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SYNTHESIS AND ANTIPROLIFERATIVE ACTIVITY *IN VITRO* OF NEW 2-THIOXOIMIDAZO[4,5-*B*]PYRIDINE DERIVATIVES

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Abstract: Two series of 2-thioxoimidazo[4,5-*b*]pyridine derivatives have been synthesized from 2,3-diaminopyridine (**1**) and 5-halogenosubstituted-2,3-diaminopyridines **2**, **3**. Mannich bases **7** – **12** and **24** – **29**, derivatives of 1-arylamino-6-halogeno-2-thioxoimidazo[4,5-b]pyridine were obtained with selected secondary amines: morpholine, piperidine, 2-methoxyphenylpiperazine, pyrimidyn-2-yl-piperazine and formaldehyde in ethanol. The structures **7** – **12** and **24** – **29** were confirmed by the results of elementary analysis and their IR, ^{1}H-NMR and MS spectra. All given structures **7** – **12** have been optimized to get the most stable low energy conformers. Synthesized compounds were of interest for biological studies or can be substrates for further synthesis. The selected compounds **7** – **10**, **12** – **17**, **22**, **25**, **27** – **29** were screened for their antiproliferative activity *in vitro* against human cancer and normal mouse fibroblast cell lines.

Keywords: 2-amino-3-arylideneamino-5-halogenopyridine, 2-thioxoimidazo[4,5-*b*]pyridine derivatives, Mannich bases, Schiff bases, IR, ^{1}H -NMR spectra, antiproliferative activity *in vitro*

There are some drugs, imidazo[4,5-*b*]pyridine derivatives, registered in the world, which exhibit diverse pharmacological activities. Antihistaminic II generation drug with selective activity to H1 receptors is represented by noberastine (1). Sulmazole (2), a new cardiotonic agent, is an A1 adenosine receptor antagonist. Tenatoprazole (3) possesses antiulcer activity and is a novel proton pump inhibitor with a prolonged plasma half-life.

Based on the review of the chemical literature, derivatives of imidazole[4,5-*b*]pyridine showed a multipharmacological effects. A number of the imidazo[4,5-*b*]pyridine possess antidepressant (4), anticancer (5, 6), antimicrobial (7, 8) activities. There were also described derivatives of imidazo[4,5-*b*]pyridine with the potential uses in the treatment of diabetes and hyperlipidemia (9). Some chemical compounds which contain in their structure the imidazo[4,5-*b*]pyridine system inhibit neurodegeneration (10) and can be used in the treatment of neurodegenerative disorders e.g., multiple sclerosis, Alzheimer's disease or Parkinson's disease. Among compounds of this class, antagonists of angiotensin II receptors that exhibit hypotensive activity are also known (11). Some of them can be used in the treatment of heart diseases.

Recently published works, showed that chemical modification of various heterocyclic compounds containing azomethine bond – Schiff base (12, 13) and aminomethyl group – Mannich base provides biological activity (14, 15). Many studies have shown that Mannich bases possess potent biological activities: antibacterial (16, 17), antiviral (18), antitumor (19, 20) properties. They may be potential drugs in epilepsy treatment (21).

In our recently published papers, we have described synthesis and antiproliferative activity *in vitro* of imidazo[4,5-*b*]pyridine derivatives (22 – 24).

The aim of this work was to synthesize the new Mannich bases, 2-thioxo-imidazo[4,5-*b*]pyridine derivatives using selected pharmacophore – secondary amines: morpholine, piperidine, 2-methoxyphenylpiperazine, pyrimidyn-2-yl-piperazine together with formaldehyde.

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Selected compounds of different chemical structures, were examined for their antiproliferative activity *in vitro* against the cells of human cancer cell lines and normal mouse fibroblasts.

EXPERIMENTAL

Chemistry

Melting points were measured with a Boethius melting point apparatus. The new products were analyzed using a Perkin Elmer 2400 analyzer. IR spectra (in KBr) were recorded with an IR 75 spectrophotometer, ^{1}H NMR spectra – on a Bruker AVANCE DRX 300 MHz using DMSO-d_6 as an internal standard. The course of reaction and the purity of products were checked by TLC (Kieselgel G, Merck) in diethyl ether : ethanol 5 : 1, v/v as eluent. The syntheses of 6-bromo- (**5**) and 2-thioxoimidazo[4,5-*b*]pyridine (**4**) have been presented in our previous paper (23).

General procedure for the synthesis of Mannich bases 7 – 12

A mixture of 0.01 mol of thioxo-1*H*,3*H*-imidazo[4,5-*b*]pyridine (**4**) or 6-halogen-2-thioxo-1*H*,3*H*-imidazo[4,5-*b*]pyridine (**5**, **6**), 0.03 mol of formaldehyde and 0.03 mol of selected secondary amine: morpholine, piperidine, 2-methoxyphenylpiperazine, pyrimidin-2-yl-piperazine in absolute ethanol was stirred at room temperature for 5 h. The precipitate formed was filtrated off and crystallized from ethanol.

1,3-Di-(morpholinemethyl)-2-thioxoimidazo[4,5-*b*]pyridine (7)

Yield: 1.64 g (47%); white solid from ethanol; m.p. 213–214°C. IR (KBr, cm^{-1}): 2910, 2850 (-CH_2-), 1450 (N-CS-N), 1125 (CS); ^{1}H NMR (DMSO, δ, ppm): 8.24 (dd, 1H, *J* = 4,8 Hz, *J* = 1.0 Hz, H-5), 8.10 (dd, 1H, *J* = 1.0 Hz, *J* = 8.1 Hz, H-7), 7.30 (dd, 1H, *J* = 4.8 Hz, *J* = 8.1 Hz, H-6), 5.20 (s, 2H, -CH_2-) 5.10 (s, 2H, -CH_2-), 3.50 (m, 8H, -CH_2-O-CH_2-), 2.70 (m, 8H, -CH_2-N-CH_2-). MS (70 eV): m/z (%): 351 (M^+ + 2, 28), 350 (M^+ + 1, 100), 348 (M^+ – 1, 15); Analysis: calcd. for $C_{16}H_{23}N_5O_2S_2$ (349.46) C, 54.99; H, 6.63; N, 20.04%; found: C, 54.56, H, 6.63; N, 19.92%.

6-Chloro-1,3-di(morpholinemethyl)-2-thioxoimidazo[4,5-*b*]pyridine (8)

Yield: 3.22 g (85%); white solid from ethanol; m.p. 220–222°C. IR (KBr, cm^{-1}): 2950, 2850 (-CH_2-), 1000 (arom.); ^{1}H NMR (DMSO, δ, ppm): 7.92 (d, 1H, H-5), 7.63 (d, 1H, H-7), 5.15 (d, 2H, -CH_2-), 5.06 (d, 2H, -CH_2-), 3.53 (m, 4H,-CH_2-O-CH_2-), 2.60 (m, 4H, -CH_2-N-CH_2-). MS (70 eV): m/z (%): 386 (M^+ – 3, 48), 385 (M^+ – 2, 36), 384 (M^+ 100), 285 (8), 186 (10). Analysis: calcd. for $C_{16}H_{22}N_5ClO_2S$ (383.90) C, 50.06; H, 5.78; N, 18.24%; found: C, 49.89; H, 5.97; N, 18.06%.

1,3-Di-(piperidinemethyl)-2-thioxoimidazo[4,5-*b*]pyridine (9)

Yield: 1.82 g (53%); white solid from ethanol; m.p. 170–172°C. IR (KBr, cm^{-1}): 2950 (CH_2), 1450 (N-CS-N), 1125 (CS); ^{1}H NMR (DMSO, δ, ppm): 8.16 (d, 1H, H-5), 7.75 (dd, 1H, *J* = 7.8 Hz, *J* = 1.2 Hz, H-7), 5.15 (s, 2H, -CH_2-), 5.02 (s, 2H, -CH_2-), 2.65 (m, 8H, -CH_2-NH-CH_2-), 1.32 (m, 12H, -CH_2-CH_2-CH_2-); Analysis: calcd. for $C_{18}H_{27}N_5S$ (345.51) C, 62.57; H, 7.88; N, 20.27%; found: C, 62.94; H, 9.29; N, 20.32%.

1,3-Di-[4-(2-methoxyphenyl)piperazinemethyl]-2-thioxoimidazo[4,5-*b*]pyridine (10)

Yield 3.18 g (57%): white solid from ethanol; m.p. 185–187°C. IR (KBr, cm^{-1}): 2950 (-CH_2-), 2825 (-OCH_3), 1500, 1450 (-CH_2-), 1175, 1060, 1000 (arom.); ^{1}H NMR ($CDCl_3$, δ, ppm): 8.20 (d, 1H, H-5), 7.60 (d, 1H, H-7), 7.10 (dd, 1H, H-6), 6.90 (m, 8H, arom.) 5.50 (s, 2H, -CH_2-), 5.20 (s, 2H, -CH_2-), 3.80 (s, 3H, -OCH_3), 3.70 (s, 3H, -OCH_3), 3.00 (m, 16H, 8× -CH_2-). Analysis: calcd. for $C_{30}H_{37}N_7O_2S$ (559.73) C, 64.38; H, 6.66; N, 17.52%; found: C, 63.91; H, 6.64; N, 17.64%.

6-Chloro-1,3-di-[4-(2-methoxyphenyl)piperazinemethyl]-2-thioxoimidazo[4,5-*b*]pyridine (11)

Yield 4.93 g (83%): white solid from ethanol; m.p. 220–222°C. IR (KBr, cm^{-1}): 2940, 2880 (-CH_2-), 2840 (-OCH_3), 1450, 1160 (N-CS-N), 1010, 880 (-CH_2-), 750 (arom.); ^{1}H NMR (DMSO, δ, ppm): 8.19 (d, 1H, H-5), 7.58 (d, 1H, H-7), 7.01 (m, 8H, Ar-H), 5.45 (s, 2H, -CH_2-), 5.14 (s, 2H, -CH_2-), 3.85 (s, 3H, -OCH_3), 3.80 (s, 3H, -OCH_3), 3.06 (m, 8H, 2× -CH_2-N-CH_2-), 2.53 (m, 8H, 2× -CH_2-N-CH_2-). Analysis: calcd. for $C_{30}H_{36}N_7ClO_2S$ (594.17) C, 60.64; H, 6.11; N, 16.50%; found: C, 60.86; H, 6.03; N, 16.41%.

1,3-Di-[4-(pyrimidin-2-yl)piperazinemethyl]-2-thioxoimidazo[4,5-*b*]pyridine (12)

1-(2-Pyrimidyl)piperazine hydrochloride (2.4 g, 0.01 mol) and 4.2 mL (0.03 mol) triethylamine in 30 mL ethanol were stirred at room temperature for 0.5 h. Next, 1.5 g (0.01 mol) of compound **2** and 2.5 mL (0.03 mol) of formaldehyde were added. A mixture was stirred at room temperature. After the com-

pletion of reaction, the solvent was evaporated and water (50 mL) was added to the dry residue. The precipitate formed was filtered off, dried decolorized with charcoal and crystallized from ethanol.

Yield 1.95 g (56%); white solid from ethanol; m.p. 165–167°C. IR (KBr, cm^{-1}): 2975 (-CH_2-), 1550 (-CH_2-), 1230, 1175 (arom.). ^{1}H NMR (DMSO, δ, ppm): 8.30 (m, 5H, H-5 + arom.), 7.90 (dd, 1H, *J* = 1.80 Hz, *J* = 8.10 Hz, H-7) 7.3 (dd, 1H, *J* = 5.10 Hz, *J* = 8.10 Hz, H-6) 6.50 (m, 2H, aromat) 5.30 (s, 2H, -CH_2-) 5.20 (s, 2H, -CH_2-) 3.70 (m, 8H, 4× -CH_2-) 2.7 (m, 8H, 4× -CH_2-). Analysis: calcd. for $C_{24}H_{29}N_{11}S$ (503.64) C, 57.24; H, 5.80; N, 30.59%; found: C, 57.59; H, 5.79; N, 30.82%.

General procedure for the synthesis of Schiff bases 13 – 15

A mixture of 0.01 mol of 2,3- diaminopyridine (**1**) or 5-bromo-2,3-diaminopyridine (**2**), 0.01 mol appropriate aldehyde: p-methoxy- or benzaldehyde and catalytic amount of triflate (trifluoromethanesulfonate indum) in 30 mL of ethanol was stirred and refluxed for 5 h. The precipitate formed was filtered off and crystallized from ethanol.

2-Amino-3-(p-methoxybenzylideneamino)pyridine (13)

Yield: 2.06 g (91%); yellow solid from ethanol; m.p. 128–130°C. IR (KBr, cm^{-1}): 3460, 3400 (NH_2), 2920 (-CH-), 2850 (-OCH_3), 1640 (CH=N, chain), 1605 (CH=N, ring), 1020, 850 (arom.); ^{1}H NMR (DMSO, δ, ppm): 8.56 (s, 1H,- CH=N-), 7.95 (d, 2H, Ar-H), 7.82 (d, 1H, *J* = 1.5 Hz, *J* = 4.8 Hz, H-6), 7.34 (d, 1H, *J* = 1.5 Hz, *J* = 7.5 Hz, H-4), 7.05 (d, 2H, Ar-H), 6.56 (dd, 1H, *J* = 4.8 Hz, *J* = 7.5 Hz, H-5), 5.88 (s, 2H,- NH_2), 3.83 (s, 3H, -OCH_3). Analysis: calcd. for $C_{13}H_{13}N_3O$ (227.27) C, 68.71; H, 5.77; N, 18.49%; found: C, 69.26; H, 5.84; N, 18.17%.

2-Amino-5-bromo-3-benzylideneaminopyridine (14)

Yield: 1.60 g (58%); yellow solid from ethanol; m.p. 126–128°C. IR (KBr, cm^{-1}): 4360, 3280 (NH_2), 1630 (CH=N, chain), 910, 780 (arom.). ^{1}H NMR ($CDCl_3$, δ, ppm): 8.44 (s, 1H, CH=N), 7.93 (d, 1H, H-6), 7.81 (m, 2H, Ar-H), 7.41 (m, 3H, Ar-H), 7.29 (d, 1H, H-4), 5.10 (br, 2H, -NH_2). Analysis: calcd. for $C_{12}H_{10}N_3Br$ (276,14) C, 52.20; H, 3.65; N, 15.22%; found: C, 52.02; H, 3.52; N, 14.93%.

2-Amino-5-bromo-3-(p-methoxybenzylideneamino)pyridine (15)

Yield: 1.98 g (65%); yellow solid from ethanol: m.p. 110–113°C. IR (KBr, cm^{-1}): 3460, 3440 (NH_2), 1650 (N=CH, chain), 1175, 1125, 830 (arom.). ^{1}H NMR ($CDCl_3$, δ, ppm): 8.41 (s, 1H, N=CH), 7.97 (d, H-6), 7.83 (m, 2H, Ar-H), 7.32 (d, 1H, H-4), 6.9 (m, Ar-H) (br, 2H, NH_2), 3.88 (s, 3H, -OCH_3). Analysis: calcd. for $C_{13}H_{12}N_3BrO$ (306,15) C, 51.00; H, 3.95; N, 13.72%; found: C, 51.36; H, 3.81; N, 13.52%.

Synthesis of 2-amino-5-chloro-3-(4-fluorobenzylidene)aminopyridine (16) and 6-chloro-2-(4-fluorophenyl)-3H-imidazo[4,5-*b*]pyridine (17)

A mixture of 5-chloro-2,3-diaminopyridine (**3**) 7.2 g (0.05 mol), 4-fluorobenzaldehyde 5.3 mL (0.05 mol) and catalytic amount of triflate in 30 mL absolute ethanol was refluxed for 5 h. After cooling, the solid of compound **16** was filtered off, the filtrate was evaporated and resulting residue was crystallized to obtain compound **17**.

2-Amino-5-chloro-3-(4-fluorobenzylidene) aminopyridine (16)

Yield: 1.99 g (80%); yellow solid from ethanol; m.p. 150–153°C. IR (KBr, cm^{-1}): 3300, 3150 (NH_2), 1660 (N=CH, chain), 1605 (N=CH, ring), 1175, 1150 (arom.). ^{1}H NMR (DMSO, δ, ppm): 8.74 (s, 1H, -N=CH-), 8.11 (m, 2H, H-6, Ar-H), 7.84 (d, 1H, Ar-H), 7.54 (d, 1H, A-H), 7.39 (m, 2H, H-4 + Ar-H), 6.22 (s, 2H, -NH_2). Analysis: calcd. for $C_{12}H_9N_3ClF$ (249.67) C, 56.73; H, 3.63; N, 16.83%; found: C, 57.71; H, 3.53; N, 16.42%.

6-Chloro-2-(4-fluorophenyl)-3H-imidazo[4,5-*b*]pyridine (17)

Yield: 0.37 g (15%); beige solid from ethanol; m.p. 347–348°C. IR (KBr, cm^{-1}): 3450, 1625 (NH), 1110, 950 (arom.). ^{1}H NMR (DMSO, δ, ppm): 13.65 (br, 1H, -NH), 8.32 (m, 4H, H-5 + Ar-H), 7.43 (m, 2H, H-6 + Ar-H). Analysis: calcd. for $C_{12}H_7N_3ClF$ (247,66) C, 58.20; H, 3.85; N, 16.97%; found: C, 58.09; H, 3.79; N, 16.82%.

General procedure for the synthesis of compounds 18 – 20

Sodium borohydride (0.01 mol) was added to the solution of Schiff base **4, 5** or **6** (0.01 mol) in ethanol (50 mL). The reaction mixture was stirred and refluxed for ca. 8–10 h. The solvent was evaporated and water (150 mL) was added. The aqueous solution was left for 24 h. The precipitate formed was filtered off, dried, decolorized with charcoal and crystallized from ethanol.

2-Amino-3-(p-methoxybenzylamino)pyridine (18)

Yield: 1.12 g (49%); beige solid from ethanol; m.p. 105–106°C. IR (KBr, cm^{-1}): 3450, 3360 (NH_2), 3110 (NH), 2840 (-OCH_3), 1650, 1590 (NH), 1460 (-CH_2-), 1050, 805, 780 (arom.); ^{1}H NMR (DMSO, δ, ppm): 7.27 (m, 3H, Ar-H), 6.88 (m, 2H, Ar-H), 6.49 (d, 1H, Ar-H), 6.37 (dd, 1H, *J* = 4.8 Hz, *J* = 7.5 Hz, H-5), 5.49 (br, 2H, NH_2), 5.31 (t, 1H, NH), 4.20 (d, 2H, -CH_2-), 3.72 (s, 3H, -OCH_3). Analysis: calcd. for $C_{13}H_{15}N_3O$ (229.28) C, 68.10; H, 6.59; N, 18.33%; found: C, 67.81; H, 6.89; N, 18.80%.

2-Amino-3-benzylamino-5-bromopyridine (19)

Yield: 2.61 g (94%); beige solid from ethanol; m.p. 112–114°C. IR (KBr, cm^{-1}): 3380, 3310 (NH_2), 2920, 2850 (CH), 1650, 1580 (NH), 1480 (CH_2), 910, 850, 750 (arom.); ^{1}H NMR (DMSO, δ, ppm): 7.31 (m, 6H, Ar-H), 6.55 (s, 1H, Ar-H), 5.77 (m, 3H, NH_2 + NH), 4.30 (d, 2H, -CH_2-). Analysis: calcd. for $C_{12}H_{12}N_3Br$ (278.15) C, 51.82; H, 4.35; N, 15.11%; found: C, 52.20; H, 4.12; N, 14.98%.

2-Amino-5-chloro-3-(4-fluorobenzyl)aminopyridine (20)

Yield: 2.81 g (87%); beige solid from ethanol; m.p. 155–158°C. IR (KBr, cm^{-1}): 1400 (NH_2), 1170 (NH), 2270 (-CH_2-), 1650 (NH), 1490 (-CH_2-), 1150, 1100 (arom.). ^{1}H NMR (DMSO, δ, ppm): 7.42 (d, 1H, H-6), 7.38 (d, 1H, H-4), 7.20 (m, 3H, Ar-H), 6.46 (d, 1H, Ar-H), 5.77 (m, 3H, -NH + -NH_2), 4.29 (d, 2H, -CH_2-). Analysis: calcd. for $C_{12}H_{11}N_3ClF$ (251.65) C, 57.27; H, 4.41; N, 16.70%; found: C, 57.04; H, 4.22; N, 16.48%.

General procedure for the synthesis of compounds 5 and 21 – 23

The mixture of compound **3, 18, 19** or **20** (0.01 mol), CS_2 (0.01 mol) and NaOH 0.4 g (0.01mol) in 30 mL of ethanol was stirred and refluxed for 8 h. After the completion of reaction, the solvent was evaporated and water (100 mL) was added to the dry residue. Aqueous solution of sodium 2-thioxoderivative was decolorized wiht charcoal. The filtrate was acidified to pH 4–5 with dilute hydrochloric acid. The precipitate formed was filtered off, dried, and crystallized from ethanol.

6-Chloro-2-thioxo-1H,3H-imidazo[4,5-*b*]pyridine (5)

Yield: 1.20 g (65%); beige solid; m.p. 345–348°C. IR (KBr, cm^{-1}): 3440, 1620 (NH), 1380 (HN-CS-NH), 1200 (C=S), 1080, 940 (arom.); ^{1}H NMR (DMSO, δ, ppm): 13.30 (br, 1H, NH), 12.91 (br, 1H, NH), 8.08 (d, 1H, H-5), 7.55 (dd, 1H, H-7). Analysis: calcd. for $C_6H_4N_3ClS$ (185.63) C, 38.82; H, 2.17; N, 22.64%; found: C, 38.53; H, 2.36; N, 22.85%.

1-(p-Methoxybenzyl)-2-thioxo-3H-imidazo[4,5-*b*]pyridine (21)

Yield: 2.54 g (94%); beige solid from ethanol; m.p. 145–147°C. IR (KBr, cm^{-1}): 3350 (NH), 2925 (-CH_2), 2850 (-OCH_3), 1650, 1560 (NH), 1425, 1350 (N-CS-N), 1025, 780 (arom.); ^{1}H NMR (DMSO, δ, ppm): 13.46 (br, 1H, NH), 8.13 (dd, 1H, *J* = 1.2 Hz, *J* = 5.1 Hz, H-5), 7.63 (dd, 1H, *J* = 1.2 Hz, *J* = 8.1 Hz, H-7), 7.36 (m, 2H, Ar-H), 7.28 (m, 2H, Ar-H), 7.14 (dd, 1H, *J* = 5.1 Hz, *J* = 8.1 Hz, H-6), 5.44 (s, 2H, -CH_2-), 3.71 (s, 3H, -OCH_3). Analysis: calcd. for $C_{14}H_{13}N_3OS$ (271.33) C, 61.87; H, 4.83; N, 15.49%; found: C, 62.15; H, 5.09; N, 15.17%.

1-Benzyl-6-bromo-2-thioxo-3H-imidazo[4,5-*b*]pyridine (22)

Yield: 2.52 g (79%); solid from ethanol; m.p. 191–194°C. IR (KBr, cm^{-1}): 3280 (NH), 2880 (-CH_2-), 1675, 1560 (NH), 1050 (C=S), 810, 720, 700 (arom.); ^{1}H NMR (DMSO, δ, ppm): 7.48 (d, 1H, Ar-H), 7.33 (m, 5H, Ar-H), 6.82 (d, 1H, Ar-H), 4.41 (s, 2H, -CH_2-), Analysis: calcd. for $C_{13}H_{10}N_3BrS$ (320.21) C, 48.76; H, 3.15; N, 13.12%; found: C, 48.51; H, 3.31; N, 13.50%.

6-Chloro-1-(4-fluorobenzyl)-3H-2-thioxoimidazo[4,5-*b*]pyridine (23)

Yield: 2.05 g (83%); solid from ethanol; m.p. 260–263°C. IR (KBr, cm^{-1}): 2850 (-CH_2-), 1605 (NH), 1480 (N-CS-N), 1325 (NH), 1240, 910, 880 (arom.); ^{1}H NMR (DMSO, δ, ppm): 13.72 (br, 1H, NH), 7.66 (m, 6H, H-5 + H-7 + Ar-H), 5.47 (s, 2H,-CH_2-). Analysis: calcd. for $C_{13}H_9N_3ClFS$ (247.66) C, 53.16; H, 3.09; N, 14.30%; found: C, 53.37; H, 2.87; N, 13.95%.

General procedure for the synthesis of Mannich bases 24 – 30

A mixture of (0.01 mol) of 6-halogeno-2-thioxo-1*H*,3*H*-imidazo[4,5-*b*]pyridine (**21**–**23**), 0.03 mol) of formaldehyde and (0.03 mol) of selected secondary amine: morpholine, piperidine, 2-methoxyphenylpiperazine or pyrimidyn-2-yl-piperazine in absolute ethanol was stirred at room temperature for 5 h. The precipitate formed was filtrated off and crystallized from ethanol.

6-Chloro-1-(4-fluorobenzyl)-3-[4-(2-methoxyphenylene)piperazinemethyl]-2-thioxoimidazo[4,5-*b*]pyridine (24)

Yield: 1.82 g (75%); white solid from ethanol; m.p. 177–180°C. IR (KBr, cm^{-1}): 2950 (CH_2), 2880

(OCH_3), 2850 (CH_2), 1450, 1160 (N-CS-N), 1010, 870 (-CH_2-); ^{1}H NMR (DMSO, δ, ppm): 8.17 (d, 1H, H-5), 7.16 (m, 9H, H-7 + Ar-H), 5.52 (s, 2H, CH_2), 5.50 (s, 2H, -CH_2-), 3.82 (s, 3H, -OCH_3), 3.09 (t, 4× -CH_2-). Analysis: calcd. for $C_{25}H_{25}N_5ClFOS$ (498.01) C, 60.29; H, 5.06; N, 14.06%; found: C, 60.47; H, 5.23; N, 13.97%.

1-(p-Methoxybenzyl)-3-[4-(2-methoxyphenyl) piperazinemethyl]-2-thioxoimidazo[4,5-*b*]pyridine (25)

Yield: 3.13 g (66%); white solid from ethanol; m.p.184–186°C; IR (KBr, cm^{-1}): 3080, 2930, 1500 (-CH_2-), 2825 (-OCH_3), 1300 (-N=), 1150 (C=S), 1000, 800, 750 (arom.); ^{1}H NMR (DMSO, δ, ppm): 8.25 (d, 1H, H-5), 7.75 (d, 1H, H-7), 7.39 (d, 1H, Ar-H), 7.25 (dd, 1H, H-6), 6.88 (m, 7H, Ar-H), 5.55 (s, 2H, -CH_2-), 5.33 (s, 2H, -CH_2-), 3.70 (s, 6H, 2× -OCH_3), 2.90 (m, 8H, 4× -CH_2-). Analysis: calcd. for $C_{26}H_{29}N_5O_2S$ (475.67): C, 65.65; H, 6.16; N, 14.73%; found: C, 65.66; H, 6.08; N, 14.37%.

1-(p-Methoxybenzyl)-3-[4-(pirymidyn-2-yl)-piperazinemethyl]-2-thioxoimidazo[4,5-*b*]pyridine (26)

Yield: 1.40 g (63%); white solid from ethanol; m.p. 167–169°C; IR (KBr, cm^{-1}): 2925 (-CH_2-), 2850 (-OCH_3), 1590 (CH_2), 1440, 1300 (N-CS-N), 1250 (C=S), 990, 805, 780 (arom.); ^{1}H NMR (DMSO, δ, ppm): 8.29 (d, 2H, Ar-H), 8.23 (d, 1H, H-5), 7.72 (d, 1H, H-7), 7.34 (d, 2H, Ar-H), 7.22 (dd, 1H, H-6), 6.85 (m, 2H, Ar-H), 6.57 (m, 1H, Ar-H), 5.51 (s, 2H, -CH_2), 5.30 (s, 2H, -CH_2), 3.69 (m, 8H, 4× -CH_2), 2.80 (s, 3H, -OCH_3). Analysis: calcd. for $C_{23}H_{25}N_7OS$ (447.55) C, 61.72; H, 5.63; N, 21.91%; found: C, 61.58; H, 5.75; N, 21.72%.

1-Benzyl-6-bromo-3-morpholinemethyl-2-thioxo-imidazo[4,5-*b*]pyridine (27)

Yield: 1,59 g (38%); white solid from ethanol; m.p.154–156°C; IR (KBr, cm^{-1}): 2940, 1480 (-CH_2-), 1430 (N-CS-N) 1205 (C=S). ^{1}H NMR (DMSO, δ, ppm): 7.79 (d, 1H, Ar-H),7.46 (d, 1H, H-5), 7.33 (m, 4H, Ar-H), 6.96 (d, 1H, H-7), 5.72 (s, 2H, CH_2), 4.99 (s, 2H, CH_2), 4.41 (m, 4H, CH_2-O-CH_2), 3.97 (m, 4H, CH_2-N-CH_2); Analysis: calcd. for $C_{18}H_{19}N_4BrOS$ (419.34) C, 51.56; H, 4.57; N, 13.36%; found: C, 51.19; H, 4.99; N, 13.35%.

1-(p-Methoxybenzyl)-3-morpholinemethyl-2-thioxoimidazo[4,5-*b*]pyridine (28)

Yield: 1.40 g (38%); white solid from ethanol; m.p. 134–137°C; IR (KBr, cm^{-1}): 2930 (-CH_2-), 2850 (-OCH_3), 1430, 1330 (N-CS-N), 1250 (C=S), 1000, 810, 750 (arom.). ^{1}H NMR (DMSO, δ, ppm): 8.22 (d, 1H, H-5), 7.73 (d, 1H, H-7), 7.36 (d, 2H, Ar-H), 7.24 (dd, 1H, J = 5.1 Hz, J = 7.8 Hz, H-6), 6.88 (d, 2H, Ar-H), 5.48 (s, 2H, -CH_2), 5.24 (s, 2H, -CH_2), 3.70 (m, 4H, CH_2-O-CH_2), 3.53 (m, 4H, -CH_2-N-CH_2-), 2.74 (s, 3H, -OCH_3). Analysis: calcd. for $C_{19}H_{22}N_4O_2S$ (370.47) C, 61.60; H, 5.99; N, 15.12%; found: C, 62.01, H, 6.02; N, 14.72%.

6-Chloro-1-(4-fluorobenzyl)-3-morpholinemethyl-2-thioxoimidazo[4,5-*b*]pyridine (29)

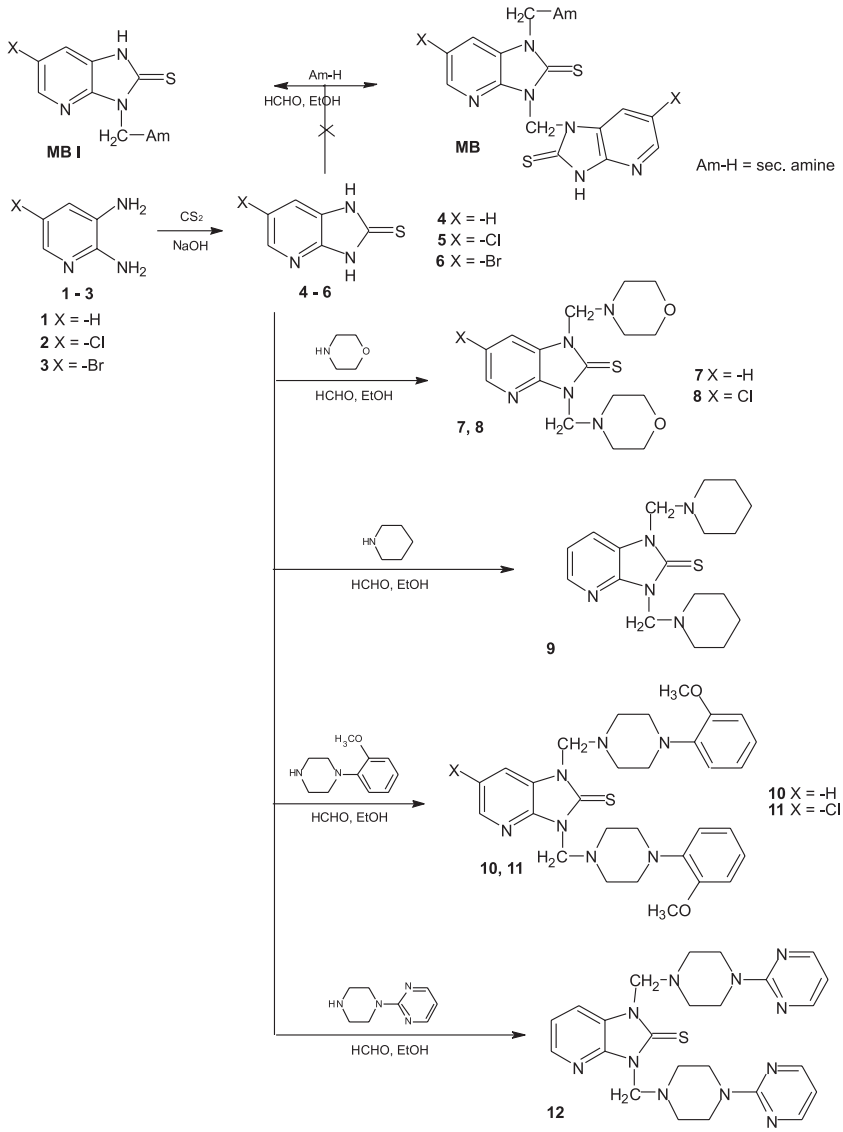
Yield: 2.66 g (70%); beige solid from ethanol; m.p. 169–172°C. IR (KBr, cm^{-1}): 2950, 2850 (-CH_2-), 1460, 1160 (N-CS-N), 1000, 750 (-CH_2-); ^{1}H NMR (DMSO, δ, ppm): 8.16 (d, 1H, H-5), 7.21 (m, 5H, H-7 + Ar-H), 5.52 (s, 2H, -CH_2), 5.36 (s, 2H, -CH_2), 3.69 (t, 4H, -CH_2-O-CH_2-), 2.88 (t, 4H, -CH_2-N-CH_2-). Analysis: calc. for $C_{17}H_{18}N_4ClFOS$ (380.87). C, 53.61; H, 4.78; N, 14.71%; found: C, 54.03; H, 4.53; N, 14.50%.

Computational methods

Potential energy surfaces have been scanned, and all minima have been optimized to find all possible conformers of the molecules. All structures have been obtained using a relaxed dihedral angle scan to generate a potential energy surface. Points were then optimized using density functional theory's (25) B3LYP (26, 27) hybrid functional with 6-31G and 6-31G(d,p) basis sets. Second order Mřller Plesset (MP2) (28) calculations have been performed to calibrate all structures. The thermodynamic parameters of all compounds have been calculated using statistical mechanics expressions. All calculations have been conducted using Gaussian 09 (29).

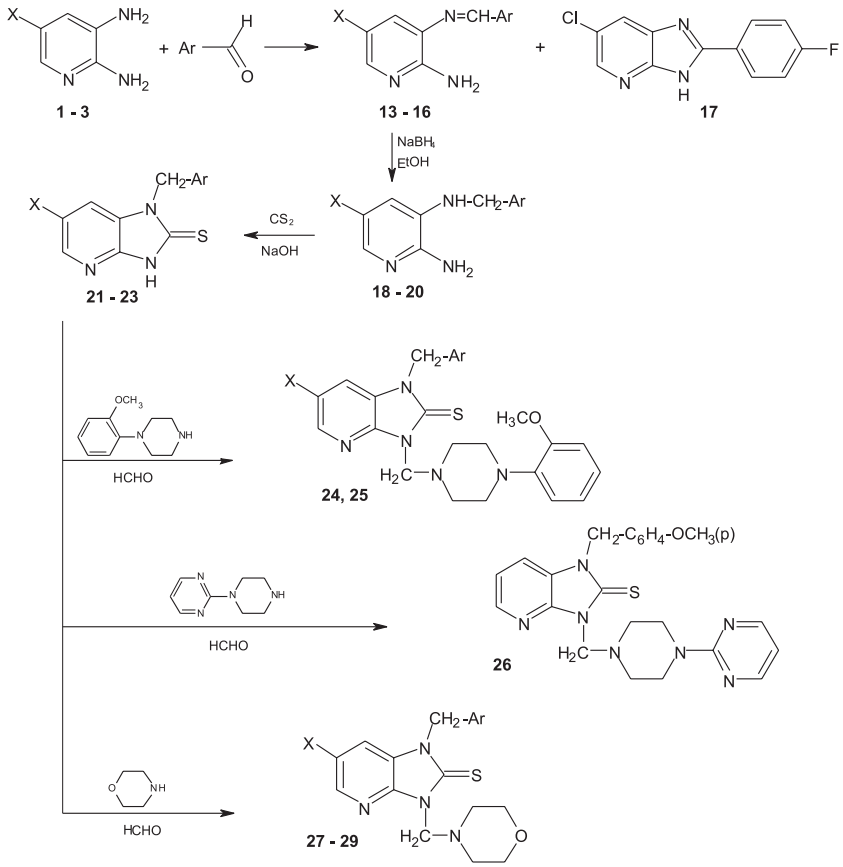
***In vitro* antiproliferative assay**

Antiproliferative tests were performed on human cancer cell lines: A549 (lung), MCF-7 (breast), leukemia MV4-11 and mouse embryonic fibroblast BALB/3T3 according to standard procedure (30). All cell lines were obtained from American Type Culture Collection (Rockville, Maryland, USA) and have been maintained in culture or frozen in thaw Cell Culture Collection of the Institute of Immunology and Experimental Therapy, Polish Academy of Sciences (IIET, PAS, Wrocław, Poland). The A549 cells were cultured in a mixture of Opti-MEM and RPMI 1640 medium (1 : 1, both from Gibco, Scotland, UK) supplemented with 2 mM L-glutamine and 5% fetal bovine serum. MCF-7 cells were cultured in Eagle medium (IIET, Wrocław, Poland), supplemented



Scheme 1. Synthesis of 1,3-di-(aminomethyl)-2-thioxoimidazo[4,5-*b*]pyridine derivatives

with 2 mM L-glutamine and 1.0 mM sodium pyruvate, 10% fetal bovine serum and with 0.8 mg/L of insulin (Sigma-Aldrich Chemie GmbH, Steinheim, Germany). MV4-11 cells were cultured in RPMI 1640 medium (Gibco, Scotland, UK) with 2 mM L-glutamine, adjusted to contain 1.5 g/L sodium bicarbonate and 1.0 mM sodium pyruvate, 10% fetal bovine serum (Sigma-Aldrich Chemie GmbH, Steinheim, Germany). Mouse fibroblasts BALB/3T3 were maintained in Dulbecco medium (DMEM, Gibco, Scotland, UK) supplemented with 2 mM L-glutamine and 10% fetal bovine serum (Sigma-Aldrich Chemie GmbH, Steinheim, Germany). All culture media were supplemented with 100 units/mL penicillin, and 100 µg/mL streptomycin (Polfa Tarchomin S.A., Warszawa, Poland). Cell lines were grown at 37°C in 5% CO_2 humidified atmosphere. The anti-proliferative effect of the tested compounds was examined after 72 h exposure of the cultured cells to varying concentrations of the tested compound (total plate incubation time: 96 h), using the sulforhodamine B (SRB) assay for adherent cells (A549, BALB/3T3 and MCF-7) and MTT assay for leukemia cells (MV4-11). The results were shown as an IC_{50} value (inhibitory concentration 50% – a concentration in µg/mL of tested agent which inhibits proliferation of 50% of cancer cells population). Each com-



No.	X	Ar
1	H	–
2	Cl	–
3	Br	–
13, **18**, **21**, **25**, **28**	H	4-C_6H_4-OCH_3
14, **19**, **22**, **27**	Br	C_6H_5
16, **20**, **23**, **24**, **29**	Cl	4-C_6H_4-F
15	Br	4-C_6H_4-OCH_3

Scheme 2. Synthesis of Mannich bases – 1-benzyl-2-thioxoimidazo[4,5-*b*]pyridine derivatives

pound was tested at every concentration in triplicate in a single experiment, which was repeated 3 times. The activity of tested compounds was compared to the activity of cisplatin used as a reference agent.

RESULTS AND DISCUSSION

Chemistry

In this paper, Mannich bases – imidazo[4,5-*b*]pyridine derivatives were prepared according to synthesis presented in Schemes 1 and 2.

In the first step of synthesis, 2-thioxo- (**4**) (23), 6-chloro- (**5**) or 6-bromo-2-thioxoimidazo[4,5-*b*]pyridine (**6**) (23) were obtained from 2,3-diaminopyridine derivatives with CS_2/NaOH in ethanol. Imidazo[4,5-*b*]pyridine derivatives **4** – **6** were substrates in Mannich condensation with triple excess of selected, secondary amines: morpholine, piperidine, 1-(2-methoxyphenyl)piperazine, 1-(2-pyridyl)piperazine and formaldehyde. The reaction was carried out in ethanol at room temperature (Scheme 1).

In all reactions single products were obtained. In this reaction products of various chemical struc-

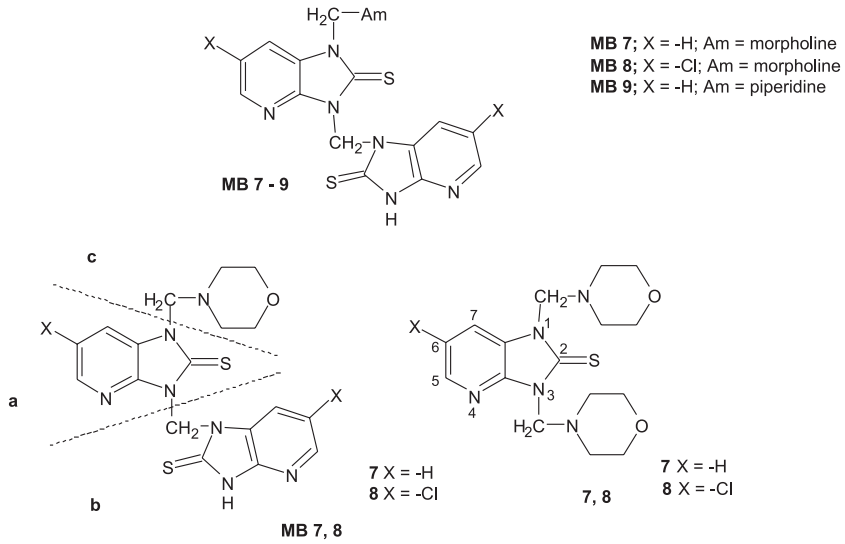


Figure 1. Exemplary structures of Mannich bases **MB 7–9** for calculated binding energies

Table 1. The antiproliferative activity of screened compounds against MV4-11 human biphenotypic B myelomonocytic leukemia cells.

Compound	IC_{50} [μg/mL]
7	19.90 ± 4.27
8	16.79 ± 12.37
9	19.66 ± 12.69
10	22.06 ± 5.59
12	12.87 ± 5.22
13	22.80 ± 6.38
14	3.98 ± 1.73
15	1.82 ± 0.84
16	7.41 ± 2.01
17	5.89 ± 0.17
22	3.61 ± 0.61
25	14.69 ± 6.68
27	2.82 ± 0.61
28	22.86 ± 7.65
29	8.60 ± 2.45
cisplatin	0.22 ± 0.07

tures could be obtained e.g., compound **MB**, 3-aminomethyl- **MB I** or 1,3-disubstituted aminomethyl compounds **7** – **12**.

Elementary analyses and MS, IR and ^{1}H NMR spectra showed that 1,3-disubstituted aminomethyl products **7** – **12** were obtained. Also quantum-chemical calculations at Density Functional Theory level have confirmed such reaction path (Fig. 1).

IR spectra of Mannich base **7** contain at 2910 and 2850 cm^{-1} bands characteristic for -CH_2- group, instead of secondary NH groups. In ^{1}H NMR spectra of compound **7** are two two protons singlets at δ = 5.10 and δ = 5.20 ppm, ascribed for methylene groups in 1 and 3 position. Protons characteristic for morpholine: -CH_2-N-CH_2- and -CH_2-O-CH_2- were observed as 8 protons two multiplets at δ = 2.70 and δ = 3.50 ppm, respectively. Additionally, number of signals for the pyridine protons in the ^{1}H NMR spectra of all Mannich bases is in good agreement with their structures.

The another route of synthesis was reaction of 5-substituted 2,3-diaminopyridines **1** – **3** with selected aromatic aldehydes: 4-methoxy-, 4-fluoro- and benzaldehyde (Scheme 2). The reactions were carried out in boiling ethanol with the presence of catalytic amounts of triflate. IR spectra of Schiff bases contain, among other absorption bands, those in the range of ~ 1650 cm^{-1} characteristic for the chain groups C=N. The presence of azamethine bond CH=N protons was confirmed by ^{1}H NMR spectra of all imines, in which one-proton singlets in the range δ = 8.41–8.74 ppm were observed. In the reaction of 5-chloro-2,3-diaminopyridine (**2**) with 4-fluorobenzaldehyde, together with Schiff base **16** cyclic product 6-chloro-2-(4-fluorophenyl)-3*H*-imidazo[4,5-*b*]pyridine (**17**) was obtained.

Table 2. The antiproliferative activity of selected compounds against human breast (MCF-7) and lung (A549) cancer and normal mouse fibroblasts (BALB/3T3) cell lines.

Compound	IC_{50} [μg/mL]/cell line		
	A549	MCF-7	BALB/3T3
14	30.67 ± 1.94	30.86 ± 0.96	73.94 ± 3.83
15	7.96 ± 1.39	10.44 ± 1.65	91.66 ± 4.22
22	11.57 ± 1.20	12.56 ± 4.78	30.41 ± 3.88
27	11.24 ± 4.03	15.80 ± 7.38	22.88 ± 7.16
cisplatin	2.47 ± 0.97	1.71 ± 1.21	1.97 ± 1.20

In the next step of synthesis, the azamethine bond in obtained Schiff bases **13**, **14** and **16** have been subjected to selective reduction using $NaBH_4$ in boiling ethanol. The extent of the hydrogenation has been monitored by TLC and decoloration of yellow solution. In ^{1}H NMR spectra of 2-amino-3-benzylaminopyridine derivatives **18** – **20** the absence of one-proton singlets at δ = 8.41–8.74 ppm was observed, whereas, two-proton doublets at δ = 4.20–4.30 ppm ascribed to NH-C**H$_2$** protons were present. Triplet or multiplet signal at δ ~ 5.50 ppm was ascribed to N**H**-CH_2 protons.

1-Benzyl-2-thioxoimidazo[4,5-*b*]pyridine derivatives **21** – **23** were obtained in cyclization of compounds **18** – **20**, using CS_2 and NaOH in ethanol.

The synthesized derivatives **21** – **23** were used as a substrates for the Mannich condensation with selected pharmacophore, secondary amines: 1-(2-methoxyphenyl)piperazine, 1-(2-pyridyl)piperazine and morpholine and formaldehyde. The reactions were carried in ethanol at room temperature. Mannich bases, 1-benzyl-3-aminomethyl-2-thioxoimidazo[4,5-*b*]pyridine derivatives, were obtained. The product structures have been confirmed by elemental analysis and IR, ^{1}H-NMR and MS spectra. The signals corresponding to aromatic protons were observed in the range of δ ~ 6.80–8.20 ppm, respectively.

Twenty four new compounds **5**, **7** – **29** of various chemical structures assigned for antiproliferative *in vitro* studies were obtained from the syntheses described here. These derivatives may also be used as starting materials for further syntheses.

Quantum-chemical calculations

All given structures **7** – **12** have been optimized to get the most stable low energy conformers. We have studied Molecular Electrostatic Potential Surfaces (MEPS) of molecules to find the reactive area of moieties. The most active area of structure **7** and **8** is located on N-3 atom and H-6 or Cl-6, respectively (Fig. 1). Adding amine moiety moves the most active region to the N-1 atom. In order to examine the affinity of the individual reactions, binding energies of the various parts of moieties were calculated. Binding energy for moieties **MB 7**, **8** consisting of part **a**–**b** is positive. Binding energy for compouds **MB 7**, **8**, consisting of part **a**–**c** is negative. The analogical results have been obtained for all synthetized structures **7** – **12**. The given results have shown that the structure of 1,3-disubstituted aminomethyl compounds **7**–**12** is more preferable than that of compounds **MB 7** – **9**.

In vitro antiproliferative assay

The compounds were screened for their antiproliferative activity using cells of MV4-11 human leukemia (Table 1). Comparing to cisplatin, the activity of tested compounds was lower, however, we selected four of them **14**, **15**, **22**, **27**, with IC_{50} value ranged between 1.82–3.98 μg/mL for further studies on the cells of breast and lung cancer, as well as on normal mouse fibroblasts to assess its selectivity towards cancer cells. The antiproliferative activity of selected compounds was remarkably lower against lung and breast cancer cells, comparing results obtained against MV4-11 cells. Interestingly, their activity towards mouse fibroblasts was much more lower, suggesting low toxicity (Table 2). Particularly, the most active on all cancer cells compound **15** was 9–50 times less cytotoxic towards normal fibroblasts than cancer cells.

CONCLUSIONS

Twenty four new compounds of different chemical structures: Schiff bases **13** – **16** and reduction of azamethine bond products **18** – **20**, 2-thioxo-

imidazo[4,5-*b*]pyridine derivatives and Mannich bases **7** – **12** and **24** – **29** were obtained from the syntheses described here. Their structures were confirmed by elemental analysis, IR, ^{1}H NMR and MS spectra. Selected new compounds **7** – **10**, **12** – **17**, **22**, **25**, **27** – **29** were screened for their antiproliferative activity *in vitro*. Four of them: 2-amino-5-bromo-3-benzylideneaminopyridine (**14**), 2-amino-5-bromo-3-(p-methoxybenzylideneamino)pyridine (**15**), 1-benzyl-6-bromo-2-thioxo-3H-imidazo[4,5-*b*]pyridine (**22**), 1-benzyl-6-bromo-3-morpholinemethyl-2-thioxoimidazo[4,5-*b*]pyridine (**27**) with high antiproliferative activity were screened on human breast (MCF-7) and lung (A549) cancer and normal mouse fibroblasts (BALB/3T3) cell lines. The most active and in parallel selective towards cancer cells was 2-amino-5-bromo-3-(p-methoxybenzylideneamino)pyridine (**15**).

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