Study of the anticonvulsant properties of new benzothienopyrimidine derivative[1]

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Abstract. New anticonvulsant compounds have been identified, which are derivatives of benzothieno pyrimidines and have anticorazole activity. Comparison with known functional analogs showed the advantages of the studied substances in terms of both anticonvulsant activity and neuro- and acute toxicity. Comparison with the structural analogue pyratidine, which is at the stage of preclinical trials, showed that the most active compound of them has therapeutic and protective indices comparable to it.

Keywords: derivatives of pyrimidines, anticonvulsant activity, corazole convulsions.

Introduction

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Derivatives of pyrimidine play an important role in many biologically important processes, and synthetic fused pyrimidine derivatives exhibit a wide range of pharmacological actions. Condensed thienopyrimidines are a new class of anxiolytics. Among them, thieno [3,2-*d*] pyrimidines, structural analogs of purines, are also biologically active substances [1-4]. On the other hand, tetracyclic fused systems including pyrano, pyridine, thiophene, and pyrimidine rings can be considered as analogs of heterosteroids, which are known to exhibit various biological effects.

The **purpose** of this study was to study the anticonvulsant effect of new benzothienopyrimidine derivatives using experimental models and seizure tests to obtain and introduce a new anticonvulsant drug into medical practice.

Materials and methods

All experiments with laboratory animals were carried out in accordance with the Guidelines for the maintenance and use of laboratory animals [5].

The anticonvulsant spectrum of action of the compounds was investigated by tests: corazole (pentylenetetrazole, PTZ) convulsions, maximum electric shock (MES), camphor, thiosemicarbazide (TSC), picrotoxin, strychnine convulsions [6-8]. The PTZ test is an experimental model for obtaining absences and myoclonic seizures and for predicting the anxiolytic properties of compounds. The PTZ test was carried out in mice by subcutaneous injection of an analeptic at a dose of 90 mg / kg, the effectiveness of the drugs is determined by the prevention of clonic seizures.

The anticonvulsant activity of the compounds was also determined by the prevention of the tonic-extensor phase of the seizure MES. The parameters of the maximum electroshock are 50 mA, the duration is 0.2 sec., The oscillation frequency is 50 imp./sec., The assessment criterion is the prevention of the tonic-extensor phase of a convulsive seizure.

Camphor clonic convulsions were obtained by intraperitoneal (i.p.) administration of camphor at a dose of 1 g/kg and the intensity of convulsions and the viability of animals were assessed by an alternative method. TSC was administered at a dose of 18 mg/kg subcutaneously, and picrotoxin at a dose of 5 mg/kg. The effectiveness of the compounds is determined by the latency of the onset of seizures, as well as their prevention and intensity. Strychnine tetanic convulsions were obtained by administering strychnine nitrate at a dose of 1.5-2 mg/kg. The presence and latency of seizures were assessed.

The substances were injected intraperitoneally at doses of 10-300 mg/kg suspended with carboxymethylcellulose (Viadi - Ingredients) and tween-80 (Ferak Berlin) 45 minutes before the

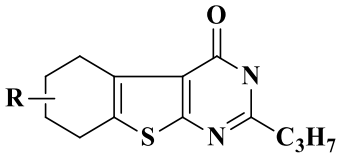
administration of convulsive agents and electrical irritation. Control animals were injected with an emulsifier. Each dose of the compounds for each test was studied in five animals. The analogs of comparison were the anticonvulsants from the group of succinimides ethosuximide and pufemide - 3- (p-isopropoxyphenyl) succinimide [9], as well as pyratidine, an antiepileptic substance with a tranquilizing effect from the group of thienopyrimidines, created at the Institute of Fine Organic Chemistry of the STC NAS RA and being a structural analogue of the studied compounds.

The side neurotoxic (muscle relaxant) effect of compounds and analogs in doses from 50 to 1000 mg/kg, as well as acute daily toxicity in doses from 500 to 2000 mg/kg with intraperitoneal administration was also studied. Myorelaxation was studied using the "rotating rod" test in mice [6, 10]. For this purpose, the mice were placed on a metal rod with a corrugated rubber coating, which was rotated at a speed of 5 rpm. The number of animals unable to stay on it for 2 min was determined.

To determine 50% effective doses - ED_{50} (causing an anticonvulsant effect in 50% of animals), as well as 50% neurotoxic - TD_{50} and 50% lethal doses - LD_{50}, we used the statistical method of probit- analysis according to Litchfield and Wilcoxon [11]. The therapeutic ($TI = LD_{50}/ED_{50}$) and protective ($GI = TD_{50}/ED_{50}$) indices were determined.

Research results

The neurotropic properties of 3 derivatives of tetrahydrobenzothienopyrimidines with the general structure were investigated:



where R= H (№ 3212-1, №1); R= 6-CH_3 (№ 3212-2, №2);

R= 7-CH_3 (№3212-3, №3)

As shown by the research results presented in table 1, the compounds have a pronounced anticorazole effect and are several times superior to their functional analogs. They have low toxicity and high therapeutic and protective indices. Of these, compound 2 is the most active, ED_{50} = 16 mg/kg for antagonism with corazole. The compound is statistically significantly superior to zarontin by 10 and puffemid by 5 times. The compound is the least toxic (LD_{50} = 2300 mg/kg) and the least neurotoxic (TD_{50} = 660 mg/kg). The therapeutic and protective indices of the compound are superior to zarontin 17 and 13 and pufemid - 6 and 8 times, respectively. In comparison with its structural analogue, pyratidine, the compound is less active, but less toxic, and as a result, the therapeutic and protective indices of the compound are comparable with the data obtained with pyratidine.

Table 1.

Corazole antagonism and toxicity of compounds **№ 1-3**, pufemide, zarontin and pyratidine

Compound №	**Corazole antagonism, ED_{50}, mg/kg**	**LD_{50} mg/kg**	**TD_{50} mg/kg**	**TI**	**ZI**
1	35 (28 ÷ 43.75)	1350(900 ÷ 2025)	480(369 ÷ 624)	8.57	13.7
2	16(10.32÷24.8)	2300(2000÷2645)	660(528 ÷ 825)	143.75	41.25
3	28(16÷49)	1150(718.7 ÷ 1840)	785(628 ÷ 981)	41	28
Zarontin	155(117.5÷204.5)	1325(1200÷1462)	520(412.6-655.2)	8.55	3.35
Pufemid	86(58.1÷127.3)	2150(1930÷2390)	450(365.8-553.5)	25	5.25
Pyratidine	1.7(1.0÷2.7)	245(207.5÷289.5)	70(60.3÷81.2)	144.1	41.1

Note: The mean values are shown, their confidence intervals (in brackets). * Statistically significant changes compared to control at a level of significance$P \leq 0.05$.

Among the above new selected compounds, the most active and promising is compound № 2 (registration № 3212), a tetrahydrobenzothienopyrimidine derivative.

To study the spectrum of anticonvulsant action comp. № 3212, we used the following additional tests: antagonism with MES, camphor, TSC, picrotoxin, strychnine (tab. 2). The studied compound in terms of antagonism with camphor surpasses pufemide and zarontin by 2.8 and 4 times, respectively (ED_{50} = 32 mg/kg). According to the test of maximum electroshock, the compound is inferior to pufemid, and zarontin is inactive. By antagonism with TSC and picrotoxin, compound № 3212 prevents clonic convulsions at the following doses - ED_50 = 84 mg/kg and 190 mg/kg, respectively, while pufemide and zarontin only increase the latent period of the onset of convulsions. Compound # 2 at a dose of 150-300 mg/kg increases the latency period of strychnine convulsions (pufemide and zarontin are active).

In general, in terms of spectrum of anticonvulsant activity, the compound is more similar to pufemide than to zarontin. In addition, a seizure model, corasole titration, was used. The change in the threshold of the extensor phase, caused by the intravenous administration of a 1% solution of corazole at a constant rate, was studied in experiments on mice after preliminary administration of

comp. № 3212, pufemis and zarontin. Studies have shown that all three drugs increase the threshold for corazole tonic extension (tab. 3).

Table 2.

Comparative anticonvulsant activity of compound **№ 3212**, **zarontin** and **pufemide** according to various convulsive tests

Compound	ANTAGONISM				
	Camphor	**MES**	**Picrotoxin**	**TSC**	**Strychnine**
№3212	32(21÷49.8)	320(278.5÷368)	190(108.6÷333)	84(65.6÷108)	Increase of the latency period by 3.0 times
Zarontin	131(100÷171)	-	Increase of the latency period by 1.5 times	–	152(109÷213)
Pufemid	90(57.0÷142)	77(52.7÷112.3)	Increase the latency period by 2 times	Increase of the latency period by 2.3 times	110(80.4÷151)

Notes: Average values and their divergence intervals are shown; * Statistically significant changes compared to control at a significance level of $P \leq 0.05$.

Table 3.

The influence of the comp. **№ 3212**, **pufemide** and **zarontin** on the threshold of corazole convulsions during intravenous titration

Compound, Dose, mg/kg		Average dose of corazole (mg/kg) inducing tonic extension	
		M with confidence intervals	**Increase of the threshold**
Control		76.73(67.94÷85.52)	1.00
№3212	**25**	94.5(80.2÷108.8)	1.23
	50	121.25(100.46÷142.04)*	1.58
	100	148.9(131.0÷166.8)*	1.94
	200	200.0(161.8÷238.2)*	2.60
Pufemid	**100**	124.16 (89.9÷158.42)*	1.61
	200	239.2 (212.94÷265.45)*	3.11
Zarontin	**200**	114.0(89.08÷138.92)*	1.48
	300	149.5 (120.0÷179.0)*	1.95

Notes: Average values and their confidence intervals are shown; * Statistically significant changes compared to control at a significance level of $P \leq 0.05$.

Pufemide at a dose of 200 mg/kg increases the threshold by 3.11 times, zarontin at a dose of 300 mg/kg - 1.95 times. Compound № 3212 was studied at various doses (25,50,100,200 mg/kg). In the study of compound № 3212 on this test, a dose-dependent increase in the seizure threshold is observed. The average dose of corazole, causing tonic extension, increases from 94.5 (80.2 ÷ 108.8) against the background of the action of compound № 3212 at a dose of 25 mg/kg to 200 (161.8 ÷

238.2) at a dose of 200 mg/kg, and the threshold compared with the control, respectively rises from 1.23 to 2.6. At all studied doses, the compound statistically significantly ($P \leq 0.05$) increases M in comparison with control animals. We also studied the duration of action № 3212 according to the test of corazole convulsions in mice, both with intraperitoneal and oral administration (into the stomach through a metal probe).

The anticonvulsant effect of effective doses № 3212 (50 mg/kg with i.p. administration, 100 mg/kg with oral administration) were tested every half hour in separate groups of animals, 5-8 in each. Determined TE_{50} (the time during which the anticonvulsant effect persists in 50% of the animals). With intraperitoneal administration, TE_{50} is 270 (243.2 ÷ 299.7), with oral administration - 300 (267.8 ÷ 336) minutes, with $P \leq 0.05$. In fact, with oral administration, the duration of the anticonvulsant action is somewhat longer than with intraperitoneal administration.

Thus, the new synthesized 3-substituted benzothienopyrimidine derivatives showed high anticonvulsant activity, especially according to the test of corasole seizures. The compounds are superior to the well-known drugs used in medical practice, pufemide and ethosuximide, and the most active compound in terms of the therapeutic and protective index is not inferior to the new antiepileptic substance pyratidine, which is at the stage of preclinical trials.

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