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Convenient Synthesis of 4-pyridinyloxy-Modified imidazo[2,1-*b*][1,3]thiazines as Potential Anti-inflammatory Agents

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Abstract: Novel imidazo[2,1-*b*][1,3]thiazine derivatives modified with 4-pyridinyloxyl moiety, as potential anti-inflammatory agents are described. Synthetic approach to the preparation of (6-((pyridin-4-yl)oxy)-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]thiazines 3a-f and their benzoanelated analogues 3g-j is based on the interaction of substituted 4-fluoropyridines with 3-hydroxy(benzo)imidazo[2,1-*b*][1,3]thiazines. The nucleophilic substitution reaction of the fluorine atom occurs selectively at position 4 of the pyridine ring. The drug-like properties of the first synthesized (4-pyridinyloxy)imidazo[2,1-*b*][1,3]thiazines were predicted *in silico* using SwissADME. Anti-inflammatory activity was studied *in vivo* using hind paw edema in white rats (carrageenan test). Compounds with satisfactory drug-like and pharmacological properties have been identified as promising for further structure optimization and in-depth research.

Keywords: 3-hydroxy-3,4-dihydro-2*H*-(benzo)imidazo[2,1-*b*][1,3]thiazines; (pyridin-4-yl)oxy) (benzo)imidazo[2,1-*b*][1,3]thiazines; structure modification; drug-like molecules; anti-inflammatory activity; ulcerogenic action.

1. Introduction

The wide spectrum of biological activities of condensed azole-azine compounds [1-9] and their prevalence in living organisms among natural substances are a strong argument for the design of new molecules based on these core as potential pharmacological agents. Application of modern methodologies and strategies allowed to identify of biologically active compounds with imidazo[2,1-*b*][1,3]thiazine motif which possess trypanocidal [10,11], antituberculous [12], antioxidant [13] antiviral [14], antitumor [15], antifungal [16] and antiparasitic [17] activities (Figure 1).

Recently [18], we developed an effective method for the synthesis of a series of new azolo-azine-type derivatives-6-[(pyridin-2-yl)oxy] -6,7-dihydro-5*H*-imidazo [2,1-*b*] [1,3] thiazines and their benzoanelated analogs, which testified to the prospects for the search for

bioactive substances by structural functionalization of the imidazo[2,1-*b*]thiazine scaffold by pyridine fragment.

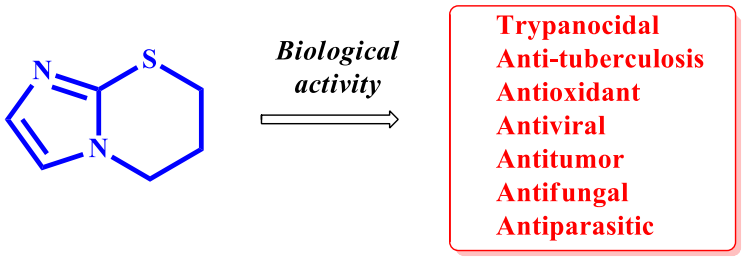


Figure 1. Pharmacology profile of imidazo[2,1-*b*][1,3]thiazine scaffold.

Indeed, synthesized for the first time, hybrid imidazo-thiazine-pyridine compounds showed moderate antibacterial, antifungal, and anti-inflammatory activity in experimental *in vitro* and *in vivo* studies [18]. It seems quite probable that this result is largely due to the diverse biological effect of the pyridine scaffold [19-21]. In particular, substituted pyridine-3-sulfonamides I (Figure 2) show a broad spectrum of inhibition of cancer cell growth in 26 lines [22], good activity, and selectivity for subpanels of leukemia colon cancer, and melanoma [23]. In turn, pyridinyl-containing vinyl ketone II inhibits the proliferation of human melanoma cells [24]. In addition, *N*-(1,2,4-triazol-3-yl)pyridine-3-sulfonamides III show greater antifungal efficacy than fluconazole [25], and pyridyl-1,4-dihydropyridines IV possess antihypertensive properties at an equal level to the nicardipine [26]. Polysubstituted pyridine derivatives are inhibitors of trypsin [27], β-lactamase [28] and phosphodiesterase (PDE2A) [29].

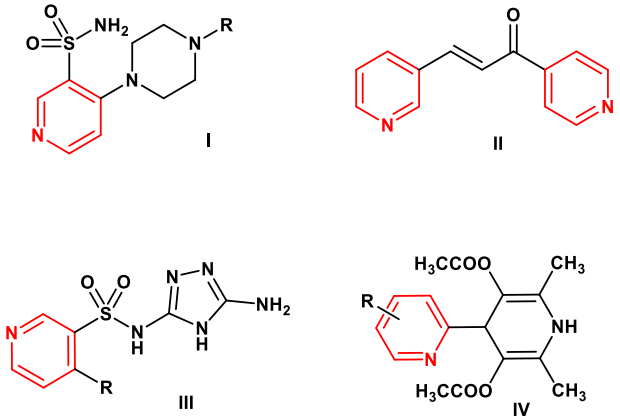


Figure 2. Some examples of the bioactive compounds containing the pyridine fragments (I-IV).

Given that the construction of molecules containing several pharmacophores is one of the most effective approaches to the search for bioactive substances, we thought it appropriate to develop a selective method for the synthesis of new derivatives of imidazo[2,1-*b*][1,3]thiazines modified in thiazine nucleus with 4-pyridinyloxy fragments and evaluate their drug-like and anti-inflammatory properties. In this context, the presented work is a development of previously published results [18], which concerned the synthesis and some biological properties of 2-pyridinyloxy-containing (benzo)imidazo[2,1-*b*][1,3]thiazines.

2. Materials and Methods

2.1. Materials.

All reagents were chemically pure and used without further purification. The solvents were purified according to standard procedures [30].

2.2. Chemistry.

Melting points were measured on a Kofler melting point-device and are uncorrected. IR spectra were recorded on Bruker Vertex 70 FT-IR spectrometer for samples in KBr pellets. ^{1}H NMR spectra were acquired in pulsed Fourier transform mode on a Varian VXR-400 spectrometer (400 MHz), while ^{13}CNMR spectra were acquired on a Bruker Avance DRX-500 spectrometer (125 MHz) using DMSO-d_6 as solvent. Mass spectra were recorded on an Agilent LC/MSD SL chromatograph equipped with Zorbax SB-C_{18} column (4.6x15mm), particle size 1.8 µm (PN 82(c)75-932), solvent DMSO, electrospray ionization at atmospheric pressure. Elemental analysis was performed on a PerkinElmer 2400 CHN Analyzer. The individuality of the obtained compounds was monitored by TLC on Silutol UV-254 plates.

2.2.1. General procedure for the synthesis of (2-pyridin-4-yloxy)substituted (benzo)imidazo[2,1-*b*][1,3]thiazines 3a-j.

To a mixture of 3-hydroxy(benzo)imidazo[2,1-*b*][1,3]thiazine 1a-c (10 mmol) and NaH (60% in mineral oil, 0.4g, 10 mmol) in dry DMF (4 ml), the 10 mmol of substituted 4-fluoropyridine 2a-d were added and stirred at room temperature for 24 h (in the case of compounds 3a,b,d,h,j) or heated at 80°C for 5 h (in the case of compounds 3c,e,g,i). The reaction mixture was poured onto the ice; the resulting precipitate was filtered off, washed with water, dried, and recrystallized from MeOH.

2.2.2. 6-[(2-Chloropyridin-4-yl)oxy]-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]thiazine (3a).

M.p.: 124-125 °C. ^{1}H NMR: δ = 8.23 (d, ^{3}J = 8.4 Hz, 1H, Ar), 7.29 (s, 1H, Ar), 7.17 (s, 1H, Ar), 7.10 (d, ^{3}J = 8.6 Hz, 1H, Ar), 6.87 (s, 1H, Ar), 5.45-5.49 (m, 1H, CH), 4.30-4.32 (m, 2H, NCH_2), 3.57-3.60 (m, 1H, SCH_2), 3.41-3.46 (m, 1H, SCH_2). ^{13}C NMR: δ = 164.42, 151.74, 150.68 (Py), 135.32 (C^{8a}), 127.82 (C^{2}), 121.41 (C^{3}), 111.58, 110.88 (Py), 67.00 (C^{6}), 47.88 (C^{5}), 28.11 (C^{7}). LC-MS: m/z = 268 [M+1] (100%). Anal. Calcd. for $C_{11}H_{10}ClN_3OS$, %: C 49.35; H 3.76; N 15.69. Found, %: C 49.10; H 3.80; N 15.77.

2.2.3. 6-[(2-Chloro-5-iodopyridin-4-yl)oxy]-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]thiazine (3b).

M.p.: 108-109 °C. ^{1}H NMR: δ = 8.53 (s, 1H, Ar), 7.51 (s, 1H, Ar), 7.16 (s, 1H, Ar), 6.86 (s, 1H, Ar), 5.56-5.61 (m, 1H, CH), 4.29-4.35 (m, 2H, NCH_2), 3.56-3.60 (m, 1H, SCH_2), 3.41-3.46 (m, 1H, SCH_2). ^{13}C NMR: δ = 163.45, 157.02, 151.99 (Py), 135.77 (C^{8a}), 128.15 (C^{2}), 121.71 (C^{3}), 110.41, 86.55 (Py), 68.50 (C^{6}), 48.13 (C^{5}), 28.50 (C^{7}). LC-MS: m/z = 394 [M+1] (100%). Anal. Calcd. for $C_{11}H_9ClIN_3OS$, %: C 33.56; H 2.30; N 10.67. Found, %: C 33.72; H 2.27; N 10.75.

2.2.4. 6-[(3-nitropyridin-4-yl)oxy]-2,3-diphenyl-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]thiazine (3c).

M.p.: 105-106 °C. ^{1}H NMR: δ = 8.97 (s, 1H, Ar), 8.65 (d, ^{3}J = 8.2 Hz, 1H, Ar), 7.60 (d, ^{3}J = 8.2 Hz, 1H, Ar), 7.46-7.48 (m, 3H, Ar), 7.33-7.35 (m, 2H, Ar), 7.28-7.30 (m, 2H, Ar), 7.16-7.20 (m, 2H, Ar), 7.12 (d, ^{3}J = 8.4 Hz, 1H, Ar), 5.59-5.63 (m, 1H, CH), 4.08-4.12 (m, 1H, NCH_2), 3.91-3.95 (m, 1H, NCH_2), 3.64-3.67 (m, 1H, SCH_2), 3.52-3.55 (m, 1H, SCH_2). ^{13}C NMR: δ = 155.71, 155.16, 146.81 (Py), 137.56 (C^{8a}), 136.89 (C^{2}), 136.77 (Ar), 134.57 (Py), 130.98 (Ar), 130.17 (C^{3}), 129.86, 129.62, 129.33, 128.55, 126.72, 126.35 (Ar), 111.75 (Py),

69.55 (C^6), 46.79 (C^5), 28.13 (C^7). LC-MS: m/z = 431 [M+1] (100%). Anal. Calcd. for $C_{23}H_{18}N_4O_3S$, %: C 64.17; H 4.21; N 13.01. Found, %: C 63.99; H 4.25; N 12.90.

2.2.5. 6-[(2-Chloropyridin-4-yl)oxy]-2,3-diphenyl-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]thiazine (3d).

M.p.: 151-152 °C. ^{1}H NMR: δ = 8.21 (d, ^3J = 8.6 Hz, 1H, Ar), 7.45-7.48 (m, 3H, Ar), 7.27-7.35 (m, 5H, Ar), 7.46-7.48 (m, 3H, Ar), 7.33-7.35 (m, 2H, Ar), 7.28-7.30 (m, 2H, Ar), 7.16-7.20 (m, 2H, Ar), 7.17-7.20 (m, 2H, Ar), 7.09-7.13 (m, 2H, Ar), 5.44-5.48 (m, 1H, CH), 4.09-4.12 (m, 1H, NCH_2), 3.84-3.87 (m, 1H, NCH_2), 3.58-3.62 (m, 1H, SCH_2), 3.46-3.51 (m, 1H, SCH_2). ^{13}C NMR: δ = 164.77, 152.09, 151.20 (Py), 136.91 (C^{8a}), 136.83 (C^2), 134.61 (Ar), 130.99 (C^3), 130.17, 129.82, 129.59, 129.27, 128.55, 126.70, 126.40 (Ar), 111.97, 111.35 (Py), 67.55 (C^6), 46.16 (C^5), 28.03 (C^7). LC-MS: m/z = 420 [M+1] (100%). Anal. Calcd. for $C_{23}H_{18}ClN_3OS$, %: C 65.78; H 4.32; N 10.01. Found, %: C 65.99; H 4.37; N 9.91.

2.2.6. 6-[(3,5-Dichloropyridin-4-yl)oxy]-2,3-diphenyl-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]thiazine (3e).

M.p.: 108-110 °C. ^{1}H NMR: δ = 8.66 (s, 2H, Ar), 7.45-7.47 (m, 4H, Ar), 7.29-7.35 (m, 6H, Ar), 7.16-7.20 (m, 4H, Ar), 5.26-5.29 (m, 1H, CH), 4.05-4.09 (m, 1H, NCH_2), 3.74-3.77 (m, 1H, NCH_2), 3.64-3.67 (m, 2H, SCH_2). ^{13}C NMR: δ = 164.57, 147.40 (Py), 137.11 (C^{8a}), 136.73 (C^2), 134.70 (Ar), 130.39 (C^3), 130.05, 129.77, 129.52, 129.15, 128.68, 126.59, 126.36 (Ar), 112. 35 (Py), 68.55 (C^6), 46.46 (C^5), 28.19 (C^7). LC-MS: m/z = 455 [M+1] (100%). Anal. Calcd. for $C_{23}H_{17}Cl_2N_3OS$, %: C 60.80; H 3.77; N 15.61. Found, %: C 60.61; H 3.73; N 15.79.

2.2.7. 6-[(2-Chloro-5-iodopyridin-4-yl)oxy]-2,3-diphenyl-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]thiazine (3f).

M.p.: 119-120 °C. ^{1}H NMR: δ = 8.52 (s, 1H, Ar), 7.44-7.50 (m, 4H, Ar), 7.34-7.38 (m, 3H, Ar), 7.26-7.29 (m, 3H, Ar), 7.15-7.19 (m, 4H, Ar), 7.10 (s, 1H, Ar), 5.54-5.57 (m, 1H, CH), 4.04-4.07 (m, 1H, NCH_2), 3.86-3.90 (m, 1H, NCH_2), 3.60-3.63 (m, 1H, SCH_2), 3.50-3.63 (m, 1H, SCH_2). ^{13}C NMR: δ = 163.32, 158.32, 156.98 (Py), 137.00 (C^{8a}), 136.67 (C^2), 134.75 (Ar), 131.34 (Ar), 130.34 (C^3), 129.75, 129.58, 129.28, 128.50, 126.58, 126.30 (Ar), 114.12, 110.43, 86.67 (Py), 68.83 (C^6), 46.71 (C^5), 28.28 (C^7). LC-MS: m/z = 546 [M+1] (100%). Anal. Calcd. for $C_{23}H_{17}ClIN_3OS$, %: C 50.61; H 3.14; N 7.70. Found, %: C 50.73; H 3.10; N 7.59.

2.2.8. 3-[(3-Nitropyridin-4-yl)oxy]-3,4-dihydro-2*H*-benzo[4,5]imidazo[2,1-*b*][1,3]thiazine (3g).

M.p.: 130-131 °C. ^{1}H NMR: δ = 8.97 (s, 1H, Ar), 8.74 (d, ^3J = 8.4 Hz, 1H, Ar), 7.74 (d, ^3J = 8.2 Hz, 1H, Ar), 7.43-7.47 (m, 2H, Ar), 7.17-7.22 (m, 2H, Ar), 5.78-5.82 (m, 1H, CH), 4.57-4.60 (m, 1H, NCH_2), 4.48-4.52 (m, 1H, NCH_2), 3.73-3.77 (m, 1H, SCH_2), 3.59-3.63 (m, 1H, SCH_2). ^{13}C NMR: δ = 162.88, 155.70 (Py), 155.28 (C^{10a}), 146.83 (Py), 146.06 (C^{9a}), 142.94 (C^{5a}), 137.60 (Py), 122.48, 121.54, 117.62, 111.87 (Ar), 109.29 (Py), 68.45 (C^3), 46.22 (C^4), 28.06 (C^2). LC-MS: m/z = 329 [M+1] (100%). Anal. Calcd. for $C_{15}H_{12}N_4O_3S$, %: C 54.87; H 3.68; N 17.06. Found, %: C 57.95; H 3.64; N 17.19.

2.2.9. 3-[(2-Chloropyridin-4-yl)oxy]-3,4-dihydro-2*H*-benzo[4,5]imidazo[2,1-*b*][1,3]thiazine (3h).

M.p.: 177-178 ºC. ^{1}H NMR: δ = 7.42-7.47 (m, 2H, Ar), 7.31 (s, 1H, Ar), 7.11-7.16 (m, 4H, Ar), 5.62-5.65 (m, 1H, CH), 4.51-4.53 (m, 1H, NCH_2), 4.44-4.46 (m, 1H, NCH_2), 3.68-3.70 (m, 1H, SCH_2), 3.55-3.58 (m, 1H, SCH_2). ^{13}C NMR: δ = 164.78 (Py), 152.16 (C^{10a}), 151.22, 146.13 (Py), 143.03 (C^{9a}), 136.19 (C^{5a}), 122.46, 121.50, 117.61, 112.03 (Ar), 111.34, 109.30 (Py), 68.78 (C^{3}), 46.42 (C^{4}), 28.11 (C^{2}). LC-MS: m/z = 318 [M+1] (100%). Anal. Calcd. for $C_{15}H_{12}ClN_3OS$, %: C 56.69; H 3.81; N 13.22. Found, %: C 56.47; H 3.77; N 13.36.

2.2.10. 3-[(3,5-Dichloropyridin-4-yl)oxy]-3,4-dihydro-2*H*-benzo[4,5]imidazo[2,1-*b*][1,3]thiazine (i).

M.p.: 135-136 ºC. ^{1}H NMR: δ = 8.60 (s, 2H, Ar), 7.40-7.44 (m, 2H, Ar), 7.12-7.15 (m, 2H, Ar), 5.43-5.46 (m, 1H, CH), 4.55-4.58 (m, 1H, NCH_2), 4.32-4.35 (m, 1H, NCH_2), 3.78-3.81 (m, 2H, SCH_2). ^{13}C NMR: δ = 166.51, 150.89 (Py), 153.42 (C^{10a}), 146.57 (Py), 142.63 (C^{9a}), 137.08 (C^{5a}), 123.00, 121.93, 117.11, 111.24 (Ar), 110.09 (Py), 67.90 (C^{3}), 46.50 (C^{4}), 28.20 (C^{2}). LC-MS: m/z = 353 [M+1] (100%). Anal. Calcd. for $C_{15}H_{11}Cl_2N_3OS$, %: C 51.15; H 3.15; N 11.93. Found, %: C 50.98; H 3.11; N 12.05.

2.2.11. 3-[(2-Chloro-5-iodopyridin-4-yl)oxy]-3,4-dihydro-2*H*-benzo[4,5]imidazo[2,1-*b*][1,3]thiazine (3j).

M.p.: 165-167 ºC. ^{1}H NMR: δ = 8.51 (s, 1H, Ar), 7.57 (s, 1H, Ar), 7.46-7.52 (m, 2H, Ar), 7.16-7.21 (m, 2H, Ar), 5.72-5.76 (m, 1H, CH), 4.56-4.59 (m, 1H, NCH_2), 4.43-4.46 (m, 1H, NCH_2), 3.69-3.72 (m, 1H, SCH_2), 3.56-3.59 (m, 1H, SCH_2). ^{13}C NMR: δ = 163.30, 157.01 (Py), 152.05 (C^{10a}), 146.27 (Py), 142.97 (C^{9a}), 136.20 (C^{5a}), 122.39, 121.43, 117.59, 110.50 (Ar), 109.27, 86.61 (Py), 67.73 (C^{3}), 46.34 (C^{4}), 28.08 (C^{2}). LC-MS: m/z = 444 [M+1] (100%). Anal. Calcd. for $C_{15}H_{11}ClIN_3OS$, %: C 40.61; H 2.50; N 9.47. Found, %: C 40.82; H 2.47; N 9.55.

2.3. Anti-inflammatory (antiexudative) activity.

2.3.1. Anti-inflammatory (antiexudative) activity.

The male albino rats weighing 180-220 g were used for anti-exudative activity studying. The animals were treated humanely throughout the study period adhering to the guideline for the use and care of animals in the declaration of Helsinki (National Research Council, 2011). The Animal Ethics Committee approved the experiment design and study protocol of the Danylo Halytsky Lviv National Medical University, protocol No.10, March 17, 2021. The carrageenan-induced hind paw edema was produced by Winter *et al*. [31]. The compounds synthesized were intraperitoneally injected in a dose 50 mg/kg (in saline solution with one drop of Tween-80™). Diclofenac (tablets “Diclofenac sodium”, “Zdorovja narodu”, Ukraine) in dose 8 mg/kg was used as reference drug. The antiexudative activity (inflammation inhibition) was expressed as a decrease of rats' paw edema, was calculated using the equation, and was given in percentage:

$$\text{Inhibition}, \% = \frac{\Delta Vcontrol - \Delta Vexperiment}{\Delta Vcontrol} * 100\ \%$$

where, $\Delta V_{control}$ and $\Delta V_{experiment}$ – the mean values of the volume difference for control and experimental animals hinds, respectively.

2.3.2. Assessment of liver function.

The serum collected from the albino rats was used to estimate biochemical parameters to determine the functional state of the liver. The levels of total alkaline phosphatase (ALP), gamma-glutamyltransferase (γ-GGT), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were estimated photometrically according to the reported methods using CORMAY ACCENT-200 automatic analyzers (PZ Cormay, Poland).

2.3.3. Ulcerogenic activity estimation.

All animals were sacrificed under deep anesthesia 6 hours after drug treatment, and then their stomachs were removed, opened along the great curvature, and rinsed with a saline solution of 0.9%. The gastric mucosa was examined using a magnifying glass (2X) to assess the incidence of redness and spot ulcers. The mucosal damage was evaluated according to the following score: 0 - no visible damage; 1 - the presence of edema or hemorrhages, 1-3 small ulcers; 2 - several (more than 3) small ulcers or 1 ulcer of considerable size; 3 - ulcer of considerable size (diameter up to 4 mm); 4 - several large ulcers; 5 - breakthrough ulcer. The gastric mucosal ulceration score was calculated by the difference between the mean score of each treated group and the control group's mean score.

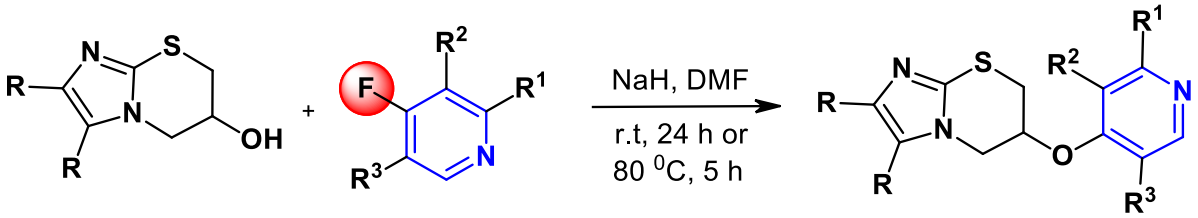
2.3.4. Statistical analysis.

All data were processed using the statistical package Statistica 10.0 (Statsoft/Dell, Tulsa, OK, USA). The descriptive statistics of the data in the tables include mean ± standard error of the mean (SEM) or mean ± standard deviation. Significance was assessed by using the one-way ANOVA followed by *t*-test. Values were considered statistically significant when *P* value was less than 0,05.

3. Results and Discussion

3.1. Chemistry.

For the synthesis of target structurally modified imidazo[2,1-*b*][1,3]thiazines with 4-pyridinyloxy fragments, we have proposed an approach based on pyridinylation of available 3-hydroxyimidazo[2,1-*b*][1,3]thiazines 1a,b [32] and their benzoanalogue 1c [12] with 4-fluoro-containing pyridines 2a-d. It was found that, despite the bi- and polyfunctional electrophilic nature of pyridines 2a-d, this reaction is characterized by high regioselectivity and is realized exclusively as a nucleophilic substitution of the fluorine atom at position 4 of the pyridine ring on oxyimidazo[2,1-*b*][1,3]thiazino fragment with the formation of 6-[(pyridin-4-yl)oxy]-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]thiazines 3a-j (Scheme 1). The LC-MS method did not detect nitro substitution products and chlorine or iodine atoms in the reaction mixtures, most likely due to the stronger polarization of the C-F bond by the sp^2-hybridized pyridine nitrogen atom. The selection of experimental conditions revealed that the optimal for the reaction is the use of DMF as a solvent and NaH as a base. Compounds 3a, b, d, f, h, j are obtained in 58-73% yields by carrying out the reaction at room temperature for 24 hours. Instead, synthesizing compounds 3c, e, g, i containing 3-nitropyridinyl- and 3,5-dichloropyridinyl fragments require heating the reaction mixtures at 80 ° C for 5 hours.



1a-c **2a-d** **3a-j**

1a R=H;
1b R=Ph;
1c RR=(CH=CH)$_2$

2a R^1=R^3=H, R^2=NO_2;
2b R^1=Cl, R^2=R^3=H;
2c R^1=H, R^2=R^3=Cl;
2d R^1=Cl, R^2=H, R^3=I

Scheme 1. Synthesis of compounds 3a-j.

The structure of the synthesized compounds was confirmed by 1H NMR, 13C NMR, and LC-MS spectra, which are presented in the experimental part. In particular, in the 1H NMR spectra of all imidazothiazines 3a-j, the pyridine cycle proton signals are presented, and derivatives 3a, b are easily identified in the range of 8.53-6.86 ppm, and their diphenyl 3c-f and benzo analogs, 3g-j are overlapped with signals of the phenyl-group protons.

The control of the reaction process and products formation was monitored by TLC. The compounds' structure characterization and yield are presented in Table 1.

Table 1. Structure characterization and yields of synthesized compounds 3a-j.

Compound	R	R	R^1	R^2	R^3	Yield, %
3a	H	H	Cl	H	H	72
3b	H	H	Cl	H	I	58
3c	Ph	Ph	H	NO_2	H	70
3d	Ph	Ph	Cl	H	H	69
3e	Ph	Ph	H	Cl	Cl	63
3f	Ph	Ph	Cl	H	I	73
3g	(-CH=CH-)$_2$		H	NO_2	H	78
3h	(-CH=CH-)$_2$		Cl	H	H	71
3i	(-CH=CH-)$_2$		H	Cl	Cl	65
3j	(-CH=CH-)$_2$		Cl	H	I	67

3.2. In silico evaluation of drug-likeness properties.

The drug-likeness properties of the derivatives 3a-j were determined based on Lipinski and Veber rules and evaluated *in silico* using the SwissAdme of the Swiss Institute of Bioinformatics website [33] (Table 2).

Table 2. Drug-likeness parameters of derivatives 3a-j according to Lipinski and Veber rules.

Compound / Parameter, descriptor	Lipinski rules				Veber rules		Fraction Csp3 ≤ 0.25	GI absorption	BBB Permeant	Leadlikeness
	MW ≤ 500	Log P ≤ 5	NHD ≤ 5	NHA ≤ 10	NBR ≤ 10	TPSA ≤ 140				
3a	267.73	1.96	0	3	2	65.24	0.27	High	Yes	Yes
3b	393.63	2.63	0	3	2	65.24	0.27	High	Yes	No
3c	430.48	3.45	0	5	5	111.06	0.13	High	No	No
3d	419.93	4.64	0	3	4	65.24	0.13	High	No	No
3e	454.37	5.07	0	3	4	65.24	0.13	High	No	No
3f	545.82	5.25	0	3	4	65.24	0.13	High	No	No
3g	328.35	1.80	0	5	3	111.06	0.20	High	No	Yes
3h	317.79	3.04	0	3	2	65.24	0.20	High	Yes	No
3i	352.24	3.48	0	3	2	65.24	0.20	High	Yes	No
3j	443.69	3.68	0	3	2	65.24	0.20	High	Yes	No

GI – gastrointestinal; BBB - the blood-brain barrier

Accordingly, with obtained data, almost all derivatives (except 3e and 3f) correspond to the Lipinski and Veber rules and possess satisfactory pharmacokinetic parameters with a high level of predicted gastrointestinal absorption.

3.3. Investigation of anti-inflammatory (anti-exudative) activity.

The anti-inflammatory (anti-exudative) activity of all synthesized compounds 3 a-j was investigated in the *in vivo* carrageenin model of the total edema of hind paws of albino rats [31]. The study results are presented in Table 3.

Table 3. *In vivo* anti-inflammatory activity of compounds 3 a-j on carrageenin-induced paw edema in white rats (intraperitoneally use; doses: carrageenin 1%, 0.1 mL; Diclofenac sodium – 8 mg/kg, tested compounds – 50 mg/kg; M±m; n=6 in each group).

Compounds/Reference drug, Doses	Rat hind limb volume increase, 4 hours, %	Inflammation inhibition, %
Carrageenin	122.9±10.8	-
Diclofenac sodium	65.9±5.3	46.3
3a	71.8±8.1	41.6
3b	87.7±7.4	28.6
3c	104.5±9.9	14.9
3d	94.0±9.3	23.5
3e	99.1±10.8	19.4
3f	103.8±11.6	15.5
3g	105.6±10.9	14.1
3h	75.1±8.3	38.9
3i	99.9±9.5	18.7
3j	96.8±.4	21.2

The synthesized compounds 3 a-j were mostly active under carrageenin-induced paw edema conditions. The inhibition index was observed in the range of 14.1 to 41.6 %. From this point of view, the "structure – anti-inflammatory activity" derivatives 3 a-j with unsubstituted imidazole ring in the imidazo[2,1-*b*][1,3]thiazine core are characterized by a total higher activity level. Such data correlate with our early obtained results and are in accordance with *in silico* predicted drug-like and pharmacokinetic properties. It should be noted that monochlorosubstituted derivatives 3a, 3h were found to be the most active, whereas the introduction of the additional chlorine, iodine atoms, or nitro-group led to activity decreasing.

The impact on the function of liver enzymes was studied for the most active derivatives 3a and 3h. Administration of tested derivatives 3a and 3h and reference drugs do not negatively impact liver function (Table 4).

Table 4. The liver enzymes activity in rats with formaldehyde-induced paw edema and treated with 3a and 3h and diclofenac sodium (intraperitoneally use, M±m, n=6 in each group)

Parameters/Time points	ALT, U/l	AST, U/l	ALP, U/l	γ-GGT, IU/l
Intact control	65.2±7.1	186.1±19.8	264.3±18.5	2.9±0.9
Carrageenin	109.5±8.9$^{\#}$	287.0±18.3$^{\#}$	398.5±41.6$^{\#}$	4.2±0.8*
3a, 50 mg/kg	92.5±8.5	223.2±21.0	312.2±31.6	5.0±0.7
3h, 50 mg/kg	91.2±7.0	193.2±20.3	274.1±22.8	5.2±0.9*
Diclofenac sodium, 8 mg/kg	95.9±6.2*	216.4±29.4*	293.8±28.4	5.1±0.6

*p<0,05; $^{\#}$p<0,001 compared with intact control group

The derivatives 3a and 3h were evaluated for ulcerogenic activity after application at a 50 mg/kg dose in rats. The results were compared with an intact control group and the diclofenac sodium group (Table 5). As a result, diclofenac sodium showed significant ulcerogenic risk with a high ulceration score. The tested compounds didn't show any ulcerogenic activity.

Table 5. The ulcerogenic pattern of rats with formaldehyde-induced paw edema and treated with 3a and 3h and diclofenac sodium (intraperitoneally use, M±m, n=6 in each group)

Parameters/Groups	Animals with ulcers, n'	Ulcer degree, points
Intact control	0	0±0.00
3a, 50 mg/kg	0	0±0.00
3h, 50 mg/kg	0	0±0.00
Diclofenac sodium, 8 mg/kg	6	1.6±0.2

4. Conclusions

A series of new (4-pyridinyloxy)modified (benzo)imidazo[2,1-*b*][1,3]thiazines 3a-j was synthesized by the interaction of 3-hydroxy-3,4-dihydro-2*H*-(benzo) imidazo[2,1-*b*][1,3]thiazines 1a-c with substituted 4-fluoropyridines 2a-d. The synthesized compounds comply with ' 'Lipinski's ""five"" rules and 'Weber's rules and have promising anti-inflammatory properties. Such drug-likeness and pharmacological features of (pyridin-4-yl)oxy(benzo)imidazo[2,1-*b*][1,3]thiazine derivatives are an important argument for their further research as potential non-steroidal anti-inflammatory drugs.

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Conflicts of Interest

The authors declare no conflict of interest.

References

1. Alizadeh, S.R.; Ebrahimzadeh, M.A. Pyrazolotriazines: Biological activities, synthetic strategies and recent developments. *Eur. J. Med. Chem.* **2021,** *223*, https://doi.org/10.1016/j.ejmech.2021.113537.
2. Ulomskiy, E.N.; Ivanova, A.V.; Gorbunov, E.B.; Esaulkova, I.L.; Slita, A.V.; Sinegubova, E.O.; Voinkov, E.K.; Drokin, R.A.; Butorin, I.I.; Gazizullina, E.R.; Gerasimova, E.L.; Zarubaev, V.V.; Rusinov, V.L. Synthesis and biological evaluation of 6-nitro-1,2,4-triazoloazines containing polyphenol fragments possessing antioxidant and antiviral activity. *Bioorg. Med. Chem. Lett.* **2020,** *30*, https://doi.org/10.1016/j.bmcl.2020.127216.
3. Abdel-Mohsen, H.T.; Abood, A.; Flanagan, K.J.; Meindl, A.; Senge, M.O.; El Diwani, H.I. Synthesis, crystal structure, and ADME prediction studies of novel imidazopyrimidines as antibacterial and cytotoxic agents. *Arch. Pharm.* **2020,** *353*, https://doi.org/10.1002/ardp.201900271.
4. Bernat, Z.; Szymanowska, A.; Kciuk, M.; Kotwica-Mojzych, K.; Mojzych, M. Review of the Synthesis and Anticancer Properties of Pyrazolo[4,3-*e*][1,2,4]triazine Derivatives. *Molecules* **2020,** *25*, https://doi.org/10.3390/molecules25173948.
5. Muhammad, Z.A.; Farghaly, T.A.; Althagafi, I.; Al-Hussain, S.A.; Zaki, M.E.A.; Harras, M.F. Synthesis of antimicrobial azoloazines and molecular docking for inhibiting COVID-19. *J. Het. Chem.* **2021**, *58*, 1286-1301, https://doi.org/10.1002/jhet.4257.
6. Aouad, M.R.; Al-Mohammadi, H.M.; Al-blewi, F.F.; Ihmaid, S.; Elbadawy, H.M.; Althagfan, S.S.; Rezki, N. Introducing of acyclonucleoside analogues tethered 1,2,4-triazole as anticancer agents with dual epidermal growth factor receptor kinase and microtubule inhibitors. *Bioorg. Chem.* **2020,** *94*, https://doi.org/10.1016/j.bioorg.2019.103446.
7. Al-Azmi, A. Pyrazolo[1,5-a]pyrimidines: A Close Look into their Synthesis and Applications. *Curr. Org. Chem.* **2019**, *23*, 721-743 https://doi.org/10.2174/1385272823666190410145238.
8. Maji, P.K. Synthesis of Pyrimidine-Annulated Five-Membered Heterocycles: An Overview. *Curr. Org. Chem.* **2019**, *23*, 20, 2204-2269, https://doi.org/10.2174/1385272823666191019111627.
9. Holota, S.; Komykhov, S.; Sysak, S.; Gzella, A.; Cherkas, A.; Lesyk R. Synthesis, Characterization and In Vitro Evaluation of Novel 5-Ene-thiazolo[3,2-b][1,2,4]triazole-6(5H)-ones as Possible Anticancer Agents. *Molecules.* **2021**, *26*, https://doi.org/10.3390/molecules26041162.

10. Volkov, O.A.; Cosner, C.C.; Brockway, A.J.; Kramer, M.; Booker, M.; Zhong, S.; Ketcherside, A.; Wei, S.; Longgood, J.; McCoy, M.; Richardson, T.E.; Wring, S.A.; Peel, M.; Klinger, J.D.; Posner, B.A.; De Brabander, J.K.; Phillips, M.A.. Identification of Trypanosoma brucei AdoMetDC Inhibitors Using a High-Throughput Mass Spectrometry-Based Assay. *ACS Infect. Dis.* **2017**; *3*, 512-26, https://doi.org/10.1021/acsinfecdis.7b00022.
11. Thompson, A.M.; Marshall, A.J.; Maes, L.; Yarlett, N.; Bacchi, C.J.; Gaukel, E.; Wringd, S.A.; Launaye, D.; Braillarde, S.; Chatelaine, E.; Mowbraye, Ch.E.; Dennya, W.A. Assessment of a pretomanid analogue library for African trypanosomiasis: Hit-to-lead studies on 6-substituted 2-nitro-6,7-dihydro-5H-imidazo[2,1-b][1,3]thiazine 8-oxides. *Bioorg. Med. Chem. Lett.* **2018**, *28*, 207–13, https://doi.org/10.1016/j.bmcl.2017.10.067.
12. Gong, J-X.; He, Y.; Cui, Zh-L.; Guo, Y.-W. Synthesis, spectral characterization, and antituberculosis activity of thiazino[3,2-A]benzimidazole derivatives. *Phosphorus Sulfur Silicon Relat. Elem.* **2016**, *191*, 1036-41, https://doi.org/10.1080/10426507.2015.1135149.
13. Rodríguez, O.A.R.; Vergaraa, N.E.M.; Sánchez, J.P.M.; Martínez, M.T.S.; Sandoval, Z.G.; Cruz, A.; Organillo, A.R. Synthesis, crystal structure, antioxidant activity and dft study of 2-aryl-2,3-dihydro-4H-[1,3]thiazino[3,2-a]benzimidazol-4-One. *J. Mol. Struct.* **2020**, *1199*, https://doi.org/10.1016/j.molstruc.2019.127036.
14. Nikolova, I.; Slavchev, I.; Ravutsov, M.; Dangalov, M.; Nikolova, Y.; Zagranyarska, I.; Stoyanova, A.; Nikolova, N.; Mukova, L.; Grozdanov, P.; Nikolova, R.; Shivachev, B.; Kuz'min, V.E.; Ognichenko, L.N.; Galabov, A.S.; Dobrikov, G.M. Anti-enteroviral activity of new MDL-860 analogues: Synthesis, in vitro/in vivo studies and QSAR analysis. *Bioorg Chem.* **2019**, *85*, 487-497, https://doi.org/10.1016/j.bioorg.2019.02.020.
15. Hamama, W.S.; Waly, M.A.; El-Hawary, I.I.; Zoorob, H.H. Utilization of 2-Chloronicotinonitrile in the Syntheses of Novel Fused Bicyclic and Polynuclear Heterocycles of Anticipated Antitumor Activity. *J. Heterocycl. Chem.* **2016**, *53*, 953-957, https://doi.org/10.1002/jhet.1631.
16. LaFleur, M.D.; Lucumi, E.; Napper, A.D.; Diamond, S.L.; Lewis, K. Novel high-throughput screen against Candida albicans identifies antifungal potentiators and agents effective against biofilms. *J. Antimicrob. Chemother.* **2011**, *66*, 820-826, https://doi.org/10.1093/jac/dkq530.
17. Thompson, A.M.; O'Connor, P.D.; Marshall, A.J.; Francisco, A.F.; Kelly, J.M.; Riley, J.; Read, K.D.; Perez, C.J.; Cornwall, S.; Thompson, R.C.A.; Keenan, M.; White, K.L.; Charman, S.A.; Zulfiqar, B.; Sykes, M.L.; Avery, V.M.; Chatelain, E.; Denny, W.A. Re-evaluating pretomanid analogues for Chagas disease: Hit-to-lead studies reveal both in vitro and in vivo trypanocidal efficacy. *Eur. J. Med. Chem.* **2020**, *207*, https://doi.org/10.1016/j.ejmech.2020.112849.
18. Saliyeva, L.; Slyvka N.; Litvinchuk, M.; Holota, S.; Grozav, A.; Yakovychuk, N.; Vovk M. Synthesis and Evaluation of Bioactivity of 6-[(2-Pyridinyloxy)](Benzo) Imidazo [2, 1-b][1, 3] Thiazine Derivatives *Biointerface Research in Applied Chemistry* **2022,** *12*, 5031–5044, https://doi.org/10.33263/BRIAC124.50315044.
19. Cordeiroa, N.D.M.; Freitas, Rosana H.C.N.; Fragab, C.A.M.; Fernandesa, P.D. New 2-amino-pyridinyl-N-acylhydrazones: Synthesis and identification of their mechanism of anti-inflammatory action. *Biomedicine and Pharmacotherapy* **2020**, *123*, https://doi.org/10.1016/j.biopha.2019.109739.
20. Leoni, A.; Frosini, M.; Locatelli, A.; Micucci, M.; Carotenuto, C.; Durante, M.; Cosconati, S.; Budriesi, R. 4-Imidazo[2,1-b]thiazole-1,4-DHPs and neuroprotection: preliminary study in hits searching. *Eur. J. Med. Chem.* **2019**, *169*, 89-102, https://doi.org/10.1016/j.ejmech.2019.02.075.
21. Bai, H.; Liu, X.; Chenzhang, P.; Xiao, Y.; Fu, B.; Qin, Z. Design, Synthesis and Fungicidal Activity of New 1,2,4-Triazole Derivatives Containing Oxime Ether and Phenoxyl Pyridinyl Moiety. *Molecules* **2020**, *25*, 5852-5864, https://doi.org/10.3390/molecules25245852.
22. S1awinski, J.; Szafranski, K.; Vullo, D.; Supuran, C.T. Carbonic anhydrase inhibitors. Synthesis of heterocyclic 4-substituted pyridine-3-sulfonamide derivatives and their inhibition of the human cytosolic isozymes I and II and transmembrane tumor-associated isozymes IX and XII. *Eur. J. Med. Chem.* **2013**, *69*, 701-710, http://dx.doi.org/10.1016/j.ejmech.2013.09.027.
23. Szafranski, K.; S1awinski, J. Synthesis of Novel 1-(4-Substituted pyridine-3-sulfonyl)-3- phenylureas with Potential Anticancer Activity. *Molecules* **2015**, *20*, 12029-12044, http://doi.org/10.3390/molecules200712029.
24. Kotowski, K.; Supplitt, S.; Wiczew, D.; Przystupski, D.; Bartosik, W.; Saczko, J.; Rossowska, J.; Drąg-Zalesińska , M.; Michel, O.; Kulbacka, J. 3PO as a Selective Inhibitor of 6-Phosphofructo2-Kinase/Fructose-2,6-Biphosphatase 3 in A375 Human Melanoma Cells. *Anticancer Research* **2020**, *40*, 2613-2625, https://doi.org/10.21873/anticanres.14232.
25. Szafranski, K.; S1awinski, J.; Kedzia, A. and Kwapisz E. Syntheses of Novel 4-Substituted N-(5-amino-1H1,2,4-triazol-3-yl)pyridine-3-sulfonamide Derivatives with Potential Antifungal Activity. *Molecules* **2017**, *22*, 1926-1943, https://doi.org/10.3390/molecules22111926.
26. Ashimori, A.; Ono, T.; Uchida, T.; Ohtaki, Y.; Fukaya, C.; Watanabe, M.; Yokoyama, K. Novel 1, 4-Dihydropyridine Calcium Antagonists. I.: Synthesis and Hypotensive Activity of 4-(Substituted Pyridyl)-1, 4-Dihydropyridine Derivatives. *Chem. Pharm. Bull.* **1990**, *38*, 2446-2458, https://doi.org/10.1248/cpb.38.2446.

27. Al-Hadhrami, N.A.; Ladwig, A.; Rahman, A.; Rozas, I.; Malthouse, J.P.G.; Evans, P. Synthesis of 2-Guanidinyl Pyridines and their Trypsin Inhibition and Docking. *Bioorg. Med. Chem.* **2020**, *28*, https://doi.org/10.1016/j.bmc.2020.115612.
28. Reddy, N.; Shungube, M.; Arvidsson, P.I.; Baijnath, S.; Kruger, H.G. Govender, T.; Naicker, T. A 2018-2019 patent review of metallo betalactamase inhibitors. Expert *Opinion on Therapeutic Patents.* **2020**, *30*, 541-555, https://doi.org/10.1080/13543776.2020.1767070.
29. Ritawidya, R.; Ludwig, F-A.; Briel, D.; Brust, P.; Scheunemann, M. Synthesis and In Vitro Evaluation of 8-Pyridinyl-Substituted Benzo[e]imidazo[2,1-c][1,2,4]triazines as Phosphodiesterase 2A Inhibitors. *Molecules* **2019**, *24*, 2791-2811, https://doi.org/10.3390/molecules24152791.
30. Armarego W.L.F.; Chai C. *Purification of Laboratory Chemicals*. 7th ed.: Elsevier: Oxford, UK, **2013**; pp. 1-1024, https://doi.org/10.1016/C2009-0-64000-9.
31. Winter, C.A.; Risley, E.A.; Nuss, G.W. Carrageenin-induced edema in hind paw of the rat as an assay for antiiflammatory drugs. *Proc. Soc. Exp. Biol. Med.* **1962**, *111*, 544-547, https://doi.org/10.3181/00379727-111-27849.
32. Alper, H.; Keung, E.C.H. New synthesis of the 1,3-thiazine ring system. *J Org Chem.* **1972**, *37*, 1464-1466, https://doi.org/10.1021/jo00974a047.
33. SwissADME. Available online: http://www.swissadme.ch/ (accessed on 28/04/2021).