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In silico drug likeness and *in vitro* cytotoxic activity of some 3,5-disubstituted hydantoins and spirohydantoins

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Dedicated to Acad. Ivan Juchnovski on the occasion of his 80th birthday

The study presents the comparative evaluation of drug-like properties of six hydantoin and spirohydantoin derivatives. Some of the compounds were designed by replacement of the hydrogen atom from NH-3(hydantoin ring) with amine group, namely 3-amino-α-tetralonespiro-5'-hydantoin, 3-amino-2-indanespiro-5'-hydantoin, 3-amino-5-methyl-5-phenylhydantoin, 3-amino-5-methyl-5-(4-pyridyl)hydantoin. The other two compounds were spirohydantoins with S-containing cycloalkane rings - 3-thiolanespiro-5'-hydantoin and 4-thio-1H-tetrahydropyranspiro-5'-hydantoin. The drug applicable properties of the reported organic compounds, essential for drug pharmacokinetics in the human body, were estimated with the Lipinski's rule of five. The value of LogP and the residual parameters of drug likeness were estimated with the method based on group contributions. The approach is used only as an initial step in drug discovery, to find the main candidates with heartening properties for further amplification. Some of the compounds were further tested for *in vitro* cytotoxicity on four human tumor cell lines SKW-3, HL-60, LAMA-84 and EJ. The tested spirohydantoins exerted concentration dependent cytotoxic activity on all human tumor cell lines. The most significant cytotoxicity was observed for 4-thio-1H-tetrahydropyranspiro-5'-hydantoin which inhibited the viability of tested cells at low micromolar concentrations.

Key words: *in silico* evaluation; LogP values; Lipinski's rule; cytotoxic activity

INTRODUCTION

Hydantoin derivatives possess a variety of pharmacological properties. Depending on the nature of the substituents in the hydantoin ring they exhibited fungicidal, herbicidal, antitumor, anti-inflammatory, anti-HIV, antiarrhythmic, and antihypertensive activities [1-7]. Hydantoins like 5-hydroxyhydantoin and 5-methyl-5-hydroxyhydantoin serve as blocking lesions for DNA polymerases [8]. 5-(2-Phenyl-3-indolal)-2-thiohydantoin have shown inhibitory activity on several cancer lines organized into subpanels representing leukemia, melanoma, and cancer of lung, colon, kidney, ovary, breast, prostate and central nervous system by the National Cancer Institute anti-cancer drug screening program [9]. Some spirohydantoin derivatives showed antimicrobial, antifungal, anti-inflammatory, antidiabetic, antiepileptic, antiproliferative activities and can act as new psychotropic agents (antidepressants, anxiolytics and antipsychotics) [10].

Modification of known bioactive structures by including many active groups and substituents is widely used approach in discovery of new potential drugs. As a result, the newly synthesized compounds tend to have higher molecular weight, high lipophilicity and low aqueous solubility which results in poor bioavailability. Another disadvantage in the development of novel "drug-like" compounds is the huge number of required *in vitro* and *in vivo* examinations. Through the last years, a lot of *in silico* methods were discovered which significantly reduce the number of *in vivo* studies required [11]. The *in silico* design allows the screening of compounds against potential targets and determines the most promising ones with applicable molecular weight, lipophilicity, hydrogen bond donors/acceptors, solubility, and other related properties. The lipophilicity is the main characteristic, affecting the membrane permeability and oral bioavailability of the compounds. An accepted measure of lipophilicity is LogP and compounds demonstrating LogP > 3.5 usually have poor aqueous solubility [12]. Decreasing of lipophilicity improves solvation potential by increasing solvent-solute interactions in aqueous media.

This paper presents the evaluation of *in silico* biological activity of six 3,5-disubstituted hydantoins and spirohydantoins. The important molecular properties were calculated to reveal how

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the incorporation of different substituents affects the lipophilicity of the compounds. Furthermore some of the derivatives were tested for *in vitro* cytotoxicity on a panel of four human tumor cell lines SKW-3, HL-60, LAMA-84 and EJ by MTT-dye reduction assay.

EXPERIMENTAL

Design of the compounds

Six substituted hydantoin and spirohydantoin derivatives were examined. 3'-amino-α-tetralonespiro-5'-hydantoin **(1)**, 3'-amino-2-indanespiro-5'-hydantoin **(2)**, 3-amino-5-methyl-5-phenylhydantoin **(3)** and 3-amino-5-methyl-5-(4-pyridyl)hydantoin **(4)** were designed by replacement of the hydrogen atom from NH-3 (hydantoin ring) with amine group. 3-thiolanespiro-5'-hydantoin **(5)** and 4-thio-1H-tetrahydro-pyranspiro-5'-hydantoin **(6)** were spirohydantoins with S-containing cycloalkane rings. All amino hydantoin derivatives **(1-4)** were synthesized by Davidson method with some modifications. The S-containing hydantoins **(5-6)** were prepared by interaction of thiolane-3-one and tetrahydro-1H-thiopyran-4-one with NaCN and $(NH_4)_2CO_3$ in aqueous ethanol. The obtaining and the characterization of the compounds by elemental analysis, IR, NMR spectra, mass spectral analysis, X-ray diffraction method etc. were described in our previously published articles [13-15].

Calculations of Molecular Characteristics

The drug applicable properties of the reported organic compounds, essential for drug pharmacokinetics in the human body, were estimated with the Lipinski's rule of five [16-17] which states that the most "drug-like" molecules have LogP ≤ 5, molecular weight (M_w) ≤ 500, number of hydrogen bond acceptors (O and N atoms) ≤ 10 and number of hydrogen bond donors (OH and NH groups) ≤ 5. Molecular volume and molecular polar surface area (PSA) are also very useful parameters for prediction of drug transport properties. The polar surface area is defined as a sum of surfaces of polar atoms (usually oxygens, nitrogens and attached hydrogens) in a molecule. The number of rotatable bonds determines flexibility of the molecules. The value of LogP and the residual parameters of drug likeness, as well as the PSA, were reckoned on the method based on group contributions [18]. These have been obtained by fitting the values of the calculated LogP with experimental LogP for a set of more than twelve thousand, mostly drug-like molecules. The percentage of absorption (% ABS) was estimated using the equation:

$$\% \text{ ABS} = 109 - (0.345 \times \text{PSA}).$$

Cell lines and Cytotoxicity assessment

The cell lines used in this study - SKW-3 (human T-cell leukemia, established from peripheral blood of a 61-year-old man with T-cell lymphocytic leukemia), HL-60 (acute myeloid leukemia, established from the peripheral blood of a patient with acute promyelocyte leukemia), LAMA-84 (human chronic myeloid leukemia, established from peripheral blood of a 29-year-old woman with chronic myeloid leukemia) and EJ (urinary bladder carcinoma, established from an invasive endometrioid adenocarcinoma of the uterine corpus in a 56-year-old patient) were purchased from the German Collection of Microorganisms and Cell Cultures. The cells were grown as a suspension-type cultures under standard conditions – RPMI 1640 liquid medium supplemented with 10% fetal bovine serum (FBS) and 2 mM L-glutamine, in cell culture flasks, housed at 37°C in an incubator "BB 16-Function Line" Heraeus with humidified atmosphere and 5% carbon dioxide. Cell cultures were maintained in logarithmic growth phase by supplementation with fresh medium two or three times weekly.

Cytotoxicity Assessment (MTT-dye Reduction Assay)

The cytotoxic activity of the tested compounds was assessed using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] dye reduction assay as described by Mossman [19]. The method is based on the reduction of the yellow tetrazolium salt MTT to a violet formazan via the mitochondrial succinate dehydrogenase in viable cells. In brief, exponentially growing cells were seeded in 96-well flat-bottomed microplates (100 μL/well at a density of 3.5×10^5 cells/mL for the adherent and 1×10^5 cells/mL for the suspension cell lines) and allowed to grow for 24 h prior the exposure to the studied compounds. Stock solutions of the organic compounds were dissolved in DMSO and diluted in RMPI-1640 growth medium. At the final dilutions the solvent concentration never exceeded 0.5%. Cells were exposed to the tested compounds for 72 h, whereby for each concentration a set of 8

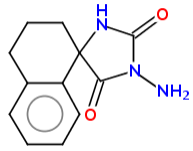
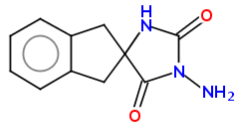
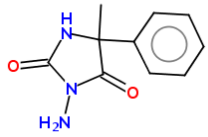
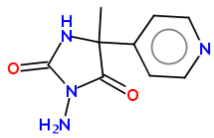
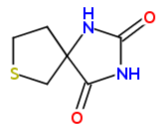
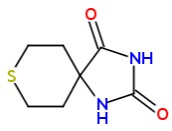
separate wells was used. Every test was run in triplicate, i.e. in three separate microplates. After the incubation with the test compounds 10 μL MTT solution (10 mg/mL in PBS) aliquots were added to each well. The microplates were further incubated for 4 h at 37°C and the MTT-formazan crystals formed were dissolved by adding 100 μL/well 5% HCOOH in 2-propanol. Absorption of the samples was measured by an ELISA reader (Uniscan Titertec) at 580 nm. Survival fraction was calculated as percentage of the untreated control. The experimental data were processed using GraphPad Prizm software and were fitted to sigmoidal concentration/response curves. Cisplatin and melphalan were used as referent cytotoxic drugs throughout the pharmacological assay

RESULTS AND DISCUSSION

In silico Evaluation of Drug Likeness

The comparative evaluation of *in silico* biological activity of six 3,5-disubstituted hydantoin and spirohydantoin derivatives was presented. The compounds and their characteristics used for evaluation of drug similarity on the base of Lipinski's rule are presented in Table 1.

Table 1. Chemical structures and parameters of evaluation of the tested hydantoin and spirohydantoin derivatives with Lipinski's rule of five.

№	Structure	LogP <5	M_w <500	O,N <10	OH,N H <5	Rot. bond	Volume	TPSA (Å²) <140	% ABS
1		0.99	231.25	5	3	0	203.71	75.43	82.98
2		0.41	217.23	5	3	0	186.91	75.43	82.98
3		0.59	205.22	5	3	1	180.71	75.43	82.98
4		-0.70	206.21	6	3	1	176.55	88.32	78.53
5		-0.35	172.21	4	2	0	138.76	58.20	88.92
6		-0.08	186.24	4	2	0	155.56	58.20	88.92

The calculations show that all derivatives observed boundary conditions of the "rule of Lipinski" and did not violate any of the listed criteria. LogP value is used in medicinal chemistry to predict the solubility of a potential drug

In general, spirohydantoins with S-containing cycloalkane ring have lower lipophilicity with negative LogP values. The LogP values of hydantoins ranged between -0.70 and 0.99. The replacement of benzene nuclei of **(3)** by pyridine nuclei in compound **(4)** notably reduces the LogP with more than 1 unit. All compounds possess low lipophilicity with values of LogP < 1, therefore they will have a good solubility in water and other polar liquids as blood and blood plasma. The molecular weight and molecular volume give information for the size of the molecules. The substituents slightly change the molecular weight but all derivatives are small drug-like molecules with M_w between 172 and 231. They are not very flexible as the number of rotatable bonds is between 0 and 1. The number of hydrogen bond donors and acceptors affects the value of polar surface area. All derivatives show a PSA of less than 140 Å², indicating a good permeability of the compounds in the cellular plasma membrane. Moreover the compounds demonstrated a PSA less than 90 Å² and thus are capable to penetrate the blood–brain barrier [20-22].

The *in silico* evaluation was used only as a first step in drug discovery, to find the leading candidates with encouraging properties for further amplification. The hydantoins, **(2)** and **(3)**, with positive LogP and the spirohydantoins **(5)** and **(6)**, with negative LogP values and lowest molecular weight were tested *in vitro* on four human tumor cell lines.

In Vitro Cytotoxicity

The derivatives, 3'-amino-2-indanespiro-5'-hydantoin **(2)**, 3-amino-5-methyl-5-phenylhydantoin **(3)**, 3-thiolanespiro-5'-hydantoin **(5)** and 4-thio-1H-tetrahydro-pyranspiro-5'-hydantoin **(6)** were tested for *in vitro* cytotoxicity. The cytotoxic potential of the compounds against the human leukemic cell lines SKW-3 (human T-cell leukemia), HL-60 (acute myeloid leukemia), LAMA-84 (human chronic myeloid leukemia) and EJ (urinary bladder carcinoma) was studied using the standard MTT-dye reduction assay for cell viability. Throughout the screening investigation the data about the compounds were compared with the referent agent cisplatin and the clinically utilized antineoplastic drug melphalan (2-amino-3-[4-bis(2-chloroethyl) amino] phenylpropanoic acid). The corresponding IC_{50} values obtained are shown in Table 2.

Table 2. Cytotoxicity of the compounds **(2), (3), (5), (6)** in some human tumor cell lines**.**

Compound	IC50 values (μM)			
	SKW-3[a]	HL-60[b]	LAMA-84[c]	EJ[d]
2	-	> 200	> 200	-
3	-	> 200	> 200	-
5	114.0	> 200	174.5	115.2
6	92.6	180.9	101.1	143.5
cisplatin	11.4	8.7	10.2	16.9
melphalan	31.3	18.5	22.1	-

[a]T-cell leukemia; [b]Acute myeloid leukemia; [c]Human chronic myeloid leukemia; [d]Urinary bladder carcinoma

IC_{50} values were calculated as concentrations of the tested compounds causing 50% decrease of cell survival. The hydantoins **(2)** and **(3)** showed lack of cytotoxic effects on tested cell lines. In contrast spirohydantoins **(5)** and **(6)** exerted concentration dependent cytotoxic activity on all human tumor cell lines. Probably the lower molecular weight and the lower lipophilicity affect positively the cytotoxicity. The most significant cytotoxicity was observed for the compound 4-thio-1H-tetrahydropyranspiro-5'-hydantoin **(6)**, which inhibited the viability of tested cells at low micromolar concentrations.

CONCLUSION

Six 3,5-disubstituted hydantoin and spirohydantoin derivatives were studied for lipophilicity using the "rule of Lipinski". The compounds have a good solubility in water and other polar liquids as blood and blood plasma and a good permeability in the cellular plasma membrane. Four of them were tested *in vitro* on some human tumor cell lines in comparison with referent drugs cisplatin and melphalan. The tested spirohydantoins exerted concentration dependent cytotoxic activity on the used human tumor cell lines. The most significant cytotoxicity was observed for the compound 4-thio-1H-tetrahydropyranspiro-5'-hydantoin.

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IN SILICO ЛЕКАРСТВЕНО ПОДОБИЕ И *IN VITRO* ЦИТОТОКСИЧНА АКТИВНОСТ НА НЯКОИ 3,5-ДИЗАМЕСТЕНИ ХИДАНТОИНИ И СПИРОХИДАНТОИНИ

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(Резюме)

Изследването представя сравнителна оценка на лекарственото подобие на шест хидантоинови и спирохидантоинови производни. Някои от съединенията са получени чрез заместване на водородния атом от хидантоиновия пръстен (NH-3) с амино група, а именно 3-амино-α-тетралонспиро-5'-хидантоин, 3-амино-2-инданспиро-5'-хидантоин, 3-амино-5-метил-5-фенилхидантоин, 3-амино-5-метил-5-(4-пиридил)хидантоин. Другите две съединения са спирохидантоини със S-съдържащи циклоалканови пръстени - 3-тиоланспиро-5'-хидантоин и 4-тио-1H-тетрахидропиран-спиро-5'-хидантоин. Важните свойства за лекарственото подобие на изследваните органични съединения, които влияят върху фармакокинетиката на съединенията в човешкото тяло, са определени с правилото на Липински. Стойността на LogP и останалите параметри на лекарствената прилика бяха оценени чрез метод, базиран на приноса на отделните групи. Теоретичният подход се използва само като начален етап в търсенето на нови лекарствени вещества. Някои от съединенията допълнително са изследвани *in vitro* за цитотоксична активност върху четири човешки туморни клетъчни линии - SKW-3, HL-60, LAMA-84 и EJ. Тестваните спирохидантоини проявяват концентрационно зависима цитотоксична активност върху всички човешки туморни клетъчни линии. Най-висока е цитотоксичността на 4-тио-1H-тетрахидропиранспиро-5'-хидантоин, който инхибира жизнеспособността на изследваните клетки при ниски микромоларни концентрации.