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Synthesis and Antiproliferative Activity *in vitro* of 1-Phenyl-2-(4-aryl-1,3,4,5-tetrahydropyrido[2,3-b][1,4]diazepin-2-ylidene)ethanone. Part I.

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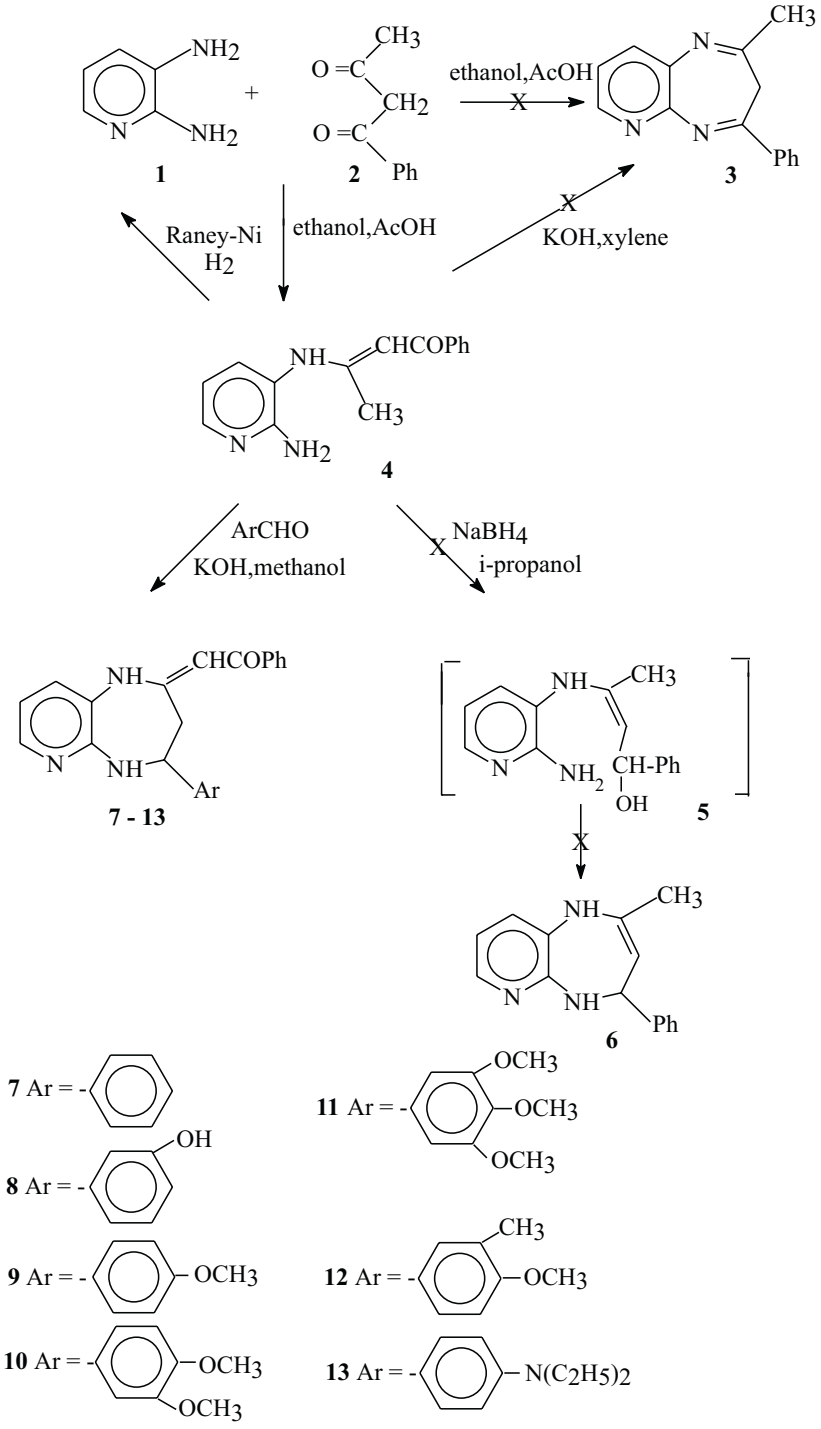
Reaction of 2,3-diaminopyridine with equimolar amount of benzoylacetone in ethanol solution in the presence of catalytic amounts of acetic acid allowed to obtain 3-(2-amino-pyridin-3-ylamino)-1-phenyl-2-buten-1-one (**4**) instead of 2-methyl-4-phenyl-3H-pyrido[2,3-b][1,5]diazepine (**3**) reported in the literature. Treatment of compound **4** with appropriate aromatic aldehydes: benzaldehyde and substituted benzaldehyde (4-methoxy-, 3,4-dimethoxy-, 3,4,5-trimethoxy-, 3-metyl-4-methoxy-, 3-hydroxy-, 4-diethyloamino-) in methanol solution in the presence of equimolar amount of potassium hydroxide gave 1-phenyl-2-(aryl-1,3,4,5-tetrahydropyrido[2,3-b][1,4]-diazepin-2-ylidene)-ethanones (**7–13**). Structures of compounds **4** and **7** were determined on a single crystal. Compounds **4** and **7–13** were examined for their antiproliferative activity *in vitro* against the cells of 7 human cancer cell lines, using SRB or MTT technique. Two out of all tested compounds revealed cytotoxic activity *in vitro*.

Key words: 3-(2-amino-pyridin-3-ylamino)-1-phenyl-2-buten-1-one, 1-phenyl-2(aryl-1,3,4,5-tetrahydropyrido[2,3-b][1,4]diazepin-2-ylidene)-ethanone, cytotoxicity, crystal structure

The derivatives of 1,4-diazepine have properties of biological and pharmacological interest [1–10]. These properties strongly depend on the nature of the heterocyclic core, particularly on the relative positions of the two nitrogen atoms and a type of ring fused to the seven-membered ring [11]. The reaction of aromatic and heterocyclic 1,2-diamines with 1,3-diketones is a versatile method for the preparation of condensed 1,4-diazepine systems [12–15]. The purpose of this work was to study the reaction of 2,3-diaminopyridine (**1**) with benzoylacetone (**2**) and aromatic aldehydes, which could allow to produce new pyridodiazepine derivatives of potential medicinal applications (Scheme 1).

Scheme 1


NH2
NH2
N
1
+
CH3
O =C
CH2
O =C
Ph
2
ethanol,AcOH
X
CH3
N
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N
Ph
3
Raney-Ni
H2
ethanol,AcOH
X
KOH,xylene
NH
CHCOPh
CH3
N
NH2
4
ArCHO
KOH,methanol
X
NaBH4
i-propanol
NH
CHCOPh
N
NH
Ar
7 - 13
NH
CH3
N
NH2
CH-Ph
OH
5
X
NH
CH3
N
NH
Ph
6
7 Ar = -
8 Ar = -
OH
9 Ar = -
OCH3
10 Ar = -
OCH3
OCH3
11 Ar = -
OCH3
OCH3
OCH3
12 Ar = -
CH3
OCH3
13 Ar = -
N(C2H5)2

RESULTS AND DISCUSSION

Chemistry: According to [16], heating of 2,3-diaminopyridine with equimolar amount of benzoylacetone in ethanol solution, in the presence of catalytic amounts of acetic acid should lead to 3-methyl-4-phenyl-3H-pyrido[2,3-b][1,5]diazepine (**3**) formation. In our treatment, the condensation of **1** with **2** gave a single product. Results of its elemental analysis revealed that the obtained compound was not pyridodiazepine (**3**).

The formula $C_{15}H_{15}N_3O_1$ of this product (**4**) was determined on the basis of its mass spectrum and elemental analysis. The molecular structure of this product was confirmed by IR and ^{1}H NMR spectra and X-ray diffraction study. Its IR spectrum displayed two sharp absorption bands at ν = 3420 and 3300 cm^{-1} characteristic for NH_2 group and the band ν =1680 cm^{-1} assigned to stretching vibrations of the C=O group. The two-proton singlet at δ = 5.9 ppm and the one-proton singlet at δ = 12.4 ppm in its ^{1}H NMR spectrum were ascribed to NH_2 and NH group, respectivetly. The singlet at δ = 6.1 ppm was attributed to the vinylene proton of =CHCOPh group and finally the 3-(2-aminopyridin-3-ylamino)-1-phenyl-2-buten-1-one (**4**) structure was ascribed. Heating of the compound **4** with NaOH in boiling ethanol or xylene did not yield diazepine **3**. Reduction of **4** by $NaBH_4$ in propanol or on Raney nickel in methanol led to the formation of 2,3-diaminopyridine **1** in both cases, it was confirmed by TLC and elemental analysis results as well as by IR and ^{1}H NMR spectral data. These results indicated that reduction of compound **4** was accompanied by its decomposition. Condensation of 3-(2-aminopyridin-3-ylamino)-1-phenyl-2-buten-1-one (**4**) with variety of substituted benzaldehydes in methanol solution and in the presence of equimolar potassium hydroxide gave pyridodiazepines **7–13**. Molecular structures of **7–13** was confirmed by elemental analysis and IR, MS and ^{1}H NMR spectra. These new compounds showed characteristic features of spectral data. The absence of an amino signal (-NH_2) in both ^{1}H NMR and IR spectra assured that this amino group participated in closing of a diazepine ring. The ^{1}H NMR spectra of these compounds showed distinguishing splitting patterns for two H-3 protons. Two doublets ranging between δ = 2.9 and δ = 2.8 ppm for compound **7** were assigned to protons H-3a and H-3b, respectively. The geminal coupling constant between them is J = 14.2 Hz and their vicinal coupling constans to H-4 indicate *trans* and *cis* geometry by J = 8.5 Hz and 1.2 Hz for H-3a and H-3b, respectively. The structural assignment of **7** was confirmed by crystallography. Coupling constants and chemical shifts are similar for hydrogen atoms located at the same relative positions in other studied compounds. Even though, compounds **9** and **11** showed less complex ^{1}H NMR spectra with a broad doublet at δ = 2.97 ppm for averaged H-3 protons. This observation revealed that a change in ring conformation for these two compounds occurs at room temperature at a rate, which is rapid on the NMR time scale.

Crystallographic part: Approximate views of the molecular structure and the intermolecular interactions of compounds **4** and **7** are shown in Fig. 1 and Fig. 2, respectively.

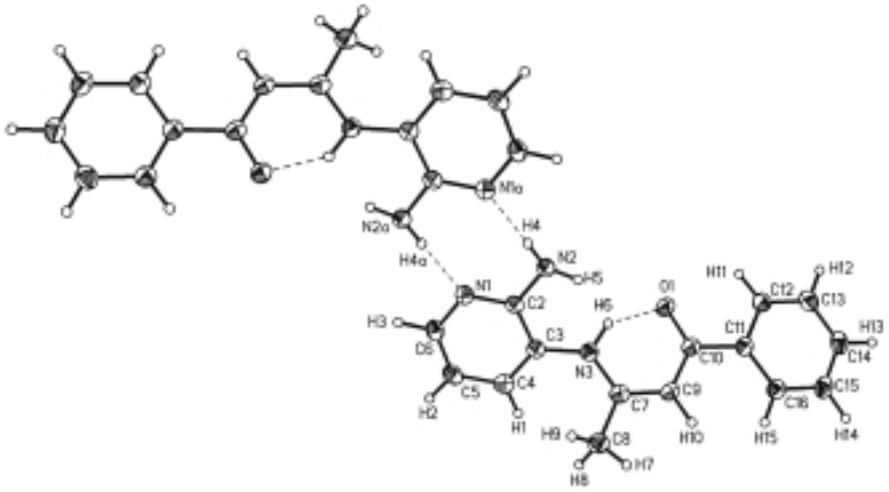

Figure 1. Molecular structure, intermolecular interactions and atoms labelling system of compound **4**.

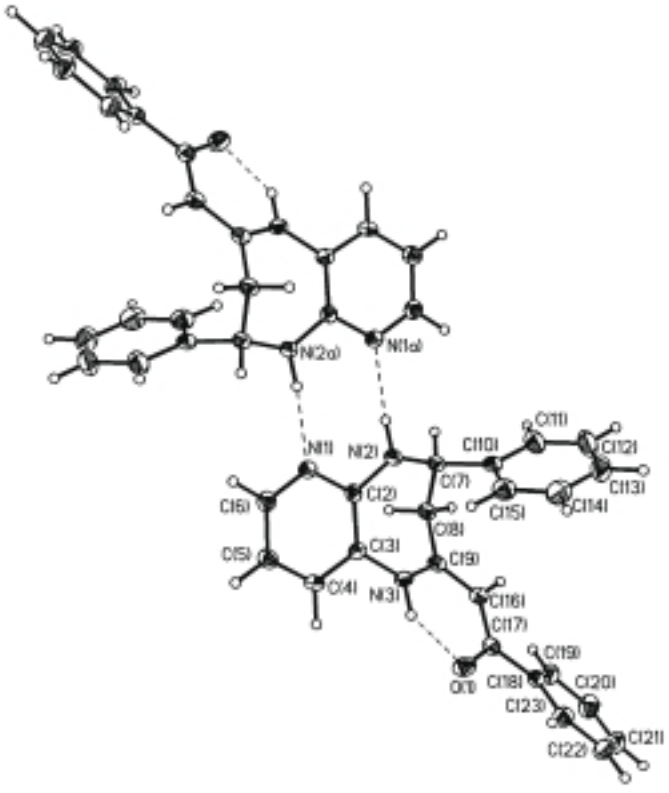

Figure 2. Molecular structure, intermolecular interactions and atoms labelling system of compound **7**.

The set of torsion angles C2–C3–N3–C7 (**4**) and C2–C3–N3–C9 (**7**) with values 137.3(1) and –35.5(3)°, respectively, indicates a possibility of rotation around the C3–N3 bond, which enables to place the reaction of **4** with benzaldehyde to pyridodiazepine **7** (Scheme 1). Values of appropriate torsion angles (Table 2) suggest a chance of rotation around C10–C12 and C17–C18 bonds in **4** and **7**, respectively. The double bonds C7–C9 and C9–C16 and intramolecular hydrogen bonds N3···O1 make rotation around these bonds impossible. The seven-membered [1,4] and/or [1,5] diazepine rings exhibit different degrees of deformation from a planar conformation. Both boat and chair conformation are possible for the diazepine rings. In compound **7** four atoms N2, C2, C3 and N3 are almost in the same plane as in other diazepines, *e.g.* [17]. Considering the distances of the remaining atoms C7, C8 and C9 from the mean N2/C2/C3/N3 plane, it should be noted that all these three atoms having the same sign are on the same side with respect to the plane [9,18–20] and their deviations are –0.495(2) for C7, –1.399(3) C8 and –0.628(2) Å for C9. Values of other geometric parameters for both studied structures remain consistent with data for related compounds [9,17].

Table 1. Crystal data and structure refinement for compounds (**4**) and (**7**).

Identification code	(**4**)	(**7**)
CCDC deposit no.	188634	188635
Empirical formula	$C_{15}H_{15}N_3O$	$C_{22}H_{19}N_3O$
Formula weight	253.30	341.40
Temperature [K]	100(1)	100(1)
Wavelength [Å]	0.71073	0.71073
Crystal system	Monoclinic	Triclinic
Space group	$P2_1/c$	$P\bar{1}$
a [Å]	13.266(3)	6.464(2)
b [Å]	12.229(2)	11.141(2)
c [Å]	7.948(2)	12.632(3)
α°		78.95(3)
β°	97.42(3)	85.33(3)
γ°		75.93(3)
V [Å^3]	1278.6(8)	865.5(4)
Z	4	2
D_c [Mg/m^3]	1.316	1.310
μ [mm^{-1}]	0.085	0.082
F(000)	536	360
Crystal size [mm]	0.15×0.15×0.20	0.20×0.20×0.20
2θ range [°]	6.6 to 52.0	6.6 to 52.0
Ranges h	–16 to 15	–7 to 7
k	–15 to 14	–13 to 9
l	–9 to 9	–15 to 15
Reflections collected	7537	5365
Reflections unique	2501	3279
R(int)	0.0231	0.0358
Data [$I > 2\delta(I)$]/parameters	2270/232	2346/311
Goodness-of-fit on F^2	1.100	1.031
R1, wR2 [$I > 2\delta(I)$]	0.0439, 0.0967	0.0469, 0.0891
R1, wR2 indices [all data]	0.0492, 0.1006	0.0804, 0.1018
Largest diff. peak and hole Δρ [eÅ^{-3}]	0.164 and –0.198	0.171 and –0.209

Table 2. Selected bond lengths [Å], angles and torsion angles [°] for compounds (**4**) and (**7**).

(4)		**(7)**	
O(1)–C(10)	1.257(2)	O(1)–C(17)	1.263(2)
N(1)–C(2)	1.334(2)	N(1)–C(2)	1.350(2)
N(1)–C(6)	1.345(2)	N(1)–C(6)	1.336(2)
N(2)–C(2)	1.372(2)	N(2)–C(2)	1.366(2)
N(3)–C(7)	1.345(2)	N(2)–C(7)	1.448(2)
N(3)–C(3)	1.410(2)	N(3)–C(9)	1.341(2)
C(2)–C(3)	1.413(2)	N(3)–C(3)	1.411(2)
C(3)–C(4)	1.375(2)	C(2)–C(3)	1.411(2)
C(4)–C(5)	1.389(2)	C(3)–C(4)	1.383(3)
C(5)–C(6)	1.369(2)	C(4)–C(5)	1.382(3)
C(7)–C(9)	1.381(2)	C(5)–C(6)	1.375(2)
C(7)–C(8)	1.492(2)	C(7)–C(10)	1.515(2)
C(9)–C(10)	1.421(2	C(7)–C(8)	1.534(2)
C(10)–C(11)	1.494(2	C(8)–C(9)	1.487(2)
		C(17)–C(18)	1.486(2)
O(1)–C(10)–C(9)	121.9(1)	O(1)–C(17)–C(16)	122.1(2)
O(1)–C(10)–C(11)	117.4(1)	O(1)–C(17)–C(18)	117.5(2)
C(2)–N(1)–C(6)	117.9(1)	C(6)–N(1)–C(2)	119.7(2)
C(7)–P(1)–O(4)	128.3(1)	C(2)–N(2)–C(7)	129.1(2)
N(1)–C(2)–N(2)	117.5(1)	C(9)–N(3)–C(3)	128.4(2)
N(1)–C(2)–C(3)	121.9(1)	N(1)–C(2)–N(2)	113.7(2)
N(2)–C(2)–C(3)	120.6(1)	N(1)–C(2)–C(3)	120.0(2)
C(4)–C(3)–N(3)	123.7(1)	N(2)–C(2)–C(3)	126.1(2)
C(4)–C(3)–C(2)	118.6(1)	N(3)–C(3)–C(4)	117.3(2)
N(3)–C(3)–C(2)	117.4(1)	N(3)–C(3)–C(2)	124.2(2)
N(1)–C(6)–C(5)	123.8(1)	N(1)–C(6)–C(5)	123.81(17)
N(3)–C(7)–C(9)	120.0(1)	N(2)–C(7)–C(10)	113.24(15)
N(3)–C(7)–C(8)	119.5(1)	N(2)–C(7)–C(8)	111.78(14)
		C(10)–C(7)–C(8)	111.77(15)
		C(9)–C(8)–C(7)	112.37(15)
		N(3)–C(9)–C(16)	120.99(16)
		N(3)–C(9)–C(8)	117.95(16)
C(2)–C(3)–N(3)–C(7)	137.3(1)	C(2)–C(3)–N(3)–C(9)	–35.5(3)
C(3)–N(3)–C(7)–C(9)	169.3(1)	C(3)–N(3)–C(9)–C(16)	–178.7(2)
N(3)–C(7)–C(9)–C(10)	–0.2(2)	N(3)–C(9)–C(16)–C(17)	–0.8(3)
C(7)–C(9)–C(10)–O(1)	–0.7(2)	C(9)–C(16)–C(17)–O(1)	0.2(3)
O(1)–C(10)–C(11)–C(12)	1.3(2)	O(1)–C(17)–C(18)–C(23)	–26.9(2)
C(3)–N(3)–C(7)–C(8)	–12.6(2)	C(3)-N(3)-C(8)-C(9)	1.7(2)

Table 3. Hydrogen-bonding geometry [Å °].

D–H...A	d(D–H)	d(H...A)	D(D...A)	<(DHA)
Compound (**4**)				
N(2)–H(4)...N(1)[–x, –y + 1, –z + 1]	0.95(2)	2.05(2)	3.006(2)	178(2)
N(3)–H(6)...O(1)	0.97(2)	1.72(3)	2.563(2)	143(2)
Compound (**7**)				
N(2)–H(4)...N(1)[–x + 1, –y + 1, –z]	0.93(2)	2.06(2)	2.963(2)	164(2)
N(3)–H(8)...O(1)	0.96(2)	1.82(2)	2.602(2)	137(2)

Table 4. Atomic coordinates and equivalent isotropic temperature factors for (**4**) and (**7**). U_{eq} is defined as one third of the trace of the orthogonalized U_{ij} tensor.

(**4**)	x	y	z	U_{eq}
O(1)	0.3155(1)	0.2474(1)	0.1854(1)	0.0305(3)
N(1)	–0.0544(1)	0.3869(1)	0.3790(2)	0.0226(3)
N(2)	0.1190(1)	0.4139(1)	0.4410(2)	0.0233(3)
N(3)	0.1586(1)	0.2056(1)	0.3315(2)	0.0221(3)
C(2)	0.0392(1)	0.3455(1)	0.3882(2)	0.0204(3)
C(3)	0.0569(1)	0.2370(1)	0.3381(2)	0.0210(3)
C(4)	–0.0253(1)	0.1711(1)	0.2864(2)	0.0251(3)
C(5)	–0.1228(1)	0.2132(1)	0.2835(2)	0.0265(3)
C(6)	–0.1332(1)	0.3205(1)	0.3276(2)	0.0247(3)
C(7)	0.2049(1)	0.1118(1)	0.3853(2)	0.0220(3)
C(8)	0.1539(1)	0.0360(1)	0.4949(2)	0.0284(3)
C(9)	0.3002(1)	0.0878(1)	0.3431(2)	0.0224(3)
C(10)	0.3532(1)	0.1586(1)	0.2431(2)	0.0218(3)
C(11)	0.4565(1)	0.1291(1)	0.2016(2)	0.0221(3)
C(12)	0.5051(1)	0.2026(1)	0.1051(2)	0.0259(3)
C(13)	0.5983(1)	0.1777(1)	0.0554(2)	0.0306(4)
C(14)	0.6441(1)	0.0782(1)	0.1005(2)	0.0300(4)
C(15)	0.5976(1)	0.0048(1)	0.1981(2)	0.0293(4)
C(16)	0.5044(1)	0.0302(1)	0.2486(2)	0.0257(3)
(**7**)	x	y	z	U_{eq}
O(1)	0.0802(2)	–0.0411(1)	0.1739(1)	0.0278(3)
N(1)	0.2775(2)	0.4609(1)	–0.0620(1)	0.0200(3)
N(2)	0.4777(2)	0.3430(1)	0.0777(1)	0.0245(4)
N(3)	0.2544(2)	0.1371(1)	0.0705(1)	0.0192(3)
C(2)	0.3278(3)	0.3492(2)	0.0053(1)	0.0185(4)
C(3)	0.2193(3)	0.2548(2)	0.0007(1)	0.0178(4)
C(4)	0.0575(3)	0.2816(2)	–0.0715(2)	0.0200(4)
C(5)	0.0005(3)	0.3983(2)	–0.1367(2)	0.0227(4)
C(6)	0.1173(3)	0.4840(2)	–0.1290(2)	0.0218(4)
C(7)	0.6221(3)	0.2336(2)	0.1348(2)	0.0208(4)
C(8)	0.6335(3)	0.1160(2)	0.0861(2)	0.0209(4)
C(9)	0.4382(3)	0.0663(2)	0.1123(2)	0.0185(4)
C(10)	0.5753(3)	0.2107(2)	0.2555(2)	0.0220(4)
C(11)	0.7412(4)	0.1619(2)	0.3260(2)	0.0317(5)
C(12)	0.7003(4)	0.1366(2)	0.4365(2)	0.0427(6)
C(13)	0.4930(4)	0.1578(2)	0.4781(2)	0.0430(6)
C(14)	0.3270(4)	0.2051(2)	0.4087(2)	0.0380(6)
C(15)	0.3673(3)	0.2329(2)	0.2980(2)	0.0290(5)
C(16)	0.4453(3)	–0.0489(2)	0.1782(2)	0.0198(4)
C(17)	0.2630(3)	–0.0990(2)	0.2071(2)	0.0196(4)
C(18)	0.2822(3)	–0.2241(2)	0.2775(2)	0.0204(4)
C(19)	0.4681(3)	–0.3181(2)	0.2799(2)	0.0256(4)
C(20)	0.4762(3)	–0.4357(2)	0.3408(2)	0.0311(5)
C(21)	0.3005(3)	–0.4593(2)	0.4035(2)	0.0323(5)
C(22)	0.1150(4)	–0.3663(2)	0.4033(2)	0.0315(5)
C(23)	0.1044(3)	–0.2491(2)	0.3396(2)	0.0262(4)

Supplementary data: CCDC No. 188634 and 188635 contain the supplementary crystallographic data for this paper. These data can be obtainet free of charge *via* www.ccdc.cam.ac.uk/conts/retreving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033; or deposit@ccdc.cam.ac.uk).

Pharmacology: ***Antiproliferative activity in vitro.*** The results of experiments, expressed as ID_{50} values, determined for each cancer cell line for both compounds studied, are summarized in Table 5. The adopted activity criterion of the new compounds in the *in vitro* screening tests was an ID_{50} level not exceeding 4 μg/ml [21]. None of the tested compounds satisfied this criterion. As shown in Table 1, the compound **4** revealed cytotoxic effect *in vitro* against the cells of all cancer cell lines used. Compound **10** was active only against the cells of both human leukemia cell lines and human bladder cancer cell line. These two compounds could be selected for further advanced studies *in vitro* using larger panel of human cancer cell lines of different tissue origin, and *in vivo* using experimental mouse tumor models.

Table 5. The cytotoxic activity *in vitro* (ID_{50} in μg/ml) of the tested compounds against the cells of various human cancer cell lines.

Cell line	Compound/ID_{50} [μg/ml]	
	4	**10**
MES-SA	27.2 ± 1.2	Neg
HCV29T	43.5 ± 1.1	84.2 ± 1.1
SW707	62.3 ± 1.1	Neg
LS-180	40.4 ± 1.1	Neg
HepG2	38.0 ± 1.0	Neg
HL-60	21.2 ± 1.1	29.5 ± 1.1
MOLT-4	28.5 ± 1.0	43.0 ± 2.3

EXPERIMENTAL

X-ray structure determination: The X-ray diffraction studies were carried out on a Kuma KM4CCD diffractometer, equipped with a graphite monochromated. The structures were solved by direct methods with SHELXS-97 [22] and refined by full-matrix least-squares methods on all F2 data using the SHELXL-97 [23] program. Non-hydrogen atoms were refined with anisotropic thermal parameters. The H-atoms were found from difference syntheses and refined with isotropic thermal parameters. A summary of data collection and structure refinement is given in Table 1; final atomic position parameters, selected bond lengths and bond angles are given in Table 2 and 3, respectively. Figures were drawn by using the XP program [24].

Biological test procedures. Test solutions of compounds **4** and **7–13** (1 mg/ml) were prepared *ex tempore* for each test by dissolving the compounds in 100 μl of DMSO + 900 μl of culture medium. After that, the tested solutions were diluted in culture medium (described below) to reach final concentrations 100, 10, 1 and 0.1 μg/ml.

Cell lines. Cells of the following human cancer lines were used: MES-SA (uterine carcinoma), SW707 and LS-180 (colon adenocarcinoma) HepG2 (hepatoma), MOLT-4 (lymphatic leukemia). All lines were obtained from American Type Culture Collection (Rockville, Maryland, U.S.A.) and cultured in Cell Culture Collection of the Department of Tumor Immunology, Institute of Immunology and Experimental Therapy, Wrocław, Poland. Human uroepithelial cell line HCV29T established in Fibiger Institute, Copenhagen, Denmark, was obtained from Dr. J. Kieler in 1982. Human promyelocytic leukaemia HL-60 cell line was obtained from 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, U.S.A.) at a density of 10^4 cells per well. The cells were cultured in the opti-MEM medium supplemented with 2 mM 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 maintained at 37°C in humid atmosphere saturated with 5% CO_2.

SRB and MTT assay. The cytotoxic assays were performed after 72-hour exposure of the cultured cells to varying concentrations (from 0.1 to 100 μg/ml) of the tested agents. The SRB method was used as described by Skehan *et al*. [25]. MTT technique was applied for the cytotoxicity screening against leukemia cells growing in suspension culture [26]. The optical densities of the samples were measured on a Multiskan RC photometer (Labsystems, Helsinki, Finland) at 570 nm (SRB) or 540 nm (MTT). The results were calculated as an ID_{50} (inhibitory dose 50%) – the dose of compound which inhibits proliferation rate of the tumor cells by 50% as compared to control untreated cells. Each compound in every concentration was tested in triplicates per experiment. Every experiment was repeated 3 times.

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. ^{1}H NMR spectra – on a Bruker ARX 300 MHz and Avance 500 MHz using DMSO-d_6 and $CDCl_3$ as solvents at room temperature and chemical shifts are referred to the residual solvent signal at δ 2.50 ppm and 7.24 ppm, respectively. Mass spectra were determined on a GCMS-LK 82091 spectrometer at the ionization energy 70 eV. The course of reaction and the purity of products were checked by TLC (Kieselgel G, Merck) in diethyl ether:ethanol = 5:1 for eluation.

*3-(2-Aminopyridin-3-ylamino)-1-phenyl-2-buten-1-one (**4**).* 2,3-Diaminopyridine (**1**) 1.09 g (10 mmol), benzoylacetone 1.62 g (10 mmol) and 1.0 ml glacial acetic acid in ethanol (30 ml) were refluxed for 6 h. The precipitate was collected by filtration, washed with cool water, dried and recrystallized from ethanol. Yield: 2.0 g (79%) yellow precipitate, m.p. 134–136°C; IR (KBr) ν cm^{-1}: 3420, 3300, 1680, 1550, 1470, 1325, 1280, 1190. ^{1}H NMR (DMSO, 300 MHz) δ (ppm): 12.42 (s, 1H, NH, D_2O exchangeable), 7.93–7.89 (m, 3H, H-6 + ArH), 7.53–7.37 (m, 4H, H-4 + ArH), 6.64–6.59 (dd, J = 4.9 Hz, 7.5 Hz, 1H, H-5), 6.09 (s, 1H, CH), 5.86 (s, 2H, NH_2, D_2O exchangeable), 1.93 (s, 3H, CH_3). Anal.: for $C_{15}H_{15}N_3O_1$ (253.70) – calcd.: C, 71.13; H, 5.97; N, 16.59 %. Found: C, 71.27; H, 5.96; N, 16.63 %. MS (70 eV) m/z (%): 254 (13), 253 (88), 239 (12), 238 (89), 149 (22), 148 (100), 147 (7), 135 (4), 134 (55), 133 (13), 109 (8), 106 (6), 105 (72), 93 (20), 78 (7), 77 (52).

*General procedure for preparation **7–13***. Compound **4** (10 mmol), appropriate aldehyde (10 mmol), KOH (10 mmol) in methanol (30 ml) were refluxed for 4 h. The solid precipitate was filtered, decolorized with charconal and recrystallized from toluene.

*1-Phenyl-2-(4-phenyl-1,3,4,5-tetrahydropyrido[2,3-b][1,4]diazepin-2-ylidene)-ethanone (**7**).* Yield: 2.05 g (60%), m.p. 220–221°C; IR (KBr) ν cm^{-1}: 3240, 1610, 1550, 1525, 1360, 1190. ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm): 13.03 (s, 1H, NH), 7.89 (dd, J = 4.8 Hz, 1.3 Hz, 1H, H-7), 7.83 (d, J = 7.9 Hz, 2H, ArH), 7.45–7.30 (m, 9H, H-9 + ArH), 6.74 (dd, J = 7.7 Hz, 4.8 Hz, 1H, H-8), 5.75 (s, 1H, =CHCOPh) 5.26 (br s, 1H, NH), 4.96 (d, J = 8.5 Hz, 1H, H-4), 2.92 (dd, J = 14.2 Hz, 8.5 Hz, 1H, H-3a), 2.80 (dd, J = 14.2, 1.2 Hz, 1H, H-3b). Anal.: for $C_{22}H_{19}N_3O_1$ (341.41) – calcd.: C, 77.40; H, 5.61; N, 12.31%. Found: C, 77.57; H, 5.60; N, 12.35%. MS (70 eV) m/z (%): 342 (20), 341 (100), 340 (7), 273 (13), 236 (83), 196 (40), 195 (18), 120 (12), 105 (27), 104 (4), 103 (4), 93 (6), 78 (6), 77 (26).

*1-Phenyl-2-(3-hydroxyphenyl)1,3,4,5-tetrahydropyrido[2,3-b][1,4]diazepin-2-ylidene)ethanone (**8**).* Yield: 2.1 g (59%), m.p. 245–247°C; IR (KBr) ν cm^{-1}: 3225, 1610, 1550, 1530, 1340, 1190; ^{1}H NMR (DMSO, 500 MHz) δ (ppm): 12.94 (s, 1H, NH), 9.32 (s, 1H, OH), 7.92 (dd, J = 4.7 Hz, 1.4 Hz, 1H, H-7), 7.82 (dd, J = 7.3 Hz, 1.4 Hz, 2H, ArH), 7.50–7.42 (m, 3H, ArH), 7.37 (dd, J = 7.7 Hz, 1.4 Hz, 1H, H-9), 7.10 (lt, J = 7.8 Hz, 1H, ArH), 6.83–6.79 (m, 3H, ArH + NH), 6.74 (dd, J = 7.7 Hz, 4.7 Hz, 1H, H-8), 6.60 (dd, J = 7.8 Hz, 1.6, 1H, ArH), 5.89 (s, 1H, =CHCOPh), 5.01–4.96 (m, 1H, H-4), 2.99 (dd, J = 13.2 Hz, ~ 0 Hz, 1H, H-3a), 2.96 (dd, J = 13.2 Hz, 7.1 Hz, 1H, H-3b). Anal.: for $C_{22}H_{19}N_3O_2$ (357.41) – calcd.: C, 73.93; H, 5.36; N, 11.76 %. Found: C, 73.66; H, 5.37; N, 11.56 %.

*1-Phenyl-2-{4-(4-methoxyphenyl)-1,3,4,5-tetrahydropyrido[2,3-b][1,4]diazepin-2-ylidene}ethanone (**9**).* Yield: 2.8 g (75%), m.p. 192–4°C; IR (KBr) ν cm^{-1}: 3220, 3020, 1620, 1600, 1550, 1520, 1360, 1170; ^{1}H NMR (DMSO, 300 MHz) δ (ppm): 12.94 (s, 1H, NH), 7.91 (dd, J = 4.7 Hz, 1.2 Hz, 1H, H-7), 7.81 (d, J = 7.5 Hz, 2H, ArH), 7.50–7.43 (m, 3H, ArH), 7.36 (d, J = 7.6 Hz, 1H, H-9), 7.29 (d, J = 8.6 Hz, 2H, ArH), 6.87 (d, J = 8.6 Hz, 2H, ArH), 6.83 (d, J = 3,9 Hz, 1H, NH), 6.74 (dd, J = 7.6 Hz, 4.7 Hz, 1H, H-8), 5.89 (s, 1H, =CHCOPh), 4.99 (dt, J = 4.5 Hz, 3.9 Hz, 1H, H-4), 3.68 (s, 3H, OCH_3), 2.97 (d, J = 4.5 Hz, 2H, H-3). Anal.: for $C_{23}H_{21}N_3O_2$ (371.44) – calcd.: C, 74.37; H, 5.70; N, 11.31%. Found: C, 74.13; H, 5.64; N,

11.11%. MS (70 eV) m/z (%): 372 (19), 371 (100), 370 (7), 267 (11), 266 (83), 250 (10), 237 (3), 236 (5), 227 (4), 226 (28), 225 (19), 134 (7), 133 (4), 132 (4), 105 (22), 93 (4), 78 (8), 77 (19).

*1-Phenyl-2{4-(3,4-dimethoxyphenyl)-1,3,4,5-tetrahydropyrido[2,3-b][1,4]-diazepin-2-ylidene}ethanone (**10**).* Yield: 2.5 g (62%), m.p. 193–194°C; IR (KBr) ν cm^{-1}: 3200, 1610, 1590, 1540, 1505, 1330, 1180. ^{1}H NMR (DMSO, 300 MHz) δ (ppm): 12.93 (s, 1H, NH), 7.91 (d, J = 4.6 Hz, 1H, H-7), 7.81 (d, J = 7.6 Hz, 2H, ArH), 7.51–7.43 (m, 3H, ArH), 7.36 (d, J = 7.6 Hz, 1H, H-9), 6.99 (s, 1H, ArH), 6.87 (s, 2H, ArH), 6.77–6.73 (m, 2H, H-8 + NH), 5.92 (s, 1H, =CHCOPh), 4.97 (m, 1H, H-4), 3.68 (s, 3H, OCH_3), 3.67 (s, 3H, OCH_3), 3.02 (dd, J = 14,6 Hz, 7.3 Hz, 1H, H-3a), 2.95 (d, J = 14.6 Hz, 1H, H-3b). Anal.: for $C_{24}H_{23}N_3O_3$ (401.46) – calcd.: C, 71.80; H, 5.77; N, 10.47%. Found: C, 71.96; H, 5.67; N, 10.32%. MS (70 eV) m/z (%): 403 (23), 402 (100), 297 (13), 296 (77), 256 (31), 255 (14), 250 (10), 151 (9), 121 (7), 105 (24), 93 (4), 78 (4), 77 (18).

*1-Phenyl-2-{4-(3,4,5-trimethoxyphenyl)-1,3,4,5-tetrahydropyrido[2,3-b][1,4]-diazepin-2-ylidene}-ethanone (**11**).* Yield: 3.0 g (70%), m.p. 200–201°C; IR (KBr) ν cm^{-1}: 3250, 3090 (CH), 1600, 1560, 1510, 1360, 1200; ^{1}H NMR (DMSO, 500 MHz) δ (ppm): 12.87 (s, 1H, NH), 7.93 (dd, J = 4.7 Hz, 1.5 Hz, 1H, H-7), 7.82 (dd, J = 7.9, 1.3 Hz, 2H, ArH), 7.52–7.40 (m, 4H, H-9 + ArH), 6.79 (d, J = 2.8 Hz, 1H, NH), 6.78 (dd, J = 8.0 Hz, 4.7 Hz, 1H, H-8), 6.74 (s, 2H, ArH), 5.94 (s, 1H, =CHCOPh), 4.98 (dt, J = 5.1 Hz, J = 2.8 Hz, 1H, H-4), 3.71 (s, 6H, 2xOCH_3), 3.57 (s, 3H, OCH_3), 2.97 (d, J = 5.1 Hz, 2H, H-3). Anal.: for $C_{25}H_{25}N_3O_4$ (431.49) – calcd.: C, 69.59; H, 5.84; N, 9.74%. Found: C, 69.39; H, 5.88; N, 9.57 %. MS (70 eV) m/z (%): 432 (20), 431 (100), 337 (9), 336 (60), 287 (4), 286 (26), 285 (8), 250 (11), 105 (20), 93 (3), 77 (12).

*1-Phenyl-2-{4-(3-methyl-4-methoxyphenyl)-1,3,4,5-tetrahydropyrido[2,3-b][1,4]-diazepin-2-ylidene}ethanone (**12**).* Yield: 2.8 g (72%), m.p. 200–202°C; IR (KBr) ν cm^{-1}: 3220, 1610, 1590, 1540, 1330, 1180. ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm): 13.07 (s, 1H, NH), 7.90 (dd, J = 4.8 Hz, 1.4 Hz, 1H, H-7), 7.85 (dd, J = 7.0 Hz, 1.2 Hz, 2H, ArH), 7.46–7.38 (m, 3H, ArH), 7.31 (dd, J = 7.7 Hz, 1.4 Hz, 1H, H-9), 7.18 (d, J = 8.3 Hz, 1H, ArH), 7.15 (s, 1H, ArH), 6.79 (d, J = 8.3 Hz, 1H, ArH), 6.73 (dd, J = 4.8 Hz, 7.7 Hz, 1H, H-8), 5.78 (s, 1H, =CHCOPh), 5.16 (br s, 1H, NH), 4.84 (d, J = 8.8 Hz, 1H, H-4), 3.80 (s, 3H, OCH_3), 2.89 (dd, J = 14.1 Hz, 8.8 Hz, 1H, H-3a), 2.74 (d, J = 14.1 Hz, 1H, H-3b), 2.20 (s, 3H, CH_3). Anal.: for $C_{24}H_{23}N_3O_2$ (385.47) – calcd.: C, 74.78; H, 6.01; N, 10.90%. Found: C, 74.66; H, 6.09; N, 10.77%. MS (70 eV) m/z (%): 386 (26), 385 (100), 384 (9), 281 (15), 280 (83), 266 (6), 265 (5), 264 (5), 251 (4), 250 (12), 240 (36), 239 (40), 246 (5), 135 (9), 133 (6), 132 (5), 131 (5), 120 (7), 106 (3), 105 (23), 93 (4), 78 (4), 77 (18).

*1-Phenyl-2-{2-(4-diethylaminophenyl-1,3,4,5-tetrahydropyrido[2,3-b][1,4]-diazepin-2-ylidene}-ethanone (**13**).* Yield: 2 g (48%), m.p. 217–219°C; IR (KBr) ν cm^{-1}: 3260, 1610, 1540, 1380, 1240, 1180. ^{1}H NMR (DMSO, 500 MHz) δ (ppm): 12.95 (s, 1H, NH), 7.91 (d, J = 4.6 Hz, 1H, H-7), 7.84 (d, J = 8.0 Hz, 2H, ArH), 7.50–7.42 (m, 3H, ArH), 7.38 (d, J = 7.6 Hz, 1H, H-9), 7.16 (d, J = 8.3 Hz, 2H, ArH), 6.75 (dd, J = 4.7 Hz, 7.6 Hz, 1H, H-8), 6.61 (d, J = 8.3 Hz, 2H, ArH), 6.52 (d, J = 2.6 Hz, 1H, NH), 5.95 (s, 1H, =CHCOPh), 4.87–4.85 (m, 1H, H-4), 3.28 (q, J = 7.0 Hz, 4H, 2×CH_2), 2.94 (dd, J = 14.2 Hz, 7.6 Hz, 1H, H-3a), 2.89 (dd, J = 14.2 Hz, 2.9 Hz, 1H, H-3b), 1.02 (t, J = 7.0 Hz, 6H, 2×CH_3). Anal.: for $C_{26}H_{28}N_4O_1$ (412.53) – calcd.: C, 75.70; H, 6.84; N, 13.58%. Found: C, 75.66; H, 6.99; N, 13.67%. MS (70 eV) m/z (%): 414 (22), 413 (84), 412 (9), 308 (20), 307 (100), 293 (10), 267 (15), 266 (10), 163 (4), 162 (28), 161 (5), 160 (11), 120 (5), 106 (4), 105 (25), 104 (3), 93 (4), 77 (14).

REFERENCES

1. Littell R. and Allen D.S., *J. Med. Chem*., **8**, 722 (1965).
2. Fiakpui C.Y., Arora V.K. and Knaus E.E., *J. Heterocycl. Chem. I,* **30**, 699 (1993).
3. Fiakpui C.Y., Phillips O.A., Murthy K.S.K. and Knaus E.E., *J. Heterocycl. Chem*., **36**, 377 (1999).
4. Grozinger K.G., Byrne D.P., Nummy L.J., Ridges M.D. and Salvagno A., *J. Heterocycl. Chem.,* **37**, 229 (2000).
5. Eberlein W.G., Engel W.W., Trummlitz G., Schmidt G. and Hammer R., *J. Med. Chem.,* **31,** 1169 (1988).
6. Watanabe T., Kakefuda A., Tanaka A., Takizawa K., Hirano S., Shibata H., Yamagiwa Y. and Yanagisawa I., *Chem. Pharm. Bull.*, **46**, 53 (1998).
7. Karton T., Bradbury B.J., Baumgold J., Paek R. and Jacobson K.A., *J. Med. Chem.*, **24**, 2133 (1991).
8. Savelli F., Boido A. and Mulé A., *Bull. Chim. Farmaceutico – Anno 128,* **4**, 132 (1989).

9. Nawrocka W., Sztuba B., Opolski A., Wietrzyk J., Kowalska M.W. and Głowiak T., *Arch. Pharm. Pharm. Med. Chem.,* **334**, 1 (2001).
10. Nawrocka W., Sztuba B. and Zimecki M., *Arch. Pharm. Pharm. Med. Chem.,* **334**, 11 (2001).
11. Blache Y., Hichour M., Di Blasi G., Chezal J.M., Viols H., Chavignon O., Teulade J.C. and Chapat J.P., *Heterocycles,* **51**, 1003 (1999).
12. Halford J.C. and Fitsch R.M., *J. Am. Chem. Soc.,* **85**, 3354 (1963).
13. Staab H.A. and Vögtle F., *Chem. Ber.,* **98**, 2701 (1963).
14. Mannschreck A., Rissmann G., Vögtle F. and Wild D., *Chem. Ber.,* **100**, 335 (1967).
15. Nawojski A. and Nawrocka W., *Roczn. Chem.,* **48**, 1073 (1974).
16. Nawojski A., *Prace Naukowe Akademii Medycznej we Wrocławiu*, *Rozprawa habilitacyjna*, *Akademia Medyczna, Wrocław*, 5(10) (1969).
17. Low J.N., Cobo J., Insuasty B., Insuasty H., Nogueras M. and Sanchez A., *Acta Cryst.*, **C58**, 103 (2002).
18. Abramenko B.L., Sergienko V.S., Garnovskii A.D. and Porai-Kochitz M.A., *Zh. Obsch. Khim.*, **60**, 1593 (1990).
19. Rodier N., Agafonov V., Dubois P., Mariand M. and Cense J.M., *Acta Cryst.*, **C49**, 156 (1993).
20. Dupont L., Liégeois I.F., Rogister F. and Delagre J., *Acta Cryst.*, **C52**, 391 (1996).
21. Geran R.I., Greenberg N.H., MacDonald M.M., Schumacher A.M. and Abbott B.J., *Cancer Chemotherapy Rep.*, **3**, 59 (1972).
22. Sheldrick G.M., SHELXS97, Program for solution of crystal structures, University of Göttingen (1997).
23. Sheldrick G.M., SHELXL97, Program for crystal structure refinement, University of Göttingen (1997).
24. Sheldrick G.M., SHELXTL-NT V5.1, Bruker AXS (1999).
25. Skehan P., Storeng R., Scudiero D., Monks A., McMahon J., Vistica D., Warren J.T., Bokesch H., Kenney S. and Boyol M.R., *J. Natl. Cancer. Inst*., **82,** 1107 (1990).
26. Opolski A., Kuduk-Jaworka J., Wietrzyk J., Wojdat E., Waszkiewicz K., Romaniewska A., Radzikowski C., *Anticancer Drugs,* **11**, 363 (2000).