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Screening Investigation of Novel 1,2,4-Triazole-3-Thione Derivatives on Anticonvulsant Activity

Dmitryi Pavlovich Kavraiskyi[1], Sergey Yurievich Shtrygol'[1], Victoria Akopovna Georgiyants[2], Narzullo Boboevich Saidov[2]

[1]Department of Pharmacology, National Pharmacy University, Kharkiv, Ukraine

[2]Department of Pharmaceutical Chemistry, National Pharmacy University, Kharkiv, Ukraine

Email address:

kavraiskyi@gmail.com (D. P. Kavraiskyi), shtrygol@mail.ru (S. Y. Shtrygol'), vgeor@ukr.net (V. A. Georgiyants), saidov_n@ukr.net (N. B. Saidov)

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Abstract: 1,2,4-triazole and its derivatives are present in many biologically active aromatic compounds that have different biological properties and clinical applications including anticonvulsant activity. The number of studies in this direction is increasing. Therefore, based on the results of previous computer predictions, 8 novel 1,2,4-triazole-3-thione derivatives have been synthesized in the Department of Pharmaceutical Chemistry at the National Pharmacy University of Ukraine. The screening test of novel 1,2,4-triazole-3-thione derivatives on anticonvulsant activity in PTZ-induced seizures was conducted on mice. Among test substances revealed were those, which in the dose of 100 mg/kg showed moderate anticonvulsive effect and which were non-inferior to "reference drug" sodium valproate by some parameters. Screening results have confirmed the reasonability of in-depth anticonvulsive activity study of substances-leaders, pharmacological study of their mechanism of action, spectrum of anticonvulsant activity, concurrent types of pharmacological activity, dose-dependence of effect and safety.

Keywords: Pharmacological Screening, Anticonvulsant Activity, Triazole Derivatives, PASS-Software, Pentylenetetrazole-Induced Seizures

1. Introduction

Epilepsy is known to be a chronic progredient disease with sudden occurrence of seizures, specific changes in development of personality and sometimes – dementia. The main feature of the disease is convulsive or non-convulsive seizures, paroxysmal conditions present, intellectual, mnestic dysfunctions and personality disorders [1]. According to the WHO data, the number of patients with different forms of epilepsy continues to increase [2].

Despite recent achievements in diagnostics and treatment of epilepsy there are still many patients, for whom the available methods of treatment fail to achieve an appropriate control of seizures and/or have serious adverse effects.

Currently there is no universal medicine effective for all types of seizures. Therefore, different patients need different medicine, often in combinations. Prescribing a second drug helps nearly 10-15% of patients with over a two-fold increase in the incidence of adverse effects [1]. The issue of the development of new original anticonvulsants with high efficacy, low toxicity and no pharmacoresistance still needs to be resolved [3].

1,2,4-triazole and its derivatives are present in many biologically active aromatic compounds that have different biological properties and clinical applications. Recently, 1,2,4-triazole derivatives have become an important class of compounds for developing new medicine with anticonvulsant action. The number of studies in this direction are increasing [4-9].

One of the modern trends in medical chemistry is the potential type of prediction of synthetic substances pharmacological activity using a preliminary virtual screening with a large number of software products developed for this purpose. One of them, which is intensively developed these days is Prediction of Activity Spectra for Substances (PASS) [10, 11]. This free access software can be

used on-line [12]. Therefore, we used it for predicting pharmacological activity of 1,2,4-triazole derivatives [13].

Based on the results of preliminary prediction 8 most promising compounds for preclinical study were selected from the synthesized original compounds of 1,2,4-triazole derivatives (Tab.1). According to the PASS prediction, all compounds of this group have a high probability to demonstrate neurotropic types of pharmacological activity, in particular antiepileptic, anticonvulsant, neuroprotective, analgesic action, and being benzodiazepine and GABA receptors agonists, etc. (Pa for anticonvulsant activity of all synthesized compounds exceeds 0.500). In addition, the prediction data (probability value) identify 1,2,4-triazole-3-thione groups with the highest probability of anticonvulsant effect: anilides [4-phenyl-5-(benztriazolyl-1-methyl)-1,2,4-triazole-4-H-3-ylthio]acetic acid (1a-c) > 2-(piperidinyl/morpholynyl -N-methyl)-4,5-diphenyl-1,2,4-triazole-3-thiones (2 a-c) > 2-(morpholynyl -N-methyl)-4-(4'-fluorobenzylidenamino)-5-trifluoromethy-l,2,4-triazole-3-thione(3) > 2'-methylanilide [4-phenyl-5-(2'-methyl-chlorphenylaminomethyl)-1,2,4-triazolyl-3-thio]acetic acid (4). However, according to the software author, the value of the effect does not always correlate with the Pa value [10, 11].

The purpose of this study was to screen the above selected 1,2,4-triazole-3-thione derivatives for anticonvulsant activity in the model of pentylenetetrazole (PTZ) induced seizures in mice and define the effect of certain structural fragments on the seizure syndrome course.

2. Materials and Methods

Substances for pharmacological screening were synthesized and purified according to the aforementioned methods [14, 15]. Prediction of pharmacological activity was carried out using a resource PASS online [16].

Investigation of anticonvulsant activity was conducted according to the guidelines on the preclinical study of medicine in PTZ-induced seizure model in mice [17, 18].

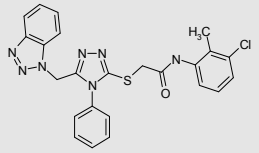
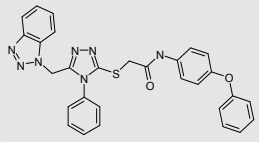
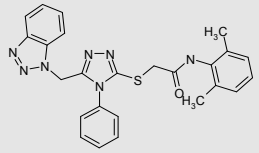
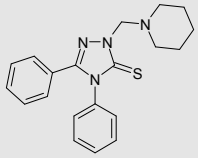
Studies were conducted in 71 albino mature non-strain male and female mice weighing 17-28 g. Animals were kept in the standard conditions of the National Pharmaceutical University's Central Scientific and Research Laboratory (NPU CSRL) Vivarium in compliance with the sanitary and hygienic norms (natural lighting, temperature 20-24^{o}C, humidity NMT 50%, standard diet with ad lib access to water) and the provisions of Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

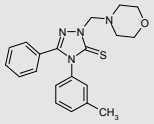
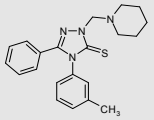
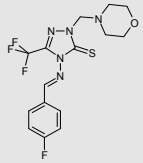
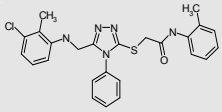
Experimental animals were randomly divided into 10 groups: (1) – control pathology, n=17; (2) – positive control, n=6; (3-10) – a group of mice which were administered the study substances, n=6.

The seizures in mice of the control pathology group were modelled by PTZ aqueous solution (PTZ, "Sigma", USA) (90 mg/kg). Animals of positive control were given sodium valproate ("Depakine", lot 472, Sanofi-Aventis, France) (300 mg/kg, per os) 30 min before administering a convulsant. The study compounds (100 mg/kg) were administered to mice of groups 3-10 through a probe into the stomach as suspended in Tween-80 30 min before administering a convulsant.

Mechanism of PTZ proconvulsant action is conditioned by an inhibitory effect on the benzodiazepine binding site of GABAa and, as a result, by reducing GABAergic inhibitory processes [18]. The anticonvulsant action was assessed using the following parameters: latency period, number of clonic and tonic seizures on 1 mouse, % of mice with clonic and tonic seizures, severity of seizures, time of convulsive period, time of death and lethality.

Table 1. *Chemical structure of triazole-derivatives and results of their PASS-prediction anticonvulsive activity.*

Code of compound	Structural formula	Name of compound	Pa – probability of anticonvulsive activity
1a		2'-methyl-3'-chloranilide [4-phenyl-5-(bentriazolyl-1-methyl)-1,2,4-triazolyl-3-thio]acetic acid	0,787
1b		4'-phenilanilid [4-phenyl-5-(bentriazolyl-1-methyl)-1,2,4- triazolyl-3-thio] acetic acid	0,665
1 c		2',6'-dimethylanilide [4-phenyl-5-(bentriazolyl-1- methyl)-1,2,4-triazolyl-3-thio] acetic acid	0,729
2 a		2-(piperidyl-N- methyl)-4,5-diphenyl-1,2,4-triazol-3-thione	0,663

Code of compound	Structural formula	Name of compound	Pa – probability of anticonvulsive activity
2 b		2-(morpholinyl-N-methyl)-4-(2'-methylphenyl)-5-phenyl-1,2,4-triazole-3-thione	0,624
2 c		2-(piperidyl-N-methyl)-4-(2'-methylphenyl)-5-phenyl-1,2,4-triazole-3- thione	0,615
3		2-(morpholinyl-N- methyl)-4-(4'-fluorobenzylidenamino)-5-trifluoromethyl -1,2,4- triazole-3-thione	0,613
4		2'-methylanilid [4-phenyl-5-(2'-methyl-3'chlorophenylaminomethyl)-1,2,4-triazolil-3-thio]acetic acid	0,568

If seizures have not been observed within 1 hour, the latency period was considered to be 60 min. Severity of seizures were evaluated according to a scale ranging as follows: 1 – shudder, 2 – running in circles, 3 – clonic seizures, 4 – clonic-tonic seizures in lateral position, 5 – tonic extension, 6 – tonic extension with resultant death of animals [19].

A Student's t-test was used for assessing statistical significance of the differences and Fisher's angular transformation – for alternative calculation (lethality, % mice with clonic and tonic seizures). The differences were considered significant at $p<0.05$.

3. Results and Their Discussion

Pharmacological screening results (table 2) justify the division of test compounds into three 3 categories: 2 compounds with moderate anticonvulsant activity (3, 4); 3 compounds with insignificant effect on seizures (1 b, c, 2 a); 3 compounds – proconvulsants (1 a, 2 b, c).

Positive control (sodium valproate) showed a perfect protective effect, ensuring survival of all animals and a statistically significant decrease in severity and duration of seizures (table 2).

Compound 4 has demonstrated the highest anticonvulsant activity – a proved decrease in the number of mice with clonic seizures by 17.7%. Also was observed an increasing latency period and time period of mice deaths, decreasing in the number of clinic-tonic seizures per one mouse but these results represent a tendency. Compound 3 showed the tendency to decrease animals' lethality by 25.5%.

According to PASS prediction compound 1a had the highest probability of anticonvulsant activity – 0.787, and compounds 2 b, c (Pa 0.624 and 0.615, respectively) had significant proconvulsant effect. They statistically significantly increased the number of mice with clonic-tonic seizures by 25%, and substances 2 b, c, in addition, significantly increased the severity of seizures by 20%, that caused the increase of lethality up to 100% (1.7-fold).

Table 2. *Screening results of triazole derivatives effect on PTZ-induced seizures in mice.*

Group of animals, compounds	n	Dose, mg/kg	Latency period, min	Number of clonic and tonic seizures on 1 mouse	% of mice with seizures		Severity of seizures, scores	Time of convulsive period, min	Time of death, min	Lethality, %
					clonic	tonic				
Control pathology	17	–	5,24±1,74	3,06±0,34	100	76,5	5,00±0,20	9,74±1,84	11,95±1,39	58,8
Sodium valproate	6	300	7,62±0,98	1,67±0,65	100	66,7	3,67±0,16*	2,43±2,02*	–	0**
Compounds, which demonstrated moderate anticonvulsant effect										
4	6	100	6,95±2,08	2,17±0,32	100	83,3	4,50±0,48	10,43±3,31	16,56±8,57	33,3
3	6	100	12,76±9,40	2,17±0,65	83,3**	83,3	4,67±0,97	9,03±3,26	14,02±4,60	66,7
Compounds, which had no significant effect on PTZ-induced seizures										
2 a	6	100	5,61±1,48	3,33±0,65	100	100**	5,17±0,32	10,76±4,70	12,67±2,35	50,0
1 б	6	100	5,19±1,73	2,50±0,48	100	100**	5,00±0,32	9,11±3,08	17,76±5,18	50,0
1 в	6	100	4,56±1,46	2,17±0,48	100	66,7	5,00±0,48	7,02±2,46	10,66±3,24	50,0
Compounds, which have demonstrated proconvulsant effect										
2 б	6	100	4,69±0,77	2,17±0,81	100	100**	6,00±0,0*	7,66±4,75	12,35±5,50	100**
2 в	6	100	5,42±1,46	2,33±0,16	100	100**	5,67±0,32	8,59±1,80	14,77±2,84	83,0
1 а	6	100	3,48±0,63	2,50±0,65	100	100**	6,00±0,0*	7,93±3,73	11,40±4,28	100**

Note. Statistically significant differences in control pathology ($p\leq0,05$): * – by Student's t-test;
** – by Fisher's angular transformation.This can be explained by the fact that PASS does not specify the exact modality of effect, i.e. tendency of effect to aggravate or relief the convulsive state, but only predicts the effect of certain pharmacophores on brain structures, that participate in convulsive activity.

The rest of the derivatives of 1,2,4-triazole-3-thione were indifferent to the course of PTZ-induced seizures. However, compounds 1b and 2a had significantly increased the number of mice with tonic seizures by 17.7%, but had not increased the integral index – mice lethality.

The results analysis of the effect of specific structure on the activity proved the highest effect to be caused by the secondary pharmacophores – substitutes in position 3, 4 and 5, rather than by the basic pharmacophore (1,2,4- triazole-3-thione). Presence of phenylaminomethyl group (compound 4) in position 5 to demonstrate the anticonvulsant activity for anilides [4-phenyl-1,2,4-triazole-(4*H*)-3-yltio] acetic acid is favorable, while bezotriazole-1-methyl fragment cannot be considered as pharmacophore for this type of activity (compounds 1 a-c). In anilid residue the 2-methyl substitute (compound 4) or its combination with chlorine atom in position 3 showed themselves in a better way. The 2-(piperidyl/morpholinil-N-methyl)-4,5-diphenyl-1,2,4-triazole-3-thiones (2 a-c) are not promising for the future search of anticonvulsants. However, phenylic radicals' replacement by trifluoromethyl and benzylidene radicals in positions 4 and 5 respectively (compound 3), resulted in substantial alleviation of convulsive activity, latency period prolongation and clonic seizures prevention in group of animals even compared to valproate. Based on the results obtained, taking into consideration the effect of the identified secondary pharmacophores and the in-depth pharmacological study of compounds-leaders (3, 4), their further chemical modification can be recommended.

4. Conclusions

(1) The screening test of 8 novel 1,2,4-triazole-3-thione derivatives (selected based on the results of previous computer prediction) on anticonvulsant activity in PTZ-induced seizures in mice was conducted.

(2) Among test substances revealed were those, which in the dose of 100 mg/kg showed moderate anticonvulsive effect and which were non-inferior to "reference drug" sodium valproate by some parameters.

(3) Screening results have confirmed the reasonability of in-depth anticonvulsive activity study of substances-leaders, pharmacological study of their mechanism of action, spectrum of anticonvulsant activity, concurrent types of pharmacological activity, dose-dependence of effect and safety.

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