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RELATIONSHIP BETWEEN THE CHEMICAL STRUCTURES AND BIOLOGICAL ACTIVITIES (TOXICITY, MUTAGENIC AND ANTITUMOR) IN NEWLY SYNTHESIZED DERIVATIVES OF PYRAZOLO PYRIMIDINES

The relationship between chemical structure, micronucleus-inducing and antitumor activities was studied in four newly synthesized pyrazolo pyrymidine compounds (DGB-216, DGB-227, DGB-228 and DGB-331). In bone marrow erythrocytes of mice no one of compounds was active. Only DGB-216 has slight antitumor activity and increases the mean life span of mice with Ehrlich ascite carcinoma by 11 %, while others were practically non-active. Changes in the chemical structures of the compounds lead to substantial changes in the acute toxicity only.

The search of antitumor compounds among the derivatives of -6-etoxycarbonyl-pyrazolo[1,5a]-pyrymidine and -2-methyl-pyrazolo[1,5a]-pyrymidine is useless, as it has been shown in the present investigation. But the search of compounds with antitumor properties among derivatives of pyrazolo pyrymidines is a perspective idea because recently some very active antitumor compounds based on mentioned strusture were synthesized in Italy and the USA.

Introduction. The pyrimidine ring appears in the structure of some antitumor drugs such as antagonists of folic acid [1], Cytarabine (Cytosine Arabinoside), Fludara (2-Fluoroadenine arabinoside), Leustatin and Xeloda (Capecitabine) [2]. The pyrimidine ring is also found in some alkylating drugs (Dopane [1], used in Russia and NIS countries) and in the antineoplastic antibiotic (Bleomycin [2]). It therefore appears quite logical to develop new drugs based upon the pyrimidine backbone.

Widely used in clinical oncology Temodar (Temozolomide) for treatment of astrocytomas and glioblastomas, is an alkylating agent that contains a bridged nitrogen atom in its structure. The drug itself is inactive while its metabolism involves rapid non-enzymatic conversion into an active compound — 5-(3-methyltriazen-1-yl)-imidazole-4-carboxamide (MTIC). Cytotoxicity of MTIC is dependent on its alkylation of DNA, where positions O^6 and N^7 of guanidine are methylated [2]. A compound of similar structure to MTIC is the antineoplastic preparation DTIC-Dome (Dacarbazine), used in the treatment of metastatic malignant melanoma and Hodgkin's disease [1—2].

Danagulyan et al [3] for the first time synthesized condensed nitrogen-containing heterocyclic structures (pyrimidine) which combine five- and six-membered rings with a bridged nitrogen atom.

The aim of the present study was to evaluate for the first time the acute toxicity, micronucleus-(MN)-inducing and antitumor activity in mice of four of these newly synthesized compounds.

Materials and methods. ***Substances.*** Four substances synthesized by G. Danagulyan and co-workers were used in the study. The chemical structures of the compounds are presented in Fig. 1.

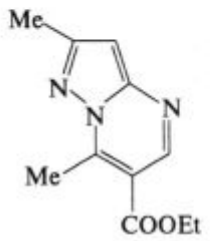
Animals. All animal experiments were approved by Ethic Commission of Ministry of Health of Armenia. The investigations were carried on Swiss albino mice (22—25 g) of both sexes obtained from Institute of Zoology, Yerevan, Armenia. Before the experiment, within two weeks, the animals were subjected to acclimatization. The temperature in the rooms made up 22 ± 2 °C, humidity 45—50 % ± ± 10 %, the artificial light regime 12:12. The animals were kept in polypropylene cages (5 animals of the same sex per cage). Drinking water and standard rodent chow were at mice disposal ad libitum.

The study of acute toxicity of the compounds. The acute toxicity and mean tolerated dose (MTD)

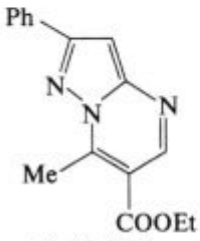
after single intraperitoineal (i. p.) injection of the substances was studied using approach of Lorke [4]. Seventy two mice were used for this experiment. The compounds were dissolved in 1 % suspension of starch and injected into mice (i. p.) at various doses.

Micronucleus assay in mouse bone marrow cells. The protocol described by Kirkhart [5] was used for evaluation of MN in mice which is routinely used in our laboratory [6, 7]. Each experimental group was consisted of 5 mice. All substances were administered to mice twice at 0 and 24 h at doses equal to $^1/_2$, $^1/_5$ and $^1/_{10}$ of LD50, and mice were sacrificed at 48 h. Using this protocol, we avoid to study MN-inducing effect at 24 h, 48 h and 72 h, because chemical agents can induce the maximum number of MN at one of mentioned time points. Application of Kirkhart's protocol can give the possibility to register MN-inducing effect of even weak mutagens and save time and animals. As positive control cyclophosphamide («Merck», Germany) was used as at a dose of 40 mg/kg (dissolved in 0.5 ml saline). It was injected i. p. once and mice were killed 24 h after. As negative control the solvent of the compounds was used — 1 % suspension of starch, 0.3 ml. Bone marrow was flushed by means of newborn calf serum (0.2 ml, «Sigma», USA) onto slides and smears were prepared. The slides were fixed with cold methanol for 20 min 24 h after the slides preparation. Slides were stained with Giemsa («Merck», Germany). After being stained, the slides were coded so that the reader was unaware of the identity of slides being scored. Each slide was assessed for MN in 2,000 polychromatic erythrocytes (PCEs). In addition, the per cent content of PCEs was calculated among 1,000 erythrocytes.

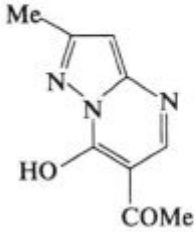
Antitumor activity of the compounds. The primary estimation of antitumor activity was carried out on a model of Ehrlich's ascitic carcinoma widely used for primary screening of new substances [8, 9]. Mice were injected i.p. with 2 • 106 tumor cells. The compounds were injected into animals i. p. at a dose equal to $^1/_2$ of the MTD 24 h after the inoculation of tumor cells, for six consecutive days. The mice of the control group received only saline 6 times. As positive control cisplatin («Sigma», USA) at the same schedule was used at dose of 1.6 mg/kg [8]. As evaluation criterion of therapeutic efficiency was an increase of the animals' life span



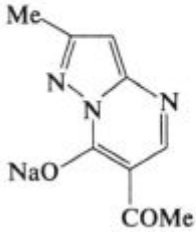
DGB-216



$C_{15}H_{15}N_3O_2$
Mol. Wt.: 281.31
DGD-231



$C_9H_9N_3O_2$
Mol. Wt.: 191.19
DGB-227



$C_9H_8N_3NaO_2$
Mol. Wt.: 213.17
DGB-28

Chemical structure of four newly synthesized compounds

in comparison with the control, and the increase by more than 25 % was considered as effective [1, 8]. The compounds for injections were prepared ex tempore and were injected to animals i. p. in the form of suspension in 1 % solution of starch glue. At least 6 mice of both sexes was in each group. The experiment was repeated using the same protocol.

Statistical analysis was performed by means of Mann-Whitney U-test (GraphPad Prism, version 3.02).

Results and discussion. The data concerning the acute toxicity and MTD of four compounds are presented in Table 1. Among the studied compounds according to LD50 three of them, namely DGB-216, DGB-227 and DGB-228 are slightly

Table 1

Acute toxicity (LD100 and LD50) and mean tolerated doses of studied compounds

Compounds	LD_{100}	LD_{50}	MTD
	mg/kg		
DGB-216	1000	510	260
DGB-227	470	329	230
DGB-228	960	650	440
DGB-231	1600	1050	700

Table 2

Micronucleus frequencies induced by four newly synthesized compounds in bone marrow cells of mice

Group of mice	Dose, mg/kg	PCEs with MN ‰	Number of MN per 1000 PCEs	Number of PCEs among 1000 erythrocytes
		(mean ±S.E.)		
DGB-216	265.0	2.2 ± 0.8	2.2 ± 0.8	52.2 ± 1.2
	100.0	1.6 ± 0.3	1.6 ± 0.3	52.8 ± 1.2
	53.0	1.6 ± 0.4	1.6 ± 0.4	54.5 ± 0.9
DGB-227	160.0	2.6 ± 0.8	2.6 ± 0.8	50.2 ± 1.6
	64.0	2.0 ± 0.3	2.0 ± 0.3	54.4 ± 1.5
	33.0	2.0 ± 0.4	2.0 ± 0.4	55.4 ± 1.2
DGS-228	325.0	2.2 ± 0.4	2.2 ± 0.4	55.2 ± 1.2
	130.0	2.2 ± 0.4	2.2 ± 0.4	53.6 ± 1.8
	65.0	1.8 ± 0.6	1.8 ± 0.6	55.4 ± 1.2
DGS-231	525.0	1.8 ± 0.2	1.8 ± 0.2	56.0 ± 1.4
	210.0	1.8 ± 0.2	1.8 ± 0.2	55.2 ± 1.2
	105.0	1.6 ± 0.4	1.6 ± 0.4	53.6 ± 1.3
Negative controlstarch	—	1.6 ± 0.3	1.6 ± 0.3	55.2 ± 1.2
Positive controlcyclophosphamide	40	36.0 ± 4.8 *	36.0 ± 4.8 *	51.6 ± 1.2

* $p < 0.001$ Mann Whitney U-test with Gaussian approximation; each group consisted of 5 mice.

Table 3

Antitumor activity of three newly synthesized compounds against Ehrlich ascitic carcinoma

Compound (number of mice under study)	Range of survival time, days	Mean survival time, days (mean ± S.D.)	Increase in life span, %
DGB-216 ($n = 10$)	14—18	16.0 ± 1.4	11.3
DGB-227 ($n = 10$)	13—16	14.3 ± 1.0	0
DGS-228 ($n = 10$)	12—17	14.6 ± 1.2	0
DGS-231 ($n = 10$)	12—16	14.2 ± 1.0	0
Positive control ($n = 10$)	16—28	23.5 ± 0.9*	63.2
Negative control ($n = 10$)	13—16	14.4 ± 1.1	—

$P < 0.001$ (Mann-Whitney U-test with Gaussian approximation).

toxic (LD_{50} is between 101 and 1000 mg/kg), and DGB-331 practically non-toxic (LD_{50} is more than 1000 mg/kg) [10].

The MN-inducing activity of the compounds is presented in Table 2. None of them induced any change either in the number of MN or in the number of PCEs compared with negative control. Cyclophosphamide was active and induced 36.0 ‰ of PCEs with MN, the datum very close to our historical controls [6, 7]. No one of the compounds was toxic at used doses for bone marrow cells because no change in the number of PCEs to NCEs was observed.

The study of antitumor activity of the compounds showed that DGB-227, DGS-228 and DGS-331 were completely non-active, because they practically did not change the mean life span of the mice. Only DGB-216 was a little active, and prolonged the MLS of mice by 11 %, which is much more less than critical 25.0 % needed to consider the compound active regarding its antitumor activity.

The data concerning the relationship between the chemical structure and biological activity of four newly synthesized compounds are presented in Table 4. Replace of methyl- group in position 2 to phenyl- one decrease the acute toxicity of DGB-231 2-fold compared with related compound DGB-216. At the same time antitumor activity of it also decreased significantly (from 11 % to 0). Both compounds have no MN-inducing activity.

Addition of Na to the chemical structure of DGB-227 increased the acute toxicity of DGB-228 2-fold without the influence of MN-inducing and antitumor activity (no activities in both studies). Actually, among the compounds studied, only DGB-216 has a weak antitumor activity. Changes in the chemical structures of the compounds lead only to substantial changes in the acute toxicity (2-fold) without the influence on mutagenic activity. The most toxic compound among studied ones, DGB-227 possesses neither mutagenic nor antitumor activities. It is well known that usually any changes in chemical structure of a substance may lead to substantial changes in the biological properties (toxicity, mutagenicity, antitumoe activity). The best examples are methyl and ethyl alcohols, and cis- and trans-platin. The first alcohol is extremely toxic while the second one is widely used as a component of many beverages. Cis-platin is a potent antitumor drug with high toxic and mutagenic potency, while the second platin compound possesses substantially less antitumor activity, toxicity and mutagenicity [13]. In addition, the first one is soluble in water and normal

Table 4

Relationship between the chemical structure and biological activity of four newly synthesized compounds

Compounds	LD_{50}, mg/kg	Main chemical structure	Radical	Radical	Radical	MN activity	Anti-tumoractivity
DGB-216	510	-6-etoxycarbonyl-pyrazolo [1,5a]-pyrymidine	7- methyl-	-2-methyl	—	None	Very weak
DGB-231	1050	-6-etoxycarbonyl-pyrazolo [1,5a]-pyrymidine	7- methyl-	2-phenyl	—	None	None
DGB-227	329	-2-methyl-pyrazolo[1,5a]-pyrymidine	6-acethyl-	-7-hydroxy-	—	None	None
DGB-228	650	-2-methyl-pyrazolo[1,5a]-pyrymidine	6-acethyl-	-7-hydroxy-	Sodium salt	None	None

saline, while the second one is soluble only in DMSO. But the only difference between two compounds is the position of Pt (cis- and trans-). Unlike mentioned, even substantial changes in chemical structure of studied compounds lead only to changes in toxicity of them.

Hence, changes of chemical structure carried out in this investigation did not influence substantially any biological activity of the compounds, except the acute toxicity. In our opinion, the search of antitumor compounds among the derivatives of -6-etoxycarbonyl-pyrazolo[1,5a]-pyrymidine and -2-methyl-pyrazolo[1,5a]-pyrymidine is useless. But the search of compounds with antitumor properties among derivatives of pyrazolo pyrymidines is a perspective idea because recently some very active antitumor compounds based on pyrazolo pyrymidines were synthesized in Italy and the USA (but with much longer radicals with addition of -Cl and -Br in the structure).

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РЕЗЮМЕ. Изучена зависимость между химической структурой, способностью индуцировать микроядра и противоопухолевым действием у четырех новосинтезированных производных пиразолопиримидинов DGB-216, DGB-227, DGB-228 и DGB-331. В эритроцитах костного мозга мышей ни одно из соединений не было активно. Только соединение DGB-216 удлинило среднюю продолжительность жизни мышей с асцитным раком Эрлиха на 11 %, в то время как другие были практически неактивны. Изменения в химической структуре соединений приводили лишь к изменению острой токсичности. Поиск противоопухолевых соединений среди производных -6-этоксикарбонил-пиразоло[1,5a]-пиримидинов и 2-метил-пиразоло [1,5a]-пиримидинов бесперспективен, как было показано в настоящем исследовании. Однако поиск активных соединений, основанных на пиразоло пиримидинах может быть успешным, так как в последнее время в США и Италии синтезированы очень перспективные соединения на их основе.

РЕЗЮМЕ. Досліджували залежність між хімічною структурою, здатністю індукувати мікроядра та протипухлинною дією у чотирьох новосинтезованих похідних піразолопіримідинів DGB-216, DGB-227, DGB-228 та DGB-331. В еритроцитах кісткового мозку мишей жодне з сполучень не було активним. Тільки сполучення DGB-216 збільшило середню тривалість життя мишей з асцитим раком Ерліха на 11 %, в той час як інші були практично неактивні. Зміни в хімічній структурі сполучень призводили до змінювання гострої токсичності. Пошук протипухлинних сполучень серед похідних -6-етоксикарбоніл-піразоло[1,5a]-піримідинів та 2-метил-піразоло[1,5a]-піримідинів не має перспективи, що було показано у даному дослідженні. Однак пошук активних сполучень, що засновані на піразолопіримідинах, може бути успішним, бо останнім часом у США та Італії синтезовані дуже перспективні сполучення на їх основі.

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