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SYNTHESIS AND ANTIPROLIFERATIVE ACTIVITY *IN VITRO* OF NEW PYRIDO[1,4-b]DIAZEPINE DERIVATIVES AND IMIDAZO[4,5-b]PYRIDINE

HANNA LISZKIEWICZ[1*], WANDA P. NAWROCKA[1], BARBARA SZTUBA[2], JOANNA WIETRZYK[3], JOANNA JAROSZEWICZ[3], ANNA NASULEWICZ[3] and MARZENA PEŁCZYŃSKA[3]

[1]Department of Technology of Drugs, [2]Department of Physical Chemistry, Wroclaw University of Medicine, 1 Nankier Sq., 50-140 Wrocław, Poland
[3] Department of Experimental Oncology, Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, 12 R. Weigla St., 53-114 Wrocław, Poland

Abstract: A novel series of esters **8-10** and hydrazones **4-6** was synthesized from 4-aryl-2-phenacylidene-1,3,4,5-tetrahydropyrido[2,3-b][1,4]diazepine (**1-3**). Subsequent treatment of hydrazone **4** with *p*-chlorbenzaldehyde furnished azine **7**. Long-standing heating of ester **8** with hydrazine hydrate afforded 3-[1-(p-chlorophenylene)-2-(5-phenyl-1H-pyrazol-3-yl)-ethyl]-1,3-dihydroimidazo[4,5-b]pyridin-2-one (**11**). The structures of **4-6** and **8-10** were identified by the results of elemental analysis and their IR, ^{1}H-NMR and MS spectra. Additionally, the structure of **11** was confirmed by X-ray diffraction method. Compounds **8–10** and **11** were examined for their antiproliferative activity *in vitro* against the cells of 5 human cancer cell lines, using SRB or MTT technique. Among tested compounds, only **11** revealed cytotoxic activity *in vitro* against all cell lines applied with ID_{50} (inhibitory dose 50%) values lower than 4 μg/mL, which is an international activity criterion for synthetic compounds. All compounds inhibit the proliferation of HL-60 human promyelocytic leukemia cell line.

Keywords: pyridodiazepine, hydrazones, transformation, imidazopyridine

The vast number of biologically active compounds includes also those with a diazepine ring. Fusion of the pure aromatic moiety and/or heteroaromatic systems, including various N-heterocycles to the different sides of the diazepine heptatomic ring modifies and broadens its pharmacological activity. The tricyclic pyridobenzodiazepinone drug, pirenzepine, shows measurable inhibitory effects exclusively toward the muscarinic receptor system (1). It is also approved to treat peptic ulcer (2) and to affect scleral metabolic changes in myopia (3). Dipyridodiazepinones were found to be not only more potent, but also more water-soluble and more resistant to metabolic N-dealkylation than pyridobenzodiazepinones. Nevirapine is the most noticeable example of dipyridodiazepinones, which is a potent and selective non-nucleoside inhibitor of HIV-1 reverse transcriptase and it was approved for use in the treatment of the HIV infection in humans (4–7).

Azines have recently received much attention due to their interesting chemical and spectral properties. For instance, they are potential nonlinear optical materials and they have been extensively used as ligands for the design of organometallic complexes (8). Among their useful properties, azines showed also antitumor activities (9).

Some of hydrazones show significant biological activities. They are used as antibacterial (10), anticonvulsant (11) and DNA-cleaving (12) agents. Recently, both diazepine derivatives (13, 14) and hydrazones (15–18) have been extensively studied due to their potent antitumor activities. However, it is quite common that anticancer drugs are associated with various adverse effects. Therefore, there is a need to search for new modified diazepines that can selectively inhibit cancer cell proliferation and exhibit biochemical stability. Our earlier report revealed that pyridodiazepine derivatives demonstrated a moderate cytotoxic activity (19). Significantly less research has been undertaken on the pyridodiazepines, compared to the benzodiazepines. Therefore, we considered it would be

* Corresponding author: e-mail:hanna@ktl.am.wroc.pl

interesting to prepare pyridodiazepines having an ester or hydrazone moiety and to investigate whether these changes could result in their ability to arrest cell division at low concentration.

EXPERIMENTAL

Chemistry

Melting points (uncorrected) were measured with a Boetius melting point apparatus. Analyses were performed on a Perkin Elmer 2400 analyzer and satisfactory results within ±0.4% calculated values were obtained for the new compounds. IR spectra (in KBr) were recorded with an IR 75 spectrophotometer and ^{1}H-NMR spectra – on a Bruker ARX 300 MHz and a Bruker Avance 500 MHz at room temperature using DMSO-d_6 or $CDCl_3$ as solvents. Chemical shifts are referred to the residual solvent signal at δ = 2.50 or 7.24 ppm, respectively. Mass spectra were determined on a GCMS-LK 82091 spectrometer at the ionization energy 70 eV and a Finnigan MAT TSQ 700 triple-stage quadrupole mass spectrometer equipped with an electrospray ion source. 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 the mobile phase.

4-(p-Chlorophenylene)-2-phenacylidene-1,3,4,5-tetrahydropyrido[2,3-b][1,4]diazepine (**1**) (20)

Yield: 2.3 g (63%); m.p. 204–206°C; IR (KBr, cm^{-1}): 3320, 3020, 1600, 1590, 1540, 1460, 1180; ^{1}H-NMR (500 MHz, DMSO-d_6, δ, ppm): 12.87 (s, 1H, NH), 7.92 (d, *J* = 4.8 Hz, 1H, H-7), 7.79 (d, *J* = 7.8 Hz, 2H, ArH), 7.50–7.34 (m, 8H, H-9 + ArH), 7.02 (d, *J* = 4.2 Hz, 1H, NH), 6.75 (dd, *J* = 7.4 Hz, 4.8 Hz, 1H, H-8), 5.84 (s, 1H, =CHCOPh), 5.10 (m, 1H, H-4), 3.04 (dd, *J* = 14.2 Hz, 3.0 Hz, 1H, H-3a), 3.00 (dd, *J* = 14.2 Hz, 6.7 Hz, 1H, H-3b); Analysis: for $C_{22}H_{18}N_3OCl$ (375.86); MS (m/z): 377 (M^++2, 33), 376 (M^++1, 25), 375 (M^+, 100).

General procedure for preparation of compounds (4–6)

A mixture of compound (**1, 2** or **3**) (0.01 mol) and 80% hydrazine hydrate (0.02 mol) in toluene was refluxd for 20 h using a Dean-Stark apparatus. The solvent was evaporated and the resulting residue was recrystallized from ethanol.

2-(2'-Hydrazone-2'-phenyl)ethylidene-4-phenyl-1,3,4,5-tetrahydropyrido[2,3-b][1,4]diazepine (**4**)

Yield: 2.7 g (77%); m.p. 167–169°C; IR (KBr, cm^{-1}): 3260, 3180, 1605, 1525, 820, 760; ^{1}H-NMR (500 MHz, DMSO-d_6, δ, ppm): 12.64 (s, 1H, NH), 7.69 (d, *J* = 7.5 Hz, 2H, Ar-H), 7.43 (d, *J* = 7.4 Hz, 2H, Ar-H), 7.38 (m, 2H, Ar-H), 7.29–7.24 (m, 4H, Ar-H), 7.17 (lt, 1H, Ar-H), 6.68 (dd, *J* = 7.3 Hz, 1.2 Hz, 1H, H-9), 6.45 (s, 1H, =CH), 6.31 (dd, *J* = 7.3 Hz, 5.0 Hz, 1H, H-8), 5.98 (d, *J* = 7.9 Hz, 1H, NH), 5.51 (m, 1H, H-4), 4.83 (s, 2H, NH_2), 3.20 (dd, *J* = 14.5 Hz, 8.7 Hz, 1H, H-3), 3.09 (dd, *J* = 14.5 Hz, 6.1 Hz, 1H, H-3); Analysis: for $C_{22}H_{21}N_5$ (355.45); MS (m/z): 355 (M^+, 13), 247 (6), 246 (2), 245 (5), 200 (5), 199 (74), 198 (100), 158 (5), 157 (6), 128 (8), 127 (6), 120 (250), 109 (16), 93 (42), 78 (7), 77 (12).

2-[2'-Hydrazone-2'-(p-chlorophenylene]ethylidene-1,3,4,5-tetrahydropyrido[2,3-b][1,4]diazepine (**5**)

Yield: 1.7 g (45%); m.p. 86–87°C; IR (KBr, cm^{-1}): 3250, 3180, 1600, 1520, 820, 750; ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 12.60 (s, 1H, NH), 7.51 (m, 3H, Ar-H), 7.27–7.10 (m, 8H, Ar-H), 6.70 (d, 1H, NH), 6.41–6.36 (dd, *J* = 7.2 Hz, 5.0 Hz, 1H, H-8), 6.23 (s, 1H, =CH), 5.51–5.37 (m, 1H, H-4), 4.40 (s, 2H, NH_2), 3.27–3.09 (d, 2H, H-3); Analysis: for $C_{22}H_{20}N_5Cl$ (389.89); MS (m/z): 389 (M^+, 3).

2-[2'-Hydrazone-2'-(p-bromophenylene]ethylidene-1,3,4,5-tetrahydropyrido[2,3-b][1,4]diazepine (**6**)

Yield: 2.3 g (53%); m.p. 130–134°C; IR (KBr, cm^{-1}): 3350, 3200, 1600, 870, 850; ^{1}H-NMR (300 MHz, DMSO-d_6, δ, ppm): 12.63 (s, 1H, NH), 7.70 (d, 2H, Ar-H), 7.50–7.27 (m, 8H, Ar-H), 6.67 (d, 1H, Ar-H), 6.48 (s, 1H, =CH), 6.34–6.29 (d, *J* = 5 Hz, 1H, H-8), 6.07 (d, 1H, NH), 5.47–5.40 (m, 1H, H-4), 4.85 (s, 2H, NH_2), 3.18–3.08 (d, 2H, H-3); Analysis: for $C_{22}H_{20}N_5Br$ (434.34).

2-[2'-(p-Chlorobenzylidenehydrazone)-2'-phenyl]ethylidene-4-phenyl-1,3,4,5-tetrahydropyrido[2,3-b][1,4]diazepine (**7**)

p-Chlorobenzaldehyde 1.4 g (0.01 mol) was added to the solution of compound **4** (3.6 g, 0.01 mol) in ethanol (50 mL). The reaction mixture was refluxed for 15 h. The solvent was evaporated and the resulting residue was recrystallized from n-butanol.

Yield: 3.1 g (64%); m.p. 97–99°C; IR (KBr, cm^{-1}): 3400, 300, 1630, 1600, 1570, 1510, 970, 840; ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 12.68 (s, 1H, NH), 8.73 (s, 1H, N=CH-Ph-Cl), 8.05–7.18 (m,18H, NH + Ar-H), 6.42 (s, 1H, =CH-C(-Ph)=N-N=), 5.51–5.48 (m, 1H, CH-Ph), 3.30 (d, 2H, >CH_2); Analysis: for $C_{29}H_{24}N_5Cl$ (477.50); MS (m/z): 477 (M^+, 6), 476 (M^+–1, 5), 472 (M^+–2, 16).

General procedure for preparation of compounds (8–10)

A mixture of compoud **1** (0.01 mol), appropriate alkyl chloroformate (ethyl, propyl, isobutyl chloroformate) (0.02 mol) and triethylamine (0.02 mol) in anhydrous toluene (50 mL) was refluxed for 10 h. The solvent was evaporated *in vacuo* and water (50 mL) was added. The resulting solid was filtered, washed with water, dried, decolorized with charconal and recrystallized from ethanol.

4-(p-Chlorophenylene)-5-ethoxycarbonyl-2-phenacylidene-1,3,4,5-tetrahydropyrido[2,3-b][1,4]diazepine (**8**)

Yield: 4.4 g (91%); m.p. 171–173°C; IR (KBr, cm^{-1}): 3380, 2980, 1725, 1660, 1570, 1550, 1380, 1310, 1270, 1200, 880; ^{1}H-NMR (300 MHz, DMSO-d_6, δ, ppm): 12.12 (s, 1H, NH), 8.41 (dd, *J* = 4.6 Hz, 1.6 Hz, 1H, H-7), 8.01 (m, 2H, Ar-H), 7.87 (d, *J* = 8.0 Hz, 1.6 Hz, 1H, H-9), 7.57–7.52 (m, 8H, Ar-H), 6.48 (s, 1H, =CH), 5.68 (t, 1H, H-4), 3.95 (q, 2H, CH_2), 2.63 (d, 2H, H-3), 1.01–0.97 (t, 3H, CH_3); Analysis: for $C_{25}H_{22}N_3O_3Cl$ (447.92).

4-(p-Chlorophenylene)-2-phenacylidene-5-propoxycarbonyl-1,3,4,5-tetrahydropyrido[2,3-b][1,4]diazepine (**9**)

Yield: 4.0 g (87%); m.p. 141–143°C; IR(KBr, cm^{-1}): 3440, 2970, 1760, 1660, 1580, 1560, 1400, 1320, 1280, 950, 830; ^{1}H-NMR (300 MHz, $CDCl_3$-d_6, δ, ppm): 12.48 (s, 1H, NH), 8.43–8.41 (dd, *J* = 4.6 Hz, 1.5 Hz, 1H, H-7), 7.95–7.92 (m, 2H, Ar-H), 7.53–7.45 (m, 6H, Ar-H), 7.33–7.24 (m, 3H, Ar- H), 6.10 (s, 1H, = CHCOPh), 5.79–5.73 (t, 1H, H-4), 3.97–3.93 (t, 2H, CH_2), 2.67 (d, 2H, H-3), 1.59–1.49 (m, 2H, CH_2), 0.73–0.67 (t, 3H, CH_3); Analysis: for $C_{26}H_{24}N_3O_3Cl$ (461.95); MS (m/z): 463 (M^++2, 36), 462 (M^++1, 29), 461 (M^+, 100).

5-*i*-Butoxycarbonyl-4-(p-chlorophenylene)-2-phenacylidene-1,3,4,5-tetrahydropyrido[2,3-b][1,4]diazepine (**10**)

Yield: 4.2 g (88%); m.p. 152–154°C; IR (KBr, cm^{-1}): 3450, 2982, 1760, 1660, 1585, 1560, 1455, 1320, 900, 830; ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 12.49 (s, 1H, NH), 8.43–8.41 (dd, *J* = 4.6 Hz, 1.4 Hz, 1H, H-7), 7.95–7.92 (m, 2H, Ar-H), 7.58–7.42 (m, 6H, Ar-H), 7.33–7.29 (m, 2H, Ar-H), 6.10 (s, 1H, =CHCOPh), 5.78–5.74 (m, 1H, H-4), 3.79–3.76 (d, 2H, CH_2), 2.77–2.66 (d, 2H, H-3), 1.73–1.64 (m, 1H, CH), 0.72–0.67 (m, 6H, 2×CH_3); Analysis: for $C_{27}H_{26}N_3O_3Cl$ (475.97); MS (m/z): 477 (M^++2, 36), 476 (M^++1, 30), 475 (M^+, 100).

3-[1-(p-Chlorophenylene)-2-(5-phenyl-1H-pyrazol-3-yl)-ethyl]-1,3-dihydroimidazo[4,5-b]pyridin-2-one (**11**)

Compoud **8** (4.48 g, 0.01 mol) and 80% hydrazine hydrate (1.30 g, 0.04 mol) in toluene (50 mL) were refluxed for 48 h. The mixure was evaporated *in vacuo*. The residue was decolorized with charcoal and recrystallized from ethanol.

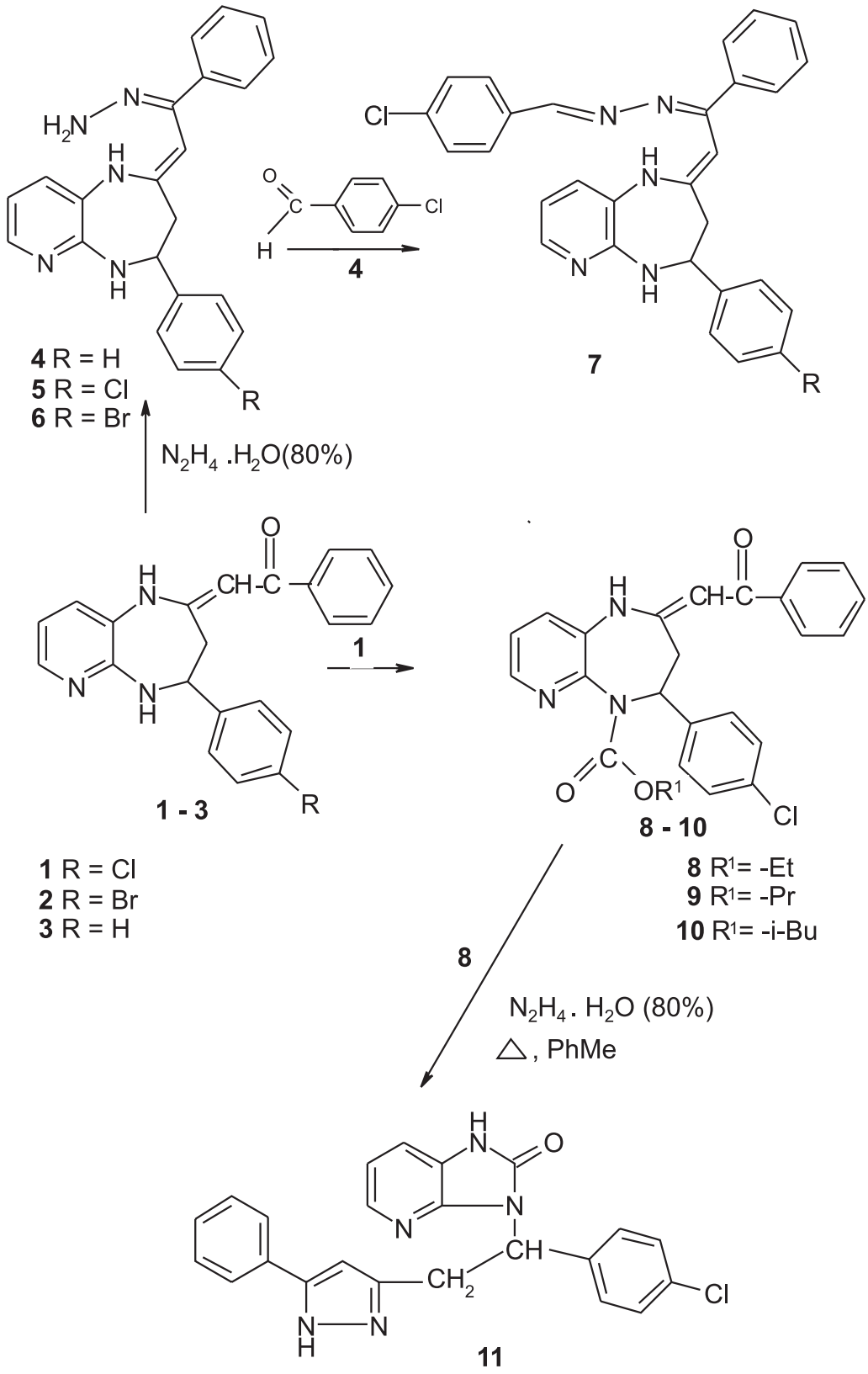
Yield: 2.7 g (64%); m.p. 236–237°C; IR (KBr, cm^{-1}): 3260, 3020, 2840, 1690, 1625, 1585, 950, 890; ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm): 12.77 (br s, 1H), 11.14 (s, 1H, pyrazole), 7.96 (d, *J* = 5.2 Hz, 1H, H-6), 7.61–7.58 (m, 4H, Ar-H), 7.42–7.24 (m, 6H, Ar-H and H-4), 7.00 (dd, *J* = 7.0 Hz, 5.2 Hz, 1H, H-5), 6.29 (s, 1H, pyrazole), 5.94 (dd, *J* = 9.7 Hz, 6.3 Hz, 1H, CH), 4.12 (dd, *J* = 14.3 Hz, 9.7 Hz, 1H, CH_2), 3.67 (dd, *J* = 14.3 Hz, 6.3 Hz, 1H, CH_2); Analysis: for $C_{23}H_{18}N_5OCl$ (415.97); MS (m/z): 415 (M^+, 4), 282 (28); ESI MS (m/z): 416 (MH^+, 100).

Biological test procedures

Cell lines

The following established *in vitro* human cancer cell lines were applied: HL-60 (leukemia), SW 707 (rectal adenocarcinoma), HCV-29T (urinary bladder cancer), MES-SA (uterine sarcoma) and HepG2 (liver cancer). All cancer cell lines (with exception of HL-60) were obtained from the American Type Culture Collection (Rockville, MD, USA) and are maintained in culture or frozen in Cell Culture Collection of the Institute of Immunology and Experimental Therapy, Wrocław, Poland. Human promyelocytic leukemia HL-60 cell line was obtained from the European Type Culture Collection by courtesy of Professor Spik and Dr. Mazurier (Laboratory of Biological Chemistry USTL, Lille, France).

Twenty-four hours before addition of the tested agents, the cells were plated in 96-well plates (Sarstedt, USA) at a density of 10^4 cells per well in 100 µL of culture medium. The cells were cultured in the opti-MEM or RPMI-1640 medium supplemented with 2 mM glutamine (Gibco, Warszawa, Poland), streptomycin (50 µg/mL), penicillin (50 U/mL) (both antibiotics from Polfa, Tarchomin, Poland) and 5% fetal calf serum (Gibco, Grand Island, USA). The cell cultures were maintained at 37°C in humid atmosphere saturated with 5% CO_2. The results of cytotoxic activity *in vitro* were expressed as ID_{50} values – the dose of compound (in µg/mL) that inhibits proliferation rate of the tumor cells by 50% as compared to the control untreated cells.


4 R = H
5 R = Cl
6 R = Br
4
7
N2H4 .H2O(80%)
1 - 3
1
8 - 10
1 R = Cl
2 R = Br
3 R = H
8 R1= -Et
9 R1= -Pr
10 R1= -i-Bu
8
N2H4 . H2O (80%)
△, PhMe
11

Scheme 1.

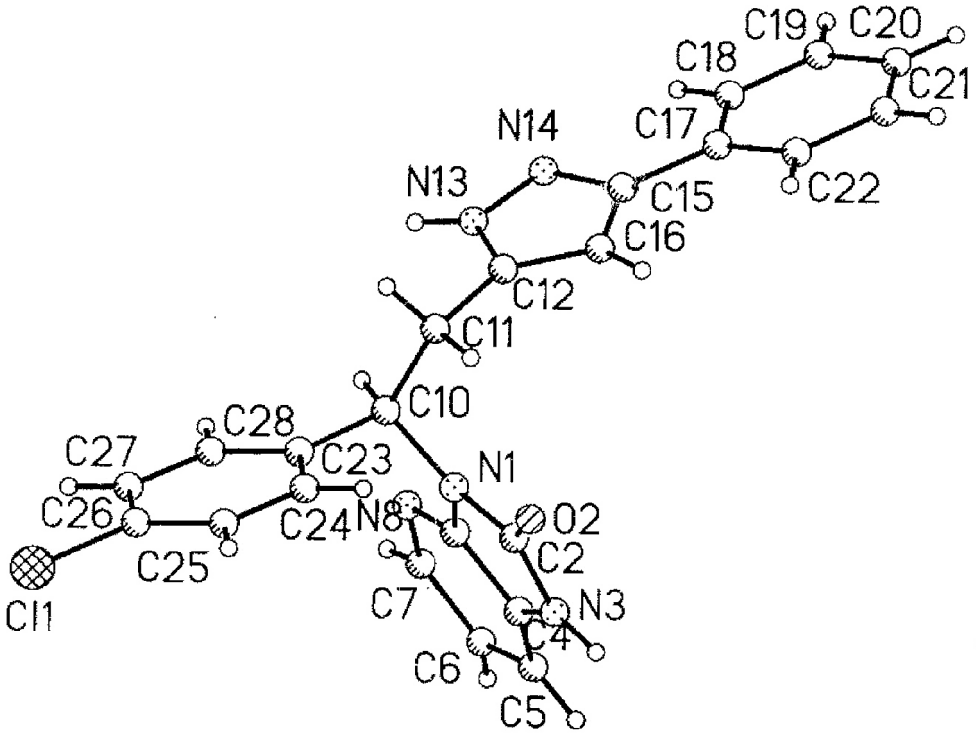


Figure 1.

Table 1. Antiproliferative activity of the compounds 8 – 11 against the cells of human cancer cell lines.

Compound	ID_{50} [μg/mL]				
	HL-60	HCV-29T	SW707	HepG2	MES-SA
8	8.1 ± 1.8	-	-	-	-
9	2.2 ± 1.1	6.1 ± 1.4	-	-	-
10	2.7 ± 1.0	9.1 ± 1.1	-	-	-
11	2.9 ± 1.8	2.9 ± 1.1	3.3 ± 1.0	3.5 ± 1.1	3.0 ± 1.1

- negative in the concentrations used

SRB (SW-707, HCV-29T, MES-SA and Hep-G2)

The details of this technique were described [24]. The cytotoxicity assay was performed after 72-h 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 the wells filled with 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 (4×) 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).

MTT (HL-60)

This technique was applied for the cytotoxicity screening against human leukemia cells growing in suspension culture. An assay was performed after 72-h exposure to varying concentrations (from 0.1 to 100 µg/mL) of the tested agents. For the last 3–4 h of incubation 20 µL of MTT solution were added to each well (MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Sigma, St. Louis, MO, USA); stock solution: 5 mg/mL). The mitochondria of viable cells reduce a pale yellow MTT to a navy blue formazan, so if more viable cells are present in well, more MTT will be reduced to formazan. When incubation time was completed, 80 µL of the lysing mixture were added to each well (lysing mixture: 225 mL dimethylformamide, 67.5 g sodium dodecyl sulfate (both from Sigma, St. Louis, MO, USA) and 275 mL of distilled water). After 24 h, when formazan crystals had been dissolved, the optical densities of the samples were read on an Multiskan RC photometer (Labsystems) at 570 nm wavelength.

Each compound in given concentration was tested in triplicates in each experiment. The experiments were repeated 3–5 times.

RESULTS, DISCUSSION AND CONCLUSION

Chemistry

Scheme 1 shows the synthetic pathways leading to the target derivatives. All the synthesized compounds were isolated in satisfactory yields (60–80%) and their molecular structures were consistent with both analytical and spectral: IR, ^{1}H-NMR and MS data..

Starting pyridodiazepines **1-3** were prepared as previously described (19, 20) and converted into hydrazones **4–6** by treatment with hydrazine hydrate in toluene solution. The absence of characteristic absorption bands corresponding to stretching vibrations of the C=O group in their IR spectra and the appearance of two-proton singlets at $\delta \approx 4.8$ ppm ascribed to the amino group of the =N-NH_2 in their ^{1}H-NMR spectra appeared particularly diagnostic for the hydrazones obtained. Their mass spectra display molecular ions at m/z (%) = 355 (M^+, 13) and m/z (%) = 389 (M^+, 3) with low intensities, however, these ions confirm their molecular weights. Subsequent treatment of hydrazone **4** with p-chlorobenzaldehyde was set for a second N-substitution step and gave azine **7**. In its IR spectrum, a band representing an azomethine group appeared at $\nu \sim 1630$ cm^{-1}. Its ^{1}H-NMR spectrum showed the absence of the NH_2 absorption of the hydrazone **4** and the presence of the one-proton singlet at δ = 8.73 ppm corresponding to the azomethine (N=C**H**) proton resonance and confirmed the azine formation. The protons of phenyls and pyridodiazepine nucleus resonated at the expected regions (19, 20).

The reaction of pyridodiazepine **1** with selected alkyl chloroformates led to the formation of corresponding carbamates **8–10** without side products. Substitution of the respective alkoxycarbonyl group to ketone **1** was confirmed by the results of elemental and spectral analysis. However, condensation reaction could take place at the N-1 or N-5 position in a diazepine ring. ^{1}H-NMR spectra of compound **1** (*vide* Experimental) contain two one-proton singlets: the signal at δ = 12.87 ppm corresponding to the NH proton at position 1 and signal at δ = 7.02 ppm, which can be ascribed to the NH proton at position 5 (20). The absence of an amino signal (-N**H**-CH=) at δ = 7.02 ppm in the ^{1}H-NMR spectra of obtained compounds assured that substitution took place at the N-5 position.

Upon long-standing heating, treatment of 4-(p-chlorophenylene)-5-ethoxycarbonyl-2-phenacylidene-1,3,4,5-tetrahydropyrido[2,3-b][1,4]diazepine (**8**) with an excess amount of hydrazine hydrate in toluene solution afforded an unexpected product. The results of elemental and MS analysis of this product indicate that the elimination of C_2H_5OH molecule leads to the compound represented by the formula $C_{23}H_{18}N_5OCl$. The mass spectrum recorded on a spectrometer equipped with an electrospray ion source showed a peak at m/z (%) = 416 (MH^+, 100) while the highest mass ion at m/z (%) = 415 (M^+, 4) appeared in the electron impact mass spectrum. Thus, both mass spectra confirmed the molecular weight determined on the base of elemental analysis data.

MS spectra recorded on different spectrometers contain two peaks corresponding to isotopic ions due to the presence of chlorine atom in the parent molecule. The low abundance of molecular ion in the electron impact mass spectrum suggested its extensive decomposition which led to the base peak at m/z (%) = 258 (100) corresponding to the elimination of 3-methyl-5-phenylpyrazole group. All spectral data supported the evaluation that obtained product has a structure of 3-[1-(p-chlorophenylene)-2-(5-phenyl-1*H*-pyrazol-3-yl)-ethyl]-1,3-dihydroimidazo[4,5-b]pyridin-2-one (**11**). Similar diazepine to imidazole ring conversions have been described earlier (21–23).

The structure of **11** was confirmed additionally by X-ray* analysis (Figure 1).

*X-ray diffraction study has been carried out by dr A. Białońska, Faculty of Chemistry, University of Wroclaw. Crystal data and structure refinement will be published.

Biology

Antiproliferative activity *in vitro*

Compounds **8–11** were examined for their antiproliferative activity in *in vitro* screening assay. The following human cancer lines were used: HL-60 (leukemia), HCV-29T (urinary bladder), SW 707 (rectal), HepG2 (liver) and MES-SA (uterine) (Table 1).

One among tested compounds, 3-[1-(p-chlorophenylene)-2-(5-phenyl-1H-pyrazol-3-yl)-ethyl]-1,3-dihydroimidazo[4,5-b]pyridin-2-one (**11**) exhibited significant antiproliferative activity against the cells of all cell lines applied. It seems that the activity of compound **11** can be attributed to the presence of pyridine, imidazole and pyrazole rings in the molecule. Carbamates **9** and **10** were active only against two cancer cell lines, namely HL-60 leukemia and HCV-29T urinary bladder cancer. Compound **8** revealed activity exclusively against HL-60 leukemia cells.

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