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ACUTE TOXICITY OF SEVERAL 6-AMINOETHIL-6H-INDOLO [2,3-B]QUINOXALINE DERIVATIVES

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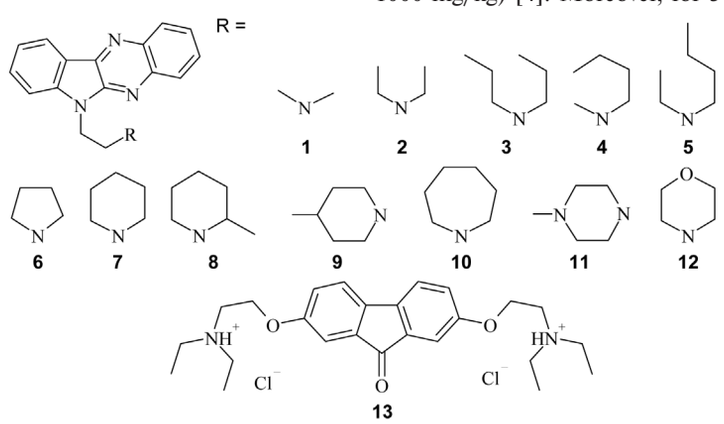
Earlier [1] we have synthesized 6-aminoethil-6H-indolo[2,3-b]quinoxalines 1 — 12 and shown their high antiviral and interferon-inducing activity. Chemotherapeutical indices of the most active substances 9 and 12, under therapeutical application in vitro [2], are 1783 and 1258, respectively, which markedly exceeds chemotherapeutical index of the reputed drug "Amixin" (13), that has a similar mechanism of action (Table 1). To evaluate expediency of further investigation, it was necessary to determine their toxicity in vivo.



Acute toxicity evaluation of compounds 1 — 12 was carried out on white outbred mice weighting 19.5 — 29.5g, received from vivarium of Odesa National Medical University. Hydrochlorides of tested substances, taking into account there low solubility in water (an average of 1 g. per 250 sm^3 H_2O), were injected intraperitoneally in the form of tween suspense. Toxic effect was studied according to Deichmann and LeBlanc [3] — one of the methods, recommended for preliminary assessment on the stage of screening research [4, p. 89]. Toxicity values, received from mice, are presented in Table 2. It should be noted, that the studied compounds widely differ in their toxic effects.

All substances can be characterized as low toxic ones (100 mg/kg < LD_{50} < 1000 mg/kg) [4]. Moreover, for 5 sub-

Table 1

Biological properties of 6-aminoethil-6H-indolo[2,3-b]quinoxalines

	-lg CC50 ±ε		-lg IC50 ±ε				T IFN		CTI			
	L929	EPT	L929		EPT		L929	EPT	L929		EPT	
			TS	PS	TS	PS			TS	PS	TS	PS
1	4.04±0.05	4.09±4.09	4.95±0.08	5.84±0.12	5.02±0.06	5.84±0.12	16	32	58	63	9	56
2	3.19±0.03	3.34±3.34	5.66±0.05	5.89±0.05	5.21±0.04	5.47±0.04	64	32	79	499	76	139
3	3.66±0.05	3.83±3.83	5.45±0.12	5.89±0.06	5.18±0.10	5.57±0.06	8	32	158	169	23	55
4	3.25±0.06	3.69±0.07	5.63±0.07	5.84±0.09	5.32±0.13	5.52±0.11	16	32	41	388	43	68
5	3.76±0.04	4.08±0.09	5.47±0.05	5.50±0.10	4.88±0.04	4.88±0.10	8	8	27	54	6	6
6	4.12±0.06	3.79±0.06	5.65±0.04	5.88±0.05	5.39±0.02	5.57±0.06	32	64	468	59	40	60
7	3.75±0.03	3.03±0.02	5.59±0.06	5.87±0.03	5.25±0.09	5.54±0.03	64	32	224	132	164	323
8	3.63±0.04	3.40±0.03	5.72±0.03	5.90±0.07	5.39±0.05	5.58±0.07	32	32	74	185	98	149
9	3.24±0.05	2.55a	5.64±0.11	5.79±0.09	4.70±0.11	5.80±0.10	8	32	78	358	142	1783
10	3.84±0.09	3.88±0.05	5.42±0.09	5.89±0.06	5.10±0.12	5.57±0.05	64	32	62	110	17	49
11	4.01±0.02	4.09±0.03	5.32±0.09	5.86±0.10	5.33±0.04	5.84±0.10	4	32	46	71	17	57
12	2.97 [a]	2.69 [a]	5.35±0.10	5.79±0.13	— [b]	5.79±0.13	8	16	17	659	—	1258
13	3.95±0.03	3.97±0.04	4.86±0.06	5.84±0.08	4.87±0.06	5.88±0.08	32	32	10	80	10	80

L929 — mice fibroblast culture; EPT — embryonic pig testicles;
lg CC_{50} — concentration logarithm (M) of the studied substance, that leads to 50 % cell monolayer destruction;
a — determination of lg LC_{50} was not possible, because in the studied concentration range the chemical did not cause 50% monolayer destruction; extrapolated data are presented;
lg IC_{50} — concentration logarithm (M) of the studied substance, that inhibits viral cytopathetic effect by 50%;
TS — therapeutic scheme, the studied substances were added to the cell monolayer straight after virus introduction;
PS — prophylactic scheme, the studied substances were added 24 hours before virus introduction;
b — determination of lg IC_{50} was not possible, in the studied concentration range the chemical inhibited viral cytopathetic effect by less than 50%;
ε — value of the confidence interval, calculated for $P < 0.05$;
T IFN — titre of induced interferon, the maximal dilution of interferon-containing culture medium that is able to prevent viral cytopathetic action.
CTI — chemotherapeutic index, calculated as a ratio CC_{50}/ IC_{50}.

Table 2

Toxicity of substances 1 — 12

Substance	LD_{50}, mg/kg*	LD_{50}, μM/kg	-lg LD^{50}, M	ClogP
1	250	690	3.16	3.33
2	437	1120	2.95	4.17
3	937	2230	2.65	4.89
4	> 1000	> 2470	2.60	4.47
5	812.5	1940	2.71	4.89
6	562.5	1450	2.84	3.83
7	687.5	1700	3.16	4.19
8	> 1000	> 2400	2.62	4.65
9	> 1000	> 2400	2.62	4.65
10	> 1000	> 2400	2.62	4.55
11	187.5	450	3.35	2.97
12	> 1000	> 2470	2.60	2.92
13	150	310		

* confidence interval is ± 30 mg/kg ($P < 0.05$)

stances LD_{50} rates were not determined, because even the highest concentrations of the tested chemicals did not lead to animals' death. Dimethylamino- and 4-methylpiperazine derivatives (1 and 11, respectively) turned out to possess the greatest toxic effect.

Comparison of acute toxicity and cytotoxicity of the substances 1 — 12 in cell cultures L929 (Fig. 1) and EPT (Fig. 2) revealed a very low correlation between two parameters.

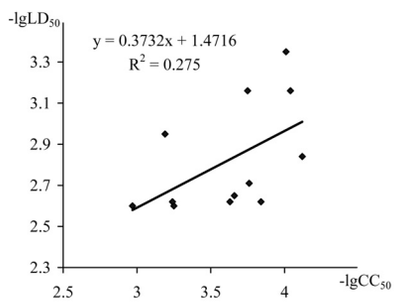


Figure 1. Poor correlation between toxicity and cytotoxicity in cell culture L929 for substances 1 – 12

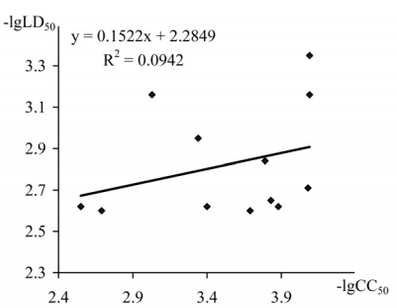


Figure 2. Poor correlation between toxicity and cytotoxicity in cell culture EPT for substances 1 – 12

Lipophilicity as logD (logarithm of the substance distribution index between n-octanol — this organic solvent is considered to modulate properties of biological membranes better than any other — and isotonic phosphate buffer, pH =7.4) is one among the fundamental physicochemical characteristics of the compounds regarding their effect on the biological properties. This parameter was first suggested and used by Hansch in his pioneer QSAR-studies [5]. Since direct experimental investigation of distribution indexes requires high labor expenditure, a number of parameters were proposed for QSAR-studies, all of which are connected with lipophilicity — chromatographic mobility of the chemicals in case of high-performance reversed-phase chromatography and paper partition chromatography, calculating methods using empirical substituent increments, etc. In the modern literature calculated lipophilicity of unionized substances (ClogP) is mentioned most frequently, which is also used as an important criteria for Lipinski's Rule of five 5 [6]. This molecular descriptor, which can be rather precisely calculated, does not coincide with logD. However the latter, with consideration for basicity of compounds, is directly linked with ClogP. Thus, exactly this parameter — ClogP — was chosen by us to evaluate the influence studied substances' structure on their acute toxicity. A great multitude of software packages and the Internet resources provides the opportunity to evaluate this parameter, and we chose a free Internet service, available at website [7].

Values of ClogP, calculated for substances 1 — 12 by means of the above mentioned resource, are presented in Table 2.

While depicting dependency for determined LD_{50} and calculated ClogP values from Table 2 for compounds 1 — 12, a linear dependency was revealed with correlation index r = — 0.93 (Fig. 3), provided that two points, representing substances 7 and 12, are excluded from the sample. The fact that 2 out of 12 points fell out as a result of approximation should not be considered as wrong model. Such instances are usual in QSAR when basic models are used. Moreover, Hansch stresses out the informative importance of these observations, which could indicate a change in mechanism of action of those chemicals, which are not consistent with the common tendency [8].

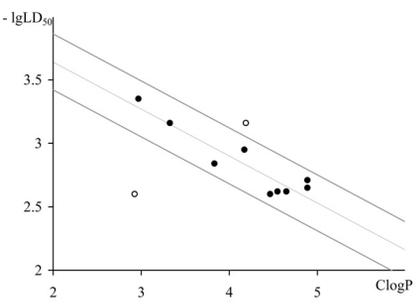


Figure 3. Dependency between lipophilicity and toxicity for substances 1 – 12
Analytical form and statistical parameters of regression:
- $lg(LD_{50})$ = 4.38 (± 0.5) – 0.37 (± 0.12) x lgP
r^2 = 0.87; r = – 0.93; n = 10;
S^2r = 3.416; S^2y = 0.066; F = 51.75;
P < 0.01

Thus, a significant and reliable reverse proportional dependency between acute toxicity and lipophilicity is observed for compounds 1 — 12, which indicates greater potential perspectives for lipophilic chemicals. On the other hand, a relatively small sample and falling out of two substances from the mentioned tendency are a premise to check model's robustness by means of new synthesis and testing new compounds.

Further investigations would require more complex and diverse testing, carried out both in vitro and in vivo, such as: chronic toxicity and mutagenic effect, chemical's distribu-

tion in organs; antitumor, immunomodulating and direct antiviral action, detailed cytokine analyses, etc.

Our final goal is to find potential preparations with stronger therapeutic characteristics than those of current drugs. Initial data for at least some of the substances are very promising, and the necessity for subsequent research is obvious.

ЛИТЕРАТУРА

1. Патент України №31885, C07D213/00, C07C209/00./Шибінська М.О., Ляхов С.А., Андронаті С.А., Жолобак Н.М., Співак М.Я. 6-Аміноетил-6Н-індоло[2,3-b]хіноксаліни як противірусні агенти та індуктори інтерферонів. Заявка № u200714028 від 13.12.2007. Опубл. 25.04.2008. Бюл. № 8.
2. Вивчення антивірусної дії потенційних лікарських засобів/ [Щербінська А.М., Дяченко Н.С., Рибалко С.Л., Носач Л.М. та ін.] // Доклінічні дослідження медичних засобів: Метод. рек. за ред. О.В.Стефанова. — К.: Авіценна, 2001. — С. 371-395. — 527 с.
3. Deichmann W.B., LeBlanc T.J. Determination of the approximate lethal dose with about six animals // J. Indust. Hygiene Toxicol. — 1943. — Vol. 25. — P. 415 — 417.
4. Вивчення специфічної активності протипухлинних засобів/ [Шарикіна Н.І., Шляховенко В.О., Мосієнко В.С., та ін.] // Доклінічні дослідження медичних засобів: Метод. рек. [за ред. О.В. Стефанова] — К.: Авіцена, 2001. — 365 с. — 527 с.
5. Kubinyi, Hugo: QSAR: Hansch analysis and related approaches / by Hugo Kubinyi. — Weinheim; New York; Basil; Cambridge; Tokyo: VCH, 1993 (Methods and principles in medicinal chemistry ; Vol. I) — 240 p.
6. The Practice of Medicinal Chemistry 2nd Ed Edited by C. G. Wermuth (University of Louis Pasteur). — Academic Press, San Diego, CA. — 2003. — 768 p.
7. Electronic resource http://intro.bio.umb.edu/111-12/OLLM/111F98/jlogp/test.html
8. Verma R.P.Hansch C. An approach toward the problem of outliers in QSAR // Bioorg. Med. Chem. — 2005. — V. 13, № 15. — P. 4597 — 4621.

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ГОСТРА ТОКСИЧНІСТЬ ПОХІДНИХ 6-АМІНОЕТИЛ-6Н-ІНДОЛО[2,3-в]ХІНОКСАЛІНУ

Новосинтезовані похідні 6-аміноетил-6Н-індоло[2,3-b]хіноксаліну є ефективними противірусними агентами та індукторами інтерферону в умовах in vitro. Для оцінки доцільності їх подальшого вивчення була досліджена їх токсичність in vivo. Виявлена значуща достовірна зворотна залежність гострої токсичності від ліпофільності сполук. Наступні дослідження передбачають вивчення хронічної токсичності та мутагенності досліджених похідних аміноетиліндолохіноксаліну, їх протипухлинної, імуномодулюючої та антивірусної дії.

Ключові слова: токсичність гостра, аміноетиліндолохіноксаліну похідні, індуктори, ліпофільність, протипухлинний, імуномоделюючий, антивірусний.

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ОСТРАЯ ТОКСИЧНОСТЬ ПРОИЗВОДНЫХ 6-АМИНОЭТИЛ-6Н-ИНДОЛО[2,3-в]ХИНОКСАЛИНА

Новосинтезированные производные 6-аминоэтил-6Н-индоло[2,3-b]хиноксалина — эффективные противовирусные агенты и индукторы интерферона в условиях in vitro. Для оценки целесообразности их дальнейшего изучения была исследована токсичность соединений in vivo. Обнаружена существенная достоверная обратная зависимость острой токсичности от липофильности исследованных производных аминоэтилиндолохиноксалина. Последующие исследования предусматривают изучение хронической токсичности, мутагенности соединений, их противоопухолевого, иммуномодулирующего и антивирусного действия.

Ключевые слова: токсичность острая, аминоэтилиндолохиноксалина производные, индукторы, липофильность, противоопухолевый, иммуномоделирующий, антивирусный.