Bioorganic & Medicinal Chemistry Letters 25 (2015) 4092–4096

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis, structure–activity relationship and biological evaluation of novel nitrogen mustard sophoridinic acid derivatives as potential anticancer agents

Dong-Dong Li *, Lin-Lin Dai, Na Zhang, Zun-Wei Tao *

Tianjin Institute of Medical and Pharmaceutical Sciences, Tianjin 300020, China

ARTICLE INFO

Article history:
Received 27 April 2015
Revised 31 July 2015
Accepted 13 August 2015
Available online 18 August 2015

Keywords:
Nitrogen mustard sophoridinic acid derivatives
Antitumor activity
Structure–activity relationship
Molecular docking

ABSTRACT

A series of novel nitrogen mustard sophoridinic acid derivatives were designed, synthesized and evaluated for their cytotoxicity. Of the newly synthesized compounds, compound **6** exhibited a potent effect against hepatocellular carcinoma in vitro and in vivo. SAR analysis indicated that introduction of a nitrogen mustard group to the structure of sophoridinic acid significantly enhance the antitumor activity. Moreover, molecular docking study exhibited benzyl group introduced to the nitrogen atom at the 12-position and aryl nitrogen mustard group at the 4′-carboxyl region for compound **6** were beneficial for the higher anticancer activity. This work provides useful information for further structural modifications of these compounds and for the synthesis of new, potent antitumor agents.

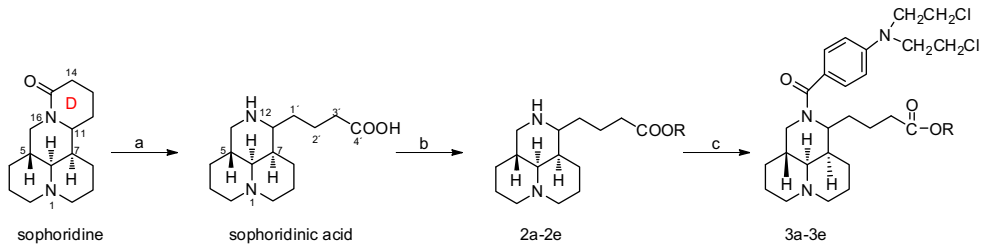
Sophoridine, which was approved by CFDA in 2005 to cure cancer patients, has been widely used to treat live cancer, gastric cancer and lung cancer in combination with other anticancer drugs used in clinic including cisplatin, vinorelbine and docetaxel et al.[1–7] The mechanism of action of sophoridine is to inhibit DNA topoisomerase I (Topo I) activity and induce cell cycle arrest at the G0/G1 phase, and then cause apoptotic cell death,[8–11] what's more, it has high solubility and good safety profiles. However, the moderate antitumor activity of sophoridine limit its use as a drug for clinical applications, and consequently, development of sophoridine derivatives is necessary to discover more effective drug candidates. Some researchers have opened the D-ring of sophoridine to generate sophoridinic acid, which is easier to modify. Li et al.[12] demonstrated that the D-ring was not be necessary for activity, and synthesized a family of *N*-substituted sophoridinic acid derivatives, then evaluated for their cytotoxicity in HepG-2 cancer cells, the mechanism of action of these compounds was to inhibit the activity of DNA topoisomerase I, followed by the S-phase arrest and then cause apoptotic cell death. Bi et al.[13,14] synthesized a series of *N*-substituted sophoridinol analogs and found that introduction of a chlorobenzyl on the 12-nitrogen atom of sophoridinol might significantly enhance the antiproliferative activity, the mode of action of these compounds was to inhibit the DNA topoisomerase I, followed by the G0/G1 phase arrest, and they also showed a moderate oral bioavailability and good safe in vivo.

Nitrogen mustard has been widely used in clinic as alkylating agent with the advantage of wide spectrum of antitumor and strong killing ability for tumor cells, but the lacking of selectivity for cells causes serious side effects. Sophoridine is a main chemical ingredient of the Chinese traditional medicine *Fufang Kushen injection*, what is more, it has many of drugable advantages such as high solubility, good safety profiles and a special chemical scaffold, suggesting that it is an ideal lead compound for further modifications and optimizations. Base on the above suggestion, we hypothesize that the introduction of nitrogen mustard group to the structure of sophoridinic acid could change the lipid–water partition coefficient, which might be beneficial to penetrate the cell, and then improve antitumor activity, however, research on the combination of nitrogen mustard and sophoridinic acid derivatives is rare.

In the present work, we modified sophoridinic acid at the 12-position and on the carboxyl group with nitrogen mustard group. In Scheme 1, we introduced nitrogen mustard group as substituent to the nitrogen atom at the 12-positition to synthesize compound **3a**–**3e**,[15] the synthetic pathway was shown in Scheme 1. Sophoridine was hydrolyzed with sodium hydroxide to produce sophoridinic acid, which was transformed to acyl chloride using sulfoxide chloride and then reacted with series of alcohols (i.e., methanol, ethanol, propanol, isopropanol, *n*-butanol) to produce intermediates **2a**–**2e**, compounds **3a**–**3e** were prepared by the amidation reactions of **2a**–**2e** with 4-(bis(2-chloroethyl)

* Corresponding authors. Tel.: +86 022 27236182.
E-mail addresses: lidongdong2010@163.com (D.-D. Li), taozunweitj@163.com (Z.-W. Tao).

http://dx.doi.org/10.1016/j.bmcl.2015.08.035
0960-894X/



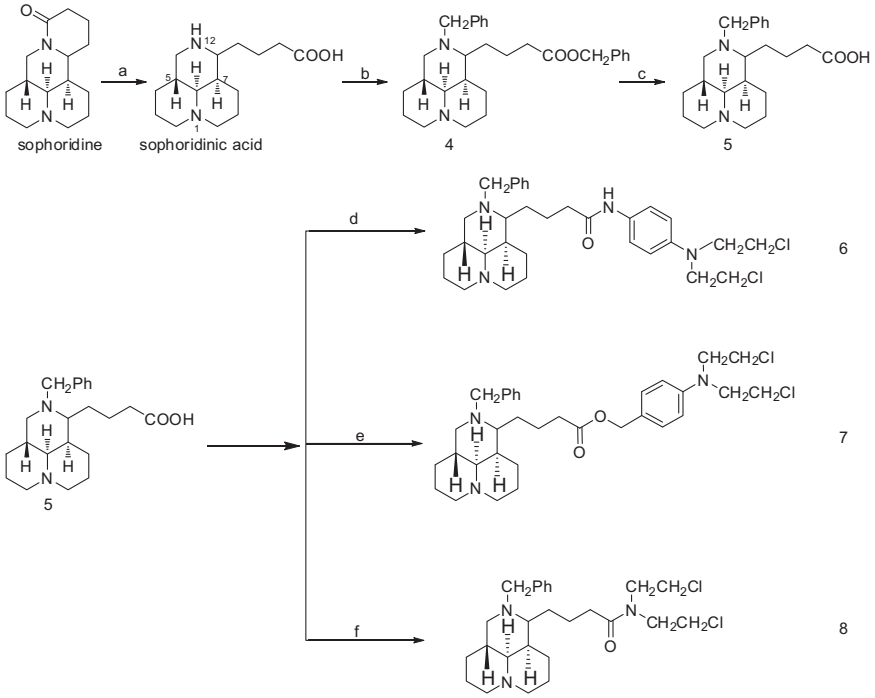
Scheme 1. Synthesis of nitrogen mustard sophoridinic acid derivatives **3a**–**3e**. Reagents and conditions: (a) 2 M NaOH/H_2O, 100 °C, 12 h; 20% H_2SO_4; (b) ROH, $SOCl_2$, K_2CO_3, 65 °C, 4 h; (c) 4-(bis(2-chloroethyl)amino)benzoic acid, $SOCl_2$, $CHCl_3$, ice-water bath; anhydrous potassium carbonate, reflux, 10 h (R = CH_3, CH_2CH_3, $CH_2CH_2CH_3$, CH $(CH_3)_2$, $CH_2CH_2CH_2CH_3$).

amino)benzoic acid, thionyl dichloride and anhydrous potassium carbonate. The structures of all products were confirmed by ^1H NMR spectroscopy and mass spectrometry.

In Scheme 2, we initially introduced benzyl as a substituent to the nitrogen atom at the 12-position, then we modified the carboxyl group with different of nitrogen mustard groups to produce target compounds. Intermediate **4** was generated through the reaction of benzyl chloride with sophoridinic acid in the present of anhydrous potassium carbonate, then converted into **5** through hydrolysis of **4** using sodium hydroxide as the base catalyst.[16,17] Target compounds **6** and **8** were obtained by amidation reactions of **5** with N^1,N^1-bis(2-chloroethyl)benzene-1,4-diamine and bis (2-chloroethyl)amine using thionyl chloride as acidylating agent under slightly alkaline conditions, while target compound **7** was prepared by esterification reactions of **5** with (4-(bis(2-chloroethyl)amino)phenyl)methanol in the present of acylation reagent.

All the desired compounds were evaluated for their cytotoxic activities in human HepG-2 hepatoma cell lines using MTT assay with melphalan as the positive control. As shown in Table 1, SAS analysis was first focused on the substituent at the 4′-carboxyl region of compound **2**, an interesting observation was that increased length of the alkyl portion of the alcohols used to form the esters enhanced the antitumor activities of the sophoridinic acid derivatives, this may be due to that the formation of ester enhances the lipophilicity of sophoridinic acid derivatives. For example, **2a**–**2e** exhibited gradually increasing inhibition activities against HepG-2 cell lines, we deduced that the possible reason for the results was that the alcohols used to form the esters changed the lipophilicity of these derivatives, which in turn influenced their interaction with tumor cells.[18] Moreover, the antitumor activity of **2d** was higher than that of **2c**, which indicated that the activity of substituent branched R group was stronger than that of substituent straight chain R group. However, when the straight chain length is long enough, the esterification is difficult due to the steric effect.

Next, the nitrogen mustard group was introduced to the sophoridinic acid ester to produce compounds **3a**–**3e**, surprisingly, the products **3a**–**3e** had significantly higher antitumor activities than that of **2a**–**2e**, it seemed that the improved antitumor activities of compounds **3a**–**3e** were consistent with their relatively higher *C*log*P* values (>4) calculated by ChemBioDraw 12.0 software. We deduced that the possible reason was that the nitrogen mustard group in the compounds increased the activities, what's more, the increased lipophilicity of sophoridinic acid ester may also enhance their permeability into cell membrane and then



Scheme 2. Synthesis of nitrogen mustard sophoridinic acid derivatives **6**–**8**. Reagents and conditions: (a) 2 M NaOH/H_2O, 100 °C, 12 h; 20% H_2SO_4; (b) benzyl chloride, DMF, K_2CO_3, 75 °C, 4 h; (c) NaOH, EtOH; (d) thionyl chloride, CH_2Cl_2, 0 °C, 1 h; N^1,N^1-bis(2-chloroethyl)benzene-1,4-diamine, K_2CO_3, $CHCl_3$, reflux, 20 h; (e) thionyl chloride, CH_2Cl_2, 0 °C, 1 h; (4-(bis(2-chloroethyl)amino)phenyl)methanol, K_2CO_3, $CHCl_3$, reflux, 20 h; (f) thionyl chloride, CH_2Cl_2, 0 °C, 1 h; bis(2-chloroethyl)amine, K_2CO_3, $CHCl_3$, reflux, 20 h.