Scientific paper

Synthesis, Anti-Inflammatory and Antioxidant Activities of Novel 3*H*-Thiazolo[4,5-*b*]Pyridines

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Abstract

5-Hydroxy-7-methyl-3*H*-thiazolo[4,5-*b*]pyridin-2-one was obtained by the reaction of 4-iminothiazolidin-2-one with acetoacetic ester. Further structural modifications include the introduction of diversity at the C^5 and C^6 positions. The anti-inflammatory action 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.

Keywords: Thiazolo[4,5-*b*]pyridines; synthesis; anti-inflammatory activity; antioxidant activity

1. Introduction

In modern theoretical and clinical medicine the inflammation problem remains one of the main research focuses.[1–4] Inflammation occurs as a defensive response which induces physiological adaptations to limit the tissue damage and remove the pathogenic infections. Deregulation of inflammatory processes leads to specific pathologies including psoriasis, rheumatoid arthritis, periodontal disease, asthma and atherosclerosis. It has also been shown that inflammation can be a fundamental contributor to other degenerative conditions, such as diabetes, cancer and cardiovascular diseases.[5–6] Furthermore, the inflammatory response can be identified as the major cause of damage related to autoimmune diseases.[5] Consequently, the regulation of inflammatory processes is an essential avenue in the treatment of various pathologies. Even if many efforts have been made in this direction in the past years,[7–11] the search for new anti-inflammatory compounds is still an important area of research, as traditional therapies involving steroidal or non-steroidal agents are often associated with a lack of efficiency and undesirable side effects. Of no lesser interest is the search for new antioxidants. There is an increasing evidence of the implication of free radicals in a variety of diseases. Free radicals are being formed during normal cellular metabolism and they are known to contribute to healthy functions in human health and development when they are not present in excessive amounts. At high concentrations free radicals can cause damage to cell structures, nucleic acids, lipids and proteins,[12] leading to age-related degenerative diseases, cancer and a wide range of different human diseases.[13] The major action of antiox-

idants in human diseases is to prevent damage caused by the action of reactive oxygen species. The development of new potent antioxidant agents is a major goal for pharmaceutical and medicinal chemistry, as a way of removing the excess of free radicals, and thus, to ameliorate their hazardous effects on human beings.

4-Azolidone core is considered to be an efficient scaffold for drug-like molecules design as the integral part of modern medicinal chemistry. Pyridine derivatives have always been among the most important research areas in the field of drug design. The thiazolidine-based heterocycles and their analogs fused to the pyridine ring were shown to posses a wide range of biological actions. The incorporation of these heterocyclic systems into a bicyclic scaffold commonly provides much more interesting analogs with the enhanced activity profile in comparison with their parent monocyclic constituents.[14–15] Thiazolo[4,5-*b*] pyridine derivatives are characterized by diverse biological activities, among which antioxidant,[16–18] tuberculostatic,[19] anticancer,[20] anti-inflammatory,[21–22] and antifungal[23] effects have been reported in the past decade.

The objective of the present work was to synthesize a series of 3*H*-thiazolo[4,5-*b*]pyridine-2-ones by the structural modification of the core heterocycle at its C^5 and C^6 positions for further pharmacological screening *in vivo* as anti-inflammatory and *in vitro* as antioxidants activities.

2. Experimental Section

2. 1. Materials

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

2. 2. Chemistry

All the melting points were determined in an open capillary and are uncorrected. ^{1}H NMR spectra of newly synthesized compounds in DMSO-d_6 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 the 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.

5-Hydroxy-7-methyl-3H-thiazolo[4,5-b]pyridin-2-one (2a)

Sodium (109 mmol) was dissolved in anhydrous methanol (125 mL) and to the solution obtained 4-iminothiazolidin-2-one (50 mmol) and acetoacetic ester (8 mL) were added at 20 °C. The mixture was left standing for 5 days with the intermittent stirring, 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. Compound **2a** was obtained as a white crystalline powdered solid, well soluble in DMF, DMSO, solutions of alkali and mineral acids, sparingly soluble in the other organic solvents. White solid; yield: 75%; mp > 300 °C with dec.; ^{1}H NMR: δ 2.33 (s, 3H, CH_3), 6.31 (s, 1H, Py), 10.84 (s, 1H, OH), 12.18 (s, 1H, NH); ^{13}C NMR: δ 19.85, 104.73, 107.84, 144.59, 147.91, 162.91, 169.55; ESI-MS: *m/z* 183 $[M+H]^+$; anal. calcd. for $C_7H_7N_2O_2S$: C 46.15, H 3.32, N 15.37; found: C 46.24, H 3.38, N 15.44.

General Procedure for the Synthesis of 6-Alkyl-5-hydroxy-7-methyl-3H-thiazolo[4,5-b]pyridin-2-ones 2b–c

Metallic sodium (200 mmol) was dissolved in anhydrous methanol (100 mL), and to the solution obtained 4-iminothiazolidin-2-one (50 mmol) and alkyl derivatives acetoacetic ester (50 mmol) were added at 20 °C. The mixture was left standing for 7 days with the intermittent stirring, 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 °C. The obtained compounds were re-crystallized from acetic acid.

5-Hydroxy-7-methyl-6-propyl-3H-thiazolo[4,5-b] pyridin-2-one (2b)

White solid; yield: 60%; mp 249–250 °C; ^{1}H NMR: δ 0.90 (t, *J* = 5.1 Hz, *J* = 7.2 Hz, 3H, CH_2-CH_2-CH_3), 1.43 (d, *J* = 7.3 Hz, 2H, CH_2-CH_2-CH_3), 2.19 (s, 3H, CH_3), 2.48 (d, *J* = 7.4 Hz, 2H, CH_2-CH_2-CH_3), 11.48 (s, 1H, OH); ^{13}C NMR: δ 14.42, 17.63, 22.39, 28.02, 108.56, 116.20, 141.73, 144.83, 160.88, 169.48; ESI-MS: *m/z* 225 $[M+H]^+$; anal. calcd. for $C_{10}H_{12}N_2O_2S$: C 53.55, H 5.39, N 12.49; found: C 53.30, H 5.44, N 12.56.

6-Benzyl-5-hydroxy-7-methyl-3H-thiazolo[4,5-b] pyridin-2-one (2c):

White solid; yield: 75%; mp 277 °C; ^{1}H NMR: δ 2.18 (s, 3H, CH_3), 3.93 (s, 2H, CH_2-C_6H_5), 7.15–7.19 (m, 3H, C_6H_5), 7.25 (t, *J* = 7.4 Hz, 2H, C_6H_5), 10.96 (s, 1H, OH), 12.18 (s, 1H, NH); ^{13}C NMR: δ 18.02, 31.46, 108.87, 114.86, 126.27, 128.44, 128.77, 140.73, 142.60, 145.62, 161.16, 169.48; ESI-MS: *m/z* 273 $[M+H]^+$; anal. calcd. for $C_{14}H_{12}N_2O_2S$: C 61.75, H 4.44, N 10.29; found: C 62.02, H 4.38, N 10.36.

General Procedure for the Synthesis Sodium Salts 5-Hydroxy-7-methyl-3H-thiazolo[4,5-b]pyridin-2-one and 6-Benzyl-5-hydroxy-7-methyl-3H-thiazolo[4,5-b]pyridin-2-one 3a–b

To 20 mL of water and (10 mmol) of potassium hydroxide was added (10 mmol) of compounds **2a** or **2b**, and the mixture was heated to complete dissolution. The solution obtained was evaporated to dryness. The residue was dried at 100 °C.

5-Hydroxy-7-methyl-3H-thiazolo[4,5-b]pyridin-2-one sodium salt (3a)

White solid; yield: 60%; mp 280 °C; ^{1}H NMR: δ 1.99 (s, 3H, CH_3), 5.41 (s, 1H, Py), 11.07 (s, 1H, NH); ^{13}C NMR:

δ 21.47, 104.13, 104.18, 144.71, 154.52, 162.27, 162.31; ESI-MS: *m/z* 205 $[M+H]^+$; anal. calcd. for $C_7H_5N_2NaO_2S$: C 41.18, H 2.47, N 13.72; found: C 41.25, H 2.52, N 13.66.

6-Benzyl-5-hydroxy-7-methyl-3H-thiazolo[4,5-b]pyridin-2-one sodium salt (3b)

White solid; yield: 86%; mp 298 °C; ^{1}H NMR: δ 2.00 (s, 3H, CH_3), 3.79 (s, 2H, CH_2), 7.11 (t, *J* = 7.0 Hz, 1H, C_6H_5), 7.17–7.23 (m, 4H, C_6H_5), 11.07 (s, 1H, NH); ^{13}C NMR: δ 18.06, 31.45, 108.87, 114.86, 126.27, 128.43, 128.78, 140.72, 142.61, 145.61, 161.15, 169.49; ESI-MS: *m/z* 295 $[M+H]^+$; anal. calcd. for $C_{14}H_{11}N_2NaO_2S$: C 57.14, H 3.77, N 9.52; found: C 57.30, H 3.74, N 9.48.

General Procedure for the Synthesis 5-yl Esters Aliphatic Derivatives 7-Methyl-3H-thiazolo[4,5-b]pyridin-2-ones 4a–d

Compounds **2a** or **2b** or **2c** (5 mmol), an appropriate aliphatic chloroanhydride (5 mmol), and triethylamine (5 mmol) were added to dioxane (20 mL). The reaction mixture was refluxed 15 min. On cooling the crystalline precipitate was filtered off, washed with methanol and dried. The obtained compounds were re-crystallized from methanol.

Acetic acid 7-methyl-2-oxo-2,3-dihydro-thiazolo[4,5-b] pyridin-5-yl ester (4a)

White solid; yield: 92%; mp 240–241 °C; ^{1}H NMR: δ 2.30 (s, 3H, CH_3), 2.36 (s, 3H, CH_3-CO), 6.88 (s, 1H, Py), 12.69 (s, 1H, NH); ^{13}C NMR: δ 12.13, 18.45, 117.33, 118.36, 143.89, 146.07, 154.01, 168.76, 169.30; ESI-MS: *m/z* 225 $[M+H]^+$; anal. calcd. for $C_9H_8N_2O_3S$: C 48.21, H 3.60, N 12.49; found: C 48.66, H 3.55, N 12.51.

Butyric acid 7-methyl-2-oxo-2,3-dihydro-thiazolo[4,5-b] pyridin-5-yl ester (4b)

White solid; yield: 56%; mp 164 °C; ^{1}H NMR: δ 0.99 (t, *J* = 7.3 Hz, 3H, CH_3-CH_2-CH_2-CO), 1.64–1.69 (m, 2H, CH_3-CH_2-CH_2-CO), 2.36 (s, 3H, CH_3), 2.59 (t, *J* = 7.1 Hz, 3H, CH_3-CH_2-CH_2-CO), 6.87 (s, 1H, Py), 12.69 (s, 1H, NH); ^{13}C NMR: δ 14.81, 19.81, 22.08, 28.15, 116.84, 118.37, 144.52, 148.15, 154.07, 166.14, 169.23; ESI-MS: *m/z* 253 $[M+H]^+$; anal. calcd. for $C_{11}H_{12}N_2O_3S$: C 52.37, H 4.79, N 11.10; found: C 52.50, H 4.74, N 11.25.

Acetic acid 7-methyl-2-oxo-6-propyl-2,3-dihydro-thiazolo[4,5-b]pyridin-5-yl ester (4c)

White solid; yield: 91%; mp 212 °C; ^{1}H NMR: δ 1.21 (t, *J* = 7.3 Hz, 3H, CH_3-CH_2-CH_2), 1.41–1.45 (m, 2H, CH_3-CH_2-CH_2), 2.32–2.36 (m, 2H, CH_3-CH_2-CH_2), 2.34 (s, 3H, CH_3), 2.47–2.49 (m, 3H, CH_3-CO), 12.56 (s, 1H, NH); ^{13}C NMR: δ 14.39, 17.99, 21.06, 22.74, 28.37, 117.61, 122.45, 143.69, 146.20, 154.23, 168.79, 169.64; ESI-MS: *m/z* 267 $[M+H]^+$; anal. calcd. for $C_{12}H_{14}N_2O_3S$: C 54.12, H 5.30 N 10.52; found: C 54.18, H 5.10, N 10.46.

Acetic acid 6-benzyl-7-methyl-2-oxo-2,3-dihydro-thiazolo[4,5-b]pyridin-5-yl ester (4d):

White solid; yield: 90%; mp 216 °C; ^{1}H NMR: δ 2.26 (s, 3H, CH_3), 2.28 (s, 3H, CH_3-CO), 3.95 (s, 2H, CH_2-C_6H_5), 7.12 (d, *J* = 7.1 Hz, 2H, C_6H_5), 7.18 (t, *J* = 6.2 Hz, *J* = 7.0 Hz, 1H, C_6H_5), 7.27 (t, *J* =7.3 Hz, *J* = 7.1 Hz, 2H, C_6H_5), 12.65 (s, 1H, NH); ^{13}C NMR: δ 18.45, 21.06, 31.84, 117.80, 121.08, 126.62, 128.49, 128.91, 139.38, 144.22, 146.77, 154.54, 168.72, 169.31; ESI-MS: *m/z* 315 $[M+H]^+$; anal. calcd. for $C_{16}H_{14}N_2O_3S$: C 61.13, H 4.49, N 8.91; found: C 61.10, H 4.45, N 8.88.

General Procedure for the Synthesis 5-yl Esters Aromatic Derivatives 7-Methyl-3H-thiazolo[4,5-b]pyridin-2-ones 4e–f

To a solution of pyridine (20 mL) and an appropriate aromatic chloroanhydride (5 mmol) was added compound **IIa** or **IIb** (5 mmol). The reaction mixture was refluxed 30 min. On cooling, the crystalline precipitate was filtered off, washed with acetic acid and dried. The obtained compounds were re-crystallized from acetic acid.

4-Nitrobenzoic acid 7-methyl-2-oxo-2,3-dihydro-thiazolo[4,5-b]pyridin-5-yl ester (4e)

White solid; yield: 72%; mp 207 °C; ^{1}H NMR: δ 2.41 (s, 3H, CH_3), 7.12 (s, 1H, Py), 8.38 (d, *J* = 8.7 Hz, 2H, C_6H_4), 8.44 (d, *J* = 8.7 Hz, 2H, C_6H_4), 12.78 (s, 1H, NH); ^{13}C NMR: δ 19.98, 111.78, 116.85, 127.67, 129.85, 132.20, 139.94, 145.60, 148.69, 155.86, 164.04, 168.88; ESI-MS: *m/z* 332 $[M+H]^+$; anal. calcd. for $C_{14}H_9N_3O_5S$: C 50.76, H 2.74, N 12.68; found: C 51.00, H 2.69, N 12.85.

Benzoic acid 7-methyl-2-oxo-6-propyl-2,3-dihydro-thiazolo[4,5-b]pyridin-5-yl ester (4f)

White solid; yield: 74%; mp 203 °C; ^{1}H NMR: δ 0.86 (t, *J* = 5.1 Hz, *J* = 7.2 Hz, 3H, CH_2-CH_2-CH_3), 1.46–1.51 (m, 2H, CH_2-CH_2-CH_3), 2.38 (s, 3H, CH_3), 2.54 (d, *J* = 7.0 Hz, 2H, CH_2-CH_2-CH_3), 7.66 (t, *J* =6.4 Hz, *J* = 7.3 Hz, 2H, C_6H_5), 7.81 (t, *J* =7.0 Hz, *J* = 6.5 Hz, 1H, C_6H_5), 8.16 (d, *J* = 7.5 Hz, 2H, C_6H_5), 12.65 (s, 1H, NH); ^{13}C NMR: δ 14.44, 18.08, 21.21, 22.74, 117.33, 118.31, 126.25, 128.34 131.15, 138.75, 144.05, 146.18, 154.25, 167.71, 168.11; ESI-MS: *m/z* 329 $[M+H]^+$; anal. calcd. for $C_{17}H_{16}N_2O_3S$: C 62.18, H 4.91, N 8.53; found: C 62.22, H 4.84, N 8.50.

General Procedure for the Synthesis 6-Arylazo-5-hydroxy-7-methyl-3H-thiazolo[4,5-b]pyridin-2-ones 5a–i

Metallic sodium (0.2 mol) was dissolved in anhydrous methanol (100 mL), and to the solution obtained 4-iminothiazolidin-2-one (50 mmol) and α-arylazo derivatives acetoacetic ester (50 mmol) were added at 20 °C. The mixture was left standing for 7 days with the intermittent stirring, 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 °C. The obtained compounds were re-crystallized from acetic acid.

5-Hydroxy-7-methyl-6-phenylazo-3H-thiazolo[4,5-b] pyridin-2-one (5a)

Red solid; yield: 97%; mp 265 °C; ^{1}H NMR: δ 2.40 (s, 3H, CH_3), 7.27 (t, *J* = 7.4 Hz, 1H, Ph), 7.48 (t, *J* = 7.9 Hz, *J* = 7.4 Hz, 2H, Ph), 7.67 (d, *J* = 7.5 Hz, 2H, Ph), 13.30 (s, 1H, OH), 14.56 (s, 1H, NH); ^{13}C NMR: δ 19.96, 112.44, 118.33, 128.87, 129.96, 133.44, 138.95, 143.98, 147.07, 163.11, 168.25; ESI-MS: *m/z* 287 $[M+H]^+$; anal. calcd. for $C_{13}H_{10}N_4O_2S$: C 54.54, H 3.52, N 19.57; found: C 54.61, H 3.49, N 19.66.

5-Hydroxy-7-methyl-6-p-tolylazo-3H-thiazolo[4,5-b] pyridin-2-one (5b)

Red solid; yield: 73%; mp 255 °C with dec.; ^{1}H NMR: δ 2.33 (s, 3H, C_6H_4-CH_3), 2.40 (s, 3H, CH_3), 7.28 (d, *J* = 7.0 Hz, 2H, C_6H_4), 7.54 (d, *J* = 7.0 Hz, 2H, C_6H_4), 13.26 (s, 1H, OH), 14.52 (s, 1H, NH); ^{13}C NMR: δ 14.44, 19.98, 112.23, 117.84, 128.15, 129.42, 132.15, 138.77, 144.32, 147.11, 163.07, 168.66; ESI-MS: *m/z* 301 $[M+H]^+$; anal. calcd. for $C_{14}H_{12}N_4O_2S$: C 55.99, H 4.03, N 18.65; found: C 55.84, H 4.00, N 18.59.

5-Hydroxy-7-methyl-6-m-tolylazo-3H-thiazolo[4,5-b] pyridin-2-one (5c)

Red solid; yield: 97%; mp 233 °C; ^{1}H NMR: δ δ 2.35 (s, 3H, C_6H_4-CH_3), 2.38 (s, 3H, CH_3), 7.07 (d, *J* = 7.6 Hz, 1H, C_6H_4), 7.35 (t, *J* = 7.8 Hz, *J* = 7.6 Hz, 1H, C_6H_4), 7.44 (d, *J* = 10.0 Hz, 2H, C_6H_4), 13.28 (s, 1H, OH), 14.58 (s, 1H, NH); ^{13}C NMR: δ 19.89, 21.54, 104.71, 107.70, 112.56, 114.58, 117.66, 119.61, 122.67, 130.06, 144.61, 148.11, 162.89, 169.8; ESI-MS: *m/z* 301 $[M+H]^+$; anal. calcd. for $C_{14}H_{12}N_4O_2S$: C 55.99, H 4.03, N 18.65; found: C 55.87, H 4.08, N 18.68.

6-(2,4-Dimethyl-phenylazo)-5-hydroxy-7-methyl-3H-thiazolo[4,5-b]pyridin-2-one (5d)

Yellow solid; yield: 97%; mp 282 °C; ^{1}H NMR: δ 2.32 (s, 3H, C_6H_3-CH_3), 2.38 (s, 3H, CH_3), 2.42 (s, 3H, C_6H_3-CH_3), 7.18–7.20 (m, 2H, C_6H_3), 7.69–7.71 (m, 1H, C_6H_3), 13.37 (s, 1H, OH), 15.10 (s, 1H, NH); ^{13}C NMR: δ 13.15, 16.23, 18.21, 102.08, 106.75, 112.12, 115.07, 118.71, 120.96, 125.12, 130.42, 144.77, 148.05, 162.44, 169.55; ESI-MS: *m/z* 315 $[M+H]^+$; anal. calcd. for $C_{15}H_{14}N_4O_2S$: C 57.31, H 4.49, N 17.82; found: C 57.45, H 4.44, N 17.77.

5-Hydroxy-6-(2-hydroxy-phenylazo)-7-methyl-3H-thiazolo[4,5-b]pyridin-2-one (5e)

Orange solid; yield: 99%; mp > 280 °C with dec.; ^{1}H NMR: δ 2.40 (s, 3H, CH_3), 6.95–6.98 (m, 2H, C_6H_4), 7.12 (t, *J* = 7.3 Hz, 1H, C_6H_4), 7.71 (d, *J* = 7.7 Hz, 1H, C_6H_4), 10.75 (s, 1H, C_6H_4-*OH*), 13.28 (s, 1H, OH), 14.89 (s, 1H, NH); ^{13}C NMR: δ 19.44, 103.12, 107.07, 113.44, 115.35, 119.33, 121.25, 124.71, 130.33, 145.12, 148.11, 163.08, 169.79; ESI-MS: *m/z* 303 $[M+H]^+$; anal. calcd. for $C_{13}H_{10}N_4O_3S$: C 51.65, H 3.33, N 18.53; found: C 51.59, H 3.40, N 18.58.

3-(5-Hydroxy-7-methyl-2-oxo-2,3-dihydro-thiazolo [4,5-b]pyridin-6-ylazo)-benzenesulfonic acid (5f)

Yellow solid; yield: 99%; mp > 280 °C with dec.; ^{1}H NMR: δ 2.41 (s, 3H, CH_3), 7.44–7.51 (m, 2H, C_6H_4), 7.60 (d, *J* = 7.0 Hz, 1H, C_6H_4), 7.86 (s, 1H, C_6H_4), 13.31 (s, 1H, OH), 14.61 (s, 1H, NH); ^{13}C NMR: δ 17.35, 114.27, 117.95, 124.07, 125.74, 129.79, 137.54, 141.28, 141.58, 150.24, 160.62; ESI-MS: *m/z* 367 $[M+H]^+$; anal. calcd. for $C_{13}H_{10}N_4O_5S_2$: C 42.62, H 2.75, N 15.29; found: C 42.33, H 2.65, N 15.38.

5-Hydroxy-6-(4-methoxy-phenylazo)-7-methyl-3H-thiazolo[4,5-b]pyridin-2-one (5g)

Red solid; yield: 99%; mp > 258 °C with dec.; ^{1}H NMR: δ 2.41 (s, 3H, CH_3), 3.80 (s, 3H, O-CH_3), 7.05 (d, *J* = 9.0 Hz, 2H, C_6H_4), 7.67 (d, *J* = 8.0 Hz, 2H, C_6H_4), 13.14 (s, 1H, OH), 14.78 (s, 1H, NH); ^{13}C NMR: δ 16.44, 19.15, 111.97, 117.75, 128.17, 129.23, 132.42, 138.92, 143.98, 146.88, 162.65, 168.07; ESI-MS: *m/z* 317 $[M+H]^+$; anal. calcd. for $C_{14}H_{12}N_4O_3S$: C 53.16, H 3.82, N 17.71; found: C 53.25, H 3.88, N 17.64.

4-(5-Hydroxy-7-methyl-2-oxo-2,3-dihydro-thiazolo [4,5-b]pyridin-6-ylazo)-benzenesulfonamide (5h)

Orange solid; yield: 100%; mp > 280 °C with dec.; ^{1}H NMR: δ 2.36 (s, 3H, CH_3), 7.38 (s, 2H, NH_2), 7.76 (d, *J* = 8.5 Hz, 2H, C_6H_4), 7.86 (d, *J* = 8.5 Hz, 2H, C_6H_4), 13.32 (s, 1H, OH), 14.32 (s, 1H, NH); ^{13}C NMR: δ 17.44, 116.88, 123.19, 125.82, 127.62, 141.33, 142.11, 146.10, 160.55, 166.84, 177.99; ESI-MS: *m/z* 366 $[M+H]^+$; anal. calcd. for $C_{13}H_{11}N_5O_4S_2$: C 42.73, H 3.03 N 19.17; found: C 42.82, H 3.14, N 19.25.

N-(5-Ethyl-[1,3,4]thiadiazol-2-yl)-4-(5-hydroxy-7-methyl-2-oxo-2,3-dihydro-thiazolo[4,5-b]pyridin-6-ylazo)-benzenesulfonamide (5i)

Orange solid; yield: 100%; mp 276–278 °C; ^{1}H NMR: δ 1.21 (t, *J* = 7.5 Hz, 3H, CH_3-CH_2-thiadiazole), 2.36 (s, 3H, CH_3), 2.80–2.85 (m, 2H, CH_3-CH_2-thiadiazole), 7.76 (d, *J* = 8.7 Hz, 2H, C_6H_4), 7.83 (d, *J* = 8.7 Hz, 2H, C_6H_4), 13.67 (s, 1H, OH), 14.32 (s, 1H, NH); ^{13}C NMR: δ 12.78, 17.43, 24.17, 117.44, 126.79, 128.03, 138.78, 140.71, 144.96, 145.01, 160.55, 168.03, 168.06; ESI-MS: *m/z* 478 $[M+H]^+$; anal. calcd. for $C_{17}H_{15}N_7O_4S_3$: C 42.76, H 3.17, N 20.53; found: C 42.79, H 3.33, N 20.46.

2. 3. Anti-Inflammatory Activity Evaluation Assays

Anti-inflammatory activity[24] was evaluated using carrageenan induced rat paw edema method in rats. Outbred (male/female) white rats weighing 180–220 g 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 10 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 administered 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:

$$\%\ \text{Inhibition} = \frac{V_{\text{control}} - V}{V_{\text{control}}} \cdot 100\ \% \qquad (1)$$

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.

2. 4. Free Radical Scavenging Assays

The antioxidant activity was determined on the basis of free radical scavenging activity of stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH). The effect of the studied compounds on DPPH radicals were estimated according to the method of Blois[25–26] with minor modifications. The solution of DPPH in ethanol with the concentration of 150 μmol/L (4 mL) was mixed with the compound or control solution in ethanol, its concentration being 250 μmol/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 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 the 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:

$$\%\ \text{Inhibition} = \frac{A_{\text{DPPH}} - A_s}{A_{\text{DPPH}}} \cdot 100\ \%\ ; \qquad (2)$$

where A_{DPPH} is the absorbance of DPPH free radicals solution, A_s is the absorbance of the sample. Each experiment was performed in triplicate and average values were recorded. Results are expressed as the means ± S.D.

3. Results and Discussion

3. 1. Chemistry

One of the efficient synthetic approaches for thiazolo[4,5-*b*]pyridine system construction used in modern medical chemistry is the protocol based on [3+3] cyclocondensation of 4-iminothiazolidin-2-one[27] (**1**) on account of its N,C-binucleophylic properties when reacting with dielectrophylic reagents.[28]

We used 4-iminothiazolidin-2-one (**1**) as the initial compound that was reacted with acetoacetic ester. We optimized the conditions of this reaction that made it possible to obtain 5-hydroxy-7-methyl-3*H*-thiazolo[4,5-*b*]pyridin-2-one (**2a**) in good yield (Scheme 1). The best results were observed in the case keeping when methanol was used and the reagents mixture was kept in the presence of sodium methylate over 7 days.

The next stage of our strategy includes the core heterocycle structural modification at its C^6 position. The direct functionalization procedure has been shown to be of a small synthetic utility owing to the low nucleophilic activity of the compound **1** at the C^6 position. We studied the behavior in reaction of [3+3] cyclocondensation of 4-iminothiazolidin-2-one with alkylated acetoacetic ester derivatives. Under the chosen reaction conditions the corresponding 6-alkyl-5-hydroxy-7-methyl-3*H*-thiazolo[4,5-*b*]pyridin-2-ones **2b–c** were isolated in good yields (Scheme 1).

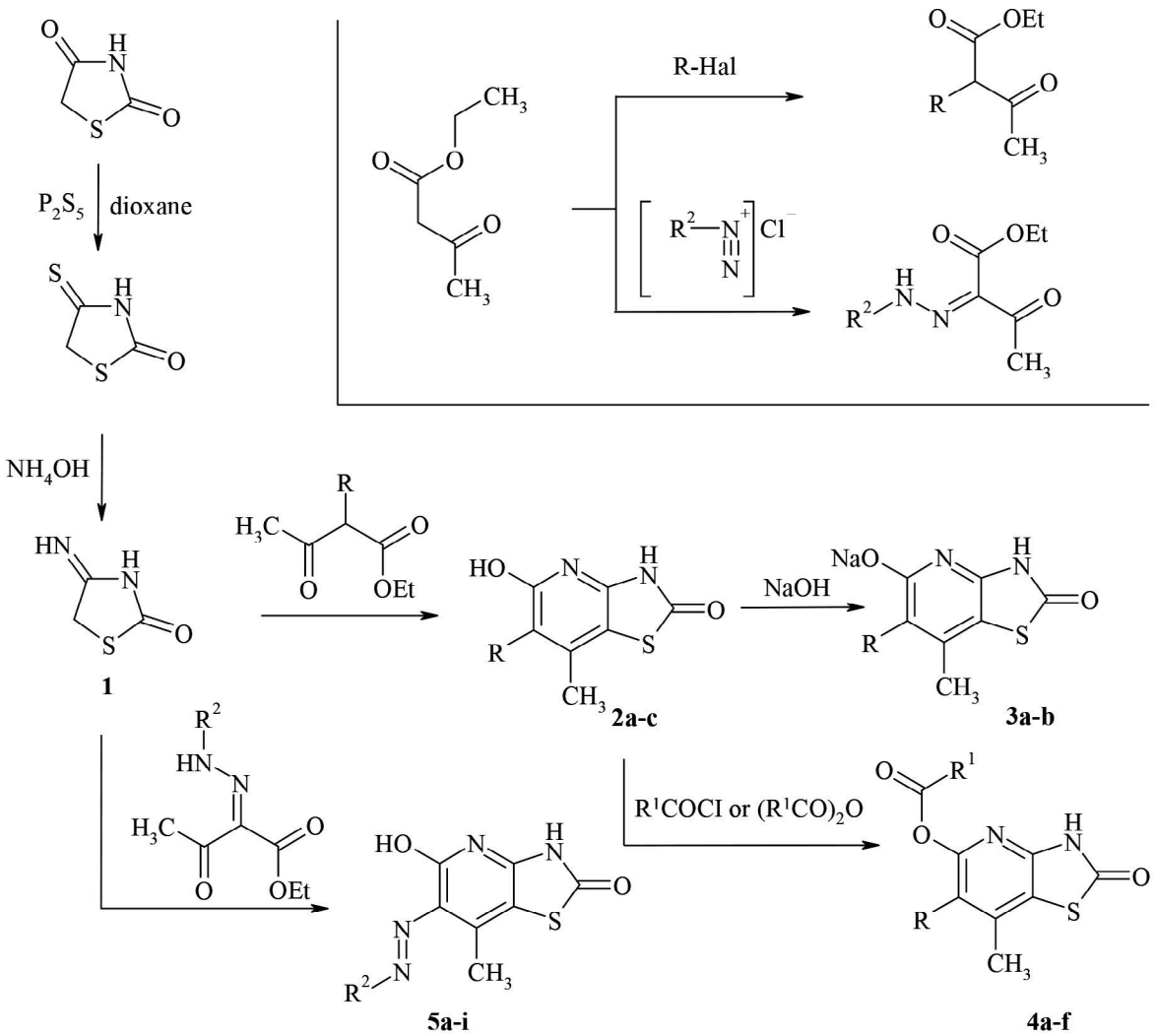
Some properties of the obtained compounds were investigated. The proton at the position 5 retained the acidic properties and the reaction with sodium hydroxide furnished the corresponding salts **3a–b** (Scheme 1).

Chloroacetamides are highly reactive chemicals which are being involved in alkylation reactions forming the basis for creating building blocks of wide utility for combinatorial chemistry, including biologically active substances, to design combinatorial libraries on their basis.

The hydroxy group present at C^5 position in compounds **2a–c** provides an entry to 5-yl ester derivatives **4a–f**. Anhydride was found to be the most suitable medium for the reaction of compounds **2a–c** with aliphatic chloroanhydrides **4a–d**. Reaction of compounds **2a–b** with chloroanhydrides of aromatic acids readily took place in pyridine medium and enabled the preparation of compounds **4e**,**f** (Scheme 1).

Literature survey revealed that some antimicrobial drugs like sulfasalazine, salazopyridazine *etc.* contain arylazo group.[29] In its turn, arylazo moiety introduction into thiazolidine ring system led to antimicrobial activity enhancement possessed by the respective thiazolidine arylazo derivatives.

In view of the results mentioned above, 5-hydroxy-7-methyl-6-arylazo-3*H*-thiazolo[4,5-*b*]pyridin-2-ones **5a–i** were synthesized following the same protocol as for α-arylazo derivatives of acetoacetic esters, by treatment with 4-iminothiazolidin-2-one (Scheme 1). Powders of these



2a-c: R = H (a), C_6H_5-CH_2 (b), C_3H_7 (c); **3a-b**: R = H (a), C_6H_5-CH_2 (b); **4a-f:** R = H, R^1 = CH_3 (a), R =H, R^1 = C_3H_7 (b), R = C_3H_7, R^1 = CH_3 (c), R = C_6H_5-CH_2, R^1 = CH_3 (d), R = H, R^1 = 4-NO_2-C_6H_4 (e), R = C_3H_7, R_1 = C_6H_5 (f), **5a-i** R^2 = C_6H_5 (a), 4-CH_3-C_6H_4 (b),3-CH_3-C_6H_4 (c), 2,4-$(CH_3)_2C_6H_3$ (d), 2-OH-C_6H_4 (e), 3-SO_3H-C_6H_4 (f), 4-OCH_3-C_6H_4 (g), 4-$SO_2NH_2C_6H_4$ (h), 4-(N-(5-ethyl-[1,3,4]thiadiazol-2-yl)-benzenesulfonamide) (i).

Scheme 1. Synthesis of novel 3*H*-thiazolo[4,5-*b*]pyridines

substances are well soluble in DMF and DMSO, sparingly soluble in water and in other organic solvents.

The structures of the obtained compounds were confirmed by ^{1}H and ^{13}C NMR spectroscopy and elemental analysis. All these new compounds gave spectroscopic data in accordance with the proposed structures. The ^{1}H NMR spectra of all compounds show the protons signals of methyl group in pyridine ring as singlets in the 1.99–2.41 ppm.

Endocyclic NH groups of basic scaffold are apparent in the region of weak magnetic field at 11.07–15.10 ppm. Proton signals of pyridine cycle for compounds are evident at 5.41–7.12 ppm and are represented by correspondent singlets. Absence of given signals confirms the origin of reaction [3+3]cyclocondensation with corresponding alkyl as well as with arylazo derivatives of acetoacetic ester.

OH group of basic scaffold is represented by relatively broad singlets at 10.84–13.67 ppm. The corresponding salts obtained are characterized by the absence of OH group signals. Conducting acylation reaction is characterized by the absence of OH group signal at position C^5 as well as by the presence of a set of singlets, doublets and multiplets at 0.99–8.44 ppm that confirm the formation of the corresponding 5-acyl derivatives.

3. 2. 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-inflammatory 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 are 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 thiazolo[4,5-*b*]pyridine-2-ones on carrageenan-induced rat paw edema (mL) *in vivo* evaluation, % protection from inflammation

Compound	Paw edema volume (mL) ± SEM*	% Inhibition	Compound	Paw edema volume (mL) ± SEM*	% Inhibition
Control	2.20 ± 0.050	–	**4f**	1.29 ± 0.025	41.2
2a	1.41 ± 0.040	36.2	**5a**	1.54 ± 0.040	30.1
2c	1.64 ± 0.035	25.3	**5g**	1.60 ± 0.035	27.2
3a	1.44 ± 0.040	34.5	**5h**	1.25 ± 0.020	43.1
4a	1.57 ± 0.035	28.5	**5i**	1.09 ± 0.025	50.5
4c	1.22 ± 0.020	44.5	**ibuprofen**	1.32 ± 0.035	40.2

Evaluation of anti-inflammatory activity indicated that six compounds (*i.e.* **2a**, **2c**, **3a**, **4a**, **5a** and **5g**) showed no significant decrease in edema, the inhibition rate for them was observed at the level of 25.3–36.2% as compared to control group. The compounds **4c**, **4f** and **5h** possessed the anti-inflammatory activity in the range of 41.2–44.5% which is comparable to the effect of Ibuprofen. The anti-inflammatory evaluation test for compound **5i** gave the result as 50.5% inhibition indicating the compound was more potent than Ibuprofen.

The results of the pharmacological tests were analyzed with respect to the compounds structure. For C^6-substituted 5-hydroxy-7-methyl-3*H*-thiazolo[4,5-*b*]pyridin-2-one derivatives prepared by [3+3] cyclocondensation of 4-iminothiazolidin-2-one with arylazo derivatives of acetoacetic ester comparison of the substituents nature on the C^6 position indicated that *N*-(5-ethyl-[1,3,4]thiadiazol-2-yl)-methanesulfonamide presence in the phenyl ring contributed to the inflammation inhibition efficiency. The presence of the acetyl or phenyl groups at the C^5 position of the basic scaffold and a propyl group at the C^6 position caused the anti-inflammatory activity which is comparable to the effect of Ibuprofen. Among the remaining test compounds no active compounds were evaluated indicating the nature and position of the substituted groups did not influence notably their anti-inflammatory activity.

3. 3. *In vitro* Antioxidant Assay

The antioxidant activity was determined on the basis of free radical scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical. DPPH radical has found many applications due to its high stability in the methanolic solutions and its intense purple color. In its oxidized form, the DPPH radical has an absorbance maximum centered at a wavelength about 540 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 a modified spectrophotometric method that makes use of the DPPH radical and its specific absorbance properties. The free radical scavenging activities of each compound were assayed using a stable DPPH and were quantified by decolorization of the solution being mixed with DHHP as observed at the wavelength of 540 nm. The absorbance of DPPH solution in ethanol (150 μmol/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.

The antioxidant activity evaluation results showed that, in general, most of the tested compounds showed that their free radical scavenging effect was insignificant being in the range of 5.50–21.10%. The pharmacological screening allowed identification of only one lead compound, namely **5i**, whose free radical scavenging activity (65.80%) exceeded that of ascorbic acid. Thus, the presence of *N*-(5-

Table 2: Values of Absorbance and % Inhibition of 3*H*-thiazolo[4,5-*b*]pyridine-2-ones

Compound or Standard	Absorbance of a Sample, A_s	% Inhibition	Compound or Standard	Absorbance of a Sample, A_s	% Inhibition
ascorbic acid	0.406 ± 0.015	47.30	**4f**	0.654 ± 0.025	15.10
2a	0.682 ± 0.025	11.50	**5a**	0.670 ± 0.025	13.00
2b	0.654 ± 0.020	15.10	**5b**	0.725 ± 0.030	5.80
2c	0.608 ± 0.020	21.10	**5c**	0.715 ± 0.030	7.10
3a	0.704 ± 0.030	8.50	**5d**	0.728 ± 0.030	5.50
3b	0.672 ± 0.025	12.70	**5e**	0.662 ± 0.020	14.00
4a	0.697 ± 0.025	9.50	**5f**	0.700 ± 0.025	9.10
4b	0.697 ± 0.025	9.50	**5g**	0.711 ± 0.020	7.70
4c	0.706 ± 0.030	8.30	**5h**	0.691 ± 0.025	10.30
4d	0.704 ± 0.030	8.50	**5i**	0.263 ± 0.010	65.80
4e	0.684 ± 0.025	11.20			

ethyl-[1,3,4]thiadiazol-2-yl)-methanesulfonamide in the phenyl ring of the core scaffold at C^6 position was essential to the antioxidant activity of these compounds.

4. Conclusions

A series of 5-hydroxy-7-methyl-3*H*-thiazolo[4,5-*b*]pyridin-2-one derivatives possessing anti-inflammatory and antioxidant activities were prepared by the structural modification of the core heterocycle at C^5 and C^6 positions. Therefore, we have shown that the proposed approaches and developed synthetic protocols provided the possibility to synthesize diverse 5-hydroxy-7-methyl-3*H*-thiazolo[4,5-*b*]pyridin-2-ones with a considerable chemical novelty involving alkylation, Japp–Klingemann condensation, [3+3] cyclocondensation and acylation reactions. Anti-inflammatory activity evaluated *in vivo* and free radicals scavenging effect determined *in vitro* allowed to identify some lead compounds causing significant decrease in edema formation or considerable antioxidant effect. The present results suggest that the core fused heterocycle can be developed as a promising scaffold for anti-inflammatory and antioxidant drug candidates.

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Povzetek

5-Hidroksi-7-metil-3*H*-tiazolo[4,5-*b*]piridin-2-on smo pripravili z reakcijo med 4-iminotiazolidin-2-onom in acetoacetatnim estrom. Nadaljnje strukturne modifikacije so vključevale uvedbo diverzitete na položajih C^5 in C^6. Anti-inflamatorno učinkovanje novih tiazolo[4,5-*b*]piridin-2-onskih derivatov smo ugotovili z *in vivo* metodo s pomočjo s karagenanom induciranega edema na podganjih tacah. Antioksidantno aktivnost sintetiziranih spojin smo določili z *in vitro* metodo s pomočjo radikalskega lovilca 2,2-difenil-1-pikrilhidrazilnega radikala (DPPH).