SYNTHESIS OF SOME NEW N^3 SUBSTITUTED 6-PHENYLAZO-3*H*-THIAZOLO[4,5-*B*]PYRIDIN-2-ONES AS POSSIBLE ANTI-INFLAMMATORY AGENTS

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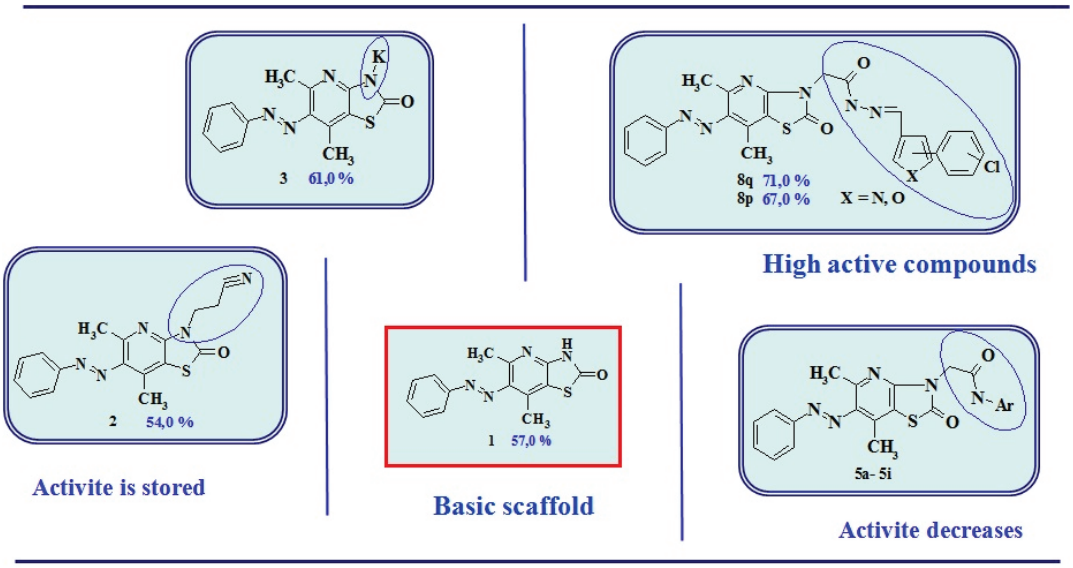
Abstract: Fused thiazolidine moiety heterocycles are an integral part of new drug discovery in anti-inflammatory research. We are reporting novel N^3 substituted 5,7-dimethyl-6-phenylazo-3*H*-thiazolo[4,5-*b*]pyridin-2-ones synthesis as the integral part of scaffold-based biologically active compounds design and their discovery as potential drug candidates using traditional organic synthesis protocols and pharmacological screening methodologies. The synthetic protocol developed for thiazolo[4,5-*b*]pyridines construction and the wide range of their biological responses highlight the therapeutic interest of designing this scaffold-based compounds. A series of novel thiazolo[4,5-*b*]pyridines derivatives testing over the carageenin induced rat paw edema revealed strong anti-inflammatory action of some compounds which exceeded that one of Diclofenac.

Graphical abstract:



Keywords: synthesis, thiazolo[4,5-*b*]pyridine-2-ones, anti-inflammatory activity

Introduction

In modern theoretical and clinical medicine inflammation problem remains one of the main.There is a significant amount of drugs used to treat inflammation. But all of them have varying degrees of ulcerogenic properties [1, 2]. Therefore, the discovery of anti-inflammatory agents is a recent problem that requires new methodological approaches development for the synthesis of novel compounds and their pharmacological activity screening, while it is also the society relevant task of life sciences, including pharmaceutical and medical chemistry.

4-Azolidone core is considered to be the efficient scaffold for drug-like molecules design as the integral part of modern medicinal chemistry. The thiazolidine based heterocycles and their analogs fused to the pyridine ring were shown to posses the wide range of biological actions. Thiazolopyridine derivatives are characterized with diverse biological activities, among which antioxidant, tuberculostatic, anticancer, anti-inflammatory and antifungal effects have been reported in the past decade [3-8]. Some of their analogues were recognized as H3 receptor antagonists [9], or act as antagonists of metabotropic glutamate receptors 5 (mGluR5) [10], another ones were revealed as potent inhibitors with respect to the receptors of the epidermal growth factor [11] and a number of other enzymes [12,13]. Thiazolopyridine derivatives have been also used as sensitive analytical reagents [14].

The objective of the present work was to synthesize a series of novel 3*H*-thiazolo[4,5-*b*]pyridine-2-ones by the structural modification of the core heterocycle in its N^3 position for further pharmacological screening *in vivo* as anti-inflammatory activity.

Experimental Part

Materials and methods

All chemicals were of analytical grade and commercially available. All reagents and solvents were used without further purification and drying.

Melting points were measured in open capillary tubes on a BUCHI B-545 melting point apparatus and are un corrected. The elemental analyses (C, H, N) were performed using the Perkin-Elmer 2400 CHN analyzer. Analyses indicated by the symbols of the elements or functions were within ±0.4% of the theoretical values.The ^{1}H NMR spectra were recorded on Varian Gemini 400 MHz in DMSO-d_6 or DMSO-d_6+CCl_4 mixtureusing tetramethylsilane (TMS) as an internal standard. Chemical shifts are reported in ppm units with use of δ scale.

Ibuprofen and Diclofenac were purchased from medical store.

Chemistry

Synthesis of 5,7-dimethyl-6-phenylazo-3H-thiazolo[4,5-b]pyridine-2-one ***(1)***.

Sodium (200 mmol) was dissolved in anhydrous methanol (100 ml), and to the solution obtained at 20°C 4-iminothiazolidin-2-one (50 mmol) and α-phenylazoacetylacetone (50 mmol) were added. The mixture was left standing for 7 days with the intermittent stirring, and then it was acidified with acetic acid to pH ~5, five-fold diluted with water. The precipitate was filtered off, washed with water, and dried at 100-110 ºC. The precipitate next was recrystallized from toluene. Brick-red crystalline powder, well soluble in DMF, DMSO, alkalis and mineral acids solutions, feebly soluble in benzene, toluene, alcohols, almostinsoluble in water.

Yield 86%, red crystals, mp 258-259 °C (decomp., toluene). ^{1}H NMR (400 MHz, DMSO-d_6+CCl_4), δ, ppm: 2.42 (s, 3H, CH_3), 2.61 (s, 3H, CH_3), 7.59-7.61 (m, 3H, C_6H_5), 7.80 (d, 2H, J=8.0 Hz, C_6H_5), 12.78 (s, 1H, NH). Calcd.for $C_{14}H_{12}N_4OS$ %: C, 59.14; H, 4.25; N, 19.70. Found %: C, 58.81; H, 4.32; N, 19.79.

Synthesis of 3 - (5,7 - dimethyl - 6 - phenylazo-2-oxo-thiazolo[4,5-b]pyridine-3-yl)-propionitryl ***(2)***.

A mixture of pyridine (50 mL) and water (10 mL) with acrylonitrile (3 mL) was added to compound **1** (10 mmol). The reaction mixture was refluxed 5 h. Oncooling the precipitation was achieved with petroleum ether-water

mixture (3:1). The precipitate was recrystallized from acetic acid, filtered off, and dried. Orange crystalline powder, well soluble in DMF, DMSO, feebly soluble in benzene, toluene, almost insoluble in water.

Yield 72%, red crystals, mp 127-128°C (decomp.,acetic acid). ^{1}H NMR (400 MHz, DMSO-d_6+CCl_4), δ, ppm: 2.44 (s, 3H, CH_3), 2.66 (s, 3H, CH_3), 3.10 (t, 2H, *J*=4.30Hz, *J*=5.70Hz CH_2), 4.27 (t, 2H, *J*=4.30Hz, *J*=5.70Hz CH_2), 7.61 (d, 3H, *J*=6.7Hz, C_6H_5), 7.87 (d, 2H, *J*=4.7Hz, C_6H_5). Calcd. for $C_{17}H_{15}N_5OS$ %: C, 60.52; H, 4.48; N, 20.76. Found %: C, 60.65; H, 4.45; N, 20.90.

Synthesis of 5,7-dimethyl-6-phenylazo-3H-thiazolo[4,5-b]pyridine-2-one potassium salt ***(3)***.

A mixture of water (50 mL) and potassium hydroxide (10 mmol) was treated with compound **1** (10 mmol), and the mixture was heated for 30 minutes to completedis solution. The solution obtained was evaporated to dryness.The residue was dried at 100°C. Red crystalline powder soluble in water and alcohols, sparingly soluble in organic solvents.

Yield quantitative (100%), mp > 300 ºC (decomp., water). ^{1}H NMR (400 MHz, DMSO-d_6+CCl_4), δ, ppm: .2.48 (s, 3H, CH_3), 2.64 (s, 3H, CH_3), 7.43 (d, 1H, *J*=6.8 Hz, C_6H_5), 7.53 (t, 2H, *J*=7.6 Hz, C_6H_5), 7.75 (d, 2H, *J*=7.6 Hz, C_6H_5). Calcd.for $C_{14}H_{11}N_4OSK$ %: C 52.34; H 3.32; N 17.41. Found %: C 52.15; H 3.44; N 17.38.

General procedure for the synthesis of N^3 - substituted 5, 7 - dimethyl - 6-phenylazo - 3H - thiazolo [4,5-b] pyridine - 2 - one derivatives ***(4a-4f; 5a-5i; 6)***.

Compound **3** (9 mmol) was dissolved in dimethylformamide (DMF) (12 mL) at heating. The obtained solution is treated with the appropriate alkylating agent (9 mmol): alkyl halides for compounds **4a-4f**, chloroacetamides for compounds **5a-5i**, and ethylchloroacetate for compound **6** obtaining. The reaction mixture was refluxed 20 min. The white precipitate of the respective N^3-substituted 5,7-dimethyl-6-phenylazo-3*H*-thiazolo[4,5-*b*]pyridine-2-one **4a-4f**, **5a-5i**, **6** was filtered hot, washed with hot DMF and cooled to 50 ºC. Water (100 mL) was added to the filtrate and the mixture was cooled to 12-15 ºC. The precipitate that formed was filtered off, washed with water and dried firstly at room temperature and then at 60 ºC. The obtained compounds were recrystallized: **4a-4f**, **6** – from acetic acid-water mixture (1:1), **5a-5i** – from isopropanole-water (1:1) mixture. The target compounds are orange crystalline powders, well soluble in alcohols, chloroform, dioxane, DMF, acetic acid, almost insoluble in water.

3-Allyl-5,7-dimethyl-6-phenylazo-3H-thiazolo[4,5-b]pyridine-2-one ***(4a)***.

Yield 61%, mp≥ 130-131 ºC (decomp., acetic acid-water (1:1)). ^{1}H NMR (400 MHz, DMSO-d_6+CCl_4), δ, ppm: 2.48 (s, 3H, CH_3), 2.65 (s, 3H, CH_3), 4.64 (d, 2H, *J*=4.8 Hz, CH_2–CH=CH_2), 5.19 (t, 2H, *J*=4.0 Hz, *J*=6.0 Hz, CH_2–CH=CH_2), 5.98-6.01 (m, 1H, *J*=6.8 Hz, CH_2–CH=CH_2), 7.62-7.88 (m, 5H,C_6H_5). Calcd.-for $C_{17}H_{16}N_4OS$ %: C61.92; H4.55; N 18.05. Found %: C 61.15; H4.51; N 17.98.

5,7-Dimethyl-3-propyl-6-phenylazo-3H-thiazolo[4,5-b]pyridine-2-one ***(4b)***.

Yield 74%, mp 107-108 ºC (decomp., acetic acid-water (1:1)). ^{1}H NMR (400 MHz, DMSO-d_6+CCl_4), δ, ppm: 0.90 (t, 3H, *J*=6.85 Hz, *J*=7.19Hz, -CH_2–CH_2-CH_3), 1,73-1,77 (m, 2H, -CH_2 –CH_2-CH_3), 2.43 (s, 3H, CH_3), 2.65 (s, 3H, CH_3), 3,96 (t, 2H, *J*=6,24 Hz, *J*=6.16 Hz, -CH_2–CH_2-CH_3),7.60 (d, 3H, *J*=5.80Hz, C_6H_5), 7.87 (d, 3H,*J*=6.50Hz, C_6H_5). Calcd.for $C_{17}H_{18}N_4OS$ %: C 62.55; H 5.56; N 17.16. Found %: C 61.98; H 5.40; N 17.10.

5,7-Dimethyl-3-isopropyl-6-phenylazo-3H-thiazolo[4,5-b]pyridine-2-one ***(4c)***.

Yield 82%, mp 104-105 ºC (decomp., acetic acid-water (1:1)). ^{1}H NMR (400 MHz, DMSO-d_6+CCl_4), δ, ppm: 1.53 (s, 3H, *J*=6.85 Hz, CH_3-CH-CH_3), 1.55 (s, 3H, CH_3-CH-CH_3), 2.45 (s, 3H, CH_3), 2.68 (s, 3H, CH_3), 5.05 (s, 1H, CH_3-CH-CH_3), 7.62 (s, 3H, C_6H_5),

7.89 (s, 2H, C_6H_5). Calcd.for $C_{17}H_{18}N_4OS$: C 61.82; H 4.86; N 17.99. Found %: C 61.78; H 4.80; N 17.85.

5,7-Dimethyl-3-pentyl-6-phenylazo-3H-thiazolo[4,5-b]pyridine-2-one ***(4d)***.

Yield 59%, mp 93-94 ºC (decomp., isopropyl-water (1:1)). ^{1}H NMR (400 MHz, DMSO-d_6+CCl_4), δ, ppm: 0.85 (t, 3H, J=7.18 Hz, J=6.91Hz, $CH_3(CH_2)_4$), 1.25-1.34 (m, 4H, CH_2), 1.67-1.70 (m, 2H, CH_2), 2.38 (s, 3H, CH_3), 2.61 (s, 3H, CH_3), 3.94 (d, 2H, J=7.23 Hz, CH_2), 7.55-7.58 (m, 3H, C_6H_5), 7.82-7.83 (m, 2H, C_6H_5). Calcd.for $C_{19}H_{22}N_4OS$: C 64.38; H 6.26; N 15.81. Found %: C 64.90; H 6.20; N15.20.

5,7-Dimethyl-3-(3-methylbutyl)-6-phenylazo-3H-thiazolo[4,5-b]pyridine-2-one ***(4e)***.

Yield 64%, mp 98-99 ºC (decomp., isopropyl-water (1:1)). ^{1}H NMR (400 MHz, DMSO-d_6+CCl_4), δ, ppm:0.95 (d, 6H, J=2.8 Hz, CH-$(CH_3)_2$), 1.58 (s, 1H, CH), 1.61 (s, 1H, N-CH_2-CH_2), 2.41 (s, 3H, CH_3), 2.61 (s, 3H, CH_3), 4.00 (t, 2H, J=7.23 Hz, N-CH_2-CH_2), 7.58-7.59 (m, 3H, C_6H_5), 7.83-7.84 (m, 2H,C_6H_5). Calcd.for $C_{19}H_{22}N_4OS$: C 64.38; H 6.26; N 15.81.Found %: C 64.85; H 6.25; N 15.38.

5,7-Dimethyl-6-phenylazo-3-(2-chloroethyl)-3H-thiazolo[4,5-b]pyridine-2-one ***(4f)***.

Yield 87%, mp 139-140 ºC (decomp., isopropyl-water (1:1)). ^{1}H NMR (400 MHz, DMSO-d_6+CCl_4), δ, ppm: 2.41 (s, 3H, CH_3), 2.67 (s, 3H, CH_3), 4.05 (t, 2H, J=6.00Hz, J=5.98 Hz, N-CH_2-CH_2), 4.36-4.40 (m, 2H,N-CH_2-CH_2), 7.61 (d, 3H, J=7.18 Hz, C_6H_5), 7.89 (d, 2H, J=7.72 Hz, C_6H_5). Calcd.for $C_{16}H_{15}ClN_4OS$: C 53.87; H 4.23; N 16.78. Found %: C 54.05; H 5.35; N 16.30.

2-(5,7-Dimethyl-2-oxo-6-phenylazo-thiazolo[4,5-b]pyridine-3-yl)-N-phenyl-acetamide ***(5a)***.

Yield 76%, mp 224-225 ºC (decomp., isopropyl-water (1:1)). ^{1}H NMR (400 MHz, DMSO-d_6+CCl_4), δ, ppm: 2.51 (s, 3H, CH_3), 2.68 (s, 3H, CH_3), 4.70 (s, 2H, CH_2), 7.28-7.34 (m, 4H, Ar), 7.61-7.64 (m, 3H, C_6H_5), 7.75 (s, 1H, Ar), 7.89-7.92 (m, 2H, C_6H_5), 11.05 (s, 1H, NH). Calcd.for $C_{22}H_{19}N_5O_2S$: C 63.29; H 4.59; N 16.77. Found %: C 63.87; H4.50; N 17.20.

2-(5,7-Dimethyl-2-oxo-6-phenylazo-thiazolo[4,5-b]pyridine-3-yl)-N-(2-nitrophenyl-acetamide ***(5b)***.

Yield 80%, mp 190-192 ºC (decomp., isopropyl-water (1:1)). ^{1}H NMR (400 MHz, DMSO-d_6+CCl_4), δ, ppm: 2.44 (s, 3H, CH_3), 2.62 (s, 3H, CH_3), 4.93 (s, 2H, CH_2), 7.61-7.64 (m, 3H,C_6H_5), 7.65-7.68 (m, 2H, Ar), 7.89-7.92 (m, 2H, C_6H_5), 8.23-8.26 (m, 2H, Ar), 11.00 (s, 1H, NH). Calcd.for $C_{22}H_{18}N_6O_4S$: C 57.14; H 3.92; N 18.17. Found %: C 56.85; H 3.85; N 17.90.

2-(5,7-Dimethyl-2-oxo-6-phenylazo-thiazolo[4,5-b]pyridine-3-yl)-N-(3-nitrophenyl-acetamide ***(5c)***.

Yield 71%, mp 194-195 ºC (decomp., isopropyl-water (1:1)). ^{1}H NMR (400 MHz, DMSO-d_6+CCl_4), δ, ppm: 2.48 (s, 3H, CH_3), 2.65 (s, 3H, CH_3), 5.36 (s, 2H, CH_2), 7.62 (t, 3H, J=7.91Hz, J=7.65Hz, C_6H_5), 7.64-7.68 (m, 2H,Ar), 7.88 (d, 2H, J=7.22 Hz,C_6H_5), 8.21 (d, 2H, J=8.60 Hz, Ar),9.23 (s, 1H, NH).Calcd.for $C_{22}H_{18}N_6O_4S$: C 57.14; H 3.92; N 18.17. Found %: C 57.48; H 3.98; N 18.55.

2-(5,7-Dimethyl-2-oxo-6-phenylazo-thiazolo[4,5-b]pyridine-3-yl)-N-(4-nitrophenyl-acetamide ***(5d)***.

Yield76%, mp 203-204 ºC (decomp., isopropyl-water (1:1)). ^{1}H NMR (400 MHz, DMSO-d_6+CCl_4), δ, ppm: 2.51 (s, 3H, CH_3), 2.62 (s, 3H, CH_3), 4.95 (s, 2H, CH_2), 7.61-7.63 (m, 3H,C_6H_5), 7.84-7.86 (m, 2H, Ar), 7.87-7.90 (m, 2H, C_6H_5), 8.20-8.23 (m, 2H, Ar), 10.89 (s, 1H, NH). Calcd.for $C_{22}H_{18}N_6O_4S$: C57.14; H 3.92; N 18.17. Found %: C 58.00; H 3.75; N 18.90.

2-(5,7-Dimethyl-2-oxo-6-phenylazo-thiazolo[4,5-b]pyridine-3-yl)-N-m-tolyl-acetamide

(5e).

Yield 74%, mp 249-250 ºC (decomp., isopropyl-water (1:1)). ^{1}HNMR (400 MHz, DMSO-d_6+CCl_4), δ, ppm: 2.26 (s, 3H, Ar-CH_3), 2.37 (s, 3H, CH_3), 2.64 (s, 3H, CH_3), 4.86 (s, 2H, CH_2), 7.13 (d, 2H, *J*=7.63 Hz, Ar), 7.46 (d, 2H, *J*=7.75 Hz, Ar), 7.62 (d, 3H, *J*=7.20Hz, C_6H_5), 7.90 (d, 2H, *J*=6.18 Hz, C_6H_5), 10.38 (s, 1H, NH). Calcd.for $C_{23}H_{21}N_5O_2S$: C 64.02; H 4.91; N 16.23. Found %: C 65.05; H 4.79; N 16.95.

2-(5,7-Dimethyl-2-oxo-6-phenylazo-thiazolo[4,5-b]pyridine-3-yl)-N-(2-hydroxy-phenyl)-acetamide ***(5f)***.

Yield 83%, mp 214-215 ºC (decomp., isopropyl-water (1:1)). ^{1}H NMR (400 MHz, DMSO-d_6+CCl_4), δ, ppm: 2.41 (s, 3H, CH_3), 2.65 (s, 3H, CH_3), 4.84 (s, 2H, CH_2), 6.88 (s, 1H, Ar), 7.10 (d, 2H, *J*=12.50 Hz, Ar), 7.61 (d, 3H, *J*=5.20 Hz, C_6H_5), 7.89 (s, 2H, C_6H_5), 7.91 (s, 1H, Ar), 9.36 (s, 1H, NH), 10.32 (s, 1H, OH). Calcd.for $C_{22}H_{19}N_5O_3S$ %: C 60.96; H 4.42; N 16.16. Found %: C 61.27; H 4.50; N 16.30.

2-(5,7-Dimethyl-2-oxo-6-phenylazo-thiazolo[4,5-b]pyridine-3-yl)-N-(3-hydroxy-phenyl)-acetamide ***(5g)***.

Yield76%, mp 218-220 ºC (decomp., isopropyl-water (1:1)). ^{1}H NMR (400 MHz, DMSO-d_6+CCl_4), δ, ppm: 2.43 (s, 3H, CH_3), 2.64 (s, 3H, CH_3), 4.95 (s, 2H, CH_2), 7.61 (s, 3H,C_6H_5), 7.64 (s, 2H, Ar), 7.87 (s, 2H, Ar), 7.91 (s, 2H, C_6H_5), 9.80 (s, 1H, NH), 12.75 (s, 1H, OH). Calcd.for $C_{22}H_{19}N_5O_3S$ %: C 60.96; H 4.42; N 16.16. Found %: C 60.10; H 4.28; N 16.45.

N-(3-Amino-phenyl)-2-(5,7-dimethyl-2-oxo-6-phenylazo-thiazolo[4,5-b]pyridine-3-yl)-acetamide ***(5h)***.

Yield79%, mp 209-210 ºC (decomp., isopropyl-water (1:1)). ^{1}H NMR (400 MHz, DMSO-d_6+CCl_4), δ, ppm: 2.43 (s, 3H, CH_3), 2.63 (s, 3H, CH_3), 4.22 (s, 2H, NH_2), 4.86 (s, 2H, CH_2), 7.28 (s, 2H, Ar), 7.59 (s, 1H, Ar), 7.61 (s, 3H, C_6H_5), 7.87 (s, 2H, C_6H_5), 8.00 (s, 1H, Ar), 10.45 (s, 1H, NH). Calcd.for $C_{22}H_{20}N_6O_2S$ %: C 61.10; H 4.66; N 19.43. Found %: C 60.35; H 4.59; N 18.90.

2-(5,7-Dimethyl-2-oxo-6-phenylazo-thiazolo[4,5-b]pyridine-3-yl)-N-(4-ethyl-phenyl)-acetamide ***(5i)***.

Yield 79%, mp 200-201 ºC (decomp., isopropyl-water (1:1)). ^{1}H NMR (400 MHz, DMSO-d_6+CCl_4), δ, ppm: 1.17 (t, 3H,*J*=7.33 Hz, *J*=7.00 Hz, OCH_2CH_3), 2.51 (s, 3H, CH_3), 2.64 (s, 3H, CH_3), 4.22 (s, 2H, OCH_2CH_3), 4.85 (s, 2H, CH_2), 7.16 (d, 2H, *J*=7.90 Hz, Ar), 7.48 (d, 2H, *J*=7.80 Hz, Ar), 7.62 (d, 3H, *J*=6.90 Hz, C_6H_5), 7.89 (d, 2H, *J*=6.80 Hz, C_6H_5), 10.38 (s, 1H, NH). Calcd.for $C_{24}H_{23}N_5O_2S$ %: C64.70; H 5.20; N 15.72. Found %: C 64.80; H 4.98; N 15.95.

(5,7-Dimethyl-2-oxo-6-phenylazo-thiazolo[4,5-b]pyridin-3-yl)-acetic acid ethyl ester ***(6)***.

Yield 66%, mp 103-104 ºC (decomp., isopropyl-water (1:1)). ^{1}H NMR (400 MHz, DMSO-d_6+CCl_4), δ, ppm: 1.23 (t, 3H, *J*=7.00 Hz, OCH_2CH_3), 2.48 (s, 3H, CH_3), 2.64 (s, 3H, CH_3), 4.19-4.23 (m, 2H, OCH_2CH_3), 4.82 (s, 2H, CH_2), 7.61-7.62 (m, 3H, C_6H_5), 7.89-7.90 (m, 2H, C_6H_5). Calcd.for $C_{18}H_{18}N_4O_3S$ %: C 58.36; H 4.90; N 15.12.Found %: C 58.88; H 4.85; N 15.25.

Synthesis of (5,7-dimethyl-6-phenylazo-2-oxo-thiazolo[4,5-b]pyridine-3-yl)-acetic acid hydrazide ***(7)***.

Compound **6** (20 mmol) was dissolved in ethanol (8mL) at heating. The obtained solution is treated with hydrazine hydrate 50% solution (30 mmol). The reaction mixture was heated in a hot water bath for 12 h. The precipitate was filtered off, washed with water and dried firstly at room temperature and then at 60 ºC. The obtained compounds were recrystallized from buthanol. White crystalline powder, well soluble in DMF, DMSO, feebly soluble in water.

Yield 65%, mp 210-211 ºC (decomp.,butanol). ^{1}H NMR (400 MHz, DMSO-d_6+CCl_4),

δ, ppm:2.47 (s, 3H, CH_3), 2.63 (s, 3H, CH_3), 4.33 (s, 2H, NH_2), 4.60 (s, 2H, CH_2), 7.61 (d, 2H, *J*=6.50 Hz, C_6H_5), 7.88 (d, 2H, *J*=6.50 Hz, C_6H_5), 9.42 (s, 1H, NH).Calcd.for $C_{16}H_{16}N_6O_2S$ %: C 53.92; H 4.53; N2 3.58. Found %: C 54.02; H 4.55; N 23.20.

General procedure for the synthesis of 5,7-dimethyl-2-oxo-6-phenylazo-thiazolo[4,5-b]pyridine-2-one hydrazide arylidene derivatives ***(8a-8q)***.

Compound **20** (5 mmol) was dissolved in ethanol (30 mL). The solution was treated with the appropriate aldehyde (5 mmol) in ethanol (20 mL) hot solution at intensive stirring. The reaction mixture was refluxed 1 h. The precipitate that formed was filtered off, washed with water, and dried firstly at room temperature, then at 100-110 ºC. The obtained compounds were recrystallized from ethanol. The target compounds are orange or brick-red crystalline powders, well soluble in DMF, DMSO, dioxane, acetic acid, alcohols (under heating), almost insoluble in water.

(5,7-Dimethyl-2-oxo-6-phenylazo-thiazolo[4,5-b]pyridine-3-yl)-acetic acid (4-nitro-bezylidene)-hydrazide ***(8a)***.

Yield 95%, mp 252-253 ºC (decomp., ethanol). ^{1}H NMR (400 MHz, DMSO-d_6+CCl_4), δ, ppm: 2.55 (s, 3H, CH_3), 2.63 (s, 3H, CH_3), 5.25 (s, 2H, CH_2), 7.61 (d, 3H, *J*=6.50 Hz, C_6H_5), 7.88 (d, 2H, *J*=6.50 Hz, C_6H_5), 8.00 (d, 2H, Ar), 8.20 (s, 1H, CH), 8.27 (d, 2H, Ar), 11.93 (s, 1H, NH).Calcd.for $C_{23}H_{19}N_7O_4S$%: C 56.93; H 3.91; N 20.03. Found %: C 54.02; H 4.55; N 23.20.

(5,7-Dimethyl-2-oxo-6-phenylazo-thiazolo[4,5-b]pyridine-3-yl)-acetic acid (3-nitro-bezylidene)-hydrazide ***(8b)***.

Yield 90%, mp 260-261 ºC (decomp., ethanol). ^{1}H NMR (400 MHz, DMSO-d_6+CCl_4), δ, ppm: 2.57 (s, 3H, CH_3), 2.63 (s, 3H, CH_3), 5.24 (s, 2H, CH_2), 7.61 (d, 3H, *J*=6.75 Hz, C_6H_5),7.75 (s, 1H, Ar),7.88 (t, 2H, C_6H_5), 8.21 (s,1H, CH), 8.26 (d, 1H, Ar), 8.55 (s, 2H, Ar), 12.02 (s, 1H, NH). Calcd.for $C_{23}H_{19}N_7O_4S$%: C 56.93; H 3.91; N 20.03. Found %: C 55.77; H 3.85; N 20.55.

(5,7-Dimethyl-2-oxo-6-phenylazo-thiazolo[4,5-b]pyridine-3-yl)-acetic acid (4-bromo-benzylidene)-hydrazide ***(8c)***.

Yield 92%, mp 274-275 ºC (decomp., ethanol). ^{1}H NMR (400 MHz, DMSO-d_6+CCl_4), δ, ppm: 2.57 (s, 3H, CH_3), 2.64 (s, 3H, CH_3), 5.20 (s, 2H, CH_2), 7.62 (d, 3H, *J*=6.75 Hz, Ar), 7.65 (d, 3H, *J*=6.50 Hz, C_6H_5),7.71 (d, 2H, *J*=7.20 Hz, Ar), 7.90 (d, 2H, *J*=5.20 Hz, C_6H_5), 8.07 (s, 1H, CH), 11.86 (s, 1H, NH).Calcd.for $C_{23}H_{19}BrN_6O_2S$%: C 52.78; H 3.66; N 16.06. Found %: C 53.25; H 3.75; N 15.85.

(5,7-Dimethyl-2-oxo-6-phenylazo-thiazolo[4,5-b]pyridine-3-yl)-acetic acid (4-hydroxy-3-methoxy-benzylidene)-hydrazide ***(8d)***.

Yield 82%, mp 257-258 ºC (decomp., ethanol). ^{1}H NMR (400 MHz, DMSO-d_6+CCl_4), δ, ppm: 2.55 (s, 3H, CH_3), 2.64 (s, 3H, CH_3), 3.85 (s, 3H, OCH_3), 5.18 (s, 2H, CH_2), 6.84 (d, 1H, *J*=5.40 Hz, Ar),7.12 (d, 1H, *J*=5.20 Hz, Ar), 7.37 (s, 1H, Ar),7.62 (d, 3H, *J*=8.50 Hz, C_6H_5), 7.92 (d, 2H, *J*=6.20 Hz, C_6H_5), 7.96 (s, 1H, CH), 9.51 (s, 1H, OH), 11.65 (s, 1H, NH). Calcd.for $C_{24}H_{22}N_6O_4S$%: C 58.76; H 4.52; N 17.13. Found %: C 58.25; H 4.28; N 17.25.

(5,7-Dimethyl-2-oxo-6-phenylazo-thiazolo[4,5-b]pyridine-3-yl)-acetic acid (4-fluoro-benzylidene)-hydrazide ***(8e)***.

Yield 90%, mp 280-281 ºC (decomp., ethanol). ^{1}H NMR (400 MHz, DMSO-d_6+CCl_4), δ, ppm: 2.55 (s, 3H, CH_3), 2.64 (s, 3H, CH_3), 5.19 (s, 2H, CH_2), 7.29 (t, 2H, *J*=7.15 Hz, *J*=6.48 Hz, Ar), 7.63 (d, 3H, *J*=7.25 Hz, C_6H_5), 7.82 (t, 2H, *J*=6.00 Hz, *J*=4.00 Hz, Ar), 7.90 (d, 2H, *J*=6.40 Hz, C_6H_5), 8.09 (s, 1H, CH), 11.73 (s, 1H, NH). Calcd.for $C_{23}H_{19}FN_6O_4S$ %: C 59.73; H 4.14; N 18.17. Found %: C 59.80; H 4.18; N 18.28.

(5,7-Dimethyl-2-oxo-6-phenylazo-thiazolo[4,5-b]pyridine-3-yl)-acetic acid (5-chloro-2-hydroxy-benzylidene)-hydrazide

(8f).

Yield 95%, mp 273-274 ºC (decomp., ethanol). ^{1}H NMR (400 MHz, DMSO-d_6+CCl_4), δ, ppm: 2.51 (s, 3H, CH_3), 2.64 (s, 3H, CH_3), 5.19 (s, 2H, CH_2), 6.94 (d, 2H, Ar), 7.30 (t, 1H, Ar), 7.63 (d, 3H, *J*=6.88 Hz, C_6H_5), 7.82 (s, 1H, Ar), 7.90 (d, 2H, *J*=6.40 Hz, C_6H_5), 8.33 (s, 1H, CH), 11.80 (s, 1H, NH). Calcd.for $C_{23}H_{19}ClN_6O_3S$ %: C 55.81; H 3.87; N 16.98. Found %: C 55.75; H 3.78; N 16.20.

(5,7-Dimethyl-2-oxo-6-phenylazo-thiazolo[4,5-b]pyridine-3-yl)-acetic acid (4-dimethylamino-benzylidene)-hydrazide ***(8g)***.

Yield 80%, mp 240-241 ºC (decomp., ethanol). ^{1}H NMR (400 MHz, DMSO-d_6+CCl_4), δ, ppm: 2.51 (s, 3H, CH_3), 2.64 (s, 3H, CH_3), 2.99 (s, 6H, N-$(CH_3)_2$), 5.13 (s, 2H, CH_2), 6.75 (d, 2H, Ar), 7.54 (d, 3H, *J*=4.70 Hz, Ar), 7.62 (m, 3H, C_6H_5), 7.90 (d, 3H, *J*=6.25 Hz, C_6H_5), 7.94 (s, 1H, CH), 11.45 (s, 1H, NH). Calcd.for $C_{25}H_{25}N_7O_2S$ %: C 61.58; H 5.17; N 20.11. Found %: C 61.90; H 5.10; N 20.20.

[5,7-Dimethyl-2-oxo-6-(1-propenyl-buta-1,3-dienylazo)-thiazolo[4,5-b]pyridine-3-yl]-acetic acid (3,4-dimethoxy-benzylidene)-hydrazide ***(8h)***.

Yield 87%, mp 267-268 ºC (decomp., ethanol). ^{1}H NMR (400 MHz, DMSO-d_6+CCl_4), δ, ppm: 2.55 (s, 3H, CH_3), 2.64 (s, 3H, CH_3), 3.82 (d, *J*=9.80 Hz, 6H, $O(CH_3)_2$), 5.20 (s, 2H, CH_2), 7.02 (d, 1H, *J*=8.10 Hz, Ar), 7.22 (d, 1H, *J*=7.60 Hz, Ar), 7.40 (d, 1H, *J*=7.95 Hz, Ar), 7.62 (d, 3H, *J*=7.58 Hz, C_6H_5), 7.90 (d, 2H, *J*=6.76 Hz, C_6H_5), 8.00 (s, 1H, CH), 11.68 (s, 1H, NH). Calcd.for $C_{25}H_{24}N_6O_4S$ %: C 59.71; H 4.59; N 16.66. Found %: C5 9.90; H 4.50; N 16.70.

(5,7-Dimethyl-2-oxo-6-phenylazo-thiazolo[4,5-b]pyridine-3-yl)-acetic acid (4-chloro-benzylidene)-hydrazide ***(8i)***.

Yield 77%, mp 280-281 ºC (decomp., ethanol). ^{1}H NMR (400 MHz, DMSO-d_6+CCl_4), δ, ppm: 2.55 (s, 3H, CH_3), 2.64 (s, 3H, CH_3), 5.19 (s, 2H, CH_2), 7.52 (d, 2H, *J*=6.95 Hz, Ar), 7.62 (d, 3H, *J*=7.45 Hz, C_6H_5), 7.79 (d, 2H, *J*=6.30 Hz, Ar), 7.90 (d, 2H, *J*=6.50 Hz, C_6H_5), 8.01 (s, 1H, CH), 11.86 (s, 1H, NH). Calcd.for $C_{23}H_{19}ClN_6O_2S$ %: C 57.68; H 4.00; N 17.55. Found %: C 57.00; H 4.15; N 17.98.

(5,7-Dimethyl-2-oxo-6-phenylazo-thiazolo[4,5-b]pyridine-3-yl)-acetic acid (3-ethoxy-4-hydroxy-benzylidene)-hydrazide ***(8j)***.

Yield 82%, mp 253-254 ºC (decomp., ethanol). ^{1}H NMR (400 MHz, DMSO-d_6+CCl_4), δ, ppm: 1.36 (s, 3H, OCH_2CH_3), 2.55 (s, 3H, CH_3), 2.64 (s, 3H, CH_3), 4.09 (s, 2H, OCH_2CH_3), 5.17 (s, 2H, CH_2), 6.95 (d, 1H, *J*=8.10 Hz, Ar), 7.10 (s, 1H, Ar), 7.34 (s, 1H, Ar), 7.62 (d, 3H, *J*=6.90 Hz, C_6H_5), 7.90 (d, 2H, *J*=7.25 Hz, C_6H_5), 7.94 (s, 1H, CH), 9.45 (s, 1H, OH), 11.66 (s, 1H, NH). Calcd.for $C_{25}H_{24}N_6O_4S$ %: C 59.98; H 5.42; N 16.14. Found %:C 59.33; H 5.37; N 16.07.

2-{[2-(5,7-Dimethyl-2-oxo-6-phenylazo-thiazolo[4,5-b]pyridine-3-yl)-acetyl]-hydrazonomethyl}-benzoic acid ***(8k)***.

Yield 90%, mp 239-240 ºC (decomp., ethanol). ^{1}H NMR (400 MHz, DMSO-d_6+CCl_4), δ, ppm: 2.55 (s, 3H, CH_3), 2.64 (s, 3H, CH_3), 5.20 (s, 2H, CH_2), 7.55-7.57 (m, 2H, Ar), 7.62 (d, 3H, *J*=6.74 Hz, C_6H_5), 7.90 (d, 2H, *J*=7.55 Hz,C_6H_5), 7.92 (s, 1H, Ar), 8.01 (s, 1H, CH), 8.84 (s, 1H, Ar), 11.91 (s, 1H, NH), 13.28 (s, 1H, COOH). Calcd.for $C_{24}H_{20}N_6O_4S$ %: C 59.01; H 4.13; N 17.20.Found %:C 59.25; H 4.25; N 17.35.

(5,7-Dimethyl-2-oxo-6-phenylazo-thiazolo[4,5-b]pyridine-3-yl)-acetic acid (2-chloro-benzylidene)-hydrazide ***(8l)***.

Yield 85%, mp 270-271 ºC (decomp., ethanol). ^{1}H NMR (400 MHz, DMSO-d_6+CCl_4), δ, ppm: 2.55 (s, 3H, CH_3), 2.64 (s, 3H, CH_3), 5.21 (s, 2H, CH_2), 7.40-7.49 (m, 2H, Ar), 7.54 (d, 1H, *J*=7.63 Hz, Ar), 7.62 (d, 3H, *J*=6.78 Hz, C_6H_5), 7.90 (d, 2H, *J*=7.53 Hz, C_6H_5), 8.01 (s, 1H, CH), 8.46 (s, 1H, Ar), 11.97 (s, 1H, NH). Calcd.for $C_{23}H_{19}ClN_6O_2S$ %: C 57.68; H 4.00;

N 17.55. Found %: C 57.85; H 4.25; N 17.75.

(5,7-Dimethyl-2-oxo-6-phenylazo-thiazolo[4,5-b]pyridine-3-yl)-acetic acid (5-(4-nitro-phenyl)-furan-2-ylmethylene)-hydrazide ***(8m)***.

Yield 82%, mp 255-256 ºC (decomp., ethanol). ^{1}H NMR (400 MHz, DMSO-d_6+CCl_4), δ, ppm: 2.55 (s, 3H, CH_3), 2.64 (s, 3H, CH_3), 5.20 (s, 2H, CH_2), 7.14 (s, 1H, Furan), 7.43 (s, 1H, Furan), 7.62 (d, 3H, J=6.57Hz, C_6H_5), 7.90 (d, 2H, J=6.45Hz, C_6H_5), 8.02 (s, 1H, CH), 8.04 (s, 1H, Ar), 8.28 (s, 2H, Ar), 11.83 (s, 1H, NH). Calcd.for $C_{27}H_{21}N_7O_5S$ %: C 58.37; H 3.81; N 17.65. Found %: C 58.70; H 3.90.; N 17.84.

(5,7-Dimethyl-2-oxo-6-phenylazo-thiazolo[4,5-b]pyridine-3-yl)-acetic acid (3-furan-2-yl-allylidene)-hydrazide ***(8n)***.

Yield 77%, mp 264-265 ºC (decomp., ethanol). ^{1}H NMR (400 MHz, DMSO-d_6+CCl_4), δ, ppm: 2.55 (s, 3H, CH_3), 2.63 (s, 3H, CH_3), 5.04 (s, 2H, CH_2), 6.60-6.74 (m, 2H, Furan), 6.95-7.01 (m, 1H, Furan), 7.62 (d, 3H, J=7.65 Hz, C_6H_5), 7.78 (s, 1H, CH), 7.84 (d, 1H, J=6.91 Hz, CH), 7.90 (d, 2H, J=6.50 Hz, C_6H_5), 7.97 (d, 1H, J=7.05 Hz, CH), 11.76 (s, 1H, NH). Calcd.-for $C_{23}H_{20}N_6O_3S$ %: C 59.99; H 4.38; N 18.25. Found %: C 59.79; H 4.51; N 18.80.

(5,7-Dimethyl-2-oxo-6-phenylazo-thiazolo[4,5-b]pyridine-3-yl)-acetic acid [5-(2-chloro-4-trifluoromethyl-phenyl)-furan-2-ylmethylene]-hydrazide ***(8o)***.

Yield 75%, mp 283-284 ºC (decomp., ethanol). ^{1}H NMR (400 MHz, DMSO-d_6+CCl_4), δ, ppm: 2.55 (s, 3H, CH_3), 2.63 (s, 3H, CH_3), 5.16 (s, 2H, CH_2), 7.15 (s, 1H, Furan), 7.41 (s, 1H, Furan), 7.62 (d, 3H, J=7.65 Hz, C_6H_5), 7.73 (s, 1H, Ar), 7.84 (d, 1H, J=6.33 Hz, Ar), 7.89 (d, 2H, J=5.75Hz, C_6H_5), 8.06 (s, 1H, CH), 8.14 (s, 1H, Ar), 11.74 (s, 1H, NH). Calcd.for $C_{28}H_{20}ClF_3N_6O_3S$ %: C 54.86; H 3.29; N 13.71. Found %: C 54.76; H 3.22.; N 13.84.

(5,7-Dimethyl-2-oxo-6-phenylazo-thiazolo[4,5-b]pyridine-3-yl)-acetic acid [5-(2,3-dichloro-phenyl)-furan-2-ylmethylene]-hydrazide ***(8p)***.

Yield 80%, mp 275-276 ºC (decomp., ethanol). ^{1}H NMR (400 MHz, DMSO-d_6+CCl_4), δ, ppm: 2.55 (s, 3H, CH_3), 2.64 (s, 3H, CH_3), 5.17 (s, 2H, CH_2), 7.07-7.15 (m, 1H, Furan), 7.36 (d, 1H, J=5.35 Hz, Furan), 7.50 (d, 2H, J=7.87 Hz, Ar), 7.62 (d, 3H, J=7.80 Hz, C_6H_5), 7.90 (d, 2H, J=6.33 Hz, C_6H_5), 8.02 (s, 1H, CH), 11.85 (s, 1H, NH). Calcd.for $C_{27}H_{20}Cl_2N_6O_3S$ %: C 55.97; H 3.48; N 14.50. Found %: C 55.85; H 3.65.; N 14.55.

(5,7-Dimethyl-2-oxo-6-phenylazo-thiazolo[4,5-b]pyridine-3-yl)-acetic acid [1-(2,5-dichloro-phenyl)-2,5-dimethyl-1H-pyrrol-3-yl-methylene]-hydrazide ***(8q)***.

Yield 79%, mp 255-256 ºC (decomp., ethanol). ^{1}H NMR (400 MHz, DMSO-d_6+CCl_4), δ, ppm: 1.92 (s, 3H, $ArCH_3$), 2.04 (s, 3H, $ArCH_3$), 2.55 (s, 3H, CH_3), 2.64 (s, 3H, CH_3), 5.08 (s, 2H, CH_2), 6.30 (s, 1H, Pyrol), 7.62 (d, 3H, J=7.77 Hz, C_6H_5), 7.68 (d, 1H, J=5.05 Hz, Ar), 7.73 (s, 1H, Ar), 7.78 (d, 1H, J=5.23 Hz, Ar), 8.04 (s, 1H, CH), 11.32 (s, 1H, NH). Calcd.-for $C_{29}H_{25}Cl_2N_7O_2S$%: C 57.43; H 4.15; N 16.16. Found %: C 57.55; H 4.18; N 16.33.

Anti-inflammatory activity

Anti-inflammatory activity [15] was evaluated using carrageenan induced rat paw edema method in rats [16]. Outbred (male/female) white rats weighing 180–220g were used for the edema test. Animals were divided into 38 groups comprising five rats per group. One group was kept as the control and remaining 37 groups (test group) were used to determine the anti-inflammatory activity elicited by the 37 drug candidates, respectively. Rats were kept in the animal house under standard conditions of light and temperature on the general diet prior to the experiment. The standard drugs, Ibuprofen (50 mg/kg body weight), Diclofenac (8 mg/kg body weight) and the test drugs (50 mg/kg body weight) were dissolved in DMSO and administrated through intraperitoneal route. DMSO was injected to the control group. 30 minutes later,

0.1 ml of 2 % carrageenan solution in saline was injected in the sub-plantar region of the right hind paw of each rat. After 4h of the carrageenan injection, the volume of paw edema (in mL) was measured using water plethysmometer and paw edema decreasing was compared between control group and drug-tested groups. Danylo Halytsky Lviv National Medical University ethics committee, constituted by the Ministry of Health of Ukraine, approved the experimental protocol. The inflammatory reaction inhibition was expressed as percent of paw volume reduction and it was calculated using the following formula:

$$\% \text{ Inhibition} = \frac{V_{\text{control}} - V}{V_{\text{control}}} \cdot 100\ \%,$$

where Vcontrol is the increase in paw volume in control group animals;

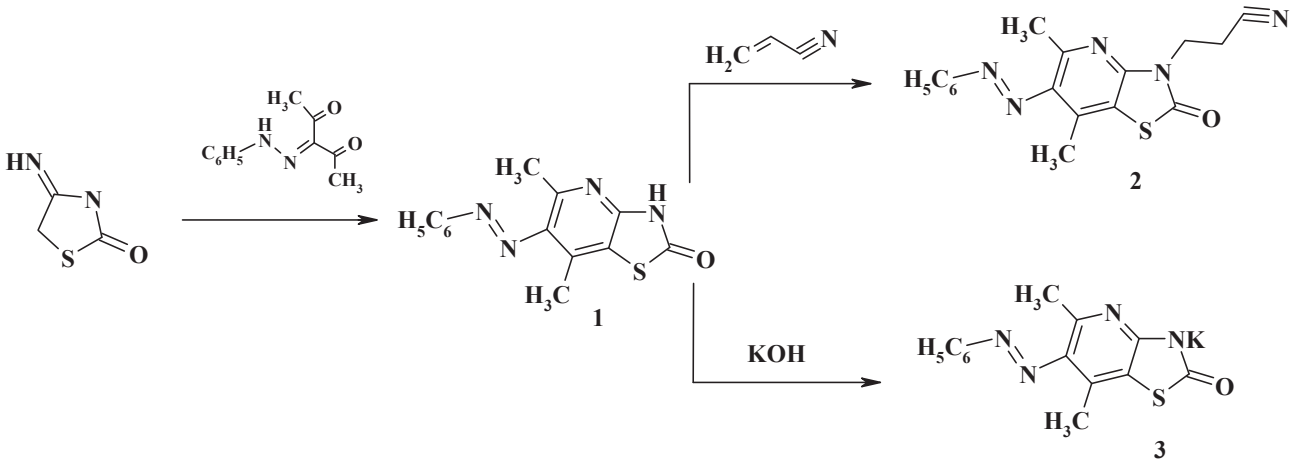
V is the increase in paw volume in animals injected with the test substances.

Results and discussion

Chemistry

The efficient synthetic approach for 3*H*-thiazolo[4,5-*b*]pyridine-2-one system construction had been developed earlier [17] as the protocol based on [3+3] cyclocondensation of 4-iminothiazolidone-2 [18] on account of its N,C-binucleophylic properties with dielectrophylic reagents like acetylacetone.

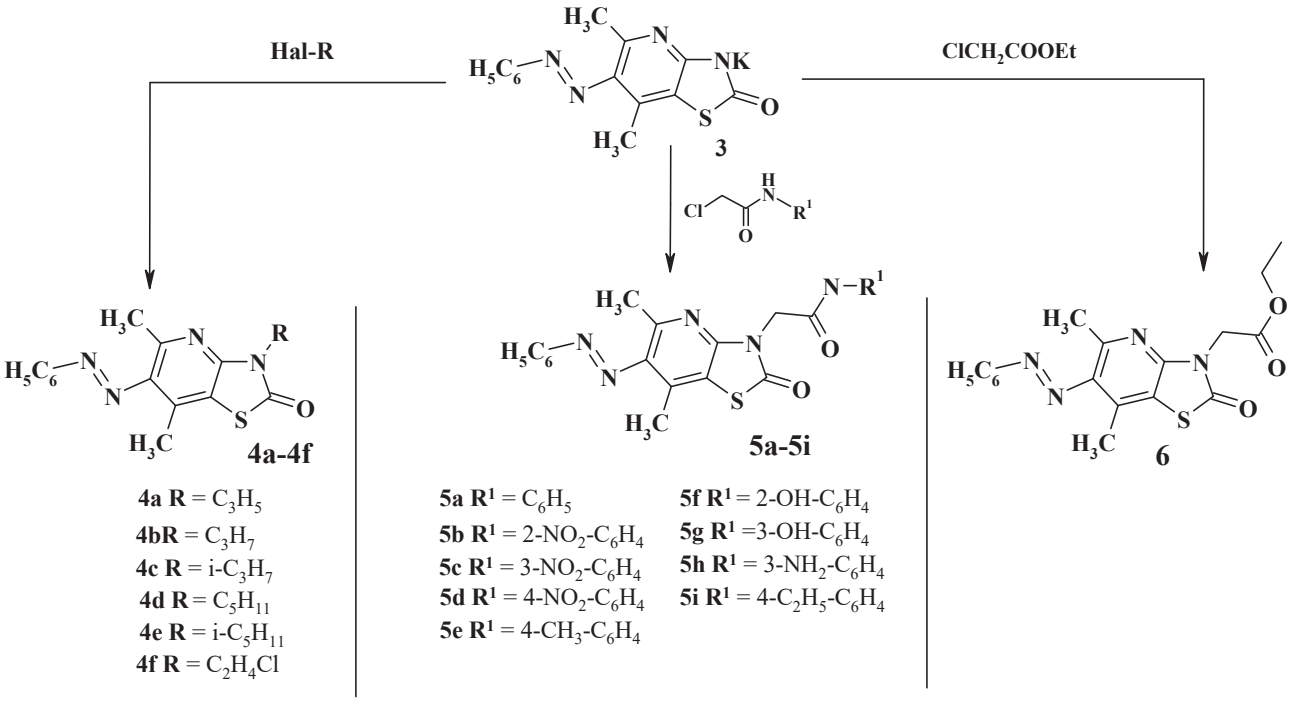
As an alternative, we looked at a possibility to use the reported method for generation 6-phenylazo derivatives of the above mentioned core fused heterocycle by involving α-phenylazoacetone as a dielectrophylic agent. It appeared that 5,7 - dimethyl - 6-phenylazo - 3*H* - thiazolo [4,5-*b*] pyridine-2-one (**1**) was accessed at the same conditions with a high yield (Scheme 1).



Scheme 1. Synthesis of 5,7-dimethyl-6-phenylazo-3*H*-thiazolo[4,5-*b*]pyridin-2-one (**1**), 3-(5,7-dimethyl-6-phenylazo-2-oxo-thiazolo[4,5-*b*]pyridin-3-yl)-propionitryl (**2**) and 5,7-dimethyl-6-phenylazo-3*H*-thiazolo[4,5-*b*]pyridin-2-one potassium salt (**3**).

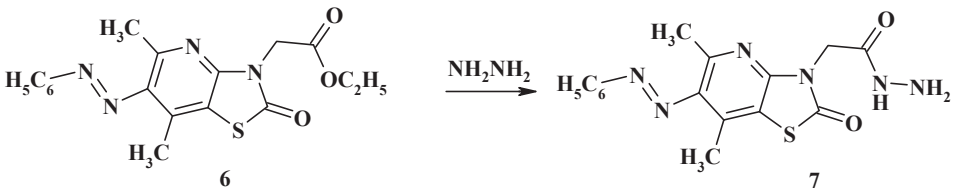
Core thiazolo[4,5-*b*]pyridine scaffold had been extensively studied as electrophilic reagent implemented on account of NH-group hydrogen atom. The good leaving property of the hydrogen atom and its strong electrophilicity advantage offered the compound **1** functionalization featuring novel N^3-substituted 5,7-dimethyl-6-phenylazo-3*H*-thiazolo[4,5-*b*]pyridine-2-one derivatives. Thus NH-center made it possible to involve compound **1** into cynoethylation reaction (Scheme 1). We discovered that the high yield of the corresponding propionitryl **(2)** can be achieved by introducing the equimolar amounts of the compound **1** and acrylonitrile in pyridine – water medium 5:1.

Further compound **1** properties studying showed the proton acidic character in the core heterocycle N^3-position which promoted the tendency of its transformation into potassium salt **3** (Scheme 1) affording by potassium hydroxide treatment. The obtained salt possessed nucleophilic properties and could be further involved into N-alkylation reaction. The alkylation mild conditions proceeding imposed to alkyl, phenylacetamide and ethyl acetate moieties introduction with the generation of corresponding N^3 substituted 5,7-dimethyl-3*H*-thiazolo[4,5-*b*]pyridine-2-ones (**4a-4f; 5a-5i**) using various alkylating agents like alkyl halides, 2-chloro-N-phenylacetamides and ethyl chloroacetate (Scheme 2).



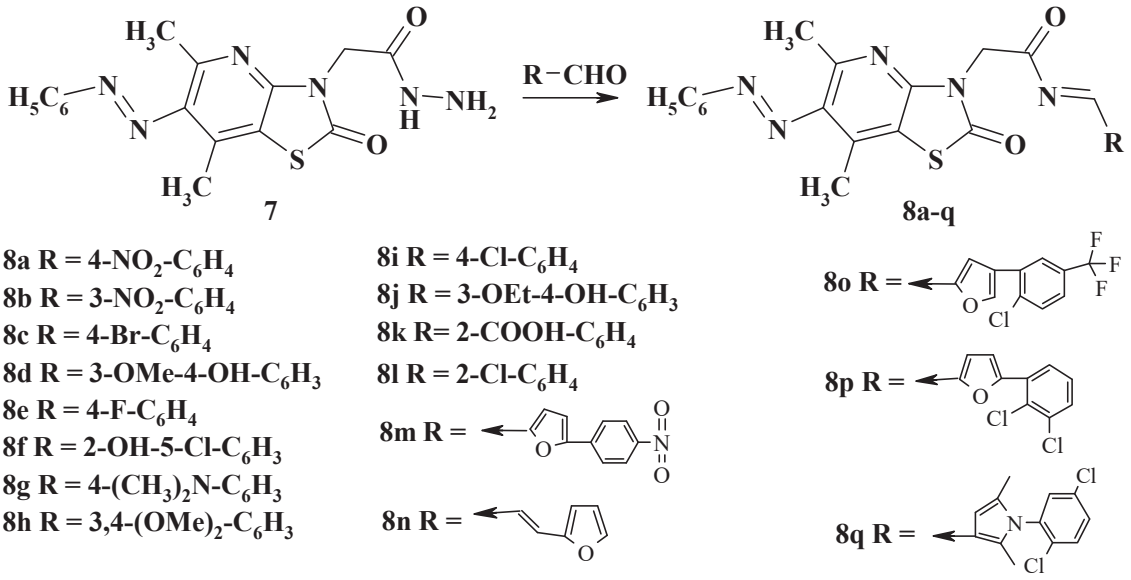
Scheme 2. Synthesis of *N*^{3}-substituted 5,7-dimethyl-6-phenylazo-3*H*-thiazolo[4,5-*b*]pyridin-2-one derivatives under the alkylation reaction.

(5,7-Dimethyl-2-oxo-6-phenylazo-thiazolo [4,5-b]pyridine-3-yl)-ethylacetate (**6**) prepared in this way represented a convenient intermediate in order to afford (5,7-dimethyl-6-phenylazo-2-oxo-thiazolo[4,5-b]pyridine-3-yl)-acetic acid hydrazide (**7**) as a building block for more elaborate functionalizations of thiazolo[4,5-b]pyridine-2-one moiety in its N^3 position (Scheme 3). Thus compound **6** was subjected to hydrazinolysis reaction with 50 % hydrazine hydrate solution proceeded in a good yield in 96 % ethanol medium under a long time hot water bath warming.



Scheme 3. Synthesis of 5,7-dimethyl-6-phenylazo-3*H*-thiazolo[4,5-*b*]pyridin-2-one hydrazide.

The hydrazide group presence in N^3 position of the compound 7 provides an entry for the series of its arylidene hydrazide derivatives **8a-8q** generation (Scheme 4) which had been achieved by compound 7 treatments with appropriate aromatic and heterocyclic aldehydes in 96 % ethanol medium.



Scheme 4. Synthesis of 5,7-dimethyl-2-oxo-6-phenylazo-3*H*-thiazolo[4,5-*b*]pyridin-2-one hydrazide arylidene derivatives.

The structures of the obtained compounds were confirmed by ^{1}H NMR spectroscopy and elemental analysis. The elemental analysis experimental data on contents of nitrogen and sulfur were within ±0.3 % of the theoretical values. In the ^{1}H-NMR spectral data, all protons were seen according to the expected chemical shift and integral values.

The ^{1}H NMR spectra of all compounds showed the protons signals of methyl groups in pyridine ring as singlets in the δ2.38–2.55 and 2.61–2.68 ppm. The phenyl moiety at C^6 position of thiazolopyridine ring was responsible for the system of doublets, triplets and multiplets at a broad range of δ7.43-7.92ppm. The N^3 H proton signal in compound **1** was recorded as a singlet at δ 12.78 ppm in low magnetic field area while it was not resolved for the rest of novel compounds that proved the cyanoethylation reaction proceeding and 5,7-dimethyl-6-phenylazo-3*H*-thiazolo[4,5-*b*]pyridine-2-one potassium salt generation. The compound **2** spectrum showed two signals of exocyclic methylene groups as triplets at 3.10 and 4.27 ppm. The ^{1}H NMR spectra of compounds **4a-4f, 5a-5i, 6, 7, 8a-8q**-contained the methylene group signal as singlet at 3.94-5.15 ppm which proved the compound **3** alkylation proceeding. The protons signals as singlets at 9.42 and 4.33 ppm were used to locate hydrazide group in the compound **7** as NH and NH_2 moieties, respectively. The protons signals due to the furan moiety in compounds **8m-8p** were recorded as characteristic singlets, dublets and multiplets at the δarea of 6.60-7.43 ppm, while the compound ***8q*** pyrrol moiety is responsible for singlet at 6.30 ppm.^{1}H NMR spectra of compounds **8a-8l** contain characteristic aromatic signals used to locate benzylidene hydrazide substituent in the δ broad area of 6.75-8.23 ppm.

Anti-inflammatory activity in vivo evaluation

Carrageenan-induced paw edema is a well-known animal model of acute inflammation which is the most widely used in the search for new anti-inflammatory drugs. The inflammatory response is usually quantified by increase in paw size (edema) which is maximal around 4-5 h postcarrageenan injection. The early phase of a biphasic edema induced by carrageenan injection into the subplantar surface of rat paw is observed around 1 h relating to the release of histamine, serotonin, bradykinin, and to a less extent prostaglandins produced by cyclooxygenase enzymes (COX), whereas the delayed phase (after 1 h) is attributed to neutrophil infiltration, and the continuing of the prostaglandin generation [15].

In vivo studies of novel 5,7-dimethyl-6-phenylazo-3*H*-thiazolo[4,5-*b*]pyridine-2-one derivatives were carried out for anti-inflammatory activity employing the carrageenan-induced rat paw edema method. Marked paw edema was produced in rats with sub-plantar injection of 0.1 ml of 2 % carrageenan. The test compounds were dissolved in DMSO and injected intraperitoneally in the dose of 50 mg/kg body weight 0.5 h prior to carrageenan injection.The NSAID drugs Ibuprofen and Diclofenac in their effective therapeutic doses were tested inparallel as an activity references. Anti-inflammatory activity was defined by measuring the paw edema volume 4 h after the carrageenan injection. Results of paw edema decreasing were expressed as the mean ± standard deviation and compared statistically with the control group using Student's t-test. A level of $p<0.05$ was adopted as the test of significance (Table 1). The percentage protection against inflammation was calculated as % inhibition by comparison between DMSO injected control group and drugs-tested groups.

Table 1. Anti-inflammatory effect of 5,7-dimethyl-6-phenylazo-3*H*-thiazolo[4,5-*b*]pyridine-2-ones on carrageenan-induced rat paw edema (mL) *in vivo* evaluation, % protection from inflammation.

Compound ID	Paw edema volume (mL) ± SEM*	% Inhibition	Compound ID	Paw edema volume (mL) ± SEM*	% Inhibition
	after 4 h	after 4 h		after 4 h	after 4 h
Control	2.20 ± 0.050	–	**7**	1.32 ± 0.035	40.2
1	0.94± 0.026	57.2	**8a**	1.28 ± 0.037	42.0
2	1.01 ± 0.045	54.2	**8b**	1.34± 0.039	39.2
3	0.86± 0.025	61.1	**8c**	1.41± 0.049	36.1
4a	1.27± 0.040	42.1	**8d**	1.36± 0.037	38.4
4b	1.21± 0.040	45.1	**8e**	1.28 ± 0.028	42.0
4c	1.16± 0.035	47.2	**8f**	1.21 ± 0.020	45.1
4d	1.10± 0.035	50.2	**8g**	1.34± 0.033	39.3
4e	1.14± 0.045	48.1	**8h**	1.38± 0.038	37.1
4f	1.40± 0.045	36.5	**8i**	0.98± 0.027	55.3
5a	1.43 ± 0.046	35.0	**8j**	1.30 ± 0.048	41.1
5b	1.58± 0.050	28.1	**8k**	1.40± 0.050	36.3
5c	1.51± 0.046	31.2	**8l**	1.09± 0.025	50.5
5d	1.47± 0.045	33.2	**8m**	1.16± 0.032	47.2

5e	1.56 ± 0.048	29.0	**8n**	1.21± 0.029	45.1
5f	1.49± 0.040	32.2	**8o**	1.03± 0.020	53.0
5g	1.34 ± 0.033	39.3	**8p**	0.72± 0.018	67.5
5h	1.47± 0.037	33.2	**8q**	0.63± 0.015	71.2
5i	1.34 ± 0.032	39.3	**Ibuprofen**	1.32 ± 0.035	40.2
6	1.23± 0.030	44.1	**Diclofenac**	1.05 ± 0.023	52.4

*SEM denotes standard error of mean.

Evaluation indicated that 6 compounds (**5b-5f**, **5h**) showed no significant decrease in edema, the inhibition rate for them was observed at the level of 28.1-33.2 % ascompared to control group. A series of the newly synthesized compounds possessed the anti-inflammatory activity in the range of 35.0-41.1 % (compounds **4f**, **5a**, **5g**, **5i**, **7**, **8b-8d**, **8g**, **8h**, **8j**, **8k**) which is comparable to the effect of Ibuprofen. The anti-inflammatory evaluation tests for compounds **4a- 4e**, **6**, **8e**, **8f**, **8l-8n** gave the results as 42.1-50.5 % inhibition indicating the compounds were more potent than Ibuprofen while possessed some weaker effect than Diclofenac. Among the synthesized compounds,5,7-dimethyl-6-phenylazo-3*H*-thiazolo[4,5-*b*]pyridine-2-one (**1**), its potassium salt (**3**) and 3-(5,7-dimethyl-6-phenylazo-2-oxo-thiazolo[4,5-*b*]pyridine-3-yl)-propionitryl (**2**) as well as5,7-dimethyl-2-oxo-6-phenylazo-thiazolo[4,5-*b*]pyridine-3-yl) - acetic acid (4-chloro - benzylidene) -hydrazide (**8i**) and derivatives containing halogens substituted phenyl-furan and phenyl-pyrrol moieties (**8o-8q**) showed the highest inflammation inhibition rate as 53.0-71.2 % protection to inflammation which exceeded the Diclofenac effect.

The results of the pharmacological tests were analyzed with respect of the compounds structure. For alkyl N^3-substituted compounds **4a-4f** an appropriate length of the alkyl chain was essential to the anti-inflammatory activity of these compounds the length increasing was resulted into activity enhancement. Among the nine phenyl-acetamide substituted derivatives **5a-5i** no active compounds were evaluated indicating the nature and position of the substituted group on the phenyl ring did not influence notably their anti-inflammatory activity. Comparison of the substituents nature on the phenyl ring of hydrazide arylidene derivatives **8a-8l** indicated that chlorine atom presence contributed to the inflammation inhibition efficiency.

Conclusions

A series of novel 5,7-dimethyl-6-phenylazo-3*H*-thiazolo[4,5-*b*]pyridine-2-one derivatives possessing anti-inflammatory activity were prepared by the structural modification of the core heterocycle in N^3 position. Evaluation indicated that a series of the novel compounds possessed inflammation inhibition rate comparable to that of Diclofenac. Compounds **1, 2, 3, 8i, 8o, 8p** and **8q** showed the highest inflammation inhibition rate as 53.0-71.2 % protection to inflammation which exceeded the Diclofenac effect.

Thus the core thiazolo[4,5-b]pyridine heterocyclic system may be regarded as a promising scaffold for the effective anti-inflammatory a drug candidates development.

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