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Derivatives of a new heterocyclic system – pyrano[3,4-c][1,2,4]triazolo[4,3-a]pyridines: synthesis, docking analysis and neurotropic activity†

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8-Hydrazino derivatives of pyrano[3,4-c]pyridines and derivatives of the new heterocyclic system 3-thioxopyrano[3,4-c][1,2,4]triazolo[4,3-a]pyridines on the basis of methanesulfonates of pyrano[3,4-c]pyridinium were synthesized by optimization of a previously used method. Derivatives of alkylsulfonyl pyrano[3,4-c][1,2,4]triazolo[4,3-a]pyridines were also synthesized. All compounds were evaluated for their neurotropic activity. Among all the compounds tested for anticonvulsant activity by pentylenetetrazole and maximal electric shock seizure (MES) tests, six compounds (**5a**, **5b**, **5e**, **5g**, **5j**, and **5p**) appeared to be active. These compounds were also evaluated for their anxiolytic as well as antidepressant activities using “open field”, “elevated plus maze” (EPM), and “forced swimming” tests, respectively. It should be mentioned that compounds tested by the “rotating rod” method did not affect neuromuscular coordination. The most active compound appeared to be **5g** in all tests. Docking studies of the most active compounds were performed on the GABA_A receptor, SERT and 5-HT_{1A} receptor.

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1. Introduction

In the last few years, with the development of information and communication technologies, and as a consequence of the effect of the deterioration of the biosphere on the population, there has been a significant increase in the number of people with neuropsychiatric disorders.¹ Neurotic disturbances, anxiety, neurosis-like disorders, and stress situations are widespread. Benzodiazepine tranquilizers have been found to be among the most effective antianxiety drugs. The pharmacological action of benzodiazepines is due to their interaction with the supra-molecular membrane GABA_A benzodiazepine receptor complex,² linked to the Cl[−]-ionophore. Benzodiazepines enhance GABA-ergic transmission and this has led to a study of the role of GABA in anxiety. The search

for anxiolytics and anticonvulsive agents has involved glutamate-ergic³ and 5HT-ergic² substances and neuropeptides.⁴

Drugs which are applicable for the treatment of neuropsychiatric diseases often have a toxic effect on the body, leading to emotional disorders, loss of memory and other side effects.^{5–7} First of all, they do not have high selectivity, and therefore have a toxic effect on vital human organs. Furthermore, many drugs active on the central nervous system can alter postural balance, increasing the risk of fractures. Anxiolytics and sedatives, probably due to an increased risk of falls, have been associated with a limited increase in the risk of fracture while neuroleptics may be associated with decreased bone mineral density. As far as antidepressants are concerned, the risk of fractures is dose dependent.⁸ In this regard, the need for new, more effective, selectively acting and low-toxicity drugs is increasing dramatically. For the same reason, it is very important to search for original drugs in new series of chemical compounds: in particular, among the derivatives of new heterocyclic systems.

Five-membered heterocycles with two or three heteroatoms, such as imidazoles, thiazoles, triazoles and others, as well as six-membered heterocycles, are key structural units in many pharmaceutical preparations. Nitrogen-containing heterocycles are biologically active compounds and are widely used in medicine.⁹ Pyridines and their condensed analogues

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possess a wide spectrum of biological activity. Thus, pyranopyridine derivatives exhibit antimicrobial, anti-inflammatory and anticonvulsant activities.^{10–13}

Triazole derivatives, in particular, alkylsulfanyl substituted triazoles and triazolopyridines, also possess a wide range of biological activities, such as antimicrobial, antitumor, antiviral, antioxidant, anti-inflammatory, anticonvulsant and analgesic activity.^{14–22} At the same time, tricyclic derivatives of triazolopyridines have been little studied. There is only one article in the literature on the synthesis of 1,2,4-triazolo[4,3-*a*]pyrano[3,2-*e*]pyridines, which show antihypertensive action.²³

The current study is a continuation of our research on the synthesis and evaluation of the biological activity of condensed pyridines. Herein we reported the design and synthesis of new 8-hydrazinoderivatives of pyrano[3,4-*c*][1,2,4]-triazolo[4,3-*a*]pyridines, by optimization of a previously published method²⁴ and their biological evaluation.

The evaluation of the neurotropic properties of the designed compounds was carried out using a computer-aided drug discovery approach with the computer program PASS.^{25–29}

In order to evaluate the probable mechanism of action, molecular docking was performed.

2. Results and discussion

2.1. Chemistry

As starting compounds for the synthesis, the pyridinium salts (2a, 2b), previously obtained from the corresponding 6-aminopyrano[3,4-*c*]pyridinethiones (1a, 1b) and dimethyl sulfate,²⁴ were used. By interaction of compounds (2a, 2b) with 80% hydrazine hydrate, 8-hydrazinopyrano[3,4-*c*]pyridines (3a, 3b) were synthesized with high yields (Scheme 1). The reaction is accompanied by the rearrangement of the pyridine ring, the mechanism of which is identical to that given in our previous paper.²⁴ A method for the synthesis of compounds (3a, 3b) by direct interaction of compounds (1a, 1b) with hydrazine hydrate (yields 75 and 78%) was reported previously.³⁰ Nevertheless, the use of pyridinium salts (2a, 2b) leads to an increase in product yields to 91 and 88% and a decrease in the reaction time from 12 to 0.5 h.

Scheme 1 Synthesis of title compounds.

In contrast to previous work,³⁰ the optimized method has allowed the exclusion of pyridine from the reaction medium and an increase in the speed of cyclization.

Next, compounds (**3a**, **3b**) were reacted with CS₂ in methanol in the presence of potassium hydroxide, with the release of 3-thioxopyrano[3,4-*c*][1,2,4]triazolo[4,3-*a*]pyridines (**4a**, **4b**). The obtained 3-thioxopyrano[3,4-*c*][1,2,4]triazolo[4,3-*a*]pyridines (**4a**, **4b**) under the action of various alkyl-halogenides turned into derivatives of alkylsulfanyl-triazolo[4,3-*a*]pyridines (**5a–5p**) (Scheme 1).

IR spectra of compounds (**5a–5p**) revealed the presence of absorption bands at 3306–3378 cm^{−1} assigned to the NH group, and strong absorption at 2210–2226 cm^{−1} for the C≡N group. At the same time, the absorption bands at 1235–1250 cm^{−1} characteristic of the C=S group were absent. The ¹H NMR spectra of compounds (**5a**, **5c–5p**) revealed the presence of signals at δ 3.15–4.92 ppm for SCH₂ protons as well as a signal at δ 5.55 ppm for the SCH proton (compound **5b**). ¹³C NMR spectra of compounds (**5a**, **5c–5p**) showed signals at δ at 36.2–41.6 ppm corresponding to the SCH₂ group and signals at δ at 54.2 ppm corresponding to the SCH group (**5b**). The data from the IR and NMR spectra confirm the formation of S-substituted products (**5a–5p**) and are in agreement with the literature.³¹ The regioselectivity of the reaction is explained by the greater polarizability of the sulfur atom compared to the nitrogen atom.³²

2.2. PASS predictions

PASS prediction of neurotropic activities was performed for the designed compounds. The neurotropic activity, mainly anticonvulsant, was predicted with Pa values in range 0.249–0.651 (Table S1†). The low calculated Pa values for some compounds are probably due to the relative novelty of the analyzed structures in comparison with the structures of substances from the PASS training set.^{33,34} Taking this into account, it may be concluded that the studied compounds have some features that are not presented in the anticonvulsant agents used in PASS for the analysis of the structure–activity relationships.

2.3. Biological assays

The study of the neurotropic activity of newly synthesized triazolopyridine derivatives was carried out according to indicators characterizing anticonvulsant, sedative, and anti-anxiety activities and side effects.

The anticonvulsive action of the tested compounds was assessed by evaluating the antagonism between the convulsive pentylenetetrazole (PTZ) action and maximal electroshock seizures (MES).^{35–39} The PTZ induced test is considered an experimental model for the clonic component of epileptic seizures and prognostic anxiolytic²⁸ activities of the compounds. The MES test is used as an animal model for the generalized tonic seizures of epilepsy. Ethosuximide was used as a control.⁴⁰ The side effects of the compounds – neurotoxicity (movement coordination disorder, myorelaxation and

ataxia) were also studied in mice using the “rotating rod” test^{35,41} and maximal tolerated dose (MTD).

To determine the 50% effective dose (ED₅₀, causing an anticonvulsant effect in 50% of animals (outbred mice), calculated by test antagonism to PTZ) and 50% neurotoxic dose (TD₅₀, causing a myorelaxide effect in 50% of animals), a statistical method of probit analysis developed by Litchfield and Wilcoxon was used.^{42,43} From a practical point of view, for the active compounds a protective index (PI = TD₅₀/ED₅₀) was identified.

The evaluation of anticonvulsant activity of all the synthesized compounds revealed that they, to varying degrees, exhibit pentylenetetrazol antagonism. Thus, the majority of compounds, at a dose of 50 mg kg^{−1}, prevented PTZ clonic seizures in only 20–40% of animals. However, compounds **5a**, **5b**, **5e**, **5g**, **5j**, **5p** among all those tested had a pronounced anticonvulsant action with activity order: **5g** > **5a** > **5e** > **5b** = **5j** > **5p**. Intraperitoneal injections of these compounds into mice, starting with a dose of 25 mg kg^{−1}, was accompanied by the prevention of PTZ seizures, and the ED₅₀ ranged from 21 mg kg^{−1} to 45 mg kg^{−1} (Table 1). It should be mentioned that the tested compounds (Table 1) are more active than ethosuximide, according to the test on PTZ but less active than diazepam. The effective dose of ethosuximide (ED₅₀, mg kg^{−1}) in antagonism with PTZ in mice was 155.0 mg kg^{−1}, while that for diazepam is 0.5 mg kg^{−1} (Table 1).

The structure–activity relationship study revealed that the presence of an *N,N*-diphenylacetamide substituent on the sulfur atom of the 1,2,4-triazolo moiety as well as the 5-phenylamino group of the dihydropyrano triazolopyridine moiety (**5g**) are beneficial for anticonvulsant activity, while replacement of the 5-phenylamino group with a 4-methylbenzeneamino group in position 5 (**5p**) had a negative effect. The replacement of an *N,N*-diphenylacetamide substituent (**5g**) by a propylbenzene group (**5a**) slightly decreased the activity. The introduction to the S atom of a triazolo moiety 1-(3-nitrophenyl)ethanone substituent (**5e**) led to a greater decrease in activity. The presence of ethyl 2-phenylacetate in combination with the 5-phenylamino group (**5b**) is not so favorable for anticonvulsant activity. The same result was obtained for the presence of 1-methoxy-4-methylbenzene as R and 4-methylbenzene as Ar (**5j**).

The compounds, tested by the “rotating rod” method, in doses of 50–100 mg kg^{−1} in mice did not interrupt the coordination of movements; no signs of muscle relaxation were observed. The TD₅₀ of the studied compounds ranged from 490 mg kg^{−1} to 610 mg kg^{−1} (Table 1). Ethosuximide in the studied doses of 100–200 mg kg^{−1} in mice also does not cause muscle relaxation.

According to the MES test, neither the compounds studied nor the reference drug exhibited an anticonvulsant effect. They did not provide protection against tonic or clonic seizures, which were caused by MES. Maximal tolerated doses of the studied compounds and ethosuximide are within the limits of 1000–1750 mg kg^{−1}. The protective indexes of the selected compounds were large, especially for compound **5g**, and far exceeded the indexes of ethosuximide and diazepam.

Table 1 Anticonvulsant activity by pentylentetrazol antagonism, myorelaxation and maximal tolerated doses of the examined all compounds (i.p. injection)

Compounds <i>n</i> = 8 (50 mg kg ⁻¹)	Antagonism with PTZ ^a		Myorelaxation ^a (TD ₅₀ , mg kg ⁻¹)	Maximal tolerated dose (MTD, mg kg ⁻¹)	PI
	%	(ED ₅₀ , mg kg ⁻¹) ^a			
5a	60	23.0 (12.2 ÷ 47.5)	560.0 (467 ÷ 672)	1250	24
5b	60	44.0 (23.5 ÷ 68.0)	520.0 (416 ÷ 650)	1200	11.8
5c	20	—	>500	1150	—
5d	40	—	>520	1250	—
5e	60	36.0 (23.8 ÷ 48.2)	490.0 (402 ÷ 598)	1000	13.6
5f	20	—	>500	1000	—
5g	80	21.0 (11.6 ÷ 37.8)	580.0 (475 ÷ 708)	1250	27.6
5h	20	—	>500	1300	—
5i	20	—	>500	1150	—
5j	60	44.0 (33.8 ÷ 57.2)	600.0 (500 ÷ 720)	1500	13.6
5k	40	—	>500	1350	—
5l	40	—	>500	1200	—
5m	40	—	>500	1250	—
5n	40	—	>500	1100	—
5o	40	—	>500	1250	—
5p	60	45.0 (37.5 ÷ 54.0)	610.0 (469 ÷ 693)	1750	13.5
Ethosuximide (200 mg kg ⁻¹)	60	155.0 (117.5 ÷ 205)	520.0 (426 ÷ 634)	1000	3.4
Diazepam (2 mg kg ⁻¹)	80	0.5 (0.4 ÷ 0.7)	2.7 (1.4 ÷ 5.5)	200	5.4

^a *P* = 0.05 at the probability level.

The six most effective compounds – 5a, 5b, 5e, 5g, 5j, and 5p – were studied on the “open field”, “elevated plus maze” (EPM) and “forced swimming” tests at a dose of 50 mg kg⁻¹, since the ED₅₀ values of these compounds were within 50 mg kg⁻¹ at the confidence intervals.

In the “open field” behavioral model,^{44–46} in rats from the control group, the number of horizontal and vertical displacements and the number of examined cells were 25.8, 5.6, and 1.8, respectively (Table 2). The compounds under study cause mild changes in the behavioral indices in comparison with the control – with the injection of the compounds, no marked changes in the horizontal and vertical movements of the animals were observed. However, all the selected compounds 5a, 5b, 5e, 5g, 5j, and 5p, were statistically significant compared with the control, especially compounds 5g and 5p, in increasing the number of sniffing cell examinations, which may be due to the manifestation of the anti-anxiety activity of the compounds (Table 2). Again, the best effect was observed for compound 5g, followed by 5j. Diazepam (2 mg kg⁻¹), in

comparison with the control, causes a significant increase in the number of cells examined, *i.e.* a pronounced anti-anxiety effect.

In order to assess fear, the methodology of the “elevated plus maze” (EPM) developed by S. Pellow *et al.* (1986) was used.⁴⁷ The “elevated plus maze” method is a behavioural assay (fear) used to estimate the anti-anxiety effect of pharmacological agents, synthetic compounds *etc.*^{48–50} In brief, rats or mice are placed at the junction of the four arms of a maze facing an open arm, followed by recording of entries/duration into each arm by a video-tracking system and observer simultaneously for 5 min.

On the EPM model, control animals (mice) are predominantly in closed arms (Table 3). After their administration, compounds 5b, 5e, 5g, and 5j statistically reliably decrease the time spent in closed arms and after intraperitoneal injection of compounds 5a and 5e, decreased numbers of entries into the closed arms were observed. All selected compounds 5a, 5b, 5e, 5g, 5j, and 5p cause a statistically significant

Table 2 Effect of compounds 5a, 5b, 5e, 5g, 5j, and 5p, in rats in the “open field” test (i.p. injection)

Compound <i>n</i> = 8	Dose, mg kg ⁻¹	Amount ^a (absolute data during 5 min)		
		Horizontal displacement	Vertical displacement	Cells
Control	—	25.8 ± 3.7	5.6 ± 2.1	1.5 ± 0.3
5a	50	20.8 ± 4.2	4.8 ± 1.2	3.4 ± 1.5 ^b
5b	50	22.6 ± 5.5	4.6 ± 0.9	3.2 ± 1.2 ^b
5e	50	30.8 ± 3.6	7.0 ± 2.2	3.0 ± 0.2 ^b
5g	50	31.6 ± 5.7	5.4 ± 2.9	4.2 ± 0.3 ^b
5j	50	23.8 ± 5.2	5.4 ± 1.5	3.8 ± 0.9 ^b
5p	50	29.4 ± 6.6	3.05 ± 1.8	4.2 ± 1.7 ^b
Diazepam	2	33.6 ± 4.2	6.4 ± 1.0	5.0 ± 0.9 ^b

^a *P* ≤ 0.05 at a probability level. ^b The differences are statistically significant compared with the control.

Table 3 Effect of compounds **5a**, **5b**, **5e**, **5g**, **5j**, and **5p** on the state of “fear and despair” of mice in the EPM model (observation time 5 min) (i.p. injection)

Compounds	Dose mg kg ⁻¹	Time spent in closed arms ^a /s/	Number of entries into the closed arms ^a	Time spent in the center ^a /s/	Time spent in the open arms ^a /s/
Control	—	278.2(262 ÷ 294.0)	7.0(5.83 ÷ 8.4)	21.8(11.0 ÷ 32.6)	—
5a	50	223(181.2 ÷ 264.8)	4.2(2.6 ÷ 5.8) ^b	67(45.2 ÷ 88.9) ^b	10.0(7.6 ÷ 13.0) ^b
5b	50	199.0(160.1 ÷ 237.9) ^b	8.8(5.0 ÷ 12.6)	95(79.8 ÷ 110.2) ^b	6.0(2.8 ÷ 9.2) ^b
5e	50	241.0(191.8 ÷ 290.2) ^b	4.6(3.8 ÷ 5.52) ^b	51.0(41.5 ÷ 62.7) ^b	8.0(4.2 ÷ 11.8) ^b
5g	50	133.0(78.2 ÷ 187.8) ^b	5.6(4.66 ÷ 6.72)	79(45.2 ÷ 110.8) ^b	88(37.3 ÷ 38.71) ^b
5j	50	200.6(171 ÷ 230.3) ^b	7.4(6.7 ÷ 8.1)	88(70.1 ÷ 105.9) ^b	11.4(6.3 ÷ 16.5) ^b
5p	50	242(203.4 ÷ 280.6)	7.2(3.5 ÷ 10.9)	58.0(44.1 ÷ 71.9) ^b	6.2(3.0 ÷ 9.4) ^b
Diazepam	2	257.5(226.2 ÷ 288.8)	5.5(4.58 ÷ 6.6)	42.5(34.8 ÷ 51.9) ^b	57(47.5 ÷ 68.4) ^b

^a $P \leq 0.05$ at a probability level. ^b The differences are statistically significant compared with the control.

increase compared with the control of the time spent by experienced animals in the center; this is especially expressed in compounds **5b**, **5g** and **5j**, which indicates some sedative activity. After the injection of the compounds, experimental animals, go to the open arms and are located there from 6 s (compound **5b**) to 88 s (compound **5g**) in contrast to the control. The time spent by mice in the open arms after administration of compound **5g** is 88 s, while control mice did not enter the open arms due to fear. Animals treated with diazepam at a dose of 2 mg kg⁻¹ also enter the open arms and stay there.

The data obtained indicate the anxiolytic activity of all selected triazolopyridine derivatives, especially expressed in compound **5g**.

The structure–activity relationships study revealed that as in the case of anticonvulsant activity, the presence of an *N,N*-diphenylacetamide substituent on the sulfur atom of the 1,2,4-triazolo moiety as well as a 5-phenylamino group of the dihydropyrano triazolopyridine moiety (**5g**) are beneficial for anxiolytic activity. Any changes in substitution in position 5 and on the sulfur atom of the molecule were not favorable, leading to compounds with decreased activity.

The “forced swimming” test (FST, also known as Porsolt's test) is one of the most commonly used assays.⁵¹ The FST is used to monitor depressive-like behavior and is based on the assumption that immobility reflects a measure of behavioral despair.

In the “forced swimming” model (Table 4) in control mice, the first immobilization occurs after 92 seconds.

All the selected compounds (**5a**, **5b**, **5e**, **5g**, **5j**, and **5p**) tested at a dose of 50 mg kg⁻¹ cause a statistically significant increase in the time of active swimming or the latent period of the first immobilization to 162 s for compound **5g** and a decrease in the total time immobilization to 43 s. This suggests that the compounds studied at a dose of 50 mg kg⁻¹ show some antidepressant effect. It was observed that compound **5g** increases the time of active swimming or latent period of the first immobilization and decreases the total immobilization time in the same manner as diazepam.

According to the data from Table 4, the activity of compounds in the FST can be presented as follows: **5g** > **5j** > **5b** > **5e** > **5a** > **5p**.

Again, according to the structure–activity relationship study, the presence of an *N,N*-diphenylacetamide substituent on the sulfur atom of the 1,2,4-triazolo moiety as well as the 5-phenylamino group of the dihydropyrano triazolopyridine moiety **5g** are favorable for antidepressant activity. Also, a good influence on activity for 1-methoxy-4-methylbenzene as R and 4-methylbenzene as Ar was observed.

2.4. Molecular docking

2.4.1. Docking to GABA_A receptor. To identify the possible mechanism of action of the compounds, docking on different targets was performed. For anticonvulsant and anxiolytic action, it is known from the bibliography that antiepileptic drugs block sodium channels or enhance the γ -aminobutyric acid (GABA) function by commonly targeting the GABA_A

Table 4 Effect of compounds **5a**, **5b**, **5e**, **5g**, **5j**, and **5p** on “forced swimming” (observation time 6 min) (i.p. injection)

Compounds	Dose mg kg ⁻¹	Time of active swimming (s), latent period I immobilization	Total time of immobilization (s)	Total time of active swimming (s)
Control	—	92(77.1 ÷ 106.9)	81(59.3 ÷ 102.7)	282(235 ÷ 338.4)
5a	50	139(107 ÷ 180.7) ^b	115(88.4 ÷ 149.5)	245(178.8 ÷ 311.2)
5b	50	156(120 ÷ 202.8) ^b	76(36.4 ÷ 115.6)	284(244.2 ÷ 323.8)
5e	50	147(117.6 ÷ 183.8) ^b	84(58.5 ÷ 109.5)	276(205.2 ÷ 346.8)
5g	50	162(128.4 ÷ 195.6) ^b	43(28.9 ÷ 57.1) ^b	317(264.2 ÷ 380.4)
5j	50	158(114.6 ÷ 201.4) ^b	71(46.3 ÷ 95.7)	289(220.3 ÷ 357.7)
5p	5	129(107.6 ÷ 154.8) ^b	106(83.2 ÷ 128.8)	254(190.7 ÷ 317.8)
Diazepam	2	174(144 ÷ 204) ^a	24(13.9 ÷ 34.1) ^b	336(282.6 ÷ 389.4)

^a $P \leq 0.05$ at a probability level. ^b The differences are statistically significant compared with the control.

receptor.^{52,53} Thus, we docked the compounds to the GABA_A receptor and the docking scores of the compounds tested are shown in Table 5.

The best docking score for binding to the GABA_A receptor is predicted for compound 5g, which is in accordance with the experimental results and with a lower free energy of binding than for diazepam (Fig. 1). The docking pose of compound 5g is shown in Fig. 2. Based on the docking results, 5g formed two hydrogen bonds. The first one is between the hydrogen atom of the N–H group of the compound and the oxygen atom of the side chain of Ser187 (distance 2.14 Å), and the second is between the N atom of the CN substituent of the compound and the hydrogen of the side chain of the residue Lys215 (distance 1.93 Å). The benzene rings showed hydrophobic interactions with the residues Thr179, Glu182, Ile181, Ile188 and Val189, while the fused rings interact hydrophobically with the residues Arg216, Asn217, Ile218 and Gly219.

2.4.2. Docking to SERT transporter and 5-HT_{1A} receptor. It is widely known that antidepressant drugs are divided into two main classes: tricyclic antidepressants (TCAs) and selective serotonin reuptake inhibitors (SSRIs).⁵⁴ These drugs target the serotonin (5-HT) transporter (SERT) by inhibiting the transport of serotonin into the presynaptic neuron. SERT is a transmembrane protein located in the membrane of presynaptic neurons and takes part in the termination of serotonergic neurotransmission by removing serotonin from the synaptic cleft. 5-HT_{1A} receptors are activated by the increase in serotonin and decrease the serotonergic neurotransmission that leads to a delay in the onset of antidepressant action.^{55–57} This delay lasts until HT_{1A} receptors become desensitised and the release of serotonin is normalised.

Taking all these factors into account, we decided to dock the synthesized compounds to the serotonin transporter (SERT) as well as to the 5-HT_{1A} receptor, to see whether they may have dual action as an inhibitors of SERT and simultaneously antagonise the presynaptic autoinhibitory 5-HT_{1A} receptors.

For docking to the SERT transporter we used the X-ray crystal structure of LeuT (PDB code: 3F3A); thus LeuT, a prokaryotic homologue of SERT, was the first, and is so far the only, sodium symporter (NSS) transporter family member to be crystallised.⁵⁸ The docking scores of the compound in a complex with the SERT transporter are shown in Table 6. The best docking score is predicted for compound 5g. The docking pose of compound 5g is presented in Fig. 3. Based on the docking results, 5g formed five hydrogen bonds. Two

Fig. 1 Docked conformation of diazepam and the GABA_A receptor complex.

are between the N atom of the triazole ring and the hydrogen atom of the side chain of Arg7 (distances 2.63 and 2.66 Å, respectively) and the other two hydrogen bonds are between the oxygen atom of the C=O group of the compound and the hydrogen of the side chain of Arg7 (distance 3.04 and 3.07 Å, respectively). The last hydrogen bond is formed between the hydrogen atom of N–H and the oxygen atom of the side chain of Asp265 (distance 2.67 Å). The fused rings showed hydrophobic interactions with the residues Ile434, Asp267, Gly433, Glu432, Lys436 and Ala5Tyr143, while the benzene rings interact hydrophobically with the residues Ala5, Trp4, His3, Arg263 and Gln266.

For docking to the 5-HT_{1A} receptor, the crystal structure of the human β₂-adrenergic receptor in complex with the beta blocker and 5-HT_{1A} receptor antagonist, alprenolol (PDB code: 3NYA) was used.^{59,60} Docking of the compounds into the orthosteric binding site of the 5-HT_{1A} receptor indicated that the compounds are good 5-HT_{1A} receptor binders. The best docking score was likewise found for compound 5g (–12.56 kcal mol^{–1}, Table 7), which formed four hydrogen bonds with the residues Tyr308, Asn312 and Tyr316. Furthermore, hydrophobic interactions between the benzene rings and the residues Thr118, Val117, Val114, Phe290, Ser204, Ser203 and Asn293 were observed. The fused rings interact hydrophobically with the residues Tyr199, Thr110, Asp113, Trp109, Trp313, Ile309, Ile94, His93 and Lys305 (Fig. 4). In comparison, the alprenolol docking score was –12.19 kcal mol^{–1} and it also formed hydrogen bonds with the same Asn312 and Tyr316 residues. This is probably a reason for the high action of compound 5g.

Table 5 GABA_A receptor: 4COF binding affinities

Comp	Est. binding energy (kcal mol ^{–1})	Binding affinity score	I–H	Residues
5a	–9.56	–30.97	2	Lys215, Asn217
5b	–8.13	–26.18	1	Lys215
5e	–9.15	–28.43	2	Lys215, Gln224
5g	–10.48	–32.86	2	Ser187, Lys215
5j	–9.11	–28.14	2	Lys215, Gln224
5p	–7.92	–25.44	1	Lys215
Diazepam	–8.72	–26.98	1	Ser205

Fig. 2 (left) Docked pose of compound **5g** and the GABA_A receptor complex; (right) 2D ligand interaction diagram for the docked ligand.

From the docking results we can infer that the compound may be dual-targeted, since it is a good inhibitor of the SERT transporter and simultaneously a good 5-HT_{1A} receptor binder.

3. Experimental

3.1. Material and methods

All chemicals, reagents, and solvents were of commercially high purity grade purchased from Sigma-Aldrich. Melting points (M.p.) were determined on a Boetius microtable. They are expressed in degrees centigrade (°C). ¹H NMR and ¹³C NMR spectra were recorded in DMSO/CCl₄, 1/3 solution on a Varian mercury 300VX 300 (¹H) and 75.462 MHz (¹³C) spectrometer. Chemical shifts are reported as δ (parts per million) relative to TMS (tetramethylsilane) as the internal standard. IR spectra were recorded on Nicolet Avatar 330-FTIR spectrophotometer and the reported wave numbers are given in cm⁻¹. Elemental analyses were performed on a Euro EA 3000 Elemental Analyzer.

The synthesis and their properties of compounds (**1a**, **1b**), (**2a**, **2b**) were described earlier.²⁴

3.2. General procedure for the synthesis of compounds (**3a**, **3b**)

A mixture of pyridinium salt (5 mmol), 80% hydrazine hydrate (10 ml) and methanol (20 ml) was refluxed for 0.5 h, and then the reaction mixture was cooled to room tempera-

ture. The obtained crystals were filtered off, washed with water, dried, and recrystallized from dioxane.

3.2.1. 6-Anilino-8-hydrazino-3,3-dimethyl-3,4-dihydro-1H-pyrano[3,4-c]pyridine-5-carbonitrile (3a). Yield 91%, m.p. 230–231 °C.

3.2.2. 8-Hydrazino-3,3-dimethyl-6-(4-methylphenyl)amino-3,4-dihydro-1H-pyrano[3,4-c]pyridine-5-carbonitrile (3b). Yield 88%, m.p. 228–229 °C.

3.3. General procedure for the synthesis of compounds (**4a**, **4b**)

To a solution of KOH (0.3 g, 5.4 mmol) in methanol (50 ml) compounds (**3a**, **3b**) (4.5 mmol) in CS₂ (5 ml) were added. The mixture was refluxed for 5 h. After cooling, the obtained solution was acidified by 10% hydrochloric acid. The resulting crystals were filtered off, washed with water and recrystallized from a mixture of EtOH–CHCl₃, 1 : 1.

3.3.1. 5-Anilino-8,8-dimethyl-3-thioxo-2,3,7,10-tetrahydro-8H-pyrano[3,4-c][1,2,4]triazolo[4,3-a]pyridine-6-carbonitrile (4a). Yield 91%, m.p. 259–260 °C.

3.3.2. 8,8-Dimethyl-5-[(4-methylphenyl)amino]-3-thioxo-2,3,7,10-tetrahydro-8H-pyrano[3,4-c][1,2,4]triazolo[4,3-a]pyridine-6-carbonitrile (4b). Yield 91%, m.p. 240–241 °C. The spectral data of compounds (**4a**, **4b**) corresponded to the data given in previous work.²⁵

3.4. General procedure for the synthesis of compounds (**5a**–**5p**)

To a solution of KOH (112 mg, 2 mmol) in a mixture of H₂O (2 ml) and EtOH (12 ml) the appropriate compounds **2a**, **2b** (2 mmol) were added. After complete dissolution, the appropriate alkyl halide (2 mmol) was added with cooling, and the reaction mixture was stirred for 12 h at room temperature. The obtained crystals were filtered off, washed with water, dried, and recrystallized from a mixture of EtOH–CHCl₃, 1 : 2.

3.4.1. 5-Anilino-8,8-dimethyl-3-[(3-phenylpropyl)thio]-7,10-dihydro-8H-pyrano[3,4-c][1,2,4]triazolo[4,3-a]pyridine-6-carbonitrile (5a). Yield 79%, m.p. 206–207 °C. IR ν /cm⁻¹: 3325 (NH), 2210 (CN). ¹H NMR (300 MHz, DMSO/CCl₄, 1/3) δ _H: 1.36 (s, 6H, C(CH₃)₂), 1.93–2.04 (m, 2H, SCH₂CH₂), 2.63 (t, *J* =

Table 6 SERT transporter: 3F3A binding affinities

Comp	Est. binding energy (kcal mol ⁻¹)	Binding affinity score	I-H	Residues
5a	−8.71	−27.15	2	Arg7
5b	−11.20	−31.66	3	Arg7, Asp265, Asp267
5c	−9.85	−29.73	2	Arg7, Asp265
5g	−13.89	−35.78	5	Arg7, Asp265
5j	−12.44	−33.62	3	Arg7, Lys436
5p	−8.02	−26.82	2	Arg7

Comp	Est. binding energy (kcal mol ⁻¹)	Binding affinity score	I-H	Residues
5a	-7.78	-26.95	1	Tyr316
5b	-10.13	-29.86	2	Asn312, Tyr316
5e	-8.93	-27.55	2	Tyr308, Asn312
5g	-12.56	-32.71	4	Tyr308, Asn312, Tyr316
5j	-11.85	-31.73	3	Asn312, Tyr316
5p	-7.71	-26.34	1	Asn312
Alprenolol	-12.19	-29.45	1	Tyr308, Asn312, Tyr316

148.8. Anal. calcd for C₂₇H₂₇N₅OS: C 69.06; H 5.80; N 14.91; S 6.83%. Found: C 69.15; H 5.85; N 14.98; S 6.74%.

3.4.2. Ethyl[(5-anilino-6-cyano-8,8-dimethyl-7,10-dihydro-8*H*-pyrano[3,4-*c*][1,2,4]triazolo[4,3-*a*]pyridine-3-yl)thio]-(phenyl)acetate (5b). Yield 78%, m.p. 195–196 °C. IR ν/cm^{-1} : 3326 (NH), 2212 (CN), 1710 (CO). ^1H NMR (300 MHz, DMSO/

CCl_4 , 1/3) δ_{H} : 1.20 (t, $J = 7.1$, 3H, OCH_2CH_3), 1.35 (s, 6H, $\text{C}(\text{CH}_3)_2$), 2.63 (s, 2H, 7- CH_2), 4.11 (q, $J = 7.1$, 2H, OCH_2CH_3), 4.85 (s, 2H, 10- CH_2), 5.55 (s, 1H, SCH), 6.82–6.86 (m, 2H, 2CH), 6.93–6.99 (m, 1H, CH), 7.20–7.36 (m, 7H, 7CH), 9.18 (br s, 1H, NH). ^{13}C NMR (75.462 MHz, DMSO/CCl_4 , 1/3) δ_{C} : 13.5, 25.9, 26.0, 36.3, 54.2, 57.8, 61.1, 69.8, 94.0, 112.9, 117.0, 117.2, 121.6, 128.0, 128.3, 128.8, 131.4, 133.9, 141.1, 141.7, 142.0, 148.2, 168.5. Anal. calcd for $\text{C}_{28}\text{H}_{27}\text{N}_5\text{O}_3\text{S}$: C 65.48; H 5.30; N 13.64; S 6.24%. Found: C 65.41; H 5.26; N 13.72; S 6.33%.

3.4.3. 5-Anilino-3-[[2-(4-chlorophenyl)-2-oxoethyl]thio]-8,8-dimethyl-7,10-dihydro-8H-pyrano[3,4-c][1,2,4]triazolo[4,3-a]pyridine-6-carbonitrile (5c). Yield 79%, m.p. 221–222 °C. IR ν/cm^{-1} : 3320 (NH), 2212 (CN), 1710 (CO). ^1H NMR (300 MHz, DMSO/CCl_4 , 1/3) δ_{H} : 1.35 (s, 6H, $\text{C}(\text{CH}_3)_2$), 2.63 (s, 2H, 7- CH_2), 4.84 (s, 2H, 10- CH_2), 4.85 (s, 2H, SCH₂), 6.87–6.98 (m, 3H, 3CH), 7.21–7.28 (m, 2H, 2CH), 7.46–7.50 (m, 2H, 2CH), 7.99–8.04 (m, 2H, 2CH), 9.32 (br s, 1H, NH). ^{13}C NMR (75.462 MHz, DMSO/CCl_4 , 1/3) δ_{C} : 25.9, 36.3, 41.3, 57.8, 69.8, 95.6, 113.0, 116.8, 121.4, 128.3, 128.4, 128.8, 129.7, 129.8, 133.7, 138.8, 141.1, 142.1, 142.4, 148.4, 191.7. Anal. calcd for $\text{C}_{26}\text{H}_{22}\text{ClN}_5\text{O}_2\text{S}$: C 61.96; H 4.40; N 13.90; S 6.36%. Found: C 61.89; H 4.46; N 13.82; S 6.25%. CAS number: 902014-79-3.

3.4.4. 5-Anilino-8,8-dimethyl-3-[[2-(4-nitrophenyl)-2-oxoethyl]thio]-7,10-dihydro-8H-pyrano[3,4-c][1,2,4]triazolo[4,3-a]pyridine-6-carbonitrile (5d). Yield 78%, m.p. 209–210 °C. IR ν/cm^{-1} : 3328 (NH), 2210 (CN), 1705 (CO), 1540, 1350 (NO_2). ^1H NMR (300 MHz, DMSO/CCl_4 , 1/3) δ_{H} : 1.35 (s, 6H, $\text{C}(\text{CH}_3)_2$), 2.64 (s, 2H, 7- CH_2), 4.84 (s, 2H, 10- CH_2), 4.89 (s, 2H, SCH₂), 6.85–6.96 (m, 3H, 3CH), 7.21–7.27 (m, 2H, 2CH), 8.20–8.25 (m, 2H, 2CH), 8.28–8.33 (m, 2H, 2CH), 9.29 (br s, 1H, NH). ^{13}C NMR (75.462 MHz, DMSO/CCl_4 , 1/3) δ_{C} : 25.9, 36.3, 41.1, 57.8, 69.8, 94.8, 113.0, 116.5, 117.6, 121.3, 123.2, 128.8, 129.4, 131.2, 134.0, 140.9, 142.4, 148.5, 149.8, 192.0. Anal. calcd for $\text{C}_{26}\text{H}_{22}\text{N}_6\text{O}_4\text{S}$: C 60.69; H 4.31; N 16.33; S 6.23%. Found: C 60.61; H 4.37; N 16.21; S 6.30%.

3.4.5. 5-Anilino-8,8-dimethyl-3-[[2-(3-nitrophenyl)-2-oxoethyl]thio]-7,10-dihydro-8H-pyrano[3,4-c][1,2,4]triazolo[4,3-a]pyridine-6-carbonitrile (5e). Yield 80%, m.p. 211–212 °C. IR ν/cm^{-1} : 3320 (NH), 2211 (CN), 1708 (CO), 1540, 1348 (NO_2). ^1H NMR (300 MHz, DMSO/CCl_4 , 1/3) δ_{H} : 1.35 (s, 6H, $\text{C}(\text{CH}_3)_2$), 2.65 (s, 2H, 7- CH_2), 4.83 (s, 2H, 10- CH_2), 4.92 (s, 2H, SCH₂), 6.86–6.96 (m, 3H, 3CH), 7.20–7.26 (m, 2H, 2CH), 7.79 (t, $J = 7.9$, 1H, CH), 8.38–8.46 (m, 2H, 2CH), 8.73 (t, $J = 1.8$, 1H, CH), 9.30 (br s, 1H, NH). ^{13}C NMR (75.462 MHz, DMSO/CCl_4 , 1/3) δ_{C} : 25.9, 36.3, 40.8, 57.8, 69.9, 94.9, 113.1, 116.4, 117.6, 121.2, 122.6, 127.0, 128.8, 129.9, 131.3, 134.1, 136.7, 140.9, 142.4, 147.8, 148.5, 191.4. Anal. calcd for $\text{C}_{26}\text{H}_{22}\text{N}_6\text{O}_4\text{S}$: C 60.69; H 4.31; N 16.33; S 6.23%. Found: C 60.78; H 4.27; N 16.45; S 6.12%.

3.4.6. 2-[[5-Anilino-6-cyano-8,8-dimethyl-7,10-dihydro-8H-pyrano[3,4-c][1,2,4]triazolo[4,3-a]pyridine-3-yl]thio]-N,N-dibutylacetamide (5f). Yield 77%, m.p. 234–235 °C. IR ν/cm^{-1} : 3306 (NH), 2226 (CN), 1675 (CO). ^1H NMR (300 MHz, DMSO/CCl_4 , 1/3) δ_{H} : 0.91 (t, $J = 7.2$, 3H, CH_2CH_3), 0.96 (t, $J = 7.2$, 3H, CH_2CH_3), 1.19–1.59 (m, 8H, 2(CH_2)₂ CH_3), 1.35 (s, 6H,

$\text{C}(\text{CH}_3)_2$), 2.63 (s, 2H, 7- CH_2), 3.22–3.31 (m, 4H, $\text{N}(\text{CH}_2)_2$), 4.22 (s, 2H, SCH₂), 4.85 (s, 2H, 10- CH_2), 6.98–7.04 (m, 3H, 3CH), 7.26–7.33 (m, 2H, 2CH), 10.05 (br s, 1H, NH). ^{13}C NMR (75.462 MHz, DMSO/CCl_4 , 1/3) δ_{C} : 13.3, 19.3, 19.4, 25.9, 29.0, 30.4, 36.4, 39.2, 45.5, 47.2, 57.4, 57.8, 69.8, 90.4, 113.1, 115.3, 117.9, 122.1, 128.6, 132.0, 140.5, 141.5, 141.9, 148.2, 166.6. Anal. calcd for $\text{C}_{28}\text{H}_{36}\text{N}_6\text{O}_2\text{S}$: C 64.59; H 6.97; N 16.14; S 6.16%. Found: C 64.51; H 7.04; N 16.02; S 6.07%.

3.4.7. 2-[[5-Anilino-6-cyano-8,8-dimethyl-7,10-dihydro-8H-pyrano[3,4-c][1,2,4]triazolo[4,3-a]pyridine-3-yl]thio]-N,N-diphenylacetamide (5g). Yield 83%, m.p. 207–208 °C. IR ν/cm^{-1} : 3306 (NH), 2225 (CN), 1673 (CO). ^1H NMR (300 MHz, DMSO/CCl_4 , 1/3) δ_{H} : 1.35 (s, 6H, $\text{C}(\text{CH}_3)_2$), 2.65 (s, 2H, 7- CH_2), 3.97 (s, 2H, SCH₂), 4.86 (s, 2H, 10- CH_2), 6.87–6.91 (m, 2H, 2CH), 6.93–6.99 (m, 1H, CH), 7.13–7.45 (m, 12H, 12CH), 9.41 (br s, 1H, NH). ^{13}C NMR (75.462 MHz, DMSO/CCl_4 , 1/3) δ_{C} : 25.9, 36.3, 38.6, 57.8, 69.8, 93.6, 113.1, 116.8, 117.0, 121.4, 126.1, 128.3, 128.7, 128.9, 129.1, 131.4, 141.1, 141.9, 142.0, 142.5, 148.4, 166.4. Anal. calcd for $\text{C}_{32}\text{H}_{28}\text{N}_6\text{O}_2\text{S}$: C 68.55; H 5.03; N 14.99; S 5.72%. Found: C 68.64; H 4.97; N 14.90; S 5.63%.

3.4.8. 2-[[5-Anilino-6-cyano-8,8-dimethyl-7,10-dihydro-8H-pyrano[3,4-c][1,2,4]triazolo[4,3-a]pyridine-3-yl]thio]-N-(3-methylphenyl)acetamide (5h). Yield 85%, m.p. 216–217 °C. IR ν/cm^{-1} : 3375, 3320 (NH), 2210 (CN), 1665 (CO). ^1H NMR (300 MHz, DMSO/CCl_4 , 1/3) δ_{H} : 1.35 (s, 6H, $\text{C}(\text{CH}_3)_2$), 2.32 (s, 3H, CH₃), 2.63 (s, 2H, 7- CH_2), 4.15 (s, 2H, SCH₂), 4.85 (s, 2H, 10- CH_2), 6.78–6.83 (m, 1H, CH), 6.94–7.02 (m, 3H, 3CH), 7.10 (t, $J = 7.7$, 1H, CH), 7.24–7.36 (m, 4H, 4CH), 9.57 (br s, 1H, NH), 10.07 (br s, 1H, NH). ^{13}C NMR (75.462 MHz, DMSO/CCl_4 , 1/3) δ_{C} : 21.0, 26.0, 36.4, 39.3, 57.8, 69.8, 92.4, 113.0, 116.1, 116.3, 117.4, 119.5, 121.8, 123.7, 127.8, 128.7, 131.7, 137.3, 138.2, 141.2, 141.3, 142.4, 148.4, 165.3. Anal. calcd for $\text{C}_{27}\text{H}_{26}\text{N}_6\text{O}_2\text{S}$: C 65.04; H 5.26; N 16.86; S 6.43%. Found: C 65.13; H 5.21; N 16.78; S 6.51%.

3.4.9. 2-[[5-Anilino-6-cyano-8,8-dimethyl-7,10-dihydro-8H-pyrano[3,4-c][1,2,4]triazolo[4,3-a]pyridine-3-yl]thio]-N-(3,4-dichlorophenyl)acetamide (5i). Yield 79%, m.p. 183–184 °C. IR ν/cm^{-1} : 3376, 3325 (NH), 2212 (CN), 1667 (CO). ^1H NMR (300 MHz, DMSO/CCl_4 , 1/3) δ_{H} : 1.35 (s, 6H, $\text{C}(\text{CH}_3)_2$), 2.63 (s, 2H, 7- CH_2), 4.15 (s, 2H, SCH₂), 4.84 (s, 2H, 10- CH_2), 6.90–7.01 (m, 3H, 3CH), 7.23–7.30 (m, 2H, 2CH), 7.35 (d, $J = 8.8$, 1H, CH), 7.44 (dd, $J_1 = 8.8$, $J_2 = 2.4$, 1H, CH), 7.85 (d, $J = 2.4$, 1H, CH), 9.43 (s, 1H, NH), 10.46 (s, 1H, NH). ^{13}C NMR (75.462 MHz, DMSO/CCl_4 , 1/3) δ_{C} : 25.9, 36.3, 38.7, 57.8, 69.8, 93.4, 113.0, 116.8, 117.1, 118.6, 120.5, 121.7, 125.4, 128.8, 129.7, 131.3, 131.6, 138.2, 141.2, 141.6, 142.5, 148.5, 165.7. Anal. calcd for $\text{C}_{26}\text{H}_{22}\text{Cl}_2\text{N}_6\text{O}_2\text{S}$: C 56.42; H 4.01; N 15.18; S 5.79%. Found: C 56.51; H 4.05; N 15.07; S 5.71%.

3.4.10. 3-[[4-Methoxybenzyl]thio]-8,8-dimethyl-5-[(4-methylphenyl)amino]-7,10-dihydro-8H-pyrano[3,4-c][1,2,4]triazolo[4,3-a]pyridine-6-carbonitrile (5j). Yield 82%, m.p. 198–199 °C. IR ν/cm^{-1} : 3326 (NH), 2210 (CN). ^1H NMR (300 MHz, DMSO/CCl_4 , 1/3) δ_{H} : 1.35 (s, 6H, $\text{C}(\text{CH}_3)_2$), 2.33 (s, 3H, CH₃), 2.60 (s, 2H, 7- CH_2), 3.74 (s, 3H, OCH_3), 4.34 (s, 2H, SCH₂), 4.85 (s, 2H, 10- CH_2), 6.70–6.77 (m, 4H, 4CH), 7.02–

7.07 (m, 2H, 2CH), 7.12–7.16 (m, 2H, 2CH), 9.01 (s, 1 H, NH). ^{13}C NMR (75.462 MHz, DMSO/ CCl_4 , 1/3) δ_{C} : 20.3, 25.9, 36.3, 37.9, 54.4, 57.8, 69.8, 91.4, 113.1, 113.3, 116.0, 117.8, 127.5, 129.2, 129.8, 131.0, 131.4, 138.8, 141.6, 142.3, 148.2, 158.5. Anal. calcd for $\text{C}_{27}\text{H}_{27}\text{N}_5\text{O}_2\text{S}$: C 66.78; H 5.60; N 14.42; S 6.60%. Found: C 66.86; H 5.55; N 14.34; S 6.56%.

3.4.11. 3-[(2-Chlorobenzyl)thio]-8,8-dimethyl-5-[(4-methylphenyl)amino]-7,10-dihydro-8H-pyrano[3,4-c][1,2,4]triazolo[4,3-a]pyridine-6-carbonitrile (5k). Yield 77%, m.p. 221–222 °C. IR ν/cm^{-1} : 3320 (NH), 2212 (CN). ^1H NMR (300 MHz, DMSO/ CCl_4 , 1/3) δ_{H} : 1.34 (s, 6H, $\text{C}(\text{CH}_3)_2$), 2.32 (s, 3H, CH_3), 2.60 (s, 2H, 7- CH_2), 4.51 (s, 2H, SCH_2), 4.85 (s, 2H, 10- CH_2), 6.70–6.75 (m, 2H, 2CH), 7.00–7.05 (m, 2H, 2CH), 7.11–7.24 (m, 2H, 2CH), 7.30–7.40 (m, 2H, 2CH), 9.07 (s, 1H, NH). ^{13}C NMR (75.462 MHz, DMSO/ CCl_4 , 1/3) δ_{C} : 20.3, 25.9, 35.7, 36.3, 57.4, 69.8, 91.5, 113.0, 116.0, 117.9, 126.4, 128.6, 128.9, 129.2, 130.9, 131.1, 131.5, 133.4, 133.8, 138.8, 141.7, 141.9, 148.4. Anal. calcd for $\text{C}_{26}\text{H}_{24}\text{ClN}_5\text{OS}$: C 63.73; H 4.94; N 14.29; S 6.54%. Found: C 63.81; H 4.90; N 14.21; S 6.47%.

3.4.12. 8,8-Dimethyl-5-[(4-methylphenyl)amino]-3-[(4-nitrobenzyl)thio]-7,10-dihydro-8H-pyrano[3,4-c][1,2,4]triazolo[4,3-a]pyridine-6-carbonitrile (5l). Yield 84%, m.p. 225–226 °C. IR ν/cm^{-1} : 3327 (NH), 2211 (CN), 1540, 1352 (NO_2). ^1H NMR (300 MHz, DMSO/ CCl_4 , 1/3) δ_{H} : 1.35 (s, 6H, $\text{C}(\text{CH}_3)_2$), 2.32 (s, 3H, CH_3), 2.62 (s, 2H, 7- CH_2), 4.52 (s, 2H, SCH_2), 4.84 (s, 2H, 10- CH_2), 6.64–6.69 (m, 2H, 2CH), 6.99–7.04 (m, 2H, 2CH), 7.54–7.59 (m, 2H, 2CH), 8.04–8.08 (m, 2H, 2CH), 8.99 (s, 1H, NH). ^{13}C NMR (75.462 MHz, DMSO/ CCl_4 , 1/3) δ_{C} : 20.2, 25.9, 36.0, 36.3, 57.8, 69.8, 94.0, 113.0, 116.6, 117.3, 122.7, 129.3, 129.8, 130.3, 131.1, 139.9, 141.1, 142.1, 144.5, 146.5, 148.5. Anal. calcd for $\text{C}_{26}\text{H}_{24}\text{N}_6\text{O}_3\text{S}$: C 62.38; H 4.83; N 16.79; S 6.41%. Found: C 62.29; H 4.88; N 16.86; S 6.48%.

3.4.13. 8,8-Dimethyl-5-[(4-methylphenyl)amino]-3-[(2-(4-nitrophenyl)-2-oxoethyl)thio]-7,10-dihydro-8H-pyrano[3,4-c][1,2,4]triazolo[4,3-a]pyridine-6-carbonitrile (5m). Yield 81%, m.p. 215–216 °C. IR ν/cm^{-1} : 3328 (NH), 2210 (CN), 1705 (CO), 1540, 1350 (NO_2). ^1H NMR (300 MHz, DMSO/ CCl_4 , 1/3) δ_{H} : 1.35 (s, 6H, $\text{C}(\text{CH}_3)_2$), 2.31 (s, 3H, CH_3), 2.61 (s, 2H, 7- CH_2), 4.83 (s, 2H, 10- CH_2), 4.89 (s, 2H, SCH_2), 6.78–6.83 (m, 2H, 2CH), 7.02–7.07 (m, 2H, 2CH), 8.20–8.25 (m, 2H, 2CH), 8.28–8.32 (m, 2H, 2CH), 9.21 (s, 1H, NH). ^{13}C NMR (75.462 MHz, DMSO/ CCl_4 , 1/3) δ_{C} : 20.2, 25.9, 36.4, 41.6, 57.8, 69.8, 92.9, 113.0, 116.6, 117.4, 123.2, 129.3, 129.4, 130.8, 131.5, 139.3, 139.9, 141.6, 142.0, 148.5, 149.8, 192.1. Anal. calcd for $\text{C}_{27}\text{H}_{24}\text{N}_6\text{O}_4\text{S}$: C 61.35; H 4.58; N 15.90; S 6.07%. Found: C 61.43; H 4.51; N 15.97; S 5.99%.

3.4.14. 2-[(6-Cyano-8,8-dimethyl-5-[(4-methylphenyl)amino]-7,10-dihydro-8H-pyrano[3,4-c][1,2,4]triazolo[4,3-a]pyridine-3-yl)thio]-N-(3-methoxyphenyl)acetamide (5n). Yield 78%, m.p. 206–207 °C. IR ν/cm^{-1} : 3376, 3323 (NH), 2210 (CN), 1667 (CO). ^1H NMR (300 MHz, DMSO/ CCl_4 , 1/3) δ_{H} : 1.36 (s, 6H, $\text{C}(\text{CH}_3)_2$), 2.33 (s, 3H, CH_3), 2.60 (s, 2H, 7- CH_2), 3.75 (s, 3H, OCH_3), 4.14 (s, 2H, SCH_2), 4.83 (s, 2 H, 10- CH_2), 6.54 (ddd, $J_1 = 8.1$, $J_2 = 2.2$, $J_3 = 1.0$, 1H, CH), 6.88–6.93 (m, 2H, 2CH), 7.00–7.14 (m, 4H, 4CH), 7.23 (t, $J = 2.2$, 1H, CH), 9.52 (s, 1H, NH), 10.15 (s, 1H, NH). ^{13}C NMR (75.462 MHz,

DMSO/ CCl_4 , 1/3) δ_{C} : 20.3, 26.0, 36.4, 39.8, 54.3, 57.7, 69.8, 90.3, 104.8, 108.7, 111.2, 113.0, 115.2, 118.4, 128.6, 129.2, 131.4, 132.0, 138.2, 139.4, 141.9, 142.0, 148.4, 159.2, 165.4. Anal. calcd for $\text{C}_{28}\text{H}_{28}\text{N}_6\text{O}_3\text{S}$: C 63.62; H 5.34; N 15.90; S 6.07%. Found: C 63.71; H 5.26; N 16.01; S 5.98%.

3.4.15. 2-[(6-Cyano-8,8-dimethyl-5-[(4-methylphenyl)amino]-7,10-dihydro-8H-pyrano[3,4-c][1,2,4]triazolo[4,3-a]pyridine-3-yl)thio]-N-(4-methoxyphenyl)acetamide (5o). Yield 76%, m.p. 209–210 °C. IR ν/cm^{-1} : 3376, 3323 (NH), 2210 (CN), 1668 (CO). ^1H NMR (300 MHz, DMSO/ CCl_4 , 1/3) δ_{H} : 1.34 (s, 6H, $\text{C}(\text{CH}_3)_2$), 2.34 (s, 3H, CH_3), 2.60 (s, 2H, 7- CH_2), 3.74 (s, 3H, OCH_3), 4.12 (s, 2H, SCH_2), 4.83 (s, 2H, 10- CH_2), 6.74–6.80 (m, 2H, 2CH), 6.89–6.95 (m, 2H, 2CH), 7.06–7.11 (m, 2H, 2CH), 7.39–7.45 (m, 2H, 2CH), 9.61 (s, 1H, NH), 10.03 (s, 1H, NH). ^{13}C NMR (75.462 MHz, DMSO/ CCl_4 , 1/3) δ_{C} : 20.3, 25.9, 36.4, 39.8, 54.5, 57.7, 69.8, 89.9, 113.0, 113.2, 115.0, 118.5, 120.4, 129.2, 131.4, 132.1, 134.0, 141.8, 142.0, 148.4, 155.2, 164.9. Anal. calcd for $\text{C}_{28}\text{H}_{28}\text{N}_6\text{O}_3\text{S}$: C 63.62; H 5.34; N 15.90; S 6.07%. Found: C 63.53; H 5.39; N 15.82; S 6.15%.

3.4.16. 2-[(6-Cyano-8,8-dimethyl-5-[(4-methylphenyl)amino]-7,10-dihydro-8H-pyrano[3,4-c][1,2,4]triazolo[4,3-a]pyridine-3-yl)thio]-N,N-diphenylacetamide (5p). Yield 81%, m.p. 222–223 °C. IR ν/cm^{-1} : 3308 (NH), 2225 (CN), 1675 (CO). ^1H NMR (300 MHz, DMSO/ CCl_4 , 1/3) δ_{H} : 1.35 (s, 6H, $\text{C}(\text{CH}_3)_2$), 2.33 (s, 3H, CH_3), 2.61 (s, 2H, 7- CH_2), 3.98 (s, 2H, SCH_2), 4.85 (s, 2H, 10- CH_2), 6.80–6.85 (m, 2H, 2CH), 7.04–7.09 (m, 2H, 2CH), 7.15–7.45 (m, 10H, 10CH), 9.39 (br s, 1H, NH). ^{13}C NMR (75.462 MHz, DMSO/ CCl_4 , 1/3) δ_{C} : 20.3, 25.9, 36.4, 39.1, 57.8, 69.8, 91.5, 113.1, 115.9, 117.8, 129.2, 129.3, 129.4, 131.0, 131.7, 138.7, 141.7, 142.1, 148.3, 166.5. Anal. calcd for $\text{C}_{33}\text{H}_{30}\text{N}_6\text{O}_2\text{S}$: C 68.97; H 5.26; N 14.62; S 5.58%. Found: C 69.08; H 5.20; N 14.54; S 5.67%.

3.5. Biological evaluation

Compounds were studied for their possible neurotropic activities (anticonvulsant, sedative, anti-anxiety activity) as well as side effects on 450 white mice of both sexes weighing 18–24 g and 50 male rats of the Wistar line weighing 120–140 g.

All groups of animals were maintained at 25 ± 2 °C in the same room, on a common food ration. All of the biological experiments were carried out in full compliance with the European Convention for the Protection of Vertebrates.

All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of “(ETS No 123, Strasbourg, 03/18/1986): Strasbourg (France). European Treaty Series – No 123, March 18, 1986. 11 P” University and Experiments were approved by the Animal Ethics Committee of the *Scientific Technological Center of Organic and Pharmaceutical Chemistry of National Academy of Science of Republic of Armenia*. P.N5 from 24.03.2016.

3.5.1. Evaluation of the anticonvulsant activity of the synthesized compounds. The anticonvulsant effect of compounds (5a–5p) was investigated by tests: pentylenetetrazole (PTZ, Acros Organics, New Jersey, USA) convulsions and

maximal electroshock (MES).^{33–39} The pentylenetetrazole (PTZ) test is an experimental model for inducing myoclonic seizures, as well as for predicting the anxiolytic properties of compounds. Outbred mice (weight 18–22 g.) were used for the study. The PTZ test was carried out in mice by subcutaneous administration of anaesthetic at a dose of 90 mg kg⁻¹ and the effectiveness of the preparations was determined by the prevention of clonic seizures.

The anticonvulsant activity of the compounds was also carried out to prevent the tonic-extensor phase of the convulsive seizure of maximal electroshock (MES). The parameters of the maximal electroshock were 50 mA, duration was 0.2 s, the oscillation frequency was 50 imp s⁻¹ and the evaluation criterion was the warning of the tonic-extensor phase of a convulsive seizure. Substances were administered intraperitoneally in doses of 10, 25, 50, 75, 100 mg kg⁻¹ in suspension with carboxymethylcellulose ("Viadi – Ingredients") with Tween-80 ("Ferak Berlin") 45 minutes before the injection of the convulsive agent pentylenetetrazole causing electrical irritation. An emulsifier was administered to the control animals. Each dose of compound for each test was studied in 8 animals. The drug ethosuximide (Neuraxpharm Arzneimittel GmbH, Germany)³³ administered intraperitoneally in doses from 100 to 300 mg kg⁻¹ was used as a reference.

3.5.2. Evaluation of the psychotropic properties of the synthesized compounds. The psychotropic properties of selected compounds **5a**, **5b**, **5e**, **5g**, **5j**, **5p** were studied with the tests: "open field", "elevated plus maze – EPM" and "forced swimming".

Open field test. The research-motor behavior of rats was studied on a modified "open field" model.^{44–46} For this purpose, an installation was used, the bottom of which was divided into squares with holes (cells). Experiments were performed in the daytime with natural light. Within 5 minutes of the experiment, indicators of sedative and activating behavior were determined – the number of horizontal movements, standing on the hind legs (vertical movements), sniffing of the cells. The number of animals in this model was 8 for each compound, control, and reference drug. The studied compounds were administered to rats at the most effective dose of 50 mg kg⁻¹ intraperitoneally as a suspension with methylcarboxymethylcellulose with Tween-80.

Elevated plus maze – EPM test. Anti-anxiety and sedative effects were studied in a model of "elevated plus maze" in mice.⁴⁷ The labyrinth was a cruciform machine raised above the floor, with a pair of open and closed arms opposed to each other. Normal animals prefer to spend most of their time in the closed (dark) arms of the labyrinth. The anxiolytic effect of the compounds is estimated by the increase in the number of entries into the open (light) arms and the time spent in them, without increasing the total motor activity. The time spent in the closed arms, and the number of attempts to enter the installation center were recorded. The test compounds and the reference drug were injected intraperitoneally before the experiments. An

emulsifier was administered to the control animals. The results were processed statistically ($P \leq 0.05$).

Forced swimming test. To assess "despair and depression" a model "forced swimming" test³⁹ was used. Experimental animals were forced to swim in a glass container (height 22 cm, diameter 14 cm), 1/3 filled with water. Intact mice swim very actively, but soon they will be forced to become immobilized. The latent period of immobilization and the total duration of active swimming immobilization were fixed for 6 minutes. The experiments were conducted under natural light.

3.5.3. Evaluation of incoordination of movements in the rotating rod test. The adverse neurotoxic (muscle relaxant) effect of compounds was studied in doses of 50 to 100 mg kg⁻¹ when administered intraperitoneally, as well as reference drugs in effective anticonvulsant doses. Miorelaxation was investigated using a "rotating rod" test on mice.^{35,41} To this end, mice were planted on a metal rod with a corrugated rubber coating, which rotated at a speed of 5 revolutions per minute. The number of animals that could stay on it for 2 minutes was determined. To determine the ED₅₀ and neurotoxic TD₅₀, the statistical method of penetration developed by Litchfield and Wilcoxon was used.^{41,42} The maximal tolerated dose (MTD) was also studied. The compounds were administered in doses from 500 to 1800 mg kg⁻¹ by i.p. injection.

3.6. Docking studies

Docking calculations were performed using AutoDock 4.0 into the 3D structures of GABA_A receptor (PDB code: 4COF), SERT transporter (PDB code: 3F3A) and 5-HT_{1A} receptor (PDB code: 3NYA). For the preparation of ligand structures, the 2D structure was sketched in ChemDraw12.0 and converted to 3D, mol2 format, for each ligand. Hydrogen atoms were added to the structures. The grid center was calculated for the GABA_A receptor: 125.711 125.735 123.318 (xyz-coordinates), for the SERT transporter: -19.7478, 22.417, -14.3006 and for the 5-HT_{1A} receptor: -8.207, 9.305, -48.61. The grid size was set to 110 × 110 × 110 xyz points with a grid spacing of 0.375 Å. For the simulation, default values of quaternation, translation and torsion steps were applied. The Lamarckian genetic algorithm with default parameters was applied for minimization. The number of docking runs was 100. The graphical representations of all ligand-protein complexes were completed with Discovery Studio 4.1.

4. Conclusion

Sixteen new pyrano[3,4-*c*][1,2,4]triazolo[4,3-*a*]pyridines were synthesized by the optimization of a previously used method and their neurotropic activity was studied. Compounds were tested for their anticonvulsive action by evaluating the antagonism between convulsive pentylenetetrazole action and maximal electroshock seizures (MES). The evaluation of anticonvulsant activity of all the synthesized compounds revealed that compounds **5a**, **5b**, **5e**, **5g**, **5j**, and **5p** among all those tested had a pronounced anticonvulsant action with activity

order: **5g** > **5a** > **5e** > **5b** = **5j** > **5p**. It should be mentioned that they appeared to be more active than ethosuximide according to the test on PTZ. These compounds were also studied for their possible anxiolytic and antidepressant activities by “open field”, “elevated plus maze” (EPM) and “forced swimming” tests. According to the “open field” behavioral model, compounds **5a**, **5b**, **5e**, **5g**, **5j**, and **5p** and especially **5g** and **5p** increase the number of sniffing cell examinations, which may be an indication of the anti-anxiety activity of the compounds. The results of the EPM model confirmed the anxiolytic effect of all tested compounds, especially compound **5g**. The increase in the time of active swimming or the latent period of the first immobilization and decrease in the total time immobilization in the “forced swimming” model is an indication of the antidepressant activity of the tested compounds. The most active again appeared to be compound **5g**. In order to predict the mechanism of action of the synthesized compounds, docking studies were performed. The general conclusion is that compound **5g** could be a lead compound for the design of new neurotropic agents.

Conflicts of interest

The authors declare no conflicts of interest.

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Notes and references

- W. Löscher, H. Klitgaard, R. E. Twyman and D. Schmidt, *Nat. Rev. Drug Discovery*, 2013, 12, 757.
- L. T. Nash and S. Hack, *Expert Opin. Pharmacother.*, 2002, 3(5), 555–571.
- J. E. Kalachnik, T. E. Hanzel, R. Evenich and S. R. Harder, *Am. J. Ment. Retard.*, 2002, 107(5), 376–410.
- A. Piulga, C. Ruzza, A. Rizzi and R. Guerrini, *Eur. J. Neurosci.*, 2012, 36(11), 3531–3537.
- B. B. Satyanarayan, S. Singhal, V. Kamat, V. Yeragani and C. Kulkarni, *J. Clin. Diagn. Res.*, 2007, 3, 205–208.
- D. K. Ziegler, Toxicity to the nervous system of diphenylhydantoin : a review, *Int. J. Neurol.*, 1978, 11(4), 383–400.
- J. Riss, J. Cloyd, J. Gates and S. Collins, *Acta Neurol. Scand.*, 2008, 118(2), 69–86.
- P. Vestergaard, *Curr. Drug Saf.*, 2008, 3(3), 185–189.
- A. F. Pozharskii, A. T. Soldatenkov and A. R. Katritzky, *Heterocycles in Life and Society*, John Wiley and Sons, Ltd., UK, 2011, p. 400.
- R. R. Kumar, S. Perumal, J. C. Menéndez, P. Yogeeswar and D. Sriram, *Bioorg. Med. Chem.*, 2011, 19, 3444.
- W.-J. Kwak, J.-H. Kim, K.-H. Ryu, Y.-B. Cho, S.-D. Jeon and C.-K. Moon, *Biol. Pharm. Bull.*, 2005, 28, 750.
- S. T. Nguyen, S. M. Kwasny, X. Ding, S. C. Cardinale, C. T. McCarthy, H.-S. Kim, H. Nikaido, N. P. Peet, J. D. Williams, T. L. Bowlin and T. J. Opperman, *Bioorg. Med. Chem.*, 2015, 23, 2024.
- E. G. Paronikyan, A. K. Hovhannisyan, A. S. Noravanyan and R. G. Paronikyan, *Chem. J. Arm.*, 1993, XLVI(1–2), 86.
- P. Polucci, P. Magnaghi, M. Angiolini, D. Asa, N. Avanzi, A. Badari, J. Bertrand, E. Casale, S. Cauteruccio, A. Cirila, L. Cozzi, A. Galvani, P. K. Jackson, Y. Liu, S. Magnuson, B. Malgesini, S. Nuvoloni, C. Orrenius, F. R. Sirtori, L. Riceputi, S. Rizzi, B. Trucchi, T. O'Brien, A. Isacchi, D. Donati and R. D'Alessio, *J. Med. Chem.*, 2013, 56, 437.
- M. A. Wafaa, A. G. Neven and S. Eman, *Acta Pharm.*, 2014, 64, 267.
- G. Elgemeie, F. Altalbawy, M. Alfaidi, R. Azab and A. Hassan, *Drug Des., Dev. Ther.*, 2017, 2017(11), 3389.
- Z. Wang, B. Wu, K. L. Kuhen, B. Bursulaya, T. N. Nguyen, D. G. Nguyen and Y. He, *Bioorg. Med. Chem. Lett.*, 2006, 16, 4174.
- H. A. M. El-Sherief, M. Abdel-Aziz and H. M. Abdel-Rahman, *Journal of Advanced Biomedical and Pharmaceutical Sciences*, 2018, 1, 1.
- F. Ahmadi, M. R. Ghayahbashi, M. Sharifzadeh, E. Alipoour, S. N. Ostad, M. Vosooghi, H. R. Khademi and M. Amini, *Med. Chem.*, 2015, 11(1), 69.
- A. Ayati, S. Emami and A. Foroumadi, *Eur. J. Med. Chem.*, 2016, 109, 380.
- M. Amir and K. Shikha, *Eur. J. Med. Chem.*, 2004, 39, 535.
- V. K. Kamboj, P. K. Verma, A. Dhanda and S. Ranjan, *Cent. Nerv. Syst. Agents Med. Chem.*, 2015, 15(1), 17.
- N. V. Kumar and U. C. Mashelkar, *Indian J. Chem., Sect. B: Org. Chem. Incl. Med. Chem.*, 2008, 47, 764.
- E. G. Paronikyan, Sh. Sh. Dashyan, A. S. Noravanyan, R. A. Tamazyan, A. G. Ayvazyan and H. A. Panosyan, *Tetrahedron*, 2015, 71, 2686.
- E. G. Paronikyan, S. S. Dashyan, N. S. Minasyan, G. M. Stepanyan, A. G. Ayvazyan and R. A. Tamazyan, *Chem. Heterocycl. Compd.*, 2016, 52(12), 1078.
- V. V. Poroikov and D. A. Filimonov, *J. Comput.-Aided Mol. Des.*, 2002, 16, 819–824.
- D. A. Filimonov, A. A. Lagunin, T. A. Glorizova, A. V. Rudik, D. S. Druzhilovskii, P. V. Pogodin and V. V. Poroikov, *Chem. Heterocycl. Compd.*, 2014, 50, 444–457.
- N. Savidov, T. A. Glorizova, V. V. Poroikov and V. M. Dembitsky, *Steroids*, 2018, 140, 114–124.
- V. M. Dembitsky, N. Savidov, V. V. Poroikov, T. A. Glorizova and A. B. Imbs, *Appl. Microbiol. Biotechnol.*, 2018, 102, 4663–4674.
- D. A. Filimonov, D. S. Druzhilovskiy, A. A. Lagunin, T. A. Glorizova, A. V. Rudik, A. V. Dmitriev, P. V. Pogodin and V. V. Poroikov, *Biomedical Chemistry: Research and Methods*, 2018, 1, e00004, DOI: 10.18097/BMCRM00004.
- F. H. Karataev and V. V. Klochkov, *NMR spectroscopy in organic chemistry, [in Russian]*, KFY, Kazan, 2013, the 1st part, p. 49.
- A. F. Pozharskii, *Theoretical fundamentals of heterocycle chemistry, [in Russian]*, Moscow, 1985, p. 159.
- A. Geronikaki, J. C. Dearden, D. Filimonov, I. Galaeva, T. L. Garibova, T. Glorizova, V. Kraineva, A. Lagunin, F. Z. Macaev, G. Molodavkin, V. V. Poroikov, S. I. Pogrebnoi, F.

- Shepeli, T. A. Voronina, M. Tsitlakidou and L. Vlad, *J. Med. Chem.*, 2004, **47**(11), 2870–2876.
- 34 A. Geronikaki, E. Babaev, J. Dearden, W. Dehaen, D. Filimonov, I. Galaeva, V. Kraineva, A. Lagunin, F. Macaev, G. Molodavkin, V. Poroikov, S. Pogrebnoi, V. Saloutin, A. Stepanchikova, E. Stingaci, N. Tkach, L. Vlad and T. Voronina, *Bioorg. Med. Chem.*, 2004, **12**(24), 6559–6568.
- 35 H. G. Vogel and W. H. Vogel, Psychotropic and neurotropic activity, in *Drug Discovery and Evaluation: Pharmacological Assays*, ed. H. E. Vogel, Springer, Berlin & N.-Y., 2008, pp. 569–874.
- 36 W. Loscher and D. Schmidt, *Epilepsy Res.*, 1988, **2**, 145.
- 37 E. A. Swinyard, in *Experimental Models of Epilepsy*, ed. D. P. Purpura, J. K. Penry, D. Tower, D. M. Woodbury and R. Walter, Raven Press, New-York, 1992, pp. 433–458.
- 38 K. A. Gevorkyan, G. L. Papayan, S. G. Chshmarityan, R. G. Paronikyan, N. E. Akopyan and A. P. Engoyan, *Pharm. Chem. J.*, 1987, **21**, 95.
- 39 B. Katzung, Drugs Used in Generalized Seizures, *Basic and Clinical pharmacology*, Large Medical Books, McGraw-Hill, 9th edn, 2003.
- 40 P. M. Patsalos, *Epilepsia*, 2005, **46**(9), 140.
- 41 N. W. Dunham and T. S. Miya, *J. Am. Pharm. Assoc., Sci. Ed.*, 1957, **46**(3), 208.
- 42 J. T. Litchfield and F. Wilcoxon, *J. Pharmacol. Exp. Ther.*, 1949, **96**, 99.
- 43 A. H. Mironov, *Manual for preclinical studies of drugs, [in Russian]*, Medicine, Moscow, the 1th part, 2012, pp. 235–250.
- 44 S. E. File, *Behav. Brain Res.*, 2001, **125**, 151.
- 45 S. C. Stanford, *J. Psychopharmacol.*, 2007, **21**, 134.
- 46 L. Prut and C. Belzung, *Eur. J. Pharmacol.*, 2003, **463**(1–3), 3.
- 47 S. Pellow and S. E. File, *Pharmacol., Biochem. Behav.*, 1986, **24**(3), 525.
- 48 S. Pelow, P. Chopin, S. E. File and M. Briley, *J. Neurosci. Methods*, 1985, **14**(3), 149.
- 49 M. C. Jardim, R. L. Nogueira, F. G. Graeff and R. L. Nunes-de-Souza, *Brain Res. Bull.*, 1999, **48**(4), 407.
- 50 F. G. Graeff, C. F. Netto and H. Zangrossi Jr., *Neurosci. Biobehav. Rev.*, 1998, **23**(2), 237.
- 51 R. D. Porsolt, G. Anton, N. Blavet and M. Jalfre, *Eur. J. Pharmacol.*, 1978, **47**(4), 379.
- 52 M. A. Rogawski and W. Löscher, *Nat. Rev. Neurosci.*, 2004, **5**(7), 553–564.
- 53 M. Kammerer, M. P. Rassner, T. M. Freiman and T. J. Feuerstein, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 2011, **384**(1), 47–57.
- 54 M. H. Saier, *Microbiol. Mol. Biol. Rev.*, 2000, **64**, 354–411.
- 55 R. Perrone, F. Berardi, N. A. Colabufo, E. Lacivita, C. Larizza and M. Leopoldo, *J. Pharm. Pharmacol.*, 2005, **57**, 1319–1327.
- 56 P. Blier, *J. Clin. Psychiatry*, 2001, **62**(Suppl 15), 12–17.
- 57 M. Gabrielsen, K. Wołosewicz, A. Zawadzka, J. Kossakowski, G. Nowak, M. Wolak, K. Stachowicz, A. Siwek, A. W. Ravna, I. Kufareva, L. Kozerski, E. Bednarek, J. Sitkowski, W. Bocian, R. Abagyan, A. J. Bojarski, I. Sylte and Z. Chilmoneczyk, *Chem. Biol. Drug Des.*, 2013, **81**(6), 695–706.
- 58 S. K. Singh, C. L. Piscitelli, A. Yamashita and E. Gouaux, *Science*, 2008, **322**, 1655–1661.
- 59 W. Kuipers, R. Link, P. J. Standaar, A. R. Stoit, I. Van Wijngaarden and R. Leurs, *Mol. Pharmacol.*, 1997, **51**, 889–896.
- 60 D. Wacker, G. Fenalti, M. A. Brown, V. Katritch, R. Abagyan and V. Cherezov, *J. Am. Chem. Soc.*, 2010, **132**, 11443–11445.