R^2 = CH_3$, $IC_{50} = 6.60$ μM) compared to **3e** ($R^2 = Ph$, $IC_{50} = 2.16$ μM), and **4a** ($R^3 = CH_3$, $IC_{50} = 4.74$ μM) compared to **4b** ($R^3 = Ph$, $IC_{50} = 1.99$ μM). Comparing the IC_{50} data for **2c**–**f** allows us to evaluate the SAR, when an electron withdrawing substituent is present on the phenyl ring of the R^1 group with the etherified berbamine derivatives. The data suggest that cytotoxicity against imatinib-resistant K562 leukemia cell line is better in most cases than when the electron donating group is present, such as with **2e** ($R^1 = 4\text{-}NO_2PhCH_2$, $IC_{50} = 0.36$ μM) and **2f** ($R^1 = 3,4,5\text{-}(CH_3O)_3PhCH_2$, $IC_{50} = 1.53$ μM). The data of **3e**–**s** likewise suggests that an electron donating substituent is present on the phenyl ring could increase the activity of esterified berbamine derivative's cytotoxicity against imatinib-resistant K562 leukemia cell line such as **3f** ($R^2 = 4\text{-}CH_3OPh$, $IC_{50} = 0.55$ μM), **3h** ($R^2 = 2\text{-}CH_3Ph$, $IC_{50} = 0.69$ μM), **3n** ($R^2 = 4\text{-}NO_2Ph$, $IC_{50} = 2.13$ μM) and **3o** ($R^2 = 3,5\text{-}diNO_2Ph$, $IC_{50} = 3.75$ μM). Finally the activity of the compounds in this series is better when R^1 or R^2 is a heterocycle containing nitrogen such as **2g** ($R^2 = (6\text{-chloropyridin-3-yl})methyl$, $IC_{50} = 0.40$ μM) and **3u** ($R^2 = 1H\text{-indol-2-yl}$, $IC_{50} = 0.46$ μM) rather



Scheme 1. Synthesis of berbamine derivatives **2**–**4**. Reagents and conditions: (a) NaH (4 equiv), R^1X (1.1 equiv or 5 equiv), DMF, 0 °C to r.t. (b) Et_3N (4 equiv), R^2COCl (1.1 equiv), 1 h, 0 °C, CH_2Cl_2. (c) 1*H*-Indole-2-carboxylic acid, DCC (2 equiv), CH_2Cl_2, overnight. (d) Et_3N (4 equiv), R^3SO_2Cl (1.1 equiv), 1 h, 0 °C, CH_2Cl_2.

Table 1
Berbamine derivatives and IC_{50} values of them for anti-leukemia activity (K562-R).

Compounds	$R^1/R^2/R^3$	Yields (%)	IC_{50} (μM) [a]	Compounds	$R^1/R^2/R^3$	Yields (%)	IC_{50} (μM) [a]
2a	CH_3CH_2	43	3.14	**3j**	2-ClPh	70	0.60
2b	$BrCH_2CH_2CH_2$	37	1.66	**3k**	4-BrPh	72	0.40
2c	$PhCH_2$	79	0.80	**3l**	2-BrPh	71	0.78
2d	4-$BrPhCH_2$	75	0.71	**3m**	3-FPh	74	1.97
2e	4-NO_2PhCH_2	77	0.36	**3n**	4-NO_2Ph	75	2.13
2f	3,4,5-$(CH_3O)_3PhCH_2$	70	1.53	**3o**	3,5-$(NO_2)_2$Ph	78	3.75
2g		78	0.40	**3p**	3-CF_3Ph	76	0.89
3a	CH_3	65	6.60	**3q**	3-$ClCH_2$Ph	72	0.50
3b	$(CH_3)_3C$	70	1.01	**3r**	$PhCH_2$	75	2.23
3c	$CH_3C{=}CH_2$	65	3.81	**3s**	3,4,6-$(F)_3PhCH_2$	75	3.02
3d	$ClCH_2$	60	2.70	**3t**		72	2.78
3e	Ph	70	2.16	**3u**		75	0.46
3f	4-CH_3OPh	70	0.55	**4a**	CH_3	77	4.74
3g	3,4,5-$(CH_3O)_3Ph$	72	0.69	**4b**	Ph	78	1.99
3h	2-CH_3Ph	76	0.69	Berbamine	H	–	8.93
3i	4-ClPh	71	0.98	EBB	$C_2H_5O(CH_2)_4$	–	4.08

[a] Mean values of three independent determinations.

than a heterocycle containing oxygen such as **3t** (R^3 = furan-2-yl, IC_{50} = 2.78 μM).

NF-κB, which is thought to be an important signal molecule downstream p210Bcr/Abl oncoprotein [17–19], is constitutively active in AML [18], MDS [20] and T-cell acute lymphoblastic leukemia (T-ALL) [21] but not in normal hematopoietic cells [22], and its inhibition is correlated with leukemia-specific cell death [20,21,23]. Therefore, we sought to investigate whether berbamine derivatives can affect NF-κB signaling pathway. We analyzed total and nuclear levels of NF-κB p65/relA subunit in leukemia cells after exposure to berbamine derivatives for 24 h. The treatment of K562 cells with berbamine derivatives led to the disappearance of NF-κB from the nuclei (Fig. 2). However, total level of NF-κB subunit p65 were not obviously affected (data not shown). These data indicate that berbamine derivatives can effectively inhibit the cytoplasm-to-nucleus translocation of NF-κB p65/relA subunit of leukemia cells.

Flow cytometry was used to evaluate the effects of compound **3h** on the cell cycles and apoptosis of K562-R cells. As summarized in Fig. 3, and Table 2, treatment of K562-R cells with compound **3h** resulted in a significant decrease of G_0/G_1 phase cells accompanied with a parallel increase of sub-G_1 phase cells. As indicated in Fig. 3, the percentage of G_0/G_1 phase cells (non-proliferating cells) before and after treatment of the compound **3h** at 1 μg/ml were 49.05% and 32.37%, respectively whereas, the percentage of sub-G_1 phase cells (apoptotic cells) were 8.0%, and 31.71%, respectively. Compound **3h** also reduced proliferating cells (S phase cells). The percentage of S phase cells was 34.99% in untreated K562-R cells, but deceased to 22.89% after treatment with the derivative **3h** at 2 μg/ml for 48 h. These observations suggest that the compound **3h** can induce apoptosis of K562-R cells and may have potential to kill leukemia stem cells that are quiescent.

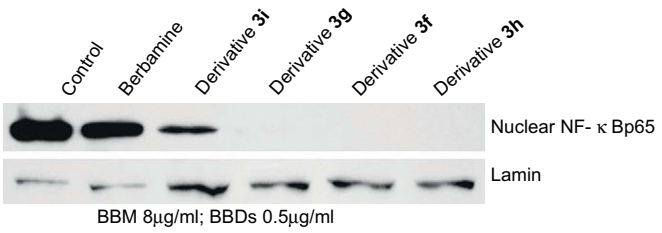


Fig. 2. Berbamine and its derivatives inhibit the cytoplasm-to-nucleus translocation of NF-kappaB in leukemia cells.

3. Conclusion

A series of berbamine derivatives were synthesized and the anti-leukemia activity of them against imatinib-resistant K562 leukemia cell line was evaluated. Etherified, esterified or sulfonylated of the phenolic hydroxyl group of berbamine increased the cytotoxic activity of berbamine against leukemia cell lines significantly. Most compounds displayed potent cytotoxic activity in the micromolar range. Compounds **2e**, **2g**, **3f**, **3k**, **3q** and **3u**, whose IC_{50} values were 0.36 μM, 0.40 μM, 0.55 μM, 0.40 μM, 0.50 μM and 0.46 μM showed the best activity. In particular, these compounds also displayed potent inhibition of the cytoplasm-to-nucleus translocation of NF-κB p65 which is critical for survival of leukemia stem cells. The cell cycle analysis on K562-R cell line indicated that these compounds can reduce quiescent (G0/G1) phase cells, suggesting that berbamine derivatives may have potential to kill leukemia stem cells.

4. Experimental protocols

Melting points were recorded on a Büchi B-540 apparatus and were uncorrected. NMR spectra were recorded at 500 MHz

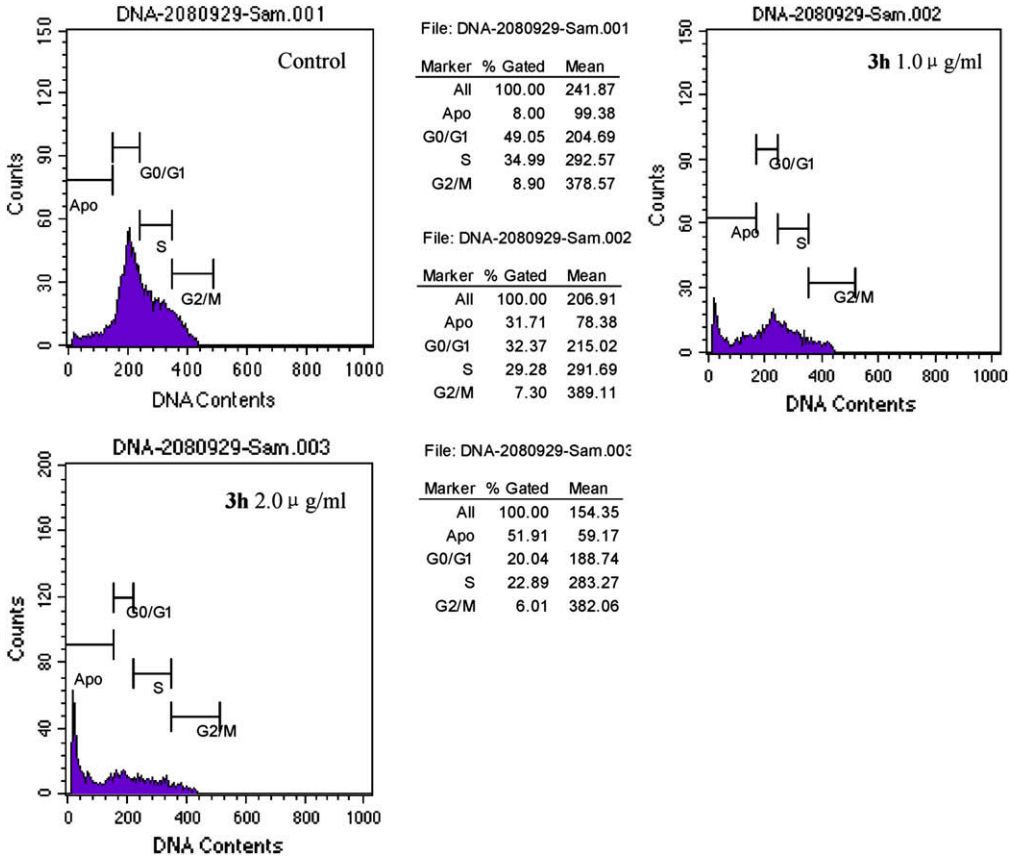


Fig. 3. Induction of apoptosis by **3h** in K562-R cells.

(^{1}H NMR) or 125 MHz (^{13}C NMR) with $CDCl_3$ as solvent and TMS as the internal standard. *J* values are in Hertz and chemical shifts are expressed in ppm downfield from internal TMS. DMF and CH_2Cl_2 were distilled from CaH_2. The other commercial reagents were used directly without further purification. Column chromatography was performed on silica gel. MS (ESI) spectra were recorded on a Thermo Finnigan LCQ Advantage mass Spectrophotometer.

4.1. General procedure for the preparation of berbamine derivatives ***2a–g***

Berbamine dihydrochloride **1** (681 mg, 1 mmol) was dissolved in 10 mL of DMF, the solution was cooled to 0 °C under N_2. NaH (96 mg, 4 mmol) was added to the solution. The mixture was stirred for 1 h and an alkyl halide (1.1 mmol, for **2a** and **2b**, 5 mmol, in 3 mL of DMF) was added dropwise over 15 min. After stirring for 1 h at 0 °C, the solution was allowed to warm to room temperature and was stirred for 1–8 h (tested by TLC). The reaction mixture was evaporated in vacuo, diluted with water and extracted with CH_2Cl_2 (3×10 mL). The combined organic phase was washed with water and brine, dried over anhydrous Na_2SO_4 and filtered. After being concentrated in vacuo, the residue was purified by flash chromatography on silica gel (CH_2Cl_2:CH_3OH = 8:1) to afford the desired product.

Table 2
Cell cycle analysis results of K562-R treated with compound **3h**.

	Control (%)	1 μg/ml (%)	2 μg/ml (%)
Apoptosis	8.0	31.71	51.91
G0/G1	49.05	32.37	20.04
S	34.99	29.28	22.89
G2/M	8.90	7.30	6.01

4.2. General procedure for the preparation of berbamine derivatives ***3a–t***

Berbamine dihydrochloride **1** (681 mg, 1 mmol) was suspended in 10 mL of CH_2Cl_2. The mixture was cooled to 0 °C under N_2. Et_3N (404 mg, 4 mmol) was added to the solution. After stirring for 1 h at 0 °C, an acyl halide (1.1 mmol, in 3 mL of CH_2Cl_2) was added dropwise to the solution over 15 min. The resulting solution was stirred for 1 h at 0 °C and was stirred another hour at room temperature. The solution was washed with water and brine, dried over anhydrous Na_2SO_4 and filtered. After being concentrated in vacuo, the residue was purified by flash chromatography on silica gel (CH_2Cl_2:CH_3OH = 8:1) to afford the desired product.

4.3. Procedure for the preparation of berbamine derivative ***3u***

1*H*-indole-2-carboxylic acid (161 mg, 1 mmol) was dissolved in 15 mL of CH_2Cl_2. The solution was cooled to 0 °C under N_2. DCC (2 mmol) was added to the solution. The solution was stirred for 1 h at 0 °C. Berbamine (1 mmol) (berbamine dihydrochloride **1** was neutralized with Et_3N and dried in vacuo) was added to the solution, and then DMAP (0.5 mmol) was added. The reaction mixture was allowed to warm to room temperature and was stirred overnight, during which time a white precipitate was formed. The reaction mixture was filtered and washed with CH_2Cl_2, the filtrate was washed with water and brine, dried over anhydrous Na_2SO_4 and filtered. After being concentrated in vacuo, the residue was purified by flash chromatography on silica gel (CH_2Cl_2:CH_3OH = 8:1) to afford the desired product.

4.4. General procedure for the preparation of berbamine derivatives **4a–b**

Berbamine dihydrochloride **1** (681 mg, 1 mmol) was suspended in 10 mL of CH_2Cl_2. The mixture was cooled to 0 °C under N_2. Et_3N (404 mg, 4 mmol) was added to the solution. The mixture was stirred for 1 h and a sulfonyl chloride (1.1 mmol, in 3 mL of CH_2Cl_2) was added dropwise over 15 min. The solution was stirred for 1 h at 0 °C and was stirred another hour at room temperature. The solution was washed with water and brine, dried over anhydrous Na_2SO_4 and filtered. After being concentrated in vacuo, the residue was purified by flash chromatography on silica gel (CH_2Cl_2:CH_3OH = 8:1) to afford the desired product.

4.5. Biology

4.5.1. Cell line, culture conditions, and growth assays

Human chronic myeloid leukemia cell line imatinib-resistant-K562 (K562-R), which constitutively express endogenous p210Bcr/Abl oncoprotein, were grown in RPMI-1640 medium supplemented with 10% fetal calf serum (FCS), 100 units/ml penicillin G, and 100 μg/ml streptomycin at 37 °C in a 95% air, 5% CO2 humidified incubator. K562-R cells expresse multipledrug resistance-1(MDR1) and are highly resistant to IM and conventional chemotherapeutic agents. Cell number and viabilities were monitored by hemocytomer and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay, respectively.

4.5.2. Detection of NF-κB p65 proteins

Leukemia cells were treated with berbamine (8 μg/ml) or berbamine derivatives (0.5 μg/ml), and total and nuclear cellular proteins were extracted using the Mammalian Protein Extraction Reagent (Pierce). NF-κB p65 protein was detected using Western blot.

4.5.3. Flow cytometry

K562-R cells at a density of 2×10^5 cells/mL were treated with various concentrations of compound **3h** (1.0 μg/ml, 2.0 μg/ml) for 48 h. The cells were harvested, washed with PBS and centrifuged. The fixation of cells was relized through the addition of 4 ml ethanol (70% ice-cold) and then keeping cells at 4 °C overnight until DNA staining. The fixed cells were treated with 100 μg/ml Rnase A in PBS for 1 h, followed by staining with 50 μg/ml propidium iodide in PBS in the dark. The DNA content of eukaryotic cells was then measured with flow cytometery.

4.6. Analytical data for compounds **2d**, **2e**, **2g**, **3h**, **3i**, **3u** and **4a**

4.6.1. O-*(4-Bromobenzyl)-berbamine (**2d**)*

White powder, m.p.: 108–111 °C. MS (ESI) *m*/*z* 778.2 ($M + H^+$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.47–7.46 (d, 2H, *J* = 7.0 Hz), 7.42–7.40 (d, 1H, *J* = 8.5 Hz), 7.36–7.33 (t, 2H, *J* = 7.5 Hz, 7.5 Hz), 7.29–7.28 (d, 1H, *J* = 10.0 Hz), 6.97–6.95 (d, 2H, *J* = 8.5 Hz), 6.81–6.79 (d, 1H, *J* = 8.5 Hz), 6.72–6.68 (m, 1H), 6.63 (s, 1H), 6.39–6.32 (m, 3H,), 5.50–5.49 (d, 1H, *J* = 1.5 Hz), 5.17 (s, 2H), 4.24–4.18 (dd, 1H, *J* = 4 Hz, 5.5 Hz), 3.78 (s, 3H), 3.63 (s, 3H), 3.37–3.31 (m, 2H), 3.19 (s, 3H), 3.15–3.09 (m, 2H), 3.07–3.00 (m, 2H), 2.91–2.71 (m, 4H), 2.67 (s, 3H), 2.55 (s, 3H), 2.37(s, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 25.5, 28.8, 37.5, 39.6, 42.2, 43.8, 45.4, 51.1, 54.9, 55.9, 60.5, 61.5, 64.0, 71.5, 105.7, 110.9, 114.3, 116.7, 117.1, 121.1, 122.2, 123.4, 123.5, 127.3, 127.5 (2C), 127.7, 127.9, 128.4 (2C), 128.6, 130.9, 131.4, 131.8, 137.0, 137.6, 139.1, 143.7, 145.7, 147.5, 148.2, 149.9, 151.5, 152.4.

4.6.2. O-*(4-Nitrobenzyl)-berbamine (**2e**)*

Yellow powder, m.p.: 131–134 °C. MS (ESI) *m*/*z* 744.4 ($M + H^+$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.22–8.20 (d, 2H, *J* = 8.8 Hz), 7.65–7.63 (d, 2H, *J* = 8.4 Hz), 7.45–7.42 (d, 1H, *J* = 8.4 Hz), 6.99–6.95 (m, 3H), 6.78–6.64 (m, 3H), 6.40–6.32 (m, 3H), 5.53–5.52 (d, 1H, *J* = 1.6 Hz), 5.25 (s, 2H), 4.26–4.18 (dd, 1H, *J* = 4.4 Hz, 5.6 Hz), 3.78 (s, 3H), 3.64 (s, 3H), 3.38–3.31 (m, 2H), 3.19 (s, 3H), 3.15–3.07 (m, 2H), 3.04–3.00 (m, 2H), 2.95–2.88 (m, 4H), 2.85–2.72 (m, 3H), 2.66 (s, 3H), 2.52 (s, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 25.3, 28.8, 37.5, 39.7, 42.1, 43.8, 45.3, 51.2, 54.9, 55.9, 60.4, 61.5, 64.0, 70.5, 105.8, 110.9, 114.5, 116.6, 117.4, 120.9, 122.1, 123.2, 123.6 (2C), 123.8, 127.5, 127.6, 127.7 (2C), 127.9, 130.9, 131.5, 132.6, 137.0, 139.3, 143.8, 145.0, 145.2, 147.4, 147.5, 148.2, 149.9, 151.5, 152.2.

4.6.3. O-*((6-Chloropyridin-3-yl)methyl)-berbamine (**2g**)*

White powder, m.p.: 112–115 °C. MS (ESI) *m*/*z* 735.2 ($M + H^+$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.43 (s, 1H), 7.82–7.79 (d, 1H, *J* = 8.0 Hz), 7.43–7.41 (d, 1H, *J* = 7.2 Hz), 7.32–7.27 (m, 2H), 7.00–6.91 (m, 2H), 6.80–6.73 (dd, 2H, *J* = 8.4 Hz, 8.0 Hz), 6.65–6.6.63 (s, 1H), 6.39–6.27 (m, 3H), 5.50–5.49 (d, 1H, *J* = 1.5 Hz), 5.13 (s, 2H), 4.22–4.18 (dd, 1H, *J* = 4.0 Hz, 5.6 Hz), 3.78 (s, 3H), 3.63 (s, 3H), 3.37–3.31 (m, 2H), 3.19 (s, 3H), 3.14–3.09 (m, 2H), 3.03–3.02 (m, 2H), 2.92–2.72 (m, 4H), 2.65 (s, 3H), 2.55 (s, 3H), 2.37(m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 25.3, 28.6, 37.5, 39.6, 42.1, 43.7, 45.2, 51.1, 54.8, 55.9, 60.4, 61.4, 63.9, 68.7, 105.7, 110.8, 115.1, 116.6, 117.3, 120.8, 122.0, 123.1, 123.7, 124.1, 127.4, 127.7, 130.9, 131.5, 132.0, 132.8, 137.0, 137.6, 138.4, 139.1, 143.7, 144.8, 147.4, 148.2, 148.8, 150.0, 150.8, 151.5, 152.1.

4.6.4. O-*(2-Methylbenzoyl)-berbamine (**3h**)*

White powder, m.p.: 122–125 °C. MS (ESI) *m*/*z* 727.6 ($M + H^+$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.15–8.13 (d, 1H, *J* = 7.6 Hz), 7.60–7.58 (d, 1H, *J* = 8.0 Hz), 7.43–7.40 (m, 2H), 7.28–7.25 (m, 3H), 7.16 (s, 1H), 7.04–6.97 (dd, 2H, *J* = 8.0 Hz, 8.4 Hz), 6.92–6.90 (d, 1H, *J* = 8.0 Hz), 6.68 (s, 1H), 6.43–6.35 (m, 2H,), 5.57–5.56 (d, 1H, *J* = 1.6 Hz), 4.39–4.37 (dd, 1H, *J* = 4 Hz, 5.5 Hz), 3.80 (s, 3H), 3.64 (s, 3H), 3.41–3.35 (m, 2H), 3.21 (s, 3H), 3.17–3.06 (m, 4H), 2.96–2.81 (m, 4H), 2.73 (s, 3H), 2.64 (brs, 6H), 2.59–2.48 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.7, 24.2, 28.2, 37.7, 41.3, 41.8, 43.5, 44.7, 50.6, 54.8, 55.7, 60.4, 61.6, 63.8, 105.7, 111.0, 116.6, 117.7, 120.8, 121.8, 122.1, 124.1, 125.7, 125.8, 126.8 128.0, 129.0, 130.7, 131.1, 131.2, 131.3, 131.6, 132.3, 136.5, 137.2, 137.7, 138.3, 140.7, 143.7, 147.6, 148.2, 151.0, 151.8, 152.4, 165.6.

4.6.5. O-*(4-Chlorobenzoyl)-berbamine (**3i**)*

White powder, m.p.: 148–151 °C. MS (ESI) *m*/*z* 748.2 ($M + H^+$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.15–8.13 (d, 2H, *J* = 8.5 Hz), 7.45–7.43 (d, 2H, *J* = 9.0 Hz), 7.41–7.39 (d, 1H, *J* = 8.0 Hz), 7.24–7.20 (m, 1H), 7.01–6.99 (d, 1H, *J* = 8.0 Hz), 6.96–6.91 (m, 2H), 6.88–6.86 (d, 1H, *J* = 8.5 Hz), 6.64 (s, 1H), 6.41–6.31 (m, 3H), 5.62–5.61 (d, 1H, *J* = 1.5 Hz), 4.29–4.18 (dd, 1H, *J* = 3.5 Hz, 5.5 Hz), 3.78 (s, 3H), 3.63 (s, 3H), 3.39–3.29 (m, 2H), 3.20 (s, 3H), 3.07–2.99 (m, 2H), 2.93–2.87 (m, 2H), 2.82–2.69 (m, 4H), 2.65 (s, 3H), 2.58 (s, 3H), 2.44–2.37(m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 24.2, 28.1, 37.8, 40.2, 41.2, 43.4, 44.5, 50.6, 54.8, 55.9, 60.4, 61.4, 63.7, 105.7, 111.9, 116.6, 117.8, 120.7, 121.5, 122.0, 124.0, 126.9, 127.9, 128.7 (2C), 128.8, 130.8, 130.9, 131.3, 131.6 (2C), 131.7, 136.9, 137.1, 137.4, 138.5, 139.7, 143.7, 147.6, 148.2, 150.9, 151.8, 152.2, 163.9.

4.6.6. O-*(1H-Indole-2-carbonyl)-berbamine (**3u**)*

White powder, m.p.: 178–181 °C. MS (ESI) *m*/*z* 752.8 ($M + H^+$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.30 (s, 1H), 7.73–7.70 (d, 1H, *J* = 8.4 Hz), 7.47–7.40 (m, 3H), 7.35–7.30 (m, 1H), 7.22–7.14 (m, 2H), 7.08–7.06 (d, 1H, *J* = 8.0 Hz), 6.98–6.87 (m, 3H), 6.67 (s, 1H), 6.43–6.33 (m, 3H,), 5.64–5.63 (d, 1H, *J* = 1.6 Hz), 4.33–4.21 (dd, 1H, *J* = 3.2 Hz, 5.2 Hz), 3.80 (s, 3H), 3.64 (s, 3H), 3.40–3.24 (m, 2H), 3.21 (s, 3H), 3.17–3.00 (m, 2H), 2.96–2.91 (m, 2H), 2.84–2.71 (m, 4H), 2.67 (s, 3H), 2.60 (s, 3H), 2.47–2.39 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 25.0, 28.7, 37.7, 39.8, 41.9, 43.8, 45.1, 51.2, 54.8, 55.8, 60.4, 61.4, 64.0, 105.7, 110.2, 110.9, 112.1, 116.5, 117.9, 120.7, 120.8, 121.6, 121.9, 122.6, 123.0, 124.1, 124.8,

125.4, 126.5, 127.3, 127.4, 127.7, 131.0, 131.3, 136.9, 140.0, 137.2, 137.3, 139.2, 143.8, 147.5, 148.2, 151.0, 151.5, 152.1, 160.0.

*4.6.7. O-(Methylsulfonyl)-berbamine (**4a**)*

White powder, m.p.: 135–138 °C. MS (ESI) *m/z* 687.8 ($M + H^+$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.48–7.46 (d, 1H, $J = 8.4$ Hz), 7.17–7.15 (d, 1H, $J = 8.0$ Hz), 6.99–6.94 (m, 2H), 6.86–6.84 (d, 1H, $J = 8.5$ Hz), 6.63 (s, 1H), 6.44–6.41 (m, 1H), 6.38 (s, 1H), 6.33–6.31 (m, 2H,), 5.60–5.59 (d, 1H, $J = 1.5$ Hz), 4.33–4.19 (dd, 1H, $J = 3.5$ Hz, 5.6 Hz), 3.78 (s, 3H), 3.64 (s, 3H), 3.41–3.27 (m, 2H), 3.23 (s, 3H), 3.20 (s, 3H), 3.13–3.01 (m, 2H), 2.97–2.92 (m, 2H), 2.87–2.82 (m, 2H), 2.79–2.72 (m, 2H), 2.68 (s, 3H), 2.57 (s, 3H), 2.40–2.37(m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 25.1, 28.9, 37.8, 38.3, 40.0, 42.0, 43.9, 45.2, 51.4, 54.9, 55.9, 60.4, 61.5, 64.0, 105.8, 111.0, 116.5, 118.1, 119.1, 120.6, 121.8, 122.9, 123.2, 124.6, 127.2, 127.8, 128.0, 131.2, 131.7, 136.0, 137.0, 138.7, 140.0, 143.8, 147.5, 148.2, 150.9, 151.5.

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