SHORT REPORT

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The Effects of Arolycoricidine and Narciprimine on Tumor Cell Killing and Topoisomerase Activity

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Abstract: In this study, narciprimine and arolycoricidine were isolated from *G. rizehensis* Stern (Amaryllidaceae). The structures of the alkaloids were elucidated by spectroscopic methods (1D NMR, EI-MS). Due to the previous reports on anti-cancer activity of this group of alkaloids, we investigated their effects on DNA topoisomerase reactions, which are known as the cellular targets of a number of chemotherapeutical drugs. The results revealed that arolycoricidine and narciprimine were effective in both type I and type II DNA topoisomerase reactions in a dose-dependent manner. Topoisomerase-interfering ability of these alkaloids partially correlated with cytostaticity assays, using HeLa (cervix adenocarcinoma), MCF7 (breast adenocarcinoma) and A431 (skin epidermoid carcinoma) cells. Our results are discussed in relation to the potential significance of these alkaloids in the course of drug-development studies.

Keywords: *Arolycoricidine; Narciprimine; Galanthus rizehensis; DNA Topoisomerases; Cytostaticity.*

1. Plant Source

The plant material was collected from Macka, Trabzon on March 11, 2007 and May 2, 2007 during both flowering and fruiting periods. The plant was identified by Prof. M. Ali Onur (Ege University, Izmir , Turkey). Voucher specimens are deposited (No's 1371, 1376) in the herbarium of

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the Department of Pharmacognosy, Faculty of Pharmacy, Ege University. The combined plant material was used for the phytochemical studies.

2. Previous Studies

Amaryllidaceae alkaloids have been shown to possess a wide spectrum of biological properties, including anti-tumor activity [1, 2]. Several compounds with anti-tumor potential interfere with the reactions of DNA topoisomerases, which are ubiquitous enzymes having a central role in many aspects of DNA metabolism [3-6]. Two types of topoisomerases are known, one able to introduce single-stranded breaks (topo I) the other able to produce double-stranded breaks (topo II) in the DNA molecules [3].

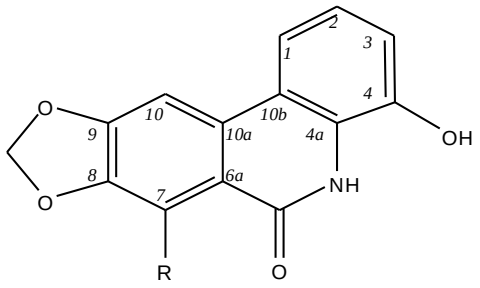
3. Present Study

Plant material (13.1 kg) was air dried, powdered and extracted with 96% ethanol. The solvent was evaporated under vacuum. The residue was dissolved in 2% hydrochloric acid and non alkaloidal content was extracted using organic solvent. The residue was basified with 26% ammonium hydroxide to pH 9-10. The basic solution was extracted with chloroform to yield the crude alkaloidal extract (10.49 g). The alkaloidal mixture was subjected to column chromatography on Silica gel 60 using chloroform gradually enriched with methanol. Narciprimine (**1**) (33.8 mg) was crystallized from the combined fractions 18-21 (0.51 g) eluted with chloroform. Combined fractions 37-46 (0.99 g) eluted with 2 % methanol in chloroform were further separated by preparative column chromatography using benzene-chloroform-methanol-ammonium hydroxide (26 %) (7:2:1:0.1) and arolycoricidine (**2**) (5.6 mg) was crystallized from frs 11-12.

*Narciprimine **(1)**:* ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 7.89 (1H, dd, J = 8.1, 0.9 Hz, H-1), 7.70 (1H, s, H-10), 7.26 (1H, t, J = 8.1 Hz, H-2), 7.11 (1H, dd, J = 8.1, 0.9 Hz, H-3), 6.34 (2H, s, OCH_2O); ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) = 165.6 (C=O), 154.3 (C-9), 145.6 (C-4), 145.1 (C-7), 133.0 (C-8), 132.6 (C-6a), 124.6 (C-4a), 123.9 (C-2), 120.1 (C-10b), 114.5 (C-1), 114.2 (C-3), 107.8 (C-10a), 103.0 (OCH_2O), 94.4 (C-10); EIMS (rel. int.): *m/z* 271 $[M]^+$ (100), 242 (7), 225 (2), 213 (16), 185 (19), 157 (7), 102 (9)

*Arolycoricidine **(2)**:* ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 8.13 (1H, s, H-10), 7.90 (1H, dd, J = 8.1, 0.9 Hz, H-1), 7.81 (1H, s, H-7), 7.20 (1H, t, J = 8.1 Hz, H-2), 7.08 (1H, dd, J = 8.1, 0.9 Hz, H-3), 6.38 (2H, s, OCH_2O); ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) = 161.6 (C=O), 153.1 (C-9), 149.2 (C-8), 146.3 (C-4), 133.0 (C-6a), 127.6 (C-10a), 124.3 (C-4a), 122.9 (C-2), 120.3 (C-10b), 114.3 (C-1), 114.3 (C-3), 107.1 (C-7), 103.1 (OCH_2O), 102.6 (C-10); EIMS (rel. int.): *m/z* 255 $[M]^+$ (100), 226 (9), 169 (6), 141 (12), 127 (7), 113 (8), 57 (7).

The structures of **1** and **2** (Figure 1) were elucidated by 1D NMR, EI-MS and also by comparison with previous literature data [7, 8].



1: R=OH, **2**: R=H

Figure 1. Structures of narciprimine (**1**) and arolycoricidine **(2).**

Previously, **1** and **2** were reported in different Amaryllidaceae species [7-9]. These alkaloids belong to the narciclasine (isocarbostyril derivative) subgroup of Amaryllidaceae alkaloids which have been shown to possess antitumor potential [2]. Furthermore, the Amaryllidaceae alkaloids, lycorine, 1-*O*-acetyllycorine and ismine isolated from *Crinum x powellii* "Album" were evaluated for their topoisomerase I inhibitory activity and only lycorine was shown to display significant DNA topoisomerase I inhibition [10]. Methods based on interfering molecular interactions, in particular topoisomerase enzyme assays have been valuable tools in assessing anti-tumor potential of natural and synthetic compounds [11]. We carried out supercoiled plasmid relaxation [12] and kinetoplast decatenation assays [13] to monitor the activities of the isolated alkaloids on type I and type II topoisomerases, respectively. A representative assay for type I topoisomerase is given in Figure 2 (See the figure legend for identification of the sample applications). As seen in Figure 2, both **1** and **2** were found to interfere with topo I reactions at the concentration of 0.25 µg/µL (Figure 2, lanes; 4 and 6, respectively) while the interference profoundly diminished upon dilution to 0.1 µg/µL (Figure 2, lanes 5 and 7, respectively). Both compounds gave rise to conflicting results of interference at higher concentrations (>1 µg/µL) (data not shown), which was a result of decreased sensitivity of the topo I reactions at sub-optimum conditions [14]. Densitometric analyses of average relative band intensities for **1** resulted in a change from 30% to 11% estimated remaining supercoiled substrate populations with a decrease in concentration from 0.25 µg/µL to 0.1 µg/µL while the same parameter changed from 42% to 4% for the compound **2** for the same intervals (Fig. 2).

Minicircle DNA decatenation assays in the presence of **1** and **2** also revealed comparable interference in human topoisomerase II reactions (Fig. 3). This method requires Etd-Br in the gel that separates open-circular (OC) monomers from covalently-closed circular (CCC) DNA [13]. As seen in Figure 3, the kinetoplast-DNA (Figure 3, lane 1) were converted into monomeric DNA rings as OC-DNA and CCC-DNA (Figure 3A, lane 2), an activity that was slightly influenced by the presence of 10 % DMSO (Figure 3A, lane 3). Decatenation was completely abolished when Etoposide (Figure 3A, lane 4) was in the reaction mixture. 1 µg/µL of either, **1** or **2** interfered with decatenating activity of topoisomerase II (Figure 3A, lane 5 and 8, respectively), however dilution of both **1** and **2** resulted in a diminished interference (Figure 3A, lanes 6 and 8, corresponding to 0.25 µg/µL for **1** and **2**, respectively; lanes 7 and 9, corresponding to 0.1 µg/µL for **1** and **2**, respectively). Densitometric calculations of percent catenated DNA population varied from 50% to 9% for **1** while the same population revealed a variation from 49% to 15% for **2** when the concentration of the test compound was diluted from 1 µg/µL to 0.1 µg/µL (Fig. 2).

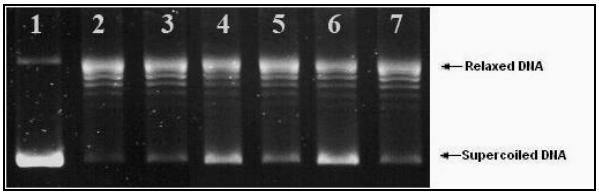


Figure 2. The effect of **1** and **2** on supercoil relaxation activity of mammalian DNA topoisomerase I. **A**. A representative agarose gel photograph of supercoil relaxation in the presence of varying concentrations of **1** and **2**; lane 1, pBR322; lane 2, pBR322 with 1 u of DNA topoisomerase I; lane 3, same as lane 2 in the presence of 10% DMSO; lanes 4-5; same as lane 2 in the presence of 0.25 µg/µL (lanes 4 and 6, for **1** and **2**, respectively) and 0.1 µg/µL (lanes 5 and 7, for **1** and **2**, respectively).

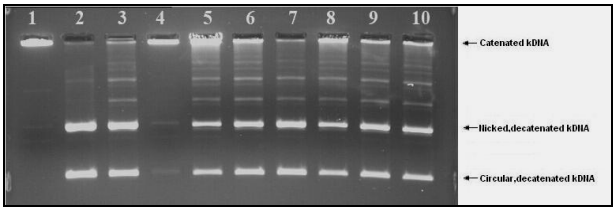


Figure 3. Decatenation assays by topoisomerase II in the presence of alkaloids. Lane 1, K-DNA; lane 2, K-DNA with 1 u of DNA topoisomerase II; lane 3, same as lane 2 in the presence of 10% DMSO; lane 4, same as lane 2 plus 0.5 mM Etoposide; lanes 5-10, same as lane 2 in the presence of **1** (lanes 5-7, corresponding to 1 µg/µL, 0.25 µg/µL and 0.1 µg/µL , respectively) and **2** (lanes 8-10, corresponding to 1 µg/µL, 0.25 µg/µL and 0.1 µg/µL , respectively).

We, then, assayed antiproliferative effects of the test compounds on the three human cell lines: HeLa (cervix adenocarcinoma), MCF7 (breast adenocarcinoma) and A431 (skin epidermoid carcinoma) by using the MTT assay [15]. Reference compound Cisplatin resulted in IC_{50} values of 12.43 µM, 9.63 µM and 2.84 µM on HeLa, MCF7 and A431 cells, respectively (Table 1). Both alkaloids exerted a limited anti-proliferative effect against the three cancer cell lines (Table 1). Though **2** seemed to be modestly effective against A431 cells at 10 µM, no concentration dependent increase was seen at 30 µM. IC_{50} values against these cell lines were above 30 µM. Since **2** did not exert at least 50 % inhibition of cell proliferation, no concentration curves were presented for this compound (Table 1).

Table 1. Antiproliferative effects of the test compounds on the HeLa, MCF7 and A431 cell lines

	Inhibition (%) ± SEM		
1	HeLa	MCF7	A431
10 µM	26.60 ± 2.68	34.66 ± 6.47	13.91 ± 3.86
30 µM	61.57 ± 3.49	52.10 ± 3.84	37.33 ± 4.92
IC_{50} (µM)	20.42	26.47	> 30
2	HeLa	MCF7	A431
10 µM	35.00 ± 4.74	28.52 ± 7.44	47.86 ± 3.09
30 µM	39.79 ± 5.58	35.42 ± 6.77	42.15 ± 3.00

Taken together, topoisomerase assays are highly valuable in assessing the pharmaceutical significance of natural compounds. Our assays showed that both **1** and **2** had a considerable effect in topoisomerase reactions. On the other hand, the enzyme assays were partially correlated with the cell culture tests. The studies to identify the exact mechanism of the interference obtained by the two compounds is currently under progress.

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