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Synthesis and Biological Evaluation of Sophoridinol Derivatives as a Novel Family of Potential Anticancer Agents

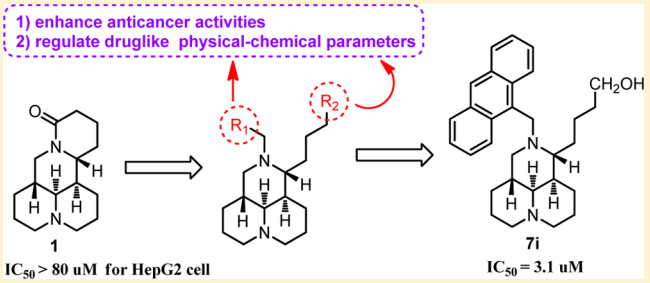
Chongwen Bi,[†,§] Caixia Zhang,[†,§] Yinghong Li,[†] Sheng Tang,[†] Shenggang Wang,[‡] Rongguang Shao,[†] Haigen Fu,[†] Feng Su,*[,‡] and Danqing Song*[,†]

[†]Institute of Medicinal Biotechnology, Chinese Academy of Medical Science & Peking Union Medical College, Beijing 100050, China

[‡]College of Chemical Engineering, Qingdao University of Science and Technology, Qingdao 266042, China

Supporting Information

ABSTRACT: New *N*-substituted sophoridinic acid/ester and sophoridinol derivatives were synthesized and evaluated for their cytotoxic activity in human HepG2 hepatoma cells from the lead sophoridine (**1**). Among the newly synthesized compounds, sophoridinol **7i** displayed a potential antiproliferative activity with an IC_{50} of 3.1 μM. Importantly, it exerted an almost equipotent effect against both wild MCF-7 and adriamycin (AMD)-resistant MCF-7 (MCF-7/AMD) breast carcinoma cell lines. Its mode of action was to arrest the cell cycle at the G0/G1 phase, consistent with that of the parent **1**. In addition, compound **7i** also showed a reasonable ClogP value and favorable pharmacokinetic property with an area under the concentration−time curve (AUC) of 10.3 μM·h in rats, indicating an ideal druggable characteristic. We consider sophoridinol derivatives to be a novel family of promising antitumor agents with an advantage of inhibiting drug-resistant cancer cells.



KEYWORDS: *Sophoridine, sophoridinol, structure−activity relationship, antiproliferative, drug resistance*

The Chinese traditional medicine *Fufang Kushen injection*, which was approved by Chinese FDA (CFDA) in 1995 as an anticancer drug, has been widely used to treat nonsmall cell lung carcinoma, live cancer, and gastric cancer in combination with other anticancer drugs, such as vinorelbine, cisplatin, and taxol et al.[1−6] The main chemical ingredients of Fufang Kushen injection are oxymatrine, matrine, and sophoridine (**1**, Figure 1),[7−9] and all of them are quinolizidine natural products

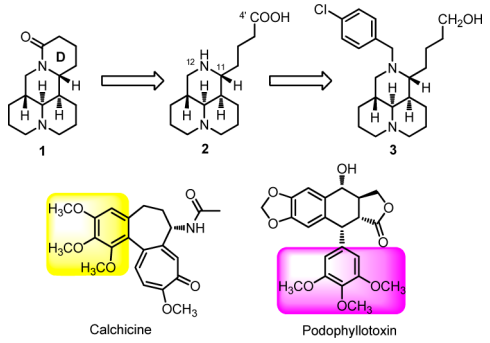


Figure 1. Chemical structures of sophoridine (**1**), sophoridinic acid (**2**), 12-*N*-*p*-chlorobenzyl sophoridinol (**3**), colchicine, and podophyllotoxin.

extracted from *Sophora flavescens*. Compound **1** alone was approved by CFDA in 2005 to cure the cancer patients with malignant trophoblastic tumors. The mechanism of action of **1** is to inhibit the DNA topoisomerase I (topo I) activity, induce cell cycle arrest at the G0/G1 phase, and cause apoptotic cell death.[10−13] What's more, it has many of druggable advantages such as special chemical scaffold, flexibility structure, high solubility, and good safety profiles, suggesting that it is an ideal lead compound for further modifications and optimizations.[14−16]

The structure−activity relationship (SAR) study of **1** as anticancer agent was carried out with the modifications of D ring opening in our laboratory,[17,18] and SAR results revealed that sophoridinic acid (**2**, Figure 1) analogues with a 3-ring core scaffold was more favorable than **1** with a 4-ring scaffold. The representative compound **3** (Figure 1), 12-*N*-*p*-chlorobenzyl sophoridinol, had a greatly improved antiproliferative activity in HepG2 hepatoma cells with an IC_{50} of 9.3 μM compared to the parent **1** (IC_{50} > 80 μM).[17] The mode of action of **3** was to inhibit DNA topo I activity and arrested the cell cycle at the G0/G1 phase, consistent with that of **1**.[17] The unique chemical scaffold and promising anticancer activity of **3** provoked our strong interest to continue SAR analysis in an effort to develop a novel class of promising anticancer candidates with high druggability.

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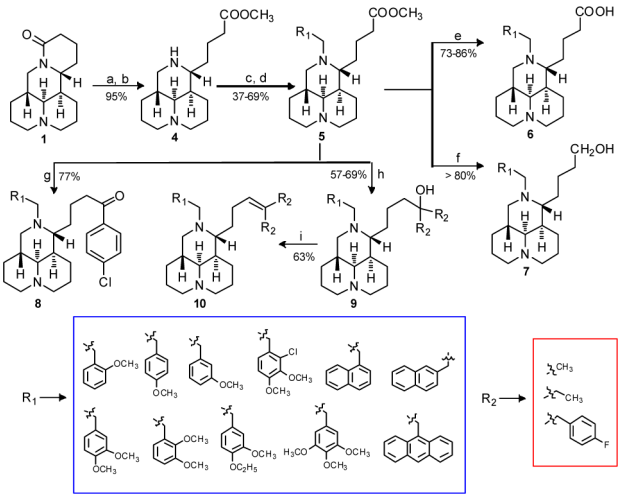
In the present study, a variety of substituents were introduced on the 12-nitrogen atom and 4′-carboxyl regions of compound **2**, respectively, from which 27 new sophoridinic acid/ester and sophoridinol derivatives were generated and examined for their anticancer activities. Herein, we describe the synthesis, *in vitro* antitumor assay, SAR analysis, primary mechanism of action, and *in vivo* pharmacokinetic (PK) evaluation of the new compounds.

Twenty-seven target compounds were prepared using commercially available **1** as the starting material as described in Scheme 1. The sophoridinic ester **5** was obtained through a

Scheme 1[a]



[a]Reagents and conditions: (a) 6 N HCl, Δ, 6 h; (b) CH_3OH, r.t., 5−6 h; (c) R_1CHO, triethylamine, 1,2-dichloroethane, Δ, 4 h; (d) sodium triacetoxyborohydride (STB), 1,2-dichloroethane, Δ, 4 h; (e) 3 N HCl, Δ, 3 h; (f) $LiAlH_4$, THF, r.t., 4−5 h; (g) *p*-Cl-PhMgBr, THF, r.t., 7 h; (h) R_2MgBr, THF, Δ, 7 h; (i) 3 N hydrochloride/ether, r.t., 4 h.

four-step procedure including hydrolysis, esterization, condensation, and reduction reactions as reported previously.[17] The sophoridinic acid **6** was acquired by the hydrolysis of **5** in 3 N HCl in a good yield, while the sophoridinol **7** was obtained via reduction of **5** with $LiAlH_4$ in THF with a yield of over 80%. The sophoridinic ketone **8** was synthesized via addition-hydrolysis reaction of **5** with an equivalent of *p*-Cl-PhMgBr in dry THF at room temperature in a 77% yield. Similarly, the products in series **9** were also gained through the addition-hydrolysis reaction of **5** with 4−5 equiv of Grignard reagents in THF at refluxing temperature with yields of 57%−69%. The product **10** was acquired by the dehydration of **9** in a 63% yield. All crude products were purified with flash column chromatography on silica gel using CH_2Cl_2 and $MeOH/NH_3{\cdot}H_2O$ as gradient eluents.

All the desired compounds were evaluated for their cytotoxic activities in human HepG2 hepatoma cell lines using MTT assay with Taxol as the positive control.[19] Structures of the 27 sophoridinic acid/ester and sophoridinol derivatives and their antiproliferative activities were depicted in Table 1.

SAR analysis was first focused on the substituents at the 12-nitrogen atom. Since trimethoxyphenyl group (Figure 1) is crucial for the anticancer activity in many natural medicines against cancer such as colchicine and podophyllotoxin, this group was introduced into the nitrogen atom at position 12 to create *N*-trimethoxybenzyl sophoridinic ester/acid (**5a** and **6a**) and sophoridinol (**7a**). However, as described in Table 1, none of them was active, indicating that the trimethoxyphenyl herein was not beneficial for the anticancer activity. Next, mono/dimethoxyphenyl groups were employed as substituents at 12-N position to generate corresponding new derivatives (**5b−f**, **6b−c**, and **7b−f**). The results showed that most of the sophoridinic acids/alcohols lost their cytotoxic activities partially or completely, while the sophoridinic esters **5b** and **5d−f** afforded moderate antiproliferative activities with IC_{50} values ranging from 8.4 to 10.7 μM. It seemed that the improved anticancer activities of compounds **5b** and **5d−f** were consistent with their relatively higher CLogP values (>4) calculated by ChemBioOffice software (version 12.0).

Then, naphthalenyl and anthracenyl with high lipophilicity were added on the 12-nitrogen atom aiming at enhancing the ClogP values, thereby enhancing the activity against tumor, with which many new corresponding compounds (**5g−i**, **6d−e**, and **7g−i**) were made and examined. As anticipated, the sophoridinic esters (**5g−i**) and sophoridinols (**7g−i**) displayed higher potency with IC_{50} values ranging from 3.1 to 7.9 μM, much better than that of Taxol (IC_{50} = 15.6 μM). Especially, compound **7i** with a 9-anthracene at the 12-N position exhibited a potent activity against HepG2 cancer with an IC_{50} of 3.1 μM. Subsequently, the 9-anthracenyl on the 12-nitrogen atom was retained, and a couple of sophoridinic ketone (**8**), sophoridinols (**9a−b**), and sophoridinic alkene (**10**) were constructed and measured. The results showed that all of them had favorable antiproliferative activities with IC_{50} between 2.0 and 5.3 μM owing to the improvement of ClogP values ranging from 6.8 and 9.5. Among the active compounds, sophoridinic esters **5g−i** had metabolic instabilities *in vivo* owing to the metabolically labile ester group. Therefore, sophoridinols **7g** and **7i** bearing potent anticancer effects as well as reasonable ClogP values were selected out as the representative compounds for further investigation.

Evaluation of compounds **7g** and **7i** against drug-resistant cancer cell lines were carried out. In this experiment, we tested their activity against human wild MCF-7 and adriamycin (AMD)-resistant MCF-7 (MCF-7/AMD) breast carcinoma cell lines,[20] with AMD as a reference control. As described in Table 2, AMD exhibited a promising activity against wild MCF-7 with an IC_{50} of 0.88 μM, compared with that of 94 μM in the MCF-7/AMD cells. Notably, compounds **7g** and **7i** afforded an almost equipotent antiproliferative effect against both MCF-7 (IC_{50} of 9.0 and 5.6 μM) and drug-resistance MCF-7/AMD cells (IC_{50} of 9.5 and 5.9 μM), suggesting a different anticancer mechanism of action from AMD.

To verify the possible change of mechanism of action after the structure modifications, flow cytometric analysis in the HepG2 cells was carried out to learn whether or not compound **7i** is able to cause G0/G1 arrest. The HepG2 cells were treated for 24 h without or with **7i** at the different concentrations of 1.25, 2.5, and 5.0 μg/mL, respectively. As shown in Figure 2, compound **7i** arrested the HepG2 cells at the G0/G1 phase as anticipated, indicating a similar mechanism of action with its parent compound **1**.

Single dose PK investigation for compound **7i** was performed in adult male Sprague−Dawley (SD) rats. Compound **7i** was administered via oral routes (25 mg/kg), and nine blood samples (0.3 mL) were collected over a 24 h period. As described in Figure 3 and Table 3, the compound **7i** was rapidly absorbed with a T_{max} of 4.6 h, a favorable half-life of 12 h, and a mean residence time (MRT) of 5.4 h. The area under the concentration−time curve (AUC) of **7i** was 10.3 μM·h, which

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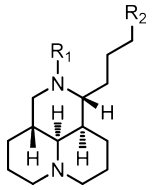
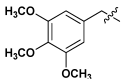
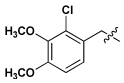
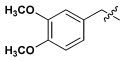
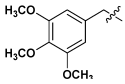
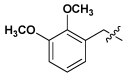
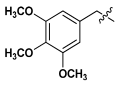
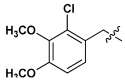
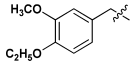
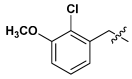
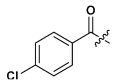
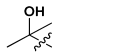
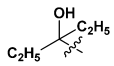
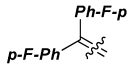
Table 1. Structure–Activity Relationships of the Newly Synthesized Compounds for Their Antiproliferative Activities in HepG2 Cells



Compd	R_1	R_2	IC_{50} (μM)	ClogP[a]
5a		$COOCH_3$	> 40	3.52
5b		$COOCH_3$	8.4 ± 4.1	4.44
5c		$COOCH_3$	> 40	3.88
5d	$CH_2C_6H_4OCH_3$-*o*	$COOCH_3$	10.3 ± 0.4	4.14
5e	$CH_2C_6H_4OCH_3$-*m*	$COOCH_3$	10.7 ± 0.3	4.14
5f	$CH_2C_6H_4OCH_3$-*p*	$COOCH_3$	10.7 ± 0.4	4.14
5g	1-methylnaphthalene	$COOCH_3$	3.3 ± 1.0	5.39
5h	2-methylnaphthalene	$COOCH_3$	5.8 ± 0.8	5.39
5i	9-methylanthracene	$COOCH_3$	4.3 ± 0.6	6.57
6a		COOH	> 40	0.87
6b		COOH	> 40	1.23
6c	$CH_2C_6H_4OCH_3$-*o*	COOH	> 40	1.49
6d	1-methylnaphthalene	COOH	> 40	2.75
6e	2-methylnaphthalene	COOH	> 40	2.75
7a		CH_2OH	> 40	3.10
7b		CH_2OH	15.7 ± 7.6	4.01
7c		CH_2OH	> 40	3.98
7d		CH_2OH	23.6 ± 2.5	4.34
7e	$CH_2C_6H_4OCH_3$-*o*	CH_2OH	34.6 ± 4.0	3.72
7f	$CH_2C_6H_4OCH_3$-*p*	CH_2OH	> 40	3.72
7g	1-methylnaphthalene	CH_2OH	6.8 ± 1.7	4.97
7h	2-methylnaphthalene	CH_2OH	7.9 ± 2.7	4.97
7i	9-methylanthracene	CH_2OH	3.1 ± 0.6	6.15
8	9-methylanthracene		2.0 ± 0.4	8.75
9a	9-methylanthracene		2.1 ± 0.2	6.86
9b	9-methylanthracene		2.7 ± 0.8	7.92
10	9-methylanthracene		5.3 ± 0.2	9.52
1			> 80	
3			9.3 ± 2.1	
Taxol			15.6 ± 2.3	

[a]ClogP value was generated by ChemBioOffice software (version 12.0).

Table 2. IC_{50} (μM) Values of 7g and 7i in MCF-7 and MCF-7/AMD Breast Carcinoma Cell Lines

	MCF-7	MCF-7/AMD
7g	9.07 ± 1.22	9.51 ± 1.85
7i	5.63 ± 0.33	5.94 ± 1.07
AMD	0.88 ± 0.15	94.1 ± 7.79

is greater than the IC_{50} value (3.1 μM) of antiproliferative activity *in vitro*. The PK profiles of **7i** were not as good as those of compound **3**,[17] and this might be due to the higher ClogP value (ClogP = 6.15) of this compound.

Taken together, 27 new sophoridinic acid/ester and sophoridinol derivatives were constructed and evaluated for their antitumor activities *in vitro*. SAR analysis revealed that (i)

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Table 3. Pharmacokinetic Profile[a] of 7i in Rats after a Single Oral Administration (n =3)

dosage (mg/kg)	T_{max} (h)	C_{max} (μM)	AUC_{0-t} (μM·h)	$AUC_{0-\infty}$ (μM·h)	MRT (h)	$t_{1/2}$ (h)
25	4.6 ± 1.2	3.4 ± 0.5	10.3 ± 1.4	10.5 ± 1.2	5.4 ± 0.1	12.7 ± 0.3

[a]PK parameters were calculated by noncompartmental analysis using WinNonlin, version 5.3.

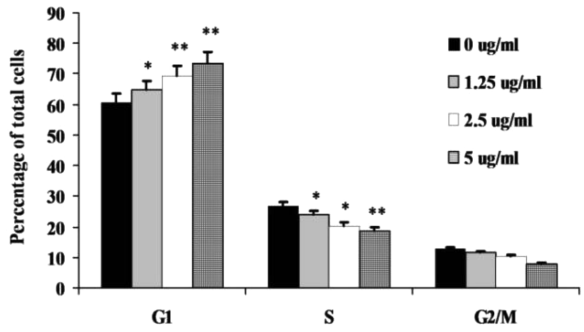

Figure 2. Cell cycle analysis of **7i**. HepG2 cells were incubated without (control) or with compounds at different concentrations (1.25, 2.5, and 5.0 μg/mL) for 24 h. Cells were then analyzed for their cell cycle distribution using flow cytometry. (*) $P < 0.05$; (**) $P < 0.01$ as compared to that of control group.

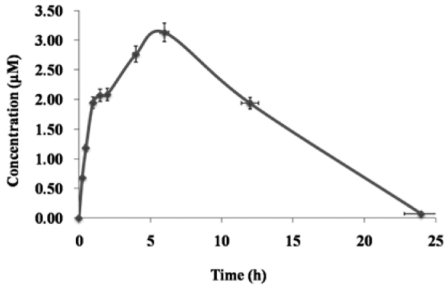

Figure 3. Mean plasma concenreation-time curve of compound **7i** after single oral administration of 25 mg/kg in SD rats (n = 3). Pharmacokinetic parameters were calculated by noncompartmental analysis using WinNonlin, version 5.3.

two suitable substituents on the 12-nitrogen atom and carboxyl region were helpful for keeping good antitumor activity. (ii) The two moieties could be introduced decorating various substituents in order to regulate the druglike physical−chemical properties of the compounds. The SAR results provide useful information on further strategic optimization. Among them, sophoridinol **7i** showed an equipotent effect against wild MCF-7 and MCF-7/AMD breast carcinoma cell lines. Its mechanism of action was to arrest the cell cycle at the G0/G1 phase consistent with that of **1**. In addition, it also showed a good PK profile *in vivo*, indicating a good druggable characteristic. Thus, we consider sophoridinol analogues to be a new family of promising antitumor agents with an advantage of inhibiting drug-resistant cancer cells.

■ ASSOCIATED CONTENT

Supporting Information

Synthetic procedure, analytical data, cytotoxicity assay, cell cycle analysis, and PK evaluation. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Authors

*(F.S.) Tel: +86 532 84023947. Fax: +86 532 84022924. E-mail: qdkdsufeng@hotmail.com.

*(D.S.) Tel: +86 10 63165268. Fax: +86 10 63165268. E-mail: songdanqingsdq@hotmail.com.

Author Contributions

§(C.B. and C.Z.) These authors contributed equally to this work.

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

SAR, structure−activity relationship; MTT, tetrazolium bromide; AMD, adriamycin; THF, tetrahydrofuran; TLC, thin layer chromatography

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