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ScienceDirect
Chinese Chemical Letters 21 (2010) 1087–1090
CHINESE CHEMICAL LETTERS
www.elsevier.com/locate/cclet

Synthesis and evaluation of *in vitro* anticancer activity of novel solasodine derivatives

Xiao Ming Zha [a,b], Fei Ran Zhang [c], Jia Qi Shan [a], Yi Hua Zhang [a], Jun O. Liu [c,*], Hong Bin Sun [a,*]

[a] *Center for Drug Discovery, School of Pharmacy, China Pharmaceutical University, Nanjing 210009, China*
[b] *Jiangsu Center for Drug Screening, China Pharmaceutical University, Nanjing 210009, China*
[c] *Department of Pharmacology and Oncology, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA*

Received 23 February 2010

Abstract

Solasodine **11** is a steroidal alkaloid with various biological activities. Herein, 8 novel solasodine derivatives were synthesized and their effect on prostate cancer cell proliferation was assessed *in vitro*. Significant improvement in antiproliferative activity was achieved among some of the synthetic analogs. In particular, **19** exhibited the most potent inhibitory effect against the proliferation of PC-3 cell line (IC_{50} = 3.91 μmol/L).

Keywords: Solasodine; Derivatives; *In vitro* anticancer

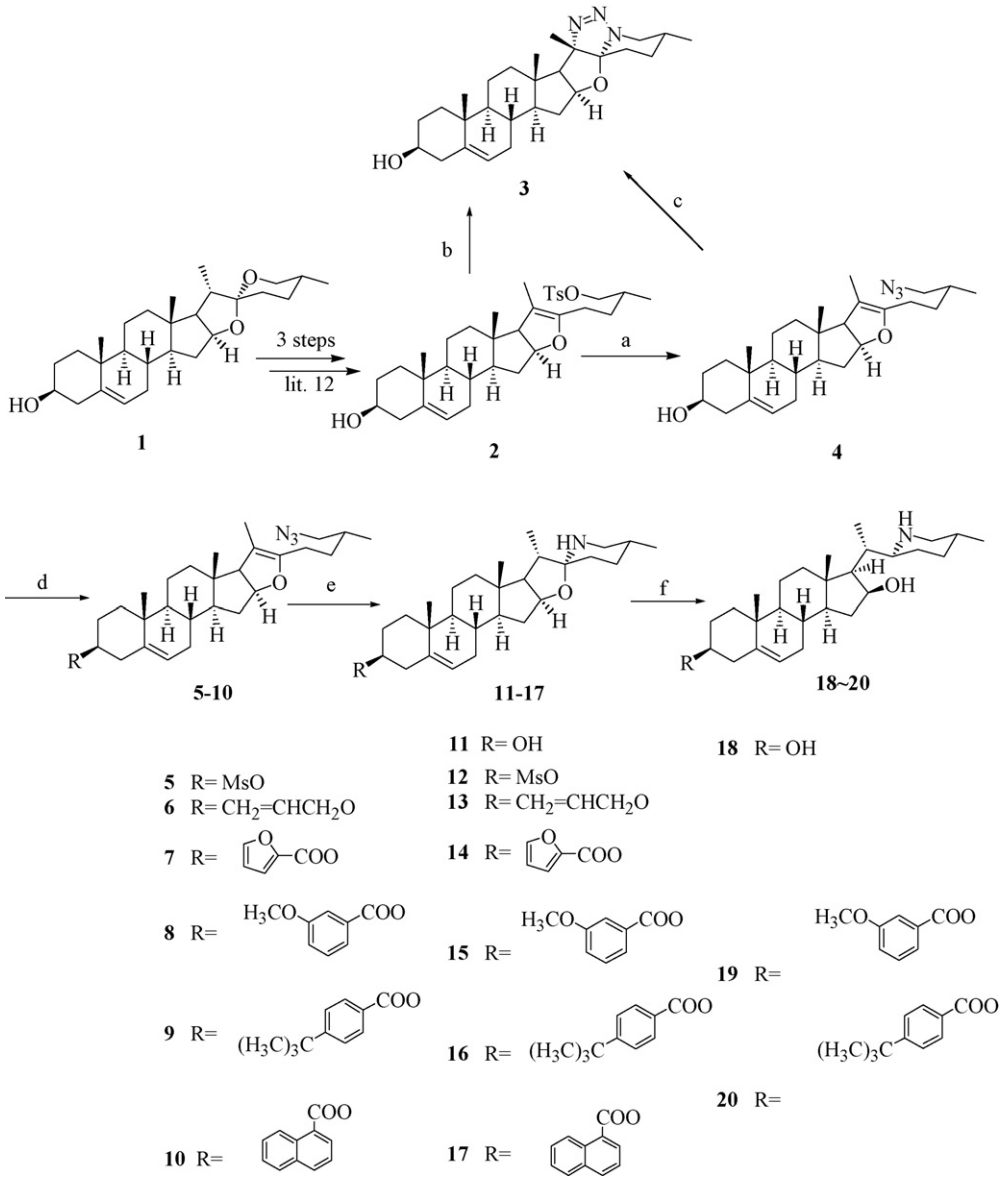
Steroidal alkaloids and their glycosides are known to possess a variety of biological activities including anti-tumor [1–4], antifungal [5], anti-inflammatory [6], teratogenic [7], antiviral [8] and antiestrogen [9] activities. Solasodine, a steroidal alkaloid extracted from *Solanum nigrum L* and *Solanum dulcamara L*, has recently attracted extensive attention due to its bioactivities. Liu reported that solasodine and its hydrochloride exhibited potent anticancer activities [10]. A phase IIA clinical trial of SBP002 (Coramsine®), a proprietary plant preparation containing solasonine and solamargine (both are solasodine glycoalkaloids), has been completed for treatment of certain skin cancers [11].

Previously we reported two efficient routes of solasodine and 12-oxosoladulcidine synthesis starting from readily available diosgenin and hecogenin, respectively [12,13]. In this manuscript, we report the synthesis of a series of novel solasodine derivatives with their anti-tumor activities and the structure of pseudodiosgenin heptacyclic triazoline **3** determined by X-ray crystallographic analysis.

The synthesis of solasodine derivatives is summarized in Scheme 1. Solasodine **11** is synthesized from readily available diosgenin **1** in 25% overall yield as described previously [12]. Uhle reported the conversion of 2 to pseudodiosgenin heptacyclic triazoline **3** with 3 equiv. of KN_3 in DMF at 100 °C in 50 h. The reaction is likely to proceed through the displacement of tosyl group by azide followed by a 1,3-dipolar cycloaddition to the

* Corresponding authors.
E-mail addresses: joliu@jhu.edu (J.O. Liu), hbsun@yahoo.com (H.B. Sun).

1001-8417/$ – see front matter
doi:10.1016/j.cclet.2010.04.020



Scheme 1. Reagents and conditions: (a) NaN_3/DMF, 50–70 °C, 97%; (b) NaN_3/DMF, 100 °C, 40 h, 87%; (c) DMF, 100 °C, 50 h, quant.; (d) RCOCl/pyridine for **5**,**7**–**10** (42–87%) or CH_2 = $CHCH_2Br$, NaH/DMF, for **6** (82%); (e) 1. TMSCl/NaI, MeCN, r.t.; 2. 10% $Na_2S_2O_3$, 5%NaOH, **11**–**17** (65–75%); (f) $NaBH_4$, MeOH/DCM, 0 °C to r.t., 1 h., **18**–**20** (77–87%).

dihydrofuranoid olefinic bond of ring E [14]. Previously, we found that the reaction temperature played a pivotal role in this reaction. No reaction took place under 50 °C [12]. When the reaction was carried out at 50–70 °C for 2 h, the azido derivative **4** was obtained in an almost quantitative yield. When the reaction temperature was elevated to 100 °C and the reaction time was prolonged to 40 h, **4** was further converted to **3**. However, the exact stereochemical structure of **3** had not been determined. Thus, we confirmed the structure of **3** using X-ray crystallographic analysis (21β-methyl) (Fig. 1) [15].

Having established the facile route from **2** to **4**, we decided to carry out a limited SAR study to explore the influence of C-3 substituents and the E ring on the activities of solasodine analogs.

Treatment of **4** with acyl chlorides or allylbromide afforded the corresponding products **5**–**10** (Scheme 1). Thus, reacting **4** with methylsulfonyl chloride in anhydrous pyridine at room temperature for 3 h afforded **5** in 87% yield. **7**–

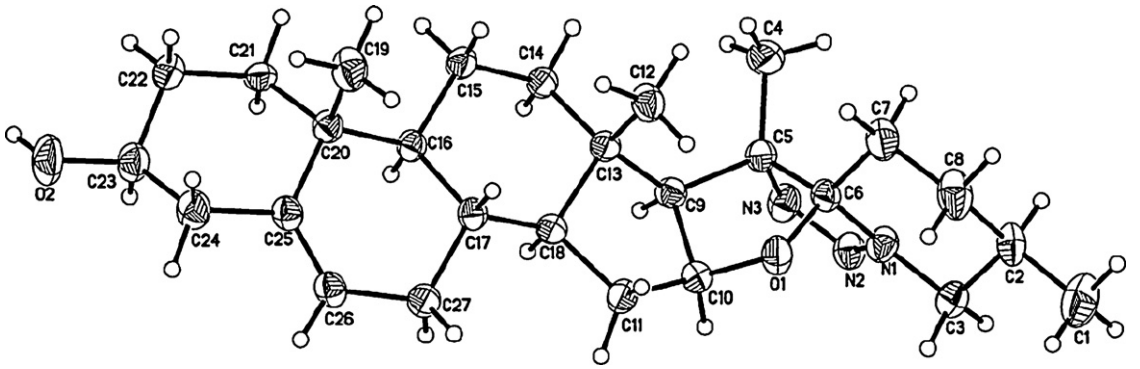


Fig. 1. The X-ray crystal structure of pseudodiosgenin heptacyclic triazoline **3**.

Table 1
The inhibitory effects of solasodine **11** and its analogues **3** and **12–20** on the proliferation of a prostate gland adenocarcinoma cell line (PC-3).

Compounds	PC-3 cell line (IC_{50}[a], μmol/L)
3	18.3 ± 3.2
11	>25
12	>20
13	>20
14	>20
15	nd[b]
16	>20
17	10–20
18	>20
19	3.91 ± 0.87
20	>20

[a] Values represent the mean ±SD of two experiments ($n = 3$).
[b] nd, not determined.

10 were prepared in a similar manner in medium to high yields. Reduction of **4** with TMSCl/NaI in MeCN at room temperature followed by an automatic cyclization of the resulting primary amine under the acidic condition and, treatment of the reaction mixture with $Na_2S_2O_3$ solution, afforded **11** (71%). **12–17** were obtained in a similar way. Bird et al. reported that reduction of solasodine **11** with $NaBH_4$ in MeOH/DCM afforded the corresponding dihydrosolasodine ((22S, 25R)-22,26-epiminocholest-5-ene-3β,16β-diol, **18**) in 95% yield (87% yield in our study [17]). Thus, **19** (81% yield) [18] and **20** (77% yield) were obtained by reduction of **15** and **16** with $NaBH_4$, respectively.

The cellular activity of compounds **3**, **11–14**, and **16–20** (each compound has a purity of above 98%), were firstly evaluated in a prostate gland adenocarcinoma cell line (PC-3) proliferation assay using incorporation of [^{3}H]-thymidine [19]. As shown in Table 1, solasodine **11** had minimal inhibitory effect on the proliferation of PC-3 cell line with an IC_{50} value above 25 μmol/L. Several esterization and etherisation of solasodine at C-3 position (**12–17**), except for **17** (1-naphthoyl), did not enhance the inhibitory activities. In comparison to solasodine, analogs **3** and **19** significantly enhanced cytotoxicity on PC-3 cell line. In particular, **19** showed the most potent inhibitory effect (IC_{50} = 3.91 μmol/L). Strangely, two other structurally related analogs, **18** (3β-hydroxyl) and **20** (3β-*p*-*tert*-butylbenzoyl) were much less active, suggesting that substituents at the C-3 position are related to the anticancer activities *in vitro*.

In summary, a series of solasodine derivatives have been synthesized and evaluated for their activities against the proliferation of PC-3 cell line. In the course of this work, the structure of **3** was determined by X-ray crystallographic analysis. Among the solasodine derivatives synthesized and tested, **19** exhibited the most potent anticancer activity. These results provided some useful information about the SAR of solasodine derivatives as novel inhibitors of prostate cancer cell proliferation.

Acknowledgment

We thank Dr Sensen Lin (Jiangsu Center for Drug Screening, China Pharmaceutical University) for discussion about the biological aspects of this manuscript.

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[15] Crystallographic data (excluding structure factors) has been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 689358 (compound **3**). Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ. UK (fax: +44 (0)1223 336033 or E-mail: deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk).
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[17] Analytical data for compound **18**: Mp 264–265 °C. IR (KBr): 3417, 2933, 1460, 1046 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 5.36 (brd, 1H), 4.42 (m, 1H), 3.53 (m, 1H), 3.00 (brd, 1H), 2.57 (brd, 1H), 1.06 (d, 3H, J = 7.1 Hz), 1.02 (s, 3H), 0.93 (s, 3H), 0.83 (d, 3H, J = 6.6 Hz). ^{13}C NMR (300 MHz, $CDCl_3$): δ 140.8; 121.6; 71.8; 71.2; 62.8; 59.8; 54.5; 54.4; 50.3; 42.8; 42.4; 40.2; 37.3; 36.6; 35.9; 35.9; 33.7; 31.9; 31.7; 31.6; 31.5; 31.5; 27.5; 20.9; 19.4; 19.0; 13.5. TOF-MS (*m/z*): 416.3 ($[M+H]^+$). (The analytical data of **18** were identical with the data reported: see Ref. [16]).
[18] Analytical data for compound 19: Mp. >300 °C. IR (KBr): 3433, 2948, 1719, 1276, 756 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.63 (m, 1H); 7.55 (m, 1H), 7.31 (m, 1H), 7.09 (m,1H), 5.42 (brd, 1H), 4.84 (m, 1H), 4.48 (m, 1H), 3.85 (s, 3H), 3.20 (brd, 1H), 2.85 (brd, 1H), 2.46 (brd, 2H), 1.13 (d, 3H, J = 7.2 Hz), 1.07 (s, 3H), 0.93 (s, 3H), 0.88 (d, 3H, J = 6.4 Hz). ^{13}C NMR (300 MHz, $CDCl_3$): δ 165.8; 159.6; 139.7; 132.3; 129.3; 122.6; 122.0; 119.2; 114.2; 77.2; 74.7; 62.3; 58.6; 55.5; 54.3; 53.3; 50.1; 42.8; 40.0; 38.2; 37.1; 36.7; 34.9; 33.0; 31.8; 31.6; 30.2; 27.9; 27.0; 20.8; 19.4; 19.1; 18.2; 13.4. TOF-HRMS (*m/z*): calcd for $C_{35}H_{52}NO_4$ ($[M+H]^+$) 550.3896, found 550.3878.
[19] PC-3 cells were obtained from ATCC and were cultured in RPMI medium, supplemented with 10% FBS and 50 units/mL penicillin plus 50 mg/mL streptomycin. Cells were grown in a humidified incubator at 37 °C in an atmosphere of 5% CO_2. In cell proliferation analysis, cultured PC-3 cells were seeded into 96-well plates before treatment with drug for 28 h. 0.5 μCi [^3H]-thymidine was added for the last 6 h before cells were trypsinized and harvested by Cell Harvester (Tomtech, CT). The scintillation counts from the isotope incorporated in the newly synthesized DNA was counted by Beta-Counter (PerkinElmer). Data were collected and analyzed by GraphPad Prism (La Jolla, CA) 4.0 software.