Recommended by Doctor of Pharmacy, professor S.V.Kolisnyk

UDC 547.398.1:547.461.3

PHYSICOCHEMICAL PARAMETERS AND THE DIURETIC ACTIVITY OF 5-(4-R)BEHZYL-1,3,4-OXADIAZOL-2-IL-THIOACETIC ACID AMIDES

V.A.Georgiyants, L.O.Perekhoda, I.A.Sych, L.O.Grinevych, O.K.Ryadnykh, A.V.Zhuravel

National University of Pharmacy

Key words: 1,3,4-oxadiazol; "drug likeness" concept; Lipinski's Rules of Five; diuretic activity; cytotoxicity

The group of aryl(heteryl)amides of 5-(4-R)benzyl-1,3,4-oxadiazol-2-il- thioacetic acid has been tested for compliance with the "drug likeness" concept. A number of physical and chemical properties that determine bioavailability according to Lipinski's Rule of Five has been calculated using the ACD/Labs computer programme. It has been found that these compounds can be recommended for further research as compounds with favourable physicochemical properties according to Lipinski's Rule of Five. The results of the experimental study of the ability of new substances to stimulate the urinary renal function predicted by the computer PASS programme have shown that some of the new compounds synthesized are promising diuretics. The results of studying cytotoxicity in vitro for the most promising compounds have shown that these compounds possess low toxicity. The pharmacological screening has allowed to select two promising compounds that are relevant for further study.

There are many approaches that assess a compound's "*drug likeness*", partially based on topological descriptors or other properties. In addition, the biological activity is also usually a function of the complex influence of a number of molecular descriptors. Lipinski's rule of Five also known as the Rule of Five is a rule of thumb to evaluate drug likeness or determine if a chemical compound with a certain pharmacological or biological activity has properties that would make it a likely orally active drug in human. The rule was formulated by Christopher A.Lipinski in 1997. It is based on the observation that active substances of most drugs are relatively small and lipophilic molecules [16]. The rule describes molecular properties that are important for drug pharmacokinetics in the human body, including their absorption, distribution, metabolism, and excretion ("ADME"). However, the rule does not predict if a compound is pharmacologically active.

Among heterocyclic compounds, 1,3,4-oxadiazole has become an important construction scaffold for development of new drugs. Compounds containing 1,3,4-oxadiazole ring have a broad spectrum of the biological activity, including the anticancer [11], anticonvulsant [17], anti-inflammatory [14], antiviral [8], antifungal [12], antimycobacterial action [13]. The oxadiazole ring occurs frequently in a variety of pharmaceutical drugs, including Furamizol, Fenadiazole, Zibotentan, Picovir, Raltegravir, Butalamine, Fasiplon and Oxolamine [10].

The results of computer prognosis of the biological activity spectrum of amides 5-(4-R)benzyl-1,3,4-oxadiazol-2-il-thioacetic acid have shown the possible diuretic activity (activity indexes of this compounds are in the range of 0.496 to 0.595) [15]. Expediency of searching potential diuretics among derivatives of 1,3,4-oxadiazoles is also confirmed by the literature data [10].

The aim of this work was to test amides of 5-(4-R) benzyl-1,3,4-oxadiazol-2-il-thioacetic acid for compliance with the concept of "drug likeness' and study their diuretic activity and toxicity.

Materials and Methods

The synthetic methods used to prepare amides of 5-(4-R)benzyl-1,3,4-oxadiazol-2-il-thioacetic acid can be found in previously reported works [1-2], characteristics of the substances synthesized are given in Tab. 1.

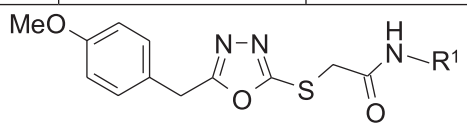
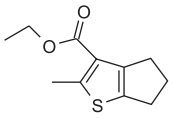
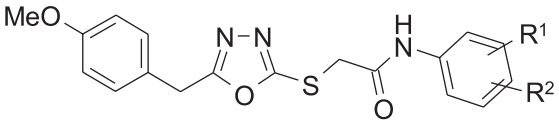
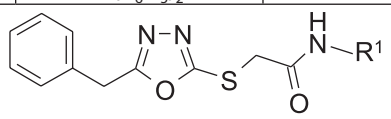
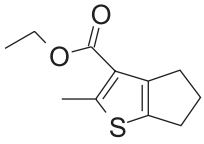
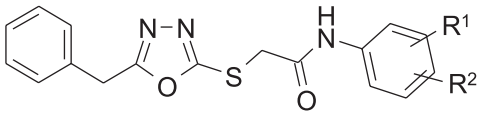
A number of physical and chemical properties determining bioavailability for aryl (heteryl)amides of 5-(4-R)benzyl-1,3,4-oxadiazol-2-il-thioacetic acid ***1.1-1.20*** was calculated using the ACD/Labs computer programme. The purpose of calculations was to compare the values obtained with the desired values of descriptors according to the concept of "drug likeness" followed by elimination of unwanted molecules from databases to optimize further screening. The results obtained are presented in Tab. 2.

The ability of the substances synthesized stimulate or, conversely, inhibit the urinary renal function was studied by the method of E.B. Berkhin in nonlinear white male rats weighing 200.0-250.0 g [4]. Hypothiazide in the dose of 40 mg/kg. was selected as the reference drug when determining the diuretic activity. The compounds studied and the reference drug were introduced as a single intragastrical injection in the form of a finely dispersed aqueous suspension stabilized by Tween-80.

All laboratory animals were received a water test in the amount of 3% of the body weight. After introduction of the substances rats were placed in the "exchange

Table 1

Characteristics of 5-(4-R)benzyl-1,3,4-oxadiazol-2-il-thioacetic acid ***1.1-1.20*** amides

Compound	R^1	R^2
		
1.1		–
		
1.2	H	H
1.3	4-C_2H_5	H
1.4	4-$COOC_2H_5$	H
1.5	2-CH_3	3-CH_3
1.6	3-Cl	4-Cl
1.7	2-Cl	5-CF_3
1.8	2-CH_3	3-Cl
1.9	4-OC_2H_5	H
1.10	3-CH_3	H
1.11	naphtyl	-
1.12	$N(C_6H_5)_2$	-
		
1.13		
		
1.14	H	H
1.15	4-C_2H_5	H
1.16	4-$COOC_2H_5$	H
1.17	2-CH_3	3-CH_3
1.18	4-CH_3	H
1.19	3-Cl	4-Cl
1.20	2-Cl	5-CH_3

cages". The amount of urine excreted by the control group of animals was taken as 100%. The control group of rats received water and Tween-80 in the same volume. The results obtained were compared with the data of the control group of animals. Indicators of the diuretic activity were the total amount of urine within 4 hours and the amount of urine per 100 g of the animal's body weight.

Recently, the so-called alternative methods are increasingly used in advanced preclinical trials along with the traditional methods. Today, alternative methods include the use of invertebrate organisms, plants, microorganisms, cell cultures, as well as a number of physical and biological methods. Using biological models (*in vitro*) can explain biological phenomena, which are difficult to investigate in the experiments with animals because of the complexity of interaction between different effectors and inhibitors.

The main preconditions for wider implementation in practice of preclinical studies of alternative methods, including methods of cell cultures – model test systems, are ethical considerations about exclusion or limitation of experiments on warm-blooded animals [3]. It should be noted that introduction of *in vitro* methods provides pharmacological and toxicological screening systems for development of new substances, allows to use biological systems derived from genetically modified animals, their certain metabolic components are close to human, and create a variety of conditions simultaneously, i.e. accelerate and increase the reliability of research, development and introduction of new drugs [5].

The study of toxicity of the most active compounds *in vitro* was conducted on the model test systems of the bone marrow cells of rats. The compounds were added to the cell suspension in the ratio of 1: 1 in the dose of 15 mg/ml. The cell activity in all samples was measured in 30 min after the start of the experiment. Counting live and dead cells was carried out with the Goryaev chamber after staining with trypan blue [7]. The proportion of dead cells was determined by the formula:

$$Pr = \frac{Nm}{N\ total} \cdot 100\%,$$

where: Nm – is the number of dead cells in the sample; N total – is the total number of cells in the sample.

Further the experimental results were statistically processed using a "StatSoft" software package [6] (Tab. 4).

Results and Discussion

All compounds studied by the complex of physical and chemical properties meet modern requirements for new compounds when testing their biological activity. The results obtained have shown that all substances can be recommended for further research as compounds with favourable physicochemical properties.

The diuretic activity of aryl (heteryl)amides of 5-(4-R)-benzyl-1,3,4-oxadiazol-2-il-thioacetic acid ***1.1-1.20*** are shown in Table 3. In terms of the total amount of urine substances ***1.3*** and ***1.4*** are the most promising. By the diuretic activity **(**the difference in % relative to the control) ***1.17*** and ***1.20*** are the most promising.

Comparing the experimental data obtained (Tab. 3) it can be concluded that substances ***1.17*** and ***1.20*** are the most active in comparison with the control.

The results of the study of cytotoxicity (Tab. 4) have shown that the compounds synthesized have low toxicity (Pr<20) and allowed to choose two substances (***1.17, 1.20)***, which are promising for further study.

Table 2

Determination of parameters of 5-(4-R)benzyl-1,3,4-oxadiazol-2-il-thioacetic acid amides ***1.1-20*** according to Lipinski's Rule of Five

Compound	Parameters of compounds				
	Molecular weight, MW	Molar refraction, MR cm	Partition coefficient, Log P	Number of the hydrogen bond donors	Number of the hydrogen bond acceptors
1.1	268.24	67.14	2.6	5	3
1.2	252.28	71.57	2.3	3	3
1.3	383.46	105.54	2.5	5	3
1.4	427.47	112.03	2.9	5	3
1.5	383.46	105.53	2.7	3	3
1.6	424.30	105.94	2.4	5	3
1.7	457.85	106.10	2.3	3	3
1.8	403.88	105.73	2.5	2	3
1.9	369.43	105.98	2.8	3	3
1.10	369.43	100.91	2.9	3	3
1.11	405.46	113.84	2.4	3	3
1.12	431.50	121.22	2.5	3	3
1.13	443.53	115.64	2.6	4	3
1.14	325.38	89.92	2.4	2	3
1.15	369.43	100.91	2.9	4	3
1.16	397.44	105.66	2.8	4	3
1.17	353.43	99.16	2.7	2	3
1.18	339.41	94.54	2.4	2	3
1.19	394.27	99.57	2.4	4	3
1.20	427.82	99.73	2.7	2	3
The range of values	252.28-457.85	67.14-121.22	2.3-2.9	2-5	3
The average value	355.07	94.18	2.6	3.5	3
The maximum permissible value	460	130	5.6	5	10
The optimal value	357	97	2.52	–	–

Table 3

The diuretic activity of 5-R-benzyl-1,3,4-oxadiazol-2-il-thioacetic acid amides

Compound	The average dose of the substance, mg/kg	The average weight of the rat, g	The average total amount of urine, ml	The amount of urine per 100 g of the animal's weight, ml	Diuretic activity, %	The difference in % relative to the control
1	2	3	4	5	6	7
1.1	34.4	213.33	3.87	1.81	58.22	-41.78
1.2	33.04	208.33	6.27	3.00	96.44	-3.56
1.3	31.84	235.00	7.33	3.10	99.66	-0.34
1.4	29.74	241.67	7.73	3.20	102.93	2.93
1.5	28.34	218.33	5.67	2.57	82.59	-17.41
1.6	29.84	243.33	6.77	2.80	117.09	17.09
1.7	28.43	230.00	6.40	2.78	116.43	16.43
1.8	28.13	230.00	5.13	2.23	93.39	-6.61
1.9	27.23	240.00	6.37	2.66	111.25	11.25
1.10	29.74	226.67	6.83	3.01	125.93	25.93
1.11	28.34	226.67	6.53	2.89	120.85	20.85
1.12	28.64	206.67	5.30	2.57	128.90	28.90
1.13	31.24	206.67	5.27	2.42	121.53	21.53

Table 3 continued

1	2	3	4	5	6	7
1.14	31.24	220.00	4.20	1.91	95.83	-4.17
1.15	34.04	220.00	4.20	1.91	129.85	29.85
1.16	40.25	211.67	5.47	2.58	137.52	37.52
1.17	29.84	246.67	5.50	2.26	157.21	57.21
1.18	29.84	228.33	5.87	2.58	131.47	31.47
1.19	32.64	228.33	4.97	2.16	120.22	20.22
1.20	38.85	233.33	6.17	2.65	161.48	61.48

Table 4

Assessment of the death rate of bone marrow cells in rats

Compound	Pr, %
Control	5.23±0.23
1.3	19.94±0.42
1.4	19.27±0.47
1.17	7.91±0.08
1.20	10.03±0.65

CONCLUSIONS

1. Testing for compliance with the concept of "drug likeness" of 20 structures of 5(4-R)benzyl-1,3,4-oxadiazol-2-il-thioacetic acid derivatives has been performed. The results obtained have shown that the compounds obtained can be recommended for further study as compounds with favourable physicochemical properties according to Lipinski's Rule.

2. The study of the diuretic activity and toxicity of the compounds synthesized (*in vitro*) has identified two active compounds, which are relevant for further study.

REFERENCES

1. *Георгіянц В.А., Перехода Л.О., Рибальченко Т.Л. та ін. // Фармац. журн. – 2010. – №6. – С. 25-31.*
2. *Георгіянц В.А., Перехода Л.О., Рядних К.С. та ін. // Тези доп. наук. конф. «Фармація України. Погляд у майбутнє: матер. VII Нац. з'їзду фармац. України». – Х., 2010. – Т. 1. – С. 86.*
3. *Доброва В.Е., Должикова Л.М., Малоштан Е.А. // Тези докл. научн. конф. «Фармация Казахстана: интеграция науки, образования и производства». – К., 2009. – С. 45-49.*
4. *Доклінічні дослідження лікарських засобів: Метод. рекоменд. / Под ред. А.В.Стефанова. – К., 2001. – С. 371-395.*
5. *Коваленко В.М., Стефанов О.В., Максимов І.М. Методичні рекомендації по експериментальному вивченню токсичної дії лікарських засобів. – К.: МОЗ України, Державний фармакологічний центр, 2000. – С. 43.*
6. *Лапач С.Н., Чубенко А.В., Бабич П.Н. Статистические методы в медико-биологических исследованиях с использованием Excel. – К.: Морион, 2000. – 320 с.*
7. *Чехун В.Ф., Кулік Г.І., Шарикіна Н.І. Методичні рекомендації з вивчення канцерогенних властивостей нових речовин та лікарських засобів. – К.: МОЗ України, Державний фармакологічний центр, 2001. – С. 19.*
8. *Farghaly A.R., El-Kashef H. // Arkivoc. – 2006. – Vol. 11. – P. 76-90.*
9. *Kalpesh P., Jayachandran E., Ravi S. et al. // Intern. J. of Pharma and Bio Sci. – 2010. – Vol. 1, №3. – P. 125-135.*
10. *Khader A.M. // Der Pharma Chemica. – 2013. – Vol. 5 (2). – P. 24-32.*
11. *Kumar A., D'Souza S.S., Gaonkar S.L. et al. // Invest. New Drugs. – 2008. – Vol. 26. – P. 425-435.*
12. *Liu F., Luo X., Song B. et al. // Bioorg. Med. Chem. – 2008. – Vol. 16. – P. 3632-3640.*
13. *Macaev F., Rusu G., Pogrebnoi S. et al. // Bioorg. Med. Chem. – 2005. – Vol. 13. – P. 4842-4850.*
14. *Narayana B., Raj K.K., Ashalatha B.V. et al. // Arch. Pharm. – 2005. – Vol. 338. – P. 373-377.*
15. *PASS: Prediction of Activity Spectra for Substances. http:///www.ibmh.msk.su/PASS.*
16. *Veber D.F., Johnson S.R., Cheng H.Y. et al. // J. Med. Chem. – 2002. – Vol. 45, №12. – P. 2615-2623.*
17. *Zarghi A., Tabatabai S.A., Faizi M. et al. // Bioorg. Med. Chem. Lett. – 2005. – Vol. 15. – P. 1863-1865.*

ФІЗИКО-ХІМІЧНІ ПАРАМЕТРИ ТА ДІУРЕТИЧНА АКТИВНІСТЬ АМІДІВ 5-(4-R) БЕНЗИЛ-1,3,4-ОКСАДІАЗОЛ-2-ІЛ-ТІОАЦЕТАТНОЇ КИСЛОТИ

В.А.Георгіянц, Л.О.Перехода, І.А.Сич, Л.О.Гріневич, О.К.Рядних, А.В.Журавель

Ключові слова: *1,3,4-оксадіазол; концепція «схожість з ліками»; Правила Ліпінські; діуретична активність; цитотоксичність*

Група арил(гетерил)амідів 5-(4-R)бензил-1,3,4-оксадіазол-2-іл-тіоацетатної кислоти була протестована на відповідність концепції «схожість з ліками», для чого за допомогою комп'ютерної програми ACD/Labs розраховані їх фізико-хімічні параметри, що визначають біодоступність за «Правилами Ліпінські». Встановлено, що зазначені сполуки можуть бути рекомендовані для подальшого вивчення як такі, що згідно з Правилами Ліпінські мають сприятливі фізико-хімічні параметри. Результати вивчення сечогінної активності, що прогнозується для даної групи похідних 1,3,4-оксадіазолу за даними комп'ютерної програми PASS, показали, що деякі досліджені аміди є перспективними діуретиками. Визначенням токсичності (in vitro) цих сполук з'ясовано, що вони мають низьку токсичність. Проведений фармакологічний скринінг дозволив відібрати для поглибленого дослідження дві найбільш активні БАР.

ФИЗИКО-ХИМИЧЕСКИЕ ПАРАМЕТРЫ И ДИУРЕТИЧЕСКАЯ АКТИВНОСТЬ АМИДОВ 5-(4-R) БЕНЗИЛ-1,3,4-ОКСАДИАЗОЛ-2-ИЛ-ТИОУКСУСНОЙ КИСЛОТЫ

В.А.Георгиянц, Л.А.Перехода, И.А.Сыч, Л.А.Гриневич, Е.К.Рядных, А.В.Журавель

Ключевые слова: *1,3,4-оксадиазол; концепция «сходство з лекарствами»; Правила Липински; диуретическая активность; цитотоксичность*

Группа арил (гетерил) амидов 5 (4-R) бензил-1,3,4-оксадиазол-2-ил-тиоацетатной кислоты была протестирована на соответствие концепции «сходство с лекарствами», для чего с помощью компьютерной программы ACD/Labs рассчитаны их физико-химические параметры, определяющие биодоступность по «Правилам Липински». Установлено, что указанные соединения могут быть рекомендованы для дальнейшего изучения как такие, которые согласно «Правил Липински» имеют благоприятные физико-химические параметры. Результаты изучения мочегонной активности, которая прогнозируется для данной группы производных 1,3,4-оксадиазола по данным компьютерной программы PASS, показали, что некоторые исследованные амиды являются перспективными диуретиками. Определением токсичности (in vitro) этих соединений установлено, что они имеют низкую токсичность. Проведенный фармакологический скрининг позволил отобрать для углубленного исследования два наиболее активных БАВ.