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COMPARATIVE ACTION OF SALSOLINE, SALSOLIDINE, AND RELATED COMPOUNDS ON KML TISSUE CULTURE AND ANIMAL TUMOR STRAINS

N. N. Kuznetsova, L. K. Abdullaeva, and A. A. Sadikov

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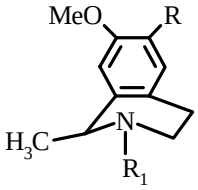
The alkaloids salsoline (**1**) and salsolidine (**6**) are derivatives of methyltetrahydroquinoline and were isolated from *Salsola richteri* L. (Chenopodiaceae) [1].

They are known to dilate peripheral arteries and lower blood pressure.

Our goal was to determine the cytotoxicity in KML cell culture, the toxicity (LD_{50}), and the antitumor activity of **1**, **6**, and eight of their derivatives (**2-5** and **7-10**) in addition to four hydrogenated quinoline derivatives (**11-14**) [2].



1 - 10

12 - 14

1: R = OH, R_1 = H
2: R = OH, R_1 = NO
3: R = OH, R_1 = CH_2CH_2CN
4: R = OH, R_1 = CH_2CH_2Cl
5: R = OH, R_1 = CH_2CH_2OH

6: R = OCH_3, R_1 = H
7: R = OCH_3, R_1 = NO
8: R = OCH_3, R_1 = CH_2CH_2CN
9: R = OCH_3, R_1 = CH_2CH_2Cl
10: R = OCH_3, R_1 = CH_2CH_2OH

12: R = NO
13: R = CH_2CH_2Cl
14: R = CH_2CH_2CN

For the initial screening, we used a murine melanoma cell line developed by us [3]. The cytotoxic activities of vinblastine and colchamine were studied first. This established that the line was sensitive to known antitumor preparations. The CE_{50} of these preparations was 1 μg/mL and less [4, 5].

Then, the activities of **1-14** toward the tested KML tumor cell line [6] were studied.

The cytotoxic test was performed with compound doses of 1, 10, and 100 μg/mL of nutrient medium. The control was KML cells without added compound.

Cells ($4 \cdot 10^4$ cells/mL) were dispersed in tubes with RPMI-1640 nutrient medium (3 mL) with fetal-calf serum (10%), glutamine (200 mM), and antibiotics and cultivated in a thermostat at 37°C. Compounds were added to the cells 24 h after dispersion. Cells were exposed to the compounds for 24 h. Then, ^{14}C-thymidine (0.03 μCi/tube) was added for 1 h. The cytotoxic activities of the compounds were calculated from the amount of ^{14}C-thymidine inclusion in cellular DNA.

The results were calculated as percent inhibition of ^{14}C-thymidine incorporation versus the control. Then, CE_{50} [7] was determined graphically from plots of the effect as a function of the dose of each compound, i.e., that concentration at which the resulting index was reduced by half (50% cell effect). A compound was considered active if a dose of less than 100 μg/mL was required to reach CE_{50} [5].

A. S. Sadykov Institute of Bioorganic Chemistry, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (99871) 162 70 73, e-mail: ibchem@uzsci.net. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 186-187, March-April, 2005. Original article submitted September 13, 2004.

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TABLE 1. Cytotoxic and Antitumor Activities of **4**, **9**, **11**, **12**, and **13***

Compound	CE_{50}, mg/mL for ^{14}C thymidine incorporation	LD_{50}, mg/kg	Animal tumor strain			
			EAC	NK/Ly	s-180	Walker's c-s
4**	30±2.5	340±3.3	25±1.1	-	35±0.8	-
9**	50±1.2	360±3.4	20±0.6	-	30±0.3	-
11	64±2.2	385±4.1	57±1.2	76±2.9	85±2.1	95±3.2
12	80±2.5	500±3.2	74±3.7	68±1.3	47±1.6	62±2.3
13**	16±0.6	250±2.3	20±0.3	-	35±1.1	-

*Data for active compounds only are included, ****4**, **9**, and **13** are inactive toward NK/Ly and Walker's c-s.

The antitumor effect was estimated in percent of the control from the increased lifespan of tumor-bearing animals by the usual method [5]. The toxicities of the compounds were determined from the LD_{50} values, also obtained using the usual method [5]. Experiments, including the controls, were repeated three times. The controls were cells or tumor-bearing animals without administered preparations. Two of the ten isoquinoline compounds with an *N*-(β-chloroethyl) group (**4** and **9**) were active in the *in vitro* system. The concentrations of these compounds, 30 and 50 μg/mL, respectively, were within the range of ^{14}C-incorporation to be considered active (Table 1).

Compounds **1** and **6** and their derivatives **2**, **3**, **5**, **7**, **8**, and **10** were inactive. Compounds **4** and **9** had mild toxicities, LD_{50} values of 340 and 360 mg/kg, respectively, or had no effect on the experimental animal tumor lines. They inhibited growth of Ehrlich's ascites cancer (EAC) and sarcoma-180 (s-180) only by from 20 to 35%.

Three of the four quinoline compounds were active in both the *in vitro* and *in vivo* systems. These were *N*-nitrosotetrahydroquinoline (**11**), *N*-nitrosodecahydroquinoline (**12**), and *N*-(β-chloroethyl)decahydroquinoline (**13**). The CE_{50} values for ^{14}C-thymidine incorporation for these compounds were 64, 80, and 16 μg/mL, respectively. The antitumor activities of **11**-**14** were studied in animals with grafted tumor strains, namely EAC, NK/Ly, s-180, and Walker's carcinosarcoma (c-s).

The highest antitumor activities were seen for **11** toward EAC, s-180, NK/Ly, and Walker's c-s (57, 85, 76, and 95% tumor-growth inhibition, respectively) and for **12** toward EAC, s-180, NK/Ly, and Walker's s-c (74, 68, 47, and 62%, respectively). Compound **13** slightly inhibited the growth of EAC (20%) and s-180 (35%). Compound **14** was inactive in both systems. Compounds **11** and **13** were toxic. The LD_{50} values for them were 385 and 500 mg/kg, respectively.

Thus, 5 active compounds were found among the 14 derivatives in the *in vitro* and *in vivo* systems.

The cytotoxicities of the salsoline and salsolidine derivatives can be explained by the formation of active three-membered immonium rings and by the labile conformation of the carrier system [7]. The biological activities of *N*-nitrosotetrahydroquinoline and *N*-nitrosodecahydroquinoline can be explained by activation of the nitrosoamines by oxidation of the C atom in the α-position to the N–NO group and subsequent hydrolysis to form the alkylating species [5].

Based on the cytotoxic activities, the LD_{50} values, and the antitumor activities, **11** and **12** can be recommended as promising cancerolytics.

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