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Synthesis, neurotropic activity and SAR of new S-alkyl derivatives of 8- pyrazol-1-yl pyrano[3,4-*c*]pyridines

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Dedicated to Prof. Girolamo Cirrincione in recognition of his outstanding contributions to the fields of organic and medicinal chemistry

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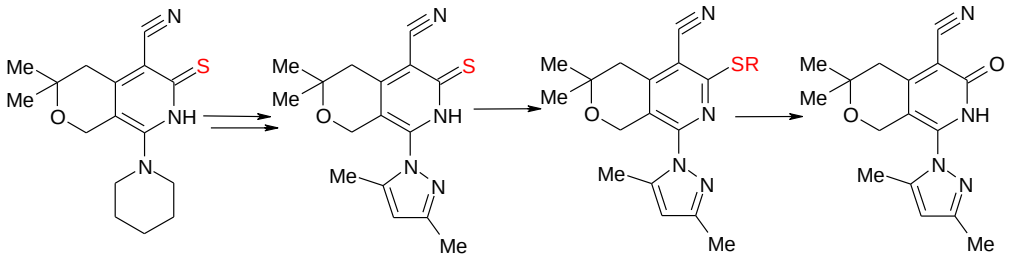
Abstract

8-Hydrazino-3,3-dimethyl-6-thioxo-3,4,6,7-tetrahydro-1*H*-pyrano[3,4-*c*]pyridine was synthesized by a newly developed method. Pyrazolo substituted 6-thioxo- and S-alkyl-derivatives of the pyrano[3,4-*c*]pyridines have also been synthesized. In this work, 8-(3,5-dimethyl-1*H*-pyrazol-1-yl)-3,3-dimethyl-6-oxo-3,4,6,7-tetrahydro-1*H*-pyrano[3,4-*c*]pyridine-5-carbonitrile was also synthesized via an intramolecular nucleophilic substitution (Smiles rearrangement). The study of the neurotropic activity of the synthesized compounds was carried out using convulsive models (pentylenetetrazole and maximal electroshock) and the rotating rod model was used to study central myorelaxation. Compounds were found to antagonize corazole, but did not exhibit muscle relaxation at the studied doses, while simultaneously, they showed anxiolytic and antidepressant psychotropic activity.



Keywords: Pyrano[3,4-*c*]pyridines, *S*-alkylation, Smiles rearrangement, neurotrophic activity, SAR

Introduction

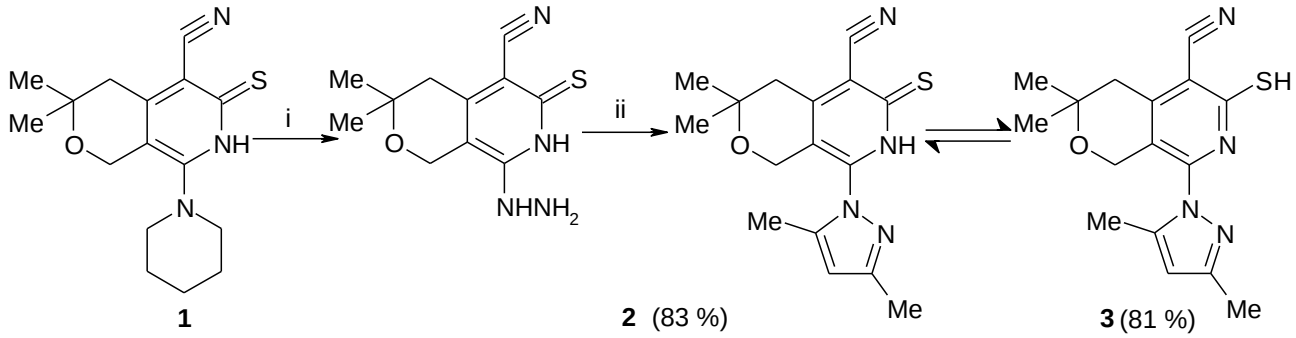
Synthesis of pharmaceuticals for the treatment of neuropsychiatric disorders, in particular epilepsy, is a serious challenge for medicinal synthetic organic chemistry. The antiepileptic agents which are used in medicine most frequently often cause toxic side responses affecting different organs and systems, which include emotional disturbances, impaired memory, etc. In this regard, the search for, and study of novel anticonvulsants possessing desirable psychotropic properties is of unquestionable interest.

The derivatives of condensed pyridines are of interest as biologically active substances. Thus, a large number of substituted pyrazolopyridine derivatives have been found to possess various biological properties including: A(1) adenosine receptor antagonists,[1] antimicrobial,[2] non-anionic antiplatelet agents[3] and compounds with psychotropic effects.[4] Some alkaloids of the pyrano[3,4-*c*]pyridine series possess universal effects, which include hypotensive, anticonvulsant, antipsychotic, anti-inflammatory and antitumor activities.[5-7]

The current study is a continuation of our research on the synthesis and evaluation of neurotropic activity of fused tri- and tetracyclic systems containing pyridine ring.[8-11] Development of new derivatives of 8-pyrazol-1-yl--6-thioalkyl-3,4,6,7-tetrahydro-1*H*-pyrano[3,4-*c*]pyridine are described here, in which we synthesized 8-(3,5-dimethyl-1*H*-pyrazol-1-yl)-3,3-dimethyl-6-oxo-3,4,6,7-tetrahydro-1*H*-pyrano[3,4-*c*]pyridine-5-carbonitriles through a Smiles rearrangement. The neurotropic properties and SAR of the synthesized compounds were also studied.

Results and Discussion

As starting compound, 3,3-dimethyl-8-piperidin-1-yl-6-thioxo-3,4,6,7-tetrahydro-1*H*-pyrano[3,4-*c*]pyridine-5-carbonitrile **1**[12] was used (Scheme 1). We employed compound **1** in the reaction with the highly nucleophilic hydrazine hydrate to obtain 8-hydrazino-3,3-dimethyl-6-thioxo-3,4,6,7-tetrahydro-1*H*-pyrano[3,4-*c*]pyridine-5-carbonitrile **2**. The transamination occurred to displace the piperidine ring and to afford 8-hydrazino analogue **2** (see Scheme 1), the structure of which was elucidated from the ^{1}H NMR spectrum which revealed the appearance of broad singlet for $NHNH_2$ moiety at 6.96 ppm and disappearance of signals for the piperidine ring.



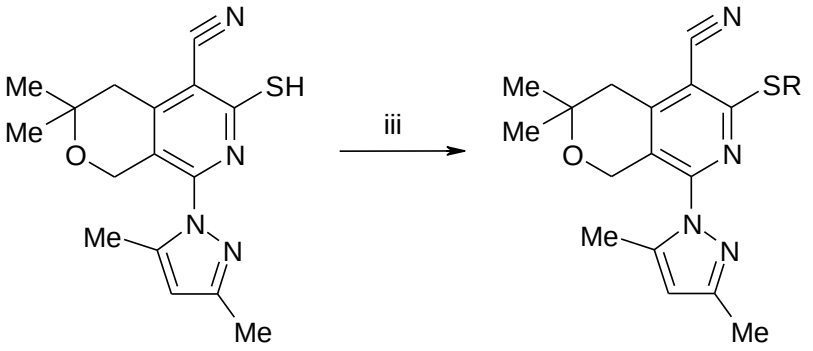
1 **2** (83 %) **3** (81 %)

Reaction conditions and reagents i: NH_2NH_2, n-butanol, heated 2h
ii: $CH_2(COMe)_2$, EtOH, heated 3h

Scheme 1. Synthesis of 8-hydrazino- (compound **2**) and 8-pyrazol-1-yl- (compound **3**) substituted thioxo-pyrano[3,4-*c*]pyridine-5-carbonitriles.

8-Hydrazino-6-thioxo-pyrano[3,4-*c*]pyridine **2**[13] was used as a key intermediate for the synthesis of 8-pyrazol-1-yl-thioxo-pyrano[3,4-*c*]pyridine-5-carbonitrile **3** (Scheme 1). The last was obtained by the interaction of compound **2** with acetylacetone. Compound **3** in the crystalline form exists in the thione tautomeric form. The absorption bands characteristic of the C=S and NH groups appear in the IR spectra of compound **3** in the regions 1230 and 3175 cm^{-1}, respectively.

However, during the reaction of thione **3** with alkyl halides the equilibrium is shifted toward the thiolactim form and *S*-alkyl derivatives **4a-e** are formed in 82-91% yield (Scheme 2). The reaction was carried out at room temperature in 80% aqueous ethanolic KOH. The formation of *S*-alkyl derivatives **4a-e** was confirmed by ^{1}H and ^{13}C NMR spectroscopy. For example, in the ^{1}H NMR spectra of compound **4a** the signal of the SCH_3 group is observed at 2.60 ppm, while the signal of the SCH_2 group of the S-alkyl derivatives **4b-e** is shifted to a lower field - 3.32-4.06 ppm. In the ^{13}C NMR spectra, the signals of SCH_3 (for compound **4a**) and SCH_2 (for compounds **4b-e**) are observed at 12.9 and 17.9-32.0 ppm, accordingly.



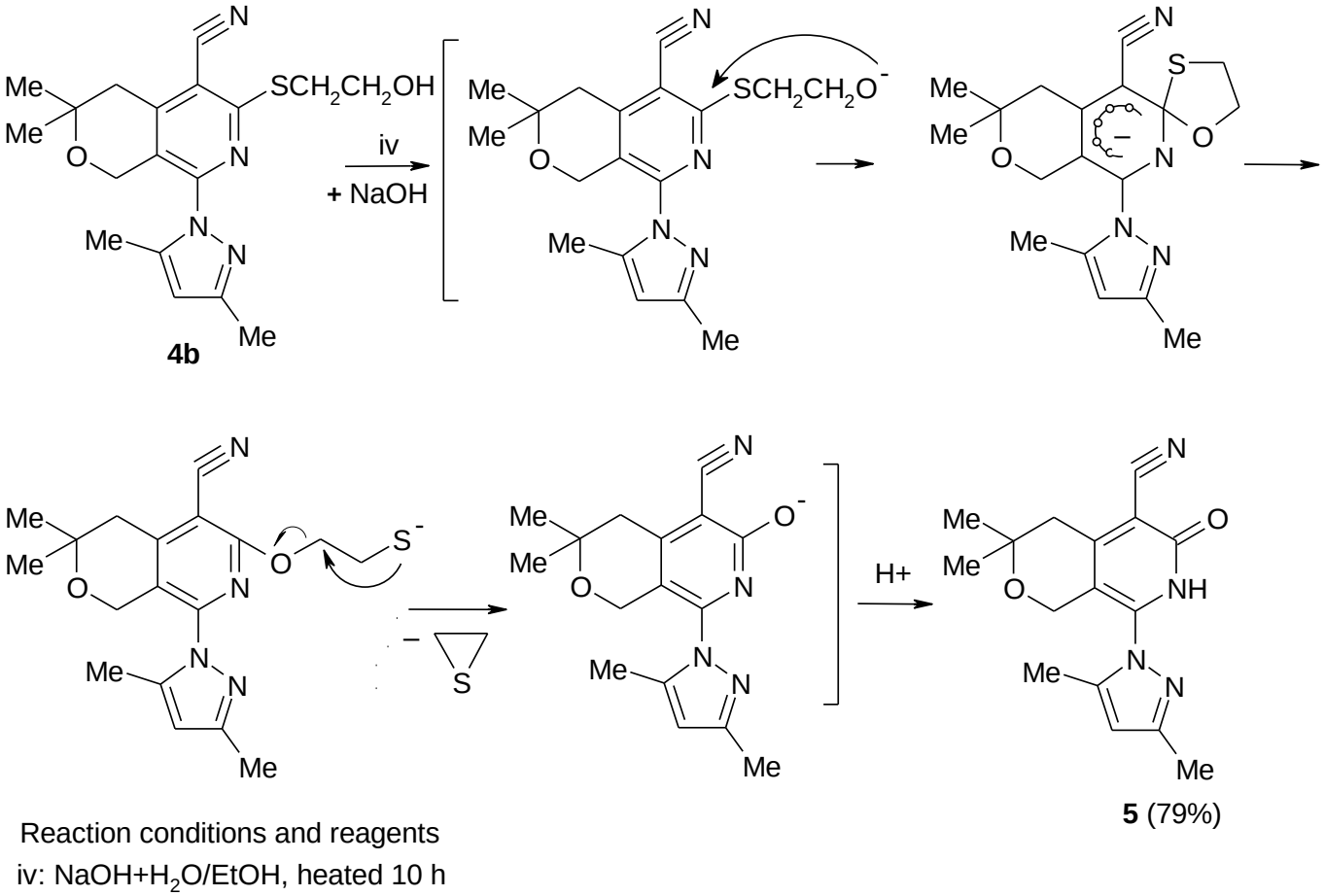
3 **4a-e**

(a) R = CH_3 (91 %)
(b) R = CH_2CH_2OH (85 %)
(c) R = CH_2COOCH_3 (82 %)
(d) R = $CH_2COOC_2H_5$ (88 %)
(e) R = CH_2CCH (90 %)

Reaction conditions and reagents
iii: KOH+EtOH, AlkHal, stirred 8 h, r.t

Scheme 2. Synthesis of 8-pyrazol-1-yl substituted thioalkyl derivatives of pyrano[3,4-*c*]pyridines (compounds **4a-e**).

Finally, a Smiles rearrangement was performed on the 8-(3,5-dimethyl-1*H*-pyrazol-1-yl)-6-[(2-hydroxyethyl)thio]-3,3-dimethyl-3,4-dihydro-1*H*-pyrano[3,4-*c*]pyridine-5-carbonitrile **4b** (Scheme 3 resulting in the formation of 8-(3,5-dimethyl-1*H*-pyrazol-1-yl)-3,3-dimethyl-6-oxo-3,4,6,7-tetrahydro-1*H*-pyrano[3,4-*c*]pyridine-5-carbonitrile **5**. Rearrangement of the compound **4b** occurred in the presence of a tenfold excess of sodium hydroxide in quite good yield (79%). The cleavage product – thiirane, is also formed in the reaction mixture and may polymerize under basic conditions. The structure of compound **5** was confirmed by IR and NMR spectroscopies. The IR spectra of compound **5** showed the amide carbonyl stretching vibration band at 1665 cm^{-1}, a nitrile group at 2222 cm^{-1}, and weak NH group vibration at 3474 cm^{-1}. In the ^{1}H NMR spectra of compound **5** the NH group signal appeared at 11.6-12.6 ppm, while in the ^{13}C NMR spectra of compound **5** the CO group signal appeared at 161.9 ppm. A similar S–O Smiles rearrangement is observed in our previous work,[14] where we also described the proposed mechanism of reaction. In this case, we suggest that the reaction proceeds by the same mechanism, as depicted in Scheme 3.



Scheme 3. Proposed mechanism for the synthesis of 8-(3,5-dimethyl-1*H*-pyrazol-1-yl)-3,3-dimethyl-6-oxo-3,4,6,7-tetrahydro-1*H*-pyrano[3,4-*c*]pyridine-5-carbonitrile (compound **5**).

The neurotropic activity of the new pyrazol-1-yl substituted pyrano[3,4-*c*]pyridine derivatives was next studied. Anticonvulsant activity was investigated by maximal electroshock (MES) and pentylenetetrazole (PTZ) convulsion tests.[15-19] The PTZ subcutaneous test is a petit mal (generalized clonic seizures) model of epilepsy, while MES is grand mal (generalized tonic seizures) model.[20] Comparison with the prominent antiepileptic drugs ethosuximide and the tranquilizer diazepam was performed.[21] The neurotoxicity of the compounds was studied on the ''rotating rod'' model[15] and the maximal tolerated dose (MTD) was also studied. To determine the ED_{50} (50% effective doses) and TD_{50} (50% neurotoxic doses), a statistical method of probity analysis by Litchfield and Wilcoxon was used.[22,23] Finally, the protective ($PI=TD_{50}/ED_{50}$) index was identified for the active compounds.

The studied compounds, at a dose of 50 mg/kg, prevented subcutaneous PTZ clonic seizures in 40-80% of animals. However, compounds **3, 4d, 4e** had more pronounced antagonism to PTZ seizures. The ED_{50} (with intraperitoneal injection) of these compounds ranged from 24 mg/kg to 34 mg/kg. It should be noted that the tested compounds are superior to ethosuximide (ED_{50}=155 mg/kg), according to the anticorazole activity, but inferior to diazepam (ED_{50}=0.5 mg/kg). The studied doses did not violate the coordination of movements on mice and the TD_{50} of the studied compounds ranged from 505 mg/kg to 590 mg/kg. Ethosuximide has the same neurotoxic effect, unlike diazepam, which causes muscle relaxation already at doses of 2-3 mg/kg. Maximal tolerated dose (MTD) of the studied compounds and ethosuximide were in the range of 1000-1350 mg/kg, and for diazepam – 200 mg/kg. The protective indexes of the selected compounds were determined as follows: **3** (PI = 21.7), **4d** (PI = 21.1), **4e** (PI = 22.3), and were found to be high and slightly exceed the indexes of

ethosuximide (PI = 3.4) and diazepam (PI = 5.4). Finally, the compounds tested, as well as the comparison drug did not exhibit an anticonvulsant effect according to the MES test.

The structure-activity relationship (SAR) study on our small set of compounds revealed that replacement of the hydrazine fragment by the pyrazole ring (**3**) in the pyranopyridine system increased the anticonvulsant activity. The same activity is observed in the presence of ethylacetate (**4d**) and prop-2-yn-1-ylsubstituent on the sulfur atom of the 6th position of the pyranopyridine ring, while replacement of these groups with methyl (**4a**), ethyl (**4b**) and methylacetate group decreased activity.

The most effective 3 compounds – **3, 4d, 4e** – were studied for psychotropic activity using the following tests: "open field", "elevated plus maze" (EPM), "forced swimming". The efficacy of the compounds was studied at a dose of 50 mg/kg, since the ED_{50} of these compounds is in the range of 50 mg/kg. In the "open field"[24-26] exploratory behavioral model test in rats, the compounds **3, 4d** and **4e** exhibiting some sedative effect and anti-anxiety activity.

The study of the anxiolytic effect of the compounds was assessed through the elevated plus-maze (EPM) methodology developed by Pellow.[27-30] The administration of compound **4e** resulted in a statistically reliable decrease in the time spent in closed arms and compound **3** also saw a decrease in the number of entries into the closed arms. All selected compounds had effects that were statistically significant, and when compared with the control increased the time spent by experienced animals in the center, which indicates sedative activity, and especially for compound **4e.** After the administration of the selected compounds, experimental animals go to the open arms. The data obtained indicate the anxiolytic activity of all selected compounds, especially expressed in the compounds **3** and **4e.**

Compounds **3, 4d** and **4e** also statistically significantly increased the time of latent period of the first immobilization and decreased the total time of immobilization in the forced swimming test (FST)[31] used to monitor depressive-like behavior. The data obtained thus indicates that compounds **3, 4d** and **4e** exhibit some antidepressant effects.

Conclusions

New pyrazolo substituted heterocyclic systems incorporating pyrano[3,4-*c*]pyridines **4a-e** were synthesized by a new method and their neurotropic activity was studied. In addition, 8-(3,5-dimethyl-1*H*-pyrazol-1-yl)-3,3-dimethyl-6-oxo-3,4,6,7-tetrahydro-1*H*-pyrano[3,4-*c*]pyridine-5-carbonitrile **5** was synthesized by a Smiles rearrangement. The structure of the synthesized compounds was confirmed by physicochemical methods. The evaluation of anticonvulsant activity of all the synthesized compounds revealed that compounds **3, 4d** and **4e** had a pronounced anticonvulsant action. It should be mentioned that replacement of the hydrazine fragment by the pyrazole ring in the pyranopyridine system appeared to increase the anticonvulsant activity, and that replacement of the sulfur atom by the oxygen in the pyranopyridine system decreased the anticonvulsant activity. Furthermore, substitution on the sulfur atom of the 6th position of the pyranopyridine ring with larger alkyl and prop-2-yn-1-yl groups increased the anticonvulsant activity (**4a≤4b≤4c<4d=4e**). The selected **3, 4d** and **4e** compounds exhibited pronounced anti-anxiety and some antidepressant activities, similar to diazepam, as well as sedative activity, the latter especially observed for compound **4e**.

Experimental Section

General. 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). ^{1}H NMR and ^{13}C NMR spectra were recorded in DMSO/CCl_4, 1/3 solution on a Varian mercury 300VX 300 (^{1}H) and 75.465 MHz (^{13}C) 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.

Synthesis of 8-hydrazino-3,3-dimethyl-6-thioxo-3,4,6,7-tetrahydro-1*H*-pyrano[3,4-*c*]pyridine-5-carbonitrile (2). A mixture of compound **1** (3.03 g, 10 mmol), hydrazine hydrate (4 mL, 80 mmol) and *n*-butanol (10 mL) was refluxed for 2 h. The formed precipitate was filtered off and recrystallized from dioxane to give the product as a pure yellow solid (2.08 g, 83%). mp: 278–279 °C. IR (ν/cm^{-1}): 3259-3212 (NH, NH_2), 2202 (CN), 1270 (C=S).^{1}H NMR (300 MHz, DMSO-d_6/CCl_4, 1/3): δ_H 1.20 (s, 6H, $C(CH_3)_2$), 2.50 (s, 2H, CH_2), 4.25 (s, 2H, CH_2), 6.96 (br.s, 3H, $NHNH_2$), 9.49 (br.s, 1H, NH). ^{13}C NMR (75.465 MHz, DMSO-d_6/CCl_4, 1/3) δ_C 25.8 ($2CH_3$), 37.6 (CH_2), 57.1 (OCH_2), 69.5 (C^3), 98.6 (C^5), 102.3 (C^{8a}), 117.6 (CN), 148.4 (C^{4a}), 149.4 (C^8), 171.7 (C^6). Anal. Calcd for $C_{11}H_{14}N_4OS$ (250.32): C, 52.78; H, 5.64; N, 22.38; S, 12.81. Found: C, 52.93; H, 5.47; N, 22.56; S, 12.94.

Synthesis of 8-(3,5-dimethyl-1*H*-pyrazol-1-yl)-3,3-dimethyl-6-thioxo-3,4,6,7-tetrahydro-1*H*-pyrano[3,4-*c*]pyridine-5-carbonitrile (3). A mixture of 8-hydrazino-3,3-dimethyl-6-thioxo-3,4,6,7-tetrahydro-1*H*-pyrano[3,4-*c*]pyridine-5-carbonitrile (**2**) (1.0 g, 4.0 mmol) and acetylacetone (18 mmol) was heated for 1 h, after which absolute ethanol (20 mL) was added and reflux continued for an additional 2 h. The obtained crystals were filtered off, washed with water, dried, and recrystallized from dioxane to give the product as a pure yellow solid (1.02 g, 81%). m.p. 140–141 °C.IR (ν/cm^{-1}): 3175 (NH), 2216 (CN), 1230 (CS).^{1}H NMR (300 MHz, DMSO-d_6/CCl_4, 1/3): δ_H 1.32 (s, 6H, $C(CH_3)_2$), 2.23 (s, 3H, CH_3), 2.32 (s, 3H, CH_3), 2.79 (t, *J* 1.2, 2H, 4-CH_2), 4.38 (t, *J* 1.2, 2H, 1-CH_2), 5.99 (s, 1H, CH), 10.7-13.2 (br, 1H, NH). ^{13}C NMR (75.465 MHz, DMSO-d_6/CCl_4, 1/3): δ_C 13.1 ($2CH_3$), 26.0 ($2CH_3$), 38.2 (CH_2), 59.8 (OCH_2), 68.8 (C^3), 107.5 (C^5), 108.7 (CH), 112.7 (CN), 122.7 (C^{8a}), 141.9 ($\underline{C}CH_3$), 149.4 (C^{4a}), 151.3 ($\underline{C}CH_3$), 152.9 (C^8), 154.6 (C^6). Anal. calcd for $C_{16}H_{18}N_4OS$ (314.41): C, 61.12; H, 5.77; N, 17.82; S, 10.20. Found: C, 61.29; H, 5.85; N, 17.96; S, 10.04.

General procedure for the synthesis of compounds (4a-e). To a solution of KOH (0.112 g, 2.0 mmol) in 80% aqueous ethanol (15 mL) the compound **3** (0.63 g, 2.0 mmol) was added. After complete dissolution, the appropriate alkyl halide (2 mmol) was added, and the reaction mixture was stirred for 8 h at room temperature. The obtained crystals were filtered off, washed with water, dried, and recrystallized from EtOH.

8-(3,5-Dimethyl-1*H*-pyrazol-1-yl)-3,3-dimethyl-6-(methylthio)-3,4-dihydro-1*H*-pyrano[3,4-*c*]pyridine-5-carbonitrile (4a). Reaction of compound **3** (0.63 g, 2.0 mmol) and methyl iodide (284 mg, 2 mmol) according to general procedure afforded 0.60 g (91%) of product **4a** isolated as a white solid: m.p. 160–161 °C. IR (ν/cm^{-1}): 2211 (CN). ^{1}H NMR (300 MHz, DMSO-d_6/CCl_4, 1/3): δ_H 1.34 (s, 6H, $C(CH_3)_2$), 2.23 (s, 3H, CH_3), 2.47 (s, 3H, CH_3), 2.60 (s, 3H, SCH_3), 2.83 (t, *J* 1.2, 2H, 4-CH_2), 4.73 (t, *J* 1.2, 2H, 1-CH_2), 6.01 (s, 1H, CH). ^{13}C NMR (75.465 MHz, DMSO-d_6/CCl_4, 1/3) δ_C: 12.7 (CH_3), 12.9 (SCH_3), 13.0 (CH_3), 26.0 ($2CH_3$), 38.0 (CH_2), 59.4 (OCH_2), 68.8 (C^3), 104.7 (C^5), 108.2 (CH), 113.3(CN), 120.1 (C^{8a}), 141.0 ($\underline{C}CH_3$), 148.8 (C^{4a}), 148.9 ($\underline{C}CH_3$), 150.4 (C^8), 159.1 (C^6). Anal. calcd for $C_{17}H_{20}N_4OS$ (328.43): C, 62.17; H, 6.14; N, 17.06; S, 9.76. Found: C, 62.29; H, 6.07; N, 16.93; S, 9.91.

8-(3,5-Dimethyl-1*H*-pyrazol-1-yl)-6-[(2-hydroxyethyl)thio]-3,3-dimethyl-3,4-dihydro-1*H*-pyrano[3,4-*c*]pyridine-5-carbonitrile (4b). Reaction of compound **3** (0.63 g, 2.0 mmol) and 2-bromoethanol (250 mg, 2 mmol) according to general procedure afforded 0.56 g (85%) of product **4b** isolated as a white solid: m.p. 65–

66 °C. IR (ν/cm^{-1}): 2215 (CN). ^{1}H NMR (300 MHz, DMSO-d_6/CCl_4, 1/3): δ_H 1.34 (s, 6H, $C(CH_3)_2$), 2.23 (s, 3H, CH_3), 2.46 (s, 3H, CH_3), 2.82 (t, *J* 1.2 Hz, 2H, 4-CH_2), 3.32 (t, *J* 6.7 Hz, 2H, SCH_2), 3.64 (dt, *J* 6.7, 5.6 Hz, 2H, $SCH_2\underline{CH_2}$), 4.71 (t, *J* 1.2 Hz, 2H, 1-CH_2), 4.73 (t, *J* 5.6 Hz, 1H, OH), 6.01 (s, 1H, CH). ^{13}C NMR (75.465 MHz, DMSO-d_6/CCl_4, 1/3) δ_C: 12.7 (CH_3), 13.0 (CH_3), 26.1 ($2CH_3$), 32.0 (SCH_2), 38.0 (CH_2), 59.4 (OCH_2), 59.6 (CH_2OH), 68.8 (C^3), 104.9 (C^5), 108.1 (CH), 113.4 (CN), 120.3 (C^{8a}), 141.1 ($\underline{C}CH_3$), 148.8 (C^{4a}), 148.9 ($\underline{C}CH_3$), 150.5 (C^6), 158.8 (C^8). Anal. calcd for $C_{18}H_{22}N_4O_2S$ (358.46): C,60.31; H, 6.19; N,15.63; S,8.95. Found: C 60.38; H 6.23; N 15.71; S 8.86.

Methyl {[5-cyano-8-(3,5-dimethyl-1*H*-pyrazol-1-yl)-3,3-dimethyl-3,4-dihydro-1*H*-pyrano[3,4-*c*]pyridin-6-yl]thio}acetate (4c). Reaction of compound **3** (0.63 g, 2.0 mmol) and methyl chloroacetate (217 mg, 2 mmol) according to general procedure afforded 0.64 g (82%) of product **4c** isolated as a white solid: m.p. 141–142 °C. IR (ν/cm^{-1}): 2220 (CN), 1756 (CO). ^{1}H NMR (300 MHz, DMSO-d_6/CCl_4, 1/3): δ_H 1.34 (s, 6H, $C(CH_3)_2$), 2.23 (s, 3H, CH_3), 2.37 (s, 3H, CH_3), 2.85 (t, *J* 1.2 Hz, 2H, 4-CH_2), 3.63 (s, 3H, OCH_3), 4.06 (s, 2H, SCH_2), 4.70 (t, *J* 1.2 Hz, 2H, 1-CH_2), 6.01 (s, 1H, CH). ^{13}C NMR (75.465 MHz, DMSO/CCl_4, 1/3) δ_C: 12.1 (CH_3), 13.0 (CH_3), 26.1 ($2CH_3$), 31.2 (SCH_2), 38.0 (CH_2), 51.8 (OCH_3), 59.3 (OCH_2), 68.9 (C^3), 104.8 (C^5), 108.0 (CH), 113.1 (CN), 121.2 (C^{8a}), 141.0 ($\underline{C}CH_3$), 148.8 (C^{4a}), 149.1 ($\underline{C}CH_3$), 150.8 (C^6), 157.1 (C^8), 167.7 (C=O). Anal. calcd for $C_{19}H_{22}N_4O_3S$ (386.47): C, 59.05; H, 5.74; N, 14.50; S, 8.30. Found: C, 59.14; H, 5.68; N, 14.58; S, 8.23.

Ethyl {[5-cyano-8-(3,5-dimethyl-1*H*-pyrazol-1-yl)-3,3-dimethyl-3,4-dihydro-1*H*-pyrano[3,4-*c*]pyridin-6-yl]-thio}acetate (4d).Reaction of compound **3** (0.63 g, 2.0 mmol) and ethyl chloroacetate (245 mg, 2 mmol) according to general procedure afforded 0.71 g (88%) of product **4d** isolated as a white solid: m.p. 120–121 °C. IR (ν/cm^{-1}): 2215 (CN), 1713 (CO). ^{1}H NMR (300 MHz, DMSO-d_6/CCl_4, 1/3): δ_H 1.17 (t, *J* 7.1 Hz, 3H, $OCH_2\underline{CH_3}$), 1.34 (s, 6H, $C(CH_3)_2$), 2.23 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 2.85 (t, *J* 1.2 Hz, 2H, 4-CH_2), 4.02 (s, 2H, SCH_2), 4.08 (q, *J* 7.1 Hz, 2H, OCH_2), 4.70 (t, *J* 1.2 Hz, 2H, 1-CH_2), 6.00 (s, 1H, CH). ^{13}C NMR (75.465 MHz, DMSO/CCl_4, 1/3) δ_C: 12.2 (CH_3), 13.0 (CH_3), 13.4 ($CH_2\underline{CH_3}$), 26.0 ($2CH_3$), 31.5 (SCH_2), 38.0 (CH_2), 59.3 (OCH_2), 60.7 (OCH_2), 68.8 (C^3), 104.7 (C^5), 108.0 (CH), 113.0 (CN), 121.1 (C^{8a}), 141.0 ($\underline{C}CH_3$), 148.7 (C^{4a}), 149.0 ($\underline{C}CH_3$), 150.8 (C^6), 157.1 (C^8), 167.1 (C=O). Anal. calcd for $C_{20}H_{24}N_4O_3S$ (400.50): C, 59.98; H, 6.04; N, 13.99; S, 8.01. Found: C, 59.81; H, 6.11; N, 14.15; S, 8.14.

8-(3,5-dimethyl-1*H*-pyrazol-1-yl)-3,3-dimethyl-6-(prop-2-yn-1-ylthio)-3,4-dihydro-1*H*-pyrano[3,4-*c*]pyridine-5-carbonitrile (4e). Reaction of compound **3** (0.63 g, 2.0 mmol) and 3-bromoprop-1-yne (238 mg, 2 mmol) according to general procedure afforded 0.64 g (90%) of product **4e** isolated as a white solid: m.p. 133–134 °C. IR (ν/cm^{-1}): 3026 (≡CH), 2218 (CN). ^{1}H NMR (300 MHz, DMSO-d_6/CCl_4, 1/3): δ_H 1.35 (s, 6H, $C(CH_3)_2$), 2.24 (s, 3H, CH_3), 2.50 (s, 3H, CH_3), 2.61 (t, *J* 2.6 Hz, 1H, ≡CH), 2.85 (t, *J* 1.2 Hz, 2H, 4-CH_2), 4.01 (t, *J* 2.6 Hz, 2H, SCH_2), 4.74 (t, *J* 1.2 Hz, 2H, 1-CH_2), 6.01 (s, 1H, CH). ^{13}C NMR (75.465 MHz, DMSO/CCl_4, 1/3) δ_C: 12.8 (CH_3), 13.0 (CH_3), 17.9 (SCH_2), 26.0 ($2CH_3$), 38.0 (CH_2), 59.3 (OCH_2), 68.8 (C^3), 72.5 (≡CH), 72.5 (C≡), 104.8 (C^5), 108.0 (CH), 113.0 (CN), 121.0 (C^{8a}), 141.0 ($\underline{C}CH_3$), 148.9 (C^{4a}), 149.1 ($\underline{C}CH_3$), 150.8 (C^8), 157.8 (C^6). Anal. calcd for $C_{19}H_{20}N_4OS$ (352.45): C, 64.75; H, 5.72; N, 15.90; S, 9.10. Found: C, 64.86; H, 5.78; N, 15.82; S, 9.17.

Synthesis of 8-(3,5-dimethyl-1*H*-pyrazol-1-yl)-3,3-dimethyl-6-oxo-3,4,6,7-tetrahydro-1*H*-pyrano[3,4-*c*]pyridine-5-carbonitrile (5). 50% Aqueous NaOH solution (4.0 g, 0.1 mol) was added to a solution of 8-(3,5-dimethyl-1*H*-pyrazol-1-yl)-6-[(2-hydroxyethyl)thio]-3,3-dimethyl-3,4-dihydro-1*H*-pyrano[3,4-*c*]pyridine-5-carbonitrile **4b** (3.60 g, 10 mmol) in EtOH (50 mL). The mixture was refluxed for 10 h. After cooling, the white precipitate of the thiirane polymer was filtered off. The filtrate was diluted with water and washed with chloroform to remove unreacted starting compound **4b**. The aqueous layer was acidified with HCl and the precipitated crystals of compound **5** were filtered off, washed with water, and recrystallized from a mixture of chloroform and ethanol (1:2) to give the product as a pure white solid (2.37 g, 79%). m.p. 159–160 °C. IR (ν/cm^{-1}): 3477 (NH), 2222 (CN), 1665 (CO).^{1}H NMR (300 MHz, DMSO-d_6/CCl_4, 1/3): δ_H 1.32 (s, 6H, $C(CH_3)_2$), 2.21 (s, 3H, CH_3), 2.43 (s, 3H, CH_3), 2.80 (t, *J* 1.2 Hz, 2H, 4-CH_2), 4.64 (t, *J* 1.2 Hz, 2H, 1-CH_2), 5.95 (s, 1H, CH), 11.6-12.6 (br,

1H, NH). ^{13}C NMR (75.465 MHz, DMSO-d_6/CCl_4, 1/3): δ_C12.7 (CH_3), 13.1 (CH_3), 26.2 ($2CH_3$), 38.3 (CH_2), 59.4 (OCH_2), 68.9 (C^3), 92.6 (C^5), 107.7 (CH), 113.8 (CN), 115.6 (C^{8a}), 141.4 ($\underline{C}CH_3$), 147.6 (C^{4a}), 148.6 ($\underline{C}CH_3$), 152.4 (C^8), 161.9 (C^6). Anal. calcd for $C_{16}H_{18}N_4O_2$ (298.34): C,64.41; H,6.08; N,18.78. Found: C 64.52; H 6.15; N 18.97.

Biological evaluation

General. 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 ºC in the same room, on a common food ration. All the biological experiments were carried out in full compliance with the European Convention for the Protection of Vertebrate. 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 Yerevan State Medical University after Mkhitar Heratsi (№7-5 from 18.03.2021).

Evaluation of the anticonvulsant activity of the synthesized compounds. The anticonvulsant effect of the new synthesized compounds was investigated by tests: PTZ convulsions (Acros organics, New Jersey, USA), MES.[15-17, 19] The PTZ test is an experimental model for inducing myoclonic seizures, as well as for predicting the anxiolytic properties of compounds. Out bred mice (weight 18-22 g.) were used for the study The PTZ test was carried out in mice by subcutaneous administration of analeptic at a dose of 90 mg/kg 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, 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 in suspension with carboxymethylcellulose ("Viadi - Ingredients") with tween-80 ("FerakBerlin") 45 minutes before the injection of the convulsive agent PTZ causing electrical irritation. The control animals were administered an emulsifier. Each dose of compounds for each test was studied in 8 animals. Analogues of comparison were an anticonvulsant drug from the group of succinimide ethosuximide (neuraxpharmArzneimittel GmbH (Germany).[21] Comparison drug, ethosuximide in doses from 100 to 300 mg/kg was administered intraperitoneally.

Evaluation of the psychotropic properties of the synthesized compounds. Psychotropic properties of selected compounds (**3, 4d, 4e**) were studied by tests: "open field", "elevated plus maze - EPM", "forced swimming".

Open field test. The research-motor behavior of rats was studied on a modified "open field" model.[24-26] For this purpose, an installation was used, the bottom of which is divided into squares with holes (cells). Experiments were performed in the daytime with natural light. Within 5 minutes of the experiment, the 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 on this model was 8 for each compound, control, and reference drug. The studied compounds were administered to rats in the most effective dose of 50 mg/kg intraperitoneally as a suspension with methylcarboxycellulose with Tween-80.

Elevated plus maze - EPM test. Anti-anxiety and sedative effects were studied on a model of the "elevated plus maze" in mice.[27] The labyrinth is a cruciform machine raised above the floor, having a pair of open and closed sleeves opposed to each other. Normal animals prefer to spend most of their time in the closed (dark) sleeves of the labyrinth. The anxiolytic effect of the compounds was estimated by the increase in the number of entries into open (light) sleeves and the time spent in, without increasing the total motor activity. This record the time spent in the closed sleeve, the number of attempts to enter the installation center. In the above model, the test

compounds and the reference drug were injected intraperitoneally before the experiments. The control animals were administered an emulsifier. Results were processed statistically ($P \leq 0.05$).

Forced swimming test. To assess "despair and depression" the model "compelling swimming"[31] was used. Experimental animals were forced to swim in a glass container (height 22 cm, diameter 14 cm), filled 1/3 with water. Intact mice swim very actively, but soon they will be forced to immobilize. The latent period of immobilization, the total duration of active swimming, immobilization is fixed for 6 minutes. The experiments were conducted under natural light.

Evaluation of incoordination of movements in the rotating rod test. Adverse neurotoxic (muscle relaxant) effect of compounds was studied in doses of 50 to 100 mg/kg when administered intraperitoneally, as well as reference drugs in effective anticonvulsant doses. Miorelaxation was investigated by the test of a "rotating rod" in mice.[15]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 cannot stay on it for 2 minutes was determined. To determine the ED_{50} and neurotoxic TD_{50}, the statistical method of penetration by Litchfield and Wilcoxon was used.[22, 23] Maximal tolerated doses (MTD) are also studied. The compounds by i.p. injection in doses from 500-1800 mg/kg were investigated.

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Supplementary Material

NMR spectra (^{1}H and ^{13}C) of products can be found in the supplementary material file.

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