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The synthesis and the anticonvulsant activity screening of new 5-substituted 2-imino-4-thiazolidinone derivatives

Abstract

Aim. To synthesize 5-ene-4-thiazolidinones containing heterocyclic rings in the molecule as potential anticonvulsants, and screen their anticonvulsant activity on a model of pentylenetetrazole (PTZ) seizures.

Results and discussion. A straightforward and convenient synthesis of novel 5-ene-derivatives of thiazol/oxazole-bearing 4-thiazolidinones as possible anticonvulsant agents was performed. Compounds were characterized using methods of spectral analysis (^{1}H NMR and LC-MS). 5-Chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde underwent the aminolysis on a chlorine atom by a molecule of monoethanolamine (MEA) in the Knoevenagel reaction with thiazole/oxazole-bearing 4-thiazolidinones. The preliminary screening of the anticonvulsant activity was performed for the compounds synthesized on the model of PTZ-induced seizures, and active derivatives were identified.

Experimental part. Commercially available 2-aminothiazole and 5-methylisoxazol-3-amine were used as starting compounds for the synthesis of 2-chloro-*N*-(hetaryl)acetamides. The latter were transformed into thiazole/oxazole-bearing 4-thiazolidinones by the treatment with ammonium isothiocyanate. Modification at C5 position of the heterocycles synthesized was performed by the Knoevenagel reaction with aromatic/heteroaromatic aldehydes and MEA as a catalyst (either equimolar or 0.1 mol% amount) in the ethanol medium. The structure of novel derivatives was confirmed by ^{1}H NMR and LC-MS spectra. The anticonvulsant activity of all derivatives synthesized was studied *in vivo* on the model of PTZ-induced seizures. Latency of the seizures, the number of clonic-tonic seizures in one mouse, the percent of animals with clonic and tonic seizures, the duration of the seizure period, and the lifetime of the animals before death were evaluated and calculated.

Conclusions. The results obtained are promising for further design of potential anticonvulsants among oxazole-bearing 4-thiazolidones with the possible mechanism of the anticonvulsant action through the GABA-ergic impact and inhibition of the prostaglandin and leukotriene synthesis.

Keywords: 4-thiazolidinones; Dimroth reaction; Knoevenagel reaction; antiepileptic drugs; epilepsy; anticonvulsant activity

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Синтез та скринінг протисудомної активності нових 5-заміщених 2-іміно-4-тіазолідинонів

Анотація

Мета. Синтезувати 5-ен-4-тіазолідинони з гетарильними фрагментами в молекулі як потенційні протисудомні засоби та провести скринінг їхньої протисудомної активності на моделі пентилентетразолових судом.

Результати та їх обговорення. Проведено простий і зручний синтез нових 5-ен-похідних тіазол/оксазоловмісних 4-тіазолідинонів як потенційних протисудомних засобів. Структуру нових сполук досліджено та підтверджено методами

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спектрального аналізу (^{1}H ЯМР та LC-MS). 5-Хлор-3-метил-1-феніл-1*H*-піразол-4-карбальдегід зазнає амінолізу за атомом хлору дією моноетаноламіну (МЕА) в реакції Кньовенагеля з тіазол/оксазолвмісними 4-тіазолідинонами. Проведено первинний скринінг протисудомної активності синтезованих сполук на моделі пентилентетразол-індукованих судом та ідентифіковано активні сполуки.

Експериментальна частина. Комерційно доступні 2-амінотіазол і 5-метилізоксазол-3-амін було використано як вихідні сполуки для одержання 2-хлор-*N*-(гетарил)ацетамідів. Останні обробленням амоній ізотіоціанатом було перетворено на тіазол/оксазолвмісні 4-тіазолідинони. Модифікацію за положенням С5 сполук проводили за реакцією Кньовенагеля з ароматичними/гетероароматичними альдегідами в присутності МЕА як каталізатора (в еквімолярній або 0,1 моль% кількості) в середовищі етанолу. Структуру нових синтезованих речовин підтверджено даними ^{1}H ЯМР-спектроскопії та LC-MS. Протисудомну активність синтезованих похідних вивчали *in vivo* на моделі PTZ-індукованих судом. Оцінювали та розраховували латентність судом; кількість клоніко-тонічних судом в однієї миші; відсоток тварин із клонічними та тонічними судомами; тривалість судомного періоду та тривалість життя тварин.

Висновки. Отримані результати є перспективними для наступного дизайну потенційних протисудомних засобів серед оксазолвмісних похідних 4-тіазолідинонів з можливим механізмом протисудомної дії через ГАМК-ергічний вплив та пригнічення синтезу простагландинів і лейкотриєнів.

Ключові слова: 4-тіазолідинони; реакція Дімрота; реакція Кньовенагеля; протиепілептичні засоби; епілепсія, протисудомна дія

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■ Introduction

The antiepileptic drug design is an important area in current medicinal chemistry [1]. Despite the significant progress in the treatment of seizures and the discovery/introduction of innovative antiepileptic drugs, there are still plenty of problems associated with adverse effects, as well as drug resistance taking place under long-term application of approved drugs [2–4]. In this regard, the search for new potent anticonvulsant agents is a logical and actual approach to achieve a positive effect on the frequency and severity of spontaneous epileptic seizures, negative cognitive and psychosomatic consequences in patients [2–4].

Nitrogen-containing heterocyclic rings are important structural elements in many known anticonvulsant drugs [5–8]. In our previous studies 4-thiazolidinones containing 2-aminothiazole moiety are reported as promising anticonvulsant agents; they are structural analogs of a potential dual cyclooxygenase-2 and 5-lipoxygenase (COX-2/5-LOX) inhibitor darbufelon (Figure 1) [9–11]. Data on the anticonvulsant activity of classical non-steroidal anti-inflammatory drugs [12–17], the polypharmacological futures of 4-thiazolidinones and our experimental findings are sound arguments for further design of novel hetarylsubstituted 2-imino/amino-4-thiazolidinones as potential anticonvulsants.

■ Results and discussion

Synthesis of the target compounds

Commercially available 2-aminothiazole (**1**) and 5-methylisoxazol-3-amine (**2**) were used as starting compounds and were converted to 4-thiazolidinones **3** and **4**, respectively (Scheme 1) [9, 18]. Initially, the appropriate 2-chloro-*N*-(hetaryl)acetamides **1.1** and **1.2** were obtained by the reaction of amines **1**, **2** with chloroacetyl chloride in the presence of triethylamine in the dry dioxane and further transformed to derivatives **3**, **4** by the interaction with ammonium isothiocyanate.

It was reported that the process mentioned above (Scheme 1) did not stop at the stage of the nucleophilic substitution and underwent a spontaneous heterocyclization to the 4-thiazolidinone cycle formation as the intramolecular Dimroth type rearrangement of substituents in positions 2 and 3 took place [18]. It should be noted that compounds **3** and **4** synthesized are characterized by the existence in the imino-form in the solution and the crystalline state as previously proven using spectral and X-ray diffraction methods [9, 18]. The structure modification of 2-(aryl/hetaryl)-imino-4-thiazolidones at C5 position leads to a significant impact on the appearance, as well potency of the anticonvulsant activity [9–11]. Derivatives **3** and **4** synthesized are active methylene compounds.

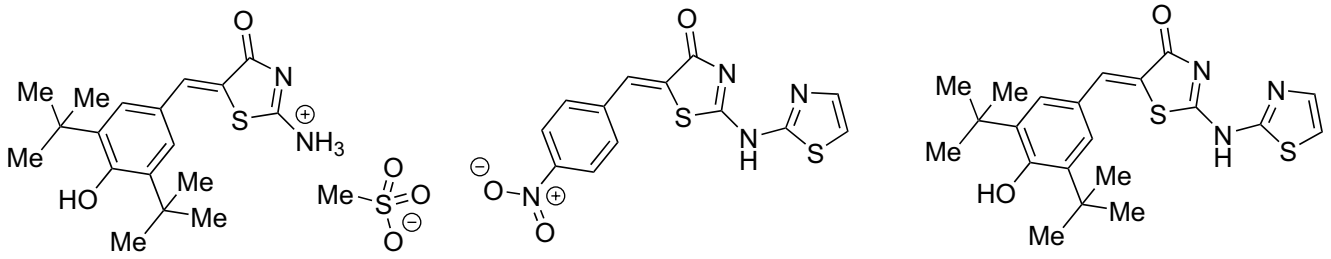


Figure 1. The structures of promising anticonvulsant agents previously found

Hence, the Knoevenagel condensation was used for further modification of the structure at C5 atom. A set of aromatic/heteroaromatic aldehydes was used, and the reaction was carried out in the soft conditions using monoethanolamine (MEA) as a catalyst (the equimolar amount in case of **3.1**, and 0.1 mol% in case of other derivatives) in the ethanol medium (Scheme 2).

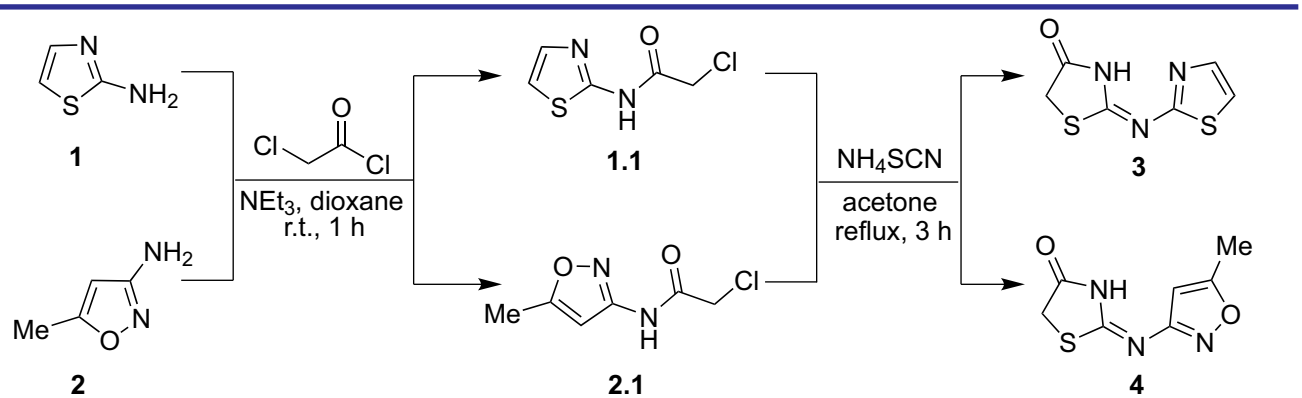
The structure of novel derivatives **3.1**–**3.3**, **4**, **4.1**–**4.3** synthesized was characterized by ^{1}H NMR, and LC-MS spectra. The aminolysis of the chlorine atom by the molecule of MEA took place during the synthesis of compound **3.1**. Two broad singlets at 3.53 and 3.59 ppm, corresponding to four methylene-groups protons, as well as broad singlet at 9.71 ppm corresponding to the aminoethanole residue of the NH-group proton were observed in the ^{1}H NMR spectrum of **3.1**. The molecular ion peak observed at m/z value of 427.0 $[M+H]^+$ in a positive ionization mode in the mass spectrum confirmed the formation of compound **3.1.** Signals of the endocyclic N-H, as well as OH-group protons for **3.1** were absent in the relevant ^{1}H NMR spectrum due to deuteroexchange. The signal of the methylidene group protons appeared at δ ~7.6–7.9 ppm and clearly indicated the *Z*-configuration of ylidene derivatives **3.1**–**3.3**, **4.1**–**4.3** synthesized [19].

It should be noted that in the ^{1}H NMR spectrum (solvent DMSO-d_6) of compound **4.2** the prototropic amino-imine tautomerism was observed (Scheme 3). The signal of the amino-form of the NH-proton of compound **4.2** was observed at 11.95 ppm, and there was the signal of that one for the imino-form at 12.35 ppm. Additional studies using variety of conditions for NMR experiments (the change of the solvent, temperature, etc.) were not performed as the feature mentioned was well-known and widely described for such type of heterocycles [20]. The molar ratio of tautomers based on the integral intensity curve was 1:6 (amine and imine forms, respectively).

The anticonvulsant activity screening

The anticonvulsant activity of derivatives **3.1**–**3.3**, **4**, **4.1**–**4.3** was studied *in vivo* on the model of PTZ-induced seizures. The results of the experiment are presented in Table and Figure 2.

Substances **3.1**, **3.2, 4.1** and **4.2** were indifferent to PTZ-induced seizures. The compounds did not have a significant effect on all parameters of the convulsive syndrome model, including the integrated indicator – mortality. The reduction in the duration of the convulsive period under the effect of compound **3.1** is not a manifestation of anticonvulsant properties in the context of other characteristics of the convulsive syndrome since it correlates with a decrease in the time from the appearance of the first paroxysms to death with a constant severity of attacks (during this



Scheme 1. The synthesis of 2-(thiazol-2-ylimino)thiazolidin-4-one (**3**) and 2-((5-methylisoxazol-3-yl)imino)thiazolidin-4-one (**4**)

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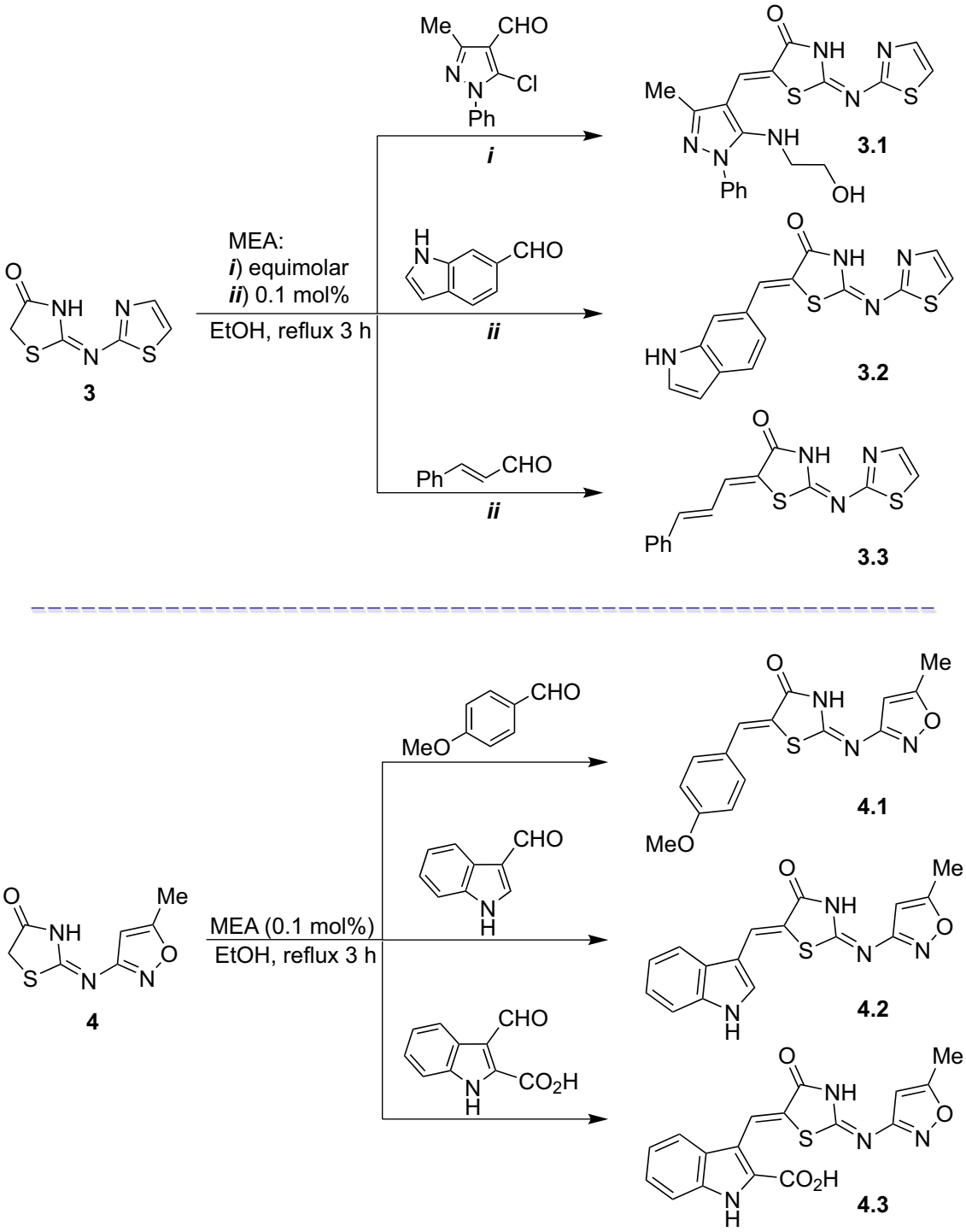
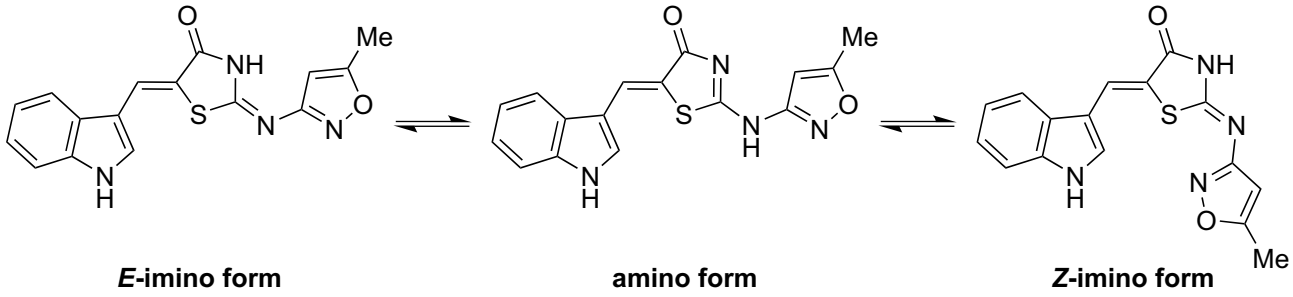


Scheme 2. The synthesis of 5-ylidene derivatives (**3.1-3.3**, **4.1-4.3**) of 2-(thiazol-2-ylimino)thiazolidin-4-one (**3**) and 2-((5-methylisoxazol-3-yl)imino)thiazolidin-4-one (**4**)

shorter time the animals have time to die from a slightly smaller number of attacks), but mortality is not reduced.

Derivative **3.3** possesses pro-convulsant properties. Under the effect of this compound, the number of seizures per one animal increased by 5.5%, in contrast to the control group; all animals, without exception, recorded a lethal tonic extension – 6 points and 100% mortality.

Compounds **4** and **4.3** showed the anticonvulsant activity, which could be determined as moderate. Under the action of **4** the number of animals with tonic convulsions decreased by 21.67% ($p < 0.05$), correlating with the animal's survival as mortality decreased by 23.33% ($p < 0.05$). Compound **4.3** significantly reduced the number of seizures in animals by 29.5% ($p < 0.05$), even reduced the percentage of clonic seizures in animals



Scheme 3. Possible tautomeric forms for compound **4.2**

by 8.33% ($p < 0.05$). Administration of the compound statistically significantly ($p < 0.05$) reduced the life expectancy of animals to death by 1.9 folds, and the duration of the convulsive period by 62.16%. The integral indicator – mortality significantly decreased compared to the control group by 23.33%.

The screening results obtained are promising for further design of potential anticonvulsants among oxazole-bearing 4-thiazolidones. It is important to note that the possible mechanism of the anticonvulsant action of the derivatives synthesized could indicate/combine GABA-ergic effects and inhibition of the prostaglandin and leukotriene synthesis [9, 10]. The new derivatives **3.1**–**3.3**, **4**, **4.1**–**4.3** synthesized and studied in this work are found less active than early found hits [9, 10], as well as darbufelon, however the data presented are extremely useful for the rational design of potential anticonvulsants among heterylsubstituted 4-thiazolidones.

■ Conclusions

In the present paper, a straightforward and convenient synthesis of novel 5-ene-derivatives of thiazol/oxazole-bearing 4-thiazolidinones as possible anticonvulsant agents has been described. Compounds have been characterized using methods of spectral analysis. The preliminary screening of the anticonvulsant activity has been performed for the compounds synthesized on the PTZ-induced seizures, and active derivatives have been identified. The results obtained are promising for further design of potential anticonvulsants among oxazole-bearing 4-thiazolidones with the possible mechanism of the anticonvulsant action through the GABA-ergic impact and inhibition of the prostaglandin and leukotriene synthesis.

■ Experimental part

Synthetic part

Melting points were measured in open capillary tubes on a BÜCHI B-545 melting point apparatus (BÜCHI Labortechnik AG, Flawil, Switzerland) and were uncorrected. The elemental analyses (C, H, N) were performed using a Perkin-Elmer 2400 CHN analyzer (PerkinElmer, Waltham, MA, USA) and were within ±0.4% of the theoretical values. ^{1}H NMR spectra (DMSO-d_6) were recorded on a Varian Unity Plus 400 (400 MHz) spectrometer (Varian Inc., Paulo Alto, CA, USA). All spectra were recorded at room temperature unless otherwise indicated and were referenced internally to the solvent reference frequencies.

Table. The anticonvulsant effect of the test compounds **3.1-3.3**, **4**, **4.1**–**4.3** in the PTZ model. Each value represents the mean ± S.E.M

Group	No.[a]	Dose, mg kg^{-1}	Number of clonic-tonic seizures per mouse	% of mice with seizures		Severity of seizures, points (0–6)	Mortality, %
				Clonic	Tonic		
Control	30	90	2.37 ± 0.26	100	96.67	5.77 ± 0.13	90
Sodium valproate	13	300	0.62 ± 0.46**	23.08**	15.38**	1.15 ± 0.64**	15.38**
3.1	6	100	1.33 ± 0.21$^{\#\#}$	100$^{\#\#}$	83.33$^{\#\#}$	5.50 ± 0.50$^{\#\#}$	83.33$^{\#\#}$
3.2	6	100	1.83 ± 0.48$^{\#\#}$	100$^{\#\#}$	100$^{\#\#}$	5.83 ± 0.17$^{\#\#}$	83.33$^{\#\#}$
3.3	6	100	2.50 ± 0.56$^{\#\#}$	100$^{\#\#}$	100$^{\#\#}$	6.00 ± 0.00$^{\#\#}$	100$^{\#\#}$
4	12	100	1.83 ± 0.42$^{\#\#}$	100$^{\#\#}$	75*$^{\#\#}$	5.08 ± 0.40$^{\#\#}$	66.67*$^{\#\#}$
4.1	6	100	3.00 ± 0.58$^{\#\#}$	100$^{\#\#}$	83.33$^{\#\#}$	5.50 ± 0.5$^{\#\#}$	83.33$^{\#\#}$
4.2	6	100	2.17 ± 0.31$^{\#\#}$	100$^{\#\#}$	83.33$^{\#\#}$	5.50 ± 0.50$^{\#\#}$	83.33$^{\#\#}$
4.3	12	100	1.67 ± 0.53*$^{\#\#}$	91.67*$^{\#\#}$	83.33$^{\#\#}$	5.00 ± 0.52$^{\#\#}$	66.67*$^{\#\#}$

Note: [a] the number of animals in each group; * $p \le 0.05$, ** $p \le 0.01$ compared to the control; $^{\#}$ $p \le 0.05$, $^{\#\#}$ $p \le 0.01$ compared to sodium valproate

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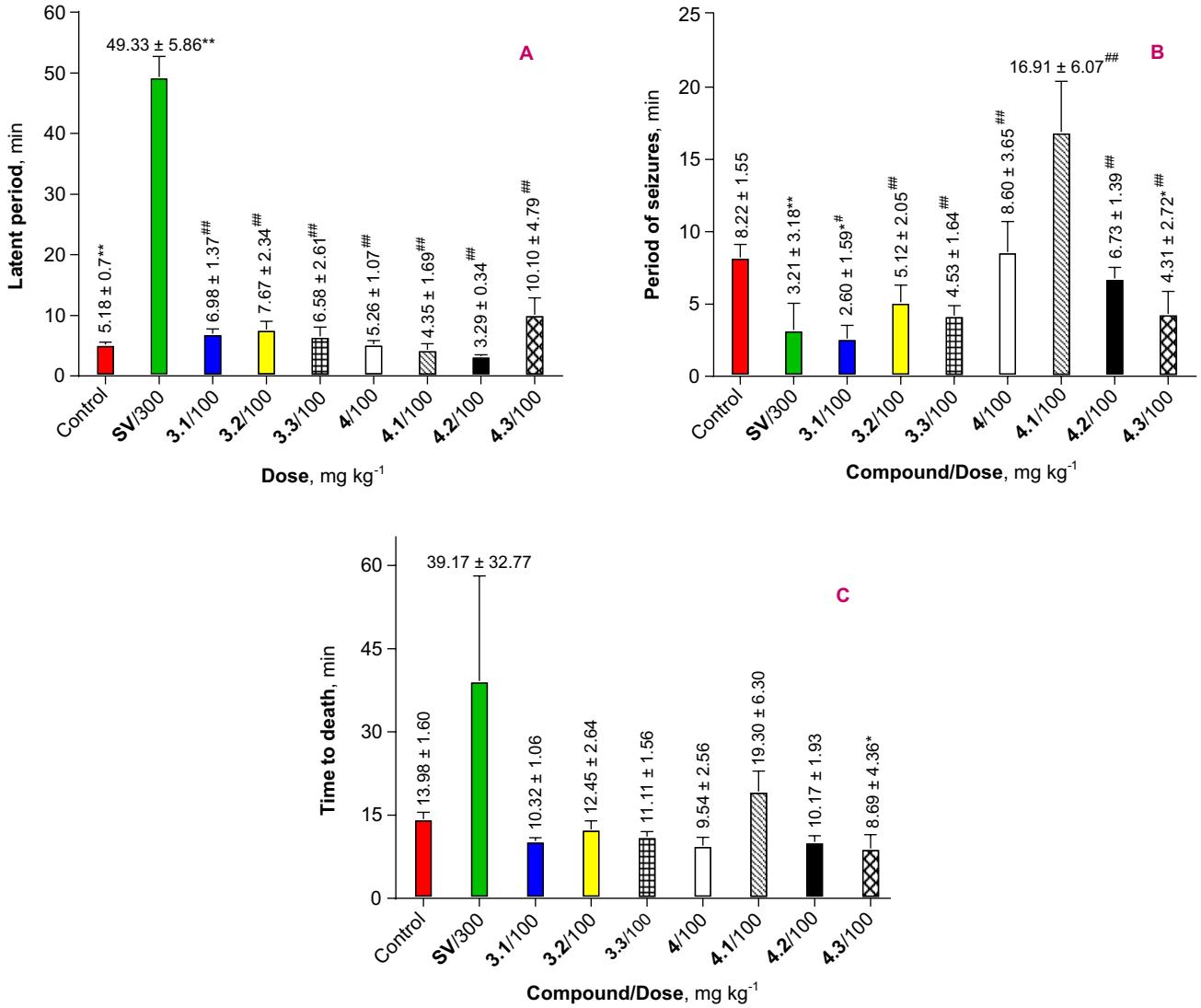

Figure 2. The anticonvulsant activity of the reference drug and derivatives **3.1**–**3.3**, **4**, **4.1**–**4.3** in the PTZ test: (**A**) latency to the first seizure episode; (**B**) duration (period) of seizures; (**C**) time to death. Each value represents the mean ± S.E.M. SV – sodium valproate; * $p < 0.05$, ** $p < 0.01$ compared to the control; # $p < 0.05$, ## $p < 0.01$ compared to sodium valproate

Chemical shifts (δ) were expressed in ppm, and coupling constants (J) were reported in Hz. LC-MS spectra were obtained on a Finnigan MAT INCOS-50 device (Thermo Finnigan LLC, San Jose, CA, USA). The reaction mixture was monitored by thin layer chromatography (TLC) using commercial glass-backed TLC plates (Merck Kieselgel 60 F_{254}). Solvents and reagents were commercially available and used without further purification. The protocol for the synthesis of compounds **3** (as well as its properties) was described in [9, 18]; compound **4** was synthesized similarly.

2-((5-Methylisoxazol-3-yl)imino)thiazolidin-4-one (**4**)

Yellow powder. Yield – 0.450 g (68%). M. p. – 230–232°C (EtOH). Anal. Calcd. for $C_7H_7N_3O_2S$, %: C 42.63; H 3.58; N 21.31. Found, %: C 42.50; H 3.51; N 21.40. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 2.36 (3H, s, CH_3); 4.04 (2H, s, CH_2); 6.09 (1H, s, isox.); 12.00 (1H, s, NH). LC-MS (ESI), m/z, peak area: 198.0 $[M+H]^+$, 100.0%.

The general procedure for the synthesis of 5-ylidene derivatives of compounds 3.1–3.3 and 4.1–4.3

To a stirred solution of compound **3** or **4** (10 mmol) in EtOH (10 mL) the appropriate aldehyde (11 mmol) and MEA (in the case of 5-chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde MEA is used in the equimolar amount, in all other cases – in 0.1 mol% amount) were added, and the mixture was then refluxed for 4 h. The resulting solids of compounds **3.1**–**3.3** and **4.1**–**4.3** were collected by filtration, washed with ethanol (5–10 mL), and crystallized from the appropriate solvent.

5-((5-((2-Hydroxyethyl)amino)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)-2-(thiazol-2-ylimino)thiazolidin-4-one (**3.1**)

A yellow powder. Yield – 0.310 g (69%). M. p. – 182–184°C (EtOH). Anal. Calcd. for $C_{17}H_{12}N_4O_4S$, %: C 53.51; H 4.25; N 19.70. Found, %: C 53.80; H 4.50; N 19.90. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 2.55 (3H, s, CH_3); 3.53 (2H, br.s, CH_2); 3.59 (2H, br. s, CH_2); 7.12 (1H, br. s, thiaz.); 7.33 (1H, t, J = 7.3 Hz, phenyl); 7.43 (1H, br. s, thiaz.); 7.60 (2H, t, J = 7.4 Hz, phenyl); 7.75 (2H, d, J = 8.0 Hz, phenyl); 7.90 (1H, s, =CH); 9.71 (1H, br. s, NH); 1H (NH, thiazolidine) and 1H (OH) are in exchange. LC-MS (ESI), m/z, peak area: 427.0 $[M+H]^+$, 100.0%.

5-((1H-Indol-6-yl)methylene)-2-(thiazol-2-ylimino)thiazolidin-4-one (**3.2**)

A brown powder. Yield – 0.450 g (71%). M. p. – 287–289°C (DMF). Anal. Calcd. for $C_{15}H_{10}N_4OS_2$, %: C 55.20; H 3.09; N 17.17. Found, %: C 55.40; H 3.30; N 17.40. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 6.51 (1H, s, thiaz.); 7.28 (1H, d, J = 8.2 Hz, indol.); 7.46 (1H, s, indol.); 7.55 (1H, s, thiaz.); 7.69 (2H, m, indol.); 7.74 (1H, s, indol.); 7.83 (1H, s, =CH); 11.55 (1H, s, NH, indol.); 12.55 (1H, s, NH, thiazolidin.). LC-MS (ESI), m/z, peak area: 327.0 $[M+H]^+$, 100.0%.

5-(3-Phenylallylidene)-2-(thiazol-2-ylimino) thiazolidin-4-one (**3.3**)

A yellow-brown powder. Yield – 0.380 g (72%). M. p. – 232–234°C (AcOH). Anal. Calcd. for $C_{15}H_{11}N_3OS_2$, %: C 57.49; H 3.54; N 13.41. Found, %: C 57.80; H 3.60; N 13.40. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 7.12 (1H, d, J = 15.0 Hz, =CH); 7.27 (1H, d, J = 15.0 Hz, =CH); 7.36-7.43 (5H, m, phenyl); 7.47 (1H, s, thiaz.); 7.65 (1H, s, thiaz.); 7.69 (1H, s, =CH); 12.50 (1H, s, NH). LC-MS (ESI), m/z, peak area: 314.0 $[M+H]^+$, 100.0%.

5-(4-Methoxybenzylidene)-2-((5-methylisoxazol-3-yl)imino)thiazolidin-4-one (**4.1**)

A yellow powder. Yield – 0.460 g (91%). M. p. – 254–256°C (dioxane). Anal. Calcd. for $C_{15}H_{13}N_3O_3S$, %: C 57.13; H 4.16; N 13.33. Found, %: C 57.50; H 4.30; N 13.40. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 2.40 (3H, s, CH_3); 3.82 (3H, s, OCH_3); 6.21 (1H, s, isox.); 7.12 (2H, d, J = 8.5 Hz, phenyl); 7.57 (2H, d, J = 8.5 Hz, phenyl); 7.66 (1H, s, =CH); 12.55 (1H, s, NH). LC-MS (ESI), m/z, peak area: 316.0 $[M+H]^+$, 100.0%.

5-((1H-Indol-3-yl)methylene)-2-((5-methylisoxazol-3-yl)imino)thiazolidin-4-one (**4.2**)

A brown-yellow powder. Yield – 0.290 g (56%). M. p. – 236–238°C (AcOH). Anal. Calcd. for $C_{16}H_{12}N_4O_2S$, %: C 59.25; H 3.73; N 17.27. Found, %: C 59.40; H 3.50; N 17.40. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 2.41 (3H, s, CH_3); 6.22 (1H, s, isox.); 7.19 (1H, t, J = 7.7 Hz, indol.); 7.24 (1H, t, J = 7.7 Hz, indol.); 7.52+7.60 (1H, d+d, J = 7.7 Hz, indol.); 7.74 (1H, s, indol.); 7.82+7.87 (1H, d+d, J = 7.7 Hz, indol.); 7.97 (1H, s, =CH); 11.86+12.05 (1H, s+s, NH); 11.95+12.35 (1H, s+s, NH). LC-MS (ESI), m/z, peak area: 325.0 $[M+H]^+$, 100.0%.

3-((2-((5-Methylisoxazol-3-yl)imino)-4-oxothiazolidin-5-ylidene)methyl)-1H-indole-2-carboxylic acid (**4.3**)

A white-yellow powder. Yield – 0.290 g (43%). M. p. – 251–253°C (dioxane). Anal. Calcd. for $C_{17}H_{12}N_4O_4S$, %: C 55.43; H 3.28; N 15.21. Found, %: C 55.60; H 3.30; N 15.40. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 3.69 (3H, s, CH_3); 5.07 (1H, s, isox.); 7.29–7.32 (3H, m, indol.); 7.54 (1H, d, J = 7.0 Hz, indol.); 7.99 (1H, s, =CH); 9.10 (1H, s, NH); 12.79 (1H, s, NH); 1H (COOH) is in exchange. LC-MS (ESI), m/z, peak area: 369.0 $[M+H]^+$, 100.0%.

Pharmacology

The study of the anticonvulsant activity of the derivatives **3.1**–**3.3**, **4**, **4.1**–**4.3** synthesized was performed on 97 randomly bred mice of both sexes weighing 18–23 g; they were kept under standard conditions in the vivarium of the Central Research Laboratory of the Educational and Scientific Institute of Applied Pharmacy of the National University of Pharmacy, Kharkiv, Ukraine.

A basic screening model of PTZ seizures was used in accordance with the guidelines in the experimental study [21]. During the experiment, the rules and principles adopted by the Helsinki Declaration on Humane Animal Welfare (2000), the Directive of the Council of the European Union on the protection of animals used for scientific purposes (2010) were observed. All animal procedures were approved by the Local Ethical Committee in the National University of Pharmacy, Kharkiv, Ukraine (Approval No: 3/2019).

Animals were randomized into the following groups: group 1 – control pathology (Control, untreated PTZ-seizures), group 2 – animals with model seizures, treated with the reference drug sodium valproate, groups 3–9 – animals with seizures, treated with derivatives **3.1**–**3.3**, **4**, **4.1**–**4.3** synthesized.

Animals were administered the test compounds once in the form of a thin suspension stabilized with polysorbate-80 (Tween-80), in the dose of 100 mg kg^{-1} intragastrically 30 min before the seizure induction. The dose was chosen on the data of 4-thiazolidinone derivatives effectiveness in the study [9–11].

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As a reference drug sodium valproate (Depakine, Sanofi-Aventis, France) was used in the dose of 300 mg kg^{-1} intragastrically as an oral syrup [21]. In the control group, animals were administered purified water intragastrically in the appropriate volume, 30 min before the PTZ seizure induction.

The mechanism of PTZ seizures is associated with inhibition of the GABA-receptor complex [21]. Animals were administered PTZ (Corazol, Sigma, USA) as an aqueous solution in the dose of 90 mg kg^{-1} subcutaneously 30 min after compounds application.

Each animal was placed in a separate cylindrical plastic container (5 L) and continuously monitored for 60 min. Latency of the seizures; the number of clonic-tonic seizures in one mouse; % of animals with clonic and tonic seizures; the duration of the seizure period (from the first to the last attack); and the lifetime of the animals before death (in mice with a lethal outcome) were calculated. The severity of seizures was evaluated according to the scale ranging from 1 to 6: 1 – trembling; 2 – circus movement; 3 – clonic seizures; 4 – clonic-tonic seizures with a lateral position; 5 – tonic extension; 6 – tonic extension leading to the animal's death. When seizures were not observed within 1 h, it was considered that the latent period was 60 min. The protection of animals from the development of clonic and tonic seizures and lethality were treated as the most significant indicators of the anticonvulsant activity of the compounds [21].

The statistical analysis was performed using a Statistica 10.0 software by the methods of variation statistics. The average values and standard errors were calculated. The significance of the differences between groups was estimated according to the Student's criterion (t) in the case of normal distribution, and according to the nonparametric Mann-Whitney criterion (U) in the case of the absence of normal distribution. The results determined in an alternative form (presence/absence of a certain feature) were evaluated using the Fisher's criterion (φ).

References

1. Sevim, D.; Karakurt, A. Recent progress in anticonvulsant drug research: strategies for anticonvulsant drug development and applications of antiepileptic drugs for non-epileptic central nervous system disorders. *Curr. Top. Med. Chem.* **2012**, *12* (9), 1033-1071. https://doi.org/10.2174/156802612800229215.
2. Löscher, W.; Potschka, H.; Sisodiya, S. M.; Vezzani, A. Drug Resistance in Epilepsy: Clinical Impact, Potential Mechanisms, and New Innovative Treatment Options. *Pharmacol. Rev.* **2020**, *72* (3), 606-638. https://doi.org/10.1124/pr.120.019539.
3. Löscher, W. Single-Target Versus Multi-Target Drugs Versus Combinations of Drugs with Multiple Targets: Preclinical and Clinical Evidence for the Treatment or Prevention of Epilepsy. *Front. Pharmacol.* **2021**, *12,* 730257. https://doi.org/10.3389/fphar.2021.730257.
4. Ciccone, L.; Cerri, C.; Nencetti, S.; Orlandini, E. Carbonic Anhydrase Inhibitors and Epilepsy: State of the Art and Future Perspectives. *Molecules* **2021**, *26* (21), 6380. https://doi.org/10.3390/molecules26216380.
5. Grover, G.; Nath, R.; Bhatia, R.; Akhtar, M. J. Synthetic and therapeutic perspectives of nitrogen containing heterocycles as anti-convulsants. *Bioorg. Med. Chem.* **2020**, *28* (15), 115585. https://doi.org/10.1016/j.bmc.2020.115585.
6. Ali, R.; Khan, M. A.; Siddiqui, N. Past, Present and Future of Antiepileptic Drug Therapy – Finding a Place for Heterocyclics. *Mini-Rev. Med. Chem.* **2015**, *15* (12), 1024-1050. https://doi.org/10.2174/1389557515121507311135 49.
7. Song, M.-X.; Deng, X.-Q. Recent developments on triazole nucleus in anticonvulsant compounds: a review. *J. Enzyme Inhib. Med. Chem.* **2018**, *33* (1), 453-478. https://doi.org/10.1080/14756366.2017.1423068.
8. Wei, C.-X.; Bian, M.; Gong, G.-H. Current Research on Antiepileptic Compounds. *Molecules* **2015**, *20* (11), 20741-20776. https://doi.org/10.3390/molecules201119714.
9. Mishchenko, M.; Shtrygol', S.; Lozynskyi, A.; Khomyak, S.; Novikov, V.; Karpenko, O.; Holota, S.; Lesyk, R. Evaluation of Anticonvulsant Activity of Dual COX-2/5-LOX Inhibitor Darbufelon and Its Novel Analogues. *Sci. Pharm.* **2021**, *89* (2), 22. https://doi.org/10.3390/scipharm89020022.
10. Mishchenko, M. V.; Shtrygol, S. Yu.; Lesyk, R. B.; Lozynskyi, A. V.; Holota, S. M. Screening study of new thiazolidinone derivatives for anticonvulsant activity. *Zaporozhye Medical Journal* **2020**, *22* (6), 840–846. https://doi.org/10.14739/2310-1210.2020.6.218463 [*in Ukrainian*].
11. Mishchenko, M.; Shtrygol, S.; Kaminskyy, D.; Lesyk, R. Thiazole-bearing 4-thiazolidinones as new anticonvulsant agents. *Sci. Pharm.* **2020**, *88* (1), 16. https://doi.org/10.3390/scipharm88010016.
12. Suemaru, K.; Yoshikawa, M.; Tanaka, A.; Araki, H.; Aso, H.; Watanabe, M. Anticonvulsant effects of acetaminophen in mice: Comparison with the effects of nonsteroidal anti-inflammatory drugs. *Epilepsy Res.* **2018**, *140*, 22–28, https://doi.org/10.1016/j.eplepsyres.2017.12.004.
13. Vieira, V.; Glassmann, D.; Marafon, P.; Pereira, P.; Gomez, R.; Coitinho, A.S. Effect of diclofenac sodium on seizures and inflammatory profile induced by kindling seizure model. *Epilepsy Res.* **2016**, *127*, 107–113, https://doi.org/10.1016/j.eplepsyres.2016.08.020.
14. Akula, K.K.; Dhir, A.; Kulkarni, S.K. Rofecoxib, a selective cyclooxygenase-2 (COX-2) inhibitor increases pentylenetetrazol seizure threshold in mice: Possible involvement of adenosinergic mechanism. *Epilepsy Res.* **2008**, *78*, 60–70, https://doi.org/10.1016/j.eplepsyres.2007.10.008.
15. Zhou, L.; Zhou, L.; Cao, S.L.; Xie, Y.J.; Wang, N.; Shao, C.Y.; Wang, Y.-N.; Zhou, J.-H.; Cowell, J.K.; Shen, Y. Celecoxib ameliorates seizure susceptibility in autosomal dominant lateral temporal epilepsy. *J. Neurosci.* **2018**, *38* (13), 3346–3357. https://doi.org/10.1523/JNEUROSCI.3245-17.2018.
16. Dhir, A.; Naidu, P. S.; Kulkarni, S. K. Effect of naproxen, a non-selective cyclooxygenase inhibitor, on pentylenetetrazol-induced kindling in mice. *Clin. Exp. Pharmacol. Physiol.* **2005**, *32* (7), 574–584. https://doi.org/10.1111/j.1440-1681.2005.04233.x.

17. Darvishi, H.; Rezaei, M.; Khodayar, M. J.; Reza Zargar, H.; Dehghani, M. A.; Rajabi Vardanjani, H.; Ghanbari, S. Differential Effects of Meloxicam on Pentylenetetrazole- and Maximal Electroshock-Induced Convulsions in Mice. *Jundishapur J. Nat. Pharm. Prod.* **2017,** *12* (2), e36412. https://doi.org/10.5812/jjnpp.36412.
18. Ostapiuk, Yu. V.; Obushak, M. D.; Matiychuk, V. S.; Naskrent, M.; Gzella, A. K. A convenient method for the synthesis of 2-[(5-benzyl-1,3-thiazol-2-yl)imino]-1,3-thiazolidin-4-one derivatives. *Tetrahedron Lett.* **2012**, *53* (5), 543–545. https://doi.org/10.1016/j.tetlet.2011.11.093.
19. Holota, S.; Komykhov, S.; Sysak, S.; Gzella, A.; Cherkas, A.; Lesyk, R. Synthesis, Characterization and In Vitro Evaluation of Novel 5-Enethiazolo [3,2-b][1,2,4] triazole-6(5H)-ones as Possible Anticancer Agents. *Molecules* **2021**, *26* (4), 1162. https://doi.org/10.3390/molecules26041162.
20. Golota, S.; Sydorenko, I.; Surma, R.; Karpenko, O.; Gzella, A.; Lesyk, R. Facile one-pot synthesis of 5-aryl/heterylidene-2-(2-hydroxyethyl- and 3-hydroxypropylamino)-thiazol-4-ones via catalytic aminolysis, *Synth. Commun.*, **2017**, *47* (11), 1071–1076. https://doi.org/10.1080/00397911.2017.1309669.
21. *Drug Discovery and Evaluation: Pharmacological Assays*, 4th Ed.; Hock, F. J., Ed.; Springer: Cham, **2016**. https://doi.org/10.1007/978-3-319-05392-9.

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