

Xinbin Yang\*, Xiaolin Qin, Qin Wang and Yu Huang\*

# Synthesis and antitumor activities of piperazine- and cyclen-conjugated dehydroabietylamine derivatives

DOI 10.1515/hc-2015-0025

Received March 7, 2015; accepted June 29, 2015; previously published online July 25, 2015

**Abstract:** A series of piperazine- and cyclen-conjugated dehydroabietylamine derivatives were synthesized and characterized by  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, and HRMS. The *in vitro* antitumor activities of conjugates **10–13** against MCF-7 and HepG-2 tumor cell lines were evaluated using CCK-8 assay. The results show that the synthesized compounds cause a dose-dependent inhibition of cell proliferation and display different antitumor activities with the  $\text{IC}_{50}$  values ranging from 23.56 to 78.92  $\mu\text{M}$ . Moreover, the antitumor activity of conjugate **10** against the MCF-7 cell line is superior to that of the positive control 5-fluorouracil. In addition, flow cytometric assay revealed that the representative conjugate **10** could induce apoptosis in MCF-7 tumor cells in a dose-dependent manner.

**Keywords:** antitumor activity; conjugate; cyclen; dehydroabietylamine; piperazine.

## Introduction

Cancer remains the primary cause of death due to the lack of effective drugs [1]. Natural compounds have played an important role in anticancer drug discovery, where the fraction of the drugs derived from natural products amounts to 60% [2]. Dehydroabietylamine is an abietane diterpenic amine that is obtained as a part of a mixture of amines derived from rosin. Recent studies have found that dehydroabietylamine derivatives demonstrate broad biological activities, such as antibacterial, antiinflammatory,

antioxidative, and antitumor activities [3–12]. These results arouse our interest in screening for new potential antitumor drugs by the introduction of various functional groups to the dehydroabietylamine skeleton.

A number of piperazine and 1,4,7,10-tetraazacyclododecane (cyclen) heterocyclic derivatives as chemotherapeutic drugs have attracted considerable attention during the past decade. Studies have shown that the introduction of piperazine and cyclen moieties can modulate the physicochemical properties and enhance the bioactivity of the compounds [13–21]. However, the studies on the synthesis and antitumor activities of heterocyclic derivatives derived from dehydroabietylamine have not been reported. Our present work is to design and synthesize a series of piperazine- and cyclen-conjugated dehydroabietylamine derivatives and to evaluate *in vitro* antitumor activities of these conjugates against HepG-2 and MCF-7 cells. Furthermore, the apoptotic effect induced by the representative conjugates is also investigated by flow cytometry.

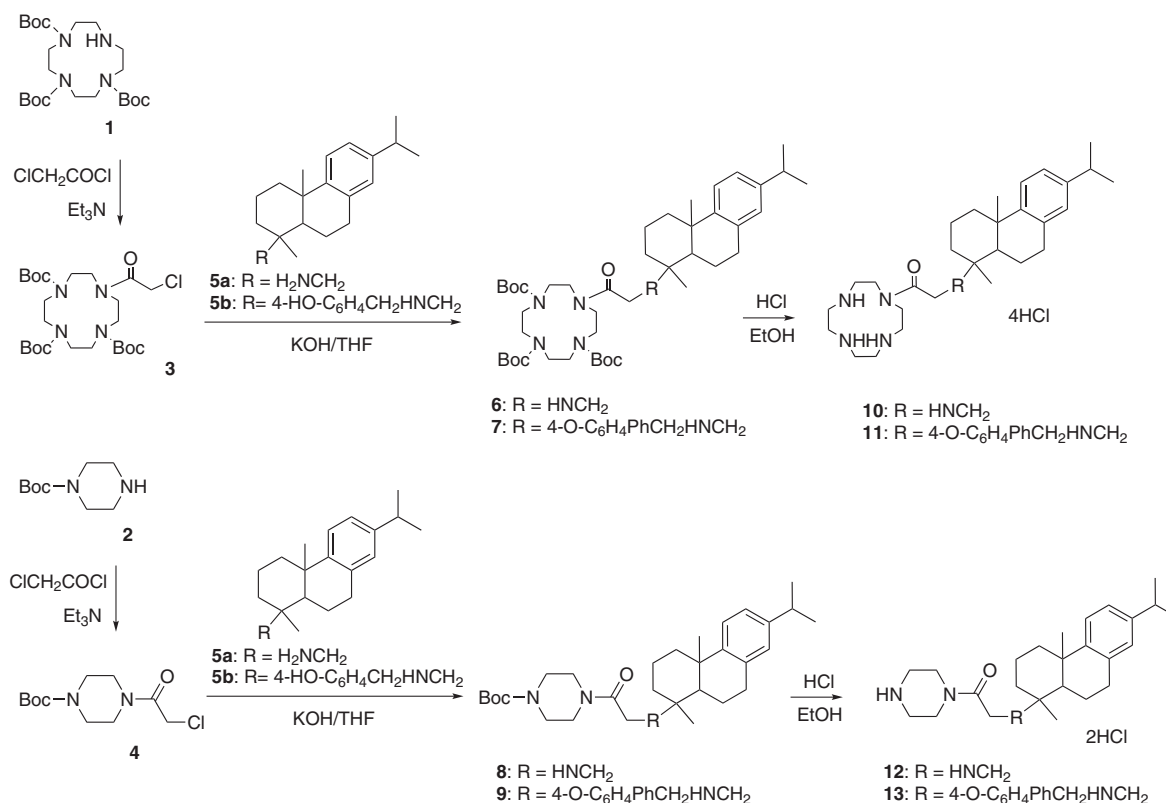
## Results and discussion

The synthetic route to the target conjugates **10–13** is shown in Scheme 1. The reaction between tri-Boc-protected cyclen **1** or Boc-protected piperazine **2** and chloroacetyl chloride afforded the respective products **3** and **4**. Compound **6** was obtained by the reaction between **3** and **5a** in the presence of KOH. Compounds **7–9** were synthesized using a similar methodology. The target compounds **10–13** were obtained by deprotection of Boc group in HCl-ethanol solution. All these products exhibit good water solubility. The structures of all synthesized conjugates were confirmed by  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, and HRMS.

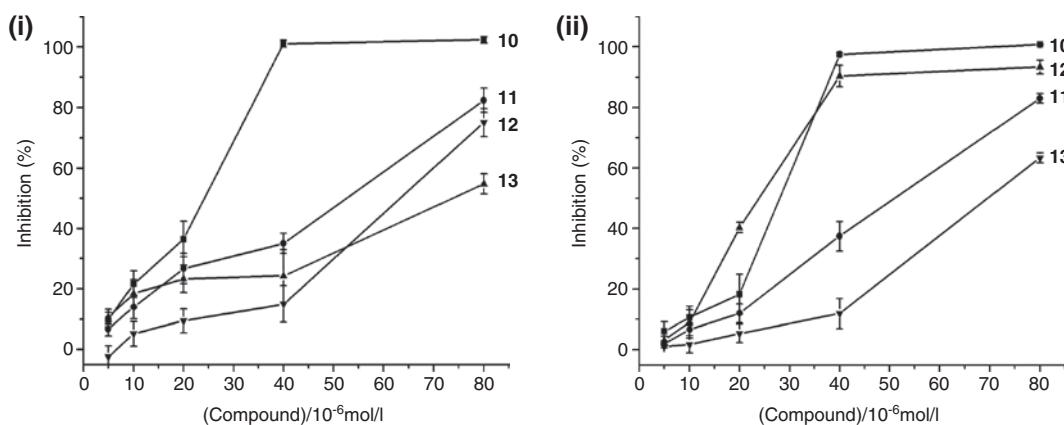
The *in vitro* antitumor activities of the conjugates **10–13** were evaluated by means of CCK-8 assay against MCF-7 and HepG-2 tumor cell lines. The inhibition rates of cell viability with different concentrations of conjugates are shown in Figure 1, and the  $\text{IC}_{50}$  values are given in Table 1. It can be seen that the treatment with increasing doses of all conjugates causes a dose-dependent inhibition of cell proliferation. As

\*Corresponding authors: Xinbin Yang, Rongchang Campus, Southwest University, Chongqing 402460, P. R. China, e-mail: yangxbqq@126.com; and Yu Huang, Pharmacy College, Ningxia Medical University, Yinchuan 750004, P. R. China, e-mail: huangyuxmu@163.com

Xiaolin Qin and Qin Wang: Rongchang Campus, Southwest University, Chongqing 402460, P. R. China



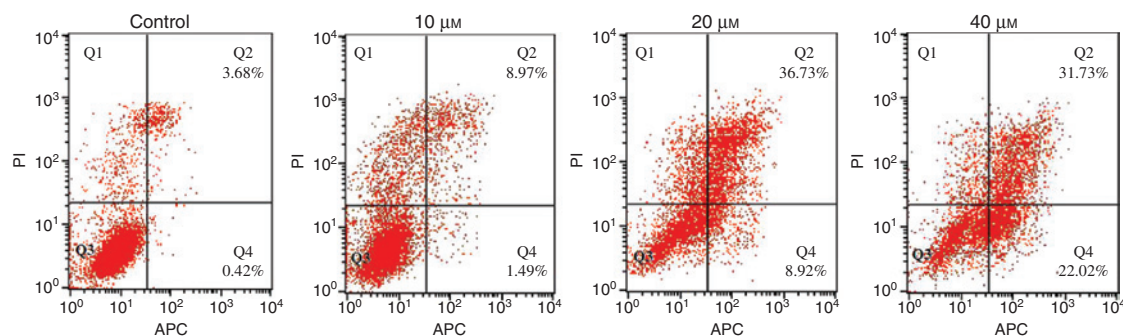
Scheme 1

**Figure 1** Antiproliferation effect of conjugates **10**, **11**, **12**, and **13** against MCF-7 (i) and HepG-2 (ii) tumor cells.

Cells were plated and incubated with the indicated concentrations of **10**, **11**, **12**, and **13** (5, 10, 20, 40, and 80  $\mu\text{M}$ ). After 24 h of treatment, cell proliferation was measured by the CCK-8 assay. Data represent the means  $\pm$  SD of triplicate experiments.

**Table 1** The  $\text{IC}_{50}$  values of the conjugates **10–13** and **5-FU** against tumor cell lines.

| Cell line | $\text{IC}_{50}$ value ( $\mu\text{M}$ ) |                  |                  |                  |                  |
|-----------|------------------------------------------|------------------|------------------|------------------|------------------|
|           | <b>10</b>                                | <b>11</b>        | <b>12</b>        | <b>13</b>        | <b>5-FU</b>      |
| MCF-7     | 25.42 $\pm$ 2.04                         | 49.61 $\pm$ 3.26 | 62.55 $\pm$ 4.01 | 78.92 $\pm$ 2.68 | 27.12 $\pm$ 1.95 |
| HepG-2    | 27.05 $\pm$ 1.88                         | 52.63 $\pm$ 2.94 | 23.56 $\pm$ 1.45 | 71.96 $\pm$ 2.33 | 6.78 $\pm$ 0.65  |



**Figure 2** Apoptosis ratio of conjugate **10** as detected by Annexin V-APC/PI double-staining assay of MCF-7 cells.

The MCF-7 cells were treated with 10, 20, and 40  $\mu\text{M}$  of conjugate **10** for 24 h. Four quadrant images were observed by flow cytometric analysis: the Q1 area represents damaged cells appearing in the process of cell collection, the Q2 region shows necrotic cells and later-period apoptotic cells, the normal cells are located in the Q3 area, and the Q4 area shows early apoptotic cells.

evident from the obtained results, conjugate **10** with cyclen moiety is the most potent cytotoxic agent against MCF-7 and HepG-2 cells among the tested conjugates. Compound **10** displays a prominent inhibitory activity by almost 100% at 40  $\mu\text{M}$ , with the  $\text{IC}_{50}$  value of 25.42  $\mu\text{M}$  for MCF-7 cell and 27.05  $\mu\text{M}$  for HepG-2 cell. Furthermore, the effect of conjugate **10** against the MCF-7 tumor cell line is slightly superior to that of the positive control 5-fluorouracil (5-FU), as indicated by the  $\text{IC}_{50}$  values. In addition, conjugate **12** with piperazine moiety shows selectivity for HepG-2 over MCF-7 with the corresponding  $\text{IC}_{50}$  values of 23.56 and 62.55  $\mu\text{M}$ . Meanwhile, conjugates **11** and **13** containing benzene ring as part of the R group exhibit only mild cytotoxic activities against MCF-7 and HepG-2 cells.

The effect of conjugate **10** on apoptosis in MCF-7 cell was investigated. Apoptosis assay may provide preliminary information about the mechanism of growth inhibition of tumor cells. The apoptosis ratios (including the early and late apoptosis rates) induced by conjugate **10** in MCF-7 cell lines were determined by flow cytometry. The results are given in Figure 2. The apoptotic rate was 4.10% of the total number of cells in the control group. The percentage of apoptotic cells were increased to 10.46, 45.65, and 53.75% by the treatment with 10, 20, and 40  $\mu\text{M}$  conjugate **10**, respectively. The results indicate that apoptosis induction of the conjugate **10** in MCF-7 tumor cells changes in a dose-dependent manner.

## Conclusion

A series of cyclic polyamine-dehydroabietylamine conjugates **10–13** were synthesized and characterized. The *in vitro* antitumor activities of these compounds against HepG-2 and MCF-7 cells were evaluated. The effect of

conjugate **10** against the MCF-7 cells was slightly superior to that of the positive control 5-FU. In addition, flow cytometric assay indicated that the representative conjugate **10** induces apoptosis in MCF-7 cells in a dose-dependent manner. These results encourage us to synthesize additional new dehydroabietylamine derivatives with the expected more potent antitumor activity.

## Experimental

All reagents were purchased from commercial sources and used without further purification. The HepG-2 (liver hepatocellular carcinoma cell) and MCF-7 (human breast adenocarcinoma cell) were obtained from ATCC. High resolution mass spectrometry (HRMS) data were recorded on a Bruker Daltonics Bio TOF instrument.  $^1\text{H}$  NMR (400 MHz) and  $^{13}\text{C}$  NMR (100 MHz) spectra were measured on a Varian INOVA-400 spectrometer. Flow cytometry was performed using a BD FASaria Cell Sorter.

### Preparation of compound **5b**

The ethanol solution (30 mL) of dehydroabietylamine (**5a**, 1.45 g, 5 mmol) and *p*-hydroxybenzaldehyde (0.61 g, 5 mmol) was heated under reflux for 4 h. After cooling, sodium borohydride (0.185 g, 5 mmol) was added and the mixture was stirred at room temperature for 12 h. Then, the mixture was concentrated under reduced pressure and quenched with water (10 mL). The aqueous phase was extracted with ethyl acetate (3×30 mL). The solvent was removed under reduced pressure and the residue was purified by column chromatography eluting with petroleum ether/ethyl acetate, 3:1, to give product **5b** as a white solid: yield 72%; mp 143–145°C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  0.95 (s, 3H), 1.21 (s, 3H), 1.26 (d, 6H,  $J = 12$  Hz), 1.35–1.79 (m, 8H), 2.24–2.31 (m, 2H), 2.49 (d, H,  $J = 10.8$  Hz), 2.80–2.84 (m, 3H), 3.63–3.72 (m, 2H), 6.72 (d, 2H,  $J = 8.0$  Hz), 6.88 (s, 1H), 6.98 (d, 1H,  $J = 8.0$  Hz), 7.14 (d, 2H,  $J = 7.0$  Hz), 7.17 (d, H,  $J = 7.0$  Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  154.8, 147.5, 145.4, 134.8, 132.3, 129.4, 126.8, 124.3, 123.8, 115.3, 60.8, 54.1, 45.5, 38.4, 37.4, 36.9, 36.2, 33.4, 30.3, 25.4, 24.0, 19.3, 18.8, 18.7. ESI-HRMS. Anal. Calcd for  $\text{C}_{27}\text{H}_{38}\text{NO}$  ( $[\text{M}+\text{H}]^+$ ):  $m/z$  392.2953. Found:  $m/z$  392.2956.

## Preparation of compounds 3 and 4

Compound **3** was prepared according to the literature [22] by the reaction of **1** with chloroacetyl chloride in the presence of Et<sub>3</sub>N at 0°C. Purification by silica gel column chromatography eluting with petroleum ether/ethyl acetate, 1:1, gave product **3** as a white solid: yield 65%; <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 1.46–1.49 (s, 27H, Boc-H), 3.38–3.56 (m, 16H, CH<sub>2</sub>), 4.06 (s, 2H); MS-ESI: *m/z* 548 (M<sup>+</sup>).

Compound **4** was synthesized using a similar procedure: yield 77%; <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 1.47 (s, 9H, Boc-H), 3.43–3.61 (m, 8H, CH<sub>2</sub>), 4.05 (s, 2H); ESI-MS: *m/z* 262 (M<sup>+</sup>).

## General procedure for the preparation of compounds 6–9

The tetrahydrofuran (THF) solution (30 mL) of **3** or **4** (0.5 mmol), **5a** or **5b** (0.5 mmol), and KOH (0.084 g, 1.5 mmol) was stirred at 60°C for 10 h. After cooling, the mixture was filtered and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography eluting with petroleum ether/ethyl acetate, 1:1, to give desired product **6–9** as a white solid.

**Compound 6** This compound was synthesized from **3** and **5a**: yield 75%; mp 108–110°C; <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 0.95 (s, 3H), 1.21 (s, 3H), 1.26 (d, 6H, *J* = 12 Hz), 1.35–1.75 (m, 35H), 2.17–2.31 (m, 2H), 2.50 (d, H, *J* = 10.8 Hz), 2.79–2.87 (m, 3H), 3.38–3.47 (m, 18H), 6.86 (s, 1H), 6.98 (d, 1H, *J* = 8.0 Hz), 7.17 (d, H, *J* = 8.0 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 170.0, 155.4, 147.4, 145.3, 134.8, 126.7, 124.3, 123.7, 80.4, 62.1, 51.7, 51.5, 49.7, 49.6, 45.5, 38.4, 37.4, 37.0, 36.1, 33.4, 30.3, 28.5, 25.3, 24.0, 19.3, 19.1, 18.8. ESI-HRMS. Calcd for C<sub>45</sub>H<sub>76</sub>N<sub>5</sub>O<sub>7</sub> ([M+H]<sup>+</sup>): *m/z* 798.5745. Found: *m/z* 798.5736.

**Compound 7** This compound was synthesized from **3** and **5b**: yield 68%; mp 99–101°C; <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 0.95 (s, 3H), 1.21 (s, 3H), 1.26 (d, 6H, *J* = 12 Hz), 1.34–1.73 (m, 35H), 2.17–2.27 (m, 2H), 2.50 (d, H, *J* = 10.8 Hz), 2.80–2.85 (m, 3H), 3.34–3.57 (m, 16H), 3.71–3.73 (m, 2H), 4.64 (s, 2H), 6.87 (d, 2H, *J* = 4.4 Hz), 6.90 (s, 1H), 6.98 (d, 1H, *J* = 8.0 Hz), 7.14 (d, H, *J* = 7.2 Hz), 7.21 (d, 2H, *J* = 6.0 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 170.1, 157.0, 155.6, 147.5, 145.4, 134.8, 132.3, 129.2, 126.7, 124.3, 123.7, 114.6, 80.5, 67.0, 60.6, 51.4, 50.4, 49.9, 49.6, 45.3, 38.5, 37.4, 36.9, 36.2, 33.4, 30.9, 28.5, 25.4, 24.0, 19.3, 18.8, 18.7. ESI-HRMS. Calcd for C<sub>52</sub>H<sub>82</sub>N<sub>5</sub>O<sub>8</sub> ([M+H]<sup>+</sup>): *m/z* 904.6163. Found: *m/z* 904.6166.

**Compound 8** This compound was synthesized from **4** and **5a**: yield 80%; mp 117–119°C; <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 0.93 (s, 3H), 1.18 (s, 3H), 1.26 (d, 6H, *J* = 12 Hz), 1.38–1.77 (m, 17H), 2.27–2.31 (m, 2H), 2.60 (d, H, *J* = 10.8 Hz), 2.81–2.87 (m, 3H), 3.02–3.20 (m, 8H), 3.45–3.51 (m, 2H), 6.86 (s, 1H), 6.95 (d, 1H, *J* = 8.0 Hz), 7.17 (d, H, *J* = 8.0 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 170.0, 154.5, 147.4, 145.4, 134.7, 126.7, 124.2, 123.7, 80.3, 62.1, 51.9, 45.5, 44.4, 41.6, 39.5, 38.4, 37.4, 36.2, 33.4, 30.2, 28.3, 25.6, 24.0, 19.1, 18.8, 18.4. ESI-HRMS. Anal. Calcd for C<sub>31</sub>H<sub>50</sub>N<sub>3</sub>O<sub>3</sub> ([M+H]<sup>+</sup>): *m/z* 512.3852. Found: *m/z* 512.3851.

**Compound 9** This compound was synthesized from **4** and **5b**: yield 78%; mp 112–114°C; <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 0.90 (s, 3H), 1.21 (s, 3H), 1.26 (d, 6H, *J* = 12 Hz), 1.37–1.65 (m, 17H), 2.17–2.28 (m, 2H), 2.51 (d, H, *J* = 12 Hz), 2.82–2.86 (m, 3H), 3.40–3.57 (m, 8H), 3.68–3.70 (m, 2H), 4.68 (s, 2H), 6.87 (d, 2H, *J* = 8.4 Hz), 6.90 (s, 1H), 6.98 (d, 1H, *J* = 8.0 Hz), 7.17 (d, H, *J* = 8.0 Hz), 7.24 (d, 2H, *J* = 8.4 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 166.8,

156.5, 154.5, 147.5, 145.4, 134.8, 132.2, 129.3, 126.7, 124.2, 123.7, 114.3, 80.3, 68.0, 60.8, 54.0, 45.3, 45.2, 42.0, 38.5, 37.4, 37.0, 36.2, 33.4, 30.3, 28.3, 25.3, 23.9, 19.3, 18.8, 18.7. ESI-HRMS. Anal. Calcd for C<sub>38</sub>H<sub>56</sub>N<sub>3</sub>O<sub>4</sub> ([M+H]<sup>+</sup>): *m/z* 618.4271. Found: *m/z* 618.4274.

## General procedure for the preparation of compounds 10–13

To a stirred solution of **6**, **7**, **8**, or **9** (0.3 mmol) in ethanol (10 mL) at room temperature was slowly added 5 mL of 3 M HCl in ethanol solution. After stirring overnight, the reaction mixture was concentrated under reduced pressure. The residue was washed by anhydrous ether to furnish a hydrochloride salt of **10**, **11**, **12**, or **13** as a white powder.

**Compound 10** Yield 87%; mp 224–226°C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 0.99 (s, 3H), 1.14 (s, 3H), 1.21 (d, 6H, *J* = 6.8 Hz), 1.50–1.72 (m, 8H), 2.17–2.31 (m, 2H), 2.50 (d, H, *J* = 10.8 Hz), 2.79–2.87 (m, 3H), 3.02–3.19 (m, 16H), 3.44–3.51 (m, 2H), 6.86 (s, 1H), 6.98 (d, 1H, *J* = 7.6 Hz), 7.17 (d, H, *J* = 8.0 Hz); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): δ 166.9, 146.7, 145.1, 134.2, 126.3, 123.8, 123.5, 58.7, 50.8, 48.2, 46.2, 45.3, 44.4, 43.1, 37.3, 36.9, 36.0, 34.6, 32.8, 28.7, 24.9, 23.9, 18.2, 18.0. ESI-HRMS. Anal. Calcd for C<sub>30</sub>H<sub>52</sub>N<sub>5</sub>O ([M+H]<sup>+</sup>): *m/z* 498.4172. Found: *m/z* 498.4174.

**Compound 11** Yield 73%; mp 244–246°C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 0.89 (s, 3H), 1.13 (s, 3H), 1.20 (d, 6H, *J* = 12 Hz), 1.38–1.61 (m, 8H), 2.24–2.28 (m, 2H), 2.51 (d, H, *J* = 12 Hz), 2.73–2.80 (m, 3H), 3.05–3.16 (m, 16H), 3.43–3.58 (m, 2H), 4.91 (s, 2H), 6.85 (s, 1H), 6.96 (d, 1H, *J* = 4.0 Hz), 7.03 (d, 2H, *J* = 8.0 Hz), 7.13 (d, H, *J* = 8.0 Hz), 7.46 (d, 2H, *J* = 8.0 Hz); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): δ 168.8, 158.8, 146.6, 145.2, 134.1, 131.8, 129.0, 126.2, 123.8, 123.4, 115.0, 65.7, 56.1, 50.4, 45.6, 44.6, 44.0, 42.9, 42.5, 37.4, 36.9, 35.7, 34.7, 32.8, 28.6, 24.7, 23.9, 18.1, 18.0. ESI-HRMS. Anal. Calcd for C<sub>37</sub>H<sub>58</sub>N<sub>5</sub>O<sub>2</sub> ([M+H]<sup>+</sup>): *m/z* 604.4591. Found: *m/z* 604.4593.

**Compound 12** Yield 75%; mp 194–196°C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 0.99 (s, 3H), 1.14 (s, 3H), 1.21 (d, 6H, *J* = 12 Hz), 1.47–1.72 (m, 8H), 2.27–2.31 (m, 2H), 2.76 (d, H, *J* = 8.0 Hz), 2.81–2.90 (m, 3H), 3.05–3.18 (m, 8H), 3.55–3.70 (m, 2H), 6.87 (s, 1H), 6.95 (d, 1H, *J* = 7.2 Hz), 7.17 (d, H, *J* = 8.0 Hz); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): δ 163.7, 146.7, 145.1, 134.4, 126.3, 123.8, 123.5, 58.7, 47.5, 44.2, 42.1, 41.1, 38.1, 37.3, 37.0, 36.1, 34.8, 32.9, 28.8, 25.3, 23.9, 18.6, 18.2. ESI-HRMS. Anal. Calcd for C<sub>26</sub>H<sub>42</sub>N<sub>3</sub>O ([M+H]<sup>+</sup>): *m/z* 412.3328. Found: *m/z* 412.3329.

**Compound 13** Yield 70%; mp 249–251°C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 0.90 (s, 3H), 1.13 (s, 3H), 1.26 (d, 6H, *J* = 12 Hz), 1.39–1.68 (m, 8H), 2.24–2.28 (m, 2H), 2.51 (d, H, *J* = 12 Hz), 2.73–2.80 (m, 3H), 3.10–3.32 (m, 8H), 3.65–3.70 (m, 2H), 4.92 (s, 2H), 6.85 (s, 1H), 6.94 (d, 1H, *J* = 4.0 Hz), 7.01 (d, 2H, *J* = 8.4 Hz), 7.13 (d, H, *J* = 8.4 Hz), 7.45 (d, 2H, *J* = 8.4 Hz); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): δ 165.9, 158.5, 146.7, 145.2, 134.2, 131.8, 129.0, 126.3, 123.8, 123.5, 115.3, 65.4, 56.2, 50.5, 44.0, 42.5, 41.1, 38.1, 37.4, 36.9, 35.7, 34.7, 32.9, 28.6, 24.8, 23.9, 18.2, 18.0. ESI-HRMS. Anal. Calcd for C<sub>33</sub>H<sub>48</sub>N<sub>3</sub>O<sub>2</sub> ([M+H]<sup>+</sup>): *m/z* 518.3747. Found: *m/z* 518.3750.

## In vitro cytotoxicity assay

Cytotoxicities of all compounds against MCF-7 and HepG-2 cell lines were determined using a cell counting kit-8 (CCK-8) assay. The cells were plated in 96-well culture plates at density of 1×10<sup>4</sup> cells per well and incubated for 24 h at 37°C in a wet atmosphere containing 5% CO<sub>2</sub>.



The tested compound was dissolved in PBS and then the diluted solution was treated with the cells for 24 h at 37°C in a 5% CO<sub>2</sub> incubator; 5-FU was used as a positive control. Then, 10 µL of a freshly diluted CCK-8 solution [5 mg/mL in phosphate buffer saline (PBS)] was added to each well for 2 h. The cell survival was evaluated by measuring the absorbance at 450 nm. The IC<sub>50</sub> value, which indicates the inhibition growth of 50% of cells relative to non-treated control cells, was calculated as the concentration of tested compound by best fit curving estimation. All experiments were carried out in triplicate.

### Assessment of cell apoptosis

The MCF-7 cells were plated in 6-well culture plates at density of 3×10<sup>5</sup> cells per well. The cells were incubated with different concentrations of compound **10** for 24 h. The cultured MCF-7 cells were washed twice with PBS (pH 7.4) and then resuspended gently in 400 µL of binding buffer. The cell solution was then stained with Annexin V-APC/PI apoptosis Kit according to the protocol of the company.

**Acknowledgments:** This study was financially supported by the National Science Foundation of China (No. 21172182 and 21362026).

## References

- [1] Son, K. H.; Oh, H. M.; Choi, S. K.; Han, D. C.; Kwon, B. M. Anti-tumor abietane diterpenes from the cones of *Sequoia sempervirens*. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2019–2021.
- [2] Newman, D. J.; Cragg, G. M.; Snader, K. M. Natural products as sources of new drugs over the period 1981–2002. *J. Nat. Prod.* **2003**, *66*, 1022–1037.
- [3] Gonzalez, M. A. Synthetic derivatives of aromatic abietane diterpenoids and their biological activities (Mini-review). *Eur. J. Med. Chem.* **2014**, *87*, 834–842.
- [4] Goodson, B.; Ehrhardt, A.; Ng, S.; Nuss, J.; Johnson, K.; Giedlin, M.; Yamamoto, R.; Moos, W. H.; Krebber, A.; Ladner, M.; et al. Characterization of novel antimicrobial peptoids. *Antimicrob. Agents Chemother.* **1999**, *43*, 1429–1434.
- [5] Wilkerson, W. W.; Galbraith, W.; DeLucca, I.; Harris, R. R. Topical antiinflammatory dehydroabietylamine derivatives. IV. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2087–2092.
- [6] Lu, Z.; Liu, C. X.; Yu, X.; Lin, Z. X. Synthesis and anti-free radical activities of several novel derivatives of dehydroabietylamine. *Chin. J. Org. Chem.* **2013**, *33*, 562–567.
- [7] Rao, X. P.; Z. Song, Z. Q.; He, L. Synthesis and antitumor activity of novel aminophosphonates from diterpenic dehydroabietylamine. *Heteroatom Chem.* **2008**, *19*, 512–516.
- [8] Lin, L. Y.; Bao, Y. L.; Chen, Y.; Sun, L. G.; Yang, X. G.; Liu, B.; Lin, Z. X.; Zhang, Y. W.; Yu, C. L.; Wu, Y.; et al. *N*-Benzoyl-12-nitrodehydroabietylamine-7-one, a novel dehydroabietylamine derivative, induces apoptosis and inhibits proliferation in HepG2 cells. *Chem. Biol. Interact.* **2012**, *199*, 63–73.
- [9] Chen, Y.; Lin, Z. X.; Zhou, A. M. Synthesis and antitumor activities of a novel class of dehydroabietylamine derivatives. *Nat. Prod. Res.* **2012**, *26*, 2188–2195.
- [10] Xing, Y. H.; Zhang, W.; Song, J. J.; Zhang, Y. X.; Jiang, X. X.; Wang, R. Anticancer effects of a novel class rosin-derivatives with different mechanisms. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 3868–3872.
- [11] Huang, X. C.; Wang, M.; Wang, H. S.; Chen, Z. F.; Zhang, Y.; Pan, Y. M. Synthesis and antitumor activities of novel dipeptide derivatives derived from dehydroabietic acid. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 1511–1518.
- [12] Huang, X. C.; Jin, L.; Wang, M.; Liang, D.; Chen, Z. F.; Zhang, Y.; Pan, Y. M.; Wang, H. S. Design, synthesis and *in vitro* evaluation of novel dehydroabietic acid derivatives containing a dipeptide moiety as potential anticancer agents. *Eur. J. Med. Chem.* **2014**, *89*, 370–385.
- [13] Kong, D. Y.; Qin, C.; Meng, L. H.; Xie, Y. Y. Synthesis, structural characterization and antitumor activity evaluations of copper complex with tetraazamacrocyclic ligand. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1087–1092.
- [14] Cholody, W. M.; Hernandez, L.; Hassner, L.; Scudiero, D. A.; Djurickovic, D. B.; Michejda, C. J. Cholody, W. M.; Hernandez, L.; Hassner, L.; Scudiero, D. A.; et al. Bisimidozaoacridones and related compounds: new antineoplastic agents with high selectivity against colon tumors. *J. Med. Chem.* **1995**, *38*, 3043–3052.
- [15] Rokosz, L. L.; Huang, C. Y.; Reader, J. C.; Stauffer, T. M.; Chelsky, D.; Sigal, N. H.; Ganguly, A. K.; Baldwin, J. J. Surfing the piperazine core of tricyclic farnesyltransferase inhibitors. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5537–5543.
- [16] Chen, J. J.; Lu, M.; Jing, Y. K.; Dong, J. H. The synthesis of l-carvone and limonene derivatives with increased antiproliferative effect and activation of ERK pathway in prostate cancer cells. *Bioorg. Med. Chem.* **2006**, *14*, 6539–6547.
- [17] Zeng, S.; Liu, W.; Nie, F. F.; Zhao, Q.; Rong, J. J.; Wang, J.; Tao, L.; Qi, Q.; Lu, N.; Li, Z. Y.; et al. LYG-202, a new flavonoid with a piperazine substitution, shows antitumor effects *in vivo* and *in vitro*. *Biochem. Biophys. Res. Commun.* **2009**, *38*, 551–556.
- [18] Kamal, A.; Kumar, P. P.; Seshadri, B. N.; Srinivas, O.; Kumar, M. S.; Sen, S.; Kurian, N.; Juvekar, A. S.; Zingde, S. M. Phosphonate-linked pyrrolo[2,1-c][1,4]benzodiazepine conjugates: synthesis, DNA-binding affinity and cytotoxicity. *Bioorg. Med. Chem.* **2008**, *14*, 3895–3906.
- [19] Liu, M. C.; Yang, S. J.; Jin, L. H.; Hu, D. Y. Xue, W.; Song, B. A. Yang, S. Synthesis and cytotoxicity of novel ursolic acid derivatives containing an acyl piperazine moiety. *Eur. J. Med. Chem.* **2012**, *58*, 128–135.
- [20] Kong, D.; Meng, L.; Ding, J.; Xie, Y.; Huang, X. New tetraazamacrocyclic ligand with neutral pendent groups 1,4,7,10-tetrakis (2-cyanoethyl)-1,4,7,10-tetraazacyclododecane (L) and its cobalt(II), nickel(II) and copper(II) complexes: synthesis, structural characterization and antitumor activity. *Polyhedron* **2000**, *19*, 217–223.
- [21] Zhang, J. X.; Li, H. G.; Chan, C. F.; Lan, R. F.; Chan, W. L.; Law, G. L.; Wong, W. K.; Wong, K. L. A potential water-soluble ytterbium-based porphyrin–cyclen dual bio-probe for Golgi apparatus imaging and photodynamic therapy. *Chem. Commun.* **2012**, *48*, 9646–9648.
- [22] Yang, X. B.; Feng, J.; Zhang, J.; Zhang, Z. W.; Lin, H. H.; Zhou, L. H.; Yu, X. Q. Synthesis, DNA binding and cleavage activities of the copper (II) complexes of estrogen-macrocyclic polyamine conjugates. *Bioorgan. Med. Chem.* **2008**, *16*, 3871–3877.