Scientia Pharmaceutica

ÖSTERREICHISCHE PHARMAZEUTISCHE GESELLSCHAFT

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Synthesis and Regularities of the Structure–Activity Relationship in a Series of *N*-Pyridyl-4-methyl-2,2-dioxo-1*H*-2λ^6,1-benzothiazine-3-carboxamides

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Received: 18 April 2019; Accepted: 11 May 2019; Published: 15 May 2019

Abstract: According to our quantum and chemical calculations 4-methyl-2,2-dioxo-1*H*-2λ^6,1-benzothiazine-3-carboxylic acid imidazolide is theoretically almost as reactive as its 2-carbonyl analog, and it forms the corresponding *N*-pyridyl-4-methyl-2,2-dioxo-1*H*-2λ^6,1-benzothiazine-3-carboxamides with many aminopyridines. However, in practice, the sulfo group introduces significant changes at times and prevents the acylation of sterically hindered amines. One of these products was 2-amino-6-methylpyridine. Thus, it has been concluded that aminopyridines interact with imidazolide in aromatic form where the target for the initial electrophilic attack is the ring nitrogen. To confirm the structure of all substances synthesized, ^{1}H-NMR spectroscopy and X-ray diffraction analysis were used. From X-ray diffraction data it follows that in the crystalline phase the carbonyl and sulfo group may occupy different positions with respect to the plane of the benzothiazine bicycle: this position may be unilateral, typical for 4-methyl-2,2-dioxo-1*H*-2λ^6,1-benzothiazine-3-carboxamides, versatile, and not yet encountered in compounds of this type. A comparison of these data with the results of the pharmacological screening conducted on the standard model of carrageenan inflammation showed that the *N*-pyridylamides of the first group demonstrated a direct dependence of their analgesic and anti-inflammatory activity on the mutual arrangement of the planes of the benzothiazine and pyridine fragments. The new molecular conformation of the benzothiazine nucleus provides a sufficiently high level of analgesic (but not anti-inflammatory) properties in all *N*-pyridylamides of the second group with an extremely weak dependence on the spatial arrangement of the pyridine cycle. All substances presented this article proved themselves in varying degrees as analgesics and antiphlogistics. Moreover, two of them—*N*-(5-methylpyridin-2-yl)- and *N*-(pyridin-3-yl)-4-methyl-2,2-dioxo-1*H*-2λ^6,1-benzothiazine-3-carboxamides—exceeded the most effective drug of oxicam type Lornoxicam by these indicators.

Keywords: 4-methyl-2,2-dioxo-1*H*-2λ^6,1-benzothiazine-3-carboxamide; 2,1-benzothiazine; aminopyridines; crystal structure; molecular conformation; analgesic activity; anti-inflammatory action

1. Introduction

Aminopyridines are widely used by the pharmaceutical industry as intermediates of the synthesis of numerous modern drugs of various pharmacological groups, including analgesics [1,2] (Figure 1). From a chemical point of view, it can be derivatives, for example, fenyramidol alkylated by the acyclic nitrogen atom. However, more often the aminopyridine fragment is present in analgesic molecules in the form of the corresponding *N*-substituted amide (flupirtine, propiram, piketoprofen). Pyridin-2-ylamide is also piroxicam—the first commercially successful non-steroidal anti-inflammatory agent with a pronounced analgesic effect in the oxicam group. Later, its more effective analogs tenoxicam and lornoxicam appeared. The series of prodrugs with improved pharmaceutical and pharmacological parameters created on the basis of piroxicam are especially noteworthy. Ampiroxicam and droxicam appeared to be the most successful [1,2].

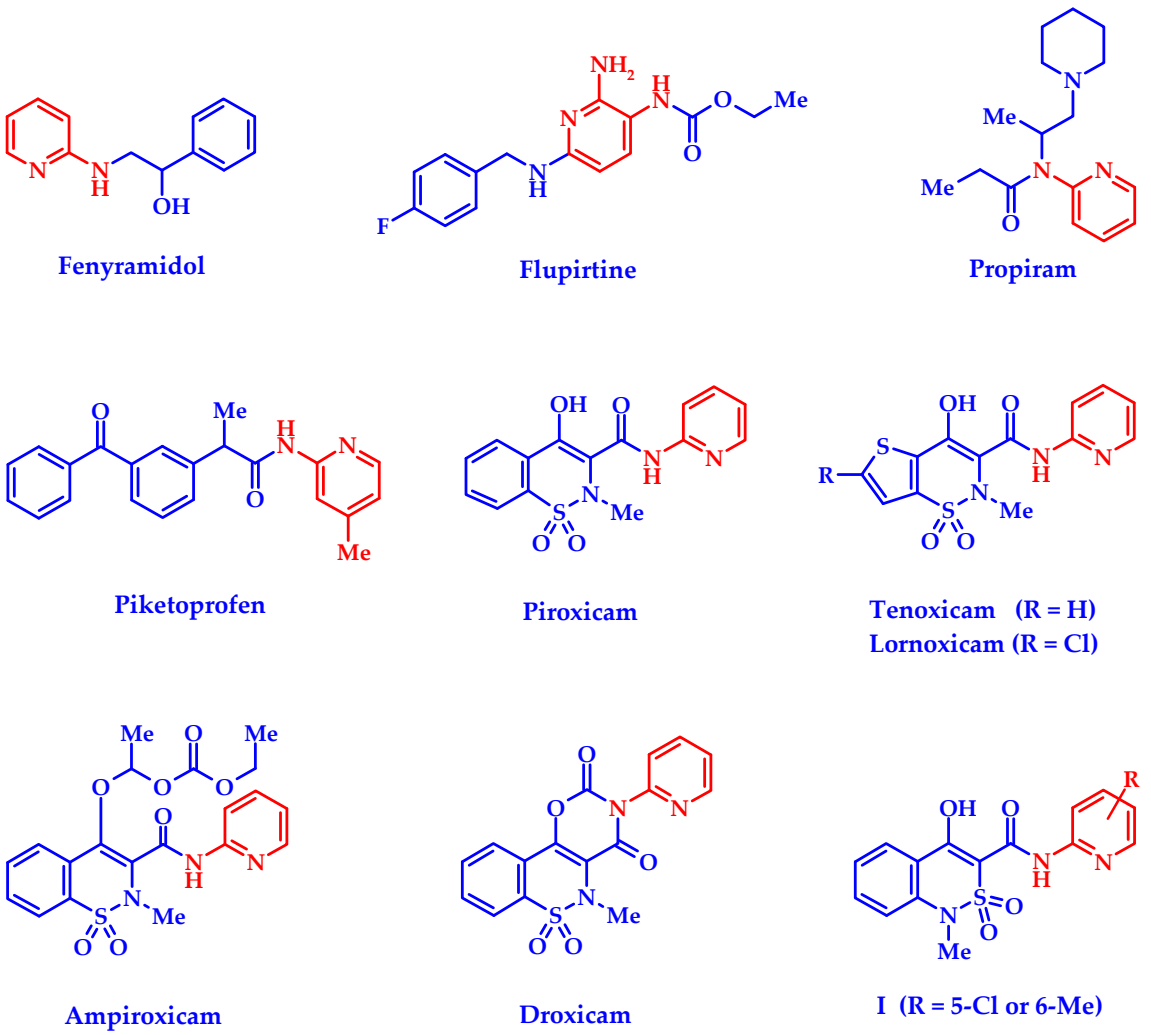


Figure 1. Analgesics and anti-inflammatory drugs with a pronounced analgesic effect based on aminopyridines [1–5].

The close structural analogs of piroxicam—*N*-(2-pyridyl)-4-hydroxy-2,2-dioxo-1*H*-2λ^6,1-benzothiazine-3-carboxamides of the general formula **1** (Figure 1) [4,5] created by the "flip-flop drugs" methodology [3] are of particular interest. They differ from piroxicam by the reciprocal arrangement of sulfo and amino groups in the benzothiazine cycle (Figure 1). At first glance, this insignificant chemical transformation of the base molecule led to a marked increase in the analgesic activity—some of pyridylamides **1** inhibit the pain reaction three times more effective than piroxicam in the same dose [4]. A logical continuation and further development of this promising direction for the search for new biologically active substances is transition to 4-methylsubstituted analogs of the compounds of formula **1**, i.e., to *N*-pyridyl-4-methyl-2,2-dioxo-1*H*-2λ^6,1-benzothiazine-3-carboxamides. In this study we tried to answer how this modification would affect the crystal structure, physicochemical and biological properties of the substances studied.

2. Materials and Methods

2.1. Chemistry

^{1}H-NMR (proton nuclear magnetic resonance) spectra were obtained on a Varian Mercury-400 (Varian Inc., Palo Alto, CA, USA) instrument (400 MHz) in hexadeuterodimethyl sulfoxide (DMSO-d_6) with tetramethylsilane as internal standard. The chemical shift values were recorded on a δ scale and the coupling constants (J) in hertz. The following abbreviations were used in reporting spectra: s = singlet, d = doublet, t = triplet. The elemental analysis was performed on a Euro Vector EA-3000 (Eurovector SPA, Redavalle, Italy) microanalyzer. The melting points were determined in a capillary using a electrothermal IA9100X1 (Bibby Scientific Limited, Stone, UK) digital melting point apparatus. In the synthesis of imidazolide **2** and all *N*-pyridyl-4-methyl-2,2-dioxo-1*H*-2λ^6,1-benzothiazine-3-carboxamides **4**–**6** described in this article the commercial *N*,*N*′-carbonyldiimidazole (CDI) and the anhydrous *N*,*N*-dimethylformamide (DMF) for peptide synthesis of Aldrich company (St. Louis, MO, USA) were used. The synthesis of the starting anhydrous 4-methyl-2,2-dioxo-1*H*-2λ^6,1-benzothiazine-3-carboxylic acid (**3**) was carried out by the method described in [6]. The quantum chemical calculations were performed using Density Functional Theory with the *m06-2x* functional [7] and standard *cc-pvtz* basis set [8] (*m06-2x/cc-pvtz*). The character of the stationary points on the potential energy surface was verified by calculations of vibrational frequencies within the harmonic approximation using analytical second derivatives at the same level of theory. All stationary points possess zero (minima) or one (saddle point) imaginary frequencies. All calculations were performed using Gaussian 09 software [9]. The atomic charges were calculated using the Natural Bonding Orbitals (NBO) theory [10] with NBO 5.0 program [11].

*2.2. 4-Methyl-2,2-dioxo-1H-2λ^6,1-benzothiazine-3-carboxylic acid imidazolide (**2**)*

N,*N*′-Carbonyldiimidazole (1.78 g, 0.011 mol) was added to a solution of the 4-methyl-2,2-dioxo-1*H*-2λ^6,1-benzothiazine-3-carboxylic acid **1** (2.39 g, 0.01 mol) in anhydrous DMF (5 mL) and protected from atmospheric moisture using a $CaCl_2$ tube. It was held for about 2 h at 80 °C until CO_2 evolution had ceased. The reaction mixture was cooled, diluted by adding cold water, and brought to pH 3 by adding hydrochloric acid. The precipitated imidazolide **2** was filtered off, washed with cold water, and dried. Yield 2.77 g (96%); yellow crystals; melting point (mp) 203–205 °C (DMF). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 11.51 (br. s, 1H, SO_2NH), 8.34 (s, 1H, 2′-H imidazole), 7.81 (d, 1H, J = 8.1, H-5), 7.75 (1H, s, H-5′ imidazole); 7.56 (t, 1H, J = 7.7, H-7), 7.28 (t, 1H, J = 7.7, H-6), 7.20 (d, 1H, J = 8.1, H-8), 7.17 (1H, s, H-4′ imidazole), 2.28 (s, 3H, 4-CH_3). This was analytically calculated (Anal. Calcd.) for $C_{13}H_{11}N_3O_3S$: C, 53.97; H, 3.83; N, 14.52; S 11.08%. We found: C, 54.06; H, 3.77; N, 14.45; S 10.99%.

*2.3. General Procedure for the Synthesis of N-pyridyl-4-methyl-2,2-dioxo-1H-2λ^6,1-benzothiazine-3-carboxamides (**4a**–**d** and **5**)*

The resulting solution of imidazolide **2** in anhydrous DMF (see Section 2.2.) was purged through a thin capillary with dry argon for 5 min to remove CO_2 residues. Then to the reaction mixture, 0.01 mol of the corresponding aminopyridine was added and kept for 24 h at the temperature of 80 °C in a tightly closed bottle made of thick glass (it is convenient to use the vials of a suitable volume from under chemicals). The reaction mixture was cooled, diluted by adding cold water, and acidified with diluted (1:1) hydrochloric acid until turbidity stops appearing. The formed precipitate was filtered, washed with cold water, dried, and recrystallized from ethanol.

N-(Pyridin-2-yl)-4-methyl-2,2-dioxo-1*H*-2λ^6,1-benzothiazine-3-carboxamide (**4a**). The yield was: 2.67 g (85%); colorless crystals; mp 274–276 °C; ^{1}H-NMR (400 MHz, DMSO-d$_6$): δ 11.69 (br. s, 1H, SO_2NH), 11.00 (s, 1H, CONH), 8.35 (d, 1H, *J* = 4.3, H-6′), 8.09 (d, 1H, *J* = 8.1, H-3′), 7.83 (t, 1H, *J* = 7.8, H-4′), 7.73 (d, 1H, *J* = 8.0, H-5), 7.47 (t, 1H, *J* = 7.7, H-7), 7.21 (t, 1H, *J* = 7.6, H-6), 7.16 (t, 1H, *J* = 6.3, H-5′), 7.13 (d, 1H, *J* = 8.1, H-8), 2.32 (s, 3H, 4-CH_3). The Anal. Calcd. was for $C_{15}H_{13}N_3O_3S$: C, 57.13; H, 4.16; N, 13.32; S 10.17%. We found: C, 57.06; H, 4.22; N, 13.40; S 10.24%.

N-(3-Methylpyridin-2-yl)-4-methyl-2,2-dioxo-1*H*-2λ^6,1-benzothiazine-3-carboxamide (**4b**). The yield was: 2.63 g (80%); colorless crystals; mp 265-267 °C; ^{1}H-NMR (400 MHz, DMSO-d$_6$): δ 11.73 (br. s, 1H, SO_2NH), 10.71 (s, 1H, CONH), 8.27 (d, 1H, *J* = 4.1, H-6′), 7.75 (d, 1H, *J* = 7.8, H-5), 7.70 (d, 1H, *J* = 7.1, H-4′), 7.47 (t, 1H, *J* = 7.2, H-7), 7.25 (d, 1H, *J* = 7.7, H-5′), 7.20 (t, 1H, *J* = 7.4, H-6), 7.13 (d, 1H, *J* = 7.8, H-8), 2.44 (s, 3H, 3′-CH_3), 2.25 (s, 3H, 4-CH_3). The Anal. Calcd. was for $C_{16}H_{15}N_3O_3S$: C, 58.35; H, 4.59; N, 12.76; S 9.73%. We found: C, 58.43; H, 4.52; N, 12.83; S 9.77%.

N-(4-Methylpyridin-2-yl)-4-methyl-2,2-dioxo-1*H*-2λ^6,1-benzothiazine-3-carboxamide (**4c**). The yield was: 2.70 g (82%); colorless crystals; mp 271–273 °C; ^{1}H-NMR (400 MHz, DMSO-d$_6$): δ 11.83 (br. s, 1H, SO_2NH), 11.00 (s, 1H, CONH), 8.19 (d, 1H, *J* = 4.9, H-6′), 7.96 (s, 1H, H-3′), 7.72 (d, 1H, *J* = 8.0, H-5), 7.46 (t, 1H, *J* = 7.6, H-7), 7.19 (t, 1H, *J* = 7.6, H-6), 7.11 (d, 1H, *J* = 8.1, H-8), 7.00 (d, 1H, *J* = 4.9, H-5′), 2.33 (s, 6H, 2 x CH_3). The Anal. Calcd. was for $C_{16}H_{15}N_3O_3S$: C, 58.35; H, 4.59; N, 12.76; S 9.73%. We found: C, 58.30; H, 4.53; N, 12.68; S 9.80%.

N-(5-Methylpyridin-2-yl)-4-methyl-2,2-dioxo-1*H*-2λ^6,1-benzothiazine-3-carboxamide (**4d**). The yield was: 2.66 g (81%); colorless crystals; mp 269-271 °C; ^{1}H-NMR (400 MHz, DMSO-d$_6$): δ 11.79 (br. s, 1H, SO_2NH), 11.03 (s, 1H, CONH), 8.17 (s, 1H, H-6′), 8.01 (d, 1H, *J* = 8.0, H-3′), 7.73 (d, 1H, *J* = 7.8, H-5), 7.65 (d, 1H, *J* = 8.5, H-4′), 7.47 (t, 1H, *J* = 7.3, H-7), 7.21 (t, 1H, *J* = 7.4, H-6), 7.12 (d, 1H, *J* = 7.8, H-8), 2.33 (s, 3H, 4′-CH_3), 2.25 (s, 3H, 4-CH_3). The Anal. Calcd. was for $C_{16}H_{15}N_3O_3S$: C, 58.35; H, 4.59; N, 12.76; S 9.73%. We found: C, 58.44; H, 4.62; N, 12.69; S 9.65%.

N-(Pyridin-3-yl)-4-methyl-2,2-dioxo-1*H*-2λ^6,1-benzothiazine-3-carboxamide (**5**). The yield was: 2.80 g (89%); colorless crystals; mp 280-282 °C; ^{1}H-NMR (400 MHz, DMSO-d$_6$): δ 11.84 (br. s, 1H, SO_2NH), 10.88 (s, 1H, CONH), 8.81 (d, 1H, *J* = 2.0, H-2′), 8.32 (d, 1H, *J* = 4.5, H-6′), 8.11 (d, 1H, *J* = 8.3, H-4′), 7.76 (d, 1H, *J* = 8.0, H-5), 7.49 (t, 1H, *J* = 7.6, H-7), 7.39 (dd, 1H, *J* = 8.1 and 4.8, H-5′), 7.22 (t, 1H, *J* = 7.6, H-6), 7.15 (d, 1H, *J* = 8.0, H-8), 2.39 (s, 3H, 4-CH_3). The Anal. Calcd. was for $C_{15}H_{13}N_3O_3S$: C, 57.13; H, 4.16; N, 13.32; S 10.17%. We found: C, 57.21; H, 4.25; N, 13.23; S 10.26%.

*2.4. N-(Pyridin-3-yl)-4-methyl-2,2-dioxo-1H-2λ^6,1-benzothiazine-3-carboxamide N,N-dimethylformamide monosolvate (**5a**)*

N-(Pyridin-3-yl)-4-methyl-2,2-dioxo-1*H*-2λ^6,1-benzothiazine-3-carboxamide (**5**) was crystallized from the mixture of DMF and acetone in the ratio of 1:5. Colorless crystals; mp 179–181 °C (decomp., −DMF).

*2.5. N-(Pyridin-4-yl)-4-methyl-2,2-dioxo-1H-2λ^6,1-benzothiazine-3-carboxamide (**6**)*

The mixture of imidazolide **2** (2.89 g, 0.011 mol), anhydrous DMF (3 mL) and 4-aminopyridine (0.94 g, 0.01 mol) was kept for 12 h at the temperature of 150 °C in a tightly closed bottle made of thick glass. The reaction mixture was cooled, diluted by adding cold water, and acidified with diluted

(1:1) hydrochloric acid until turbidity stops appearing. The precipitate formed was filtered, washed with cold water, dried, and recrystallized from ethanol. The yield was: 2.33 g (74%); colorless crystals; mp 288–290 °C; ^{1}H-NMR (400 MHz, DMSO-d$_6$): δ 11.73 (br. s, 1H, SO_2NH), 11.11 (s, 1H, CONH), 8.48 (d, 2H, *J* = 5.4, H-2′,6′), 7.75 (d, 1H, *J* = 8.1, H-5), 7.65 (d, 2H, *J* = 5.4, H-3′,5′), 7.48 (t, 1H, *J* = 7.7, H-7), 7.20 (t, 1H, *J* = 7.5, H-6), 7.12 (d, 1H, *J* = 8.1, H-8), 2.31 (s, 3H, 4-CH_3). The Anal. Calcd. was for $C_{15}H_{13}N_3O_3S$: C, 57.13; H, 4.16; N, 13.32; S 10.17%. We found: C, 57.05; H, 4.23; N, 13.26; S 10.10%.

*2.6. X-ray Structural Analysis of 4-Methyl-2,2-dioxo-1H-2λ^6,1-benzothiazine-3-carboxylic acid imidazolide (**2**)*

The crystals of imidazolide **2** ($C_{13}H_{11}N_3O_3S$) were monoclinic, colourless. At 20 °C: *a* 8.7215(4), *b* 11.3141(5), *c* 13.1930(7) Å; *β* 97.249(5)°, *V* 1291.4(1) Å^3, *Z* 4, space group P2$_1$/c, d_{calc} 1.488 g/cm^3, μ(MoK$_\alpha$) 0.262 mm^{-1}, *F*(000) 600. The unit cell parameters and intensities of 13,563 reflections (3710 independent reflections, R_{int} = 0.055) were measured on an Xcalibur-3 diffractometer (Oxford Diffraction Limited, Oxford, UK) using MoK$_\alpha$ radiation, a CCD detector, graphite monochromator, and ω-scanning to 2θ$_{max}$ 60°. The structure was solved by the direct method using the SHELXTL program package (Institute of Inorganic Chemistry, Göttingen, Germany) [12]. The positions of the hydrogen atoms were found from the electron density difference maps and refined using the "riding" model with U_{iso} = nU_{eq} for the non-hydrogen atom bonded to a given hydrogen atom (*n* = 1.5 for methyl, and *n* = 1.2 for the other hydrogen atoms). The hydrogen atoms involved in hydrogen bonds formation were refined using isotropic approximation. The structure was refined using F^2 full-matrix least-squares analysis in the anisotropic approximation for non-hydrogen atoms to wR_2 0.099 for 3710 reflections (R_1 0.052 for 2056 reflections with $F > 4\sigma(F)$, *S* = 0.919). The final atomic coordinates, and the crystallographic data for the molecule of imidazolide **2** have been deposited to with the Cambridge Crystallographic Data Centre, 12 Union Road, CB2 1EZ, UK (fax: +44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk) and are available on request quoting the deposition number CCDC 1910363 [13].

*2.7. X-ray Structural Analysis of N-(Pyridin-2-yl)-4-methyl-2,2-dioxo-1H-2λ^6,1-benzothiazine-3-carboxamide (**4a**)*

The crystals of *N*-(pyridin-2-yl)-amide **4a** ($C_{15}H_{13}N_3O_3S$) were monoclinic, colourless. At 20 °C: *a* 7.8257(8), *b* 16.600(2), *c* 11.031(1) Å; *β* 90.3(1)°, *V* 1433.1(2) Å^3, *Z* 4, space group P2$_1$/c, d_{calc} 1.462 g/cm^3, μ(MoK$_\alpha$) 0.243 mm^{-1}, *F*(000) 656. The unit cell parameters and intensities of 14,548 reflections (4098 independent reflections, R_{int} = 0.095) were measured on an Xcalibur-3 diffractometer (Oxford Diffraction Limited) using MoK$_\alpha$ radiation, a CCD detector, graphite monochromator, and ω-scanning to 2θ$_{max}$ 60°. The structure was solved by the direct method using the SHELXTL program package (Institute of Inorganic Chemistry) [12]. The positions of the hydrogen atoms were found from the electron density difference maps and refined using the "riding" model with U_{iso} = nU_{eq} for the non-hydrogen atom bonded to a given hydrogen atom (*n* = 1.5 for methyl, and *n* = 1.2 for the other hydrogen atoms). The hydrogen atoms involved in hydrogen bonds formation were refined using isotropic approximation. The structure was refined using F^2 full-matrix least-squares analysis in the anisotropic approximation for non-hydrogen atoms to wR_2 0.132 for 4098 reflections (R_1 0.062 for 1981 reflections with $F > 4\sigma(F)$, *S* = 0.905). The final atomic coordinates, and the crystallographic data for the molecule of *N*-(pyridin-2-yl)-amide **4a** have been deposited to with the Cambridge Crystallographic Data Centre, 12 Union Road, CB2 1EZ, UK (fax: +44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk) and are available on request quoting the deposition number CCDC 1910364 [14].

*2.8. X-ray Structural Analysis of N-(3-Methylpyridin-2-yl)-4-methyl-2,2-dioxo-1H-2λ^6,1-benzothiazine-3-carboxamide (**4b**)*

The crystals of *N*-(3-methylpyridin-2-yl)-amide **4b** ($C_{16}H_{15}N_3O_3S$) were triclinic, colourless. At 20 °C: *a* 7.990(2), *b* 10.041(1), *c* 10.922(2) Å; *α* 112.71(1)°, *β* 103.75(2)°, *γ* 95.07(1)°,*V* 769.1(2) Å^3, *Z* 2, space group P$\bar{1}$, d_{calc} 1.392 g/cm^3, μ(MoK$_\alpha$) 0.227 mm^{-1}, *F*(000) 342. The unit cell parameters and intensities of 7511 reflections (4364 independent reflections, R_{int} = 0.093) were measured on

an Xcalibur-3 diffractometer (Oxford Diffraction Limited) using MoK_α radiation, a CCD detector, graphite monochromator, and ω-scanning to $2\theta_{max}$ 60°. The structure was solved by the direct method using the SHELXTL program package (Institute of Inorganic Chemistry) [12]. The positions of the hydrogen atoms were found from the electron density difference maps and refined using the "riding" model with $U_{iso} = nU_{eq}$ for the non-hydrogen atom bonded to a given hydrogen atom (n = 1.5 for methyl, and n = 1.2 for the other hydrogen atoms). The structure was refined using F^2 full-matrix least-squares analysis in the anisotropic approximation for non-hydrogen atoms to wR_2 0.127 for 4364 reflections (R_1 0.069 for 1730 reflections with $F > 4\sigma(F)$, S = 0.841). The final atomic coordinates, and the crystallographic data for the molecule of *N*-(3-methylpyridin-2-yl)-amide **4b** have been deposited to with the Cambridge Crystallographic Data Centre, 12 Union Road, CB2 1EZ, UK (fax: +44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk) and are available on request quoting the deposition number CCDC 1910362 [15].

*2.9. X-ray Structural Analysis of N-(4-Methylpyridin-2-yl)-4-methyl-2,2-dioxo-1H-2λ⁶,1-benzothiazine-3-carboxamide (**4c**)*

The crystals of *N*-(4-methylpyridin-2-yl)-amide **4c** ($C_{16}H_{15}N_3O_3S$) were monoclinic, colourless. At 20 °C: a 8.357(1), b 16.791(2), c 11.0098(9) Å; β 93.655(8)°, V 1541.8(3) Å^3, Z 4, space group $P2_1/c$, d_{calc} 1.419 g/cm^3, μ(MoK_α) 0.229 mm^{-1}, F(000) 688. The unit cell parameters and intensities of 15,546 reflections (4427 independent reflections, R_{int} = 0.129) were measured on an Xcalibur-3 diffractometer (Oxford Diffraction Limited) using MoK_α radiation, a CCD detector, graphite monochromator, and ω-scanning to $2\theta_{max}$ 60°. The structure was solved by the direct method using the SHELXTL program package (Institute of Inorganic Chemistry) [12]. The positions of the hydrogen atoms were found from the electron density difference maps and refined using the "riding" model with $U_{iso} = nU_{eq}$ for the non-hydrogen atom bonded to a given hydrogen atom (n = 1.5 for methyl, and n = 1.2 for the other hydrogen atoms). The structure was refined using F^2 full-matrix least-squares analysis in the anisotropic approximation for non-hydrogen atoms to wR_2 0.133 for 4427 reflections (R_1 0.068 for 1688 reflections with $F > 4\sigma(F)$, S = 0.841). The final atomic coordinates, and the crystallographic data for the molecule of *N*-(4-methylpyridin-2-yl)-amide **4c** have been deposited to with the Cambridge Crystallographic Data Centre, 12 Union Road, CB2 1EZ, UK (fax: +44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk) and are available on request quoting the deposition number CCDC 1910367 [16].

*2.10. X-ray Structural Analysis of N-(5-Methylpyridin-2-yl)-4-methyl-2,2-dioxo-1H-2λ⁶,1-benzothiazine-3-carboxamide (**4d**)*

The crystals of *N*-(5-methylpyridin-2-yl)-amide **4d** ($C_{16}H_{15}N_3O_3S$) were triclinic, colourless. At 20 °C: a 7.631(3), b 9.515(5), c 11.588(6) Å; α 67.75(5)°, β 84.86(4)°, γ 76.69(4)°, V 757.8(7) Å^3, Z 2, space group $P\bar{1}$, d_{calc} 1.422 g/cm^3, μ(MoK_α) 0.234 mm^{-1}, F(000) 344. The unit cell parameters and intensities of 4751 reflections (2551 independent reflections, R_{int} = 0.096) were measured on an Xcalibur-3 diffractometer (Oxford Diffraction Limited) using MoK_α radiation, a CCD detector, graphite monochromator, and ω-scanning to $2\theta_{max}$ 50°. The structure was solved by the direct method using the SHELXTL program package (Institute of Inorganic Chemistry) [12]. The positions of the hydrogen atoms were found from the electron density difference maps and refined using the "riding" model with $U_{iso} = nU_{eq}$ for the non-hydrogen atom bonded to a given hydrogen atom (n = 1.5 for methyl, and n = 1.2 for the other hydrogen atoms). The structure was refined using F^2 full-matrix least-squares analysis in the anisotropic approximation for non-hydrogen atoms to wR_2 0.179 for 2551 reflections (R_1 0.084 for 1100 reflections with $F > 4\sigma(F)$, S = 0.898). The final atomic coordinates, and the crystallographic data for the molecule of *N*-(5-methylpyridin-2-yl)-amide **4d** have been deposited to with the Cambridge Crystallographic Data Centre, 12 Union Road, CB2 1EZ, UK (fax: +44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk) and are available on request quoting the deposition number CCDC 1910361 [17].

*2.11. X-ray Structural Analysis of N-(Pyridin-3-yl)-4-methyl-2,2-dioxo-1H-2λ⁶,1-benzothiazine-3-carboxamide-N,N-dimethylformamide monosolvate (**5a**)*

The crystals of *N*-(pyridin-3-yl)-amide-*N*,*N*-dimethylformamide monosolvate **5a** ($C_{15}H_{13}N_3O_3S \cdot C_3H_7O$) were orthorhombic, colourless. At 20 °C: *a* 10.649(4), *b* 15.029(8), *c* 23.297(10) Å; *V* 1687.2(2) Å^3, *Z* 8, space group Pbca, d_{calc} 1.384 g/cm^3, μ(MoK$_\alpha$) 0.206 mm^{-1}, *F*(000) 1632. The unit cell parameters and intensities of 25,391 reflections (5352 independent reflections, R_{int} = 0.102) were measured on an Xcalibur-3 diffractometer (Oxford Diffraction Limited) using MoK$_\alpha$ radiation, a CCD detector, graphite monochromator, and ω-scanning to 2θ$_{max}$ 60°. The structure was solved by the direct method using the SHELXTL program package (Institute of Inorganic Chemistry) [12]. The positions of the hydrogen atoms were found from the electron density difference maps and refined using the "riding" model with $U_{iso} = nU_{eq}$ for the non-hydrogen atom bonded to a given hydrogen atom (n = 1.5 for methyl, and n = 1.2 for the other hydrogen atoms). The hydrogen atoms involved in hydrogen bonds formation were refined using isotropic approximation. The structure was refined using F^2 full-matrix least-squares analysis in the anisotropic approximation for non-hydrogen atoms to wR_2 0.356 for 5352 reflections (R_1 0.073 for 1063 reflections with $F > 4\sigma$ (F), S = 0.665). The final atomic coordinates, and the crystallographic data for the molecule of *N*-(pyridin-3-yl)-amide *N*,*N*-dimethylformamide monosolvate **5a** have been deposited to with the Cambridge Crystallographic Data Centre, 12 Union Road, CB2 1EZ, UK (fax: +44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk) and are available on request quoting the deposition number CCDC 1910365 [18].

*2.12. X-ray Structural Analysis of N-(Pyridin-4-yl)-4-methyl-2,2-dioxo-1H-2λ⁶,1-benzothiazine-3-carboxamide (**6**)*

The crystals of *N*-(pyridin-4-yl)-amide **6** ($C_{15}H_{13}N_3O_3S$) were orthorhombic, colourless. At 20 °C: *a* 17.573(2), *b* 9.227(1), *c* 18.431(1) Å; *V* 2988.4(5) Å^3, *Z* 8, space group Pbca, d_{calc} 1.402 g/cm^3, μ(MoK$_\alpha$) 0.233 mm^{-1}, *F*(000) 1312. The unit cell parameters and intensities of 29,394 reflections (4305 independent reflections, R_{int} = 0.107) were measured on an Xcalibur-3 diffractometer (Oxford Diffraction Limited) using MoK$_\alpha$ radiation, a CCD detector, graphite monochromator, and ω-scanning to 2θ$_{max}$ 60°. The structure was solved by the direct method using the SHELXTL program package (Institute of Inorganic Chemistry) [12]. The positions of the hydrogen atoms were found from the electron density difference maps and refined using the "riding" model with $U_{iso} = nU_{eq}$ for the non-hydrogen atom bonded to a given hydrogen atom (n = 1.5 for methyl, and n = 1.2 for the other hydrogen atoms). The hydrogen atoms involved in hydrogen bonds formation were refined using isotropic approximation. The structure was refined using F^2 full-matrix least-squares analysis in the anisotropic approximation for non-hydrogen atoms to wR_2 0.179 for 4305 reflections (R_1 0.065 for 1618 reflections with $F > 4\sigma$ (F), S = 0.851). The final atomic coordinates, and the crystallographic data for the molecule of *N*-(pyridin-4-yl)-amide **6** have been deposited to with the Cambridge Crystallographic Data Centre, 12 Union Road, CB2 1EZ, UK (fax: +44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk) and are available on request quoting the deposition number CCDC 1910366 [19].

2.13. Pharmacology

Analgesic and Anti-Inflammatory Tests

All biological experiments were performed in accordance with the European Convention on the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes and the Ukrainian Law No. 3447-IV "On protection from cruelty to animals" [20] (project ID 3410U14, approved in October 15, 2015). The pharmacological study was conducted with the permission and under the supervision of the Commission on Bioethics (N.I. Pirogov Vinnitsa National Medical University, Vinnitsa, Ukraine).

The analgesic action with the simultaneous assessment of the anti-inflammatory effect of the initial imidazolide **2** and all *N*-pyridyl-4-methyl-2,2-dioxo-1*H*-2λ⁶,1-benzothiazine-3-carboxamides

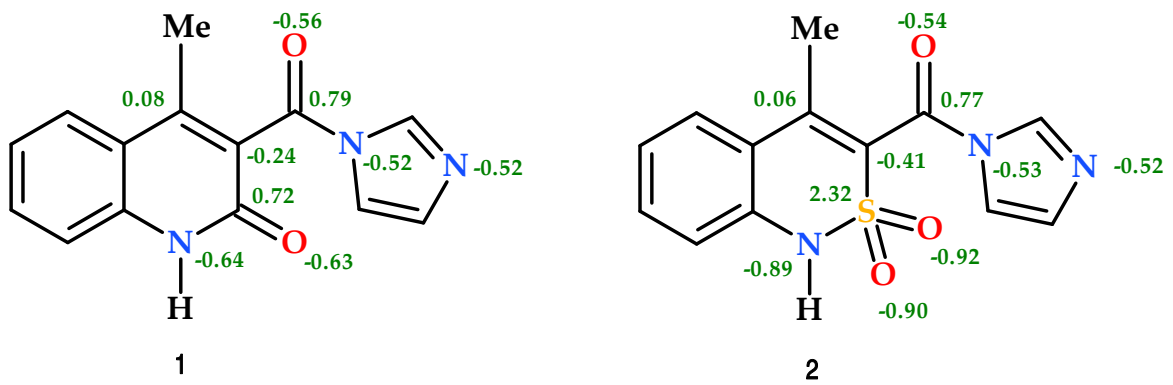
4–6 synthesized was studied using the standard carrageenan edema model [21,22]. The experiments were conducted on white Wistar male rats weighing 200–250 g. The substances under research and Lornoxicam (Wasserburger Arzneimittelwerk GmbH, Wasserburger, Germany) were injected intraperitoneally as fine aqueous suspensions stabilized with Tween-80 in the screening dose of 20 mg/kg. The control group received an equivalent amount of water with Tween-80. A detailed description of the biological experiments and the methods of statistical processing of the results obtained are given in the work [6].

3. Results and Discussion

3.1. Chemistry

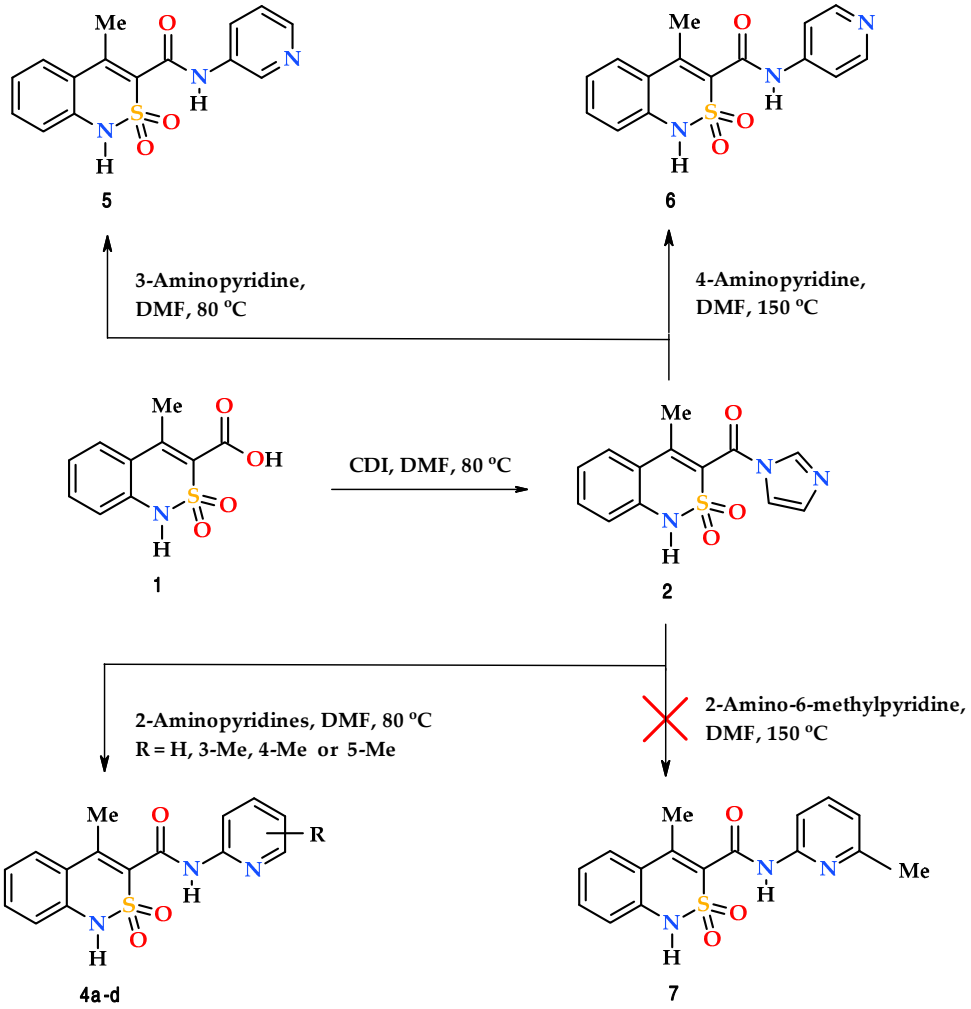
Theoretically, the synthesis of *N*-pyridyl-4-methyl-2,2-dioxo-1*H*-2λ^6,1-benzothiazine-3-carboxamides by a series of successive chemical transformations of the 4-hydroxy derivatives of formula **I** already known is obviously possible (for example, see the replacement of the 4-hydroxy group to the methyl group previously described in structurally close 4-hydroxyquinolin-2-ones [23,24]). However, it will not be easy to practically implement such a multistage synthetic scheme. In addition, we should not forget that sometimes supposedly canonical reactions occur in a completely different direction, and even not feasible [25]. It is not for nothing that among synthetic chemists there is a half-joking and half-serious expression that chemical reactions have a weak sense of duty! Therefore, to obtain the target *N*-pyridylamides of 4-methyl-2,2-dioxo-1*H*-2λ^6,1-benzothiazine-3-carboxylic acid, the imidazolide method that has proven itself in the synthesis of benzyl-, hetarylalkyl- and 1-phenylethylamides of this acid is more rational [26,27].

The majority of *N*-acylimidazoles (imidazolides of carboxylic acids) are highly active acylating agents, and their wide application in organic synthesis is based on this fact [28]. However, sometimes imidazolides of aromatic and heterocyclic carboxylic acids exhibit amazing resistance to the action of nucleophiles. In particular, one of these compounds is imidazolide of 4-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (**1**), which reacts with anilines and aminopyridines only with prolonged boiling in DMF [29,30]. Its inertness to water was also noted although usually *N*-acylimidazoles are hydrolyzed very easily even under the action of air moisture. We gave this example for a reason. Planned as a starting reagent imidazolide of 4-methyl-2,2-dioxo-1*H*-2λ^6,1-benzothiazine-3-carboxylic acid (**2**) is structurally very close 2-sulfo analog of imidazolide **1** (Scheme 1). Quantum chemical calculations show that this modification is accompanied by a very insignificant decrease in the positive charge (and hence electrophilicity) of the 3-carbonyl carbon atom. However, in reality, due to the large volume and proximity to the reaction center, the sulfo group together with other substituents can create very serious steric obstacles for many nucleophiles. As a result, the real acylating potential of imidazolide **2** may be significantly lower compared to its 2-carbonyl analog **1**.



Scheme 1. The charges on some atoms of imidazolides **1** and **2**.

Nevertheless, our first experiment showed that imidazolide **2** easily obtained from 4-methyl-2,2-dioxo-1*H*-2λ^6,1-benzothiazine-3-carboxylic acid (**3**) without isolation from the reaction mixture further with 2-aminopyridine in the anhydrous DMF solution at 80 °C gives the final *N*-(pyridin-2-yl)-amide **4a** with good yield and purity (Scheme 2). Quite satisfactory results were obtained in reactions with 3-, 4- and 5-monomethyl substituted 2-aminopyridines (amides **4b–d**, respectively), as well as with 3-aminopyridine (amide **5**). Against this background the relative inertness of 4-aminopyridine is very unexpected although it is usually acylated much easier and faster than its *ortho*- and *meta*-isomers [31]. After all *N*-(pyridin-4-yl)-amide **6** was obtained, but on the basis of imidazolide **2** pre-isolated in pure form and at much higher temperature (150 °C). It is noteworthy that imidazolide **2** does not react with 2-amino-6-methylpyridine even in such rigid conditions.



Scheme 2. Synthesis of *N*-pyridyl-4-methyl-2,2-dioxo-1*H*-2λ^6,1-benzothiazine-3-carboxamides **4**–**6**. **4**: **a** R = H; **b** R = 3-Me; **c** R = 4-Me; **d** R = 5-Me.

The amino group in 3-aminopyridine, as is known [32], is largely similar in properties to that in aniline. This explains the unambiguous behavior of 3-aminopyridine in reactions with various acylating agents (including imidazolide **2**), which results in formation of exocyclic-*N*-acyl substituted products [33,34], for example amide **5**. In the case of 2- and 4-aminopyridines prone to prototropic tautomerism the pattern is quite different. Here, the reaction center may vary depending on the tautomeric form of aminopyridine. Thus, in aromatic forms, due to the mutual influence of the pyridine nitrogen atom and the amino group, the latter largely loses its basic properties (as in carboxamides) while increasing nucleophilicity and basicity of the ring nitrogen, which becomes a target for the initial

electrophilic attack. The resulting endocyclic-*N*-acyl derivatives are generally unstable and rapidly regrouped into conventional amides. Conversely, in tautomeric imino forms of 2- and 4-aminopyridines the nucleophilic center is exocyclic nitrogen, on which acylation undergoes [35–37].

The comparison of these data with the results of our experiments suggests that 2-aminopyridines react with imidazolide **2** in the aromatic aminoform. The reason for this conclusion was the fact that we failed to obtain the corresponding amide **7** from 2-amino-6-methylpyridine (Scheme 2). Such extreme resistance to acylation is possible only if in the conditions of the reaction studied 2-amino-6-methylpyridine is in the aminoform, the access to its reaction center (pyridine nitrogen atom) is blocked by the neighboring 2-amino and 6-methyl groups. For the imino form with exocyclic nitrogen as the reaction target the opposite effect would occur, in which 2-amino-6-methylpyridine would react with imidazolide **2** much easier and faster than 2-amino-3-methylpyridine, but this is not true.

Steric obstacles significantly complicating or even blocking the course of reactions with 2-amino-6-methylpyridine were repeatedly noted earlier [38,39]. It should be added that imidazolide **2** does not also differ in the availability of its reaction center—carbonyl carbon atom, as is very clearly evidenced by X-ray diffraction analysis (Figure 2). It is clear that a carbonyl carbon atom (highlighted in green) located in such a dense surrounding is unlikely to interact with ring nitrogen of a very volumetric 2-amino-6-methylpyridine and form a tetrahedral intermediate **8** required for the further successful course of the reaction studied [40] (Scheme 3).

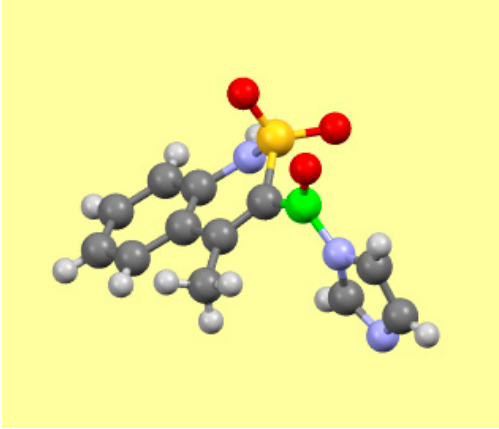
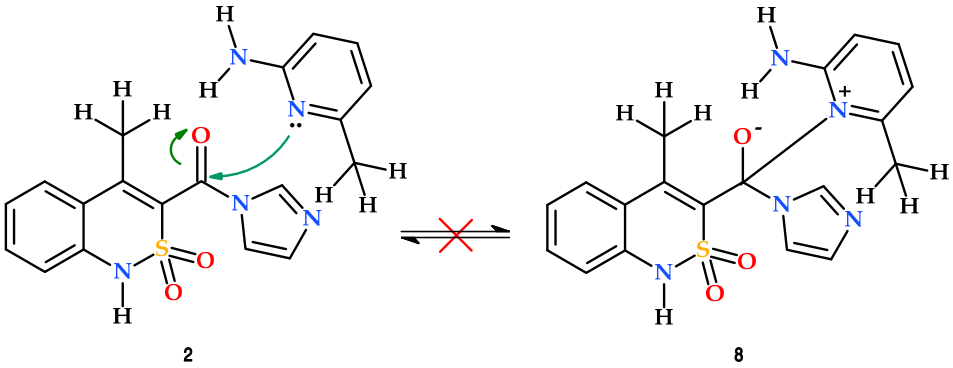


Figure 2. The molecular structure of imidazolide **2**. The carbonyl group carbon atom is highlighted in green.



Scheme 3. The first stage of the reaction of imidazolide **2** and 2-amino-6-methylpyridine.

The situation with 4-aminopyridine requires separate consideration. A much lower reactivity unexpectedly demonstrated by it in the experiment compared to *ortho*- and *meta*-isomers with steric obstacles is clearly not related. In our opinion, the cause for the relative inertness of this heterocyclic

amine is most likely to lie in its strongly basic properties: pK_a = 9.12 (for comparison, the pK_a values of 2-aminopyridine and 3-aminopyridine are 6.86 and 6.04, respectively [37]). Being such a strong base, 4-aminopyridine obviously forms a rather stable pyridinium salt with imidazolide **2** by the sulfamide group; however, very strict conditions are required for its transformation in *N*-(pyridin-4-yl)-amide **6**. A similar decrease in reactivity was also observed in transition from structurally close alkyl 4-hydroxy-2-oxo-1,2-dihydroquinoline-3-carboxylates to their salts by the 4-hydroxy group [41].

N-pyridyl-4-methyl-2,2-dioxo-1*H*-2λ^6,1-benzothiazine-3-carboxamides **4–6** synthesized are colorless crystalline substances with distinct melting points (see Sections 2.3–2.5). They are all moderately soluble in dimethyl sulfoxide (DMSO) and DMF, slightly soluble in ethanol, practically insoluble in water at room temperature.

A characteristic feature of ^{1}H NMR spectra of all *N*-pyridyl-amides **4–6** is a powerful paramagnetic shift of the signals of the pyridine nucleus protons compared to the aromatic protons of the benzothiazine fragment (Figure 3).

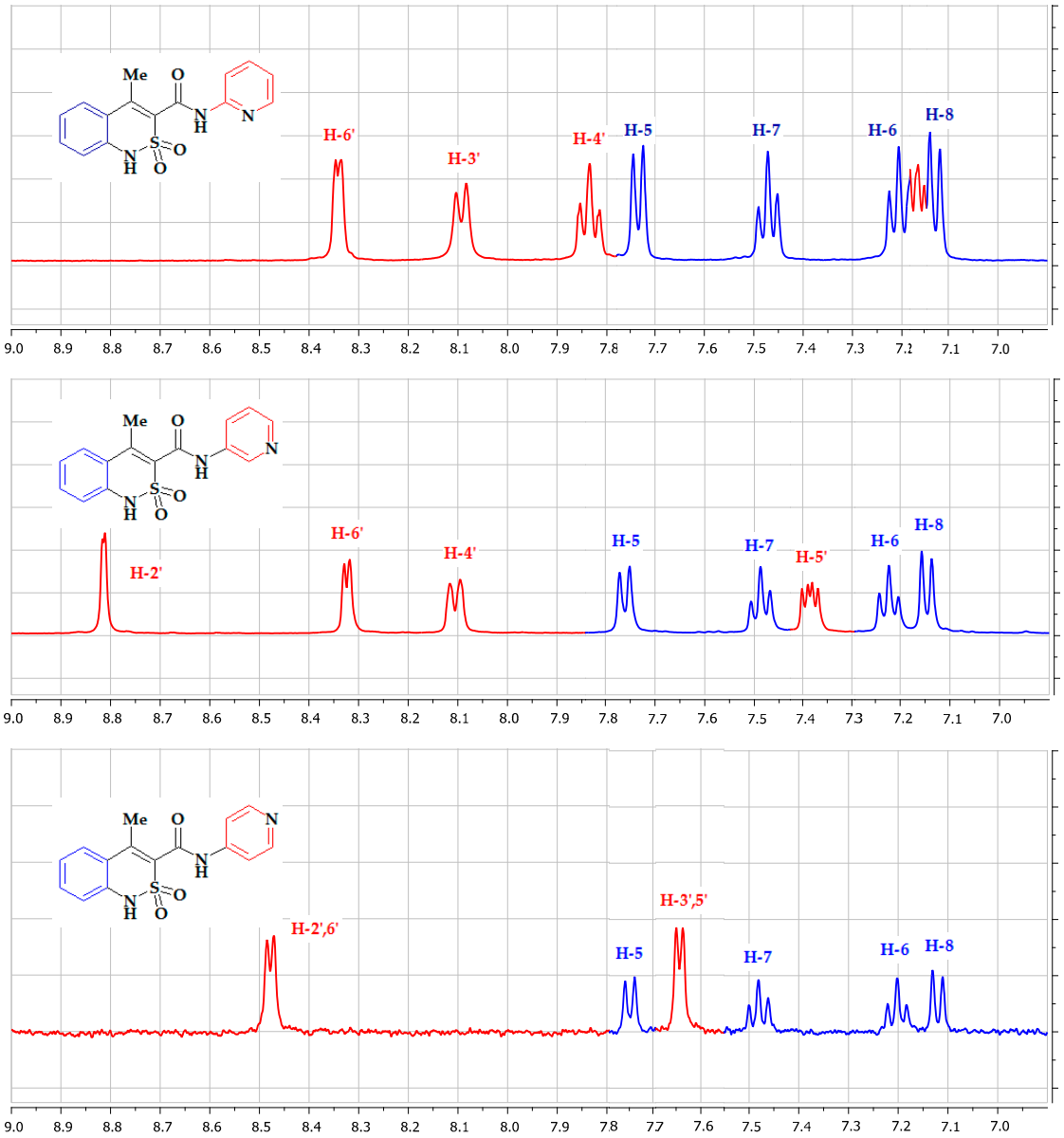


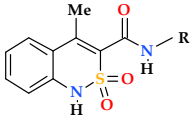
Figure 3. Fragments of the ^{1}H NMR spectra (signals of aromatic protons) of isomeric *N*-pyridyl-amides **4a**, **5 and 6**.

As we might expect, this effect is most pronounced with respect to protons that are in *para*- and especially in *ortho*-positions to the ring nitrogen. It is interesting that in ^{1}H NMR spectra of *N*-pyridyl-amides **4–6** the multiplicity of signals of all aromatic protons fully corresponds to their chemical surrounding. In the structure of imidazolide **2**, there is no NH-bridge; thus, both heterocyclic fragments of the molecule are located in space much closer to each other than in amides **4–6**. As a result, in the ^{1}H NMR spectrum of imidazolide **2**, there is distortion of signals of protons in positions 4 and 5 of the imidazole nucleus instead of doublets with the spin-spin coupling constant value of about 1.5–1.8 Hz [29] each of them appears a singlet with the intensity of ^{1}H (see Section 2.2). In the ^{1}H NMR spectrum of 2-carbonyl analog **1** these anomalies were not observed [29]. Therefore, their cause is the influence of a sulfo group with a powerful magnetic anisotropy.

3.2. Evaluation of the Analgesic and Anti-Inflammatory Activity

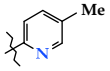
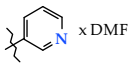
The results of our pharmacological experiments (Tables 1 and 2) indicate that biologically the transition from 4-hydroxy derivatives to *N*-pyridyl-4-methyl-2,2-dioxo-1*H*-2λ^6,1-benzothiazine-3-carboxamides proved to be very interesting and productive. All pyridylamides **4–6** without exception and even the starting imidazolide **2** exhibit analgesic and anti-inflammatory properties of moderate to high level. Noteworthy is the fact that *meta*-isomer **5** is the most potent analgesic and anti-inflammatory agent in the series of unsubstituted pyridylamides **4a**, **5** and **6**. The presence of 3-substituent of the pyridine nucleus has been repeatedly noted by us earlier as a factor contributing to the enhancement of analgesic properties [3,42–45]. However, the modification of *N*-(pyridin-3-yl)-amide **5** in *N*,*N*-dimethylformamide monosolvate **5a** causes a considerable decline in the biological activity probably due to a significant conformational rearrangement of the molecule because of solvation. The methyl group noticeably increases the analgesic effect regardless of the position in the pyridine nucleus (amides **4b–d**). Moreover, the presence of the methyl substituent is reflected differently on the anti-inflammatory properties: in position 3 relative to the ring nitrogen (amide **4b**) it virtually has no effect, in position 4 (amide **4c**) it reduces these properties twice, while in position 5 (amide **4d**)—on the contrary, increases by about 20% compared to unsubstituted *N*-(pyridin-2-yl)-amide **4a**. In general, half of all substances presented in this article as analgesics surpassed Lornoxicam, one of the most effective drugs of oxicam series. Two samples from this group—*N*-(5-methylpyridin-2-yl)-amide **4d** and *N*-(pyridin-3-yl)-amide **5**—were also more active than Lornoxicam by their anti-inflammatory properties.

Table 1. The analgesic activity of imidazolide **2**, pyridylamides **4–6**, and reference drug.



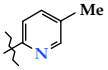
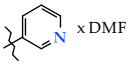
Entry	Product	R	Pain Threshold on Damaged Extremity (g/mm^2)	Pain Threshold on Non-Damaged Extremity (g/mm^2)	Δ Pain Threshold	Analgesic Activity, Compared to Control (%)
1	**2**		358.0 ± 25.5	216.0 ± 15.6	142.0 ± 14.0 [1]	+55.3
2	**4a**		449.0 ± 20.5	291.0 ± 11.6	158.0 ± 4.6 [1,2]	+50.3
3	**4b**		350.0 ± 26.1	281.0 ± 27.4	69.0 ± 6.4 [1,2]	+78.3
4	**4c**		346.0 ± 27.0	282.0 ± 14.9	64.0 ± 4.9 [1]	+79.9

Table 1. *Cont.*

Entry	Product	R	Pain Threshold on Damaged Extremity (g/mm^2)	Pain Threshold on Non-Damaged Extremity (g/mm^2)	Δ Pain Threshold	Analgesic Activity, Compared to Control (%)
5	**4d**		362.0 ± 33.2	341.0 ± 35.3	21.0 ± 4.0 [1,2]	+93.4
6	**5**		390.0 ± 34.9	353.0 ± 29.9	37.0 ± 4.3 [1,2]	+88.4
7	**5a**		419.0 ± 38.3	206.0 ± 12.6	213.0 ± 29.9	+33.0
8	**6**		412.0 ± 30.9	172.0 ± 16.0	240.0 ± 15.7 [1]	+24.5
9	Lornoxicam	–	441.0 ± 33.1	346.0 ± 30.2	95.0 ± 12.7 [1]	+70.1
10	Control	–	593.0 ± 21.1	275.0 ± 32.1	318.0 ± 18.6 [1]	0

[1] Differences statistically significant for $p \leq 0.05$ vs. non-damaged extremity; [2] Differences statistically significant for $p \leq 0.05$ vs. Lornoxicam.

Table 2. The anti-inflammatory activity of imidazolide **2**, pyridylamides **4–6**, and reference drug.

Entry	Product	R	Volume of Damaged Extremity (mm^3)	Volume of Non-Damaged Extremity (mm^3)	Δ Volume (Volume Increase)	Anti-Inflammatory Activity, Compared to Control (%)
1	**2**		469.1 ± 33.6	180.7 ± 65.5	288.4 ± 22.0 [1]	+30.3
2	**4a**		349.4 ± 33.5	197.9 ± 43.2	151.4 ± 16.9 [1,2]	+63.4
3	**4b**		330.5 ± 27.3	159.7 ± 8.31	170.8 ± 6.5 [1]	+58.7
4	**4c**		493.5 ± 45.0	218.7 ± 29.4	274.7 ± 21.6 [1,2]	+33.6
5	**4d**		288.3 ± 53.5	212.5 ± 23.9	75.8 ± 7.9 [1,2]	+81.7
6	**5**		243.4 ± 37.5	148.4 ± 28.2	94.9 ± 4.8 [1,2]	+77.1
7	**5a**		473.7 ± 35.3	209.7 ± 41.0	263.9 ± 14.8	+36.2
8	**6**		427.9 ± 44.3	129.5 ± 36.1	298.4 ± 15.01	+27.9
9	Lornoxicam	–	360.5 ± 82.5	263.9 ± 60.9	96.6 ± 5.7 [1]	+76.7
10	Control	–	568.7 ± 27.3	154.9 ± 11.4	413.9 ± 32.2 [1]	0

[1] Differences statistically significant for $p \leq 0.05$ vs. non-damaged extremity; [2] Differences statistically significant for $p \leq 0.05$ vs. Lornoxicam.

3.3. The Molecular and Crystal Structure Study

In a series of our previous publications it was convincingly shown that the biological properties of *N*-R-2,2-dioxo-1*H*-2λ^6,1-benzothiazine-3-carboxamides were largely determined by their molecular conformations fixed in crystals [26,27,46–48]. Continuing research in this interesting and largely still unpredictable field of pharmacy we studied the molecular and crystal structure of

N-pyridyl-4-methyl-2,2-dioxo-1*H*-2λ^6,1-benzothiazine-3-carboxamides presented in this work and then attempted to link these data with the results of pharmacological tests.

First of all, we have found that in contrast to 4-hydroxy derivatives of the general formula **I** (Figure 1) characterized by the existence in the form of internal salts [4,5] their 4-methyl substituted analogs **4**–**6** in a crystal represent only conventional hetarylamides although their cyclic sulfamide group has acidic properties that are sufficient for salt formation (all of these amides are readily soluble in aqueous solutions of Na_2CO_3, K_2CO_3, imidazole, aminopyridine and other amines).

In addition, it should be noted that a number of the samples analyzed (amides **4a**, **b**, **c**) have been found to have a versatile orientation of the carbonyl and sulfo groups in relation to the plane of the benzothiazine cycle (Figure 4, left-hand side), which has not yet been found in this type of compounds. In this case, the other part of the compounds (amide **4d**, solvate **5a**, and amide **6**) retains the one-sided orientation of these fragments, which is typical for *N*-hetaryl(aryl)alkyl substituted analogs [26,27] (Figure 4, right-hand side).

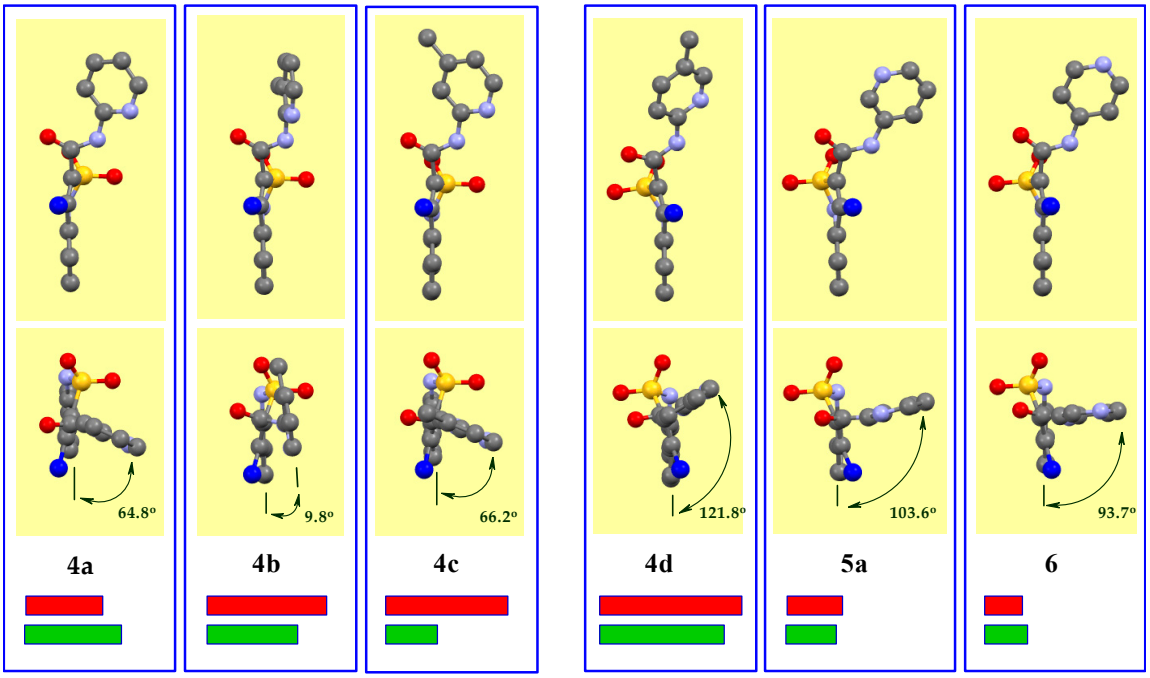


Figure 4. Crystal conformers of *N*-(pyridin-2-yl)-amides **4a**–**d**, *N*-(pyridin-3-yl)-amide *N*,*N*-dimethyl formamide monosolvate **5a**, and *N*-(pyridin-4-yl)-amide **6** in two angles; a relative level of their analgesic and anti-inflammatory action is shown by red and green stripe, respectively (the experimental data of X-ray structural and biological studies). The C-atom of the benzothiazine 4-CH_3 group is painted in a non-standard blue color for easier perception.

Despite this, the packing of molecules in the crystal of compounds of both groups is usually caused by two intermolecular hydrogen bonds: between the cyclic sulfamide group and the pyridine nitrogen atom, as well as between the carbamide group and the oxygen atom of the sulfo group (amides **4a**, **4c**, **4d**), carbonyl (amide **6**) or DMF (solvate **5a**). As a result, molecules of *N*-(pyridin-2-yl)-amide **4a** (Figure 5, left-hand side) and its 4-methylsubstituted analog **4c** in crystals form similar zigzag chains along the crystallographic direction [001]: $N_{(1)}$–H … $N_{(3')}$ (x, 0.5 − y, −0.5 + z, H … N 2.04 Å, N–H … N 176°) and $N_{(2)}$–H … $O_{(2')}$ (x, 0.5 − y, 0.5 + z, H … O 2.34 Å, N–H … O 153°) for amide **4a**, and $N_{(1)}$–H … $N_{(3')}$ (x, 1.5 − y, 0.5 + z, H … N 1.93 Å, N–H … N 174°) and $N_{(2)}$–H … $O_{(2')}$ (x, 1.5 − y, −0.5 + z, H … O 2.29 Å, N–H … O 164°) for amide **4c**.

Crystal packing of *N*-(5-methylpyridin-2-yl)-amide **4d** molecules is completely different—centrosymmetric dimers (Figure 5, right-hand side)—although it is formed at the

expense of the same intermolecular hydrogen bonds: $N_{(1)}$–H … $N_{(3')}$ (1 − *x*, 1 − *y*, 1 − *z*, H … N 2.18 Å, N–H … N 135°) and $N_{(2)}$–H … $O_{(2')}$ (1 − *x*, 1 − *y*, 1 − *z*, H … O 2.33 Å, N–H … O 158°).

In the systems of the intermolecular hydrogen bonds of *N*-(pyridin-4-yl)-amide **6** and solvate **5a**, which provide construction of their molecules into zigzag chains along the crystallographic direction [010] and [100], respectively (Figure 6), the same $N_{(1)}$–H … $N_{(3')}$ bonds (0.5 + *x*, 1.5 − *y*, 1 − *z*, H … N 2.03 Å, N–H … N 168° for amide **6**, and 0.5 + *x*, 0.5 − *y*, 1 − *z*, H … N 2.02 Å, N–H … N 138° for solvate **5a**) play the key role. A significant contribution is made by the intermolecular hydrogen bonds that are specific for each of these substances, for example $N_{(2)}$–H … $O_{(1')}$ (1.5 − *x*, 0.5 + *y*, *z*, H … O 2.12 Å, N–H … O 151°) for amide **6**. The solvate DMF molecules are bound with the molecules **5a** by the $N_{(2)}$–H … $O_{(1S')}$ intermolecular hydrogen bond (H … O 2.09 Å, N–H … O 156°).

The only exception is *N*-(3-methylpyridin-2-yl)-amide **4b** (Figure 7), which molecules form chains along the crystallographic direction [011] due to the intermolecular hydrogen bonds: $N_{(1)}$–H … $O_{(1')}$ (1 − *x*, 1 − *y*, − *z*, H … O 2.07 Å, N–H … O 157°) and $N_{(2)}$–H … $N_{(3')}$ (2 − *x*, 2 − *y*, 1 − *z*, H … N 2.24 Å, N–H … N 173°). They are completely different and atypical for other compounds of the group studied.

A comparative analysis of molecular conformations, crystal packaging and biological properties of *N*-pyridyl-4-methyl-2,2-dioxo-1*H*-2λ^6,1-benzothiazine-3-carboxamides with the versatile orientation of the carbonyl and sulfo groups with respect to the plane of the benzothiazine cycle (amides **4a**, **b**, **c**, Figure 4) unexpectedly found the absence of any significant influence of the mutual spatial arrangement of both heterocyclic fragments of the samples studied on the analgesic activity of the molecule as a whole. All *N*-pyridylamides of this group were approximately the same substances by the strength of their analgesic effect although amides **4a**, **c** and **4b** differ very significantly conformationally and by the type of crystal packaging (Figures 4, 5 and 7). Probably, a close relationship with the biological target and, as a consequence, high activity is provided by an unusual conformation of the benzothiazine bicycle, in which the presence of a molecule of the pyridine nucleus in the carbamide fragment is important, but not its fixed position in the crystal. However, the presence of the pyridine substituent and not some other heterocyclic or aromatic substituent may also be not mandatory; there are too little experimental data for such conclusions.

A very different picture is observed in the group of derivatives with the unilateral orientation of the carbonyl and sulfo groups (amides **4d**, **5a**, **6**, Figure 4), which is typical for many *N*-R-4-methyl-2,2-dioxo-1*H*-2λ^6,1-benzothiazine-3-carboxamides. The packaging of molecules of these amides in the crystal phase is also different. However, now there is a clear relationship between the level of the biological action and the position of the pyridine nucleus in space. The angle between the planes of two heterocycles, which are part of the molecule—benzothiazine and pyridine is very indicative in this respect. The closer it is to 90° (for example, pyridin-4-yl-amide **6**), the lower the activity (Figure 4).

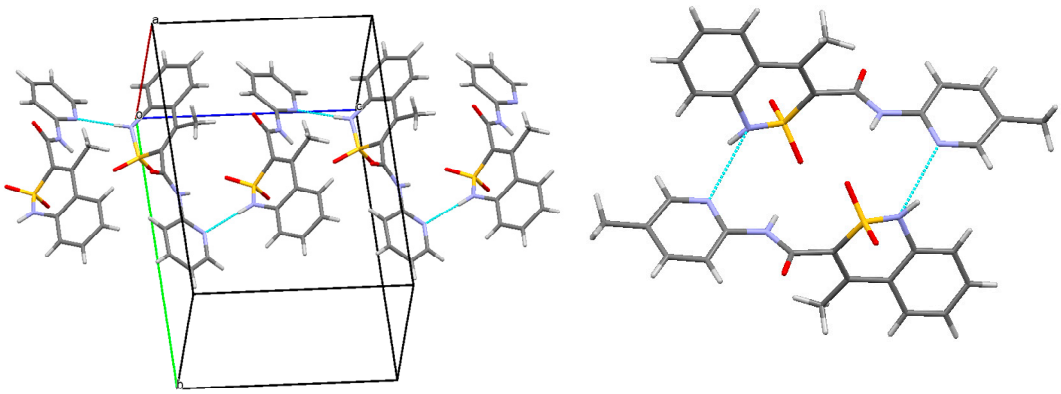


Figure 5. Packing of molecules of *N*-(pyridin-2-yl)-amide **4a** (left) and *N*-(5-methylpyridin-2-yl)-amide **4d** (on right) in the crystal phase.

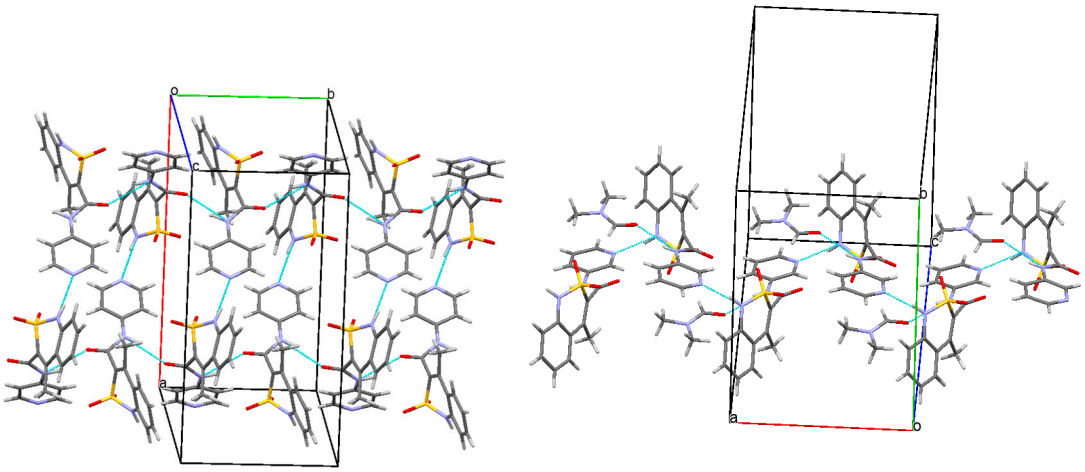


Figure 6. Packing of molecules of *N*-(pyridin-4-yl)-amide **6** and *N*-(pyridin-3-yl)-amide *N*,*N*-dimethylformamide monosolvate **5a** in the crystal phase.

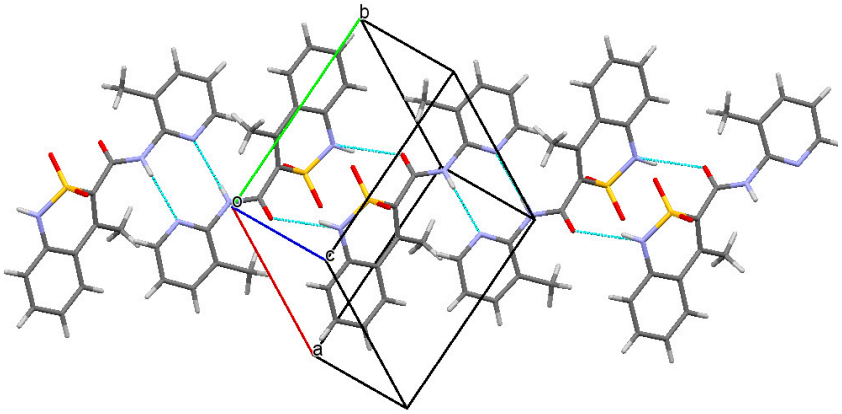


Figure 7. Packing of molecules of *N*-(3-methylpyridin-2-yl)-amide **4b** in the crystal phase.

Interestingly, in this case, the spatial structure directly determines not only the analgesic action, but also the anti-inflammatory one, and it was not previously observed [26,27]. Unfortunately, due to tiny crystals, it was not possible to study the spatial structure of a highly active analgesic and antiphlogistic—*N*-(pyridin-3-yl)-amide **5**. However, useful information that was important for structural and biological relationships was obtained in the study of its solvate **5a**. As a result of solvation, naturally, the molecule of amide **5** underwent an inevitable conformational restructuring. In solvate **5a**, the planes of the benzothiazine and pyridine fragments were located at an angle of 103.6° to each other (Figure 4), which obviously caused some increase in analgesic and anti-inflammatory properties compared to the "rectangular" *N*-(pyridin-4-yl)-amide **6** and at the same time their significant decline compared to the original amide **5**.

Thus, in the course of this study, it has been found that the replacement of the 4-hydroxy group in *N*-pyridyl-4-hydroxy-2,2-dioxo-1*H*-2λ^6,1-benzothiazine-3-carboxamides by methyl group is accompanied with a significant restructuring of the crystal structure, as well as the appearance of new molecular conformations of the benzothiazine nucleus, which ultimately determine the strength of the analgesic and anti-inflammatory action of the substances studied.

4. Conclusions

This article analyzes the reactivity of 4-methyl-2,2-dioxo-1*H*-2λ^6,1-benzothiazine-3-carboxylic acid imidazolide and aminopyridines; on their basis, a series of new *N*-pyridyl-4-hydroxy-

2,2-dioxo-1*H*-2λ^6,1-benzothiazine-3-carboxamides has been synthesized as potential analgesic and anti-inflammatory agents. The features of ^{1}H NMR spectra of all of the compounds obtained, as well as their molecular and crystal structure are discussed. According to the results of X-ray diffraction and pharmacological studies, it has been found that the benzothiazine cycle in some of *N*-pyridylamides obtained exists in a conformation that is atypical for the compounds of this group with a versatile orientation of carbonyl and sulfo groups, in which the spatial position of the pyridine nucleus affects the biological properties extremely weakly. Conversely, in the group of *N*-pyridylamides with the normal, i.e., one-sided, orientation of these substituents, there is a direct relationship between the level of analgesic and anti-inflammatory properties and the mutual arrangement of the planes of benzothiazine and pyridine heterocycles.

Author Contributions: The synthesis of the compounds presented in this work and analysis of their characteristics were performed by I.V.U., G.M.H., and A.A.B. The X-ray structural studies and quantum chemical calculations were performed by S.V.S., L.V.S., K.O.B., and G.S. The pharmacological studies were conducted by N.I.V., and O.V.M. The manuscript was written by I.V.U., G.M.H., and O.V.M.

Funding: This research received no external funding.

Acknowledgments: We are grateful to Candidate of Chemistry Evgene S. Gladkov (SSI "Institute for Single Crystal" National Academy of Sciences of Ukraine, Kharkiv, Ukraine) for his help in registration of NMR spectra of the compounds synthesized.

Conflicts of Interest: The authors declare no conflicts of interest.

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