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SYNTHESIS AND *IN VITRO* ANTIPROLIFERATIVE SCREENING OF NEW 2,7-NAPHTHYRIDINE-3-CARBOXYLIC ACID HYDRAZIDE DERIVATIVES

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Abstract: The new pyrrolo[3,4-*c*]pyridines and 2,7-naphthyridine derivatives have been synthesized. 4-Hydroxy-8-methyl-1-oxo-6-phenyl-1,2-dihydro-2,7-naphthyridine-3-carboxylic acid hydrazide (**4**) was the key intermediate for the synthesis of the novel derivatives of various chemical structures: Schiff bases, 1,3,4-oxadiazoles, pyrazoles, carbohydrazides, semi- and thiosemicarbazides. The structures of these new compounds were confirmed by elemental analysis and IR, NMR and MS spectra. The antitumor activities of the obtained derivatives were examined. Eight of the twenty one newly synthesized compounds were qualified by the NCI (Bethesda, MD, USA) for *in vitro* screening against 60 different human tumor cell lines. The most active proved to be the Schiff bases.

Keywords: pyrrolo[3,4-*c*]pyridine-1,3-dione, 2,7-naphthyridine derivatives, Schiff bases, antiproliferative activity *in vitro*

2,7-Naphthyridine is one of the six structural isomers of pyridopyridine. The reviews (1, 2) showed that natural alkaloids and synthetic compounds, containing the 2,7-naphthyridine scaffold, exhibit a broad spectrum of biological activities. Most of them have been studied as antitumor agents (3-7). Antibacterial (3, 8, 9), antifungal (10, 11), anti-inflammatory (12), antimalarial (13, 14), analgesic, and anticonvulsant (15, 16) activities were also examined. The various biological properties of 2,7-naphthyridines encourage the search for new methods of their preparation.

In our previous paper (17), a way of synthesizing 2,7-naphthyridine ring has been determined by alkoxide-induced rearrangement of pyrrolo[3,4-*c*]pyridines. The structure of new compounds was determined by X-ray crystallography to prove the presence of 2,7-naphthyridine isomer (17). Most of the newly synthesized 6-phenyl-2,7-naphthyridine derivatives were evaluated against the different human tumor cell lines, representing leukemia, melanoma, and CNS, breast, colon, kidney, ovary, prostate, and non-small cell lung cancers. In our studies, we have found that the most active compounds were the 4-hydroxy-1-oxo-6-phenyl-2,7-naphthyridine-3-carboxylic acid hydrazide derivatives (GI_{50} values between 0.24–3.48 μmol) (18). The present work is a follow-up study to our recent articles (17, 18).

The aim of this paper was to synthesize the new 4-methyl-6-phenyl-pyrrolo[3,4-*c*]pyridine-1,3-diones **2a**-**c** and 8-methyl-4-hydroxy-1-oxo-6-phenyl-1,2-dihydro-2,7-naphthyridine-3-carboxylate derivatives **3a**-**b**, **5**-**13**, according to the method reported earlier (17–20). Selected compounds were tested for their antiproliferative activity *in vitro*.

EXPERIMENTAL

Chemistry

Melting points were measured in open glass capillaries with a MEL-TEMP apparatus (Barnstead International, Dubuque, IO, USA) and were uncorrected. The new products were analyzed using a Perkin Elmer 2400 analyzer (Waltham, MA, USA). IR spectra were performed on a Specord M80 spectrometer (Zeiss/Analytic Jena, Germany) using KBr pellets. ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 with a Bruker Avance ARX-300 MHz spectrometer (Bruker Analytic, Karlsruhe, Germany) with TMS as the internal standard. MS spectra were determined on a GCMS-LK82091 spectrometer at the ionization energy 70 eV. The course of the reactions and the purity of the com-

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pounds were checked by TLC using aluminum sheet silica gel 60 F_{254} (Merck KGaA, Darmstadt, Germany). The chemicals for the syntheses were purchased from Chempur, Alfa Aesar, and Lancaster. Compounds **1** and **4** were prepared according to the methods presented in our previous papers (17, 18).

General procedure for the synthesis of 4-methyl-6-phenyl-pyrrolo[3,4-*c*]pyridine-1,3-dione derivatives (2a-c)

To a solution of 4-methyl-6-phenyl-pyrrolo[3,4-*c*]pyridine-1,3-dione **1** (0.01 mol) in anhydrous *N*,*N*-dimethylformamide (100 mL), sodium hydride (0.01 mol) was added. The mixture was stirred at room temperature for 2 h. To obtained sodium salt, methyl bromoacetate (0.01 mol), or benzyl bromoacetate (0.01 mol), or 2-bromoacetophenone (0.01 mol) was dropped. The mixture was stirred at room temperature for 4-6 h, and next, it was diluted with water. The obtained solid was filtered, dried and crystallized.

Methyl 2-(4-methyl-1,3-dioxo-6-phenyl-pyrrolo[3,4-*c*]pyridin-2-yl)acetate (2a)

Yield 2.82 g (91%), yellow solid, crystallized from methanol, m.p. 152-154°C. IR (KBr, cm^{-1}): 1780, 1740, 1720 (C=O), 1260 (CO), 750 (CH arom.). ^{1}H NMR (DMSO-d_6, δ, ppm): 2.82 (s, 3H, CH_3), 3.71 (s, 3H, OCH_3), 4.46 (s, 2H, CH_2), 7.52 (m, 3H, phenyl), 8.26 (m, 3H, pyridine, phenyl). MS (70 eV): m/z (%) : 311 [(M + 1)+, 5], 310 (M^+, 37), 252 (12), 251 (100), 250 (3), 195 (2), 154 (5), 153 (3), 127 (6), 126 (4), 104 (2), 77 (4), 59 (2). Analysis: calcd. for $C_{17}H_{14}N_2O_4$ (310.31): C, 65.80; H, 4.55; N, 9.03%; found: C, 65.71; H, 4.61; N, 9.15%.

Benzyl 2-(4-methyl-1,3-dioxo-6-phenyl-pyrrolo[3,4-*c*]pyridin-2-yl)acetate (2b)

Yield 3.24 g (84%), white solid, crystallized from ethanol, m.p. 182-184°C. IR (KBr, cm^{-1}): 1740, 1720, 1620 (C=O), 1220 (CO), 750, 700 (CH arom.). ^{1}H NMR (DMSO-d_6, δ, ppm): 2.85 (s, 3H, CH_3), 4.53 (s, 2H, CH_2), 5.20 (s, 2H, CH_2), 7.37 (m, 5H, phenyl), 7.54 (m, 3H, phenyl), 8.27 (m, 2H, phenyl), 8.32 (s, 1H, pyridine). Analysis: calcd. for $C_{23}H_{18}N_2O_4$ (386.41): C, 71.49; H, 4.70; N, 7.25%; found: C, 71.11; H, 4.58; N, 7.22%.

4-Methyl-2-phenacyl-6-phenyl-pyrrolo[3,4-*c*]pyridine-1,3-dione (2c)

Yield 2.56 g (72%), white solid, crystallized from ethanol, m.p. 217-218°C. IR (KBr, cm^{-1}): 1710, 1700 (C=O), 750, 680 (CH arom.). ^{1}H NMR (DMSO-d_6, δ, ppm): 2.86 (s, 3H, CH_3), 5.26 (s, 2H, CH_2), 7.54 (m, 3H, phenyl), 7.60 (m, 2H, phenyl), 7.74-8.10 (m, 3H, phenyl), 8.28 (m, 2H, phenyl), 8.31 (s, 1H, pyridine). Analysis: calcd. for $C_{22}H_{16}N_2O_3$ (356.38): C, 74.15; H, 4.53; N, 7.86%; found: C, 74.12; H, 4.62; N, 8.11%.

General procedure for the synthesis of 4-hydroxy-8-methyl-1-oxo-6-phenyl-1,2-dihydro-2,7-naphthyridine derivatives (3a,b)

To a solution of sodium ethoxide (0.04 mol) in anhydrous ethanol the appropriate 4-methyl-6-phenyl-pyrrolo[3,4-*c*]pyridine-1,3-dione derivatives **2a-c** (0.01 mol) were added. The mixture was heated at 60°C with stirring for 1 h. After cooling, the mixture was diluted with ice-water and acidified with 10% hydrogen chloride to pH = 5-6. The obtained solid was filtered, dried and crystallized.

Ethyl 4-hydroxy-8-methyl-1-oxo-6-phenyl-1,2-dihydro-2,7-naphthyridine-3-carboxylate (3a)

Yield: 2.11 g (65%), beige solid, crystallized from methanol, m.p. 232-234°C (lit. 233-235°C) (17). IR (KBr, cm^{-1}): 3440 (NH), 2900 (OH), 1650 (C=O), 1280 (C-O), 770 (CH, arom.). ^{1}H NMR (DMSO-d_6, δ, ppm): 1.38 (t, J = 7.0 Hz, 3H, CH_3), 3.05 (s, 3H, CH_3), 4.41 (q, J = 8.9 Hz, 2H, CH_2), 7.54 (m, 3H, phenyl), 8.18 (m, 3H, phenyl, pyridine), 8.41 (s, 1H, OH), 10.52 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, δ, ppm): = 13.8, 26.4, 62.3, 109.5, 112.8, 119.5, 127.0 (2C), 128.9 (2C), 129.9, 137.5, 140.2, 141.7, 156.5, 158.5, 161.7, 163.2. Analysis: calcd. for $C_{18}H_{16}N_2O_4$ (324.34): C, 66.66; H, 4.97; N, 8.64%; found: C, 66.81; H, 4.80; N, 8.69%.

3-Benzoyl-4-hydroxy-8-methyl-6-phenyl-2*H*-2,7-naphthyridin-1-one (3b)

Yield 2.49 g (70%), yellow solid, crystallized from ethanol, m.p. 252-255°C. IR (KBr, cm^{-1}): 3450 (NH), 1650, 1620 (C=O), 1280 (CO), 780, 690 (CH arom.). ^{1}H NMR (DMSO-d_6, δ, ppm): 3.12 (s, 3H, CH_3), 7.55 (m, 4H, phenyl), 7.66 (m, 2H, phenyl), 7.91 (m, 2H, phenyl), 8.21 (m, 3H, phenyl, pyridine), 8.41 (s, 1H, OH), 11.10 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, δ, ppm): 27.3, 112.5, 118.1, 121.9, 124.5, 126.8 (2C), 127.1, 128.3 (2C), 128.5, 128.9 (2C), 129.8 (2C), 130.2, 132.5, 136.9, 137.1, 138.2, 154.3, 159.1. Analysis: calcd. for $C_{22}H_{16}N_2O_3$ (356.38): C, 74.15; H, 4.53; N, 7.86%; found: C, 73.96; H, 4.45; N, 7.45%.

General procedure for the synthesis of Schiff bases (5a-i)

To a solution of 4-hydroxy-8-methyl-1-oxo-6-phenyl-1,2-dihydro-2,7-naphthyridine-3-carboxylic acid hydrazide **4** (0.01 mol) in ethanol (100 mL) the appropriate aldehyde (0.01 mol) and catalytic amount of indium (III) trifluoromethanesulfonate were added. The mixture was refluxed with stirring for 2-4 h. After cooling, the precipitate was filtered off. Recrystallization from the proper solvents afforded the Schiff bases **5a-i**.

4-Hydroxy-8-methyl-1-oxo-6-phenyl-1,2-dihydro-2,7-naphthyridine-3-carboxylic acid phenethylidene-hydrazide (5a)

Yield 1.69 g (41%), yellow solid, crystallized from toluene, m.p. 270-272°C. IR (KBr, cm^{-1}): 3350, 3200 (NH), 3000 (CH), 1650 (C=O), 1580 (C=N), 1280 (CO), 770, 690 (CH arom.). ^{1}H NMR (DMSO-d_6, δ, ppm): 3.05 (s, 3H, CH_3), 3.33 (d, J = 7.0 Hz, 2H, CH_2), 7.51-7.59 (m, 5H, phenyl), 8.01-8.17 (m, 5H, phenyl), 8.42 (s, 1H, CH), 8.86 (s, 1H, pyridine), 9.37 (s, 1H, OH), 10.68 (br, 1H, NH), 12.21 (s, 1H, NH). Analysis: calcd. for $C_{24}H_{20}N_4O_3$ (412.44): C, 69.89; H, 4.89; N, 13.38%; found: C, 69.98; H, 4.59; N, 13.26%.

4-Hydroxy-8-methyl-1-oxo-6-phenyl-1,2-dihydro-2,7-naphthyridine-3-carboxylic acid propylidene-hydrazide (5b)

Yield 1.57 g (45%), yellow solid, crystallized from ethanol, m.p. 284-285°C. IR (KBr, cm^{-1}): 3430, 3240 (NH), 2950 (CH), 1650 (C=O), 1580 (C=N), 1350 (CO), 750, 700 (CH arom.). ^{1}H NMR (DMSO-d_6, δ, ppm): 0.97 (t, J = 6.4 Hz, 3H, CH_3), 1.63-1.78 (q, J = 6.8 Hz, 2H, CH_2), 3.07 (s, 3H, CH_3), 5.45 (t, J = 8.7 Hz, 1H, CH), 6.35 (s, 1H, phenyl), 7.47-7.56 (m, 3H, phenyl), 8.17-8.21 (m, 3H, phenyl, pyridine, OH), 10.14 (s, 1H, NH), 12.34 (s, 1H, NH). Analysis: calcd. for $C_{19}H_{18}N_4O_3$ (350.37): C, 65.13; H, 5.18; N, 15.99%; found: C, 65.08; H, 5.28; N, 15.79%.

4-Hydroxy-8-methyl-1-oxo-6-phenyl-1,2-dihydro-2,7-naphthyridine-3-carboxylic acid (2-hydroxybenzylidene)-hydrazide (5c)

Yield 1.60 g (39%), yellow solid, crystallized from toluene, m.p. 272-273°C. IR (KBr, cm^{-1}): 3330, 3000 (NH), 1630 (C=O), 1580 (C=N), 1360, 1270 (CO), 750, 690 (CH arom.). ^{1}H NMR (DMSO-d_6, δ, ppm): 3.07 (s, 3H, CH_3), 6.95 (m, 2H, phenyl), 7.33 (s, 1H, CH), 7.53-7.66 (m, 5H, phenyl), 8.20-8.24 (m, 3H, phenyl, pyridine), 8.40 (s, 1H, OH), 8.63 (s, 1H, OH), 10.91 (s, 1H, NH), 12.17 (s, 1H, NH). Analysis: calcd. for $C_{23}H_{18}N_4O_3$ (414.42): C, 66.69; H, 4.38; N, 13.50%; found: C, 66.31; H, 3.98; N, 13.88%.

4-Hydroxy-8-methyl-1-oxo-6-phenyl-1,2-dihydro-2,7-naphthyridine-3-carboxylic acid (2-chlorobenzylidene)-hydrazide (5d)

Yield 1.50 g (35%), yellow solid, crystallized from toluene, m.p. 286-288°C. IR (KBr, cm^{-1}): 3350 (NH), 2950 (CH), 1640 (C=O), 1580 (C=N), 1360 (CO), 750, 690 (CH arom.). ^{1}H NMR (DMSO-d_6, δ, ppm): 3.06 (s, 3H, CH_3), 7.51-7.59 (m, 5H, phenyl), 8.00 (s, 1H, CH), 8.17-8.20 (m, 4H, phenyl), 8.71 (s, 1H, pyridine), 10.65 (s, 1H, OH), 11.29 (s, 1H, NH), 12.23 (s, 1H, NH). Analysis: calcd. for $C_{23}H_{17}ClN_4O_3$ (432.86): C, 63.82; H, 3.96; N, 12.94%; found: C, 64.21; H, 3.89; N, 12.65%.

4-Hydroxy-8-methyl-1-oxo-6-phenyl-1,2-dihydro-2,7-naphthyridine-3-carboxylic acid (4-fluorobenzylidene)-hydrazide (5e)

Yield 1.99 g (48%), yellow solid, crystallized from toluene, m.p. 312-314°C. IR (KBr, cm^{-1}): 3330 (NH), 2930 (CH), 1650 (C=O), 1580 (C=N), 1240 (CO), 780, 690 (CH arom.). ^{1}H NMR (DMSO-d_6, δ, ppm): 3.09 (s, 3H, CH_3), 7.22-7.33 (m, 4H, phenyl), 7.54-7.56 (m, 2H, phenyl), 7.71-7.80 (m, 4H, phenyl, pyridine), 8.13 (s, 1H, CH), 8.59 (s, 1H, OH), 8.69 (s, 1H, NH), 12.19 (s, 1H, NH). Analysis: calcd. for $C_{23}H_{17}FN_4O_3$ (416.40): C, 66.34; H, 4.11; N, 13.45%; found: C, 66.27; H, 3.90; N, 13.64%.

4-Hydroxy-8-methyl-1-oxo-6-phenyl-1,2-dihydro-2,7-naphthyridine-3-carboxylic acid (3-phenylallylidene)-hydrazide (5f)

Yield 2.41 g (57%), yellow solid, crystallized from toluene, m.p. 232-234°C. IR (KBr, cm^{-1}): 3340 (NH), 2950 (CH), 1640 (C=O), 1590 (C=N), 1240 (CO), 750, 700 (CH arom.). ^{1}H NMR (DMSO-d_6, δ, ppm): 3.07 (s, 3H, CH_3), 7.10-7.21 (m, 2H, CH), 7.34-7.40 (m, 3H, phenyl), 7.49-7.55 (m, 3H, phenyl), 7.63-7.65 (m, 2H, phenyl), 8.14-8.22 (m, 4H, phenyl, pyridine, CH), 10.61 (s, 1H, OH), 11.90 (s, 1H, NH), 12.22 (s, 1H, NH). Analysis: calcd. for $C_{25}H_{20}N_4O_3$ (424.45): C, 70.74; H, 4.75; N, 13.20%; found: C, 70.69; H, 4.51; N, 13.41%.

4-Hydroxy-8-methyl-1-oxo-6-phenyl-1,2-dihydro-2,7-naphthyridine-3-carboxylic acid (2,4-dihydroxybenzylidene)-hydrazide (5g)

Yield 2.58 g (60%), beige solid, crystallized from toluene, m.p. 286-288°C. IR (KBr, cm^{-1}): 3380 (NH), 1630 (C=O), 1580 (C=N), 1360, 1340, 1230 (CO), 770, 690 (CH arom.). ^{1}H NMR (DMSO-d_6, δ, ppm): 3.06 (s, 3H, CH_3), 6.29-6.33 (m, 2H, OH), 7.38-7.41 (d, J = 8.1 Hz, 1H, CH), 7.51-7.60 (m, 4H, phenyl), 8.17-8.23 (m, 4H, phenyl), 8.46 (s, 1H, pyridine), 10.05 (s, 1H, OH), 11.18 (s, 1H, NH),

12.20 (s, 1H, NH). Analysis: calcd. for $C_{23}H_{18}N_4O_5$ (430.41): C, 64.18; H, 4.22; N, 13.02%; found: C, 64.43; H, 3.95; N, 13.14%.

4-Hydroxy-8-methyl-1-oxo-6-phenyl-1,2-dihydro-2,7-naphthyridine-3-carboxylic acid (2,4,6-trimethoxybenzylidene)-hydrazide (5h)

Yield 2.04 g (42%), yellow solid, crystallized from toluene, m.p. 280-282°C. IR (KBr, cm^{-1}): 3340 (NH), 2930 (CH), 1640 (C=O), 1580 (C=N), 1360, 1320, 1230, 1120 (CO), 780, 700 (CH arom.). ^{1}H NMR (DMSO-d_6, δ, ppm): 3.04 (s, 3H, CH_3), 3.69 (s, 3H, CH_3), 3.82 (s, 6H, CH_3), 6.94-6.97 (m, 2H, phenyl, CH), 7.51-7.58 (m, 3H, phenyl), 8.18-8.26 (m, 4H, phenyl, pyridine), 10.68 (s, 1H, OH), 12.00 (s, 1H, NH), 12.53 (s, 1H, NH). Analysis: calcd. for $C_{26}H_{24}N_4O_6$ (488.49): C, 63.90; H, 5.00; N, 11.50%; found: C, 64.27; H, 4.65; N, 11.32%.

4-Hydroxy-8-methyl-1-oxo-6-phenyl-1,2-dihydro-2,7-naphthyridine-3-carboxylic acid (3-nitrophenylbenzylidene)-hydrazide (5i)

Yield 3.01 g (68%), orange solid, crystallized from toluene, m.p. 284-285°C. IR (KBr, cm^{-1}): 3350 (NH), 2930 (CH), 1660 (C=O), 1580 (C=N), 1540, 1360 (NO), 1250 (CO), 740, 690 (CH arom.). ^{1}H NMR (DMSO-d_6, δ, ppm): 3.03 (s, 3H, CH_3), 7.16 (s, 1H, CH), 7.50-7.71 (m, 4H, phenyl), 8.14-8.52 (m, 5H, phenyl), 8.71 (s, 1H, pyridine), 10.11 (br, 2H, NH, OH), 12.61 (s, 1H, NH). Analysis: calcd. for $C_{23}H_{17}N_5O_5$ (443.41): C, 62.41; H, 4.58; N, 16.35%; found: C, 62.69; H, 4.45; N, 15.98%.

4-Hydroxy-8-methyl-6-phenyl-3-(5-phenyl-1,3,4-oxadiazol-2-yl)-2*H*-2,7-naphthyridin-1-one (6)

To the solution of 4-hydroxy-8-methyl-1-oxo-6-phenyl-1,2-dihydro-2,7-naphthyridine-3-carboxylic acid hydrazide **4** (0.01 mol) in anhydrous DMF (30 mL), benzoic acid (0.01 mol) and phosphorous oxychloride (3 mL) were added. The mixture was refluxed with stirring for 3 h. After cooling, the mixture was diluted with ice-water and then neutralized with sodium bicarbonate. The obtained solid was filtered, dried and crystallized.

Yield 1.62 g (41%), beige solid, crystallized from ethanol, m.p. 296-297°C. IR (KBr, cm^{-1}): 3400 (NH, OH), 2930 (CH), 1660, 1580 (C=N, C=O), 1190 (CO), 790, 690 (CH arom.). ^{1}H NMR (DMSO-d_6, δ, ppm): 3.05 (s, 3H, CH_3), 7.51-7.54 (m, 6H, phenyl, pyridine), 8.03-8.20 (m, 5H, phenyl), 11.60 (br, 1H, OH), 12.93 (s, 1H, NH). Analysis: calcd. for $C_{23}H_{16}N_4O_3$ (396.40): C, 69.78; H, 4.10; N, 14.11%; found: C, 70.16; H, 4.08; N, 14.50%.

4-Hydroxy-8-methyl-3-(1,3,4-oxadiazol-2-yl)-6-phenyl-2*H*-2,7-naphthyridin-1-one (7)

The mixture of 4-hydroxy-8-methyl-1-oxo-6-phenyl-1,2-dihydro-2,7-naphthyridine-3-carboxylic acid hydrazide **4** (0.01 mol) and triethyl orthoformate (0.01 mol) in ethanol (50 mL) was refluxed with stirring for 5 h. After cooling, the obtained solid was filtered, dried and crystallized.

Yield 2.02 g (63%), yellow solid, crystallized from ethanol, m.p. 300-302°C. IR (KBr, cm^{-1}): 3450, 3200, 2900 (OH, NH), 1650 (C=O), 1580 (C=N), 1300 (CO), 760 (CH arom.). ^{1}H NMR (DMSO-d_6, δ, ppm): 2.99 (s, 3H, CH_3), 7.50-7.55 (m, 3H, phenyl), 8.02-8.29 (m, 4H, phenyl, pyridine, oxadiazole), 11.55 (s, 1H, OH), 12.12 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, δ, ppm): 26.96, 108.43, 110.32, 117.31, 127.07 (2C), 128.07, 128.88 (2C), 130.35, 136.78, 138.01, 139.46, 154.79, 156.74, 160.19, 162.52. Analysis: calcd. for $C_{17}H_{12}N_4O_3$ (320.30): C, 63.70; H, 3.80; N, 17.51%; found: C, 63.86; H, 4.05; N, 17.11%.

5-(4-Hydroxy-8-methyl-1-oxo-6-phenyl-2*H*-2,7-naphthyridin-3-yl)-3*H*-1,3,4-oxadiazol-2-one (8)

To solution of 4-hydroxy-8-methyl-1-oxo-6-phenyl-1,2-dihydro-2,7-naphthyridine-3-carboxylic acid hydrazide **4** (0.01 mol) in anhydrous tetrahydrofuran (50 mL) 1,1-carbonyldiimidazole (0.02 mol) was added. The mixture was stirred for 18 h. The solvent was evaporated under reduced pressure. The precipitate was washed with ethanol and filtered, dried and crystallized.

Yield 1.31 g (38%), beige solid, crystallized from ethanol, m.p. 327-330°C. IR (KBr, cm^{-1}): 3100 (OH), 2900 (NH), 1650, 1600 (C=O), 1500 (CN), 820 (CH arom.). ^{1}H NMR (DMSO-d_6, δ, ppm): 3.02 (s, 3H, CH_3), 7.48-7.50 (m, 3H, phenyl), 8.08-8.15 (m, 3H, phenyl, pyridine), 11.61 (br, 3H, NH, OH). Analysis: calcd. for $C_{17}H_{12}N_4O_4$ (336.30): C, 60.71; H, 3.80; N, 16.72%; found: C, 60.94; H, 4.07; N, 16.90%.

***N*-formyl-4-hydroxy-8-methyl-1-oxo-6-phenyl-2*H*-2,7-naphthyridine-3-carbohydrazide (9)**

A solution of 4-hydroxy-8-methyl-1-oxo-6-phenyl-1,2-dihydro-2,7-naphthyridine-3-carboxylic acid hydrazide **4** (0.01 mol) in formic acid (30 mL) was heated under reflux for 1 h. After cooling, the obtained solid was filtered, dried and crystallized.

Yield 1.65 g (49%), beige solid, crystallized from ethanol, m.p. 296-297°C. IR (KBr, cm^{-1}): 3300 (OH), 2900 (NH), 2750 (CH), 1650, 1600 (C=O), 770 (CH arom.). ^{1}H NMR (DMSO-d_6, δ, ppm): 3.05 (s, 3H, CH_3), 7.52-7.56 (m, 3H, phenyl), 8.19-8.22

(m, 3H, phenyl, pyridine), 8.40 (s, 1H, CHO), 10.35 (br, 1H, OH), 10.68 (s, 1H, NH), 11.61 (s, 1H, NH), 12.12 (s, 1H, NH). Analysis: calcd. for $C_{17}H_{14}N_4O_4$ (338.33): C, 60.35; H, 4.13; N, 16.56%; found: C, 60.71; H, 3.73; N, 16.85%.

_N_'-acetyl-4-hydroxy-8-methyl-1-oxo-6-phenyl-2_H_-2,7-naphthyridine-3-carbohydrazide (10)

A solution of 4-hydroxy-8-methyl-1-oxo-6-phenyl-1,2-dihydro-2,7-naphthyridine-3-carboxylic acid hydrazide **4** (0.01 mol) in acetic acid anhydride (30 mL) was heated under reflux for 2 h. After cooling, the obtained solid was filtered, dried and crystallized.

Yield 1.65 g (47%), beige solid, crystallized from methanol, m.p. 316-318°C. IR (KBr, cm^{-1}): 3300 (OH), 2850 (NH), 1650, 1600 (C=O), 740 (CH arom.). ^{1}H NMR (DMSO-d_6, δ, ppm): 1.96 (s, 3H, CH_3), 3.07 (s, 3H, CH_3), 7.52-7.55 (m, 3H, phenyl), 8.19-8.25 (m, 3H, phenyl, pyridine), 10.35 (s, 1H, OH), 10.51 (s, 1H, NH), 10.80 (s, 1H, NH), 11.90 (s, 1H, NH). Analysis: calcd. for $C_{18}H_{16}N_4O_4$ (352.35): C, 61.36; H, 4.58; N, 15.90%; found: C, 61.50; H, 4.25; N, 15.77%.

3-(3,5-Dimethylpyrazole-1-carbonyl)-4-hydroxy-8-methyl-6-phenyl-2_H_-2,7-naphthyridin-1-one (11)

To solution of 4-hydroxy-8-methyl-1-oxo-6-phenyl-1,2-dihydro-2,7-naphthyridine-3-carboxylic acid hydrazide **4** (0.01 mol) in ethanol (50 mL), pentanedione (0.01 mol) and acetic acid (3 mL) were added. The mixture was refluxed with stirring for 5 h. After cooling, the obtained solid was filtered, dried and crystallized.

Yield 2.69 g (72%), yellow solid, crystallized from ethanol, m.p. 219-220°C. IR (KBr, cm^{-1}): 3500 (OH), 3000 (NH), 1660 (C=O), 1580 (C=N), 770 (CH arom.). ^{1}H NMR (DMSO-d_6, δ, ppm): 1.88 (s, 3H, CH_3), 2.05 (s, 3H, CH_3), 3.03 (s, 3H, CH_3), 6.86 (s, 1H, CH), 7.50-7.52 (m, 3H, phenyl), 8.13-8.17 (m, 3H, phenyl, pyridine), 11.15 (s, 1H, OH), 11.92 (s, 1H, NH). Analysis: calcd. for $C_{21}H_{18}N_4O_3$ (374.39): C, 67.36; H, 4.80; N, 15.02%; found: C, 67.06; H 4.96; N, 15.07%.

4-Phenyl-1-(4-hydroxy-8-methyl-1-oxo-6-phenyl-2_H_-2,7-naphthyridine-3-carbonyl)thiosemicarbazide (12)

To solution of 4-hydroxy-8-methyl-1-oxo-6-phenyl-1,2-dihydro-2,7-naphthyridine-3-carboxylic acid hydrazide **4** (0.01 mol) in ethanol (50 mL) phenyl isothiocyanate (0.01 mol) was added. The mixture was refluxed with stirring for 6 h. After cooling, the separated solid was filtered, dried and crystallized.

Yield 3.07 g (69%), white solid, crystallized from ethanol, m.p. 327-330°C. IR (KBr, cm^{-1}): 3300 (OH), 2800 (NH), 2300 (NCS), 1650, 1600, 1500, 1350, 1300 (C=O, NH, CN), 820, 700 (CH arom.). ^{1}H NMR (DMSO-d_6, δ, ppm): 3.05 (s, 3H, CH_3), 7.15 (s, 1H, phenyl), 7.32-7.35 (m, 2H, phenyl), 7.52-7.60 (m, 5H, phenyl), 8.12-8.21 (m, 4H, phenyl, pyridine, OH), 9.65-9.95 (m, 2H, NH), 11.72 (br, 2H, NH). Analysis: calcd. for $C_{23}H_{19}N_5O_3S$ (445.50): C, 62.01; H, 4.30; N, 15.72%; found: C, 61.82; H, 4.06; N, 15.96%.

4-Phenyl-1-(4-hydroxy-8-methyl-1-oxo-6-phenyl-2_H_-2,7-naphthyridine-3-carbonyl)semicarbazide (13)

To a solution of 4-hydroxy-8-methyl-1-oxo-6-phenyl-1,2-dihydro-2,7-naphthyridine-3-carboxylic acid hydrazide **4** (0.01 mol) in ethanol (30 mL), phenyl isocyanate (0.02 mol) was added. The mixture was refluxed with stirring for 4 h. After cooling, the obtained solid was collected. The obtained solid was filtered, dried and crystallized.

Yield 1.72 g (40%), beige solid, crystallized from ethanol, m.p. 280-282°C. IR (KBr, cm^{-1}): 3350, 3200 (OH), 2900 (NH), 1670, 1580 (C=O), 750, 690 (CH arom.). ^{1}H NMR (DMSO-d_6, δ, ppm): 3.04 (s, 3H, CH_3), 6.93-7.23 (m, 3H, phenyl), 7.46-7.53 (m, 5H, phenyl), 7.95 (s, 1H, phenyl), 8.10-8.15 (m, 2H, phenyl, pyridine), 8.20 (m, 2H, NH, OH), 8.76 (s, 1H, NH), 10.10 (br, 2H, NH). Analysis: calcd. for $C_{23}H_{19}N_5O_4$ (429.44): C, 64.37; H, 4.46; N, 16.31%; found: C, 64.76; H, 4.18; N, 16.37%.

Biology

Anti-proliferative *in vitro* tests were performed at the National Cancer Institute (Bethesda, MD, USA) on 60 different human tumor cell lines, representing nine cancer diseases: leukemia, melanoma, cancers of the breast, lung, brain, colon, prostate, ovary, renal. The cancer cell lines were grown in RPMI 1640 medium containing fetal bovine serum (5%) and L-glutamine (2 mM). After cell inoculation (densities from 5000 to 40000 cells/well), the microtiter plates were incubated (37°C, 5% CO_2, 95% air, 100% humidity) for 24 h. Next, cell lines were fixed *in situ* with trichloroacetic acid to represent a measurement of the cell population for each cell line at the time of the compound addition. Experimental compounds were solubilized in DMSO at 400-fold the desired final maximum test concentration. The samples were stored frozen. The aliquot was thawed and diluted to the appropriate

test concentration with complete medium containing gentamicin (50 µg/mL), prior to use. Following compound addition, the microtiter plates were incubated (37°C, 5% CO_2, 95% air, 100% humidity) for 48 h. Next, cells were fixed *in situ* with cold 50% trichloroacetic acid (50 µL) and incubated at 4°C for 60 min. The supernatant was discarded and the microtiter plates were washed with tap water and dried. The 0.4% solution of sulforhodamine B (100 µL) in 1% acetic acid was added to each well. The plates were incubated at room temperature for 10 min. and next, washed with 1% acetic acid and dried. After solubilization with 10 mM trizma base, the absorbance was read on an automated plate reader at a wavelength of 515 nm. Using 7 absorbance measurements the percentage growth was calculated for each of the compounds. The results were shown as percentage of growth of the treated cells (21-23).

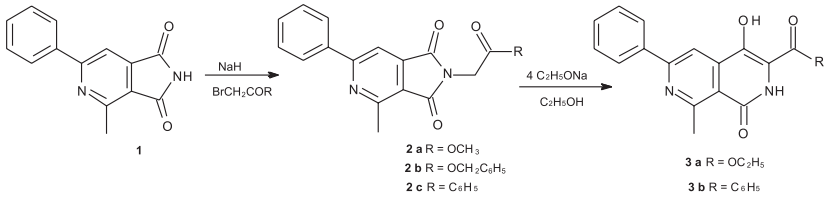
RESULT AND DISCUSSION

Chemistry

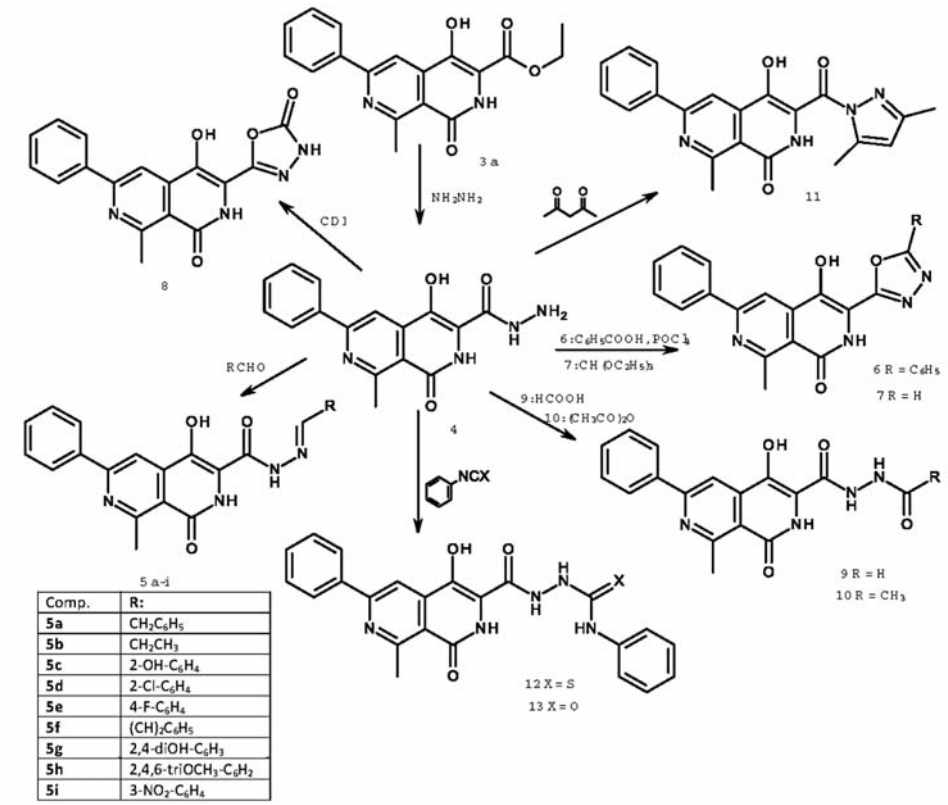
In our previous paper the method of rearrangement of pyrrolo[3,4-*c*]pyridine derivatives to the corresponding 2,7-naphthyridines has been described (17). In the present study, the obtained earlier 4-methyl-6-phenyl-pyrrolo[3,4-*c*]pyridine-1,3-dione **1** was alkylated with methyl or benzyl bromoacetates and bromoacetophenone (Scheme 1), according to the method described by us earlier (17). The new pyrrolo[3,4-*c*]pyridine derivatives **2a**-**c** were isolated with very good yield (72-91%). IR spectra of the obtained compounds **2a**-**c** displayed absorption bands within the range ν = 3000-3400 cm^{-1} characteristic for the NH. ^{1}H NMR spectra contained two-protons singlets at δ = 4.46 ppm for compound **2a**, δ = 5.20 ppm for compound **2b** and δ = 5.26 ppm for compound **2c**, corresponding to protons of the CH_2 group instead of one-proton singlets of pyrrole NH. Stoichiometric amount of sodium ethoxide or sodium methoxide, did not yield the expected 2,7-naphthyridine derivatives. Treatment of pyrrolo[3,4-*c*]pyridine-1,3-dione derivatives with sodium methoxide in a molar ratio of 1 : 4 gave 3-[(carboxymethyl)carbamoyl]-2-alkyl-6-phenylpyridine-4-carboxylic acids. Products isolated in these reactions were described in our previous paper (17). Treatment of pyrrolo[3,4-*c*]pyridine derivatives **2a**-**c** with fourfold excess of sodium ethoxide resulted in the rearrangement to the 2,7-naphthyridine ring. However, the reaction did not yield the expected methyl ester from compound **2a**, and benzyl ester from compound **2b**, but the product of alkoholysis **3a** was isolated. The results of elemental analysis and spectra indicated that the obtained compound **3a** was the same as the ethyl ester synthesized by us earlier from the corresponding ethyl 2-(4-methyl-1,3-dioxo-6-phenyl-pyrrolo[3,4-*c*]pyridin-2-yl)acetate (17). In the ^{1}H NMR spectra of the newly synthesized 3-benzoyl-4-hydroxy-8-methyl-6-phenyl-2H-2,7-naphthyridin-1-one **3b**, two singlets at δ = 8.41 ppm and at δ = 11.10 ppm, corresponding to the OH and NH protons, were observed.

The 4-hydroxy-8-methyl-1-oxo-6-phenyl-1,2-dihydro-2,7-naphthyridine-3-carboxylic acid hydrazide **4**, obtained from **3a** according to the method described in our previous paper (18), was found to be useful as the key intermediate for further synthesis. The reactions were illustrated in Scheme 2. The synthesis of Schiff bases **5a**-**i** involved the reaction between appropriate aldehydes and hydrazide **4** in a presence of catalytic amount of indium (III) trifluoromethanesulfonate. In the ^{1}H NMR spectra of obtained Schiff bases the two-protons signal at δ = 4.51 ppm due to the NH_2 group, which was observed in the ^{1}H NMR spectra of hydrazide **4**, disappeared. The appearance of the signals between δ = 6.35 and 7.51 ppm indicates the formation of imines (CH=N).

1,3,4-Oxadiazole derivatives **6**–**8** were produced as the products of cyclocondensation. 4-Hydroxy-8-methyl-6-phenyl-3-(5-phenyl-1,3,4-oxadiazol-2-yl)-2*H*-2,7-naphthyridin-1-one **6** was synthesized from hydrazide **4** with benzoic acid, in the presence of an excess of phosphorous oxychloride. 4-Hydroxy-8-methyl-3-(1,3,4-oxadiazol-2-yl)-6-phenyl-2*H*-2,7-naphthyridin-1-one **7** was obtained from the hydrazide **4** and an equimolar amount of



Scheme 1. Synthesis of 4-hydroxy-8-methyl-1-oxo-6-phenyl-1,2-dihydro-2,7-naphthyridine-3-carboxylic acid derivatives



Comp.	R:
5a	$CH_2C_6H_5$
5b	CH_2CH_3
5c	2-OH-C_6H_4
5d	2-Cl-C_6H_4
5e	4-F-C_6H_4
5f	$(CH)_2C_6H_5$
5g	2,4-diOH-C_6H_3
5h	2,4,6-triOCH$_3$-C_6H_2
5i	3-NO_2-C_6H_4

Scheme 2. Synthesis of 4-hydroxy-8-methyl-1-oxo-6-phenyl-1,2-dihydro-2,7-naphthyridine derivatives

triethyl orthoformate. In the reaction of hydrazide **4** with 1,1-carbonyldiimidazole, 5-(4-hydroxy-8-methyl-1-oxo-6-phenyl-2H-2,7-naphthyridin-3-yl)-3*H*-1,3,4-oxadiazol-2-one **8** was isolated.

In the next synthesis, carbohydrazide derivatives were produced. The reaction of hydrazide **4** with formic acid or acetic acid anhydride resulted in the formation of *N*-formyl-4-hydroxy-8-methyl-1-oxo-6-phenyl-2*H*-2,7-naphthyridine-3-carbohydrazide **9** and *N*'-acetyl-4-hydroxy-8-methyl-1-oxo-6-phenyl-2*H*-2,7-naphthyridine-3-carbohydrazide **10**, respectively. The number of signals for the protons in the ^{1}H NMR spectra of obtained compounds is in good agreement with their structures.

Cyclocondensation of hydrazide **4** with pentanedione in the presence of catalytic amount of acetic acid resulted in the formation of 3-(3,5-dimethylpyrazole-1-carbonyl)-4-hydroxy-8-methyl-6-phenyl-2*H*-2,7-naphthyridin-1-one **11** in good yield (72%). ^{1}H NMR spectra exhibited three three-protons singlets at δ = 1.88 ppm, δ = 2.05 ppm, and δ = 3.03 ppm for the methyl groups.

The reaction of hydrazide **4** with phenyl isocyanate or phenyl isothiocyanate in boiling ethanol gave 4-phenyl-1-(4-hydroxy-8-methyl-1-oxo-6-phenyl-2*H*-2,7-naphthyridine-3-carbonyl)semicarbazide **13** and 4-phenyl-1-(4-hydroxy-8-methyl-1-oxo-6-phenyl-2*H*-2,7-naphthyridine-3-carbonyl) thiosemicarbazide **12**, respectively. ^{1}H NMR spectra of the obtained compounds contain five additional signals for the aromatic protons at δ = 7.46-7.60 ppm and one more signal corresponding to proton of NH. Additionally, IR spectrum of the thiosemicarbazide **12** contains among other absorption bands, those within the range of ν = 2300 cm^{-1} characteristic for the NC=S group.

Biology

In our previous works, 8-ethoxy-4-hydroxy-1-oxo-6-phenyl-1,2-dihydro-2,7-naphthyridine-3-carboxylic acid derivatives were evaluated for their antitumor activity *in vitro* (17, 18). The tested compounds demonstrated variable antitumor activity. Among all derivatives, the hydrazide derivatives showed the better antiproliferative activity *in vitro*. The 8-ethoxy-4-hydroxy-1-oxo-6-phenyl-1,2-dihydro-2,7-naphthyridine-3-carboxylic acid (2,4-dihydroxy-bezylidene)-hydrazide (24) and 8-ethoxy-4-

Table 1. *In vitro* percent growth of some selected tumor cell lines caused by the tested compounds.

Compd.	Panel	Tumor cell line	Percent growth
5a	Leukemia	SR	46.06
	Leukemia	HL-60(TB)	76.79
	Leukemia	K-562	76.63
5b	Leukemia	MOLT-4	52.92
	Leukemia	CCRF-CEM	68.37
	Leukemia	K-562	44.47
	Leukemia	SR	52.96
	Colon Cancer	HCT-15	67.73
	Renal Cancer	A498	65.89
	Renal Cancer	CAKI-1	39.95
	Renal Cancer	UO-31	66.57
5g	Leukemia	CCRF-CEM	49.97
	Non-small Cell Lung Cancer	NCI-H322M	67.90
	Renal Cancer	A498	68.29

Data obtained from the NCI's *in vitro* human tumor cell screen.

hydroxy-3-(1,3,4-oxadiazol-2-yl)-6-phenyl-2*H*-2,7-naphthyridin-1-one (25) were active against most of the 60 different subpanel tumor cell lines. The results have been encouraging to the preparation of new hydrazide derivatives.

Eight of the newly synthesized compounds: **2a, 2c, 5a, 5b, 5f, 5g, 6, 11** were qualified by the National Cancer Institute in Bethesda (USA) for antiproliferative *in vitro* screening. These compounds were tested against 60 different human tumor cell lines, representing leukemia, melanoma, and breast, lung, colon, ovary, renal, prostate, central nervous system cancers in a single dose of 10 μmol. Antitumor activity was reported as percentage of growth of the treated cells. A value of 100 means no growth inhibition and a value of 0 means no net growth over the course of the experiment. Values below 0 designate percentage of lethality.

Unfortunately, compounds **2a**, **2c**, **5f**, **6** and **11** were inactive (growth higher than 50% in all cell lines). Only Schiff bases **5a**, **5b**, **5g** showed the moderate growth inhibitory activity against a few of the cell lines. The most interesting results are depicted in Table 1. The most sensitive to their antitumor activity were found to be human leukemia and renal cancer cells.

CONCLUSIONS

The aim of the present research was to synthesize the novel pyrrolo[3,4-*c*]pyridines and their rearrangement to the corresponding 2,7-naphthyridines (Scheme 1). Next step of this work was to obtain 4-hydroxy-8-methyl-1-oxo-6-phenyl-1,2-dihydro-2,7-naphthyridine-3-carboxylic acid hydrazide **4** derivatives of various chemical structure, according to the syntheses illustrated in Scheme 2. Twenty-one new compounds were isolated as the result of these reactions. Their structures were confirmed by IR, NMR, MS spectra and elemental analysis. Eight of the prepared compounds were evaluated against the 60 different human tumor cell lines for their antiproliferative activity *in vitro*. Among the tested compounds the Schiff bases: 4-hydroxy-8-methyl-1-oxo-6-phenyl-1,2-dihydro-2,7-naphthyridine-3-carboxylic acid phenethylidene-hydrazide **5a**, 4-hydroxy-8-methyl-1-oxo-6-phenyl-1,2-dihydro-2,7-naphthyridine-3-carboxylic acid propylidene-hydrazide **5b**, and 4-hydroxy-8-methyl-1-oxo-6-phenyl-1,2-dihydro-2,7-naphthyridine-3-carboxylic acid (2,4-dihydroxy-benzylidene)-hydrazide **5g** showed the moderate antitumor activity *in vitro*.

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