Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2016, 8(19):534-542
(http://derpharmachemica.com/archive.html)

Synthesis of novel thiazolo[4,5-b]pyridines as potential biologically active substances

Taras I. Chaban, Olena V. Klenina, Borys S. Zimenkovsky, Igor G. Chaban, Volodymyr V. Ogurtsov and Lesya S. Shelepeten

Danylo Halytsky Lviv National Medical University, Pekarska Str, 69, Lviv, 79010, Ukraine

---

ABSTRACT

In an afford to develop novel anti-exudative and anti-oxidant agents, a series of thiazolo[4,5-b]pyridines were prepared by alcylation of the core scaffold in its N^3 position and further ethoxy group nucleophilic replacement with hydrazine one. Hydrazine group functionalization included (5,7-Dimethyl-2-oxo-thiazolo[4,5-b]pyridine-3-yl)-acetic acid hydrazide treatment with thionyl-bis-glycolic acid leading to 2-(5,7-Dimethyl-2-oxo-thiazolo[4,5-b]pyridine-3-yl)-N-(4-oxo-2-thioxo-thiazolydine-3-yl)-acetamide generation and its interaction with carbon disulfide and KOH in equimolar amounts employed for 3-(5-mercapto-[1,3,4]oxodiazole-2-yl-methyl)-5,7-dimethyl-3H-thiazolo[4,5-b]pyridine-2-one potassium salt preparation. The presence of active methylene group in C^5 position of 2-(5,7-Dimethyl-2-oxo-thiazolo[4,5-b]pyridine-3-yl)-N-(4-oxo-2-thioxo-thiazolydine-3-yl)-acetamide thiazolydine ring provided an entry for Knoevenagel condencation carrying out with the respective aryliden derivatives obtaining. More elaborate functionalization proceeding leaded to hydrazide group acylation with further hetarylsulfanyl moiety introducing. The anti-exudative effect of novel thiazolo[4,5-b]pyridine-2-one derivatives was evaluated in vivo employing the carrageenan-induced rat paw edema method. The antioxidant activity of the synthesized compounds was evaluated in vitro by the method of scavenging effect on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals.

Key words: thiazolo[4,5-*b*]pyridines; alkylation; nucleophilic replacement; Knoevenagel condensation; acylation.

INTRODUCTION

Condensed bicyclic systems with thiazolydine core being annulated to pyridine one occupy prominent place in medicinal chemistry because of their broad spectrum of pharmacological activities. The combination of these heterocyclic systems into a bicyclic scaffold commonly provides much more interest in the enhanced activity profile of its analogs than their parent monocyclic constituents.

Thiazolo[4,5-*b*]pyridine derivatives are characterized with diverse biological activities, among which antioxidant, tuberculostatic, anticancer, antimicrobial, anti-inflammatory and antifungal effects have been reported in the past decade [1-7]. Some of their analogues are capable of reducing and preventing the occurrence of hyperproliferative disorders in living organisms due to the inhibitory effect on the integrin linked kinase [8-10]. Thiazolopyridine derivatives have been known to exhibit the beneficial effects associated with a reduction in sexual dysfunction [11]. Some of their analogues were recognized as H3 receptor antagonists [12], or act as antagonists of metabotropic glutamate receptors 5 (mGluR5) [13], another ones were revealed as potent inhibitors with respect to the receptors of the epidermal growth factor [12] and a number of other enzymes [15,16]. Thiazolopyridine derivatives have been also used as sensitive analytical reagents [17].

Thus, the research to explore different avenues of chemical modifications of thiazolo[4,5-*b*]pyridine-2-ones to obtain novel active compounds should be continued. For continual discovery of potential agents with anti-

inflammatory and antioxidant activities, we are reporting the synthesis, anti-exudative and free radicals scavenging effects evaluation for the series of novel thiazolo[4,5-*b*]pyridine-2-ones in the present paper using earlier obtained 5,7-dimethyl-3*H*-thiazolo[4,5-*b*]pyridine-2-one [18] as a precursor.

MATERIALS AND METHODS

Materials

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

Chemistry

All the melting points were determined in an open capillary and are uncorrected. ^{1}H NMR spectra of newly synthesized compounds in DMSO-d6 solutions were recorded on a spectrometer Varian Mercury VX-400 (400 MHz) at 298 K. Chemical shifts are reported as δ (ppm) relative to TMS as internal standard, coupling constant *J* are expressed in Hz. The elemental analysis experimental data on contents of sulfur and nitrogen were within ±0.4 % of the theoretical values.

Ethyl ester of (5,7-dimethyl-2-oxo-thiazolo[4,5-b]pyridine-3-yl)-acetic acid, 1.

Potassium salt of 5,7-dimethyl-3*H*-thiazolo[4,5-*b*]pyridine-2-one (0.003 mol) was dissolved in DMF (20 ml) at heating. To the solution obtained the equimolar amount of monochloroacetic acid ethyl ester was added. The reaction mixture was refluxed 2 h accompanied by white precipitate formation in significant amount. The hot reaction mixture was filtered off, the precipitate was washed with DMF through folded filter. The filtrate was cooled down to the temperature of about 50 °C, treated with 100 ml of water and then cooled to 12-15 °C. The resulting precipitate was filtered off again, washed with water and dried. The precipitate next was re-crystallized from acetic acid. Compound **1** was obtained as a beige crystalline powdered solid.

Yield 87 %, m. p. 94-95 ºC. ^{1}H NMR, δ (ppm): 1.23 t (3H, *J* = 7.0 Hz, $OCH_2\underline{CH_3}$), 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, N-CH_2), 7.01 (1H, Py). Anal. Calcd. for $C_{12}H_{14}N_2O_3S$ (%): N 10.52, S 12.04. Found (%): N 10.36, S 11.98.

4-(5,7-Dimethyl-2-oxo-thiazolo[4,5-*b*]pyridine-3-yl) butyric acid, 2.

The synthetic route to afford **2** is similar to that one for the compound **1** starting from potassium salt of 5,7-dimethyl-3*H*-thiazolo[4,5-*b*]pyridine-2-one treated with butyrolactone. The resulting precipitate was re-crystallized from acetic acid. Compound **2** was successively obtained as a beige crystalline powdered solid.

Yield 74 %, m. p. 88-89 ºC. ^{1}H NMR, δ (ppm): 2.00 t (2H, *J* = 6.8 Hz, CH_2), 2.28 t (2H, *J* = 7.1 Hz, CH_2), 2.35 s (3H, CH_3), 2.51 s (3H, CH_3), 4.06 t (2H, *J* = 6.3 Hz, N-$\underline{CH_2}$), 6.91 s (1H, Py.), 11.97 s (1H , COOH). Anal. Calcd. for $C_{10}H_{14}N_2O_3S$ (%): N 10.52, S 12.04. Found (%): N 10.59, S 12.10.

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

Compound **2** (0.02 mol) was dissolved in methanol (8 ml), and to the solution obtained 0.03 mol of 50 % hydrazine hydrate solution were added. The reaction mixture was heated for 2 h at boiling water bath and then cooled to afford compound **3**. The resulting precipitate was obtained at cooling, filtered off, water-washed and dried. The obtained compound **3** was re-crystallized from water as a white crystalline powder.

Yield 82 %, m. p. 198-199 ºC. ^{1}H NMR, δ (ppm): 2.33 s (3H, CH_3), 2.45 s (3H, CH_3), 4.41 s (2H, NH_2), 4.82 s (2H, N-$\underline{CH_2}$), 7.02 s (1H, Py.), 11.51 bs (1H , NH). Anal. Calcd. for $C_{10}H_{12}N_4O_2S$ (%): N 22.21, S 12.71. Found (%): N 22.51, S 12.56.

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

Compound **3** (0.02 mol) was dissolved in ethanol (10 ml). Thionyl-bis-glycolic acid (0.02 mol) was then added to he obtained solution and the reaction mixture was refluxed for 3 h. The solid which was precipitated at cooling was filtered off, washed with ethanol and dried. Compound **4** was successively obtained as a beige crystalline powdered solid after its re-crystallization from acetic acid.

Yield 70 %, m. p. 180-181 ºC. ^{1}H NMR, δ (ppm): 2.32 s (3H, CH_3), 2.45 s (3H, CH_3), 4.38 s (2H, CH_2),), 4.87 s (2H, N-$\underline{CH_2}$), 7.05 s (1H, Py.), 11.48 s (1H , NH). Anal. Calcd. for $C_{13}H_{12}N_4O_3S_3$ (%): N 15.21, S 26.11. Found (%): N 15.25, S 26.35.

Potassium salt of 3-(5-mercapto-[1,3,4]oxodiazole-2-yl-methyl)-5,7-dimethyl-3*H*-thiazolo[4,5-*b*]pyridine-2-one, 5.

Potassim hydroxide (0.006 mol) was dissolved in ethanol (10 ml). The prepared solution was treated with carbon disulfide (0.006 mol) and compound **3** (0.005 mol). The reaction mixture was heated for 6 h at boiling water bath. The resulting precipitate was obtained at cooling, filtered off, water-washed and dried. The obtained compound **5** was re-crystallized from acetic acid as a beige crystalline powder.

Yield 60 %, m. p. 154-155 ºC. ^{1}H NMR, δ (ppm): 2.32 s (3H, CH_3), 2.44 s (3H, CH_3), 5.11 s (2H, CH_2),), 7.03 s (1H, Py.). Anal. Calcd. for $C_{11}H_9KN_4O_2S_2$ (%): N 16.85, S 19.29. Found (%): N 16.72, S 19.35.

N-[5-(4-fluorobenzylidene)-4-oxo-2-thioxo-thiazolydine-3-yl]-2-(5,7-dimethyl-2-oxo-thiazolo[4,5-*b*]pyridine-3-yl)-acetamide, 6.

Compound **4** (0.005 mol), 4-fluorobenzaldehyde (0.005 mmol) and a few drops of ethanolamine were added to acetic acid (15 ml). The reaction mixture was refluxed 30 min. On cooling the crystalline precipitate was filtered off, washed with water and dried. Compound **6** was obtained after its re-crystallization from acetic acid as a beige crystalline powdered solid.

Yield 72 %, m. p. 186-187 ºC. ^{1}H NMR, δ (ppm): 2.39 s (3H, CH_3), 2.59 s (3H, CH_3), 4.90 s (2H, N-$\underline{CH_2}$), 6.97 s (1H, Py.), 7.34 t (2H, J = 8.7 Hz, J = 8.1 Hz, C_6H_4), 7.74 t (2H, J = 6.4 Hz, J = 6.3 Hz, C_6H_4), 7.93 s (1H, CH), 11.77 s (1H , NH). Anal. Calcd. for $C_{20}H_{15}FN_4O_3S_3$ (%): N 11.81, S 20.27. Found (%): N 11.74, S 20.27.

N-[5-(4-Nitro-benzylidene)-4-oxo-2-thioxo-thiazolydine-3-yl]-2-(5,7-dimethyl-2-oxo-thiazolo[4,5-*b*]pyridine-3-yl)-acetamide, 7.

The synthetic route to afford compound **7** is similar to that one for compound **6** starting from compound **4** treated with 4-nitrobenzaldehyde. The obtained compound **7** was re-crystallized from acetic acid as a pale yellow crystalline powdered solid.

Yield 75 %, m. p. 193 ºC. ^{1}H NMR, δ (ppm): 2.38 s (3H, CH_3), 2.55 s (3H, CH_3), 4.90 s (2H, N-$\underline{CH_2}$), 6.96 s (1H, Py.), 7.92 d (2H, J = 8.2 Hz, C_6H_4), 8.03 s (1H, CH), 8.36 d (2H, J = 8.2 Hz, C_6H_4), 11.71 s (1H , NH). Anal. Calcd. for $C_{20}H_{15}N_5O_5S_3$ (%): N 13.96, S 19.18. Found (%): N 13.88, S 19.18.

N'-[2-(5,7-Dimethyl-2-oxo-thiazolo[4,5-*b*]pyridine-3-yl)-acetyl]-acetic acid hydrazide, 8.

Compound **3** (0.01 mol) was dissolved in dioxane (10 ml) in a flat-bottomed flask. To the solution obtained in this way another solution prepared by chloroacetic anhydride (0.01 mol) and triethylamine (0.01 mol) dissolving in dioxane (10 ml) was added. After 10 min at 100 °C the reaction mixture was poured into water. A precipitate formed was filtered from the solution. The crystalline precipitate of compound **8** was dissolved in acetic acid and re-crystallized as a greyish-white solid powder.

Yield 78 %, m. p. 178-179 ºC. ^{1}H NMR, δ (ppm): 2.32 s (3H, CH_3), 2.43 s (3H, CH_3), 2.51 s (3H, CO$\underline{CH_3}$), 4.64 s (2H, CH_2), 7.01 s (1H, Py), 10.45 s (1H, CO$\underline{NH}$), 10.56 s (1H, $\underline{NH}$CO). Anal. Calcd. for $C_{12}H_{14}N_4O_3S$ (%): N 19.04, S 10.89. Found (%): N 19.32, S 11.30.

N'-[2-(5,7-Dimethyl-2-oxo-thiazolo[4,5-*b*]pyridine-3-yl)-acetyl]-chloro-acetic acid hydrazide, 9.

The synthetic protocol for obtaining compound **9** is similar to that one for compound **8** starting from compound **3** and monochloroacetic acid chloro anhydride. Compound **9** was obtained after its re-crystallization from ethanol as a greyish-white crystalline powdered solid.

Yield 74 %, m. p. 165-167 ºC. ^{1}H NMR, δ (ppm): 2.34 s (3H, CH_3), 2.44 s (3H, CH_3), 4.12 s (2H, CH_2), 4.67 s (2H, N-$\underline{CH_2}$), 7.02 s (1H, Py), 10.44 s (2H, CO$\underline{NH}$), 10.53 s (2H, $\underline{NH}$CO). Anal. Calcd. for $C_{12}H_{13}ClN_4O_3S$ (%): N 17.04, S 9.75. Found (%): N 16.84, S 10.01.

N'-[2-(5,7-Dimethyl-2-oxo-thiazolo[4,5-*b*]pyridine-3-yl)-acetyl] butyric acid hydrazide, 10.

The synthetic route to afford compound **10** is similar to that one for compound **9** starting from compound **3** and butyric acid chloro anhydride. Compound **10** was obtained after its re-crystallization from ethanol as a greyish crystalline powdered solid.

Yield 74 %, m. p. 165-167 ºC. ^{1}H NMR, δ (ppm): 0.86 t (3H, J = 7.4 Hz, CH_2-CH_2 -$\underline{CH_3}$), 1.51-1.55 m (2H, CH_2-$\underline{CH_2}$ -CH_3), 2.09 t (2H, J = 7.2 Hz, $\underline{CH_2}$-CH_2 -CH_3), 2.34 s (3H, CH_3), 2.44 s (3H, CH_3), 4.64 s (2H, N-$\underline{CH_2}$), 7.02 s (1H, Py.), 9.87 s (2H, CO$\underline{NH}$), 10.24 s (2H, $\underline{NH}$CO). Anal. Calcd. for $C_{14}H_{18}N_4O_3S$ (%): N 17.38, S 9.95. Found (%): N 17.62, S 9.74.

(5-Phenyl[1,3,4]oxodiazole-2-ylsulfanyl)-N'-(5,7-dimethyl-2-oxo-thiazolo[4,5-*b*]pyridine-3-yl)- acetyl hydrazide acetic acid, 11.

Compound 8 (0.005 mol), 5-phenyl-[1,3,4]oxadiazolidine-2-thiol (0.005 mol) and ethanol (20 ml) were mixed in a round bottom flask. The reaction mixture was refluxed 1 h. The precipitate which formed on cooling was filtered off, washed with ethanol, and dried. The precipitate was re-crystallized from methanol. Compound **11** was isolated as a white crystalline powdered solid.

Yield 70 %, m. p. 180-181 ºC. ^{1}H NMR, δ (ppm): 2.25 s (3H, CH_3), 2.39 s (3H, CH_3), 4.07 s (2H, CH_2), 4.68 s (2H, N-$\underline{CH_2}$), 7.03 s (1H, Py), 7.39 s (2H, C_6H_5), 7.56 s (1H, C_6H_5), 7.83 s (2H, C_6H_5), 9.70 s (1H, CO-$\underline{NH}$), 10.89 s (1H, $\underline{NH}$-CO). Anal. Calcd. for $C_{20}H_{18}N_6O_4S_2$ (%): N 17.86, S 13.63. Found (%): N 17.71, S 13.69.

(Benzothiazole-2-ylsulfanyl)-N'-(5,7-dimethyl-2-oxo-thiazolo[4,5-*b*]pyridine-3-yl)- acetyl hydrazide acetic acid, 12.

The synthetic protocol for obtaining compound **12** is similar to that one for compound **11** starting from compound **8** and benzothiazol-2-thiol. Compound **12** was obtained after its re-crystallization from methanol as a white crystalline powdered solid.

Yield 74 %, m. p. 165-167 ºC. ^{1}H NMR, δ (ppm): 2.18 s (3H, CH_3), 2.36 s (3H, CH_3),), 4.11 s (2H, CH_2), 4.61 s (2H, N-$\underline{CH_2}$), 7.03 s (1H, Py), 7.39 t (1H, J = 7.0 Hz, J = 6,7 Hz, Ar), 7.50 t (1H, J = 7.2 Hz, J = 6,7 Hz, Ar), 7.84 d (1H, Ar), 8.04 d (1H, Ar), 9.74 s (1H, CO-$\underline{NH}$), 10.95 s (1H, $\underline{NH}$-CO). Anal. Calcd. for $C_{19}H_{17}N_5O_3S_3$ (%): N 15.24, S 20.93. Found (%): N 15.17, S 20.78.

Pharmacological screening evaluations

Anti-exudative activity evaluation assays

Anti-inflammatory activity [19] was evaluated using carrageenan induced rat paw edema method in rats [20]. Outbred (male/female) white rats weighing 180–220g were used for the edema test. Animals were divided into 13 groups comprising five rats per group. One group was kept as the control and remaining 12 groups (test groups) were used to determine the anti-inflammatory activity elicited by the 12 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 drug, Ibuprofen (50 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 4 h 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 V_{control} is the increase in paw volume in control group animals;
V is the increase in paw volume in animals injected with the test substances.

Free radical scavenging assays

The antioxidant activity was determined on basis of free radical scavenging activity of stable 2,2-diphenyl-1-picrylhydrazyl (DPPH). The effect of the studied compounds on DPPH radicals were estimated according to the method of Blois [21, 22] with minor modifications. The solution of DPPH in ethanol with the concentration of 150 μmoles/l (4 ml) was mixed with the compound or control solution in ethanol its concentration been 250 μmoles/l (0.2 ml). The reaction mixture was vortex mixed thoroughly and incubated at room temperature in the dark for 60 min. Simultaneously, a control was prepared as ascorbic acid solution in ethanol (0.2 ml) mixed with of DPPH solution in ethanol (4 ml) without sample fraction. Reduction in the absorbance of the mixture was measured at 517 nm using ethanol as blank. Ascorbic acid was used as a standard. Also the absorbance of DPPH solution was measured. Percentage of free-radical-scavenging activity was expressed as percent inhibition and it was calculated using the following formula:

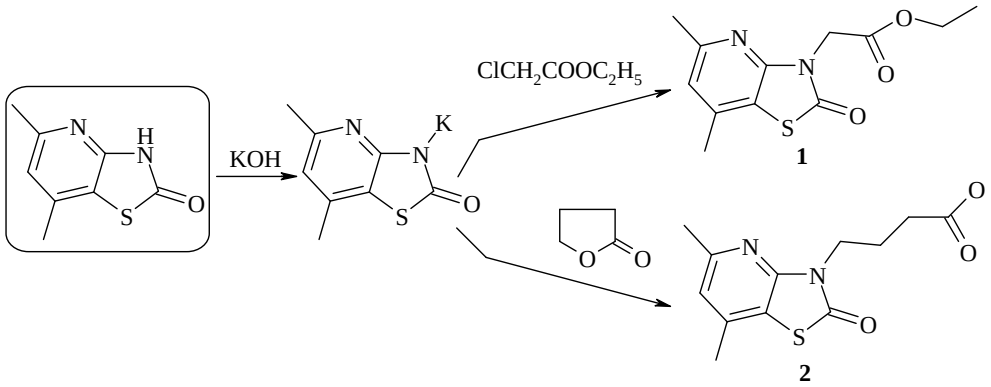
$$\%\ \text{Inhibition} = \frac{\text{A}_{\text{DPPH}} - \text{A}_{\text{s}}}{\text{A}_{\text{DPPH}}} \cdot 100\,\%$$

where A_{DPPH} is the absorbance of DPPH free radicals solution, A_s is the absorbance of a sample.

Each experiment was performed in triplicate and average values were recorded. Results are expressed as the means ± S.D.

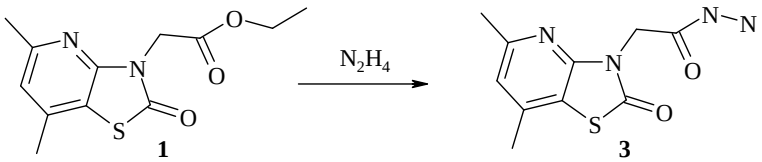
RESULTS AND DISCUSSION

With the view of thiazolo[4,5-*b*]pyridines systematic study continuing as potential drug candidates we introduced structural modification of 5,7-dimethyl-3*H*-thiazolo[4,5-*b*]pyridine-2-one as the synthetic protocol development in order to afford ethyl ester of (5,7-dimethyl-2-oxo-thiazolo[4,5-*b*]pyridine-3-yl)-acetic acid (**1**). Such structural modification covers alcylation of the core scaffold in its N^3 position with monochloroacetic acid ethyl ester *via* the intermediate stage of 5,7-dimethyl-3*H*-thiazolo[4,5-*b*]pyridine-2-one potassium salt obtaining. The reaction mixture reflux for 2 hours in DMF medium were optimal conditions for compound **1** formation proceeding in good yields. The similar synthetic route with butyrolactone involving leaded to 4-(5,7-dimethyl-2-oxo-thiazolo[4,5-*b*]pyridine-3-yl) butyric acid (**2**) successive obtaining (Scheme 1).



Scheme 1. Synthesis of (5,7-dimethyl-2-oxo-thiazolo[4,5-b]pyridine-3-yl)-acetic acid ethyl ester (2)

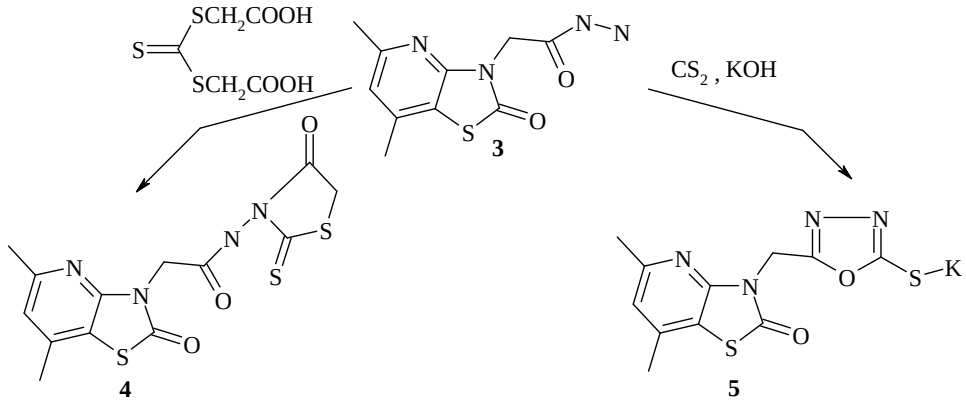
For broadening the scope of N^3 substituted 3*H*-thiazolo[4,5-*b*]pyridine-2-ones we involved compound **1** into the reaction with hydrazine hydrate leading to ethoxy group nucleophilic replacement with hydrazine one and the corresponding hydrazide of (5,7-dimethyl-2-oxo-thiazolo[4,5-*b*]pyridine-3-yl)-acetic acid (**3**) formation. We discovered that the high yield of the product can be achieved by introducing 50 % hydrazine hydrate solution as a hydrazinolysis agent in 96 % ethanol medium and the reaction mixture heating for 2 hours at boiling water bath. Unlike most azolidones, for which boiling with hydrazine hydrate results in azolidone ring destruction, thiazolo[4,5-*b*]pyridine fused core was stable under these conditions (Scheme 2).



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

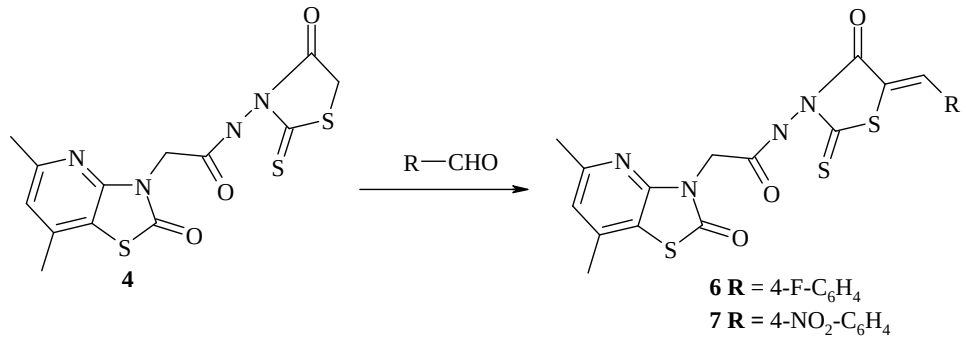
For the compound **3** further structural modification on account of its hydrazide center it was treated with thionyl-bis-glycolic acid leading to compound **4** generation. Ethanol was found to be the most suitable medium for the reaction when equimolar amounts of reagents were refluxed for 3 hours.

The next stage of the hydrazide group functionalization was based on the synthetic protocol of compound **2** treatment with carbon disulfide and KOH in equimolar amounts employed for compound **5** preparation. The reaction mixture heating for 6 hours at boiling water bath in 96% ethanol medium were optimal conditions for compound **5** formation proceeding in good yield (Scheme 3).



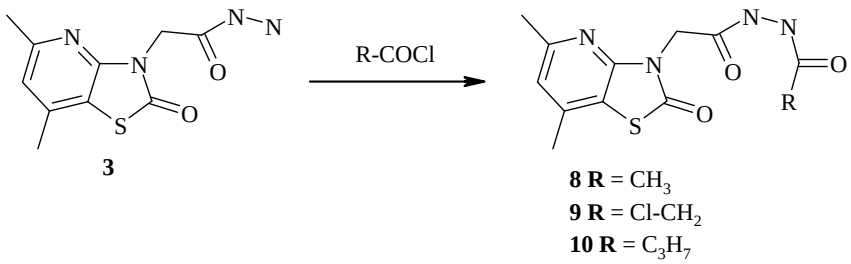
Scheme 3. Synthesis of 2-(5,7-Dimethyl-2-oxo-thiazolo[4,5-*b*]pyridine-3-yl)-N-(4-oxo-2-thioxo-thiazolydine-3-yl)-acetamide (4) and 3-(5-mercapto-[1,3,4]oxodiazole-2-yl-methyl)-5,7-dimethyl-3*H*-thiazolo[4,5-*b*]pyridine-2-one potassium salt (5)

The presence of active methylene group in C^5 position of the compound **4** thiazolydine ring provided an entry for Knoevenagel condencation carrying out with the respective aryliden derivatives (**6-7**) obtaining. The developed synthetic strategy showed the target compounds high yielding may be achieved by the reaction proceeding in acetic acid medium by introducing the equimolar amounts of compound **4** and the appropriate aromatic aldehydes. Monoamine ethanol was assayed as a catalyst for the reaction (Scheme 4).



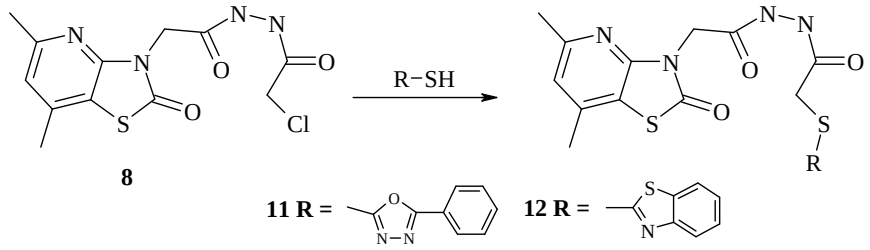
Scheme 4. Synthesis of 2-(5,7-Dimethyl-2-oxo-thiazolo[4,5-*b*]pyridine-3-yl)-N-(4-oxo-2-thioxo-thiazolydine-3-yl)-acetamide arylidene derivatives (6, 7)

For more elaborate functionalization proceeding of compound **4** hydrazide group it was subjected to acylation with carboxylic acids chloroanhydrides. The synthetic strategy developed showed 3-acylhydrazides **(8 - 10)** high yielding may be achieved in anhydrous dioxane medium in the presence of triethylamine (Scheme 5).



Scheme 5. Synthesis of N'-[2-(5,7-Dimethyl-2-oxo-thiazolo[4,5-*b*]pyridine-3-yl)-acetyl] carboxylic acids hydrazides, (8 – 10)

Compound **9** represents a convenient intermediate in order to afford hetarylsulfanyl derivatives of N-[2-((5,7-dimethyl-2-oxo-thiazolo[4,5-*b*]pyridine-3-yl)-acetyl hydrazide acetic acid. The synthetic protocol was based on compound **9** treatment with the appropriate heterocyclic thiols. We discovered that the high yield of the target products (**11**, **12**) can be achieved by introducing the equimolar amounts of the reactants in ethanol medium and 1 hour refluxing (Scheme 6).



Scheme 6. Synthesis of hetarylsulfanyl derivatives of N'-(5,7-dimethyl-2-oxo-thiazolo[4,5-*b*]pyridine-3-yl)- acetyl hydrazide acetic acid (11, 12)

The structures of the obtained compounds were confirmed by ^{1}H NMR spectroscopy and elemental analysis. All new compounds gave spectroscopic data in accordance with the proposed structures, all protons were seen according to the expected chemical shifts 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 δ 2.18–2.48 and 2.36–2.64 ppm, respectively. The singlet signals derived from methine group in 5th position of the core scaffold appeared around 6.91-7.05 ppm. The ^{1}H NMR spectra of compounds **1** and **2** contained signals of methylene group as a singlet at 4.82 ppm and as a triplet at 4.06 ppm, respectively, that proved the alkylation reaction proceeding as potassium salt of 5,7-dimethyl-3*H*-thiazolo[4,5-*b*]pyridine-2-one treatment with either monochloroacetic acid ethyl ester or butyrolactone. The signals of NH- and NH_2-group as singlets at 11.51 and 4.41 ppm, respectively, proved ethoxy group nucleophilic replacement with hydrazine one and the appropriate hydrazone formation. Compound **4** obtaining was characterized with the free methylene group presence which showed the singlet signal at 4.38 ppm in its spectrum, as well as further Knoevenagel condensation proceeding featured by the absence of methylene group and the presence of methyne group. Compound **3** acylation proceeding was proved by the absence of hydrazide group signals as well as further alkylation of compound **8** with the appropriate thiols proceeding.

Anti-inflammatory activity *in vivo* evaluation

Carrageenan-induced paw edema is the most widely used animal model of acute inflammation. *In vivo* studies of novel thiazolo[4,5-*b*]pyridine-2-one derivatives were carried out for anti-exudative activity employing the carrageenan-induced rat paw edema method. Marked paw edema was produced in rats with sub-planter 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 drug Ibuprofen in its effective therapeutic dose was tested in parallel as an activity reference. 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-exudative effect of 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	–	**5**	1.65 ± 0.035	**25.2**
1	1.45 ± 0.040	**34.2**	**8**	1.67 ± 0.035	**24.0**
2	0.97 ± 0.025	**56.1**	**9**	1.34 ± 0.030	**39.1**
3	1.30 ± 0.040	**41.0**	**12**	1.76 ± 0.045	**20.0**
4	1.82 ± 0.045	**17.3**	**Ibuprofen**	1.32 ± 0.035	**40.2**

**SEM denotes standard error of mean.*

Evaluation indicated that 4 compounds (**4**, **5**, **8** and **12**) showed no significant decrease in edema, the inhibition rate for them was observed at the level of 17.3-25.2 % as compared to control group. A series of the newly synthesized compounds possessed the anti-inflammatory activity in the range of 34.2-41.0 % (compounds **1**, **9**, **3**) which is comparable to the effect of Ibuprofen. The anti-inflammatory evaluation test for compound **2** gave the result as 56.1 % inhibition indicating the compound was more potent than Ibuprofen.

The results of the pharmacological tests were analyzed with respect of the compounds structure. The presence of carboxylic group substituents (**2**, **9**, **1**) and carboxylic group hydrazide substituent (**3**) in the core scaffold N^3 position was essential to the anti-exudative activity of these compounds the length increasing resulted into activity enhancement.

***In Vitro* Antioxidant Assay**

The antioxidant activity was determined on basis of free radical scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical. DPPH radical had found many applications due to its high stability in a methanolic solution and intense purple color. In its oxidized form, the DPPH radical has an absorbance maximum centered at a wavelength about 517 nm. The absorbance decreases when the radical is reduced by antioxidants. Its reduction affords 2,2-diphenyl-1-picrylhydrazine (DPPH-H), or the corresponding anion ($DPPH^-$) in basic medium. The DPPH radical acts as a scavenger for other odd-electron species which afford *para*-substitution products at phenyl rings.

The DPPH method is described as a simple, rapid and convenient method for screening of many samples for radical scavenging activity. These advantages make the DPPH method interesting for testing newly synthesized compounds to scavenge radicals and to find out promising antioxidant drug candidates.

In the present paper we demonstrate modified spectrophotometric method making use of the DPPH radical and its specific absorbance properties. The free-radical-scavenging activity of each compound was assayed using a stable DPPH and was quantified by decolorization the solution being mixed with DHHP at a wavelength of 517 nm. The absorbance of DPPH solution in ethanol (150 μmoles/l) was measured as 0.770. The absorbances and free-radical-scavenging activities % inhibitions of standard (ascorbic acid) and each compound are listed in Table 2.

Table 2. Values of Absorbance and % Inhibition of thiazolo[4,5-*b*]pyridine-2-ones

The Compound or Standard	Absorbance of a Sample, A_s	% Inhibition	The Compound or Standard	Absorbance of a Sample, A_s	% Inhibition
Ascorbic acid	0.580±0.015	**24.68**	**8**	0.492±0.025	**36.14**
3	0.720±0.030	**6.50**	**9**	0.707±0.020	**8.16**
4	0.704±0.020	**8.52**	**10**	0.506±0.015	**34.27**
6	0.693±0.015	**10.05**	**12**	0.697±0.025	**9.50**

For the series of the newly synthesized compounds (**3**, **4**, **6**, **9** and **12**) their free radical scavenging effect was insignificant (6.50-10.05 %). The pharmacology screening allowed identifying two lead compounds (**8** and **10**) whose free radical scavenging activities exceed that one for ascorbic acid. Thus acylhydrazide substituents presence in N^3 position of the core heterocycle resulted into their antioxidant activity enhancement. However, the presence of chlorine atom in acyl moiety was undesirable.

CONCLUSION

A series of novel 5,7-dimethyl-thiazolo[4,5-*b*]pyridine-2-one derivatives possessing anti-excudative and antioxidant activities were prepared by the structural modification of the core heterocycle in N^3 position. Therefore we have shown that the proposed approaches and developed synthetic protocols provided the possibility to design 5,7-dimethyl-thiazolo[4,5-*b*]pyridine-2-ones diversity with a considerable chemical novelty involving alcylation, nucleophilic replacement, [2+3] cyclocondensation, Knoevenagel condensation and acylation reactions. Anti-exudative activity *in vivo* evaluation and free radicals scavenging effect *in vitro* evaluation allowed identifying lead compounds possessing significant decrease in edema or considerable antioxidant effect. The present results suggested the core fused heterocycle as a promising scaffold for anti-inflammatory and antioxidant drug candidates development.

REFERENCES

[1] T.I. Chaban, V.V. Ogurtsov, I.G. Chaban, O.V. Klenina, J.D. Komarytsia, *Phosphorus, Sulfur, and Silicon and the Related Elements*, **2013**, 188, 1611.
[2] O. Klenina, I. Drapak, T. Chaban, V. Ogurtsov, I. Chaban, I. Golos, *Chemistry & chemical technology*, **2013**, 7, 397.
[3] Chaban T., Klenina O., Drapak I., Ogurtsov V., Chaban I., Novikov V., *Chemistry & chemical technology*, **2014**, 89, 287.
[4] T.I. Chaban, R.R. Panchuk, O.V. Klenina, N.R. Skorokhyd, V.V. Ogurtsov, I.G. Chaban, *Biopolymers and Cell*, **2012**, 28, 389.
[5] T.I. Chaban, V.V. Ogurtsov, I.G. Chaban, J.D. Komarytsia, *Journal of org. and pharm. chemistry*, **2012**, 10, 2, 70-76.
[6] O.V. Klenina, T.I. Chaban, V.V. Ogurtsov, I.G. Chaban, I. Ya. Golos, *Journal of org. and pharm. chemistry*, **2014**, 12, 1, 60-68.
[7] S. Marzoog, Al-Thebeiti, *Il Farmaco*, **2000**, 55, 109.
[8] Z. Zhang, T. S. Daynard, S. Wang et al., Canada, №WO2003CA01078 (**2004)**.

[9] F. Parlati, U.V. Ramesh, R. Singh et al., USA, №WO2004US34397 (**2005)**.
[10] M. Hemmerling, T. Klingstedt, Sweden, №WO2004099204 (**2004)**.
[11] M. Cowart, USA, №WO2003US16878 (**2004)**.
[12] K. Walczynski, P. Zuiderveld, H. Timmerman, *European J. of Med. Chem.*, **2005,** 40, 15.
[13] S.S. Kulkarni , A.H. Newman, *Bioorg. Med. Chem. Lett.*, **2007,** 17, 2987.
[14] R. Lin, S. G. Johnson, P. J. Connolly, S. K. Wetter, E. Binnun, T. V. Hughes, W. V.Murray, N. B. Pandey, S. J. Moreno-Mazza, M. Adams, A. R. Fuentes-Pesquera, S. A. Middleton, *Bioorg. Med. Chem. Lett.*, **2009**, 19, 2333.
[15] S. Komoriya, S. Kobayashi, K. Osanai, T. Yoshino, T. Nagata, N. Haginoya, Y. Nakamoto, A. Mochizuki, T. Nagahara, M. Suzuki, T.Shimada, K. Watanabe, Y. Isobe, T. Furugoori, *Bioorg. Med. Chem.*, **2012,** 14, 1309.
[16] B. Singh, E. R. Bacon, E. R. Lesher, S. Robinson, P. O. Pennock, D. C. Bode, E. D. Pagani, R. G. Bentley, M. J. Connell, L. T. Hamel, P. J. Silver, *J. Med. Chem.*, **2012**, 38, 2546.
[17] Lesya Lozynska, Oleksandr Tymoshuk and Taras Chaban, *Acta Chim. Slov*., **2015**, 62, 159.
[18] T.I. Chaban, B.S. Zimenkovskii, J.D. Komaritsa, I.G. Chaban, *Rus. J. Org. Chem*., **2012**, 48, 268.
[19] Pillai, A. D., Rathod, P. D., Franklin, P. X., Padh, H., Vasu, K. K., Sudarsanam, V., *Biochem. Biophys. Res. Commun*. **2004**, 317, 1067.
[20] M. M. Suleiman, S. G. Isaev, O.V. Klenina, V. V. Ogurtsov, *J. Chem. Pharm. Res.*, **2014**, 6(5), 1219.
[21] Blois M.S., *Nature*, **1958**, 181, 1199.
[22] Molyneux P., *J. Sci. Technol.*, **2004**, 26, 211.