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Original article

Novel *N*-substituted sophoridinol derivatives as anticancer agents

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ABSTRACT

Using sophoridine (**1**) as the lead compound, a series of new *N*-substituted sophoridinic acid derivatives were designed, synthesized and evaluated for their cytotoxicity. SAR analysis indicated that introduction of a chlorobenzyl on the 12-nitrogen atom of sophoridinol might significantly enhance the antiproliferative activity. Of the newly synthesized compounds, sophoridinol analogue **9k** exhibited a potent effect against six human tumor cell lines (liver, colon, breast, lung, glioma and nasopharyngeal). The mode of action of **9k** was to inhibit the DNA topoisomerase I activity, followed by the G0/G1 phase arrest. It also showed a moderate oral bioavailability and good safety *in vivo*. Therefore, compound **9k** has been selected as a novel-scaffold lead for further structural optimizations or as a chemical probe for exploring anticancer pathways of this kinds of compounds.

1. Introduction

Sophoridine (**1**, Fig. 1), a quinolizidine natural product extracted from *Sophora flavescens*, was approved by Chinese FDA in 2005 as an anticancer drug against malignant trophoblastic tumors [1]. Nowadays, it has been widely used to elevate the therapeutic efficacy against gastric cancer, liver cancer, and lung cancer in combination with other anticancer drugs used in clinic including vinorelbine, cisplatin and docetaxel et al. [2–6]. The mechanism of action of **1** is to inhibit the DNA topoisomerase I (topo I) activity, and arrest the cell cycle at the S-phase, then cause apoptotic cell death [7–10]. Compared to topo I inhibitors in clinical use, the camptothecin family drugs such as camptothecin and Topotecan (TPT), compound **1** has couple of advantages, for example, special scaffold, simple structure, good solubility and considerable opportunities of formulation for oral administration, suggesting a novel family of antitumor agents with ideal druggable characteristics.

The structure–activity relationship (SAR) of **1** has been recently initiated with the D ring analysis with **1** as the lead compound [11]. The SAR results revealed that sophoridinic acid (**2**, Fig. 1) with a 3-ring structure scaffold was more favorable than **1** with a 4-ring scaffold. Compound **3** (Fig. 1), 12-*N*-bromoacetyl sophoridinic acid, had an improved antiproliferative activity with IC_{50} of 15.4 μg/ml in HepG2 cells, compared to its parent **1** ($IC_{50} > 20$ μg/ml), with a mode of action as consistent with that of parent **1**. The simple and unique chemical scaffold and promising antiproliferative activity of **3** provoked our strong interest to further develop structural modifications and optimizations in an effort to discover a novel class of anticancer candidates with a 3-ring scaffold. As shown in Fig. 1, in the present study we would continue to carry out the SAR analysis on the substituents on the 12-nitrogen atom, carboxyl acid group and the configuration of the chiral center at position 5, respectively. Based on this strategy, 47 new sophoridinic acid derivatives were synthesized and screened for their antiproliferative activity in human HepG2 cell line.

Herein, we describe the synthesis, *in vitro* antitumor assessment, SAR analysis, primary mechanism of action as well as pharmacokinetic and safety evaluation *in vivo* of the newly synthesized compounds.

2. Results and discussion

2.1. Chemistry

Forty-seven target compounds were synthesized and grouped into sophoridinic core (5*R*-configuration) and matrinic core (5*S*-configuration) as described in Schemes 1 and 2, respectively. Compound **1** was hydrolyzed in 6 N HCl at the refluxing temperature to give **2**, which was then esterified in methanol to provide the

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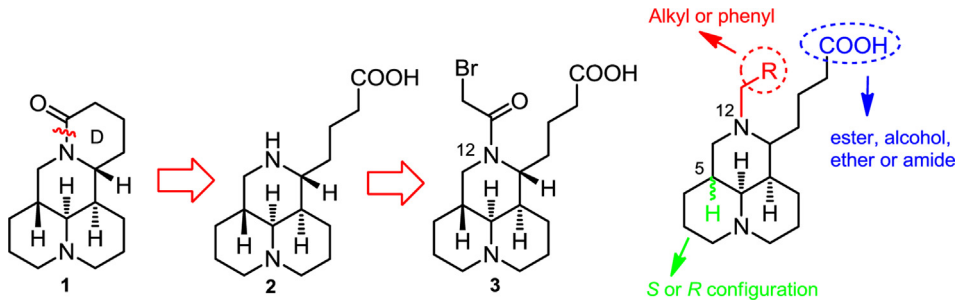
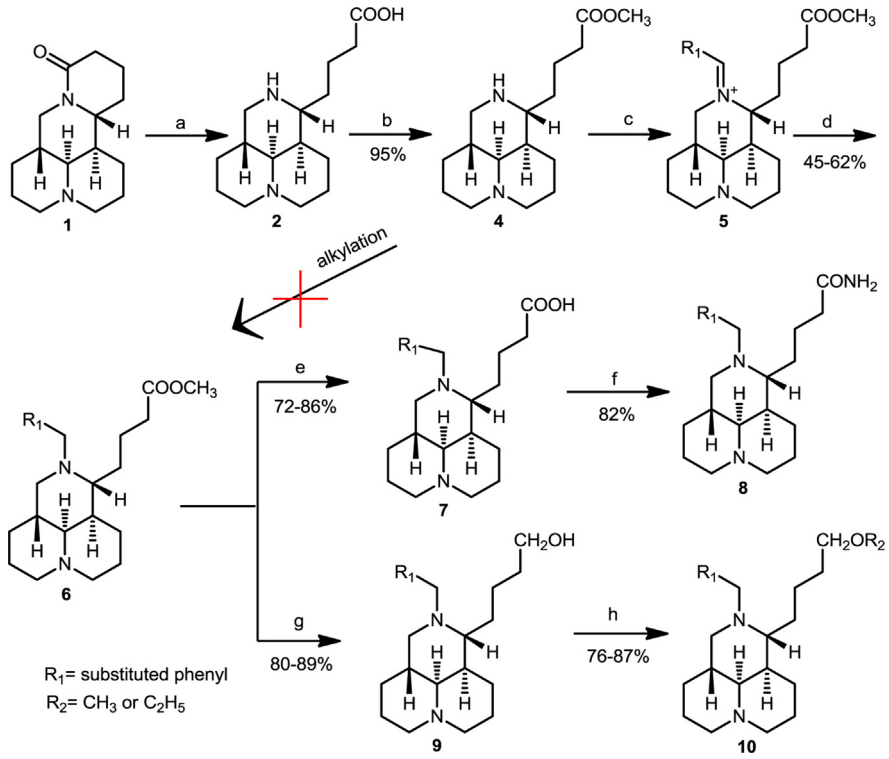


Fig. 1. Structures of sophoridine (**1**), sophoridinic acid (**2**), compound **3** and the modification sites of constructed new scaffold (right).



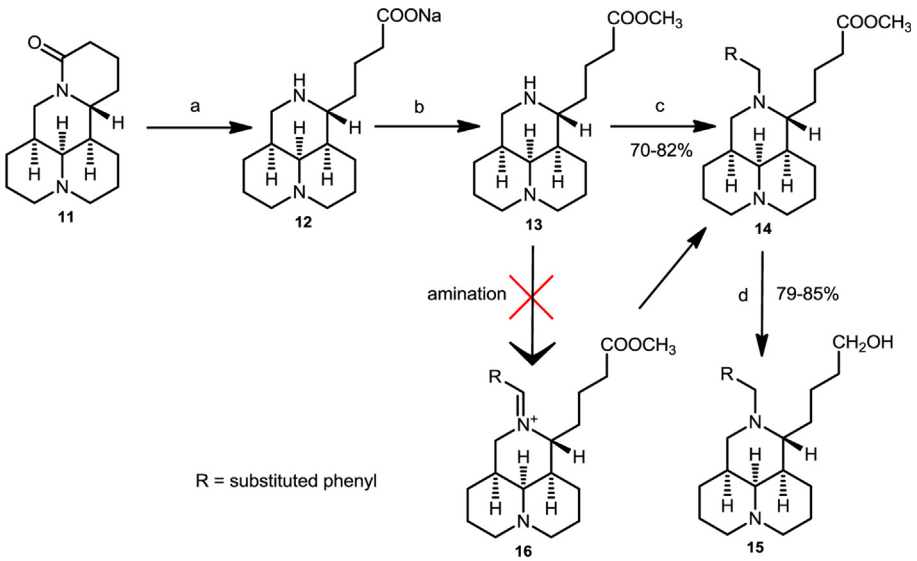
Scheme 1. (a) 6 N HCl, reflux, 4 h; (b) CH_3OH, 2 h; (c) R_1CHO, triethylamine, 1,2-dichloroethane, reflux, 2 h; (d) sodium triacetoxyborohydride, reflux, 2 h; (e) 3 N HCl, reflux, 3 h; (f) NH_4HCO_3/DIBOC, pyridine, CH_3CN; (g) $LiAlH_4$, THF; (h) methyl/ethyl benzenesulfonate, THF.

key intermediate **4** with a high yield of 95% [12]. Intermediate **4** was condensated with the corresponding aldehydes in alkaline condition to give the Schiff base **5**, which was reduced selectively using sodium triacetoxyborohydride (STB) as a reducing agent to afford the desired product **6** in 45%–62% yields. As shown in Scheme 1, product **6** could not be obtained through alkylation of **4** with the corresponding alkyl halide directly [13]. The product in series **7** was gained by the hydrolyzation of **6** in 3 N HCl at the refluxing temperature in a good yield. The amide product **8** was gained by amination of **7** with NH_4HCO_3/DIBOC as a NH_2-donator using pyridine as a catalyst [14]. The alcohol derivative **9** was prepared via reduction of **6** with $LiAlH_4$ as a reducing agent in THF with over 80% yields. Another ether product in series **10** was obtained via etherification of **9** with methyl/ethyl benzenesulfonate in THF in yields of 76–87%.

The second synthetic route used commercially available matrine (**11**) as the starting material. The key intermediate **13** was gained by hydrolyzation and esterification with the methods reported previously [15]. As described in Scheme 2, the product **14** was readily acquired through alkylation of **13** with the alkyl halide in 70–82% yields; but it could not be obtained by condensation with the corresponding aldehydes owing to transferring into raw material **11** in the amination conditions of **13**. The final product **15** was gained through the reduction of **14** with $LiAlH_4$ as a reducing agent mentioned above. All crude products were purified with flash column chromatography on silica gel using CH_2Cl_2 and MeOH as gradient eluents.

2.2. SAR analysis for the antiproliferative activity

All of the newly synthesized compounds were initially screened for their cytotoxicity activities in human HepG2 hepatoma cells with TPT as a positive reference using MTT assay. Structures of the 47 analogues and their antiproliferative activity at the concentration of 10 μg/ml were summarized in Table 1.



Scheme 2. (a) 3 N NaOH, reflux, 8 h; (b) 3 N HCl, MeOH, 2 h; (c) RCH_2X, K_2CO_3, CH_3CN; (d) $LiAlH_4$, THF.

SAR analysis was first focused on the substituents at the 12-nitrogen atom. The SAR study for a group of *N*-acyl sophoridinic acid analogues has been carried out in our previous report [11]. In this part, a variety of alkyl groups including aliphatic, benzyl, picolyl were introduced into the nitrogen atom at the 12-position respectively, by which 10 new *N*-substituted sophoridinic acids (**7a**–**j**) were generated and tested. As indicated in Table 1, all of them showed a weak activity with inhibition rates less than 26% at the concentration of 10 μg/ml, regardless of the size of the side-chains. The results suggested that the improvement of ClogP in this kind of compounds (ClogP = 0.07–2.5) might be helpful for the activity. Hence, a series of *N*-substitued sophoridinic esters (**4**, **6a**–**j**) with the improved ClogP value (ClogP = 1.94–5.08) was then gained, aiming to enhance the activity against cancer. As expected, a couple of compounds (**6d, 6f**–**h**) afforded significantly higher cytotoxicity with inhibition rates ranging from 81.8% to 97.6%, much higher than TPT. Similarly, among the new family of *N*-substituted sophoridinol (**9a**–**n**), compounds **9c** and **9j**–**k** showed pleasing antiproliferative effects with over 85% inhibition rate. The results indicated that the introduction of a fluorobenzyl or chlorobenzyl on the 12-nitrogen atom could significantly improve the anticancer activity. As indicated in Fig. 2, p-π conjugation between Cl atom and phenyl might be helpful for the binding of compound **9k** and target topo I residues.

Next, we retained the fluorobenzyl or chlorobenzyl at the 12-position as a pharmacophore for activity, and replaced the carboxylic acid group with ether or amide to explore the SAR of the carboxyl variation. Therefore, several *N*-substituted sophoridinic ether (**10a**–**f**) and amide (**8**) were made and tested. The results showed that all of them lost their anticancer activity completely with less than 7% inhibition rate at the same concentration. The results revealed that sophoridinic ester/alcohol exhibited potent antiproliferative effect among this kind of compounds.

In another variation, SAR study was carried out to investigate the (*R*)- or (*S*)-configuration of the 5-chiral center for the activity, and a group of *N*-substituted matrinic ester/alcohol (**14**, **15a**–**c**) were prepared. The results showed that most of them lost the activity partly or completely, compared to their corresponding sophoridinic ester/alcohol. As shown in Fig. 2, the docking analysis showed that multiple hydrogen bonds formed between **9k** and topo I residues, while there was no obvious interaction between **15c** and the aimed targets. It was deduced that conformational alteration into matrinic core might cause decreased activities, and sophoridine scaffold was probably essential for the activity against cancer. Among the newly synthesized compounds, sophoridinol **6g** and sophoridinic ester **9k** were selected as the representative compounds for further investigation.

2.3. Antiproliferative activities of compounds **6g** and **9k** in six human tumor cell lines

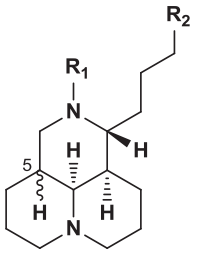
Compounds **6g** and **9k** were further examined their antiproliferative effect in six human tumor cell lines including HepG2, HCT116 (colon cancer), H1299 (lung cancer), U87 (malignant glioma), MCF-7 (breast cancer) and KB (nasopharyngeal epidermoid carcinoma). As described in Table 2, both of them showed ideal cytotoxicities for the six solid tumor lines with IC_{50} values ranging from 4 μM to 20 μM, indicating a broad antiproliferative spectra. Especially, compounds **6g** and **9k** had potent activities for the KB tumor cells with IC_{50} of 6.1 and 19.7 μM respectively, much higher than that of TPT ($IC_{50} > 200$ μM). Thus, both of them were chosen to carry out for further druggable evaluations *in vivo* studies.

2.4. In vivo pharmacokinetic and safety assessment of **6g** and **9k**

The *in vivo* pharmacokinetic (PK) behaviors of compound **6g** and **9k** were investigated in rat model. The tested compounds were administered via oral (ig, 25 mg/kg) and intravenous (iv, 5 mg/kg) routes to adult male Sprague–Dawley (SD) rats. As anticipated, ester compound **6g** was rapidly hydrolyzed into the acid metabolite **7h** *in vivo* (data not shown), which had no anticancer activity *in vitro* (Table 1). As indicated in Table 3, the actual oral bioavailability of alcohol **9k** in this experiment was moderate ($F = 18\%$) in the rats.

Single-dose toxicity tests were carried out in mice. After **6g** and **9k** were given by intragastric administration (i.g) at a dose of 250, 500 or 1000 mg/kg respectively, no death of the mice was observed. In addition, this treatment with **6g** and **9k** showed no effect on body weight of mice as well (data not shown). The results suggested that both of them were considerably safe *in vivo*.

Table 1
Structure–activity relationships of the sophoridinic acid derivatives for their antiproliferative activities in HepG2 cells.



Compd	5-H	R_1	R_2	Inhibition rate[a]	ClogP[b]
4	*R*	H	$COOCH_3$	33.4 ± 1.5	1.94
6a	*R*	$CH_2CH_2CH_3$	$COOCH_3$	24.9 ± 0.6	3.53
6b	*R*	$CH_2CH(CH_3)_2$	$COOCH_3$	26.8 ± 0.7	3.79
6c	*R*	CH_2PhF-*o*	$COOCH_3$	60.8 ± 1.0	4.36
6d	*R*	CH_2PhF-*m*	$COOCH_3$	81.8 ± 1.0	4.36
6e	*R*	CH_2PhF-*p*	$COOCH_3$	73.1 ± 2.4	4.36
6f	*R*	CH_2PhCl-*m*	$COOCH_3$	84.9 ± 1.9	4.93
6g	*R*	CH_2PhCl-*p*	$COOCH_3$	97.6 ± 2.4	4.93
6h	*R*	CH_2PhBr-*p*	$COOCH_3$	89.6 ± 2.8	5.08
6i	*R*	4-picolyl	$COOCH_3$	29.9 ± 3.6	2.72
6j	*R*	2-furfuryl	$COOCH_3$	16.6 ± 5.1	3.39
7a	*R*	$CH_2CH_2CH_3$	COOH	25.3 ± 4.7	0.88
7b	*R*	$CH_2CH(CH_3)_2$	COOH	10.7 ± 2.4	1.28
7c	*R*	CH_2Ph	COOH	1.5 ± 1.0	1.57
7d	*R*	CH_2PhF-*o*	COOH	1.6 ± 1.7	1.71
7e	*R*	CH_2PhF-*m*	COOH	25.2 ± 0.8	1.71
7f	*R*	CH_2PhF-*p*	COOH	2.0 ± 2.0	1.71
7g	*R*	CH_2PhCl-*m*	COOH	22.8 ± 3.9	2.28
7h	*R*	CH_2PhCl-*p*	COOH	3.5 ± 1.9	2.28
7i	*R*	CH_2PhBr-*p*	COOH	24.9 ± 0.3	2.43
7j	*R*	4-picolyl	COOH	22.3 ± 3.5	0.07
8	*R*	CH_2PhCl-*p*	$CONH_2$	1.7 ± 3.2	3.63
9a	*R*	$CH_2CH_2CH_3$	CH_2OH	0.9 ± 0.4	3.11
9b	*R*	$CH_2CH_2CH_2CH_3$	CH_2OH	19.7 ± 8.0	3.64
9c	*R*	$CH_2C(CH_3)_3$	CH_2OH	95.7 ± 3.3	3.91
9d	*R*	$CH_2CH(CH_3)_2$	CH_2OH	9.1 ± 2.6	3.79
9e	*R*	CH_2Ph	CH_2OH	18.7 ± 5.7	3.80
9f	*R*	CH_2PhCH_3-*p*	CH_2OH	17.8 ± 0.7	4.30
9g	*R*	CH_2PhF-*o*	CH_2OH	30.9 ± 3.4	3.94
9h	*R*	CH_2PhF-*m*	CH_2OH	24.3 ± 4.2	3.94
9i	*R*	CH_2PhF-*p*	CH_2OH	35.7 ± 5.8	3.94
9j	*R*	CH_2PhCl-*o*	CH_2OH	88.1 ± 1.9	4.51
9k	*R*	CH_2PhCl-*p*	CH_2OH	87.7 ± 2.1	4.51
9l	*R*	4-picolyl	CH_2OH	1.2 ± 0.3	2.30
9m	*R*	2-furfuryl	CH_2OH	33.2 ± 2.0	2.98
9n	*R*	CH_2CH_2Ph	CH_2OH	34.6 ± 6.5	2.72
10a	*R*	CH_2Ph	CH_2OCH_3	4.4 ± 1.6	4.51
10b	*R*	CH_2Ph	$CH_2OCH_2CH_3$	2.1 ± 2.4	4.90
10c	*R*	CH_2PhF-*p*	CH_2OCH_3	6.8 ± 3.7	4.66
10d	*R*	CH_2PhF-*p*	$CH_2OCH_2CH_3$	5.1 ± 3.2	5.04
10e	*R*	CH_2PhCl-*p*	CH_2OCH_3	0.3 ± 0.1	5.23
10f	*R*	CH_2PhCl-*p*	$CH_2OCH_2CH_3$	0.2 ± 0.1	5.61
14	*S*	CH_2PhF-*p*	$COOCH_3$	0.8 ± 4.4	4.36
15a	*S*	CH_2Ph	CH_2OH	31.0 ± 4.8	3.80
15b	*S*	CH_2PhF-*p*	CH_2OH	0.1 ± 14.1	3.94
15c	*S*	CH_2PhCl-*p*	CH_2OH	4.9 ± 6.8	4.51
TPT				72.6 ± 2.1	

[a] % of inhibition. Cells were untreated (control) or treated with test compounds (10 μg/ml) for 48 h, respectively.
[b] ClogP value was generated by software Chembiooffice (2010).

2.5. Primary mechanism of action of the representative compounds

We extended our work to the primary mechanism investigation of compounds **6g** and **9k**. Flow cytometric analysis in the HepG2 cells was carried out. As shown in Fig. 3A, compound **9k** attested HepG2 cells at the G0/G1 phase as anticipated, indicating a similar mechanism of action with its parent compound **1** or **2**. However, as indicated in Fig. 3B, different from the parent, compound **6g** caused a major shift of the cell population from G0/G1 to G2/M phase, indicating a significant change of the action mode after the esterification of carboxyl group. Then, we accessed the inhibitory activity of **6g** and **9k** on DNA Top I. Compound **6g** did not show significant inhibitory activity of Top I at the concentration of 15 mg/ml (data not shown), while compound **9k** significantly inhibited the activity of Top I at the same concentration as shown in Fig. 4, which was consistent with that of the parent compound **1** or **2**. The mode of action of **6g** is further being investigated in our laboratory.

3. Conclusion

Taken together, 47 new sophoridinic acid derivatives were designed, synthesized and evaluated for their antitumor activities. SAR analysis indicated that (i) *R*-configuration at the 5-position was crucial; (ii) introduction of a chlorobenzyl on the 12-nitrogen atom of the sophoridinol might significantly enhance the activity. Among them, compound **9k** showed a potent anticancer effect in the six tested tumor cell lines. Its mode of action was to inhibit the activity of DNA topo I, followed by the G0/G1 phase arrest. In addition, it displayed a good PK profile and safety *in vivo*. We consider *N*-substituted sophoridinol derivatives to be a novel scaffold of topo I inhibitors against cancer with good druggable characteristics. Compound **9k** was selected as a novel-scaffold lead for further structural optimizations or a chemical probe for revealing the biological pathways against cancer of this kind of compounds.

4. Experimental section

4.1. Chemistry

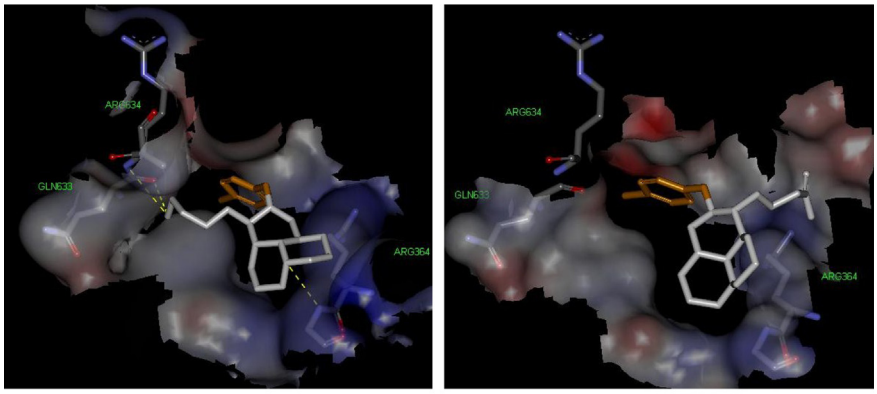
Melting points (mp) were obtained with CXM-300 melting point apparatus. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance 400 spectrometer using deuterated DMSO or CH_3OH as a solvent and tetramethylsilane (TMS) as an internal standard. ESI high-resolution mass spectra (HRMS) were recorded on an AutospecUltima-TOF spectrometer. Flash column chromatography was performed on Combiflash Rf 200, particle size 0.038 mm. HPLC analyses were performed on an Agilent 1200 using a 5.0 μm C18 reversed phase column. For purities obtained by HPLC, the major peak accounted for ≥95% of the combined total peak area monitored by a MWD detector at 220 nm.

4.1.1. Methyl sophoridinate dihydrochloride (*4*)

Compound **1** (4.96 g, 20 mmol) was evenly dispersed in 6 N hydrochloric acid solution (50 ml) and heated to reflux for 4 h. Then the solvent was evaporated under vacuum and anhydrous methanol (50 ml) was added into the residue. The reaction mixture was stirred at room temperature for 2 h, and then the solvent was evaporated under vacuum. The resultant solid was purified by flash chromatography over silica gel to obtain white solid **4** (6.72 g, 95%). Mp 112–114 °C; ^{1}H NMR (DMSO-d_6) δ: 11.61 (s, 1H), 9.67 (s, 1H), 9.29 (s, 1H), 3.61 (s, 3H), 3.60–3.30 (m, 3H), 3.22–2.93 (m, 3H), 2.86–2.69 (m, 1H), 2.54–2.49 (m, 1H), 2.48–2.31 (m, 4H), 2.12 (m, 1H), 1.98–1.58 (m, 9H), 1.51–1.27 (m, 2H); ^{13}C NMR δ: 172.8, 56.1, 55.4, 51.6, 51.3, 44.1, 41.6, 33.2, 32.8, 26.8, 24.9, 24.8, 21.8, 21.2, 20.8, 17.0; HRMS: calcd for $C_{16}H_{30}N_2O_2Cl_2$ $[M-2HCl+H]^+$, 281.2223; found, 281.2222.

4.1.2. General procedures for the synthesis of the compounds *6a–j*

To a stirred solution of **4** (2.81 g, 10.0 mmol) and triethylamine (2.8 ml, 20.0 mmol) in 1,2-dichloroethane (50 ml), aldehyde (15.0 mmol) was added dropwise. The reaction mixture was refluxed for 2 h and STB (3.16 g, 15.0 mmol) was added into the



sophoridinic core (5*R*) matrinic core (5*S*)

Fig. 2. Stereo viewing docked models of **9k** (left) and **15f** (right) in the active site of DNA topo I complex (from PDB: 1K4T). Molecular figures were generated by Discovery Studio Client.

reaction mixture slowly. The reaction solution was refluxed till TLC analysis showed completion of the reaction. After cooling down, the mixture was separated and washed successively with water (20 ml), brine (20 ml). The organic layer was evaporated under vacuum, and the residue was purified by flash chromatography over silica gel or acidized with 3 N hydrochloride/ether (10 ml) to obtain the desired product.

*4.1.2.1. Methyl 12-N-n-propylsophoridinate dihydrochloride (**6a**).* The title compound was prepared from **4** and propanal in the same manner as described above. Yield: 51%; Yellow solid; mp 100–103 °C; ^{1}H NMR (DMSO-d_6) δ: 11.59 (s, 1H), 10.72 (s, 1H), 3.61 (s, 3H), 3.43–3.23 (m, 2H), 3.23–2.99 (m, 5H), 2.98–2.74 (m, 3H), 2.69–2.47 (m, 4H), 2.41–2.19 (m, 2H), 1.87–1.49 (m, 10H), 1.37–1.19 (m, 2H), 0.97–0.81 (m, 3H); ^{13}C NMR δ: 172.8, 60.1, 55.1, 54.2, 51.4, 51.3, 51.1, 44.1, 33.3, 32.7, 25.5, 24.8, 21.9, 21.6, 21.2, 21.0, 17.0, 16.6, 10.8; HRMS: calcd for $C_{19}H_{36}N_2O_2Cl_2$ $[M-2HCl+H]^+$, 323.2693; found, 323.2696.

*4.1.2.2. Methyl 12-N-isobutylsophoridinate dihydrochloride (**6b**).* The title compound was prepared from **4** and isobutyraldehyde in the same manner as described above. Yield: 62%; Yellow solid; mp 89–92 °C; ^{1}H NMR (DMSO-d_6) δ: 11.69 (s, 1H), 10.09 (s, 1H), 3.63 (s, 3H), 3.41–3.33 (m, 2H), 3.32–3.01 (m, 5H), 3.00–2.77 (m, 3H), 2.71–2.47 (m, 4H), 2.47–2.30 (m, 2H), 1.99–1.50 (m, 10H), 1.46–1.30 (m, 1H), 1.04–0.83 (m, 6H); ^{13}C NMR δ: 172.8, 60.0, 58.8, 54.9, 52.3, 51.4, 51.3, 44.1, 33.0, 32.7, 25.5, 24.7, 22.9 (2), 21.8, 21.6, 20.9, 20.8, 20.7, 17.2; HRMS: calcd for $C_{20}H_{38}N_2O_2Cl_2$ $[M-2HCl+H]^+$, 337.2849; found, 337.2852.

*4.1.2.3. Methyl 12-N-2-fluorobenzylsophoridinate (**6c**).* The title compound was prepared from **4** and 2-fluorobenzaldehyde in the same manner as described above. Yield: 57%; Thick yellow solid; mp 52–54 °C; ^{1}H NMR (DMSO-d_6) δ: 7.42–7.25 (m, 2H), 7.21–7.06 (m, 2H), 3.61 (s, 3H), 2.90–2.73 (m, 3H), 2.63–2.41 (m, 4H), 2.39–2.26 (m, 3H), 2.26–2.15 (m, 1H), 2.11–1.94 (m, 2H), 1.86–1.29 (m, 10H), 1.28–1.18 (m, 1H), 1.10–0.91 (m, 2H); ^{13}C NMR δ: 173.7, 162.3 (d, J = 242 Hz), 130.8, 128.9, 127.1, 124.6, 115.3, 64.2, 58.4, 54.5, 51.6,

Table 2
Antiproliferative activities (IC_{50}, μM) of the compounds **6g** and **9k** in six human tumor cell lines.

	HepG2	HCT116	H1299	U87	MCF-7	KB
6g	4.05 ± 0.46	4.59 ± 0.42	4.06 ± 0.33	12.60 ± 0.94	20.02 ± 0.77	6.11 ± 0.17
9k	9.37 ± 0.49	10.64 ± 0.22	5.55 ± 0.31	4.52 ± 10.04	5.73 ± 0.48	19.71 ± 2.02
TPT	1.66 ± 0.06	4.32 ± 2.59	10.97 ± 0.62	0.16 ± 0.02	14.3 ± 1.03	>200

HepG2: hepatoma. HCT116: colon cancer. H1299: lung cancer. U87: malignant glioma. MCF-7: breast cancer. KB: nasopharyngeal epidermoid carcinoma.

Table 3
Pharmacokinetic parameters[a] of **9k** in rats after single oral and intravenous dosing (n = 3).

Route	Dosage (mg/kg)	C_{max} (μM)	T_{max} (h)	AUC $_{0-t}$ (μM·h)	MRT (h)	$t_{1/2}$ (h)	F (%)
iv	5	3.43 ± 1.58	0.25 ± 0.00	20.51 ± 1.28	2.39 ± 1.13	4.46 ± 1.06	
ig	25	5.25 ± 1.66	0.66 ± 0.29	18.60 ± 2.95	3.28 ± 0.37	3.87 ± 0.53	18

[a] PK parameters were calculated by non-compartmental analysis using WinNonlin, version 5.3.

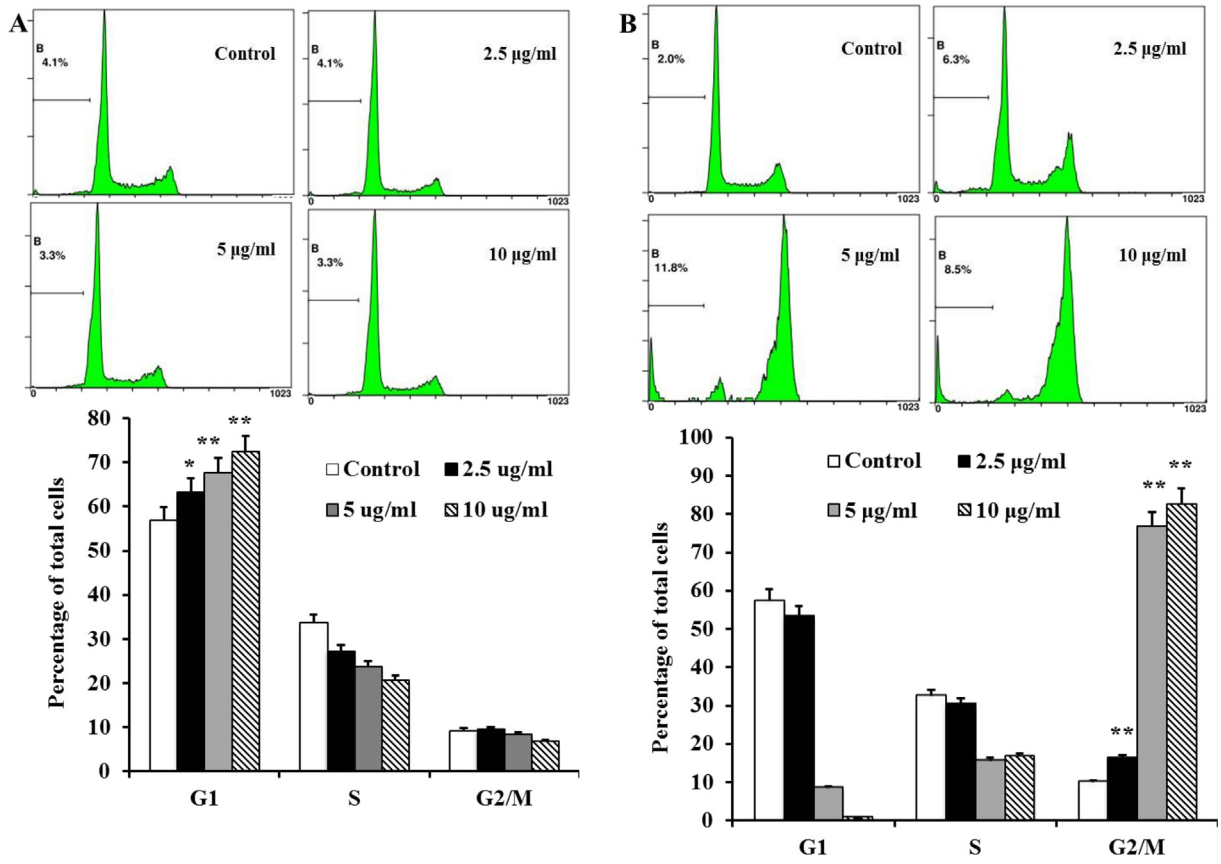


Fig. 3. Cell cycle analysis of **9k** (A) and **6g** (B). HepG2 cells were incubated without (control) or with compounds at different concentrations (2.5, 5, 10 μg/ml) for 24 h. Cells were then analyzed for their cell cycle distribution using flow cytometry.

51.5, 51.3, 45.4, 38.1, 33.8, 29.9, 26.6, 26.1, 25.6, 23.3, 23.0, 19.2; HRMS: calcd for $C_{23}H_{33}N_2O_2F$ $[M+H]^+$, 389.2598; found, 389.2593.

*4.1.2.4. Methyl 12-N-3-fluorobenzylsophoridinate dihydrochloride (**6d**).* The title compound was prepared from **4** and 3-fluorobenzaldehyde in the same manner as described above. Yield: 51%; White solid; mp 98–101 °C; ^{1}H NMR (DMSO-d_6) δ: 11.47 (s, 1H), 11.11 (s, 1H), 7.90–7.68 (m, 2H), 7.50–7.29 (m, 2H), 4.42–4.21 (m, 2H), 3.94–3.74 (m, 1H), 3.60 (s, 3H), 3.35–3.01 (m, 6H), 3.01–2.80 (m, 3H), 2.75–2.42 (m, 4H), 1.98–1.62 (m, 5H), 1.60–1.35 (m, 4H), 1.27–1.01 (m, 1H); ^{13}C NMR δ: 172.6, 162.1 (d, J = 242 Hz), 131.9, 130.8, 128.8, 117.3, 116.3, 60.9, 55.5, 54.6, 51.5, 51.3, 44.1, 33.4, 32.5, 25.5, 24.7, 21.8, 21.6, 21.0, 20.7, 17.1; HRMS: calcd for $C_{23}H_{35}N_2O_2FCl_2$ $[M-2HCl+H]^+$, 389.2598; found, 389.2599.

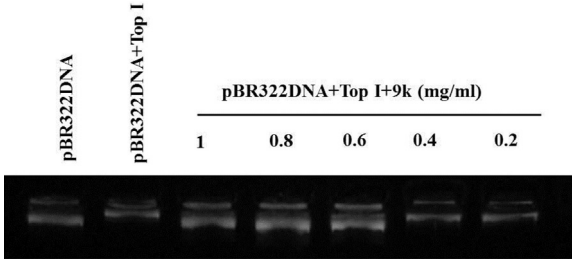


Fig. 4. DNA Top I inhibitory activity of **9k** at different concentrations (0.2, 0.4, 0.6, 0.8 and 1.0 mg/ml).

*4.1.2.5. Methyl 12-N-4-fluorobenzylsophoridinate (**6e**).* The title compound was prepared from **4** and 4-fluorobenzaldehyde in the same manner as described above. Yield: 59%; Thick yellow solid; mp 59–61 °C; ^{1}H NMR (DMSO-d_6) δ: 7.43–7.32 (m, 2H), 7.23–7.03 (m, 2H), 3.63 (s, 3H), 3.17–3.01 (m, 2H), 2.92–2.69 (m, 3H), 2.57–2.40 (m, 3H), 2.36–2.18 (m, 3H), 2.06–1.86 (m, 3H), 1.80–1.60 (m, 3H), 1.60–1.41 (m, 5H), 1.40–1.31 (m, 1H), 1.31–1.23 (m, 1H), 1.19–0.94 (m, 2H); ^{13}C NMR δ: 173.7, 160.3 (d, J = 242 Hz), 136.6, 130.2 (2), 115.2 (2), 63.5, 58.6, 57.5, 54.1, 51.6, 49.0, 45.3, 37.8, 33.8, 29.4, 26.9, 25.7, 25.2, 23.2, 22.7, 19.1; HRMS: calcd for $C_{23}H_{33}N_2O_2F$ $[M+H]^+$, 389.2598; found, 389.2593.

*4.1.2.6. Methyl 12-N-3-chlorobenzylsophoridinate dihydrochloride (**6f**).* The title compound was prepared from **4** and 3-chlorobenzaldehyde in the same manner as described above. Yield: 49%; Yellow solid; mp 96–98 °C; ^{1}H NMR (DMSO-d_6) δ: 11.52 (s, 1H), 11.12 (s, 1H), 8.10 (s, 1H), 7.85 (d, J = 7.1 Hz, 1H), 7.56–7.43 (m, 2H), 4.41–4.20 (m, 2H), 3.94–3.74 (m, 1H), 3.60 (s, 3H), 3.31–3.00 (m, 6H), 2.96–2.71 (m, 3H), 2.58–2.13 (m, 6H), 1.97–1.77 (m, 5H), 1.42–1.13 (m, 3H); ^{13}C NMR δ: 172.6, 133.5, 131.9, 130.6, 130.5, 129.4, 127.3, 60.3, 55.0, 54.5, 51.5, 51.3, 44.1, 40.1, 38.8, 33.5, 32.6, 25.6, 24.7, 21.7, 21.1, 20.8, 17.1; HRMS: calcd for $C_{23}H_{35}N_2O_2Cl_3$ $[M-2HCl+H]^+$, 405.2303; found, 405.2309.

*4.1.2.7. Methyl 12-N-4-chlorobenzylsophoridinate (**6g**).* The title compound was prepared from **4** and 4-chlorobenzaldehyde in the same manner as described above. Yield: 53%; Yellow thick solid; mp 62–64 °C; ^{1}H NMR (DMSO-d_6) δ: 8.16–6.76 (m, 4H), 3.69–3.43 (m, 4H), 3.43–3.22 (m, 2H), 3.22–2.70 (m, 5H), 2.61–2.39 (m, 5H), 2.39–2.17 (m, 2H), 2.19–1.86 (m, 2H), 1.71–1.41 (m, 5H), 1.41–1.12

(m, 3H), 1.14–0.92 (m, 1H); ^{13}C NMR δ: 173.7, 139.6, 131.5 (2), 130.3 (2), 128.6, 81.8, 63.6, 58.6, 57.5, 53.9, 51.6, 51.3, 49.0, 45.2, 33.8, 27.0, 25.4, 24.9, 23.2, 22.7, 19.0; HRMS: calcd for $C_{23}H_{33}N_2O_2Cl$ $[M+H]^+$, 405.2303; found, 405.2307.

*4.1.2.8. Methyl 12-N-4-bromobenzylsophoridinate dihydrochloride (**6h**).* The title compound was prepared from **4** and 4-bromobenzaldehyde in the same manner as described above. Yield: 45%; White solid; mp 88–91 °C; ^{1}H NMR (DMSO-d_6) δ: 11.49 (s, 1H), 11.08 (s, 1H), 7.86 (d, J = 8.2 Hz, 2H), 7.65 (d, J = 8.3 Hz, 2H), 4.37–4.18 (m, 2H), 3.93–3.78 (m, 1H), 3.60 (s, 3H), 3.36–3.20 (m, 2H), 3.15–3.00 (m, 2H), 2.99–2.81 (m, 2H), 2.74–2.41 (m, 5H), 2.31–2.14 (m, 2H), 1.88–1.62 (m, 6H), 1.60–1.34 (m, 3H), 1.21–1.09 (m, 1H); ^{13}C NMR δ: 172.7, 132.8 (2), 131.8, 129.0 (2), 123.0, 60.1, 55.0, 54.6, 51.5, 51.3, 48.5, 44.1, 33.4, 32.5, 25.6, 24.7, 21.8, 21.6, 21.0, 20.7, 17.1; HRMS: calcd for $C_{23}H_{35}N_2O_2Cl_2Br$ $[M-2HCl+H]^+$, 449.1798; found, 449.1806.

*4.1.2.9. Methyl 12-N-4-picolylsophoridinate dihydrochloride (**6i**).* The title compound was prepared from 4 and 4-pyridinecarboxaldehyde in the same manner as described above. Yield: 56%; White solid; mp 89–91 °C; ^{1}H NMR (DMSO-d_6) δ: 11.71 (s, 1H), 9.14–8.92 (m, 2H), 8.75–7.99 (m, 2H), 4.86–4.03 (m, 2H), 3.60 (s, 3H), 3.47–3.35 (m, 2H), 3.24–2.66 (m, 6H), 2.57–2.33 (m, 3H), 2.34–2.05 (m, 3H), 2.05–1.76 (m, 4H), 1.76–1.12 (m, 5H), 1.10–0.74 (m, 1H); ^{13}C NMR δ: 173.9, 145.9, 143.0 (2), 125.0 (2), 61.7, 51.5, 51.3, 48.5, 44.5, 44.2, 33.7, 33.1, 32.8, 25.8, 25.0, 21.9, 21.4, 21.3, 21.0, 17.2; HRMS: calcd for $C_{22}H_{35}N_3O_2Cl_2$ $[M-2HCl+H]^+$, 372.2645; found, 372.2644.

*4.1.2.10. Methyl 12-N-2-furfurylsophoridinate dihydrochloride (**6j**).* The title compound was prepared from **4** and 2-furaldehyde in the same manner as described above. Yield: 56%; Yellow solid; mp 98–111 °C; ^{1}H NMR (DMSO-d_6) δ: 11.60 (s, 1H), 11.10 (s, 1H), 7.75 (d, J = 7.1 Hz, 1H), 6.87 (m, 1H), 6.55 (d, J = 7.1 Hz, 1H), 4.62–4.20 (m, 5H), 3.62–3.28 (m, 1H), 3.15 (s, 3H), 3.06–2.84 (m, 3H), 2.78–2.60 (m, 2H), 2.41–2.14 (m, 2H), 1.96–1.69 (m, 5H), 1.65 (m, 4H), 1.43–1.30 (m, 3H), 1.28–1.15 (m, 1H); ^{13}C NMR δ: 172.7, 144.6, 143.9, 114.7, 111.2, 61.2, 54.9, 51.5, 51.3, 50.5, 48.5, 48.3, 44.1, 33.4, 32.7, 25.7, 24.8, 22.0, 21.8, 21.1, 21.0; HRMS: calcd for $C_{21}H_{34}N_2O_3Cl_2$ $[M-2HCl+H]^+$, 361.2485; found, 361.2487.

*4.1.3. General procedures for the synthesis of compounds (**9a**–**n**)*

After the suspension of $LiAH_4$ (0.5 g, 12.0 mmol) in anhydrous tetrahydrofuran, Compound **6** (4.0 mmol) in anhydrous tetrahydrofuran was added dropwise under strict moistureless conditions. Then the reaction solution was stirred at room temperature till TLC analysis showed completion of the reaction. The reaction system was deactivated successively with water (0.5 ml), 3 N NaOH aqueous solution (0.5 ml) under ice bath condition. The reaction mixture was filtered and the filtrate was evaporated to afford crude target compound, which was purified by flash chromatography over silica gel or acidized with 3 N hydrochloride/ether (10 ml) to obtain desired compounds.

*4.1.3.1. 12-N-n-Propylsophoridinol dihydrochloride (**9a**).* The title compound was prepared in the same manner as described above. Yield: 81%; Yellow solid; mp 123–125 °C; ^{1}H NMR (DMSO-d_6) δ: 11.55 (s, 1H), 10.63 (s, 1H), 3.71–3.39 (m, 7H), 3.23–2.74 (m, 6H), 2.52–2.20 (m, 3H), 1.97–1.20 (m, 14H), 0.97–0.75 (m, 3H); ^{13}C NMR δ: 61.2, 60.8, 55.6, 54.7, 52.0, 51.4, 44.6, 33.8, 32.5, 32.4, 26.0, 25.2, 22.7, 22.4, 21.8, 17.5, 17.2, 11.3; HRMS: calcd for $C_{18}H_{36}N_2OCl_2$ $[M-2HCl+H]^+$, 295.2743; found, 295.2745.

*4.1.3.2. 12-N-n-Butylsophoridinol dihydrochloride (**9b**).* The title compound was prepared in the same manner as described above. Yield: 83%; Yellow solid; mp 118–121 °C; ^{1}H NMR (DMSO-d_6) δ: 11.65 (s, 1H), 10.72 (s, 1H), 4.71–4.35 (m, 2H), 3.78–3.26 (m, 6H), 3.21–2.75 (m, 5H), 2.73–2.08 (m, 5H), 1.69–1.52 (m, 3H), 1.53–1.35 (m, 6H), 1.34–1.10 (m, 4H), 1.00–0.65 (m, 4H); ^{13}C NMR δ: 60.9, 56.4, 53.0, 52.0, 49.0, 44.6, 36.0, 35.5, 33.1, 32.6, 28.0, 25.7, 25.3, 23.7, 22.5, 20.0, 19.0, 17.8, 14.1; HRMS: calcd for $C_{19}H_{38}N_2OCl_2$ $[M-2HCl+H]^+$, 309.2900; found, 309.2901.

*4.1.3.3. 12-N-Neopentylsophoridinol (**9c**).* The title compound was prepared in the same manner as described above. Yield: 86%; White solid; mp 81–83 °C; ^{1}H NMR (DMSO-d_6) δ: 3.15–2.97 (m, 4H), 2.63–2.51 (m, 2H), 2.48–2.27 (m, 4H), 2.24–2.01 (m, 4H), 2.02–1.74 (m, 4H), 1.74–1.26 (m, 7H), 1.27–0.98 (m, 3H), 0.82 (s, 9H); ^{13}C NMR δ: 66.0, 65.9, 61.1, 59.1, 53.9, 52.3, 44.8, 35.9, 34.1, 33.1, 28.2, 27.8 (3), 26.5, 24.3, 24.1, 23.4, 22.9, 18.1; HRMS: calcd for $C_{20}H_{38}N_2O$ $[M+H]^+$, 323.3056; found, 323.3058.

*4.1.3.4. 12-N-Isobutylsophoridinol dihydrochloride (**9d**).* The title compound was prepared in the same manner as described above. Yield: 84%; White solid; mp 119–121 °C; ^{1}H NMR (DMSO-d_6) δ: 11.68 (s, 1H), 10.14 (s, 1H), 3.55–3.22 (m, 7H), 3.23–2.80 (m, 8H), 2.78–2.36 (m, 4H), 2.23–2.09 (m, 2H), 1.26–1.12 (m, 2H), 1.05–0.96 (m, 3H), 0.96–0.87 (m, 3H), 0.83–0.77 (m, 6H); ^{13}C NMR δ: 61.1, 60.8, 59.2, 55.4, 44.6, 33.5, 32.5, 26.0, 23.5, 22.5 (2), 22.4, 22.3, 21.4, 21.3, 21.1, 21.0, 20.9, 17.7; HRMS: calcd for $C_{19}H_{38}N_2OCl_2$ $[M-2HCl+H]^+$, 309.2900; found, 309.2898.

*4.1.3.5. 12-N-Benzylsophoridinol dihydrochloride (**9e**).* The title compound was prepared in the same manner as described above. Yield: 80%; White solid; mp 104–107 °C; ^{1}H NMR (DMSO-d_6) δ: 11.58 (s, 1H), 11.02 (s, 1H), 7.88–7.51 (m, 2H), 7.43–7.21 (m, 3H), 4.42–4.19 (m, 2H), 3.74–3.42 (m, 1H), 3.37–3.22 (m, 4H), 3.09–2.74 (m, 4H), 2.75–2.53 (m, 2H), 2.54–2.32 (m, 2H), 2.00–1.55 (m, 7H), 1.55–1.34 (m, 1H), 1.33–1.15 (m, 4H), 0.90–0.66 (m, 1H); ^{13}C NMR δ: 131.0 (2), 130.2, 129.8, 129.3 (2), 61.2, 60.6, 55.8, 55.6, 52.1, 52.0, 44.6, 33.8, 32.1, 26.1, 25.2, 22.6, 22.3, 22.2, 21.8, 17.6; HRMS: calcd for $C_{22}H_{36}N_2OCl_2$ $[M-2HCl+H]^+$, 343.2744; found, 343.2743.

*4.1.3.6. 12-N-4-Methylbenzylsophoridinol dihydrochloride (**9f**).* The title compound was prepared in the same manner as described above. Yield: 89%; White solid; mp 104–107 °C; ^{1}H NMR (DMSO-d_6) δ: 11.50 (s, 1H), 10.90 (s, 1H), 7.73 (d, J = 7.7 Hz, 2H), 7.22 (d, J = 7.7 Hz, 2H), 4.43–4.24 (m, 1H), 4.13–3.71 (m, 1H), 3.43–3.26 (m, 3H), 2.98–2.75 (m, 2H), 2.67–2.40 (m, 3H), 2.39–2.03 (m, 4H), 2.00–1.53 (m, 7H), 1.53–1.14 (m, 8H), 0.90–0.68 (m, 2H); ^{13}C NMR δ: 139.2, 131.0, 129.8 (2), 127.1 (2), 61.1, 60.6, 55.6, 52.0, 49.0, 48.1, 44.6, 33.8, 32.2, 27.9, 26.1, 25.2, 22.6, 22.3, 21.8, 21.3, 17.6; HRMS: calcd for $C_{23}H_{38}N_2OCl_2$ $[M-2HCl+H]^+$, 357.2900; found, 357.2904.

*4.1.3.7. 12-N-2-Fluorobenzylsophoridinol dihydrochloride (**9g**).* The title compound was prepared in the same manner as described above. Yield: 80%; White solid; mp 107–110 °C; ^{1}H NMR (DMSO-d_6) δ: 11.57 (s, 1H), 11.14 (s, 1H), 7.93–7.68 (m, 2H), 7.49–7.29 (m, 2H), 4.42–4.21 (m, 3H), 3.74–3.58 (m, 1H), 3.42–3.18 (m, 4H), 3.10–2.74 (m, 4H), 2.75–2.54 (m, 2H), 2.55–2.31 (m, 2H), 2.05–1.58 (m, 6H), 1.54–1.13 (m, 5H), 0.99–0.51 (m, 1H); ^{13}C NMR δ: 163.7 (d, J = 242 Hz), 132.8, 131.4, 127.3, 117.8, 117.0, 61.3, 60.6, 55.5, 55.0, 52.4, 52.0, 49.0, 44.6, 33.8, 32.1, 26.0, 25.1, 22.6, 22.3, 21.8, 17.6; HRMS: calcd for $C_{22}H_{35}N_2OFCl_2$ $[M-2HCl+H]^+$, 361.2649; found, 361.2654.

*4.1.3.8. 12-N-3-Fluorobenzylsophoridinol dihydrochloride (**9h**).* The title compound was prepared in the same manner as described

above. Yield: 82%; Yellow solid; mp 101–104 °C; ^{1}H NMR (DMSO-d_6) δ: 11.53 (s, 1H), 11.14 (s, 1H), 7.93–7.70 (m, 1H), 7.68–7.50 (m, 2H), 7.29–7.15 (m, 1H), 4.48–4.35 (m, 1H), 4.31–4.16 (m, 2H), 3.81–3.68 (m, 1H), 3.38–3.02 (m, 6H), 3.02–2.79 (m, 2H), 2.75–2.41 (m, 4H), 2.01–1.58 (m, 6H), 1.57–1.17 (m, 5H), 0.94–0.71 (m, 1H); ^{13}C NMR δ: 163.2 (d, J = 242 Hz), 132.3, 130.9, 126.8, 117.5, 116.3, 60.9, 60.1, 55.0, 54.5, 51.5, 48.5, 44.1, 33.4, 31.7, 25.5, 24.6, 23.5, 22.1, 21.8, 21.3, 17.1; HRMS: calcd for $C_{22}H_{35}N_2OFCl_2$ [M−2HCl+H]$^+$, 361.2649; found, 361.2652.

*4.1.3.9. 12-N-4-Fluorobenzylsophoridinol dihydrochloride (**9i**).* The title compound was prepared in the same manner as described above. Yield: 83%; White solid; mp 102–104 °C; ^{1}H NMR (DMSO-d_6) δ: 11.56 (s, 1H), 10.91 (s, 1H), 8.21–7.77 (m, 2H), 7.14–7.07 (m, 2H), 3.64–3.45 (m, 3H), 3.21–2.90 (m, 5H), 2.70–2.23 (m, 2H), 2.22–2.02 (m, 4H), 1.98–1.56 (m, 6H), 1.55–1.02 (m, 7H), 1.02–0.71 (m, 1H); ^{13}C NMR δ: 159.2 (d, J = 242 Hz), 133.5 (2), 130.2, 116.7 (2), 63.3, 61.0, 59.1, 57.3, 52.2, 50.3, 44.7, 35.6, 33.1, 28.0, 26.5, 23.8, 23.1, 22.8, 22.4, 18.1; HRMS: calcd for $C_{22}H_{35}N_2OFCl_2$ [M−2HCl+H]$^+$, 361.2649; found, 361.2647.

*4.1.3.10. 12-N-2-Chlorobenzylsophoridinol dihydrochloride (**9j**).* The title compound was prepared in the same manner as described above. Yield: 88%; Thick yellow solid; mp 107–110 °C; ^{1}H NMR (DMSO-d_6) δ: 11.60 (s, 1H), 11.06 (s, 1H), 8.77–8.10 (m, 1H), 7.79–6.98 (m, 3H), 4.56–4.31 (m, 1H), 3.70–3.54 (m 1H), 3.40–2.97 (m, 7H), 2.88–2.34 (m, 6H), 2.03–1.77 (m, 4H), 1.79–1.58 (m, 2H), 1.58–1.12 (m, 6H), 0.94–0.71 (m, 1H); ^{13}C NMR δ: 133.9, 132.7, 131.7, 130.6, 128.3, 127.9, 62.1, 60.6, 55.5, 52.6, 52.2, 52.0, 44.6, 33.9, 32.2, 28.2, 26.0, 25.2, 22.8, 22.3, 21.7, 17.6; HRMS: calcd for $C_{22}H_{35}N_2OCl_3$ [M−2HCl+H]$^+$, 377.2354; found, 377.2358.

*4.1.3.11. 12-N-4-Chlorobenzylsophoridinol (**9k**).* The title compound was prepared in the same manner as described above. Yield: 86%; Yellow solid; mp 69–71 °C; ^{1}H NMR (DMSO-d_6) δ: 7.61–7.19 (m, 4H), 3.60–3.20 (m, 5H), 3.17–2.99 (m, 2H), 2.91–2.42 (m, 3H), 2.40–1.98 (m, 5H), 1.89–1.67 (m, 4H), 1.64–1.27 (m, 7H), 1.26–1.04 (m, 2H); ^{13}C NMR δ: 139.3, 131.6, 130.3 (2), 128.6 (2), 63.5, 61.0, 59.0, 57.3, 52.4, 50.5, 44.7, 35.8, 33.1, 27.8, 26.8, 23.9, 23.3, 23.1, 22.6, 18.2; HRMS: calcd for $C_{22}H_{33}N_2OCl$ [M+H]$^+$, 377.2354; found, 377.2358.

*4.1.3.12. 12-N-4-Picolylsophoridinol dihydrochloride (**9l**).* The title compound was prepared in the same manner as described above. Yield: 82%; Yellow solid; mp 117–119 °C; ^{1}H NMR (DMSO-d_6) δ: 11.62 (s, 2H), 11.06 (s, 1H), 8.99 (d, J = 6.8 Hz, 2H), 8.52 (d, J = 6.8 Hz, 2H), 4.81–3.55 (m, 4H), 3.48–3.23 (m, 3H), 3.23–2.68 (m, 4H), 2.49–2.28 (m, 3H), 2.00–1.75 (m, 4H), 1.75–1.53 (m, 3H), 1.53–1.10 (m, 5H), 1.08–0.75 (m, 1H); ^{13}C NMR δ: 149.0 (2), 148.3, 143.4 (2), 62.8, 61.8, 56.0, 55.6, 55.5, 55.4, 55.3, 54.9, 54.3, 53.6, 52.0, 37.9, 33.3, 32.1, 26.2, 25.5; HRMS: calcd for $C_{21}H_{35}N_3OCl_2$ [M−2HCl+H]$^+$, 344.2696; found, 344.2696.

*4.1.3.13. 12-N-2-Furfurylsophoridinol dihydrochloride (**9m**).* The title compound was prepared in the same manner as described above. Yield: 85%; Yellow solid; mp 121–124 °C; ^{1}H NMR (DMSO-d_6) δ: 11.51 (s, 1H), 11.01 (s, 1H), 7.78 (d, J = 7.4 Hz, 1H), 6.88–6.56 (m, 2H), 3.70–3.42 (m, 2H), 3.36–3.25 (m, 1H), 3.23–3.09 (m, 3H), 3.0–2.85 (m, 2H), 2.75–2.58 (m, 2H), 2.55–2.04 (m, 4H), 1.99–1.75 (m, 5H), 1.75–1.58 (m, 2H), 1.52–1.21 (m, 5H), 1.18–1.08 (m, 2H); ^{13}C NMR δ: 144.6, 144.0, 114.6, 111.3, 61.8, 60.2, 54.9, 51.5, 50.5, 48.2, 44.1, 33.4, 31.9, 25.7, 24.7, 22.3, 22.2, 21.8, 21.3, 17.0; HRMS: calcd for $C_{20}H_{34}N_2O_2Cl_2$ [M−2HCl+H]$^+$, 333.2464; found, 333.2538.

*4.1.3.14. 12-N-2-Phenethylsophoridinol dihydrochloride (**9n**).* The title compound was prepared in the same manner as described above. Yield: 83%; Yellow solid; mp 110–112 °C; ^{1}H NMR (DMSO-d_6) δ: 11.45 (s, 1H), 10.98 (s, 1H), 7.60–6.93 (m, 5H), 3.67–3.23 (m, 8H), 3.22–2.92 (m, 5H), 2.87–2.53 (m, 4H), 1.90–1.80 (m, 4H), 1.77–1.59 (m, 4H), 1.59–0.99 (m, 5H); ^{13}C NMR δ: 137.5, 129.2 (2), 129.0 (2), 127.2, 61.6, 60.8, 55.5, 54.2, 51.9, 51.3, 49.0, 44.6, 33.9, 32.4, 29.9, 26.1, 22.7, 22.5, 22.4, 21.9, 17.5; HRMS: calcd for $C_{23}H_{38}N_2OCl_2$ [M−2HCl+H]$^+$, 357.2900; found, 357.2901.

*4.1.4. General procedures for the synthesis of sophoridinic acid (**7a**–**j**)*

Compound **6** (4.0 mmol) was dissolved in 3 N HCl (15 ml) and heated to reflux for 3 h. Then the pH of reaction solution was adjusted to 8–9 by addition of NH_4OH. The reaction solvent was removed under reduced pressure. Then the residue was dissolved with MeOH and filtered to remove the inorganic salts. The solution was concentrated to obtain crude targeted compound, which was further separated by flash column chromatography on silica gel to afford purified title compounds.

*4.1.4.1. 12-N-n-Propylsophoridinic acid (**7a**).* The title compound was prepared in the same manner as described above. Yield: 81%; White solid; mp 127–130 °C; ^{1}H NMR (DMSO-d_6) δ: 3.60–3.40 (m, 2H), 3.31–3.02 (m, 3H), 2.76–2.35 (m, 5H), 2.35–2.23 (m, 3H), 2.22–2.05 (m, 2H), 1.98–1.95 (m, 2H), 1.93–1.82 (m, 1H), 1.80–1.59 (m, 3H), 1.59–1.39 (m, 6H), 1.37–1.20 (m, 1H), 1.16–0.91 (m, 3H); ^{13}C NMR δ: 180.1, 64.1, 61.1, 56.8, 52.4, 46.3, 37.3, 37.0, 29.2, 27.7, 24.7, 24.3, 24.0, 23.0, 21.8, 21.6, 19.2, 11.9; HRMS: calcd for $C_{18}H_{32}N_2O_2$ [M+H]$^+$, 309.2536; found, 309.2538.

*4.1.4.2. 12-N-Isobutylsophoridinic acid (**7b**).* The title compound was prepared in the same manner as described above. Yield: 84%; White solid; mp 133–135 °C; ^{1}H NMR (DMSO-d_6) δ: 3.47–3.29 (m, 1H), 3.27–3.13 (m, 1H), 3.13–2.83 (m, 3H), 2.62–2.45 (m, 3H), 2.45–2.36 (m, 1H), 2.33–2.05 (m, 6H), 2.07–1.91 (m, 2H), 1.91–1.70 (m, 3H), 1.69–1.48 (m, 3H), 1.48–1.28 (m, 3H), 1.18–0.83 (m, 7H); ^{13}C NMR δ: 180.1, 64.1, 61.1, 56.8, 52.4, 46.3, 37.3, 37.0, 29.2, 27.7, 25.1, 24.7, 24.3, 24.0, 23.0, 21.8 (2), 19.2, 11.9; HRMS: calcd for $C_{19}H_{34}N_2O_2$ [M+H]$^+$, 323.2693; found, 323.2696.

*4.1.4.3. 12-N-Benzylsophoridinic acid (**7c**).* The title compound was prepared in the same manner as described above. Yield: 84%; White solid; mp 139–142 °C; ^{1}H NMR (DMSO-d_6) δ: 7.47–7.36 (m, 2H), 7.33–7.17 (m, 3H), 3.59–3.43 (m, 3H), 3.28–2.79 (m, 2H), 2.78–2.52 (m, 2H), 2.47–2.04 (m, 6H), 2.01–1.53 (m, 9H), 1.53–1.35 (m, 2H), 1.35–1.09 (m, 2H); ^{13}C NMR δ: 172.3, 139.4, 135.8 (2), 129.4 (2), 126.5, 62.7, 60.0, 57.9, 52.6, 52.5, 50.3, 44.9, 36.1, 28.0, 26.4, 23.9, 22.8, 22.7, 21.7, 17.8; HRMS: calcd for $C_{22}H_{32}N_2O_2$ [M+H]$^+$, 357.2536; found, 357.2539.

*4.1.4.4. 12-N-2-Fluorobenzylsophoridinic acid (**7d**).* The title compound was prepared in the same manner as described above. Yield: 86%; White solid; mp 127–130 °C; ^{1}H NMR (DMSO-d_6) δ: 7.43–7.25 (m, 2H), 7.16–6.99 (m, 2H), 3.75–3.43 (m, 4H), 3.37–3.21 (m, 3H), 3.03–2.54 (m, 3H), 2.43–2.04 (m, 5H), 2.03–1.82 (m, 3H), 1.78–1.56 (m, 5H), 1.46–1.20 (m, 3H); ^{13}C NMR δ: 180.8, 162.3 (d, J = 242 Hz), 132.2, 129.8, 127.6, 125.1, 117.1, 64.6, 61.2, 53.8, 52.1, 51.6, 46.2, 37.6, 37.3, 29.4, 27.8, 24.9, 24.1, 24.0, 23.3, 19.2; HRMS: calcd for $C_{22}H_{31}N_2O_2F$ [M+H]$^+$, 375.2442; found, 375.2450.

*4.1.4.5. 12-N-3-Fluorobenzylsophoridinic acid (**7e**).* The title compound was prepared in the same manner as described above. Yield: 81%; Yellow solid; mp 121–133 °C; ^{1}H NMR (DMSO-d_6) δ: 7.81–7.51 (m, 1H), 7.43–7.23 (m, 1H), 7.23–6.95 (m, 2H), 3.75–3.42 (m, 4H), 3.35–3.06 (m, 3H), 2.68–2.35 (m, 3H), 2.36–2.16 (m, 4H), 2.14–1.80 (m, 4H), 1.78–1.36 (m, 7H), 1.34–1.20 (m, 1H); ^{13}C NMR δ: 180.8,

165.7 (d, J = 242 Hz), 144.0, 131.0, 126.2, 118.7, 115.8, 66.9, 61.4, 58.8, 54.0, 51.6, 46.3, 37.6, 36.9, 29.5, 27.7, 24.6, 24.2, 24.0, 23.3, 19.2; HRMS: calcd for $C_{22}H_{31}N_2O_2F$ $[M+H]^+$, 375.2442; found, 375.2449.

*4.1.4.6. 12-N-4-Fluorobenzylsophoridinic acid (**7f**).* The title compound was prepared in the same manner as described above. Yield: 82%; White solid; mp 133–135 °C; ^{1}H NMR (DMSO-d_6) δ: 7.45–7.24 (m, 2H), 7.05–6.95 (m, 2H), 3.56–3.45 (m, 3H), 3.41–3.33 (m, 1H), 3.26–3.04 (m, 2H), 2.71–2.59 (m, 1H), 2.54–2.34 (m, 2H), 2.33–2.13 (m, 4H), 2.13–1.82 (m, 4H), 1.73–1.52 (m, 5H), 1.51–1.33 (m, 2H), 1.34–1.20 (m, 2H); ^{13}C NMR δ: 179.7, 163.1 (d, J = 242 Hz), 135.3 (2), 129.8, 114.5 (2), 62.6, 59.8, 57.0, 52.3, 50.2, 47.8, 36.4, 35.8, 28.0, 26.4, 23.5, 22.6, 21.6, 21.1, 17.8; HRMS: calcd for $C_{22}H_{31}N_2O_2F$ $[M+H]^+$, 375.2442; found, 375.2447.

*4.1.4.7. 12-N-3-Chlorobenzylsophoridinic acid (**7g**).* The title compound was prepared in the same manner as described above. Yield: 79%; White solid; mp 130–133 °C; ^{1}H NMR (DMSO-d_6): δ 7.42 (s, 1H), 7.39–7.25 (m, 2H), 7.25–7.11 (m, 1H), 3.68–3.42 (m, 2H), 3.30–3.14 (m, 1H), 3.15–2.84 (m, 4H), 2.69–2.30 (m, 3H), 2.25–2.07 (m, 4H), 2.05–1.94 (m, 1H), 1.88–1.56 (m, 4H), 1.56–1.44 (m, 4H), 1.42–1.21 (m, 3H); ^{13}C NMR: δ 175.6, 133.4, 132.9, 130.4, 130.0, 128.2, 127.1, 63.6, 58.1, 57.6, 52.7, 51.1, 44.6, 36.4, 35.3, 28.2, 27.3, 24.2, 24.1, 23.4, 22.6, 18.6; HRMS: calcd for $C_{22}H_{31}N_2O_2Cl$ $[M+H]^+$, 391.2147; found, 391.2152.

*4.1.4.8. 12-N-4-Chlorobenzylsophoridinic acid (**7h**).* The title compound was prepared in the same manner as described above. Yield: 82%; White solid; mp 127–130 °C; ^{1}H NMR (DMSO-d_6) δ: 7.39 (d, J = 7.5, 2H), 7.30 (d, J = 7.5, 2H), 3.52–3.42 (m, 2H), 3.33–3.26 (m, 1H), 3.26–3.04 (m, 2H), 2.64–2.49 (m, 2H), 2.44–2.26 (m, 2H), 2.27–2.13 (m, 3H), 2.11–2.02 (m, 3H), 2.02–1.80 (m, 4H), 1.67–1.50 (m, 4H), 1.50–1.35 (m, 2H), 1.33–1.18 (m, 1H); ^{13}C NMR δ: 179.2, 138.3, 132.2, 130.5 (2), 127.9 (2), 62.7, 59.7, 57.1, 52.4, 50.3, 44.8, 36.0, 35.9, 28.0, 26.4, 23.4, 22.7, 21.7, 17.8; HRMS: calcd for $C_{22}H_{31}N_2O_2Cl$ $[M+H]^+$, 391.2147; found, 391.2152.

*4.1.4.9. 12-N-4-Bromobenzylsophoridinic acid (**7i**).* The title compound was prepared in the same manner as described above. Yield: 72%; White solid; mp 137–139 °C; ^{1}H NMR (DMSO-d_6) δ: 7.44 (d, J = 8.1 Hz, 2H), 7.27 (d, J = 8.1 Hz, 2H), 3.68–3.44 (m, 4H), 3.40–3.33 (m, 1H), 3.29–3.19 (m, 2H), 3.06–2.66 (m, 2H), 2.57–2.35 (m, 2H), 2.35–2.15 (m, 4H), 2.11–1.70 (m, 5H), 1.70–1.36 (m, 5H), 1.28 (d, J = 3.9 Hz, 1H); ^{13}C NMR δ: 170.1, 130.7, 122.9 (2), 122.0 (2), 112.1, 57.4, 54.7, 52.0, 49.1, 44.6, 42.2, 36.9, 28.2, 27.1, 20.0, 18.2, 15.0, 14.8, 13.7; HRMS: calcd for $C_{22}H_{31}N_2O_2Br$ $[M+H]^+$, 435.1659; found, 435.1658.

*4.1.4.10. 12-N-4-Picolylsophoridinic acid (**7j**).* The title compound was prepared in the same manner as described above. Yield: 80%; White solid; mp 151–154 °C; ^{1}H NMR (DMSO-d_6) δ: 8.72–8.37 (m, 2H), 7.66–7.46 (m, 2H), 3.81–3.69 (m, 3H), 3.57–3.35 (m, 3H), 3.18–2.73 (m, 2H), 2.59–2.24 (m, 6H), 2.01–1.81 (m, 5H), 1.73–1.41 (m, 5H), 1.39–1.21 (m, 2H); ^{13}C NMR δ: 177.4, 153.5, 150.6 (2), 125.4 (2), 64.9, 61.5, 58.2, 54.3, 51.7, 46.5, 38.0, 34.7, 29.4, 27.5, 24.3, 24.0, 23.9, 23.8, 19.2; HRMS: calcd for $C_{21}H_{31}N_3O_2$ $[M+H]^+$, 358.2489; found, 358.2494.

*4.1.5. General procedures for the synthesis of compounds (**10a**–**f**)*

To a stirred solution of compound **9** (5.0 mmol) in anhydrous tetrahydrofuran, methyl/ethyl benzenesulfonate (20.0 mmol) was added dropwise under strict moistureless conditions. Then the reaction solution was stirred at room temperature till TLC analysis showed completion of the reaction. The reaction mixture was deactivated with methanol and evaporated to afford crude product, which was purified by flash chromatography over silica gel.

*4.1.5.1. 12-N-Benzylsophoridinic methyl ether (**10a**).* The title compound was prepared in the same manner as described above. Yield: 87%; Yellow thick solid; mp 56–59 °C; ^{1}H NMR (DMSO-d_6) δ: 7.62–7.49 (m, 3H), 7.30–7.11 (m, 2H), 4.44–4.26 (m, 2H), 3.60–3.39 (m, 3H), 3.39–3.07 (m, 6H), 3.07–2.75 (m, 3H), 2.75–2.42 (m, 5H), 2.14–1.88 (m, 2H), 1.87–1.50 (m, 5H), 1.40–1.13 (m, 5H); ^{13}C NMR δ: 137.9, 128.6 (2), 128.4 (2), 127.5, 74.6, 67.5, 62.0, 60.8, 57.8, 54.8, 53.2, 40.8, 37.9, 30.9, 30.5, 28.2, 27.4, 26.2, 24.1, 23.4, 23.2; HRMS: calcd for $C_{23}H_{36}N_2O$ $[M+H]^+$, 357.2900; found, 357.2904.

*4.1.5.2. 12-N-Benzylsophoridinic ethyl ether (**10b**).* The title compound was prepared in the same manner as described above. Yield: 81%; Yellow thick solid; mp 62–65 °C; mp 127–130 °C; ^{1}H NMR (DMSO-d_6) δ: 7.65–7.58 (m, 3H), 7.37–7.32 (m, 2H), 4.44–4.25 (m, 2H), 4.05–3.71 (m, 2H), 3.69–3.40 (m, 3H), 3.40–3.13 (m, 5H), 3.13–2.63 (m, 5H), 2.53–2.45 (m, 2H), 2.14–1.87 (m, 2H), 1.70 (m, 5H), 1.37–1.12 (m, 7H); ^{13}C NMR δ: 137.9, 128. 7 (2), 128.5 (2), 127.6, 71.9, 67.6, 66.8, 62.0, 60.8, 54.8, 53.2, 40.9, 37.9, 30.9, 30.4, 28.3, 26.3, 25.5, 24.2, 23.4, 23.3, 14.8; HRMS: calcd for $C_{24}H_{38}N_2O$ $[M+H]^+$, 371.3057; found, 371.3056.

*4.1.5.3. 12-N-4-Fluorobenzylsophoridinic methyl ether (**10c**).* The title compound was prepared in the same manner as described above. Yield: 85%; Yellow thick solid; mp 60–62 °C; ^{1}H NMR (DMSO-d_6) δ: 7.61–7.58 (m, 2H), 7.30–7.20 (m, 2H), 4.48–4.25 (m, 2H), 3.60–3.10 (m, 10H), 3.09–2.75 (m, 3H), 2.66–2.42 (m, 5H), 1.89–1.12 (m, 11H); ^{13}C NMR δ: 163.6 (d, J = 242 Hz), 132.3, 130.8 (2), 115.3 (2), 74.6, 67.5, 62.0, 60.1, 57.8, 54.8, 53.1, 40.8, 37.9, 35.4, 30.9, 30.5, 28.2, 26.2, 24.1, 23.4, 23.2; HRMS: calcd for $C_{23}H_{35}N_2OF$ $[M+H]^+$, 375.2806; found, 375.2802.

*4.1.5.4. 12-N-4-Fluorobenzylsophoridinic ethyl ether (**10d**).* The title compound was prepared in the same manner as described above. Yield: 76%; Yellow thick solid; mp 55–57 °C; ^{1}H NMR (DMSO-d_6) δ: 7.62–7.57 (m, 2H), 7.28–7.15 (m, 2H), 4.47–4.21 (m, 2H), 3.99–3.41 (m, 5H), 3.40–3.11 (m, 4H), 3.09–2.80 (m, 3H), 2.75–2.26 (m, 5H), 2.14–1.54 (m, 6H), 1.55–0.69 (m, 8H); ^{13}C NMR δ: 163.5 (d, J = 242 Hz), 132.2, 130.7 (2), 115.2 (2), 71.9, 67.5, 66.8, 62.0, 60.8, 54.8, 53.2, 40.8, 37.9, 30.9, 30.4, 28.2, 26.2, 25.1, 24.1, 23.4, 23.2, 14.8; HRMS: calcd for $C_{24}H_{37}N_2OF$ $[M+H]^+$, 389.2962; found, 389.2958.

*4.1.5.5. 12-N-4-Chlororobenzylsophoridinic methyl ether (**10e**).* The title compound was prepared in the same manner as described above. Yield: 81%; Yellow thick solid; mp 62–65 °C; ^{1}H NMR (DMSO-d_6) δ: 7.69–7.57 (m, 2H), 7.41–7.29 (m, 2H), 4.48–4.18 (m, 2H), 4.03–3.71 (m, 2H), 3.71–3.42 (m, 3H), 3.42–3.12 (m, 5H), 3.12–2.83 (m, 3H), 2.78–2.55 (m, 3H), 1.89–1.56 (m, 5H), 1.54–1.11 (m, 8H); ^{13}C NMR δ: 136.4, 132.0, 129.3 (2), 128.2 (2), 74.0, 67.5, 62.0, 60.8, 57.8, 54.4, 53.2, 40.8, 37.9, 30.9, 30.5, 28.2, 26.2, 25.8, 24.1, 23.4, 23.2; HRMS: calcd for $C_{23}H_{35}N_2OCl$ $[M+H]^+$, 389.2510; found, 391.2509.

*4.1.5.6. 12-N-4-Chlororobenzylsophoridinic methyl ether (**10f**).* The title compound was prepared in the same manner as described above. Yield: 82%; Yellow thick solid; mp 60–62 °C; ^{1}H NMR (DMSO-d_6) δ: 7.69–7.58 (m, 2H), 7.39–7.28 (m, 2H), 4.47–4.21 (m, 2H), 3.99–3.41 (m, 5H), 3.40–3.11 (m, 4H), 3.09–2.80 (m, 3H), 2.75–2.26 (m, 5H), 2.14–1.54 (m, 6H), 1.55–0.69 (m, 8H); ^{13}C NMR δ: 136.9, 132.2, 129.5 (2), 128.2 (2), 71.9, 67.5, 66.8, 62.2, 60.1, 54.4, 53.1, 40.7, 37.5, 30.3, 30.4, 28.9, 26.8, 25.2, 24.7, 23.4, 23.5, 14.4; HRMS: calcd for $C_{24}H_{37}N_2OCl$ $[M+H]^+$, 405.2666; found, 405.2667.

*4.1.6. 12-N-4-chlorobenzylsophoridinic amide dihydrochloride (**8**)*

To a solution of the mixture **7h** (160 mg, 0.41 mmol) and NH_4HCO_3 (40 mg, 0.49 mmol) in acetonitrile (10 ml), di-tert-butyl dicarbonate (115 μL, 0.53 mmol) and pyridine (20 μL, 0.25 mmol) were added. Then the reaction solution was stirred at room temperature till TLC analysis showed completion of the reaction. The reaction mixture was filtered and the filtrate was evaporated to afford crude compound **8**, which was further purified with flash column chromatography and acidized with 3 N hydrochloride/ether. Yield: 82%; White solid; mp 127–130 °C; ^{1}H NMR (DMSO-d_6) δ: 11.54 (s, 1H), 11.05 (s, 1H), 7.95 (d, J = 8.4 Hz, 2H), 7.52 (d, J = 8.4 Hz, 2H), 4.38–4.19 (m, 2H), 3.32–3.19 (m, 3H), 3.14–2.76 (m, 5H), 2.70–2.36 (m, 5H), 2.07–1.94 (m, 2H), 1.94–1.59 (m, 7H), 1.56–1.30 (m, 3H), 1.18–1.07 (m, 1H); ^{13}C NMR δ: 179.2, 138.3, 132.2, 130.5 (2), 127.9 (2), 62.7, 59.7, 57.1, 52.4, 50.3, 44.8, 36.0, 35.9, 28.0, 26.4, 23.4, 22.7, 21.7, 17.8; HRMS: calcd for $C_{22}H_{34}N_3OCl_3$ [M−2HCl+H]$^+$, 390.2307; found, 390.2306.

*4.1.7. General procedures for the synthesis of compounds **14** and **15a–c***

Compound **11** (4.96 g, 20 mmol) was evenly dispersed in 3 N NaOH solution (50 ml) and heated to reflux for 8 h. Then the solvent was evaporated under vacuum and anhydrous methanol (50 ml) and 10 ml 12 N HCl solution were added into the residue. The reaction mixture was stirred at room temperature for 2 h and the solvent was evaporated under vacuum. To a solution of residue in CH_3CN (30 ml), anhydrous K_2CO_3 (3.5 eq) and benzyl chloride (1.5 eq) were added and then the reaction solution was stirred at room temperature till TLC analysis showed completion of the reaction. The reaction mixture was filtered and the filtrate was evaporated to afford crude compound **14**, which was further purified via flash column chromatography on silica gel with dichloromethane/methanol as eluents.

After the suspension of $LiAH_4$ (0.5 g, 12.0 mmol) in anhydrous THF, **14** (4.0 mmol) in anhydrous THF was added dropwise under strict moistureless conditions. Then the reaction solution was stirred at room temperature till TLC analysis showed completion of the reaction. The reaction system was deactivated successively with water (0.5 ml), 3 N NaOH aqueous solution (0.5 ml) under ice bath condition. The reaction mixture was filtered and the filtrate was evaporated to afford crude product, which was purified by flash chromatography over silica gel or acidized with 3 N hydrochloride/ether to obtain ***15a–c***.

*4.1.7.1. Methyl 12-N-4-fluorobenzylmatrinate dihydrochloride (**14**).* The title compound was prepared in the same manner as described above. Yield: 82%; mp 99–101 °C; ^{1}H NMR (DMSO-d_6) δ: 11.59 (s, 1H), 11.08 (s, 1H), 7.80–7.58 (m, 2H), 7.38–7.25 (m, 2H), 4.25–4.15 (m, 2H), 4.05–3.84 (m, 2H), 3.60–3.43 (m, 3H), 3.31–3.10 (m, 3H), 3.05–2.78 (m, 3H), 2.63–2.53 (m, 2H), 2.17–1.90 (m, 3H), 1.85–1.65 (m, 7H), 1.63–1.30 (m, 4H); ^{13}C NMR δ: 173.7, 164.2 (d, J = 242 Hz), 134.2 (2), 126.6, 116.0 (2), 60.7, 56.8, 54.6, 52.0, 51.8, 49.0, 36.3, 32.8, 30.4, 27.9, 26.6, 24.4, 23.9, 21.7, 18.2; HRMS: calcd for $C_{22}H_{35}N_2O_2F$ [M−2HCl+H]$^+$, 389.2958; found, 389.2564.

*4.1.7.2. 12-N-Benzylmatrinol dihydrochloride (**15a**).* The title compound was prepared in the same manner as described above. Yield: 82%; mp 92–95 °C; ^{1}H NMR (MeOD) δ: 7.71–7.54 (m, 2H), 7.53–7.36 (m, 3H), 4.28–3.87 (m, 6H), 3.67–3.50 (m, 1H), 3.37–3.15 (m, 2H), 3.07–2.80 (m, 2H), 2.75–2.36 (m, 5H), 2.01–1.62 (m, 6H), 1.63–1.33 (m, 6H); ^{13}C NMR δ: 132.6, 131.1 (2), 130.0 (2), 129.4, 61.9, 61.4, 61.3, 58.7, 55.4, 55.3, 49.9, 37.2, 33.0, 31.2, 30.4, 29.5, 25.2, 24.8, 24.0, 19.1; HRMS: calcd for $C_{22}H_{34}N_2O$ [M−2HCl+H]$^+$, 389.2958; found, 389.2958.

*4.1.7.3. 12-N-4-Fluorobenzylmatrinol dihydrochloride (**15b**).* The title compound was prepared in the same manner as described above. Yield: 79%; mp 99–101 °C; ^{1}H NMR (DMSO-d_6) δ: 11.51 (s, 1H), 11.08 (s, 1H), 7.75–7.58 (m, 2H), 7.36–7.19 (m, 2H), 4.28–4.10 (m, 2H), 4.07–3.76 (m, 6H), 3.67–3.52 (m, 1H), 3.32–3.17 (m, 2H), 3.10–2.78 (m, 3H), 2.73–2.53 (m, 3H), 1.97–1.64 (m, 5H), 1.64–1.38 (m, 6H); ^{13}C NMR δ: 164.2 (d, J = 242 Hz), 134.3 (2), 126.6, 116.2 (2), 61.0, 60.6, 59.0, 57.0, 54.6, 54.6, 49.0, 36.5, 32.5, 30.4, 28.7, 24.4, 24.0, 23.4, 23.2, 18.3; HRMS: calcd for $C_{22}H_{35}N_2OFCl_2$ [M−2HCl+H]$^+$, 361.2649; found, 361.2647.

*4.1.7.4. 12-N-4-Chlorobenzylmatrinol dihydrochloride (**15c**).* The title compound was prepared in the same manner as described above. Yield: 85%; mp 96–99 °C; ^{1}H NMR (DMSO-d_6) δ: 11.63 (s, 1H), 11.05 (s, 1H), 7.67 (d, J = 8.4 Hz, 2H), 7.53 (d, J = 8.4 Hz, 2H), 4.24–4.10 (m, 1H), 4.04–3.83 (m, 2H), 3.71–3.53 (m, 1H), 3.48–3.34 (m, 8H), 3.09–2.83 (m, 3H), 2.75–2.54 (m, 2H), 2.54–2.39 (m, 2H), 1.64–1.43 (m, 9H); ^{13}C NMR δ: 133.0, 131.5, 125.4 (2), 124.8 (2), 64.9, 61.5, 58.2, 54.3, 51.7, 46.5, 38.0, 34.7, 29.4, 28.4, 27.5, 24.3, 24.0, 23.9, 23.8, 19.2; HRMS: calcd for $C_{22}H_{35}N_2OCl_3$ [M−2HCl+H]$^+$, 377.2354; found, 377.2352.

4.2. Tumor cell lines

The human tumor cell lines HepG2, HCT116, H1299, U87, MCF-7 and KB 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% penicillin–streptomycin and incubated at 37 °C with 5% CO_2.

4.3. Cell growth inhibition assay

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 MTT assay as previous described [16]. The growth inhibition rate (%) was calculated at each concentration and IC_{50} value was calculated with Sigmaplot software [17,18]. Results were obtained from triplicate determinations and shown as mean SD.

4.4. Flow cytometric analysis

Flow cytometric analysis was performed as previously described [19]. HepG2 cells were treated with **6g** and **9k** at different concentrations (0, 2.5, 5 and 10 μg/ml) 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).

4.5. TOP I enzyme activity

A gel assay as previously described [20], was used to determine if **9k** inhibited Top I enzyme activity by measuring the decreased mobility of the relaxed isomers of supercoiled pBR322 DNA. Human Top I and pBR322 were obtained from Beyotime (Haimen, Jiangsu, China). One unit of human Top I and the different concentrations compound were mixed and incubated at 37 °C for 20 min, and then 0.5 μg pBR322 DNA was added and the mixture was continued to incubate at 37 °C for 30 min. The reaction was terminated by adding 0.5% SDS, 0.25 μg/ml bromophenol blue, and 15% glycerol and the reaction products were separated on a horizontal 1% agarose gel in tris-acetate/EDTA buffer at 4 V/cm for 40 min at room temperature.

The gel was stained with GelRed I and imaging was conducted by Gel Documentation system (Bio-Rad, USA).

4.6. Pharmacokinetic studies

Male SD rats were obtained from SLAC Laboratory Animal Inc. (Shanghai, China). Three male ICR rats were used in each study. Each of them was dosed with a tested compound at 25 mg/kg via oral administration or 5 mg/kg via intravenous injection. Nine blood samples were collected at 0, 0.25, 0.50, 1.0, 2.0, 4.0, 6.0 and 24 h and were immediately centrifuged to separate the plasma fractions. The separated plasma samples were stored at −20 °C for analysis. Concentration-versus-time profiles were obtained for each analyte, and standard non-compartmental analysis was performed on the data using WinNonlin software, version 5.3, to recover the AUC and other non-compartmental parameters.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.04.069.

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