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SYNTHESIS AND ANTIPROLIFERATIVE ACTIVITY *IN VITRO* OF NEW 3–SUBSTITUTED AMINOPYRAZOLO[3,4–*b*]PYRIDINES

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Abstract: The synthesis of several new 3–substituted aminopyrazolo[3,4–*b*]pyridines is described. The obtained compounds were tested for their antiproliferative activity *in vitro*. Two of them: 3–chloroacetylaminopyrazolo[3,4–*b*]pyridine **[II]** and *3–(2–*bromopropionyl–amino)pyrazolo[3,4–*b*]pyridine **[III]** revealed cytotoxic activity against the cells of 5 human tumor cell lines applied. Their ID_{50} values were in the range of the international activity criterion for synthetic agents (4 μg/ml). The structures of the products **II – XVII** were established on the basis of elemental analysis and spectral data (IR, ^{1}H NMR and MS).

Keywords: 3–aminopyrazolo[3,4–*b*]pyridine derivatives, synthesis, antitumor activity *in vitro*

The derivatives of pyrazolo[3,4–*b*]pyridine showed in many earlier studies a very broad spectrum of pharmacological activity, from bacteriostatic, analgesic, anti–inflammatory, antidiabetic, cardiotonic, anxiolytic, antilipidemic, plateled antiaggregatory, immunotropic to cytostatic (1–9). From among these derivatives, six were investigated in clinical studies: Cartazolate, Etazolate, Tracazolate, SQ2006, Glicaramide (10) and Y25510 (11). O– and N–alkylamino–p–hydroxypropyl derivatives of 3–hydroxy– and 3–aminopyrazolo[3,4–*b*]pyridine synthesized by us earlier, revealed antiarrhytmic and hypotensional activity (12) and 4–phenyl–2–(3,4,5–trimethoxy–β–styrylo) pyrido[2',3':3,4] pyrazolo[1,5–*a*]pyrimidine was found to have antiproliferative activity *in vitro* against cancer cells (13).

This multidirectional biological activity of the pyrazolopyridines was the reason of our further studies on this group of chemical compounds. The chemical structure of pyrazolopyridines is close to that of natural purine bases and, if suitably modified, they may act as the antimetabolites (14). It seemed quite probable that the introduction of such groups as chloro(bromo)alkyl(aryl)amido–, aminoalkylamido– into the molecule of pyrazolo[3,4–*b*] pyridine may lead to the potentially effective antitumor compounds. Peplomycine, Mitoxantron, Ledakrin or Flutamid, known antitumor agents, contain in their structures amide, alkyloamino or chloroalkyl groups determining their biological activity (15). Only a few papers dealing with the synthesis of pyrazolo[3,4–*b*]pyridine derivatives with antitumor activity have been found in the literature. Therefore, it seems important to start synthesis and biological studies of new compounds of that heterocyclic system.

In this paper we describe the synthesis of 3–aminopyrazolo[3,4–*b*]pyridine derivatives of halogenoalkyl(aryl)amide and aminoalkylamide type as well as the results of studies on their antiproliferative activity *in vitro* against the cells of human cancer cell lines.

EXPERIMENTAL

Chemistry

Melting points (uncorrected) were measured with a Boethius melting point apparatus. Analyses were performed on a Perkin Elmer 2400 analyser and satifactory results within ±0.4% of calculated values were obtained for the new compounds. IR spectra (in KBr) were recorded with an IR 75 spectrophotometer. ^{1}H NMR spectra on a Tesla BS 587 (80 MHz). The position of the resonances were referred to the residual solvent peak (DMSO – d_6, δ 2,5 ppm). Mass spectra were determined on a GC MS–LKB 2091 spectrometer at an ionization energy of 15 or 70 eV. The course of reaction and the purity of products were checked by TLC on a Merck aluminium foil with silica gel F_{254}.

Reaction 3–aminopyrazolo[3,4–*b*]pyridine **[I]** with selected halogeno acid halides: chloroacetyl–, 2–bromopropionyl bromides, 2–chloro– i 3–chloro-

propionyl, 4–chlorobutyryl chlorides **[II–VI]** and substituted benzoyl chlorides: *p*–methoxy– and 3,4,5–trimethoxybenzoyl chlorides **[VII–VIII]** and crotonoyl chloride **[IX]**

General procedure.

To a solution of 0.01 mole of 3–aminopyrazolo[3,4–*b*]pyridine **[I]** (16) in 100 ml of tetrahydrofurane 0.7 g of anhydrous K_2CO_3 and 0.01 mole of the appropriate halogeno acid halides, *p*–methoxybenzoyl, 3,4,5–trimethoxybenzoyl or crotonoyl chlorides were added. The reaction mixture was heated under reflux for 4–10 h, the solvent was then removed in vacuo and the residue obtained was triturated with water, filtered, dried and recrystallized.

3–Chloroacetyl–**[II]**, 2–bromopropionyl–**[III]**, 2–chloropropionyl–**[IV]**, 3–chloropropionyl–**[V]**, 4–chlorobutyryl–**[VI]**, 4–methoxybenzoyl–**[VII]**, 3,4,5–trimethoxybenzoyl–**[VIII]**, and crotonoylaminopyrazolo[3,4–*b*]pyridines **[IX]**

Reaction 3–aminopyrazolo [3,4–*b*]pyridine **[I]** with selected substituted benzoyl chlorides: (*p*–chloro–, *p*–nitro– and 3,5–dinitrobenzoyl chlorides).

To a solution of 0.01 mole of 3–aminopyrazolo[3,4–*b*]pyridine **[I]** in 100 ml of dry toluene few drops of pyridine and 0.01 mole of *p*–chloro–, *p*–nitro– and 3,5–dinitrobenzoyl chlorides were added. The reaction mixture was heated under reflux for 10 h, the solvent was then removed in vacuo and the residue obtained was triturated with water, filtered, dried and recrystallized.

3–(4–Chlorobenzoyl–**[X]**, 3–(4–nitrobenzoyl–**[XI]** and 3–(3,5–dinitrobenzoylamino)pyrazolo[3,4–*b*]pyridines **[XII]**

General procedure for synthesis of 3–aminoacetyloaminopyrazolo[3,4–*b*]pyridines **[XIII–XV]**

To a solution of 0.01 mole of 3–chloroacetylaminopyrazolo[3,4–*b*]pyridine **[II]** in 50 ml of n–butanol, 0.02 mole of the appropriate amine: morpholine, N–methylpiperazine or N–diethyl–amine in 10 ml of n–butanol was added dropwise. The reaction mixture was heated under reflux for 16 h. The solvent was removed under reduced pressure and the residue was suspended in 50 ml water. The precipitated solid was collected by filtration, washed with water, dried and recrystallised.

3–Morpholino–**[XIII]**, 3–(N–methylpiperazine)–**[XIV]** and 3–(N–diethylamino)acetylamino–pyrazolo[3,4–*b*]pyridines **[XV]**

6–[3–(3–Propionylamino)pyrazolo[3,4–*b*]pyridylo]–1,3,4,10*b*–tetrahydropyrido[2,3:3,4]pyrazolo[1,5–*a*]–pyrimidine–2–one **[XVI]**

To a solution of 0.6 g (2.5 mmol) of 3–(3–chloropropionylamino)pyrazolo[3,4–*b*]pyridine (V) in 50 ml of isopropanol 0.37 g (0.01 mole) of $NaBH_4$ was added. The reaction mixture was heated under reflux for 8 h with mechanical stirring. Then, isopropanol was evaporated in vacuo and water (100 ml) was added. The product was filtered off, washed with water, dried and recrystallized.

3–(2–Pyrrolidinon–1–yl)pyrazolo[3,4–*b*]pyridine **[XVII]**

To a solution of 0.6 g (2.5 mmol) 3–(4–chlorobutyrylamino)pyrazolo[3,4–*b*]pyridine **(V)** in 50 ml of isopropanol 0.37 g (0.01 mole) of $NaBH_4$ was added. The reaction mixture was heated under reflux for 10 h with mechanical stirring. Then isopropanol was evaporated in vacuo and water (100 ml) was added. The product was filtered off, washed with water, dried and recrystallized.

The analytical data for the new compounds **II–XVII** are given in the Table 1.

Biology

Antiproliferative assay *in vitro*

Cells

The following established *in vitro* human cancer cell lines were applied: SW707 (rectal adenocarcinoma), A549 (non–small cell lung carcinoma), MCF–7 (breast carcinoma), KB (cervix carcinoma) and HCV29T (bladder cancer). The first four lines were obtained from the American Type Culture Collection (Rockville, Maryland, U.S.A.) and maintained in the Cell Culture Collection of the Institute of Immunology and Experimental Therapy, Wrocław, Poland. Human uroepithelial cell line HCV29T, established in the Fibiger Institute, Copenhagen, Denmark, were obtained from Dr. J. Kieler in 1982 and maintained at the Institute of Immunology and Experimental Therapy, Wrocław, Poland.

Twenty–four hours before addition of the tested agents, the cells were plated in 96–well plates (Sarstedt, U.S.A.) at a density of 10^4 cells per well in 100 µl of culture medium.

The cells were cultured in an opti–MEM medium supplemented with 2mM glutamine (Gibco, Warsaw, Poland), streptomycin (50 µg/ml), penicillin (50 U/ml) (both antibiotics from Polfa, Tarchomin, Poland) and 5% fetal calf serum (Gibco, Grand Island, U.S.A.). The cell cultures were

Table 1. Physicochemical and spectral properties of 3–substituted aminopyrazolo[3,4–*b*]pyridines

No.	Formula molecular weight	M.p. °C Yield % (solvent)	IR (KBr) ν [cm^{-1}]	^{1}H NMR (DMSO–d$_6$) δ [ppm] MS (70 eV): *m/z* (%)
II	$C_8H_7ClN_4O$ (210.62)	264–5 75.23 (ethanol)	3100, 2950 (CH, NH), 1670, 1580 (CONH), 1530, 1490 (C=C, C=N), 1280 (CH_2Cl)	4.41 (s, 2H, CH_2), 7. 17 (dd, 1H, J = 4.27 Hz, J = 8.23 Hz, H–5), 8.35 – 8.56 (m, 2H, H–6,4), 11.05 (s, br, 1H, NH), 13.34 (s, br, 1H, NH pyrazole) 212 (8), 211 (3), 210 (28) [M$^+$], 134 (100), 135 (6), 104 (5), 79 (11), 78 (8), 77 (9), 51 (6), 52(4), 49(5), 44(17)
III	$C_9H_9BrN_4O$ (269.10)	260–1 59.47 (benzene)	3100, 2900 (CH, NH), 1665, 1580 (CONH), 1550, 1450 (C=C, C=N)	1.81 (d, J = 6.7 Hz, 3H, CH_3), 4.89 (q, J = 6.7 Hz, 1H, CH), 7.18 (dd, J = 4.27 Hz, J= 8.54 Hz, 1H, H–5), 8.34–8.55 (m, 2H, H–6,4), 11.08 (s, br, 1H, NH), 13.32 (s, br, 1H, NH pyrazole) 271 (2), 270 (10), 268 (11) [M^1], 224 (4), 134 (100), 135 (11), 107 (5), 79 (8), 57 (20), 55 (17), 45 (15), 44 (26), 43 (23), 41 (17), 39 (6).
IV	$C_9H_9ClN_4O$ (224.65)	216–7 69.19 (ethanol)	3100, 2900 (CH, NH), 1675, 1580 (CONH), 1550, 1450 (C=C, C=N)	1.80 (d, 3H, J = 6.4 Hz, CH_3), 4.95 (q, 1H, J = 6.4 Hz, CH), 7.20 (dd, 1H, J = 4.24 Hz, J = 8.32 Hz, H–5), 8.30 – 8.65 (m, 2H, H–6,4), 11.00 (s, br, 1H, NH), 13.25 (s, br, 1H, NH pyrazole)
V	$C_9H_9ClN_4O$ (224.65)	187–8 87.05 (ethanol)	3100, 2900 (CH, NH), 1660, 1590 (CONH), 1550, 1450 (C=C, C=N), 1230 (CH_2Cl), 770 (CH_2)	2.96 (t, J = 5.8 Hz, 2H, CH_2), 3.96 (t, J = 5.8 Hz, 2H, CH_2), 7.15 (dd, J = 4.49 Hz, J = 8.30 Hz, 1H, H–5), 8.45 (m, 2H, H–6,4), 10.83 (s, br, 1H, NH), 13.25 (s, br, 1 H, NH pyrazole) 226 (9), 225 (3), 224 (34) [M$^+$], 134 (100), 135 (25), 104 (13), 105 (10), 77 (10), 63 (49), 55 (59), 51 (25), 44 (25), 38 (17), 36 (15)
VI	$C_{10}H_{11}ClN_4O$ (238.06)	187–8 83.19 (ethanol)	3100, 2900 (CH, NH), 1660, 1590 (CONH), 1550,1450(C=C, C=N), 1230(CH_2Cl), 770 (CH_2)	2.09 (m, 2H, J = 6.34 Hz, J = 6.83 Hz, CH_2), 2.6 (t, 2H, J = 6.83 Hz, CH_2), 3.78 (t, 2H, J = 6.34 Hz, CH_2), 7. 10 (dd, 1H, J = 4.39 Hz, J = 8.30 Hz, H–5), 8.42 (m, 2H, H–6,4), 10.67 (s, br, 1H, NH), 13.18 (s, br, 1H, NH pyrazole) 240 (6), 239 (2), 238 (19) [M$^+$], 202 (38), 147 (24), 135 (22), 134 (100), 105 (16), 77 (25), 78 (11), 79 (22), 41 (50), 44 (13), 36 (13), 39 (10)
VII	$C_{14}H_{12}N_4O_2$ (268.27)	254–5 73.13 (ethanol)	3200, 2910 (CH, NH), 1670, 1560 (CONH), 1510, 1450 (C=C, C=N), 1240 (C–O–C)	3.86 (s, 3H, CH_3), 7.13 (dd, 1H, J = 4.3 Hz, J = 8.3 Hz, H–5), 8.06 – 8.54 (m, 6H, H–6,4 & Ph), 10.89 s, br, 1H, NH), 13.32 (s, br, 1H, NH pyrazole) 269 (5), 268 (28) [M$^+$], 136 (13), 135 (100), 107 (10), 92 (13), 77 (19), 69 (12), 45 (8)
VIII	$C_{16}H_{16}N_4O_4$ (328.33)	249–250 88.41 (ethanol)	3200, 2910 (CH, NH), 1670, 1560 (CONH), 1510, 1450 (C=C, C=N), 1240 (C–O–C)	3.78 (s, 3H, CH_3), 3.91 (s, 6H, 2 x CH_3), 7.19 (dd, 1H, J = 4.39 Hz, J = 8.3 Hz, H–5), 7.5 (s, 2H, H–2,6 Ph), 8.32–8.56 (m, 2H, H–6,4), 11.08 (s, br, 1H, NH), 13.37 (s, br, 1H, NH pyrazole) 329 (8), 328 (57) [M$^+$], 196 (29), 194 (100), 167 (6), 152 (13), 122 (7), 78 (6), 77 (12), 66 (8), 53 (6)
IX	$C_{10}H_{10}N_4O$ (202.22)	228–9 79.20 (ethanol)	3200, 2910 (CH, NH), 1670, 1560 (CONH), 1510, 1450 (C=C, C=N), 1640 (CH=CH)	1.89 (d, J = 6.8 Hz, 3H, CH_3), 6.26 (d, J = 15.6 Hz, 1H, CH), 6.95 (m, J = 6.8 Hz, 15.6 Hz, 1H, CH), 7.13 (dd, 1H, J = 4.39 Hz, J = 8.3 Hz, H–5), 8.45 (m, 2H, H–6, 4), 10.70 (s, br, 1H, NH), 13.20 (s, br, 1H, NH pyrazole) 203 (2), 202 (20) [M$^+$], 187 (22), 135 (5), 134 (67), 79 (5), 77 (5), 69 (100), 55 (5), 44 (50), 43(8), 41(42), 39(12)

Table 1. – continued

No.	Formula molecular weight	M.p. °C Yield % (solvent)	IR (KBr) ν [cm^{-1}]	^{1}H NMR (DMSO–d_6) δ [ppm] MS (70 eV): *m/z* (%)
X	$C_{23}H_9ClN_4O$ (272.69)	307–8 80.88 (butanol)	3200, 2910 (CH, NH), 1670, 1560 (CONH), 1510, 1450 (C=C, C=N)	7.19 (dd, 1H, J = 4.2 Hz, J = 8.23 Hz, H–5), 7.57–8.55 (m, 6H, H–6,4 & Ph), 11.15 (s, br, 1H, NH), 13.38 (s, br, 1H, NH pyrazole) 274 (15), 273 (7), 272 (47) [M$^+$], 141 (56), 140 (11), 139 (100), 111 (52), 113 (16), 75 (15), 77 (6), 69 (10), 44 (9)
XI	$C_{13}H_9N_5O_3$ (283.25)	190–2 81.27 (butanol)	3200, 2910 (CH, NH), 1670, 1560 (CONH), 1510, 1450 (C=C, C=N)	7.02 (dd, 1H, J = 4.25 Hz, J = 7.86 Hz, H–5), 8.22–8.55 (m, 6H, H–6,4 & Ph), 10.70 (s, br, 1H, NH), 13.20 (s, br, 1H, NH pyrazole) 284 (5), 283 (29) M$^+$, 255 (7), 151 (8), 150 (100), 134 (14), 120 (16), 104 (36), 92 (11), 76 (22), 69 (16), 50 (11), 44 (18)
XII	$C_{13}H_8N_6O_3$ (328.24)	325–6 54.87 (butanol)	3200, 2910 (CH, NH), 1670, 1560 (CONH), 1510, 1450 (C=C, C=N)	7.17 (dd, 1H, J = 4.39 Hz, J = 7.85 Hz, H–5), 8.34 – 8.56 (m, 2H, H–6,4), 9.03 (s, 1H, H–4 Ph), 9.28 (s, 2H, H–2,6 Ph), 11.80 (s,br, 1H,NH), 13.43 (s, br, 1H, NH pyrazole)
XIII	$C_{12}H_{15}N_5O_2$ 261.28	192–3 86.59 (toluene)	2940, 3190 (CH, NH), 1670, 1580 (CONH), 1530, 1490 (C=C, C=N), 1280 (C–O–C)	2.34 (m, 6H, CH_2N & CH_2 morpholine), 3.52 (m, 4H, CH_2O), 7.14 (dd, 1H, J = 4.39 Hz, J = 8.3 Hz, H–5), 8.32 – 8.51 (m, 2H, H–6,4), 10.34 (s, br, 1H, NH), 13.25 (s, br, 1H, NH pyrazole) 262 (5), 261 (41) [M$^+$], 176 (8), 161 (6), 135 (3), 134 (17), 104 (4), 101 (39), 100 (100), 98 (7), 78 (10), 77 (8), 70 (11), 57 (5), 56 (92), 43 (21), 42 (49), 41 (18)
XIV	$C_{13}H_{18}N_6O$ (274.33)	164–5 67.51 (toluene)	2940, 3190 (CH, NH), 1670, 1580 (CONH), 1530, 1490 (C=C, C=N)	2.02 (s, 3H, CH_3), 3.24 (m, 8H, CH_2 piperazine), 3.95 (s, 2H, CH_2), 7.09 (dd, 1H, J = 4.37 Hz, J = 8.31 Hz, H–5), 8.34–8.55 (m, 2H, H–6,4), 10.30 (s, br, 1H, NH), 13.24 (s, br, 1H, NH pyrazole)
XV	$C_{12}H_{17}N_5O$ (247.30)	145–6 71.25 (toluene)	2940, 3190 (CH, NH), 1670, 1580 (CONH), 1530, 1490 (C=C, C=N)	1.02 (t, 6H, J = 7.2 Hz), 2xCH_3), 2.65 (q, 4H, J = 7.2 Hz, 2xCH_2), 4.00 (s, 2H, CH_2), 7.09 (dd, 1H, J = 4.35 Hz, J = 8.3 Hz, H–5), 8.32–8.56 (m, 2H, H–6,4), 10.35 (s, br, 1H, NH), 13.20 (s, br, 1H, NH pyrazole) 248 (4), 247 (41) [M$^+$], 175 (54), 146 (100), 135 (5), 134 (20), 77 (33), 72 (83), 44 (26)
XVI	$C_{18}H_{18}N_8O_2$ (378.39)	239–240 74.46 (ethanol)	3200 (NH, CH), 1680, 1570 (NHCO), 1530, 1450 (C=C, C=N)	1.15 (t, 2H, CH_2), 2.55 (m, 3H, CH_2 + CH), 3.42 (t, 2H, CH_2), 7.35 (m, 2H, H–β), 8.63 (m, 4H, H–α,γ), 11.20 (s, br, 2H, NHCO), 13.65 (s, br, NH pyrazole) 379 (3), 378 (13) [M$^+$], 254 (34), 244 (8), 203 (39), 191 (16), 189 (22), 188 (20), 161 (19), 160 (11), 147 (100), 135 (22), 134 (93), 105 (12), 104 (15), 78 (22), 57 (30), 44 (18)
XVII	$C_{10}H_{10}N_4O$ (202.22)	196–7 67.30 (ethanol)	3120, 2900 (NH, CH), 1710, 1620, 1320 (NCO, lactam), 1590, 1500, 1410 (C=C, C=N).	2.26 (m, 2H, CH_2), 2.65 (t, 2H, CH_2), 4.01 (t, 2H, CH_2), 7.27 (dd, 1H, H–5), 8.57–9.00 (m, 2H, H–4 i H–5), 13.54 (s, br, 1H, NH pyrazole) 203 (6), 202 (58) [M$^+$], 147 (100), 134 (5), 119 (17), 103 (3), 92 (9), 77 (5), 64 (7), 39 (9)

maintained at 37°C in a humid atmosphere saturated with 5% CO_2.

SRB assay

The details of this technique were described by Skehan et al. (17). The cytotoxicity assay was performed after 72–hour exposure of the cultured cells to varying concentrations (from 0.1 to 100 μg/ml) of the tested agents. The cells attached to the plastic were fixed by gently layering cold 50% TCA (trichloroacetic acid, Aldrich–Chemie, Germany) on the top of the culture medium in each well. The plates were incubated at 4°C for 1 h and then washed five times with tap water. The background optical density was measured in wells filled with the culture medium, without the cells. The cellular material fixed with TCA was stained with 0.4% sulforhodamine B (SRB, Sigma, Germany), dissolved in 1% acetic acid (POCh, Gliwice, Poland) for 30 min. Unbound dye was removed by rinsing (4x) with 1% acetic acid. The protein–bound dye was extracted with 10 mM unbuffered Tris base (POCh, Gliwice, Poland) for determination of optical density (at 540 nm) in a computer–interfaced, 96–well microtiter plate reader Multiskan RC photometer (Labsystems, Helsinki, Finland). Each compound in a given concentration was tested in triplicates in each experiment, which was repeated 3–5 times. The results of cytotoxic activity *in vitro* were expressed as an ID_{50} – the dose of compound (in μg/ml) that inhibits proliferation rate of the tumor cells by 50% as compared to the control untreated cells.

RESULTS AND DISCUSSION

Chemistry

Synthesis of new 3–substituted aminopyrazolo[3,4–*b*]pyridine derivatives **II** – **XVII** is presented in Scheme I. 3–Aminopyrazolo[3,4–*b*]pyridine [**I**] was prepared by the reaction of 2–chloro–3–cyanopyridine (18) with hydrazine hydrate according to the Hatt and Vass method (16). By acylating amine **I** with selected acid halides in tetrahydrofurane in the presence of anhydrous potassium carbonate or in toluene in the presence of pyridine the respective monoacyloamides **II** – **XII** were obtained. Such a structure of the prepared amides is confirmed by the H^1NMR spectra where a broad single signal at d ≅ 11 ppm represents the NHCO group protons, while the other broad singlet at d ≅ 13 ppm corresponds to the proton linked to the pyrazol ring. Then, the selected 3–chloroacetylaminopyrazolo[3,4–*b*]pyridine [**II**] was subjected to ammonolysis with secondary amines in n–butanol and 3–morpholino–, 3–N–methylpiperazine–, 3–N–diethylaminoacetylaminopyrazolo[3,4–*b*]pyridine [**XIII** – **XV**] were obtained. Some additional signals representing the alkyl group protons, appear in the H^1NMR spectra in the low intensity field.

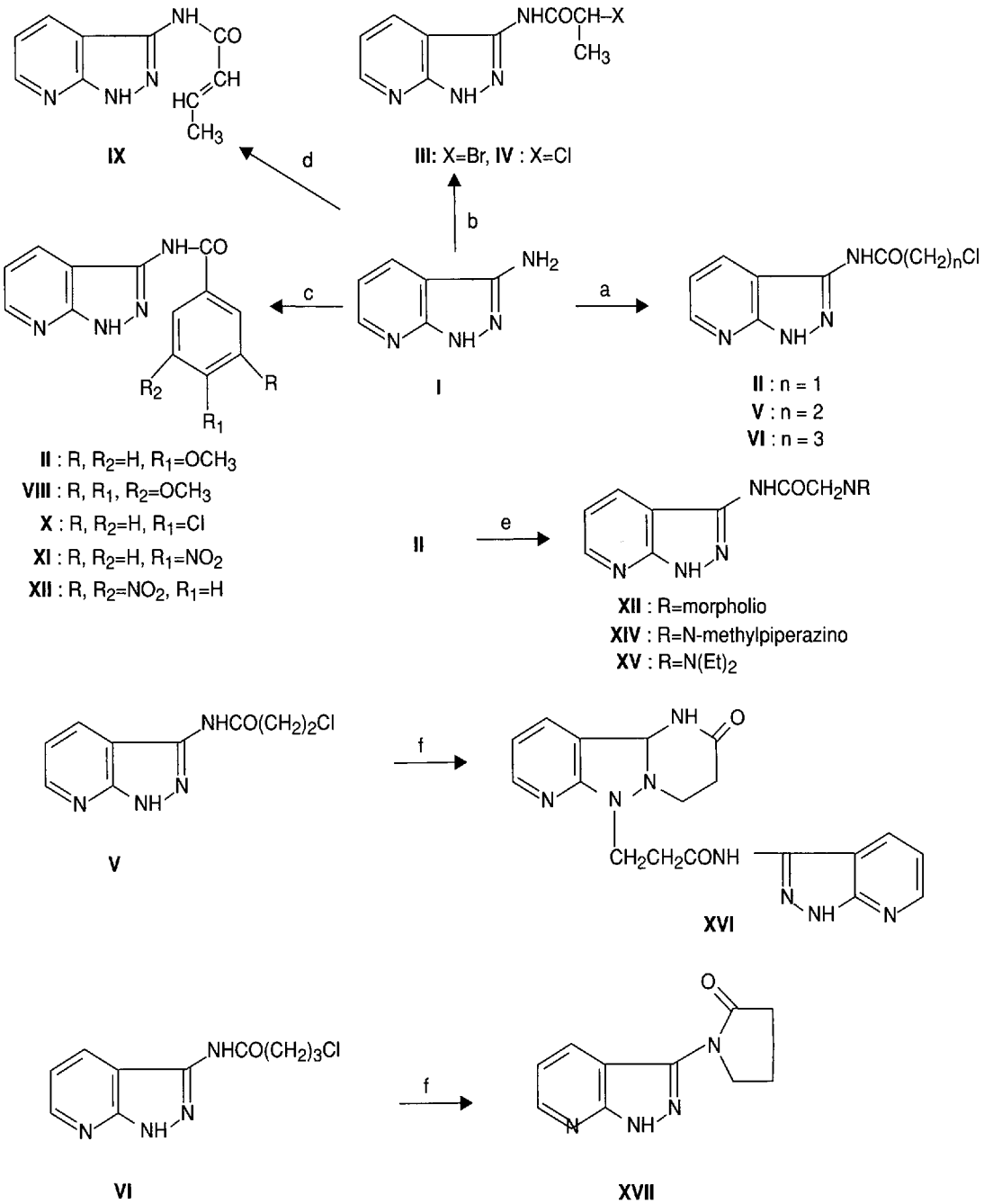
Then, attempts have been made to cyclize chloroalkylamides **V** and **VI** with sodium boro–hydride in isopropanol. Under the influence of this reactant 3–(3–chloropropionyl–amino)pyrazolo[3,4–*b*] pyridine (**V**) was transformed into a product having the molecular weight of 378, which indicated a dimeric structure of the synthesized compound. The H^1NMR spectrum contains four signals corresponding to the methylene groups and two broad singlets with the surface area ratio of 2:1. The singlet d ≅ 11.20 ppm was assigned to two protons of the NHCO groups, while the second singlet at d ≅ 13.65 ppm was assigned to the proton linked to the pyrazol ring. Hence, a conclusion was drawn that 3–chloropropionylamine **V** in the presence of $NaBH_4$ becomes cyclized to form tetrahydropyridopyrazolpyrimidine with simultaneous condensation with the second molecule of amide **V** forming a product defined by formula **XVI**.

On the other hand, 3–(4–chlorobutyrylamino)pyrazolo[3,4–*b*] pyridine (**VI**) treated with $NaBH_4$ under similar reaction conditions is transformed into 3–(2–pyrrolidinon–1–yl)pyrazolo[3,4–*b*] pyridine (**XVII**). The IR spectrum shows some bands which are characteristic of a five–member lactam system. In the H^1NMR spectrum, apart from the signals of aromatic protons and methylene groups, one can find a broad singlet of the N–1 pyrazol proton.

Pharmacology

Antiproliferative activity *in vitro*

The compounds 3–chloroacetylaminopyrazolo[3,4–*b*]pyridine [**II**] and 3–(2–bromo–propionylamino)pyrazolo[3,4–*b*]pyridine [**III**] were tested for their antiproliferative activity *in vitro* against the cells of 5 human cancer cell lines: SW707 (rectal adenocarcinoma), A549 (non–small cell lung carcinoma), MCF–7 (breast carcinoma), KB (cervix carcinoma) and HCV29T (bladder cancer). All of them revealed cytotoxic activity against the cells of all 5 lines applied (Table 2 and Figure 1). The ID_{50} value for this compound varied from 0.8 (MCF–7) to 10.0 μg/ml (KB). These values are in the range of the international activity criterion for synthetic agents (4 μg/ml) (19). Thus, both compounds may be considered as the agents with potential antitumor activity and seem to be good



Scheme 1. Synthesis of 3-substituted aminopyrazolo[3,4-*b*]pyridines.

Reagents: **a:** chloroacetyl-, 3-chloropropionyl-, 4-chlorobutyrylchlorides; **b:** 2-bromopropionyl bromides, 2-chloropropionyl chlorides; **c:** *p*-methoxy-, 3,4,5-trimethoxy-, *p*-chloro-, *p*-nitro-, 3,5-dinitrobenzoylchlorides; **d:** crotonoyl chloride; **e:** morpholine N-methylpiperazine, N-diethylamine; **f:** $NaBH_4$.

Table 2. Antiproliferative activity *in vitro* of the compounds coded **II**, **III**, **V** and **IX** against the cells of human cancer cell lines

Compound	Cell line / ID_{50} [μg/ml] ± SD				
	KB	MCF-7	A-549	HCV29T	SW707
II	3.54 ± 1.09	0.80 ± 0.40	3.50 ± 1.00	1.80 ± 1.30	1.80 ± 1.03
III	10.00 ± 1.00	3.40 ± 1.00	5.70 ± 1.60	3.90 ± 1.03	3.90 ± 1.20
V				53.90 ± 1.10	
IX				48.00 ± 1.10	

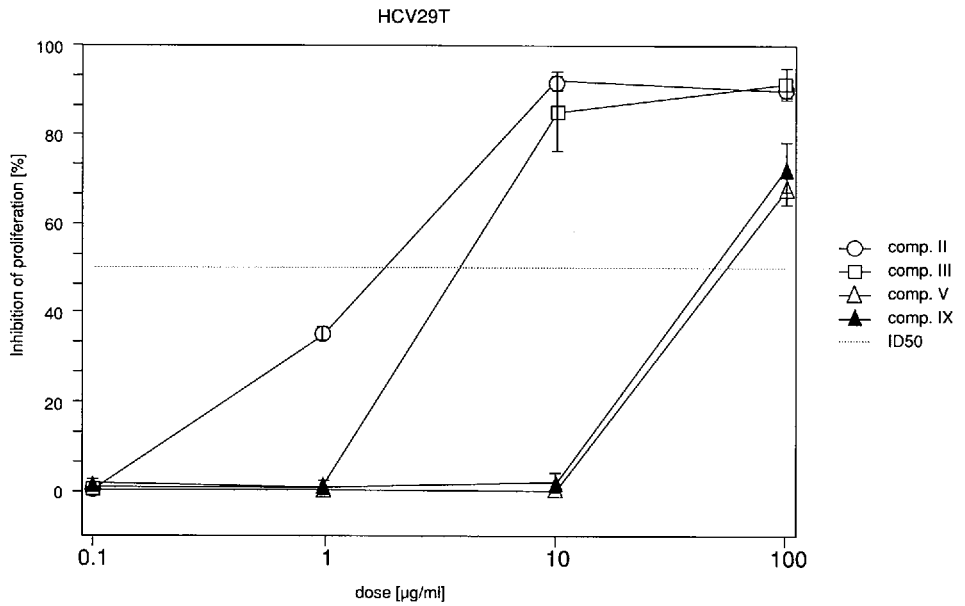


Figure 1. Antiproliferative activity *in vitro* of the compounds coded **II, III, V** and **IX** against the cells of human bladder cancer cell line HCV29T.

candidates for further stages of screening *in vitro* and/or *in vivo*.

The compounds 3–(3–chloropropionylamino)pyrazolo[3,4–*b*]pyridine **[V]** and 3–crotono–ylaminopyrazolo[3,4–*b*]pyridine **[IX]** were tested for their antiproliferative activity *in vitro* against the cells of human bladder cancer line HCV29T. Both compounds revealed a very weak cytotoxic activity, with ID_{50} values (54 and 48 μg/ml, respectively) not satisfying an activity criterion (Table 2 and Figure 1). All other compounds tested did not reveal any cytotoxic activity.

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