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 hydrazone (4) was the key intermediate for the synthesis of the novel derivatives of various chemical structures: Schiff bases, 1,3,4-oxadiazoles, pyrazoles, carboxyhydrazides, 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 hydrazone derivatives (GI50 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. 1H and 13C NMR spectra were recorded in DMSO-d6 with a Bruker Avance ARX-300 MHz spectrometer (Bruker Analytica, 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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297
pounds were checked by TLC using aluminum sheet silica gel 60 F254 (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 OC. IR (KBr, cm -1): 1780, 1740, 1720 (C=O), 1260 (CO), 750 (CH arom.). 1H NMR (DMSO-d 6, δ, ppm): 2.82 (s, 3H, CH3), 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 (154), 153 (13), 127 (6), 126 (4), 104 (2), 77 (4), 59 (2).
Analysis: calcd. for C17H14N2O4 (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): 3440 (NH), 2900 (OH), 1650 (C=O), 1280 (C-O), 770 (CH arom.). 1H NMR (DMSO-d 6, δ, ppm): 2.82 (s, 3H, CH3), 7.31 (s, 3H, OCH 3), 4.46 (s, 2H, CH2), 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 (154), 153 (13), 127 (6), 126 (4), 104 (2), 77 (4), 59 (2).
Analysis: calcd. for C23H18N2O4 (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): 3100, 1700 (C=O), 750, 680 (CH arom.). 1H NMR (DMSO-d 6, δ, ppm): 2.86 (s, 3H, CH3), 5.26 (s, 2H, CH2), 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 C22H16N2O3 (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 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 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.). 1H NMR (DMSO-d 6, δ, ppm): 1.38 (t, J = 7.0 Hz, 3H, CH 3), 3.05 (s, 3H, CH3), 4.41 (q, J = 8.9 Hz, 2H, CH2), 7.54 (m, 3H, phenyl), 8.18 (m, 3H, phenyl, pyridine), 8.41 (s, 1H, OH), 10.52 (s, 1H, NH). 13C NMR (DMSO-d 6, δ, ppm): 13.8, 26.4, 62.3, 109.5, 112.8, 119.5, 127.0 (2C), 128.9 (2C), 129.2, 137.5, 140.2, 141.7, 156.5, 158.5, 161.7, 163.2.
Analysis: calcd. for C18H16N2O4 (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-2H-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.). 1H NMR (DMSO-d 6, δ, ppm): 3.12 (s, 3H, CH3), 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). 13C 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 C22H16N2O3 (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 hydrazone 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 (O=C), 770, 690 (NH). ¹H NMR (DMSO-d₆, δ, ppm): 3.05 (s, 3H, CH₃), 3.33 (d, J = 7.0 Hz, 2H, CH₂), 7.51-7.59 (m, 5H, phenyl), 8.01-8.17 (m, 5H, phenyl), 8.42 (s, 1H, pyridine), 9.37 (s, 1H, OH), 12.21 (s, 1H, NH). Analysis: calcd. for C₂₄H₂₀N₄O₃ (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 (O=C), 780, 690 (NH). ¹H NMR (DMSO-d₆, δ, ppm): 0.97 (t, J = 6.4 Hz, 3H, CH₃), 1.63-1.78 (q, J = 6.8 Hz, 2H, CH₂), 3.07 (s, 3H, CH₃), 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, 1H, phenyl, pyridine, OH), 10.14 (s, 1H, NH), 12.34 (s, 1H, NH). Analysis: calcd. for C₁₉H₁₈N₄O₃ (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-hydroxy-benzylidene)-hydrazide (5c)

Yield 1.60 g (39%), yellow solid, crystallized from toluene, m.p. 272-273°C. IR (KBr, cm⁻¹): 3330, 3240 (NH), 2950 (CH), 1650 (C=O), 1580 (C=N), 1350 (O=C), 750, 700 (CH). ¹H NMR (DMSO-d₆, δ, ppm): 3.07 (s, 3H, CH₃), 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 C₂₃H₁₈N₄O₃ (414.37): C, 66.38; 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-chloro-benzylidene)-hydrazide (5d)

Yield 1.50 g (35%), yellow solid, crystallized from toluene, m.p. 286-288°C. IR (KBr, cm⁻¹): 3350 (NH), 2930 (CH), 1640 (C=O), 1580 (C=N), 1280 (O=C), 750, 690 (CH). ¹H NMR (DMSO-d₆, δ, ppm): 3.06 (s, 3H, CH₃), 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 C₂₃H₁₇ClN₄O₃ (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 (O=C), 780, 690 (CH). ¹H NMR (DMSO-d₆, δ, ppm): 3.09 (s, 3H, CH₃), 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 C₂₃H₁₇F₃N₄O₃ (416.40): C, 66.32; 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, 2930 (NH), 2950 (CH), 1650 (C=O), 1580 (C=N), 1340 (O=C), 750, 700 (CH). ¹H NMR (DMSO-d₆, δ, ppm): 3.07 (s, 3H, CH₃), 6.10-6.14 (m, 2H, CH), 7.34-7.36 (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 C₂₅H₂₀N₄O₃ (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), 2930 (CH), 1630 (C=O), 1580 (C=N), 1340, 1230 (CO), 770, 690 (CH). ¹H NMR (DMSO-d₆, δ, ppm): 3.06 (s, 3H, CH₃), 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_{17}H_{12}N\textsubscript{4}O\textsubscript{4} (336.30): C, 63.70; H, 4.10; N, 14.11%; 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-nitrophenylbenzylidine)-hydrazide (5i)

Yield 3.01 g (68%), orange solid, crystallized from toluene, m.p. 284-285°C. IR (KBr, cm\textsuperscript{-1}): 3100 (OH), 2900 (NH), 1650, 1600 (C=O), 1540, 1380, 1390, 1540, 1560, 1600 (CH arom.). \textsuperscript{1}H NMR (DMSO-d\textsubscript{6}, δ, ppm): 3.03 (s, 3H, CH\textsubscript{3}), 7.48-7.50 (m, 3H, phenyl), 8.71 (s, 1H, pyridine), 10.11 (br, 2H, NH, OH), 12.61 (s, 1H, NH). Analysis: calcd. for C\textsubscript{26}H\textsubscript{24}N\textsubscript{4}O\textsubscript{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-nitrophenylbenzylidine)-hydrazide (5j)

Yield 1.31 g (38%), beige solid, crystallized from ethanol, m.p. 327-330°C. IR (KBr, cm\textsuperscript{-1}): 3100 (OH), 2900 (NH), 1650, 1600 (C=O), 1500 (CN), 820, 790 (CH arom.). \textsuperscript{1}H NMR (DMSO-d\textsubscript{6}, δ, ppm): 3.02 (s, 3H, CH\textsubscript{3}), 7.48-7.50 (m, 3H, phenyl), 8.08-8.15 (m, 3H, phenyl, pyridine), 11.61 (br, 2H, NH, OH), 12.61 (s, 1H, NH). Analysis: calcd. for C\textsubscript{23}H\textsubscript{18}N\textsubscript{4}O\textsubscript{5} (430.41): C, 64.18; H, 4.22; N, 13.02%; found: C, 64.27; H, 4.65; N, 11.32%.

4-Hydroxy-8-methyl-1-oxo-6-phenyl-1,2-dihydro-2,7-naphthyridin-1-one (7)

Yield 1.65 g (49%), beige solid, crystallized from ethanol, m.p. 296-297°C. IR (KBr, cm\textsuperscript{-1}): 3300 (OH), 2900 (NH), 1750 (C=O), 1770 (CH arom.). \textsuperscript{1}H NMR (DMSO-d\textsubscript{6}, δ, ppm): 3.05 (s, 3H, CH\textsubscript{3}), 7.52-7.56 (m, 3H, phenyl), 8.19-8.22 ppm.
N’-acetyl-4-hydroxy-8-methyl-1-oxo-6-phenyl-2H,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. Aftercooling, 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.). ¹H NMR (DMSO-d₆, δ, ppm): 1.96 (s, 3H, CH₃), 3.07 (s, 3H, CH₃), 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 C₁₈H₁₆N₄O₄ (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-2H,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), 2900 (NH), 1670, 1580 (C=O), 750, 690 (CH arom.). ¹H NMR (DMSO-d₆, δ, ppm): 1.88 (s, 3H, CH₃), 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 C₂₁H₁₈N₄O₃ (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-2H,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. 330°C. IR (KBr, cm⁻¹): 3300 (OH), 2800 (NH), 1650, 1600, 1500, 1350 (C=O, NH, CN), 820, 700 (CH arom.). ¹H NMR (DMSO-d₆, δ, ppm): 3.05 (s, 3H, CH₃), 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 C₁₉H₁₆N₅O₃S (445.50): C, 61.82; H, 4.06; N, 15.96%.

4-Phenyl-1-(4-hydroxy-8-methyl-1-oxo-6-phenyl-2H,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.). ¹H NMR (DMSO-d₆, δ, ppm): 3.04 (s, 3H, CH₃), 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 C₂₃H₁₉N₅O₄ (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% CO₂, 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% CO₂, 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. 1H 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 CH₂ group instead of one-proton singlets of pyrrole NH. Stoichiometric amount of sodium ethoxide or sodium methoxide, did not yield the expected methyl ester from compound 2a, and benzyl ester from compound 2b, but the product of alcoholysis 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 ¹H NMR spectra of the newly synthesized 3-benzoyl-4-hydroxy-8-methyl-6-phenyl-2H-2,7-naphthyridine-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 ¹H NMR spectra of obtained Schiff bases the two-protons signal at δ = 4.46 ppm 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)-2H-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-2H-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](image-url)
triel ethyl 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)-3H-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 anhydride resulted in the formation of N-formyl-4-hydroxy-8-methyl-1-oxo-6-phenyl-2H-2,7-naphthyridine-3-carbohydrazide 9 and N-acetyl-4-hydroxy-8-methyl-1-oxo-6-phenyl-2H-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-2H-2,7-naphthyridin-1-one 11 in good yield (72%). $^1$H NMR spectra exhibited three three-protons singlets at $\delta = 1.88$ ppm, $\delta = 2.05$ ppm, and $\delta = 3.03$ 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-2H-2,7-naphthyridine-3-carbonyl)semicarbazide 13 and 4-phenyl-1-(4-hydroxy-8-methyl-1-oxo-6-phenyl-2H-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 $\delta = 7.46$-$7.60$ 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 $\nu = 2300$ 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 (24) and 8-ethoxy-4-
hydroxy-3-(1,3,4-oxadiazol-2-yl)-6-phenyl-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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REFERENCES


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