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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Design, synthesis, and anticonvulsant evaluation of 4-GABA-3-nitrocoumarines, 1-thiocoumarines, quinolone-2-ones, and their derivatives

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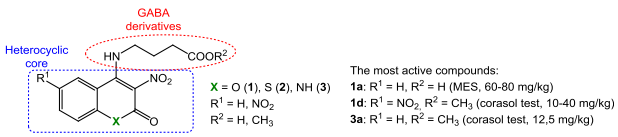
Abstract

The novel group of 4-GABA-3-nitrocoumarines, 1-thiocoumarines, quinolone-2-ones, and their derivatives was designed as potential anticonvulsants using GABA pharmacophore and corresponding heterocyclic moieties. A number of compounds of this group were synthesized and studied in the maximum electroshock seizure (MES) test and in the model of primary-generalized convulsions caused by subcutaneous pentylenetetrazole (scPTZ) in mice. The most active compound in the MES test was found to be **1a** (*N*-(3-nitrocoumarin-4-yl)-4-aminobutyric acid) at a dose range of 60–80 mg/kg that increased the number of survived animals up to 60% in comparison with the control group, whose survival rate was 10%. Compounds **1d** (*N*-(3,6-dinitrocoumarin-4-yl)-4-amino-butyric acid methyl ester) at doses of 10–40 mg/kg and **3a** (*N*-(3-nitro-2-oxo-1,2-dihydroquinolin-4-yl)-4-amino-butyric acid methyl ester) at a dose of 12.5 mg/kg had the most pronounced anticonvulsant effect in scPTZ test.

Graphical Abstract



Keywords Anticonvulsant · Coumarines · 1-Thiocoumarines · Quinolone-2-ones · GABA derivatives

Introduction

The creation of highly active anticonvulsant drugs is an important problem of modern pharmacology. Epilepsy is a chronic disease characterized by repeated unprovoked attacks of motor disorders, sensory, autonomic, or mental functions, resulting from excessive neural discharges in the gray matter of the cerebral cortex. The prevalence of epilepsy in the population is high and affects nearly 1% of the world's population (World Health Organization 2005). Seizures are the same for one patient but significantly differ between patients. According to the international classification of epilepsy, epileptic syndromes and similar diseases, epilepsy is not considered as one single disease with various types of attacks, but it is divided into separate types (epileptic syndromes) with their own electroclinical features and differ in their prognosis and approaches to therapy (Fisher et al. 2017).

Supplementary information The online version of this article (https://doi.org/10.1007/s00044-019-02422-5) contains supplementary material, which is available to authorized users.

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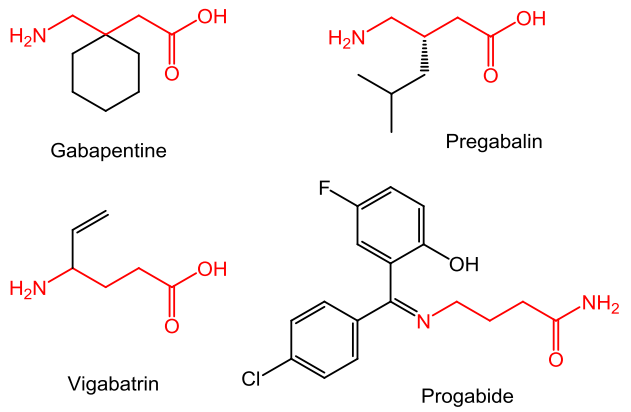


Fig. 1 GABA-containing drugs with anticonvulsant activity

The derivatives of gamma-aminobutyric acid (GABA) can be allocated in a special group among the existing anticonvulsants. The most widely used drugs among them are: gabapentin, pregabalin, vigabatrin, and progabide (Fig. 1). The compounds of this group have significantly different mechanisms of action, and for some of them are not fully clear.

Gabapentin and pregabalin are the GABA analogues, but they do not bind to the GABA receptors and do not directly modulate GABA transport or metabolism (Uchitel et al. 2010). These compounds are the ligands of the auxiliary α2δ subunit site of certain voltage-dependent calcium channels (VDCCs), and thereby act as an inhibitors of α2δ subunit-containing VDCCs (Calandre et al. 2016). Vigabatrin is an irreversible mechanism-based inhibitor of gamma-aminobutyric acid aminotransferase, the enzyme responsible for the catabolism of GABA, which increases the level of GABA in the brain (Rogawski and Löscher 2004). Progabide is an analogue and prodrug of GABA. Via conversion into GABA progabide behaves as an agonist of the GABA receptors (Fromm et al. 1985).

Thus, existence of many drugs with GABA pharmacophore suggests that its use is very attractive for designing of new compounds with a potential anticonvulsant activity.

Result and discussion

Design

The purpose of the research was synthesis and study of anticonvulsant activity of compounds with general formula **1**–**3**, containing unsubstituted GABA pharmacophore or its methyl ester, linked through an amino group to coumarin, thioumarin, or quinolin-2-one heterocycle with one or two nitro groups (Fig. 2). According to the literature, coumarin and quinolin-2-one heterocycles are often used as a core to create compounds with anticonvulsant activity (Srikrishna et al. 2018, Proisl et al. 2017). It was assumed that the combination of GABA and these heterocyclic systems would give compounds with pronounced anticonvulsant effect.

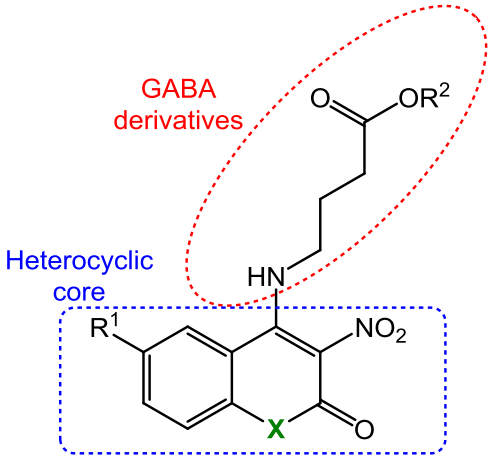
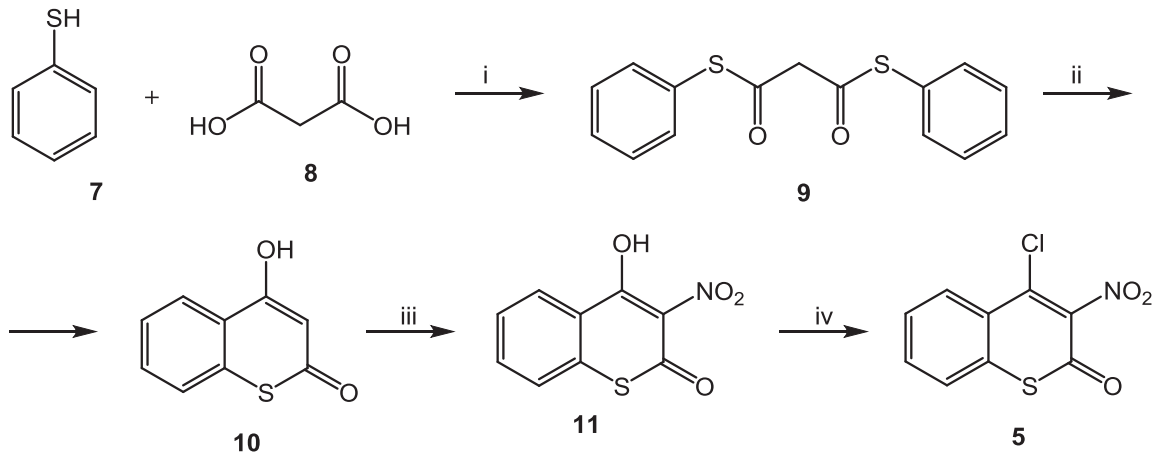


Fig. 2 Design of 4-GABA-3-nitrocoumarines, 1-thiocoumarines, quinolone-2-ones, and their derivatives

Chemistry

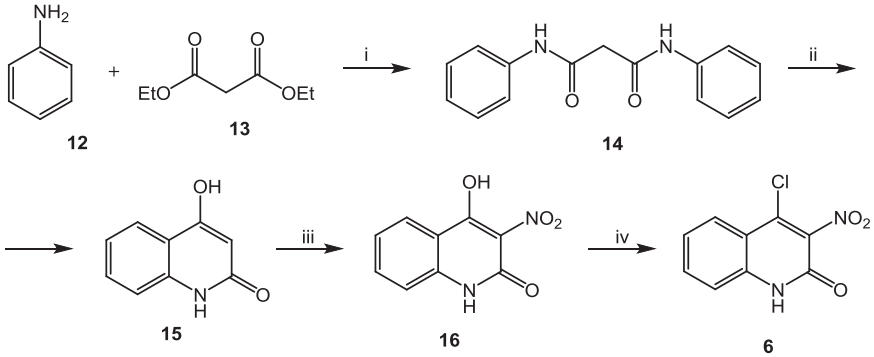
The corresponding starting halogen-substituted heterocyclic compounds were required to obtain the desired compounds. 4-Chloro-3-nitrocoumarin **4a** is a commercially available reagent. 4-Chloro-3,6-dinitrocoumarin **4b** was obtained in accordance with the literature method (Kuleš et al. 1984). 3-Nitro-4-chloro-1-thioumarin **5** was obtained on the basis of thiophenol **7** (Scheme 1). On the first stage thiophenol **7** reacted with malonic acid **8** to give malonic acid dithiophenyl ester **9**. Cyclocondensation of the obtained compound **9** occurs in the presence of $AlCl_3$ as a Lewis acid on the second stage resulted in 4-hydroxy-1-thioumarin **10**. The nitration of this compound was carried out with nitric acid in the presence of sulfuric and acetic acids to give 4-hydroxy-3-nitro-1-thioumarin **11** (Savel'ev et al. 1978). Then hydroxyl group in compound **11** was replaced by a chlorine atom by the action of phosphoryl chloride in DMF, it gave the target compound **5** (Savel'ev et al. 1978).

3-Nitro-4-chloroquinolin-2-one **6** was obtained according to a similar scheme based on aniline **12** (Scheme 2). On the first stage the reaction of malonic acid diethyl ester **11** with aniline **10** gave malonic acid danilide **12**. On the second stage cyclocondensation of the obtained dianilide **12** in 4 hydroxyquinolin-2-one **13** occurs in the presence of



Scheme 1 Reagents and conditions: (i) $POCl_3$, reflux, 40 min; (ii) $AlCl_3$, NaCl, 195 °C, 25 min; (iii) HNO_3, H_2SO_4, AcOH, RT., 3 h; (iv) $POCl_3$, DMF, 2 h

Scheme 2 Reagents and conditions: (i) 210 °C, 5 h; (ii) polyphosphoric acid, 150 °C, 5 h; (iii) HNO_3 (54%), 75 °C, 12 min; (iv) $POCl_3$, TBBAB, MeCN, 40 °C, 30 min, then reflux, 22 min

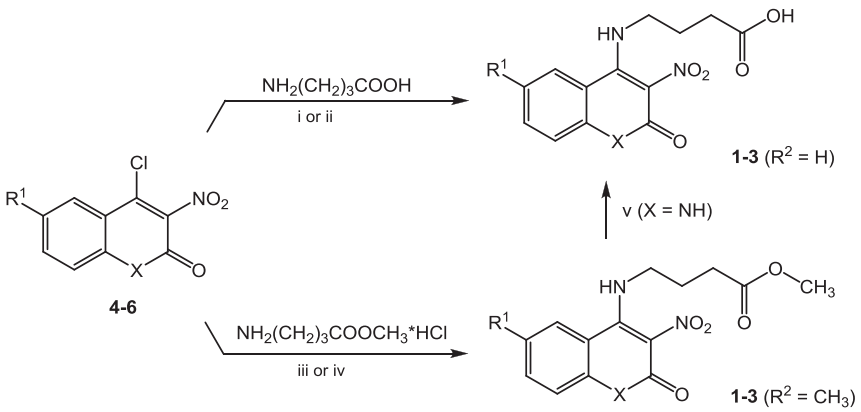


freshly prepared polyphosphoric acid when heated for 5 h (Shobana et al. 1989). The nitration of 4-hydroxyquinolin-2-one **13** was carried out in 54% nitric acid for 20 min at 75 °C to give 4-hydroxy-3-nitroquinolin-2-one **14** (Dolle et al. 1995). 3-Nitro-4-chloroquinolin-2-one **15** was obtained by replacing the hydroxyl group of 4-hydroxy-3-nitroquinolin-2-one **14** with a chlorine atom by the action with phosphoryl chloride in the presence of a quaternary ammonium salt tri-n-butyl benzylammonium bromide (Dolle et al. 1995).

There is scant information in the literature regarding reactions of nitrocoumarines, 1-thiocoumarines, quinolone-2-ones like **4**–**6** with amino acids, and their derivatives to date. Stunić described the interaction of 4-chloro-3-nitrocoumarin **4a** with glycine and alanine and their esters (Stunić et al. 1981). Reactions with unsubstituted amino acids were carried out either by boiling in absolute ethanol for 2 h, or in ethanol in the presence of triethylamine at room temperature with stirring for 6 h. Reactions with hydrochlorides of amino acids esters were carried out in benzene in the presence of ammonia or triethylamine at room temperature or heated for 30 min. The yields of the corresponding products ranged from 50 to 99%. Jung presented the interaction of 3-nitro-4,7-dichloroquinolin-2-one with beta-alanine ethyl ether by refluxing in pyridine for 2 h (Jung et al. 2003). A heterocyclic product with an amino acid substituent was formed in 68% yield.

The target 4-GABA-3-nitrocoumarines, 1-thiocoumarines, quinolone-2-ones, and their derivatives was obtained by reacting the corresponding chlorine-substituted heterocyclic compounds **4**–**6** with GABA or its methyl ester (Scheme 3).

Several reaction conditions have been developed to obtain compounds **1**–**3** with unsubstituted carboxyl group (R^2=H). The first method consisted in the interaction of 4-chlorocoumarins **4** and 4-chlorothycoumarin **5** with a 2.2-fold excess of GABA when boiling in dioxane. Selection of this solvent based on the fact that it dissolves the starting materials and poorly dissolves the reaction product. As the second method, it was proposed to interact equimolar amounts of 4-chlorocoumarins **2** and 4-chlorothycoumarin **5** with GABA in the presence of triethylamine in boiling ethanol. Regardless of the proposed conditions, reactions come with high yields. It should be noted that the reaction



Scheme 3 Reagents and conditions: (i) Dioxane, reflux, t min; (ii) TEA, EtOH, reflux, t min; (iii) TEA, toluene, RT, t min; (iv) Py, reflux, t min; (v) NaOH, H_2O, MeOH, reflux, 40 min

Compound	Conditions	t, min	X	R^1	R^2
1a	i	120	O	H	H
	ii	90			
1b	iii	30	O	H	CH_3
1c	i	60	O	NO_2	H
1d	iii	25	O	NO_2	CH_3
2a	i	120	S	H	H
	ii	180			
2b	iii	30	S	H	CH_3
3a	iv	120	NH	H	CH_3
3b	v	-	NH	H	H

of 3-nitro-4-chloroquinolin-2-one **6** with GABA did not lead to the desired product both under these conditions and when carrying out in other solvents.

The interaction of 4-chlorocoumarins **4** and 4-chlorothycoumarin **5** with GABA methyl ester to form compounds **1**–**2** (R^2=CH_3) occurred much faster than with unsubstituted GABA and under milder conditions: at room temperature in toluene in the presence of triethylamine. Pyridine was chosen for the reaction of 3-nitro-4-chloroquinolin-2-one **6** with GABA ester as the solvent due to the low solubility of the starting material in other solvents. This reaction successfully proceeded by boiling in pyridine for 2 h with the formation of the target ester **3a**. *N*-(3-nitroquinolin-2-one-4-yl)-4-aminobutyric acid **3b** was obtained by ether **3a** saponifying with alkali action in aqueous methanol.

Biological evaluation

The compounds obtained were evaluated in the in vivo rodent models of epilepsy within the order of the Ministry of Health of Russian Federation "Statement of the proper laboratory practice rules" of April 1, 2016, N°199 and according to Anticonvulsant Screening Program (ASP) of the National Institute of Neurological Disorders and Stroke (NINDS).

Anticonvulsant activity in maximal electroshock seizure test (MES)

Initially the compounds were screened in the MES test (Voronina and Nerobkova 2012, Löscher et al. 1991;8(2)). Compound's protection in the MES test indicates the ability of a substance to prevent seizure spread. The MES test caused tonic extension in 87.5–100% and death in 75–90% of mice in control group. Compound **1a** at a dose range of 20–40 mg/kg did not demonstrate protection from seizure and did not increase survival rate in the MES test, while at dose of 60–80 mg/kg it possessed significant protective effect against MES in comparison with the control group (Table 1). Compounds **1b** and **2a** had a dome-shaped dose dependence of activity. Thus, compound **1b** at a dose range of 1–60 mg/kg had the weakest protection, whereas the maximum number of survived animals was recorded at doses of 10 mg/kg and 30 mg/kg. Compound **2a** at doses of 20 and 40 mg/kg increased the survival rate of mice up to 50% and 63% relatively in comparison with the control group, while at a dose range of 60–120 mg/kg the effectiveness of **2a** compound decreased to 38% in terms of survival. The results obtained are presented in Table 1. Compound **3a** at a dose of 12.5 mg/kg increased the survival rate by 63% in comparison with the control group and reduced the percentage of animals with tonic extensions up

Table 1 Anticonvulsant activity of coumarin, thiocoumarin, and quinolinone derivatives in the maximum electroshock test in mice

Group	Dose, mg/kg	Number of animals in the group	% of animals with tonic extensions	% of survived animals
Control, MES	–	10	100	10
Compound **1a** + MES	80	10	60 ($p = 0087$)	60*
	60	10	70 ($p = 0211$)	60*
	40	10	80	30
	20	10	100	10
Compound **1b** + MES	60	8	100	14
	30	8	63 ($p = 0069$)	38 ($p = 0275$)
	10	8	75	38 ($p = 0275$)
	5	8	100	13
	1	8	100	13
Control, MES	–	10	90	20
Compound **1c** + MES	40	8	75	25
	20	8	75	25
	10	8	88	25
Compound **1d** + MES	40	8	75	25
	20	8	100	13
	10	8	100	0
Control, MES	–	8	87, 5	25
Compound **2a** + MES	120	8	50	38
	60	8	50	38
	40	8	75	63 ($p = 0315$)
	20	8	63	50
	10	8	75	25
Compound **2b** + MES	60	8	88	25
	40	8	100	0
	20	8	100	25
	10	8	88	25
Control, MES	–	10	90	20
Compound **3a** + MES	50	8	100	0
	25	8	100	13
	12, 5	8	50 ($p = 0118$)	63 ($p = 0145$)

Asterisk indicates significant difference between the test group and the control group, with $p \le 0.05$ (Fisher's exact test)

to 50%, but with the dose increase (up to 25 and 50 mg/kg) its anticonvulsant effect was not recorded (Table 1). Compounds **1c**, **1d** at doses of 10–40 mg/kg, and compound **2b** at doses of 10–60 mg/kg were identified as MES inactive displaying no significant protection.

Thus, it was identified that in the MES test **1a** at a dose range of 60–80 mg/kg increased the number of survived animals up to 60% in comparison with the control group, whose survival rate was 10%. Compounds **1b**, **2a**, and **3a** showed activity at doses of 10–30, 40, and 12.5 mg/kg at the trend level, respectively.

Among known GABA-derived anticonvulsants in the MES test pregabalin shows activity in the doses of 32–320 mg/kg (mice, i.p.) (Lotarski et al. 2014), vigabatrin significant increase survival of mice (i.p.) at the dose of 50 mg/kg (Sills et al. 1999), and gabapentin at the dose of 419 mg/kg (Tutka et al. 2019). Thus the activity of compound **1a** in the MES test at least not inferior the actions of known GABA derivatives.

Anticonvulsant activity in the model of primary-generalized convulsions caused by subcutaneous pentylenetetrazole (scPTZ)

Anticonvulsant activity of the tested compounds was evaluated against scPTZ to identify the agents rising the seizure threshold (Voronina and Nerobkova 2012, Löscher et al. 1991;8(3)). Administration of scPTZ at a dose of 100 mg/kg to control group developed convulsive manifestations in the following sequence:

- One or more myoclonic twitches of the whole body—100% of mice.
- Repeated clonic seizures of the fore and/or hind limbs with a duration of more than 3 s without loss of the inversion reflex—100% of mice.
- Generalized clonic seizures of the fore and hind limbs with the loss of the inversion reflex—90% of mice.
- The death of animals—100% of mice.

Compound **1a** at a dose range of 10–80 mg/kg and compound **1c** at a dose range of 10–40 mg/kg did not prevent the development of chemical-induced seizures and animal's death (Table 2). Compound **1b** increased the mice survival only at a dose of 40 mg/kg (up to 38%) in comparison with the control group. Compound **1d** showed an outstanding protective effect at all tested doses (10–40 mg/kg), preventing death in 50–63% of animals. Compounds **2a** and **2b** exhibited a maximum anticonvulsant effect at a dose of 10 mg/kg via increasing the survival of mice by maximum 50%. Compound **3a** showed anticonvulsant activity at a dose of 12.5 mg/kg, increasing the survival rate of mice up to 56% (Table 2). Dose increase of compounds **2a**, **2b**, and **3a** diminished the anticonvulsant activity.

Thus, it can be noted that compound **1d** at doses of 10–40 mg/kg and **3a** at a dose of 12.5 mg/kg had the most pronounced anticonvulsant effect in the test with scPTZ. Compounds **1b**, **2a**, and **2b** demonstrated activity in the test at the trend level.

In accordance with the literature data, gabapentin exhibits anticonvulsant activity in scPTZ test in the doses of 25–200 mg/kg, pregabalin in the doses of 10–40 mg/kg, and vigabatrin in the doses of 200–600 mg/kg (mice, i.p.) (Akula et al. 2009, Nieoczym et al. 2012). Synthesized compounds **1d** and **3a** not inferior in its effect to these drugs.

Table 2 Anticonvulsant effect of coumarin, thiocumarin, and quinolinone derivatives in test with subcutaneous pentylenetetrazole in mice

Compound	Dose, mg/kg	Number of animals in the group	% of survived animals
Subcutaneous pentylenetetrazole	100	10	0
1a	80	8	0
	60	8	0
	20	8	0
1b	40	8	38
	20	8	0
	10	8	0
1c	40	8	13
	20	8	13
	10	8	0
1d	40	8	63*
	20	8	63*
	10	8	50#
2a	40	8	25
	20	8	38
	10	8	50#
2b	40	8	38
	20	8	38
	10	8	50#
3a	50	8	0
	25	6	17
	12, 5	9	56*

Asterisk indicates significant difference between the test group and the control group, with $p \le 0.05$ (Fisher's exact test); The hash symbol indicates the tendency to reliability of the difference between the test group and the control group, with $p \le 0.08$ (Fisher's exact test)

Structure activity relationship analysis of synthesized compounds was conducted based on the obtained data of the anticonvulsant activity screening in mice and it indicated that substance's activity essentially depended on their structure. In the MES test, compound **1a** showed the highest activity, it contains a coumarin heterocycle with one nitro group in the 3rd position and GABA pharmacophore. The change of **1a** to its methyl ester (**1b**) resulted in a decrease of anticonvulsant activity. The additional nitro group in compounds **1a** and **1b** (compounds **1c** and **1d**) led to a complete disappearance of the anti-seizure protection. The change of the heteroatom in the coumarin heterocycle demonstrated the decrease in the activity of the compounds. In addition, thiocumarin derivative with GABA pharmacophore **2a** had the anticonvulsant activity, whereas this compound with methyl ester **2b** did not demonstrate efficacy. The activity of the quinolone derivative **3a** was weak, while it was the only compound in this derivatives that possessed the activity with the presence of an ester group.

In the test with scPTZ, compound **1d** was the most active, it contains GABA pharmacophore in the form of methyl ester and a coumarin heterocycle with two nitro groups. The existence of an ester group in this compound was critical for the presence of activity, since such a compound without an ester group (**1c**) was not active. The activity of compound **1b**, that differs from **1d** by the absence of one nitro group, decreased. Coumarin derivative with one nitro group and unsubstituted GABA moiety (compound **1a**) was inactive in the screening test. Replacing of heteroatom in the heterocyclic core with a sulfur atom led to the fact that the compounds with unsubstituted GABA pharmacophore (**2a**) and with methyl ester of GABA pharmacophore (**2b**) had the moderate activity. The quinoline compound **3a** with GABA methyl ester had even more pronounced activity.

As a result, we identified the following trends: compounds with a coumarin heterocyclic core and with unsubstituted GABA pharmacophore were more active in the MES test; compounds with GABA methyl ester exhibited more activity in the test with scPTZ.

Conclusion

The novel group of 4-GABA-3-nitrocoumarines, 1-thiocoumarines, quinolone-2-ones, and their derivatives was designed with GABA pharmacophore and corresponding heterocyclic moieties as potential anticonvulsants. A number of compounds of this group were synthesized and studied in the MES test and in the model of primary-generalized convulsions caused by scPTZ in mice. The most active compound in the MES test was found to be **1a** at dose range of 60–80 mg/kg that increased the number of survived animals up to 60% in comparison with the control group, whose survival rate was 10%. Compounds **1d** at doses of 10–40 mg/kg and **3a** at a dose of 12.5 mg/kg had the most pronounced anticonvulsant effect in scPTZ test.

Experimental section

Chemistry

The chemical reagents used in the synthesis were obtained from commercial supplier and used without purification. TLS was performed on silica gel F_{254} plates (Merck). Melting points are uncorrected and were measured in open capillary tubes, using OptiMelt melting point apparatus. ^{1}H NMR spectral data were recorded on Bruker Fourier 300 spectrometer (300 MHz) in DMSO-d6 and $CDCl_3$ using

the signals of the residual protons of the solvents (δ 2.50 and 7.25, respectively) as the internal standard. High-resolution mass spectra (HRMS) were recorded on a «Bruker micrOTOF II» MS equipment.

We used commercial 4-chloro-3-nitrocoumarin **4a** (AlfaAesar). 4-Chloro-3,6-dinitrocoumarin **4b** was obtained in accordance with the literature method (Kuleš et al. 1984).

Malonic acid dithiophenyl ester (9)

The mixture of 75 mmol (7.81 g) malonic acid **8**, 80 mmol (12.27 g, 7.46 ml) phosphoryl chloride, and 150 mmol (16.52 g, 15.35 ml) thiophenol **7** was refluxed for 40 min. Eighty milliliters of ethanol was added to the cooled mixture. The precipitate formed was filtered and washed with 50 ml of ethanol to obtain a pale blue powder. After recrystallization from 30 ml of ethanol, 17.9 g of a white crystalline substance was obtained (yield 82%). M.p. 94–95 °C. (Lit: M.p. 94.6–95.3 °C (Matsuo and Shindo 2011)). ^{1}H NMR (DMSO, δ, ppm, J/Hz): 4.33 (s, 2H, CH_2); 7.36–7.54 (m, 10H, 2 Ph).

4-Hydroxy-1-thioumarin (10)

60 mmol (8.00 g) aluminum chloride and 40 mmol (2.34 g) sodium chloride were added to 20 mmol (5.76 g) dithiophenyl malonic acid ester **9** and mixed thoroughly. The mixture was heated to 195 °C, and the temperature was maintained for 25 min. After cooling, 100 ml of 1% hydrochloric acid and 100 ml of ethyl acetate were poured into the reaction mass. The organic layer was separated, and the product was extracted with 100 ml of a 10% sodium hydroxide solution. The aqueous solution was separated and acidified to pH = 4, the precipitate was filtered and washed twice with 20 ml of distilled water, obtaining 1.85 g of gray powder (yield 73%). M.p. 211–212 °C (Lit: M.p. = 210 °C (Jamkhandi and Rajagopal 1963)). ^{1}H NMR (DMSO, δ, ppm, J/Hz): 6.07 (s, 1H, HC(3)); 7.48 (m, 2H, HC(6), HC (8)); 7.60 (m, 1H, HC(7)); 8.13 (d, 1H, HC(5), $^3J = 8.1$); 12.26 (br.s, 1H, OH).

4-Hydroxy-3-nitro-1-thioumarin (11) (Savel'ev et al. 1978)

A mixture of 19.8 mmol (1.4 ml) of 65% nitric acid and 19.8 mmol (1.14 ml) of 96% sulfuric acid was added to a suspension of 18 mmol (3.21 g) 4-hydroxy-1-thiocoumarin **10** in 80 ml of glacial acetic acid. The mixture was stirred at room temperature for 3 h. The precipitation was filtered off, washed twice with 10 ml of distilled water, dissolved in boiling toluene, passing the hot solution through activated carbon and evaporated to dryness, yielding 3.78 g of product as a yellow powder (yield 92%). M.p. 149–152 °C (Lit: M.p. 142–143 °C (Savel'ev et al. 1978)). ^{1}H NMR (DMSO, δ, ppm, J/Hz): 7.38 (m, 2H, HC(6), HC(8)); 7.44 (t, 1H, HC (7), $^3J = 8.1$); 8.17 (d, 1H, HC(5), $^3J = 8.1$).

3-Nitro-4-chloro-1-thiocumarin (5) (Savel'ev et al. 1978)

4 mmol (0.373 ml) of phosphoryl chloride was dissolved in 2.5 ml of dimethylformamide and mixed for 30 min. 4 mmol (0.89 g) of 4-hydroxy-3-nitrothiocoumarin (**11**) in 9.6 ml of dimethylformamide was added to the solution, and the mixture was stirred for 2 h. 40 ml of cold water are added to the reaction mixture, the precipitate was filtered off, washed three times with 10 ml of distilled water, and recrystallized from 10 ml of ethanol to obtain 0.85 g of dark yellow crystals (yield 88%). M.p. 171–173 °C (Lit: M.p. 176–177 °C (Savel'ev et al. 1978)). ^{1}H NMR (DMSO, δ, ppm, J/Hz): 7.77, 7.89, 7.94 (three m, each 1H, HC(6), HC (7), HC(8)); 8.38 (d, 1H, HC(5), $^2J = 8.1$).

Malonic acid bis-anilide (14) (Shobana et al. 1989)

A mixture of 200 mmol (18.64 g) aniline (**12**) and 100 mmol (16.02 g) of malonic acid diethyl ester (**13**) was heated at 210 °C for 5 h. After cooling the reaction mixture, the precipitate formed was filtered and recrystallized from ethanol to obtain 19.3 g of a white powder (yield 76%). M. p. 227–229 °C (Lit: M.p. 226–227 °C (Shobana et al. 1989)). ^{1}H NMR (DMSO, δ, ppm, J/Hz): 3.48 (s, 2H, CH_2); 7.07 (dd, 2H, 2H(4), $^3J_1 = 7.6$, $^3J_2 = 7.4$); 7.32 (dd, 4H, 2H (3), 2 H(5), $^3J_1 = 7.6$, $^3J_2 = 8.1$); 7.60 (d, 4H, 2H(2), 2 H (6), $^3J = 8.1$), 10.17 (s, 2 H, 2 NH).

4-Hydroxyquinolin-2-(1H)-one (15) (Shobana et al. 1989)

A mixture of 50 mmol (12.72 g) of malonic acid bis-anilide (14) and 80 g of polyphosphoric acid was heated at 150 °C for 5 h and poured into 400 g of ice. The precipitate formed was filtered, washed with water until neutral and dried, giving 5.88 g of a pale yellow powder (yield 73%). M.p. > 300 °C (Lit: M.p. > 300 °C (Shobana et al. 1989)). ^{1}H NMR (DMSO, δ, ppm, J/Hz): 5.74 (s, 1H, HC(3)); 7.13 (dd, 1H, H(6), $^3J_1 = 8.0$, $^3J_2 = 7.1$); 7.27 (d, 1H, H(8), $^3J = 8.0$); 7.48 (dd, 1H, H(7), $^3J_1 = 8.0$, $^3J_2 = 7.1$); 7.77 (d, 1H, H(5), $^3J = 8.0$); 11.96 (s, 1 H, NH).

4-Hydroxy-3-nitroquinolin-2-(1H)-one (16) (Dolle et al. 1995)

A suspension of 20 mmol (3.22 g) 4-hydroxyquinolin-2-(1H)-one (15) in 30 ml of nitric acid ($d = 1.33$ g/ml) was stirred at room temperature for 10 min and at a temperature

of 75 °C for 20 min The resulting solution was poured into 150 ml of ice-cold water. The precipitate formed was filtered, washed with water until neutral and dried, yielding 3.83 g of an orange powder (yield 93%). M.p. 243–245 °C (Lit: M.p. 250 °C (Dolle et al. 1995)). ^{1}H NMR (DMSO, δ, ppm, J/Hz): 7.27 (m, 2H, H(6), H(8)); 7.65 (m, 1H, H(7)); 8.02 (d, 1H, H(5), $^3J = 7.6$); 11.96 (s, 1 H, NH).

3-Nitro-4-chloroquinolin-2-(1H)-one (6) (Dolle et al. 1995)

15 mmol (3.09 g) 4-hydroxy-3-nitroquinolin-2-(1H)-one (16) and 36 mmol (5.52 g) phosphoryl chloride were added to a solution of 60 mmol (21.38 g) of tri-n-butylbenzylammonium bromide in 60 ml of acetonitrile. The mixture was stirred at 40 °C for 30 min, then heated under reflux for 22 min and evaporated to dryness. 60 ml of water was added to the residue and the mixture was stirred at room temperature for 3 h. The precipitate formed was filtered, washed with water until neutral, and recrystallized from 20 ml of acetone to give 2.02 g of a yellow powder (yield 60%). M.p. 231–233 °C. ^{1}H NMR (DMSO, δ, ppm, J/Hz): 7.46 (m, 2H, H(6), H(8)); 7.80 (m, 1H, H(7)); 8.01 (d, 1H, H(5), $^3J = 7.9$); 11.03 (s, 1 H, NH).

Synthesis of 4-GABA-3-nitrocoumarines and 1-thiocoumarines (1 and 2, R^2=H). General method

Method A

22 mmol (2.27 g) of GABA was added to a solution of 10 mmol of substituted 4-chlorocoumarin (**4**) or nitro-4-chloro-1-thioumarin (**5**) in 20 ml of dioxane. The reaction mass was refluxed for ***t*** minutes, then cooled. The resulting precipitate was filtered, washed with 10 ml of dioxane, and three times with 10 ml of distilled water. The resulting substance was recrystallized from ethanol.

Method B

10 mmol (1.03 g) of GABA and 11 mmol (1.11 g) of triethylamine was added to a solution of 10 mmol of substituted 4-chlorocoumarin (**4**) or nitro-4-chloro-1-thioumarin (**5**) in 200 ml of ethanol. The reaction mass was refluxed for ***t*** minutes, then evaporated to dryness. The residue was washed three times with 50 ml of distilled water and recrystallized from ethanol.

N-(3-nitrocoumarin-4-yl)-4-aminobutyric acid (1a)

Obtained using method A (**t** = 120 min, yield 59%) and method B (**t** = 90 min, yield 69%). Yellow powder. M.p. 210–211 °C. ^{1}H NMR (DMSO, δ, ppm, J/Hz): 1.90 (tt, 2 H, $NHCH_2CH_2$, $^3J_1 = {}^3J_2 = 7.1$); 2.30 (t, 2 H, CH_2COOH, $^3J = 7.1$); 3.18 (t, 2H, $NHCH_2$, $^3J = 7.1$); 7.41 (d, 1 H, H(8), $^3J = 8.2$); 7.45 (dd, 1H, HC(6), $^3J_1 = {}^3J_2 = 8.2$); 7.73 (dd, 1H, HC(7), $^3J_1 = {}^3J_2 = 8.2$); 8.33 (d, 1H, HC(5), $^3J = 8.2$); 8.44 (br.s, 1H, NH); 12.00 (br.s, 1 H, COOH). Anal. Calcd for $C_{13}H_{13}N_2O_6$: C, 53.63; H, 4.14; N, 9.59. Found: C, 53.46; H, 4.24; N, 9.61. HRMS (ESI+) *m/z* calcd for $C_{13}H_{13}N_2O_6$ $[M+H]^+$ 293.0768; found 293.0764.

N-(3,6-dinitrocoumarin-4-yl)-4-aminobutyric acid (1c)

Obtained using method A, **t** = 60 min, yield 81%. Yellow powder. M.p. 183–185 °C. ^{1}H NMR (DMSO, δ, ppm, J/Hz): 1.91 (tt, 2 H, $NHCH_2CH_2$, $^3J_1 = 7.2$, $^3J_2 = 6.8$); 2.30 (t, 2 H, CH_2CO, $^3J = 7.2$); 3.17 (dt, 2 H, $NHCH_2$, $^3J_1 = 6.8$, $^3J_2 = 5.0$); 7.62 (d, 1 H, H(8), $^3J = 9.1$); 8.49 (dd, 1 H, H(7), $^3J = 9.1$, $^4J = 2.3$); 8.83 (br.s, 1 H, NH); 9.39 (d, 1 H, H(5), $^4J = 2.3$), 12.14 (br.s, 1 H, COOH). Anal. Calcd for $C_{13}H_{11}N_3O_8$: C, 46.30; H, 3.29; N, 12.46. Found: C, 46.21; H, 3.50; N, 12.62.

N-(3-nitro-1-thioumarin-4-yl)-4-aminobutyric acid (2a)

Obtained using method A (**t** = 120 min, yield 60%) and method B (**t** = 180 min, yield 74%). Yellow powder. M.p. 215–216 °C. ^{1}H NMR (DMSO, δ, ppm, J/Hz): 1.90 (m, 2 H, $NHCH_2CH_2$); 2.30 (t, 2 H, CH_2COOH, $^3J = 7.1$); 3.18 (m, 2H, $NHCH_2$); 7.62 (m, 3H, HC(6), HC(7), HC(8)); 8.40 (d, 1H, HC(5), $^3J = 8.1$); 8.43 (br.s, 1H, NH); 12.21 (br.s, 1 H, COOH). Anal. Calcd for $C_{13}H_{11}N_2O_5S$: C, 50.65; H, 3.92; N, 9.09; S, 10.40. Found: C, 50.64; H, 3.69; N, 9.33; S, 10.41.

Synthesis of 4-GABA-3-nitrocoumarines and 1-thiocoumarines methyl esters (1 and 2, R^2=CH_3). General method

A thoroughly mixed mixture of 10 mmol (1.54 g) GABA methyl ester hydrochloride solution in 8 ml of water and 20 mmol (2.02 g) of triethylamine was added to a solution of 10 mmol of substituted 4-chlorocoumarin (**4**) or nitro-4-chloro-1-thioumarin (**5**) in 80 ml of toluene with stirring. The mixture was stirred at room temperature for ***t*** minutes. The precipitate was filtered, washed with 50 ml of 1% HCl and 100 ml of distilled water and dried.

N-(3-Nitrocoumarin-4-yl)-4-amino-butyric acid methyl ester (1b)

Yield 79%. **t** = 30 min. Light yellow powder. M.p. 125–126 °C. ^{1}H NMR (DMSO, δ, ppm, J/Hz): 1.92 (tt, 2 H, $NHCH_2CH_2$, $^3J_1 = {}^3J_2 = 7.1$); 2.38 (t, 2 H, CH_2COOH, $^3J = 7.1$); 3.19 (m, 2H, $NHCH_2$); 3.55 (s, 3H, OCH_3); 7.39 (d,

1 H, H(8), $^3J = 8.7$); 7.44 (t, 1H, HC(6), $^3J = 8.0$); 7.73 (t, 1H, HC(7), $^3J = 7.8$); 8.36 (d, 1H, HC(5), $^3J = 8.1$); 8.47 (br.s, 1H, NH). Anal. Calcd for $C_{15}H_{15}N_2O_6$: C, 54.90; H, 4.61; N, 9.15. Found: C, 54.78; H, 4.72; N, 9.06. HRMS (ESI+) *m/z* calcd for $C_{13}H_{13}N_2O_6$ $[M+H]^+$ 307.0925; found 307.0921.

N-(3,6-dinitrocoumarin-4-yl)-4-amino-butyric acid methyl ester (1d)

Yield 79%. **t** = 25 min. Light yellow powder. M.p. 130–131 °C. ^{1}H NMR (DMSO, δ, ppm, J/Hz): 1.94 (tt, 2 H, $NHCH_2CH_2$, $^3J_1 = 7.3$, $^3J_2 = 6.8$); 2.40 (t, 2 H, CH_2CO, $^3J = 7.3$); 3.17 (dt, 2 H, $NHCH_2$, $^3J_1 = 6.8$, $^3J_2 = 5.0$); 3.57 (s, 3 H, OCH_3); 7.62 (d, 1 H, H(8), $^3J = 9.2$); 8.49 (dd, 1 H, H (7), $^3J = 9.2$, $^4J = 2.5$); 8.81 (t, 1 H, NH, $^3J = 5.0$); 9.39 (d, 1 H, H(5), $^4J = 2.5$). Anal. Calcd for $C_{14}H_{13}N_3O_8$: C, 47.87; H, 3.73; N, 11.96. Found: C, 48.08; H, 3.62; N, 12.11.

N-(3-nitrothiocoumarin-4-yl)-4-amino-butyric acid methyl ester (2b)

Yield 77%. **t** = 30 min. Light yellow powder. M.p. 125–126 °C. ^{1}H NMR (DMSO, δ, ppm, J/Hz): 1.92 (tt, 2 H, $NHCH_2CH_2$, $^3J_1 = {}^3J_2 = 7.1$); 2.38 (t, 2 H, CH_2COOH, $^3J = 7.1$); 3.19 (m, 2H, $NHCH_2$); 3.55 (s, 3H, OCH_3); 7.58 (t, 1 H, H(6), $^3J = 8.0$); 7.64 (m, 1H, HC(6)); 7.68 (m, 1H, HC (8)); 8.40 (d, 1H, HC(5), $^3J = 8.5$); 8.36 (br.s, 1H, NH). Anal. Calcd for $C_{14}H_{14}N_2O_5S$: C, 52.17; H, 4.38; N, 8.69; S, 9.95. Found: C, 52.06; H, 4.22; N, 8.87; S, 9.87. HRMS (ESI+) *m/z* calcd for $C_{13}H_{13}N_2O_6$ $[M+H]^+$ 323.0696; found 323.0694.

N-(3-nitro-2-oxo-1,2-dihydroquinolin-4-yl)-4-amino-butyric acid methyl ester (3a)

5 mmol (0.77 g) of 4-aminobutyric acid methyl ester hydrochloride was added to a solution of 1.25 mmol (0.28 g) 3-nitro-4-chloroquinolin-2(1H)-one (**6**) in 10 ml of pyridine. The mixture was heated under reflux for 2 h. The solvent was evaporated to dryness, 15 ml of water was added to the residue, and the product was extracted twice with 15 ml of dichloromethane. The organic layer was evaporated to dryness, and the residue was recrystallized from ethanol to give 0.20 g of a dark yellow powder (yield 63%). M.p. 208–210 °C. ^{1}H NMR (DMSO, δ, ppm, J/Hz): 1.98 (m, 2 H, $NHCH_2CH_2$); 2.36 (t, 2 H, CH_2COOH, $^3J = 7.1$); 3.11 (m, 2H, $NHCH_2$); 3.55 (s, 3 H, OCH_3); 7.24 (t, 1 H, H(6), $^3J = 7.6$); 7.28 (d, 1 H, H(8), $^3J = 7.6$); 7.42 (t, 1 H, $NHCH_2$, $^3J = 4.3$); 7.58 (t, 1H, HC(7), $^3J = 7.5$); 8.20 (d, 1H, HC(5), $^3J = 8.2$); 11.53 (br.s, 1H, NH(quinoline)). Anal. Calcd for $C_{14}H_{15}N_3O_5$: C, 55.08; H, 4.95; N, 13.76. Found: C, 55.01; H, 4.78; N, 13.61.

N-(3-nitro-2-oxo-1,2-dihydroquinolin-4-yl)-4-amino-butyric acid (3b)

A mixture of 0.66 mmol (0.2 g) of N-(3-nitro-2-oxo-1,2-dihydroquinolin-4-yl)-4-amino-butyric acid methyl ester (**3a**), 1.3 mmol (52 mg) sodium hydroxide, 20 ml water, and 20 ml of methanol was refluxed for 40 min, cooled to room temperature and acidified with 1% hydrochloric acid until acidic. The precipitate was filtered, washed with water and dried, yielding 0.12 g of a dark yellow powder (yield 64%).M.p. 119–120 °C. 1H NMR (DMSO, δ, ppm, J/Hz): 1.85 (m, 2 H, $NHCH_2CH_2$); 2.28 (t, 2 H, CH_2COOCH_3, $^3J = 7.2$); 3.08 (m, 2H, $NHCH_2$); 7.24 (m, 1H, HC(6)); 7.27 (d, 1 H, H(8), $^3J = 7.6$); 7.43 (m, 1 H, $NHCH_2$); 7.58 (m, 1H, HC(7)); 8.21 (d, 1H, HC(5), $^3J = 8.2$); 11.53 (br.s, 1H, NH(quinoline)), 12.16 (br.s, 1 H, COOH). Anal. Calcd for $C_{13}H_{13}N_3O_5$: C, 53.61; H, 4.50; N, 14.43. Found: C, 53.58; H, 4.45; N, 14.40.

Pharmacology

Animals

Experiments were performed on white outbred male mice weighing 20–26 g. Experimental animals were obtained from the Stolbovaya nursery of the Federal State Budgetary Institution of Science "Scientific Center for Biomedical Technologies of the Federal Medical-Biological Agency" (Moscow Region). The maintenance of animals complied with the guidelines of laboratory practice and the regulatory documents "Sanitary guidelines for vivarium organization, equipment and maintenance" N° 708n.

The maximal electroshock seizure test

In the MES test, an electrical stimulus of 0.3 s in duration (144 mA, 500/300 V/mA) was delivered via corneal electrodes (Rodent Shocker RS installation, type 221, HarvardApparatus, GmbH, Germany) primed with an electrolyte solution containing an anesthetic agent. Mice were tested in 40 min after intraperitoneal (ip) administration of test compound (Voronina and Nerobkova 2012, Löscher et al. 1991;8(2)). Each dose of the compound was tested on 8–10 animals. The following indicators were recorded: the tonic extension of the hind and forelimbs and the death of animals. The anticonvulsant effect of the tested compounds was assessed by their ability to prevent the development of tonic extension and animal death.

The model of primary-generalized convulsions caused by subcutaneous pentylenetetrazole

Each dose of test compound was tested on groups of at least eight animals. Tested compounds were suspended in saline

and ip administrated 40 min before the introduction of subcutaneous pentylenetetrazole (Sigma-Aldrich, USA). Control group of animals was ip pretreated with saline in an equivalent volume. To obtain a convulsive seizure, pentylenetetrazole was administered subcutaneously to the cervical region of the back at a dose of 100 mg/kg, what produced clonic seizures lasting for a period of at least 5 s in 97% (CD_{97}) of mice in the control group. Animals were observed during 30–60 min after subcutaneous pentylenetetrazole introduction. The number of dead animals was recorded (Voronina and Nerobkova 2012, Löscher et al. 1991;8(3)).

Statistical analysis

Statistical processing of the results was performed using MS Excel 2010 and BioStat 2009 (Analyst Soft Inc.). The normal distribution of the data was determined by the Shapiro–Wilk criterion. The significance of differences between groups was determined using nonparametric criteria: Kruskal–Walis and Fisher's exact test.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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