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ANTIMONOAMINOOXIDASE AND ANTITUMOR ACTIVITY OF HYBRID MOLECULES BASED ON INDOLE

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The effect of substituted spirooxoindolines on the activity of monoamine oxidase (MAO) in bovine brain *in vitro* was studied. Serotonin (5-hydroxytryptamine, 5-OT) creatinine sulfate was used as a substrate. Studies of antimonoamine oxidase activity have shown that most compounds of this class have a pronounced inhibitory effect on 5-OT deamination, inhibiting MAO activity within 50-75%, while the two compounds have an opposite effect on the enzyme, activating MAO by 6 and 12%. A derivative with a dimethylcyclohexanone fragment exhibited weak antitumor activity in a 180 mouse sarcoma model.

Spirooxoindolines – bovine brain monoamine oxidase – antimonoamine oxidase activity – activating effect - antitumor effect.

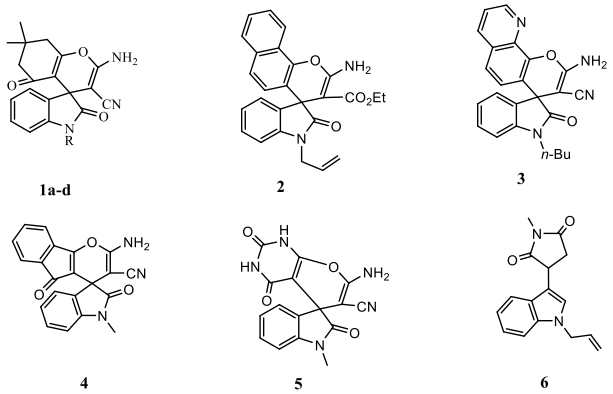
Ուսումնասիրվել է տեղակալված սպիրոօքսոինդոլինների ազդեցությունը ցուլի ուղեղի մոնոամինօքսիդազի /ՄԱՕ/ ակտիվության վրա *in vitro* պայմաններում: Որպես սուբստրատ օգտագործվել է սերոտոնին (5-OT) կրեատին սուլֆատը: Ամինոմո-նորօքսիդազային ակտիվության ուսումնասիրությունը ցույց է տվել, որ նշված դասի միացությունների մեծ մասը ցուցաբերում է արտահայտված ճնշող ազդեցություն 5-OT-ի դեզամինացման վրա՝ արգելակելով ՄԱՕ-ի ակտիվությունը 50-75 տոկոսով, միաժամանակ երկու միացություններ ցուցաբերում են հակառակ ազդեցություն ֆերմենտի վրա՝ ակտիվացնելով ՄԱՕ-ն 6 և 12 տոկոսով: Դիմեթիլցիկլոհեքսանոնի հատված պարունակող ածանցյալները ցուցաբերում են թույլ հակաուռուցքային ազդեցություն մկների 180 սարկոմայի մոդելի վրա:

Սպիրոօքսոինդոլիններ – ցուլի ուղեղի մոնոամինօքսիդազա – հակամոնոամինօքսիդազային ակտիվություն – ակտիվացնող ազդեցություն – հակաուռուցքային ազդեցություն

Исследовано влияние замещенных спирооксоиндолинов на активность моноаминооксидазы (МАО) бычьего мозга *in vitro* . В качестве субстрата использовали серотонин (5-окситриптамин, 5-ОТ) креатинин сульфат. Исследования антимоноаминооксидазной активности показали, что большинство соединений указанного класса оказывают выраженное угнетающее влияние на дезаминирование 5-ОТ, тормозя активность МАО в пределах 50-75 %, в то же время два соединения оказывают противоположное влияние на фермент, активируя МАО на 6 и 12 %. Производное с фрагментом диметилциклогексанона проявило слабую противоопухолевую активность на модели саркомы 180 мышей.

Спирооксоиндолины – моноаминоксидаза бычьего мозга – антимоноаминооксизная активность – активирующее действие – противоопухолевое действие

Substituted indoles exhibit a wide range of biological activities, including antibacterial, antiviral, anti-inflammatory, analgesic, anticancer, etc. [3]. Earlier, we have synthesized a number of hybrid spiroindolines, in the molecule of which, in addition to the indole ring system, fragments of other ring systems are present [4, 5]. In this communication, we present the results of studies on the antimonoamineoxidase and antitumor properties of a small library of spiroindolines of the above type **1a-d** -**5** and indoline **6** and made an attempt to correlate the biological activity of compounds with the calculated parameters of the main physicochemical descriptors.



1a-d **2** **3**

4 **5** **6**

1a-e: R = CH_3 (**a**), C_3H_7 (**b**), C_4H_9 (**c**), CH_2Ph (**d**).

***Materials and methods*.** The source of monoamine oxidase (MAO) was a 50% bovine brain homogenate, which was obtained by homogenizing the brain in a glass homogenizer with an equal (by weight) volume of 2.5 % Arkopal solution. In the resulting 50 % homogenate, MAO activity was determined. Experimental samples contained 0.2 ml of homogenate, 0.18 ml of a solution of the test compound and 0.18 ml of a substrate solution [2]. The sample volume was adjusted to 1.8 ml with 0.1 M K-Na-phosphate buffer to pH 7.4. Serotonin (5-OT) creatinine sulfate monohydrate was used as a substrate, which was added to the samples after 30 minutes of preincubation of the enzyme with the test substance at room temperature. The serotonin content in the sample is 1 μmol / sample. Oxygen saturation was carried out for 5 min at 37^0C, and then the samples were incubated for 45 min at 37^0C in an oxygen atmosphere. The reaction was stopped by adding 0.2 ml of 50 % trichloroacetic acid. The protein precipitate was separated by centrifugation at 3000 rpm. In the protein-free supernatant, the ammonia content was determined by isometric distillation for 24 h, followed by non-serration and photometry using an FEK-56-2 nephelometer photometer. MAO activity is expressed as % of control. Data on the antimonoamine oxidase and anticancer activity of the compounds are presented in the table.

The acute toxicity and antitumor activity of the compounds was studied according to the generally accepted method [1]. The toxicity of the compounds was determined on white outbred mice of both sexes weighing 20-22 g with a single intraperitoneal injection. The study of antitumor activity was carried out on mice with a transplantable tumor of sarcoma 180. In chemotherapeutic experiments, the compounds were administered intraperitoneally daily for 6 days at doses equal to 1/15 of the LD100.

The experiments were carried out in full compliance with the European Convention and the directives of the European Parliament for the protection of vertebrate animals used for experimental and other scientific purposes. (Strausbourg No123. 18.03.1986. Directive 2010/63 / EU of the European Parlament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. Statistical processing of the results was carried out according to the Student-Fisher method.

Molecular descriptors were obtained using the computer program ACD / ChemSketch (version ACD / Labs 6.00).

Results and Discussion. Studies of the antimonoamine oxidase activity of the studied compounds showed that, in a series of derivatives with a cyclohexanone fragment **1a-c**, compounds with a methyl, butyl, and benzyl substituent in position 1 of the isatin fragment (compounds **1a**, **b**, **d**) have a pronounced inhibitory effect on 5-OT deamination, inhibiting enzyme activity in the range of 70-75%. At the same time, derivative **1b** with a propyl substituent under the same conditions exhibits an MAO-activating effect, increasing the enzyme activity by 6% (tab.1).

Table 1. Antimonoamine oxidase and antitumor activities of compounds **1a-d**, **2-6** and the values of the calculated physicochemical descriptors

Com-pounds	MAO*	p	T, %	logP	MR*	P*	MV	ST	IR
1a	75	$\leqslant 0.05$	38.5	5.35±0.75	93.79	37.18	252.9	66.8	1.663
1b	+6	$\leqslant 0.05$	_	6.42±0.75	103.05	40.85	285.2	62.9	1.642
1c	70	$\leqslant 0.05$	_	6.95±0.75	107.68	42.68	301.4	61.3	1.633
1d	72	$\leqslant 0.05$	_	7.15±0.75	118.50	46.97	311.5	68.7	1.685
2	20	$\leqslant 0.05$	_	6.46±0.64	121.68	48.23	312.5	66.7	1.706
3	52	$\leqslant 0.05$	_	5.29±0.75	113.16	44.86	284.7	76.9	1.725
4	24	$\leqslant 0.05$	_	6.86±0.79	95.33	37.79	230.0	85.0	1.767
5	32	$\leqslant 0.05$	0	3.45±0.75	82.68	32.77	199.7	98.0	1.766
6	_	$\leqslant 0.05$	0	0.94±0.63	77.88	30.87	219.0	46.5	1.629
индопан	86±6	$\leqslant 0.05$		1.73±0.22	56.20	22.28	155.2	50.3	1.643

MR* (Molar Refractivity): N ± 0.5sm^3; P*(Polarizability): N ± 0.5 10^{-24} sm^3;
MV* (Molar Volume): N ± 7.0 sm^3; ST*(Surface Tension): N ± 0.07 dyne/sm; IR* (Index of Refraction): N ± 0,05

* Concentration of compounds – 1 μmol / ml sample, substrate - serotonin (5-OT). Over 100% the intensity of serotonin deamination in control samples is taken.
MR * (Molar Refractivity): N ± 0.5 sm^3; P* (Polarizability): N ± 0.5 10^{-24} cm^3;
MV * (Molar Volume): N ± 7.0 cm^3; ST* (Surface Tension): N ± 0.07 dyne / sm;
IR * (Index of Refraction): N ± 0.05;

This result seems unexpected, since the electronic characteristics and the size of the substituent propyl group in compound **1b**, and hence the entire molecule, are not significantly different from other derivatives of this series, in particular from the butyl derivative **1c**. This is also confirmed by a comparison of the main molecular descriptors of compounds **1a-c**, the values of which for compounds **1b** and **1c** are quite close. Other compounds of a series of spiro-fused indolines **2,4,5** with fragments of naphthalene, inden-1-one and pyrimidine, respectively, are much weaker MAO inhibitors, inhibiting the enzyme activity within 20-32 %, and the activity of the derivative with a quinoline fragment is slightly higher - 52%. There is no clear relationship between the antimonoamine oxidase activity and the values of the main physicochemical descriptors in the series of substituted spiroindolines. We can only note a tendency towards a slight increase in the refractive index (IR) of compounds (1.6 for compounds **1a**, **c**, **d**, 1.7 for compounds **2-5**), which correlates with a decrease in anti-MAO properties.

At the same time, the studied compounds are significantly inferior to the control drug furazolidone in terms of anti-MAO activity (inhibition of the enzyme by 86%).

When studying the acute toxicity of compounds **1a**, **5**, **6**, it was found that the lethal dose (LD_{100}) for compound **5** is 1500 mg / kg, and for compounds **1a**, **6** - 2000 mg / kg. Chemotherapy experiments showed that compound **1a** with a cyclohexanone fragment exhibits weak antitumor activity, inhibiting the growth of sarcoma 180 by 38.5%.

Spiroindoline **5** with pyrimidine fragments and pyrrolidine-2,5-dione derivative **6** are completely devoid of antitumor action.

Thus, as a result of studies of the antimonoamine oxidase activity of spiroindolines of various structures, it was shown for the first time that derivatives of this class of compounds with cyclohexanone fragments exhibit pronounced anti-MAO activity, and the compound with a methyl radical, along with inhibition of MAO activity by 75%, also exhibits a weak antitumor effect. In the investigated series, the compound with the propyl radical unexpectedly showed a small MAO-activating effect. Other derivatives of the studied series with fragments of naphthalene, indene-1-one, pyrimidine, and quinoline exhibited weak anti-MAO activity. When comparing the values of physicochemical descriptors of 7 studied compounds, with one exception, a negative correlation was revealed between the values of anti-MAO activity and the refractive index (IR).

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