Natural Inhibitors of DNA Topoisomerase I with Cytotoxicities

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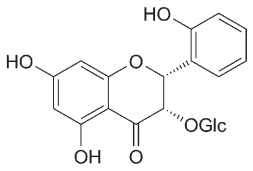
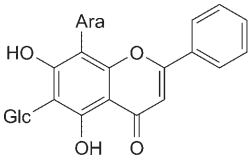
DNA Topoisomerase I can cause DNA breaks and play a key role during cell proliferation and differentiation. It is an important target for anticancer agents. While screening for anticancer compounds, seven natural compounds, **1**–**7**, showed potent cytotoxicities against a panel of ten cancer cell lines. Moreover, an inhibition assay demonstrated that they are also DNA topoisomerase I inhibitors, in which inhibitors **1**–**5** are new ones.

Introduction. – DNA Topoisomerases (Topos) are enzymes that alter DNA conformation through a concerted breaking and rejoining of the DNA molecule, thereby controlling the topological state of DNA. Topos-caused DNA breaks are essential for uncoiling of the DNA helix during DNA replication, transcription, and recombination, which made them play a key role during cell proliferation and differentiation. There are two types of DNA topoisomerases: type-I DNA topoisomerase (Topo I) acts by making a transient break in one strand of DNA, while type-II DNA topoisomerase (Topo II) introduces transient double-strand breaks [1]. Because of the involvement in many vital cellular processes, both Topo I and Topo II have been target enzymes for chemotherapy, especially for cancer treatment, and inhibitors of Topos represent a major class of anticancer drugs in the clinic [1][2]. Many anticancer agents, such as amsacrine, etoposide, teniposide, and doxorubicin have emerged, which are inhibitors of Topo II. Compared with Topo-II inhibitors, fewer inhibitors of Topo I have been discovered, in which the most widely studied and characterized inhibitors are camptothecin (CPT) and its derivatives [3][4].

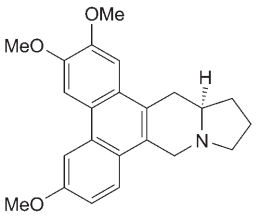
Natural products comprise broad chemical structural types, and, therefore, there is a lot of interest in discovering novel inhibitors from natural products as potential lead compounds for drug development. In our previous work, ten novel inhibitors targeting osteoclast-mediated bone resorption have been identified through random screening our compound library of *ca.* 1800 compounds [5]. These inhibitors include six biflavones, two alkaloids, one sesquiterpene lactone, and one lignan. To search for new anticancer agents, we performed a cell-growth inhibition assay on all the compounds in our compound library with ten cancer cell lines. Results indicated that 324 compounds showed cytotoxicity ($IC_{50} \leq 10$ μg/ml) against at least two cancer cell lines. Among



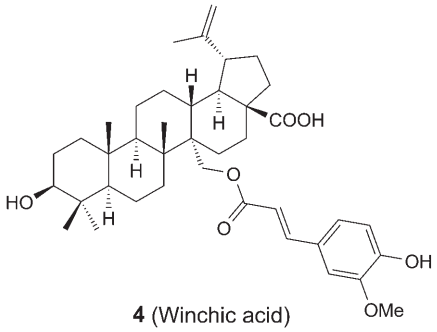
1 (*cis*-2',5,7-Trihydroxyflavanonol 3-*O*-β-D-glucopyranoside)



2 (Chrysin 6-*C*-β-D-glucopyranosyl 8-*C*-α-L-arabinopyranoside)



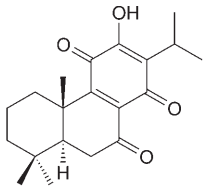
3 (Antofine)



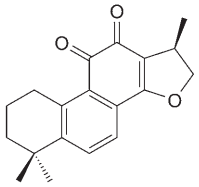
4 (Winchic acid)

Plant sources:

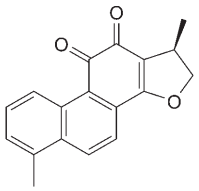
1, **2** from *Scutellaria amoena* [13]
3 from *Cynanchum komarovii* [15]
4 from *Winchia calophylla* [14]
5 from *Orthosiphon wulfenioides* [17]
6, **7** from *Salvia castanea* f. *tomentos* [16]



5 (7-Ketoroyleanone)



6 ((–)-Cryptotanshinone)



7 (Dihydrotanshinone I)

these cytotoxic compounds, seven demonstrated inhibitory activities in the topoisomerase-I assay. Here, we report their cytotoxicities and inhibitory effects on Topo I.

Results and Discussion. – Seven cytotoxic compounds with Topo-I inhibitory activity were found through screening (*Table*), which include two flavonoids, **1** and **2**, one alkaloid, **3**, one triterpenoid, **4**, and three diterpenoids, **5**–**7**. Among these compounds, **3** and **5** showed broad-spectrum cytotoxic activity. Compound **3** showed potent cytotoxic activity against nine cancer cell lines, but had no inhibition to BGC-823. Compound **5** showed cytotoxic activity to all the cell lines but SGC-7901. Although the cytotoxic effects of the other five compounds were not very strong, they all showed cytotoxic activity against at least two cell lines (*Table*).

Table. *Cytotoxicities against Ten Cancer Cell Lines and Topo-I Inhibitory Activities of Compounds* **1**–**7**

Compound	IC_{50}[a]) [μM]										
	A549	BEL-7402	BGC-823	SGC-7901	DU-145	HT-29	MCF 7	MDA-MB-231	U251	B16	Topo I
1	10.24 ± 0.45	NA	18.94 ± 1.51	NA	NA	NA	NA	NA	NA	NA	20.00 ± 1.93
2	13.09 ± 0.07	NA	11.10 ± 0.73	NA	NA	NA	NA	NA	NA	NA	16.39 ± 1.15
3	0.08 ± 0.01	0.33 ± 0.15	NA	3.55 ± 0.73	0.22 ± 0.01	0.25 ± 0.07	1.57 ± 0.02	0.80 ± 0.07	1.62 ± 0.30	0.39 ± 0.26	5.17 ± 0.36
4	13.16 ± 0.25	NA	4.48 ± 0.42	NA	NA	NA	NA	NA	NA	NA	15.41 ± 2.53
5	13.14 ± 0.36	24.42 ± 0.84	15.01 ± 2.72	NA	16.86 ± 0.14	13.35 ± 0.17	22.79 ± 1.66	25.67 ± 0.72	24.24 ± 3.77	15.59 ± 1.39	38.92 ± 8.72
6	6.31 ± 0.40	26.55 ± 0.83	9.51 ± 1.63	NA	24.26 ± 1.04	NA	28.24 ± 2.99	NA	NA	NA	22.77 ± 4.35
7	5.46 ± 0.10	NA	11.32 ± 0.57	33.06 ± 2.06	NA	NA	NA	8.62 ± 0.20	NA	13.94 ± 0.94	66.83 ± 6.13
Taxol	0.02 ± 0.01	0.68 ± 0.10	0.04 ± 0.01	6.75 ± 4.29	0.05 ± 0.03	0.04 ± 0.01	1.08 ± 0.21	–	–	0.08 ± 0.03	–
HCPT	–	–	–	–	–	–	–	8.62 ± 0.44	–	–	–
VCR	–	–	–	–	–	–	–	–	3.04 ± 0.06	–	–
CPT	–	–	–	–	–	–	–	–	–	–	127.66 ± 84.37

[a]) Each value is the average of three experiments (average ± SD). HCPT, VCR, and CPT represent 10-hydroxycamptothecin, vincristine, and camptothecin, resp. NA: No activity at 10 μg/ml; – not tested.

The cytotoxicities and Topo-I inhibitory activities of (−)-cryptotanshinone (**6**) and dihydrotanshinone I (**7**) have been reported previously [6–8]. Flavonoids have been found to be an important group of topoisomerase inhibitors [9–11]. In this study, two flavonoids, **1** and **2**, were found to be new topoisomerase-I inhibitors. Additionally, one diterpenoid, **5**, one alkaloid, **3**, and one triterpenoid, **4**, were also found to suppress the activity of topoisomerase I effectively, which was not reported previously.

DNA topoisomerase I inhibitors represent an important group of anticancer agents [1][12]. CPT, a well studied Topo-I inhibitor, could induce high level of DNA breaks both *in vitro* and *in vivo*, and lead to cell death eventually [3][4]. In this study, we identified seven topoisomerase-I inhibitors with potent cytotoxic activities. Except compound **6** and **7**, the other five compounds were all new topoisomerase-I inhibitors. These seven inhibitors are compounds from various plants with natural chemotypes of flavonoid, diterpenoid, triterpenoid, and alkaloid. They provided us new insights into the study of Topo-I inhibitors as new anticancer drugs, which deserve further research in the future.

This work was financially supported by the *National Natural Science Foundation of China* (30725048) and the *Foundation of Chinese Academy of Sciences* (West Light Program).

Experimental Part

General. The DNA topoisomerase I and substrate DNA pBR322 were from *MBI Fermentas, Inc.* (Vilnius, Lithuania). Seven pure compounds, **1**–**7**, were isolated from various plants by us with purities > 95%. Detailed isolation and identification of these compounds have been described previously [13–17]. The positive drugs taxol, 10-hydroxycamptothecin, vincristine, camptothecin, and the compounds **1**–**7** under study were dissolved in DMSO at 2.5 mg/ml as stock soln. and stored at −20° in aliquots. The dye sulphorhodamine B (SRB) was from *Sigma Chemicals Co., Ltd.* (St. Louis, MO, USA). All other chemicals were of anal. reagent grade. Ten cell lines including A549 (non-small-cell lung carcinoma), BGC-823 (gastric carcinoma), SGC-7901 (gastric carcinoma), DU-145 (prostate carcinoma), MDA-MB-231 (breast carcinoma), HT-29 (colon adenocarcinoma), BEL-7402 (hepatic carcinoma), MCF7 (breast adenocarcinoma), U-251 (glioma), and B16 (murine melanoma) were obtained from the Shanghai Institute of Materia Medica, Chinese Academy of Sciences (Shanghai, China), or the Cell Culture Centre of Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences (Peking, China).

Cell-Growth Inhibition Assay. The sulphorhodamine B (SRB) assay has been adopted for a quant. measurement of cell growth and viability [18]. First, cells were seeded in 96-well microtiter plates at 3000–7000 cells per well. Twenty-four h later, compounds were added to a final concentration of 10 μg/ml. After incubation for 48 h, cells were fixed by the addition of 50% ice-cold Cl_3CCOOH and then left at 4° for 1 h. After washing, air-drying, and staining for 15 min with 100 μl 0.4% SRB in 1% glacial AcOH, excessive dye was removed by washing with 1% glacial AcOH. Finally, the OD values of re-suspended SRB in 10 mM *Tris* buffer were read at 560 nm on a plate reader (*Molecular Devices, SPECTRA MAX 340*); and further assessment was carried out with at least four diluted concentrations (dilution ratio 1:2) if the inhibition is upon 50% at this concentration.

Topo-I Assay. The Topo-I activity was determined by DNA relaxation assay according to the instruction of *MBI Fermentas, Inc.* with some modification. Supercoiled pBR322 DNA (0.25 μg) was incubated with 0.6 unit calf thymus topoisomerase I at 37° for 30 min in 20 μl relaxation buffer (pH 8.0, 35 mM *Tris*·HCl, 72 mM KCl, 5mM $MgCl_2$, 5mM DTT, 0.1 mg/ml BSA) in the absence or presence of tested compounds under study. The reaction was terminated by the addition of 5 μl of dye soln. containing 0.17% bromophenol blue, 20% glycerol, and 3.3% SDS. Reaction products were separated on a 1.0% agarose gel, whereafter photographs of the ethidium bromide-stained gel were taken under UV light and quantified through the Quantity One software (*Bio-Rad Ltd.*).

Data Analysis. Inhibition data were expressed as IC_{50} values, which were calculated by dose-response curves with at least four concentrations (dilution ratio 1:2). The highest test concentrations for cell growth inhibition assay and Topo-I assay are 10 and 20 μg/ml, resp. Results are expressed as mean IC_{50} values ± standard deviation (SD).

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Received August 28, 2007