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Synthesis, structure–activity relationship and biological evaluation of anticancer activity for novel N-substituted sophoridinic acid derivatives

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ABSTRACT

Sophoridine (**1**), a natural anticancer drug, has been used in China for decades. A series of novel N-substituted sophoridinic acid derivatives were synthesized and evaluated for their cytotoxicity with **1** as the lead. The structure–activity relationship indicated that introduction of an aliphatic acyl on the nitrogen atom might significantly enhance the anticancer activity. Among the compounds, **6b** bearing bromoacetyl side-chain afforded a potential effect against four human tumor cell lines (liver, colon, breast, and lung). The mechanism of action of **6b** is to inhibit the activity of DNA topoisomerase I, followed by the S-phase arrest and then cause apoptotic cell death, similar to that of its parent **1**. We consider **6b** promising for further anticancer investigation.

Sophoridine (**1**, Fig. 1), which was extracted from the traditional medicine herb *Sophora alopecuroides* L., has been used to treat cancer in China for decades with low side-effects.[1,2] In particular, it exhibited the potential therapeutic efficacy on malignant tumors in the digestive tract such as gastric cancer, colon cancer, and esophageal cancer.[3,4] The mechanism of action of **1** is to inhibit the activity of DNA topoiosmerase I (topo I), to arrest at S-phase, and then to cause apoptotic cell death.[5]

The DNA topo I inhibitors such as 10-hydroxycamptothecin (HCPT) and topotecan have become the important chemotherapeutic drugs in clinical use.[6,7] These drugs, however, have major limitations. First, they have poor water solubility. Second, they are difficult targets for de novo synthesis because they are large natural products (Fig. 1).[8a,b] Therefore, searching for simple topo I inhibitors has become an effective strategy for discovering new anticancer drugs.[9]

In contrast to the camptothecin derivatives used in clinic, compound **1** has simple and small structure, and provides advantages in chemical synthesis and opportunities of formulation for oral administration. The special structure and biological features of **1** provoked our strong interest for a further structure–activity relationship (SAR) study. Ring D of **1** was opened, and the resultant sophoridinic acid **2** (Fig. 1) still retained moderate antiproliferative activity (Table 1). It was deduced that ring D might not be required for activity. The results suggested that a novel family of

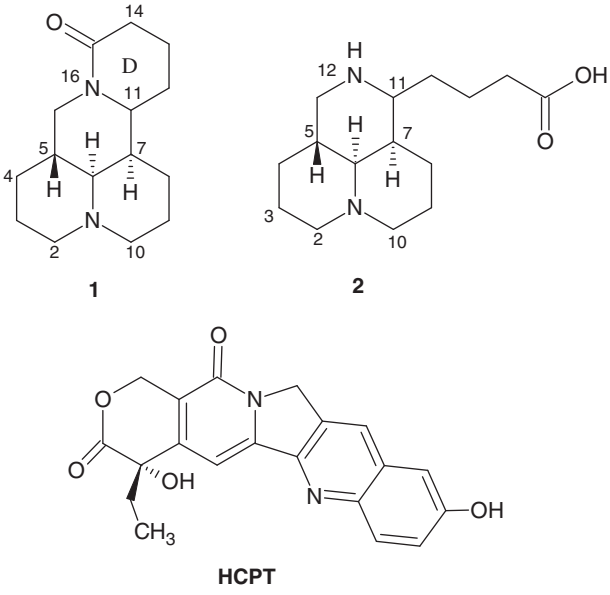

Figure 1. Chemical structures of **1**, **2**, and HCPT.

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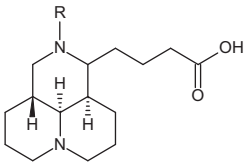
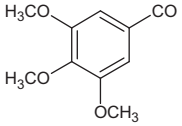
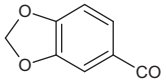
Table 1
Structures and cytotoxicity of the 22 N-substituted sophoridinic acid derivatives



Compd	R	Inhibitory ratio[a] (%)
1		13.8 ± 2.5
2	H	10.3 ± 1.7
6a	$COCH_3$	11.2 ± 2.1
6b	$COCH_2Br$	88.0 ± 2.1
6c	$COCHBrCH_3$	15.6 ± 3.2
6d	$COC(CH_3)_3$	41.1 ± 2.8
6e	COC_6H_5	<5
6f	COC_6H_4F-*p*	<5
6g	$COC_6H_4CH_3$-*p*	8.5 ± 4.4
6h	$COC_6H_4OCH_3$-*p*	<5
6i	$COC_6H_4NO_2$-*m*	<5
6j	$COC_6H_3NO_2$-*m*-CH_3-*o*	8.9 ± 6.4
6k	$COC_6H_3NO_2$-*m*-F-*p*	7.7 ± 1.6
6l	$COC_6H_3NO_2$-*m*-OCH_3-*p*	<5
6m	$COOCH_3$	7.8 ± 1.2
6n	$COOC_2H_5$	19.9 ± 7.2
6o	$COOCH_2C_6H_5$	8.4 ± 4.1
6p	$SO_2C_6H_5$	<5
6q	$SO_2C_6H_4CH_3$-*p*	13.4 ± 5.6
6r	$CH_2C_6H_4OCH_3$-*p*	<5
6s	$CH_2C_6H_4CH_3$-*p*	<5
6t		11.8 ± 2.7
6w		11.4 ± 1.5
HCPT		92.0 ± 0.3
3		91.6 ± 0.5

[a] % of inhibition. HepG2 cells were treated with the tested compounds (30 μg/mL), HCPT (30 μg/mL) or compound **3** (0.6 μg/mL) for 48 h, respectively. The antiproliferative activity was determined with SRB method. The data shown were mean ± SD of three separate experiments for % of the inhibition of cell proliferation.

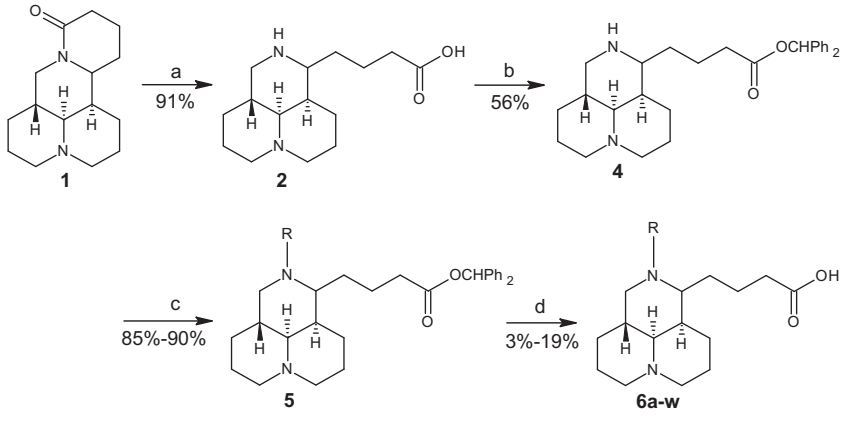
N-substituted sophoridinic acid derivatives could be generated by introducing a variety of substituents into the nitrogen atom at the 12-position of **2**. In particular, this novel of analogs might enhance water solubility owing to introducing a carboxyl group. On the basis of this strategy, 21 novel N-substituted sophoridinic acid derivatives were subsequently designed, synthesized and evaluated for their cytotoxicity in the present paper.

Twenty-two N-substituted sophoridinic acid analogs were synthesized with commercially available **1** as the starting material as depicted in Scheme 1. Compound **2** was prepared via the hydrolysis of **1** in aqueous KOH,[10,11] and then acidification with dilute HCl (3 N) with a good yield of 91%. The intermediate **4** was obtained through carboxyl protection of **2**, in which diphenyldiazomethane was used as a protective agent and petroleum ether (30–60 °C) as the solvent in a good yield.[12] Then the key intermediate **5** was acquired by the substitution reaction of **4** with acyl, sulfonyl or benzyl, respectively, under slightly alkaline conditions. Finally, *m*-cresol was used as a de-protective reagent as well as solvent to transform compound **5** into desired products **6**. Purity estimation was done with TLC (thin layer chromatography), and only those with purity around 95% were tested for biological activity.

The antiproliferative activity of aimed compounds in human HepG2 hepatoma cells was examined using the sulforhodamine B (SRB) assay with HCPT as a positive control.[13] Structures of 22 N-substituted sophoridinic acid derivatives and their cytotoxicity were shown in Table 1.

The SAR studies for antiproliferative activity were first focused on the substituents on the nitrogen atom at the 12-position. A group of aliphatic acyl including acetyl (**6a**), bromoacetyl (**6b**), 2-bromopropionyl (**6c**) and pivaloyl (**6d**) was introduced into the nitrogen atom of **2**, respectively, with which four N-substituted sophoridinic acids were made and tested. The results showed that compounds **6b** and **6d** (30 μg/mL) afforded the potential antiproliferative activities with inhibition rate of 88% and 41%, respectively, much stronger than that of **1** or **2**. It was suggested that attachment of a substituent at the 12-position might significantly enhance the anticancer activity of this kind of compounds.

Similarly, eight compounds **6e–l** possessing substituted benzoyl at the same position were also prepared and evaluated. The results showed that all of them lost their activity partially or completely. Furthermore, methoxycarbonyl (**6m**), ethoxycarbonyl (**6n**), benzyloxycarbonyl (**6o**), phenylsulfonyl (**6p**) and *p*-tosyl (**6q**) were also introduced into the nitrogen atom at the 12-position, respectively,



Scheme 1. Synthesis of the aimed compounds. Reagents and conditions: (a) 12% KOH, reflux, 10 h; (b) diphenyldiazomethane, petroleum ether, rt, 24 h; (c) RX, K_2CO_3/CH_2Cl_2, rt, 24 h; (d) *m*-cresol, 100 °C, 6–8 h.

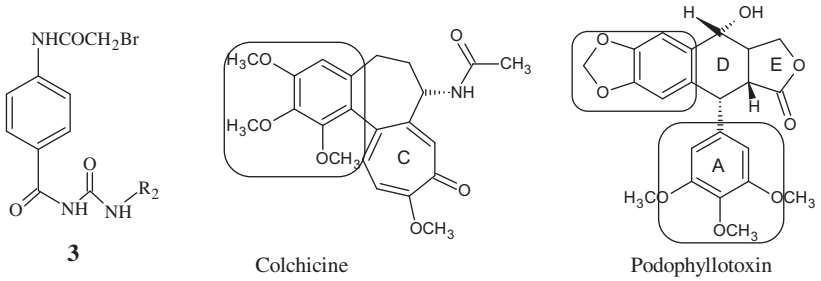


Figure 2. Chemical structures of **3**, colchicine and podophyllotoxin.

by which five analogs were generated. Compounds **6n** and **6q** retained moderate cytotoxicity, similar to that of the lead **1**. In addition, benzyl was added at the same position as well, and the resultant compounds **6r** and **6s** almost lost their anticancer activity. It was deduced that the larger size side-chains at the 12-position might not be beneficial for the anticancer activity.

Among the tested analogs, compound **6b** possessing bromoacetyl, a functional group of benzoylurea derivatives for the cytotoxicity in our previous reports,[14] afforded the most potent anticancer activity. As a trimethoxyphenyl is dispensable to the anticancer activity of colchicine or podophyllotoxin (Fig. 2), the well-known tubulin-active agents,[15,16] it was linked at the 12-nitrogen atom, from which compound **6t** was synthesized. Similarly, a methylenedioxyphenyl of podophyllotoxin was also coupled at the same position, and thus compound **6w** was produced. The results showed that compounds **6t** and **6w** exhibited moderate antiproliferative activity, much weaker than that of **6b**.

Since compounds **6b** and **6d** afforded more potent anticancer activity in comparison to the parent **1**, their dose-dependent curves in HepG2 hepatoma were further carried out. As shown in Figure 3, compounds **6b** and **6d** showed a potent anticancer activity with an IC_{50} value of 15.4 and 43.6 μg/mL, respectively. Thus, compound **6b**[17] was selected to perform anticancer evaluation in three human tumor cell lines including colon cancer (HCT-1116), lung cancer (A549) and breast cancer (MCF-7).

As shown in Figure 4, it appears that **6b** was a potent killer for three tumor cells with inhibition rate in the range of 40–73% at the concentration of 30 μg/mL. The least sensitive cell line was A549 with inhibition of 40%. The most sensitive cell line was MCF-7 with inhibition of 73%. Since compound **6b** afforded a potent anticancer activity, and its mechanism was further carried out to learn whether the chemical modification retains the mode of action of its parent **1**.

Flow cytometric analysis of DNA profile in the HepG2 cells (Fig. 5) showed that **6b** treatment (20 μg/mL) produced a major shift of the cell population from G_0/G_1 to S phase, revealing a sig-

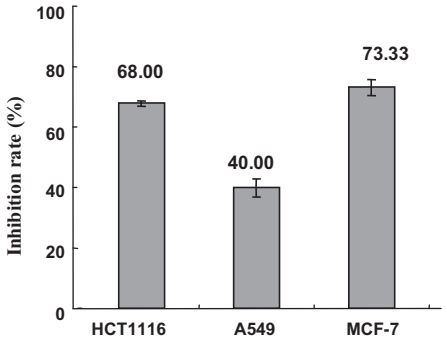


Figure 4. Antiproliferative activities of **6b** in three human tumor cell lines. The cells were treated with **6b** (30 μg/mL) for 48 h, and antiproliferative activity was done with SRB assay.

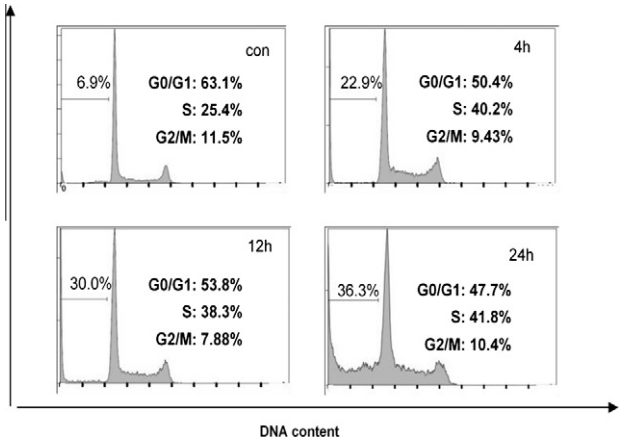


Figure 5. Cell cycle analysis of **6b**. HepG2 cells were incubated without (control) or with **6b** (20 μg/mL) for 4, 12, or 24 h, respectively. Cells were then analyzed for their cell cycle distribution using flow cytometry.

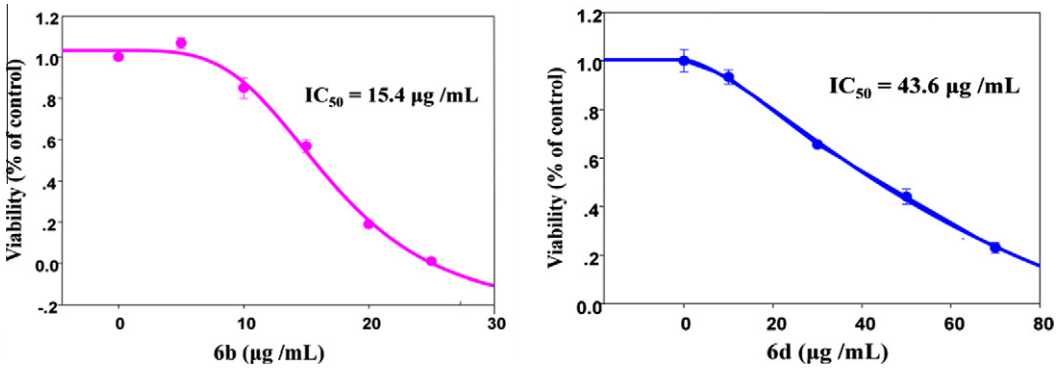


Figure 3. Dose-dependent killing of HepG2 Hepatoma by **6b** and **6d**. HepG2 cells were treated with **6b** or **6d** at different concentrations for 48 h and the cell death was measured using SRB assay.

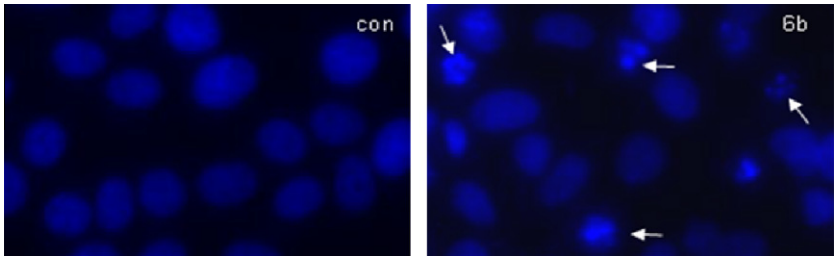


Figure 6. Morphological examination. HepG2 cells were untreated or treated with **6b** (20 μg/mL) for 48 h. Cells were then harvested on slides for morphology observation: (left) untreated; (right) **6b** treated (20 μg/mL).

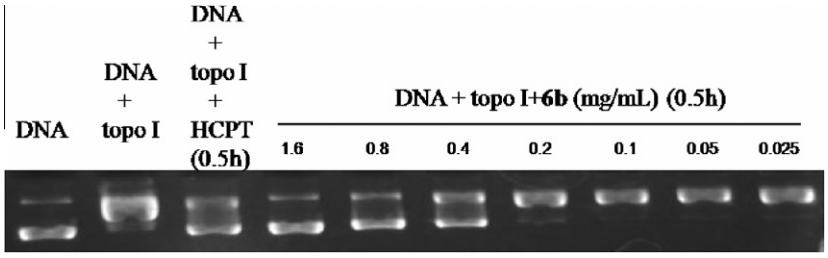


Figure 7. DNA topo I inhibitory activity of **6b** at different concentration using HCPT (50 M) as a positive control.

nificant accumulation of cells in the S-phase. To further define the cell cycle arrest, morphology examination was also performed. HepG2 hepatoma cells treated with **6b** for 48 h displayed the characteristic features of apoptosis (Fig. 6), that is, the disappearance of nuclear membrane and appearance of apoptotic body. Next, we accessed the inhibitory activity of **6b** on the DNA topo I with HCPT as a reference drug. As shown in Figure 7, compound **6b** significantly inhibited the activity of topo I, which was consistent with that of its parent **1**.[5] The results indicated that the chemical modifications retained the mode of action of the parent compound **1**.

In conclusion, we have designed, synthesized and evaluated for the antiproliferative activity of 22 novel N-substituted sophoridinic acid derivatives with **1** as the lead. The preliminary SAR analysis revealed that (i) the ring D of **1** is not indispensable for activity; (ii) introduction of a substituent on the nitrogen atom of **2**, especially aliphatic acyl, might significantly enhance the anticancer effect. Among these analogs, compound **6b** exhibited a promising antiproliferative activity in a variety of cell lines including liver, colon, lung, and breast. The mechanism study showed that compound **6b** inhibits the activity of DNA topo I, suspends the cell cycle at S-phase, and eventually leads the tumor cells to apoptosis, indicating a mechanism similar to its parent **1**. The in vivo anticancer efficacy of **6b** is currently under investigation in our laboratories.

Acknowledgment

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Supplementary data

Supplementary data (HRMS of the final compounds) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.07.038.

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17. *Analytical data for selected final compound* ***6b***. Mp: 171.4–173.2 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.62 (s, 1H), 4.45–4.41 (m, 1H), 4.24–4.12 (m, 2H), 4.08–4.00 (m, 1H), 3.73–3.63 (m, 2H), 3.51–3.39 (m, 1H), 3.21 (s, 2H), 3.01 (s, 1H), 2.87–2.80 (m, 1H), 2.49–2.32 (m, 1H), 2.27–2.18 (m, 2H), 2.01–1.98 (m, 1H), 1.90–1.67 (m, 6H), 1.55–1.27 (m, 5H). IR: ν 3417 (OH), 2944 (CH_2), 1728, 1636 (C=O) cm^{-1}. HRMS-ESI calcd for $C_{17}H_{27}BrN_2O_3$: 387.1283 $(M+H)^+$. Found: 387.1300.