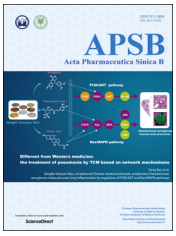
Acta Pharmaceutica Sinica B 2016;6(3):222–228

Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

www.elsevier.com/locate/apsb
www.sciencedirect.com



ORIGINAL ARTICLE

Synthesis and biological evaluation of 12-*N*-*p*-chlorobenzyl sophoridinol derivatives as a novel family of anticancer agents

Chongwen Bi[a,b], Cheng Ye[a], Yinghong Li[a], Wuli Zhao[a], Rongguang Shao[a], Danqing Song[a,*]

[a]*Institute of Medicinal Biotechnology, Chinese Academy of Medical Science & Peking Union Medical College, Beijing 100050, China*
[b]*Tianjin Medical University General Hospital, Tianjin 300052, China*

Received 16 February 2016; received in revised form 7 March 2016; accepted 10 March 2016

KEY WORDS

Sophoridinic derivatives;
Synthesis;
Structure–activity relationship;
Anticancer;
Drug resistance

Abstract Taking 12-*N*-*p*-chlorobenzyl sophoridinol **2** as a lead, a series of novel sophoridinic derivatives with various 3′-substituents at the 11–side chain were synthesized and evaluated for their anticancer activity from sophoridine (**1**), a natural antitumor medicine. Among them, the sophoridinic ketones **5a–b**, alkenes **7a–b** and sophoridinic amines **14a–b** displayed reasonable antiproliferative activity with IC_{50} values ranging from 3.8 to 5.4 μmol/L. Especially, compounds **5a** and **7b** exhibited an equipotency in both adriamycin (AMD)-susceptible and resistant MCF-7 breast carcinoma cells, indicating a different mechanism from AMD. The primary mechanism of action of **5a** was to arrest the cell cycle at the G0/G1 phase, consistent with that of parent compound **1**. Thus, we consider 12-chlorobenzyl sophoridinic derivatives with a tricyclic scaffold to be a new class of promising antitumor agents with an advantage of inhibiting drug-resistant cancer cells.

*Corresponding author. Tel./fax: +86 10 63165268.
E-mail address: songdanqingsdq@hotmail.com (Danqing Song).

Peer review under responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association.

http://dx.doi.org/10.1016/j.apsb.2016.03.004

1. Introduction

Several quinolizidine alkaloids extracted from Chinese herb Kushen have demonstrated potential antitumor activity[1–4]. Sophoridine (**1**, Fig. 1), the main active ingredient of Kushen extracts, was approved by the Chinese FDA to treat malignant neoplasm in 2005[5,6]. Its mechanism is to inhibit the DNA topoisomerase I (topo I) activity and induce cell cycle arrest at the G0/G1 phase[7–9]. From compound **1**, as depicted in Fig. 1, we have successfully identified that 12-*N*-substitued sophoridinol derivatives were a promising class of anticancer agents with a novel chemical scaffold and ideal druggable parameters[10,11]. A representative compound, 12-*N*-*p*-chlorobenzyl sophoridinol (**2**, Fig. 1) displayed a reasonable antiproliferative activity with an IC_{50} of 9.3 μmol/L, much better than that of the parent **1** (>80 μmol/L)[10]. Furthermore, it has a couple of advantages such as special scaffold, flexibility structure, high solubility and good safety profiles. In addition, it has an excellent potential for further chemical modifications and optimization[10,11].

The previous structure–activity relationship (SAR) revealed that 12-*N*-*p*-cholobenzyl substituent was beneficial for the antitumor activity[10]. Thus, taking compound **2** as a lead, SAR investigation was continuously developed on the variations of 3′-CH_2OH at the 11–side chain in an effort to discover more potent antitumor candidates. In the present study, as illustrated in Fig. 1, the 12-*N*-*p*-cholobenzyl was retained as a required group for activity, and 3′-CH_2OH at 11–attachment was replaced with various substituents and different sophoridinic derivatives were produced. Based on this strategy, a series of sophoridinic ketone, alkene, imine and amine analogs were then constructed and measured. Herein, presented in this study were the synthesis, antitumor assessment, SAR analysis and primary mechanism of action of the representative compounds.

2. Results and discussion

2.1. Synthetic routes

The synthesis of all the newly synthesized compounds was respectively described in Schemes 1 and 2. Each target compound was prepared using commercially available **1** with purity over 98% as the starting material, which was purchased from the Yanchi Dushun Biological and Chemical Co., Ltd. (Shanxi, China).

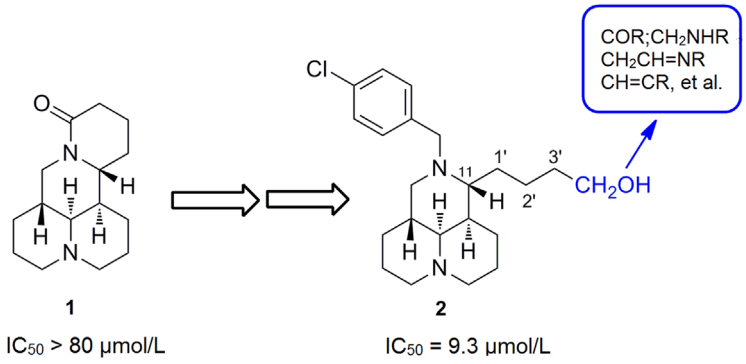
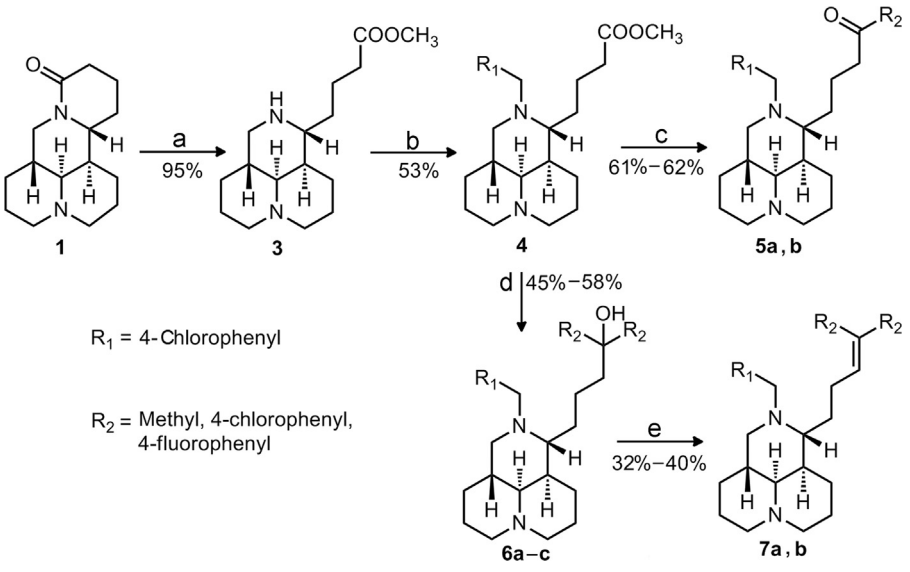
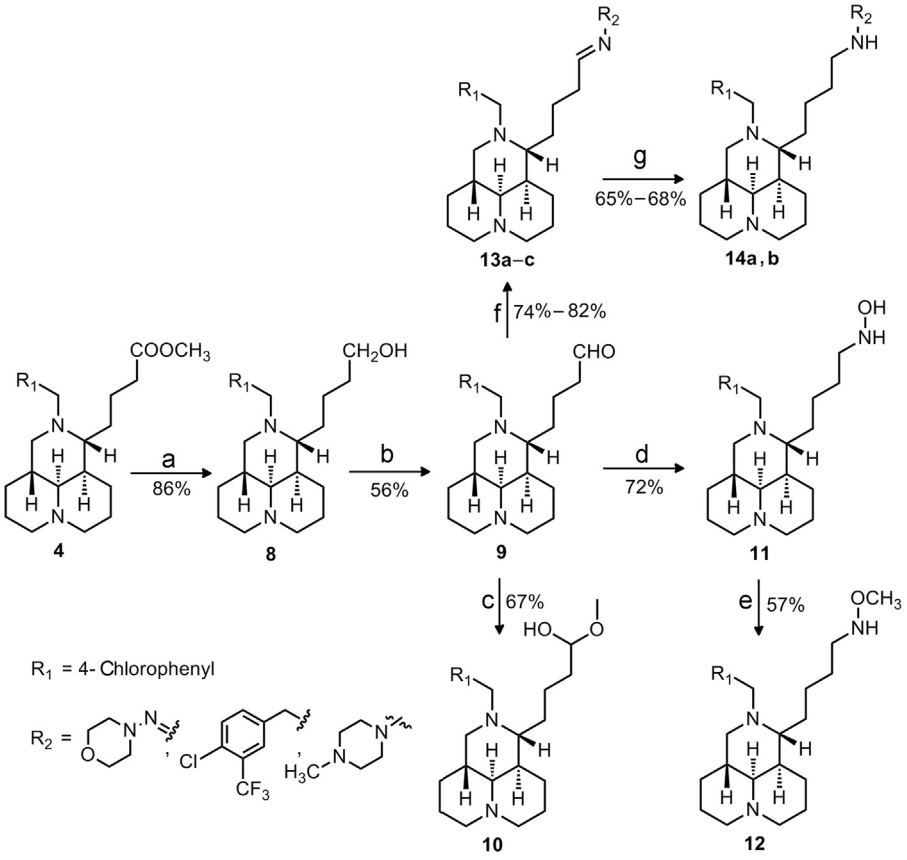


Figure 1 Chemical structures of sophoridine (**1**) 12-*N*-*p*-cholobenzyl sophoridinol (**2**) and the structural fragments in **2** to be modified.



Scheme 1 Reagents and conditions: (a) HCl, reflux, 6 h; CH_3OH, r.t., 5–6 h, in 2 steps; (b) R_1CHO, TEA, 1,2-dichloroethane, reflux, 4 h; STB, reflux, 4 h, in 2 steps; (c) R_2MgBr (1.0 equiv.), THF, r.t., 7 h; (d) R_2MgBr (excessive), THF, reflux, 7 h; (e) hydrochloride/ether, r.t., 4 h.



Scheme 2 Reagents and conditions: (a) $LiAlH_4$, THF, r.t.; (b) DMSO, $(COCl)_2$, TEA, DCM, –78 °C, 1 h; (c) HCl/MeOH, r.t., 10 min; (d) $NH_2OH.HCl$, 20% NaOH, r.t., 12 h; (e) methyl benzenesulfonate, THF, r.t.; (f) R_2NH_2, MeOH, reflux, 4 h; (g) $NaBH_3CN$, MeOH, reflux, 4 h.

The synthesis of the desired products 12-*N*-*p*-cholobenzyl sophoridinic ketones **5a**–**b** and 12-*N*-*p*-cholobenzyl sophoridinic alkenes **7a**–**b** was displayed in Scheme 1. The intermediate **3** was obtained through hydrolysis and esterization of **1** in an overall yield of 95%[10,11]. Then the condensation of **3** with 4-chlorobenzaldehyde followed by the reduction with sodium triacetoxyborohydride (STB) afforded the key intermediate **4** in 53% yield[12]. The nucleophilic-addition reaction of **4** with one molar equivalent of *p*-Cl-PhMgBr and *p*-F-PhMgBr in dry THF at room temperature provided products **5a**–**b** with yields of 61%–62%[13], while the reaction of **4** with excessive amount of Grignard reagents at reflux temperature generated tertiary alcohols **6a–c** in 45%–58% yields, which were dehydrated under acidic conditions to give the target alkenes **7a**–**b** with yields of 32%–40%.

As illustrated in Scheme 2, the reduction of **4** in the presence of $LiAlH_4$ generated sophoridinol **8** in 86% yield, which was converted to sophoridinic aldehyde **9** through a Swern oxidation reaction in 56% yield[14]. The aldol condensation of **9** in HCl/CH_3OH afforded hemiacetal **10** at room temperature with the yield of 67%, while the condensation of **9** with hydroxylamine hydrochloride afforded hydroxyl oxime **11** in a 72% yield, which went through a methyl etherification to give the oxime ether **12** in 57% yield. The sophoridinic imines **13a**–**c** were obtained *via* the condensation of **9** and three amines, which were then reduced by sodium cyanoborohydride ($NaBH_3CN$) to afford **14a**–**b** in good yields. All desired products were purified by flash chromatography using CH_2Cl_2/CH_3OH as the gradient eluent.

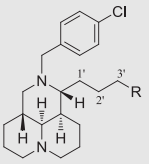
2.2. *SAR analysis for antitumor activity*

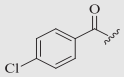
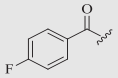
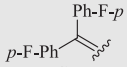
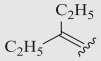
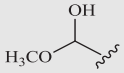
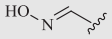
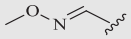
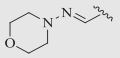
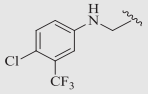
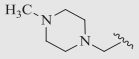
All the target compounds were evaluated for their cytotoxic activity in human HepG2 hepatoma cell line using Taxol as a positive control using the MTT assay[15]. Structures of the 11-sophoridinic derivatives and their anti-proliferative activity were shown in Table 1. The *C*log*P* values were calculated by ChemBioOffice software (Version 12.0).

Taking compound **2** as a lead, the SAR investigation was mainly focused on the variation of 3'-CH_2OH at the 11–side chain with 12-*N*-*p*-cholobenzyl as a required group for antitumor activity. Firstly, in replacing the CH_2OH group with ketones, the resultant compounds **5a**–**b** with higher lipophilicity (*C*log*P* > 7.0) displayed improved cytotoxic activity with the IC_{50} values of 4.9 μmol/L and 4.0 μmol/L, respectively. Introduction of two methyl groups on the α-carbon adjacent to the hydroxy group led to a tertiary butyric alcohol **6a,** which showed a decreased activity with an IC_{50} value of 16.3 μmol/L. The replacement of 3′-CH_2OH with an alkene group on the 3'-position created more lipophilic **7a**–**b**, which showed significant improvement on the antiproliferative activity with the IC_{50} values of 4.3 and 3.8 μmol/L, respectively, much better than that of **2** and Taxol. The results suggested that a higher *C*log*P* values might be beneficial for good potency against cancer.

Secondly, the generated hemiacetal **10**, hydroxyl oxime **11**, oxime ether **12** and imines **13a**–**c** abolished cytotoxic activity partially or completely with IC_{50} between 11.8 and > 50 μmol/L.

Table 1 SAR of the target compounds for their antiproliferative activities in HepG2 cells.



Compd.	R	IC_{50} (μmol/L)	*C*log*P*
2		9.3±0.4	4.5
5a		4.9±1.2	7.1
5b		4.0±0.9	7.0
6a		16.3±2.8	5.9
7a		4.3±1.3	10.0
7b		3.8±1.0	8.0
10		17.3±2.1	4.5
11		11.2±1.2	4.6
12		>50	4.8
13a		11.8±0.7	4.9
14a		4.3±0.1	7.6
14b		5.4±0.2	4.5
Taxol		15.6±2.3	4.7

The constructed aromatic amine **14a** and aliphatic amine **14b** showed a significantly improved activity with IC_{50} values of 4.3 and 5.4 μmol/L, respectively. It seemed that the introduction of a suitable methyleneamine group on the 3′-position might greatly enhance the activity against cancer.

Considering their potent anticancer effects as well as reasonable *C*log*P* values, compounds **5a** and **7b** were chosen as the representative compounds for further investigation.

2.3. *Anti-resistant tumor effect of **5a** and **7b***

Evaluation of compounds **5a** and **7b** against drug-resistant cancer cell lines were then carried out. We measured their activity against human wild MCF-7 and adriamycin (AMD)-resistant MCF-7 (MCF-7/AMD) breast carcinoma cells using AMD as a reference control[16]. As depicted in Fig. 2, AMD was active against wild type MCF-7, and completely inactive in the MCF-7/AMD cells. Meanwhile, compounds **5a** and **7b** were equipotent or almost equipotent in both MCF-7 cell lines, suggesting a different mode of action from AMD. As compound **5a** exhibited an equivalent antiproliferative effect against both MCF-7 cell lines, thus was selected for the next study.

2.4. *Mechanism of action of **5a***

To verify the possible change of mechanism of action after the structure modifications, flow cytometric analysis in the HepG2 cells was conducted. The HepG2 cells were treated for 24 h without or with **5a** at concentrations of 1.25, 2.5 and 5.0 μg/mL, respectively. As shown in Fig. 3, compound **5a** arrested the HepG2 cells at the G0/G1 phase as anticipated, indicating a similar mechanism of action to that of its parent compound **1**[11].

3. Conclusions

A variety of novel sophoridinic derivatives, such as sophoridinic ketones, alkenes, imines and amines, were synthesized and evaluated for their antitumor activity. SAR analysis indicated that the introduction of a suitable methyl amine group on the 3'-position could greatly improve the potency. Compounds **5a** and **7b** exhibited an equipotent effect in both AMD-susceptible and AMD-resistant breast carcinoma cells, indicating a different mechanism from AMD. The mechanism of action of **5a** was to arrest the cell cycle at the G0/G1 phase, consistent with that of **1**. The SAR results provided powerful information for further modifications and optimization of this novel scaffold to identify anticancer candidates that might be active in drug-resistant cancer cells.

4. Experimental section

4.1. *Chemistry*

Melting point (m.p.) was obtained with a CXM-300 melting point apparatus and uncorrected. The ^{1}H NMR spectra were performed

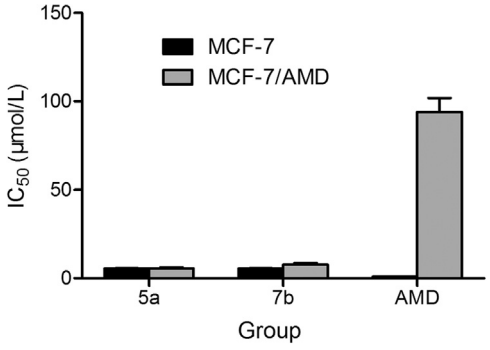


Figure 2 IC_{50} (μmol/L) value comparison of **5a** and **7b** in MCF-7 and MCF-7/AMD breast carcinoma cell lines.

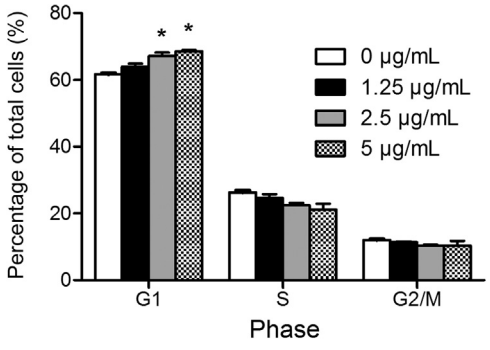


Figure 3 Blockage of cell cycle in HepG2 cells treated without or with **5a** at different concentrations for 24 h.

on a Varian Inova 400 MHz spectrometer (Varian, San Francisco, CA, USA) and ^{13}C NMR on a Bruker Avance III 400 spectrometer in CD_3OD or $(CD_3)_2SO$, using Me_4Si as a internal standard. ESI high-resolution mass spectra (HR-MS) were recorded on an Autospec UItima-TOF mass spectrometer (Micromass UK Ltd., Manchester, UK). Flash chromatography was performed on a CombiflashRf 200 machine (Teledyne, Nebraska, USA) using silica gel having particle size of 0.038 mm.

*4.1.1. General procedures for the synthesis of compound **5***

To a solution of **4** (0.50 g, 1.2 mmol) in anhydrous THF (20 mL), 1 mol/L 4-chlorophenylmagnesium bromide or 4-fluorophenylmagnesium bromide (1.2 mL) was slowly added at 0 °C and then stirred at room temperature until TLC analysis showed the completion of the reaction. Then the reaction was quenched by the addition of saturated ammonium chloride solution (5 mL). After the solvent was removed by condensation, dichloromethane (30 mL) was added, and the resultant mixture was washed by water (20 mL) and brine (20 mL), dried over anhydrous sodium sulfate, concentrated, and the residue was purified by flash column chromatography on silica gel with CH_2Cl_2/CH_3OH as the eluents to afford target compounds.

*4.1.1.1. 12-N-p-Chlorobenzyl sophoridinic-4′,4-chlorophenyl ketone (**5a**).* The title compound was prepared from **4** and 4-chlorophenylmagnesium bromide using the same manner as described above. Yield: 62%; white solid; m.p. 83–84 °C; ^1H NMR (500 MHz, DMSO-d_6) δ 7.97 (d, J = 8.1 Hz, 2H), 7.60 (d, J = 8.5 Hz, 2H), 7.38–7.29 (m, 4H), 3.61 (d, J = 14.1 Hz, 1H), 3.54–3.42 (m, 2H), 3.41–3.32 (m, 4H), 3.20–3.08 (m, 1H), 3.06–2.93 (m, 2H), 2.65–2.57 (m, 1H), 2.40–2.25 (m, 2H), 2.25–2.14 (m, 2H), 2.13–2.03 (m, 1H), 1.94–1.74 (m, 3H), 1.73–1.53 (m, 3H), 1.52–1.35 (m, 3H), 1.28–1.14 (m, 1H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 198.8, 138.6, 137.9, 135.1, 131.1, 129.8 (2), 129.75 (2), 128.7 (2), 128.1 (2), 62.2, 58.5, 56.8, 51.6, 49.9, 44.1, 38.0, 35.0, 27.3, 26.0, 22.4, 22.2, 21.6, 21.2, 17.6; HR-MS: Calcd. for $C_{28}H_{34}ON_2Cl_2$ $[M+H]^+$, 485.2121, Found, 485.2123.

*4.1.1.2. 12-N-p-Chlorobenzyl sophoridinic-4′,4-fluorophenyl ketone (**5b**).* The title compound was prepared from **4** and 4-fluorophenyl magnesium bromide using the same manner as described above. Yield: 61%, m.p.: 87–89 °C; ^1H NMR (400 MHz, DMSO-d_6) δ 7.98 (d, J = 8.5 Hz, 2H), 7.60 (d, J = 8.5 Hz, 2H), 7.43–7.20 (m, 4H), 3.74–3.43 (m, 2H), 3.41–3.32 (m, 4H), 3.20–2.87 (m, 5 H), 2.73–2.55 (m, 1H), 2.41–2.26 (m, 2H), 2.27–2.09 (m, 2H), 1.99–1.74 (m, 3 H), 1.74–1.55 (m, 3H), 1.55–1.33 (m, 3H), 1.27–1.11 (m, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 199.3, 139.2, 138.4, 135.7, 131.7, 130.3 (2), 130.3 (2), 129.3 (2), 128.6 (2), 62.8, 59.0, 57.3, 52.2, 50.5, 44.7, 38.5, 35.5, 27.9, 26.5, 23.0, 22.7, 22.2, 21.7, 18.1; HR-MS: Calcd. for $C_{28}H_{34}ON_2FCl$ $[M+H]^+$, 469.2416, Found, 469.2416.

*4.1.2. General procedures for the synthesis of compounds **6** and **7***

*4.1.2.1. -4′,4′ dimethyl sophoridinol (**6**).* To a solution of **4** (0.50 g, 1.2 mmol, 1.0 equiv.) in anhydrous THF (20 mL), solution of methylmagnesium bromide (4.0 equiv.) in THF was slowly added at 0 °C and then stirred at room temperature until TLC analysis showed completion of the reaction. Then the reaction was quenched by the addition of saturated ammonium chloride solution (5 mL). After the solvent was removed by condensation, dichloromethane (30 mL) was added, and the resultant mixture was washed by water (20 mL) and brine (20 mL), dried over anhydrous sodium sulfate, concentrated, and the residue was purified by flash column chromatography on silica gel with CH_2Cl_2/CH_3OH as the eluents to afford target compound **6**. Yield: 45%, m.p.: 91–93 °C; ^1H NMR (400 MHz, DMSO-d_6) δ 8.28–7.76 (m, 2 H), 7.66–7.42 (m, 2 H), 4.28–4.05 (m, 1 H), 3.89–3.61 (m, 1 H), 3.37–3.02 (m, 5 H), 3.00–2.74 (m, 3 H), 2.76–2.54 (m, 2 H), 2.06–1.79 (m, 5 H), 1.74–1.58 (m, 5 H), 1.60–1.27 (m, 4 H), 1.00–0.76 (m, 6 H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 134.7, 133.0, 132.9 (2), 129.3 (2), 80.6, 60.9, 55.6, 52.0, 51.9, 44.1, 39.4, 34.0, 33.4, 26.0, 25.2, 22.7, 21.7, 21.1, 17.6, 13.0, 9.0 (2); HR-MS: Calcd. for $C_{24}H_{37}ON_2Cl$ $[M+H]^+$, 405.2667, Found, 405.2677.

*4.1.2.2. 12-N-p-Chlorobenzyl-4′,4′-di-4-fluorophenyl sophoridinene (**7a**).* Compound **7a** was obtained from 4-fluorophenylmagnesium bromide and **4** stirring at refluxing temperature following the similar procedure with **6** and acidification by HCl/diethyl ether as a white solid. Yield: 32%, m.p.: 64–66 °C; ^1H NMR (600 MHz, CD_3OD) δ 7.67–7.57 (m, 2H), 7.55–7.48 (m, 1H), 7.45–7.25 (m, 3H), 7.20–7.03 (m, 6H), 4.59–4.39 (m, 1H), 4.41–4.16 (m, 1H), 3.97–3.79 (m, 1H), 3.66–3.49 (m, 1H), 3.49–3.33 (m, 3H), 3.21–3.02 (m, 2H), 3.00–2.77 (m, 2H), 2.35–2.23 (m, 2H), 2.14–1.92 (m, 5H), 1.92–1.80 (m, 2H), 1.79–1.56 (m, 1H), 1.53–1.36 (m, 2H), 1.39–1.21 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.2 (2), 134.5, 129.3 (2), 128.7(2), 128.6 (4), 126.7 (2), 125.5, 115.4, 115.1, 114.9 (4), 54.7, 53.0, 51.5, 50.8, 48.6, 44.1, 34.5, 26.5, 26.0, 24.9, 21.7, 20.8, 17.4, 15.1; HR-MS: Calcd. for $C_{34}H_{37}N_2F_2Cl$ $[M+H]^+$, 547.2686, Found, 547.2686.

*4.1.2.3. -4′,4′-diethyl sophoridinene bihydrochloride (**7b**).* Compound **7a** was obtained from ethylmagnesium bromide and **4** stirring at refluxing temperature following the similar procedures with **6** and acidification by HCl/diethyl ether as a white solid. Yield: 40%, m.p.: 77–79 °C; ^1H NMR (500 MHz, DMSO-d_6) δ 11.67 (s, 1H), 11.20 (s, 1H), 8.29–7.79 (m, 2H), 7.66–7.19 (m, 2H), 4.62–4.47 (m, 4H), 4.46–4.33 (m, 1H), 4.31–4.09 (m, 1H), 3.96–3.67 (m, 1H), 3.35–3.23 (m, 2H), 3.23–3.02 (m, 3H), 3.01–2.79 (m, 1H), 2.77–2.54 (m, 1H), 2.51–2.43 (m, 1H), 2.02–1.77 (m, 5H), 1.78–1.61 (m, 5H), 1.61–1.29 (m, 4H), 0.94–0.84 (m, 4H), 0.84–0.67 (m, 2H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 143.6, 140.4 (2), 134.1 (2), 132.4, 128.8, 117.3, 80.1, 60.3, 55.0, 54.3, 51.5, 44.0 (2), 38.8, 32.4, 25.4, 24.6, 22.1, 21.1, 17.0, 12.9, 12.4, 8.5 (2); HR-MS: Calcd. for $C_{26}H_{39}N_2Cl{\cdot}2HCl$ $[M-2HCl+H]^+$, 415.2874, Found, 415.2871.

*4.1.3. 12-N-p-Chlorobenzyl sophoridinic hemiacetal (**10**)*

A dry, 250-mL, three-necked, round-bottomed flask equipped with a magnetic stirrer and a dropping funnel was charged with 100 mL of dry dichloromethane under an atmosphere of nitrogen. After cooling to −78 °C, oxalyl chloride (1.0 mL, 12 mmol) and dry dimethyl sulfoxide (1.5 mL, 20 mmol) were added dropwise to the stirred solution. After 5 min, **8** (3.7 g, 10 mmol) in 20 mL of dichloromethane was added with stirring. The mixture was stirred for an additional 0.5 h at −78 °C, and freshly distilled triethylamine (5.6 mL, 40 mmol) was added. The mixture was then allowed to warm to room temperature over 0.5 h, whereupon 50 mL of water is added. The phases are separated, and the aqueous phase is extracted three times with 50 mL of diethyl ether or dichloromethane. The combined organic phases were washed successively with water (50 mL) and brine (50 mL). The organic layer was dried over anhydrous magnesium sulfate, and the solvent was removed under reduced pressure, and the residue was purified by flash column chromatography on silica gel with

CH_2Cl_2/CH_3OH as the eluents to afford compound **9** as a yellow viscous solid, to which 1 mol/L hydrochloric acid-methanol solution (10 mL) was added and stirred at room temperature for 10 min, removal of the solvent generated target compound **10** as a white solid. Yield: 67%, m.p.: 64–66 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 7.38–7.28 (m, 4 H), 4.91 (s, 3H), 3.68–3.46 (m, 2H), 3.20–3.05 (m, 1H), 3.00–2.74 (m, 2H), 2.65–2.46 (m, 1H), 2.39–2.28 (m, 1H), 2.25–1.97 (m, 4H), 1.94–1.69 (m, 4H), 1.72–1.48 (m, 6H), 1.47–0.94 (m, 6H); ^{13}C NMR (126 MHz, CD_3OD) δ 140.2, 133.4, 131.0 (2), 129.2 (2), 99.6, 65.0, 64.9, 60.2, 58.9, 55.1, 52.6, 46.2, 39.1, 30.2, 28.4, 26.4, 26.0, 23.8, 23.7, 23.5, 20.0; HR-MS: Calcd. for $C_{23}H_{35}O_2N_2Cl$ $[M+H]^+$, 407.2459, Found, 407.2459.

*4.1.4. 12-N-p-lorobenzyl sophoridinic hydroxyl oxime bihydrochloride (**11**)*

To a mixture of hydroxylamine hydrochloride (0.25 g, 3.6 mmol) in 20% sodium hydroxide solution (70 mL), **9** (1.1 g, 3.0 mmol) was added and the mixture was stirred at room temperature until TLC analysis showed the completion of the reaction. The insoluble solid was filtered off and the solvent was removed by condensation, and the residue was purified by flash column chromatography on silica gel with CH_2Cl_2/CH_3OH as the eluents to obtain the free form of **11** as colorless oil, which was then acidified by 3 mol/L HCl/ether (10 mL) to afford **11** as a white solid. Yield: 72%, m.p.: 91–92 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.81 (s, 1H), 10.44 (s, 1H), 7.53–7.14 (m, 4H), 3.64–3.48 (m, 2H), 3.42–3.33 (m, 3H), 3.22–3.05 (m, 2H), 3.01–2.89 (m, 1H), 2.66–2.57 (m, 1H), 2.41–1.97 (m, 7H), 1.93–1.71 (m, 3H), 1.68–1.33 (m, 6H), 1.32–1.14 (m, 2H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 149.7, 139.3, 131.7, 130.3 (2), 128.6 (2), 63.2, 58.9, 57.3, 50.3, 44.6, 35.5, 29.6, 27.9, 26.5, 25.1, 24.4, 23.0, 22.8, 22.3, 18.1; HR-MS: Calcd. for $C_{22}H_{32}ON_3Cl{\cdot}2HCl$ $[M-2HCl+H]^+$, 390.2306, Found, 390.2305.

*4.1.5. 12-N-p-Chlorobenzyl sophoridinic methyl ether (**12**)*

To a solution of free **11** (1.95 g, 5.0 mmol) in anhydrous THF (50 mL) under nitrogen, methyl benzenesulfonate (2.64 g, 20.0 mmol) was added. The mixture was stirred at room temperature until TLC analysis showed the completion of the reaction. The reaction was quenched by methanol (5 mL), and condensed, the residue was purified by flash column chromatography on silica gel with CH_2Cl_2/CH_3OH as the eluents to obtain the free form of **12** as a yellow solid. Yield: 57%, m.p.: 83–85 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.49–7.14 (m, 5H), 3.52 (s, 3H), 3.42–3.23 (m, 3H), 3.23–3.02 (m, 2H), 3.03–2.91 (m, 1H), 2.76–2.56 (m, 1H), 2.43–1.89 (m, 7H), 1.91–1.65 (m, 4H), 1.71–1.36 (m, 6H), 1.34–0.99 (m, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 150.6, 149.7, 139.3, 131.7, 130.3 (2), 128.6 (2), 63.2, 59.0, 57.3, 52.2, 50.3, 44.6, 35.6, 29.6, 27.9, 26.5, 24.4, 23.0, 22.8, 22.3, 18.1; HR-MS: Calcd. for $C_{23}H_{34}ON_3Cl$ $[M+H]^+$, 404.2463, Found, 404.2482.

*4.1.6. 12-N-p-Chlorobenzyl-4′-N-morpholin-sophoridinic imine (**13**)*

To a mixture of **9** (1.1 g, 3.0 mmol) in anhydrous methanol (50 mL), 4-amine-morpholin (0.43 mL, 4.5 mmol) was added and the mixture was refluxed for 4 h before cooled to room temperature. The solvent was condensed and dichloromethane (50 mL) was added, and the mixture was washed successively with water (30 mL) and saturated ammonium chloride solution (30 mL). The mixture was evaporated and the residue was purified by flash column chromatography on silica gel with CH_2Cl_2/CH_3OH as the eluents to obtain the target compound **13** as a white solid. Yield: 74%, m.p.: 59–61 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.38–7.30 (m, 4H), 6.93 (t, J = 5.2 Hz, 1H), 3.73–3.65 (m, 4H), 3.61–3.44 (m, 2H), 3.01–2.89 (m, 1H), 2.88–2.75 (m, 6H), 2.71–2.60 (m, 1H), 2.50–2.35 (m, 2H), 2.25–1.87 (m, 6H), 1.82–1.62 (m, 3H), 1.58–1.37 (m, 5H), 1.31–1.21 (m, 2H), 1.17–1.10 (m, 1H), 1.01 (dd, J = 20.5, 11.7 Hz, 1H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 140.4, 139.7, 131.4, 130.2 (2), 128.5 (2), 66.1 (2), 63.9, 58.5, 57.7, 54.4, 52.5 (2), 52.4, 51.6, 45.4, 38.0, 32.9, 29.8, 26.8, 26.1, 25.5, 22.6, 19.2; HR-MS: Calcd. for $C_{26}H_{39}ON_4Cl$ $[M+H]^+$, 459.2885, Found, 459.2885.

*4.1.7. General procedures for the synthesis of **14***

*4.1.7.1. 12-N-p-Chlorobenzyl-4′-(4-chloro-3-trifluoromethylphenyl)sophoridinic amine trihydrochloride (**14a**).* To a mixture of **9** (1.1 g, 3.0 mmol) in anhydrous methanol (50 mL), 4-chloro-3-trifluoromethylphenylamine (0.8 g, 4.5 mmol) was added and the mixture was refluxed for 4 h, then sodium cyanoborohydride (0.9 g, 4.5 mmol) was added portion wise, and the mixture was refluxed for another 4 h. The mixture was cooled to room temperature and evaporated, then dichloromethane (50 mL) was added, and the mixture was washed successively with water (30 mL) and saturated ammonium chloride solution (30 mL). The mixture was evaporated and the residue was purified by flash column chromatography on silica gel with CH_2Cl_2/CH_3OH as the eluents, then acidified by 3 mol/L HCl/ether (10 mL) and filtrated to obtain the target compound **14a** as a white solid. Yield: 68%, m.p.: 71–73 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.55 (s, 1H), 11.12 (s, 1H), 10.93 (s, 1H), 8.12–7.77 (m, 2H), 7.01 (d, J = 8.7 Hz, 3H), 6.91–6.74 (m, 2H), 4.52–4.34 (m, 1H), 4.34–4.12 (m, 1H), 3.88–3.69 (m, 1H), 3.65–3.25 (m, 4H), 3.24–2.76 (m, 3H), 2.76–2.55 (m, 3H), 2.43–1.98 (m, 2H), 1.99–1.29 (m, 8H), 1.29–1.14 (m, 2H), 1.03–0.79 (m, 1H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 153.9, 146.3, 139.7, 131.5, 130.4, 130.2, 128.9 (2), 128.5 (2), 90.3, 64.1, 60.6, 58.6, 57.7, 56.0, 54.2, 51.5, 45.4, 43.6, 37.8, 30.2, 29.5, 26.9, 25.5, 25.4, 23.1, 23.1, 19.1; HR-MS: Calcd. for $C_{29}H_{36}N_3F_3Cl_2{\cdot}3HCl$ $[M-3HCl+H]^+$, 554.2311, Found, 554.2313.

*4.1.7.2. 12-N-p-Chlorobenzyl-4′-(N-methypiperazine)sophoridinic amine tetrahydrochloride (**14b**).* Compound **14b** was obtained from *N*-methypiperazine and **9** following the similar procedure with **14a**. Yield: 65%, m.p.: 73–75 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.25 (s, 1H), 12.15 (s, 1H), 11.61 (s, 1H), 11.16 (s, 1H), 7.99 (d, J = 7.9 Hz, 2H), 7.57 (d, J = 7.9 Hz, 2H), 5.07 (s, 3H), 4.50–4.16 (m, 1H), 3.92–3.68 (m, 3H), 3.66–3.35 (m, 5H), 3.34–2.75 (m, 11H), 2.73–2.53 (m, 2H), 2.21–1.58 (m, 9H), 1.60–1.23 (m, 3H), 1.16–0.99 (m, 1H), 1.01–0.80 (m, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 134.7, 133.1, 129.5 (2), 129.2 (2), 65.3, 60.9, 55.5, 55.0, 52.1 (2), 51.9 (2), 49.9, 48.5, 44.6, 42.5, 26.1, 25.1, 23.0, 22.8, 22.3, 21.9, 21.7, 17.6, 15.6; HR-MS: Calcd. for $C_{27}H_{43}N_4Cl{\cdot}4HCl$ $[M-4HCl+H]^+$, 459.3249, Found, 459.3249.

4.2. Biological evaluation

4.2.1. MTT assay

The human tumor cell lines, HepG2 and MCF-7, were obtained from American Type Culture Collection. Cells were routinely cultured in the MEM-EBSS medium (Hyclone, UT) with 10% FBS (Gibco, USA) and 1% penicillinestreptomycin and incubated at 37 °C with 5% CO_2. Cells were counted and plated at a density

of 4000 cells per well in 96-well plates. After 24 h, cells were treated with different compounds at different concentrations for 48 h. The effect on cell growth inhibition was determined by the MTT assay as previous described. The growth inhibition rate (%) was calculated at each concentration and IC_{50} value was calculated with Sigmaplot software. Results were obtained from triplicate determinations and shown as mean ± SD.

4.2.2. Cell cycle distribution assay

HepG2 cells were treated with **5a** at different concentrations for 24 h and then cells were harvested. Cells were fixed with 70% ethanol and stored at –20 °C overnight. The fixed cells were incubated with 200 μg/mL RNase at 37 °C for 30 min and then stained with 50 μg/mL propidium iodide in the dark for 30 min. Cell cycle distribution was then analyzed by flow cytometry using FACS analysis (BD FACSCalibur, USA).

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 81473248) and Tianjin Medical University General Hospital Funding (ZYYFY2015030).

References

1. Kyogoku K, Hatayama K, Komatsu M. Constituents of Chinese crude drug "Kushen" (the root of *Sophora flavescens* Ait.). Isolation of five new flavonoids and formononetin. *Chem Pharm Bull* 1973;**21**:2733–8.
2. Liang L, Wang XY, Zhang XH, Ji B, Yan HC, Deng HZ, et al. Sophoridine exerts an anti-colorectal carcinoma effect through apoptosis induction *in vitro* and *in vivo*. *Life Sci* 2012;**91**:1295–303.
3. Liu B, Shi RB. Constituents in the alkaloid fraction of Kushen decoction. *China J Chin Mater Med* 2006;**31**:557–60.
4. Zhang H, Gu J. Progress of experimental study on treatment of psoriasis by Chinese medicinal monomer and single or compound recipe in Chinese materia medica. *Chin J Integr Med* 2007;**13**:312–6.
5. Yan JG, Yang YQ, Wang YJ, Kan J. Study on effect of sophoridine against bone cancer pain and its mechanism. *China J Chin Mater Med* 2013;**38**:4134–7.
6. Yong JP, Wu XY, Lu CZ. Anticancer advances of matrine and its derivatives. *Curr Pharm Des* 2015;**21**:3673–80.
7. Wang WX, Sun ZH, Chen HM, Xu BN, Wang FY. Role and mechanism of sophoridine on proliferation inhibition in human glioma U87MG cell line. *Int J Clin Exp Med* 2015;**8**:464–71.
8. Wang QR, Li CH, Fu XQ, Liu YW, Tang J, Fan Q, et al. Effects of sophoridine on the growth and expressions of p53 and vascular endothelial growth factor of transplanted solid tumor SW480 in nude mice. *J South Med Univ* 2010;**30**:1593–6.
9. Li XM, Wu YG, Chen SL, Pan DX, Wu JN, Yu YH. Antitumor action of sophoridine. *Acta Pharmacol Sin* 1987;**8**:153–8.
10. Bi CW, Zhang CX, Li YH, Tang S, Deng HB, Zhao WL, et al. Novel *N*-substituted sophoridinol derivatives as anticancer agents. *Eur J Med Chem* 2014;**81**:95–105.
11. Bi CW, Zhang CX, Li YH, Tang S, Wang SG, Shao RG, et al. Synthesis and biological evaluation of sophoridinol derivatives as a novel family of potential anticancer agents. *ACS Med Chem Lett* 2014;**5**:1225–9.
12. Chun BK, Du JF, Zhang HR, Chang W, Ross BS, Jiang Y, et al. Synthesis of stable isotope labeled analogs of the anti-hepatitis C virus nucleotide prodrugs PSI-7977 and PSI-352938. *Nucleosides Nucleotides Nucleic Acids* 2011;**30**:886–96.
13. Bhansali P, Hanigan CL, Perera L, Casero Jr. RA, Tillekeratne LMV. Synthesis and biological evaluation of largazole analogues with modified surface recognition cap groups. *Eur J Med Chem* 2014;**86**:528–41.
14. Dietz J, Martin SF. Novel entry to the tricyclic core of stemofoline and didehydrostemofoline. *Tetrahedron Lett* 2011;**52**:2048–50.
15. Zhang H, Xin MH, Xie XX, Mao S, Zuo SJ, Lu SM, et al. Synthesis and antitumor activity evaluation of PI3K inhibitors containing 3-substituted quinazolin-4(3*H*)-one moiety. *Bioorg Med Chem* 2015;**23**:7765–76.
16. Kumar R, Kaur M, Bahia MS, Silakari O. Synthesis, cytotoxic study and docking based multidrug resistance modulator potential analysis of 2-(9-oxoacridin-10(9*H*)-yl)-*N*-phenyl acetamides. *Eur J Med Chem* 2014;**80**:83–91.