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Research Article

Cytotoxic activity of Quinoline alkaloids and their derivatives

Zilola Kabirova, Nargiz Iskandarova, Ekaterina Terentieva,
Farkhod Eshboev and Shakhnoz Azimova
Department of Molecular genetics,
S.Yu. Yunusov Institute of the Chemistry of Plant Substances,
Academy of Sciences of the Republic of Uzbekistan,
Mirzo Ulugbek Str. 77, 100170, Tashkent, Uzbekistan,
email: genlab_icps@yahoo.com

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Abstract
Quinoline alkaloids, important class of N-based heterocyclic aromatic compounds, have attracted tremendous attention from researchers worldwide, because of their wide-ranging biological activities and their varied applications. In the present work, the cytotoxic activities of dubamine and its 15 derivatives against HBL-100 and CCRF-CEM cancer cells and healthy blood mononuclear cells were studied. Among the tested dubamine derivatives, four compounds showed cytotoxic activity. The LD_{50} values of the substances was determined.

Keywords: alkaloids, dubamine, cytotoxic, cancer cells, blood mononuclear cells.

INTRODUCTION

One of the urgent problems of world medicine and pharmacology is the search for new antitumor compounds. Despite the fact that at present experimental and clinical oncology has a wide range of carcinolytic and anticancer drugs, a significant drawback of most of them is high toxicity to healthy tissues and organs and limitation to a specific malignant neoplasm [1, 2].

Nowadays, experimental and clinical oncology has a wide range of carcinolytic and anticancer drugs for treatment of cancer. According to their mechanism of action, they are all divided into cytotoxins (anticancer drugs that cause damage to the membrane, nucleus and other components of it, which leads to cell death) and cytostatics, which, unlike cytotoxins, trigger the process of apoptosis inside the malignant cell - self-destruction programs, embedded in any cell from the moment of its birth. Among the cytostatic drugs, the most famous are Doxorubicin, Cisplatin, Fluorouracil, Hydroxyurea, Cyclophosphamide [4-7].

Moreover, intensive research is underway in the world to search for new cytostatic compounds among substances of plant origin. To date, cytostatic activity has been detected in almost all groups of chemical compounds that make up plants: alkaloids, coumarins, polyphenols, lignins, etc [8, 9]. Quinoline alkaloids and their synthetic modifications are a very promising group for the search for anticancer agents among them, since they exhibit a wide spectrum biological activity, including cytotoxic properties [10]. However, industrial production of biologically active substances from natural

sources of plant or other origin is difficult due to the complexity of their purification from impurities or due to their low content. In such cases, the attention of researchers is drawn to its synthetic preparation, as well as to its chemical modification, aimed at enhancing physiological activity and/or reducing side effects. For instance, tetrahydroisoquinolines, the molecules of which have one or more reaction centers, contain wide synthetic possibilities and, therefore, have attracted the attention of specialists in the field of organic chemistry for a long time [11, 12]. It has been established that various quinoline derivatives exhibit antitumor activity against cancer of the breast, prostate, cervix and colon, leukemia, and lung cancer. Among these isoquinoline alkaloids cicleapeltin, zefirantine, and methyltelobin-N have a pronounced cytostatic activity and inhibit the growth of cervical carcinoma cells at low concentrations [13].

The aim of the study is to identify new quinoline compounds with high cytotoxicity to cancer cells and low toxicity to healthy cells.

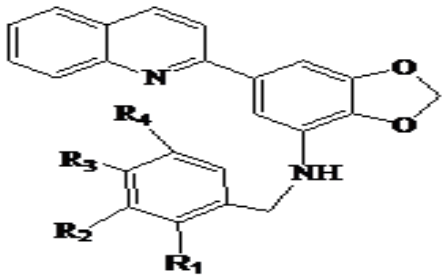
Materials and methods

Cell lines and culture medium.

The HBL-100 cell line (breast adenocarcinoma) was obtained from the Institute of Cytology, Russian Academy of Sciences; cell line CCRF-CEM (T-lymphoblastic leukemia) obtained from Halderberg University, Germany. Suspension of mononuclear cells was obtained from the whole blood of conditionally healthy people. We used culture medium RPMI-1640 (Gibco, Canada), fetal calf serum (Gibco, Canada), L-glutamine (Sigma, USA), and antimycotic antibiotic (Sigma, USA) for cultivation of the cells.

Structure of the studied compounds

Compounds were represented by the general formula:



Deravatives	Structure of substituents to the main formula
1	R_1= H , R_2= OCH_3 , R_3= OCH_3, R_4= H
2	R_1= H , R_2= H , R_3= H, R_4= H
3	R_1= H , R_2= H , R_3= NO_2, R_4= H
4	R_1= H , R_2= H , R_3= OCH_3, R_4= H
5	R_1= H , R_2= H , R_3= OH, R_4= H
6	R_1= H , R_2= H , R_3= $N(CH_3)_2$, R_4= H
7	R_1= H , R_2= OH , R_3= OCH_3, R_4= H
8	R_1= Br , R_2= OH , R_3= OCH_3, R_4= H
9	R_1=Br , R_2= H , R_3= OCH_3, R_4= OH
10	R_1= H, R_2= OCH_3, R_3= OH, R_4= H
11	R_1= H , R_2= OCH_2-Ar , R_3= OCH_3, R_4= H
12	R_1= H , R_2=R_3 = O-CH_2O- , R_4= H
13	R_1= OH , R_2= H , R_3= H, R_4= Br
14	R_1=OH , R_2= H , R_3= H, R_4= H
15	R_1=OH , R_2=NH_2 , R_3= H, R_4= H

Study of cytotoxicity by trypan blue exclusion method

HBL-100 and CCRF-CEM cells were seeded into 96-well plates at a concentration of 20

thousand cells/ml, 100 µl per well, blood mononuclear cells - in the amount of 50 thousand cells/ml. Cells were cultured in a CO_2 incubator for 24 hours. After the day, substances previously dissolved in DMSO were added at concentrations of 1, 10, and 100 µM and left for 24 hours in a CO_2 incubator. Cells without exposure to substances served as control. The well-known antitumor drugs Cisplatin (Fresenius Kabi, India), which is used in the treatment of breast cancer, and Cytarin, which is used in blood cancers, were used as a reference drug. Then, HBL-100 cells were harvested with Versen's solution and CCRF-CEM cells and blood mononuclear cells were carefully pipetted. 50 µl of cell suspension was taken from the wells and mixed with an equal amount of trypan blue. Finally, the mixture was carefully and rapidly pipetted and an aliquot was transferred to a Goryaev chamber for cell counting.

Cell count by MTT method.

The cells were seeded into 96-well plates, 2000 cells per 100 µl per well, and cultured in a CO_2 incubator. After the day, substances were added at concentrations as described above, and the cells were cultured with the substance for 24 hours in a CO_2 incubator. Then the dye MTT was added in the amount of 20 µl (5 mg/ml) per well and incubated for 3-4 hours. At the end of the incubation time, the entire medium was decanted from the plate with HBL-100 cells and 50 µl of DMSO was added. In the case of CCRF-CEM cells and blood mononuclear cells, a portion of the medium was carefully removed and DMSO was injected into the remaining medium with the cells. Cells were stirred for 10 minutes on a shaker. Optical density of cells was measured on a spectrophotometer at a wavelength of 620 nm. Cells without substances served as control. The anticancer drug cisplatin was used as a reference drug.

Statistical analysis

Statistical analysis and exponential curve fitting were performed using Origin 8.6 software (Microcal Software Inc., Northampton, MA). Results were expressed as mean ± S.E.M. To determine the statistical significance of the results One-Way ANOVA and two-tailed *t*-test were performed.

RESULTS AND DISCUSSION

Study of the cytotoxic activity of the alkaloid dubamine and its quinoline derivatives by the MTT method.

In the present work, derivatives of the quinoline alkaloid dubamine were obtained from Laboratory of Chemistry of Alkaloids of the Institute of Chemistry of Plant Substances and the cytotoxicity of the compounds was studied by all the methods listed above. The results on the cytotoxicity of quinoline derivatives obtained by the MTT method are presented in Table 1.

According to the Table 1, the alkaloid dubamine itself in the studied concentration range does not show cytotoxicity on the cell lines of breast adenocarcinoma and T-lymphoblastic leukemia - suppression of growth of breast cancer cells in the studied concentration range did not exceed 9%, T-lymphoblastic leukemia cells - 14 % compared to the control value.

The introduction of an N-phenyl radical to ring C of the dubamine molecule (derivative 2) led to a slight increase in the cytotoxicity of the molecule against CCRF-CEM cells only at a concentration of 100 µM - 28.7% inhibition of cell growth. With a decrease in concentration by 10 and 100 times under the action of this derivative, cell death was 11.0-19.3%.

Further modification of the N-phenyl substituent by introducing it to the ortho-, para-, and meta-positions led to ambiguous results. Thus, the appearance of the OH- hydroxyl group in the R3 position of the ring (5) enhanced the inhibitory properties of quinoline derivatives on CCRF-CEM cells - 35.7 - 36.7% of cell death. In relation to breast cancer cells, these substances were absolutely inactive.

The replacement of hydroxyl by the NO_2 nitro group in the same position (3) contributed to the activity of the compound on breast cancer cells (at 100 µM, 35.3% of HBL-100 cell death is observed), which is enhanced by the introduction of the OCH3 methoxy group (4) instead of NO_2 - 42.3% inhibition of breast cell growth. There was no significant difference in the manifestation of cytotoxicity from the presence of the nitrogen-ethylene substituent $N(CH_3)_2$ in position R3 (6) of the ring.

Table 1: Cytotoxicity of dubamine alkaloid and its derivatives on cancer cell lines (% cell death, MTT method)

	HBL-100			**CCRF-CEM**		
	1 μM	**10 μM**	**100 μM**	**1 μM**	**10 μM**	**100 μM**
Dubamine	9,0 ± 4,3	0,0 ± 0,0	0,0 ± 0,0	3,0 ± 2,2	14.0 ± 5,3	12,0 ± 6,9
1	8,7 ± 4,1	**46,0±12,6**	**68,7 ± 1,3**	0,0 ± 0,0	**25,5 ± 13,4**	**35.0 ± 2,8**
2	0,0 ± 0,0	9,0 ± 4,4	11,0 ± 2,7	11,0 ± 2,2	19,3 ± 13,4	28,7 ± 1,3
3	0,0 ± 0,0	2,7 ± 1,6	35,3 ± 2,5	4,5 ± 3,2	7,0 ± 1,7	26,7 ± 7,6
4	3,3 ± 1,2	14,3± 7,4	42,3 ± 4,4	13,7 ± 2,8	25,5 ± 6,1	23,7 ± 10,4
5	0,0 ± 0,0	7,0 ± 2,1	17,0 ± 7,8	4,3 ± 10,7	35,7 ± 3,8	36,7 ± 15,5
6	10,0 ± 5,9	8,5 ± 6,2	39,5 ± 2,8	28,5 ± 4,4	27,0 ± 8,4	39,0 ± 2,4
7	18,0 ± 8,4	21,1± 1,8	**58,0 ± 9,5**	18,5 ± 6,2	18,5 ± 6,1	16,5 ± 9,7
8	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0	14,0 ± 7,9	13.0 ± 14,1	9,0 ± 2,7
9	3,5 ± 1,4	13,5± 4,7	**41,0 ± 4,2**	0,0 ± 0,0	0,0 ± 0,0	**60,0 ± 6,8**
10	6,5 ± 3,2	8,5 ± 4,2	0,0 ± 0,0	11,0 ± 5,6	20,0 ± 12,5	17,0 ± 4,8
11	0,0 ± 0,0	11.0± 7,6	**54,0 ± 7,9**	13,0 ± 8,3	13,5 ± 9,1	20,0 ± 8,2
12	12,3 ± 5,6	7,3 ± 3,7	17,0 ± 6,7	3,0 ± 1,1	30,3 ± 6,0	26,3 ± 4,3
13	9,0 ± 4,3	2,0 ± 0,7	5,0 ± 2,3	23,7 ± 4,2	24,7 ± 1,9	**39,0 ± 4,8**
14	7,5 ± 4,6	0,0 ± 0,0	17,3 ± 7,6	34,0 ± 1,5	18,7 ± 9,6	10,5 ± 6,3
15	0,0 ± 0,0	0,0 ± 0,0	25,0 ± 8,4	15,5 ± 6,9	0,0 ± 0,0	26,0 ± 6,5
Citarin	-	-	-	20,0 ± 5,3	16,8 ± 5,6	43,0 ± 14,8
Cisplatin	16,5 ± 6,5	22,0± 8,2	61,8 ± 7,4	-	-	-
Control	0	0	0	0	0	0

The additional addition of an OH group to the R2 position, while retaining the methoxyl substituent (7), led to an increase in the cytotoxic properties of quinoline - 58% death of HBL-100 cells, but the activity on leukemia cells decreased. The joint association in the quinoline molecule of three substituents OCH_3-, OH- and the Br atom (8) led to the complete disappearance of cytotoxic activity in two lines of cancer cells. It is interesting to note that if all these substituents are interchanged, then we can observe a sharp increase in the % of cell death of two cell cultures - at 100 μM 41.0 ± 4.2% inhibition of the growth of HBL-100 cells and 60.0 ± 6.8% growth inhibition of CCRF-CEM cells.

Thus, not only the nature of the substituents, but also their localization is important for the manifestation of cytotoxicity by quinolines. This is probably due to the location of the substance structure in space and interaction with the target in the cell. This can be confirmed by the example of substance 10: this sample differs from derivative 7 in that the methoxy group OCH_3- and the hydroxy group OH- were swapped, and as a result, we can observe, according to Table 1, the disappearance of damaging properties on cancer cells.

If two methoxyl radicals, OCH_3 (1), are added to the molecule, then this quinoline acquires the highest cytotoxic activity among the studied compounds: suppression of HBL-100 cell growth under the action of this quinoline is 68.7 ± 1.3% at 100 μM and 46, 0±12.6% at 10 μM. Additional addition to the methoxyl radical Ar (derivative 11) retained the activity of the derivative only on breast cancer cells. It is interesting to note that the introduction of two methylenedioxy substituents O-CH_2O- into the structure instead of methoxy groups (12) contributes to a sharp decrease in inhibitory properties.

Table 2: IC_{50} value of quinoline derivatives on HBL-100 and CCRF-CEM cancer cell lines (MTT method).

Compounds	**Значение IC_{50}, μM**	
	HBL-100	**CCRF-CEM**
1	56,2	>100
7	80,5	>100
9	>100	84,6
11	91,8	>100
Cisplatin	73,7	-

For the test substances, we calculated the dose IC-concentration at which 50% of the cells die, using the Origin 8.6 statistics program. The data is presented in the table below. According to the Table 2, the IC_{50} value for quinoline 1 on breast adenocarcinoma cells was lower than that of the reference drug cisplatin - 56.2 μM versus 73.7 μM, which indicates a high activity of this compound. The IC_{50} value for derivatives not listed in Table 2 was above 100 μM, which indicates the absence of their pronounced cytotoxic activity.

Thus, the study of the cytotoxicity of 15 derivatives of the quinoline alkaloid dubamine by the MTT method and the study of their structural and functional relationship showed that quinoline with two methoxyl radicals, OCH_3, exhibits the highest cytotoxicity against breast cancer cells and T-lymphoblastic leukemia.

When searching for anticancer compounds, it is important to consider the effect of their toxicity on healthy cells in the body. Since chemotherapy drugs are usually administered intravenously, the blood is the first barrier to their toxicity. In connection with the above, we investigated the effect of dubamine and its derivatives on healthy blood cells. The data obtained by the MTT method are presented in Table 3.

As can be seen from Table 3, quinolines that showed cytotoxicity on cancer cell lines caused the death of healthy blood cells to a lesser extent. It was not possible to calculate their IC_{50} values, since the inhibition of cell growth under the action of these substances at the highest concentration (100 μM) did not exceed 49% compared with the control.

Table 3: Cytotoxicity of dubamine alkaloid and its derivatives on blood mononuclear cells (% cell death, MTT method)

	Healthy blood mononuclear cells, %		
	1 μM	**10 μM**	**100 μM**
Dubamine	1,0 ± 0,3	7,0 ± 0,0	10,0 ± 1,0
1	**18,5 ± 5,1**	**34,0±2,4**	**49,0 ± 2,3**
2	0,0 ± 0,0	5,0 ± 0,4	11,0 ± 2,0
3	10,0 ± 1,0	32,7 ± 1,5	49,1 ± 2,0
4	13,2 ± 2,2	31,3 ± 3,5	47,0 ± 3,4
5	10,2 ± 1,1	27,0 ± 2,3	42,0 ± 3,1
6	5,0 ± 1,9	10,5 ± 1,2	29,1 ± 2,0
7	**10,0 ± 2,6**	**13,1 ± 2,8**	**41,0 ± 2,5**
8	35,2 ± 1,9	40,2 ± 1,0	40,0 ± 3,1
9	**0,5 ± 0,1**	**10,5 ± 4,0**	**35,0 ± 3,2**
10	0,5 ± 0,2	1,5 ± 1,0	2,3 ± 0,2
11	**0,5 ± 0,1**	**13.3 ± 4,0**	**34,1 ± 3,0**
12	15,5 ± 0,8	27,0 ± 3,0	29,0 ± 1,9
13	9,0 ± 4,3	12,0 ± 1,8	42,0 ± 3,3
14	7,0 ± 2,6	20,0 ± 1,0	30,3 ± 1,6
15	0,0 ± 0,0	0,0 ± 0,0	15,1 ± 1,4
Cytarine	18,6±2.0	29,8±1.9	43,6±2,5
Cisplatin	-	-	-
Control	0	0	0

As for the cytotoxically active quinolines, the most active derivative 1 at 100 μM inhibited the growth of 68.7% of HBL-100 cells, 35% of CCRF-CEM cells and 49% of healthy blood leukocytes. A 10-fold decrease in the concentration of the substance also led to a

decrease in its cytotoxicity: 46% death of HBL-100 cells, 25.5% of CCRF-CEM cells and 34% death of blood mononuclear cells. Compound 9, active on two lines at a concentration of 100 μM (41 and 60%), inhibited the growth of leukocytes only by 39%, which is one third lower than in cancer cells of the same nature. As for substances 7 and 11, which are active only against laryngeal adenocarcinoma cells - 58 and 54% of death, on healthy cells, the suppression on average turned out to be 33% lower and amounted to only 41 and 34%, respectively, compared with the control. It is interesting to note that the reference drug cytarin did not show selectivity in relation to the presented cell lines in the studied concentration range. Thus, using the MTT method, it was found that cytotoxically active quinolines exhibit less toxicity on healthy blood cells.

Conclusion

Among the tested dubamine derivatives, substances 1, 7, 9 and 11 were exhibited cytotoxic activity against cancer cells at a concentration of 100 μm. This means that cytotoxic activity depends on the structure of substances. The LD_{50} values of these substances were also found to be higher than the control drug.

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Conflict of Interest: No potential conflict of interest declared.

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