HL ISSN: 2231 – 3087(print) / 2230 – 9632 (Online)
http://heteroletters.org Vol. 2: (2), 2012, 198-205

SYNTHESIS AND CYTOTOXICITY OF 4-[(*E*)-HETARYL-VINYL]-6,6-DIMETHYL-2-OXO-1,2,5,6-TETRAHYDRO-PYRIDINE-3-NITRILES

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ABSTRACT:

A detailed investigation of condensation of 4,6,6-trimethyl-2-oxo-1,2,3,4-tetrahydropyridine-3-nitrile with heteroaromatic aldehydes in the presence of catalytic amounts of NaOH in EtOH was presented. 4-[(E)-Hetaryl-vinyl]-6,6-dimethyl-2-oxo-1,2,5,6-tetrahydro-pyridine-3-nitriles were isolated in 50-97 % yields. The cytotoxicity of studied compounds towards HT-1080 (human fibrosarcoma), MG22A (mouse hepatoma) and 3T3 (mouse embryonic fibroblasts) was described. 4-[(E)-2-(6-Bromo-2-pyridyl)-vinyl]-6,6-dimethyl-2-oxo-1,2,5,6-tetrahydro-pyridine-3-nitrile exhibit high activity against MG-22A cancer cell line.

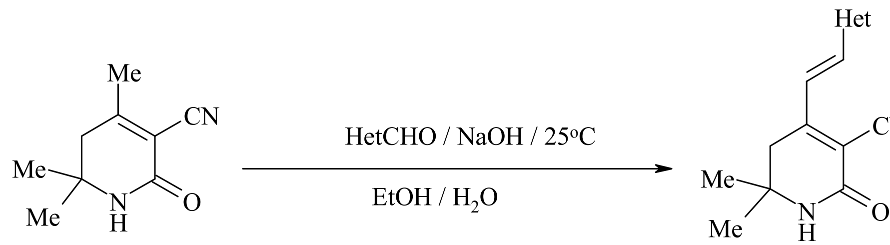
Keywords: 4,6,6-trimethyl-2-oxo-1,2,3,4-tetrahydropyridine-3-nitrile, 4-[(E)-hetaryl-vinyl]-6,6-dimethyl-2-oxo-1,2,5,6-tetrahydro-pyridine-3-nitriles, heteroaromatic aldehydes, condensation, cytotoxicity

INTRODUCTION

2-Pyridone derivatives are of interest as anticancer and cytotoxic agents [1]. Beside this some recent publications were dedicated to investigation of antitumoral activity of unsaturated derivatives of pyridones [2-4]. Among these works synthesis of cytotoxic and anticancer derivatives of Citridone A [2] and camptothecin [3] were presented. It is well known that combretastatins [5] and bis-styrylpyridines [6] exhibit high anticancer activity. The present work is carried out in the continuation of our previous work dedicated to the synthesis and investigation of cytotoxicity of 4-[(*E)*-aryl-vinyl]-6,6-dimethyl-2-oxo-1,2,5,6-tetrahydro-pyridine-3-nitriles [7]. Condensation of 4,6,6-trimethyl-2-oxo-1,2,3,4-tetrahydropyridine-3-nitrile with heteroaromatic aldehydes was not investigated till now and therefore is one of aim of present work. The second aim is investigation of cytotoxicity of obtained 4-[(*E*)-hetaryl-vinyl]-6,6-dimethyl-2-oxo-1,2,5,6-tetrahydro-pyridine-3-nitriles.

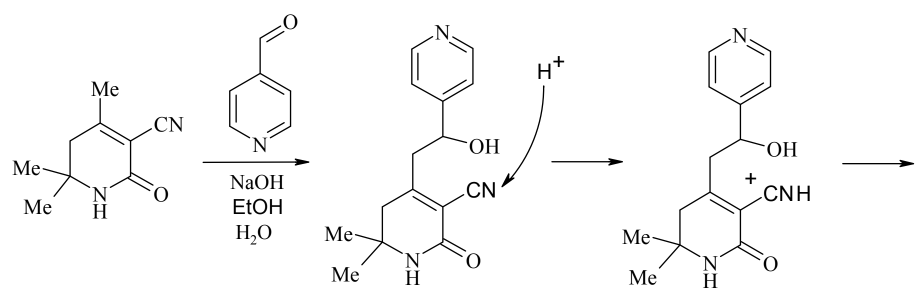
RESULTS AND DISCUSSION

Herein we report a detailed synthesis of novel 4-[(*E*)-hetaryl-vinyl]-6,6-dimethyl-2-oxo-1,2,5,6-tetrahydro-pyridine-3-nitriles (**2-8**) from 4,6,6-trimethyl-2-oxo-1,2,3,4-tetrahydropyridine-3-nitrile (**1**) and heteroaromatic aldehyde in the presence of catalytic amounts of NaOH. Thus, treatment of pyridone **1** with 2,3-dihydro-benzo[1,4]dioxin-6-carboxaldehyde in the presence of catalytic amount of NaOH (molar ratio **1** : aldehyde : NaOH = 1 : 1.5 :0.25) at room temperature leads to 4-[(*E*)-2-(2,3-dihydro-benzo[1,4]dioxin-6-yl)-vinyl]-6,6-dimethyl-2-oxo-1,2,5,6-tetrahydro-pyridine-3-nitrile (**2**) in 72 % yield. Similarly were prepared products **3-5** and **7** (yields 50-97%). Reaction of 4,6,6-trimethyl-2-oxo-1,2,3,4-tetrahydropyridine-3-nitrile (**1**) with 3-pyridinecarboxaldehyde in NaOH ethanolic solution (molar ratio **1** - aldehyde – NaOH is 1 : 1.5 :0.25) afforded 4-[(*E*)-2-(3-pyridyl)-vinyl]-6,6-dimethyl-2-oxo-1,2,5,6-tetrahydro-pyridine-3-nitrile **(6)** only in 22 % yield. However, when this reaction was carried out using pyridone **1** - 3-pyridinecarboxaldehyde - NaOH molar ratio of 1 :1 : 0.075 yield of product **6** was increased to 35 %.

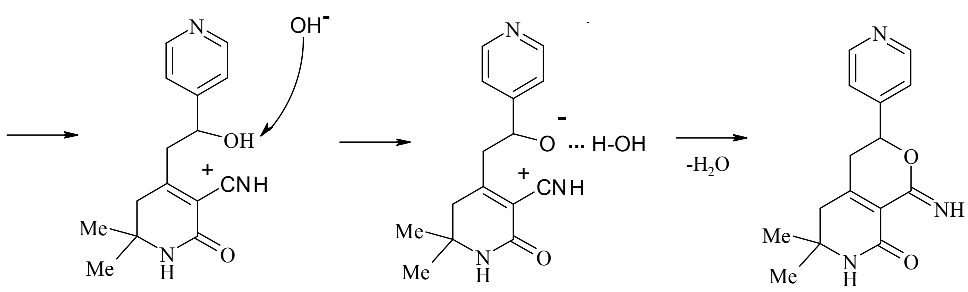


1 **2-8**

Interesting results were obtained when carrying reaction of pyridone (**1**) with 4-pyridinecarboxaldehyde in the presence of different amounts of NaOH. The best results in the synthesis aldol condensation product **8** was obtained when reaction was carried out in equimolar amounts of pyridone and aldehyde in the presence of 25 mol.% of NaOH. In this case 4-[(*E*)-2-(4-pyridyl)-vinyl]-6,6-dimethyl-2-oxo-1,2,5,6-tetrahydro-pyridine-3-nitrile **(8)** was isolated in 65 % yield**.**



1 **9** **9a**

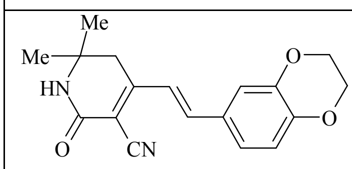
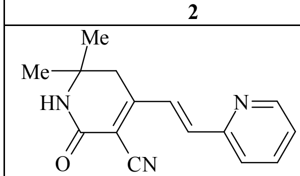
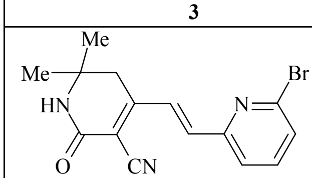
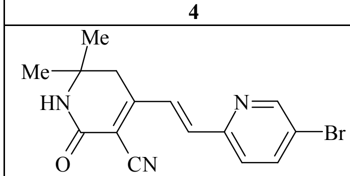
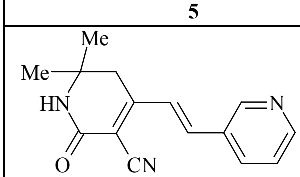
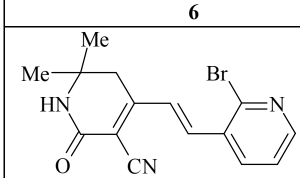


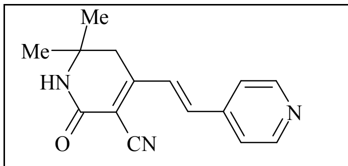
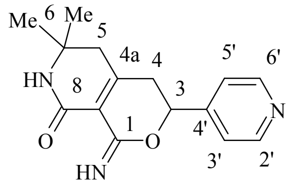
9b **9c** **8a**

In the case, when the pyridone **1** react with an excess of 4-pyridinecarboxaldehyde (1.25 equivalents) in the presence of 7.5 mol.% of NaOH in ethanol, instead of the expected crotonic condensation product, 1-imino-6,6-dimethyl-3-(pyridin-4-yl)-1,3,4,5,6,7-hexahydro-pyrano[3,4-

c]pyridin-8-one (**8a**) was isolated in 71 % yield**.** We carried out a quantum-chemical study of mechanism of this reaction. In the first step of the reaction occurred formation of the intermediate **9** as the result of aldol condensation. According to usual reaction scheme, addition of proton to the oxygen atom of the hydroxy group proceeds. Then follows *trans*-position proton elimination from the methylene group. It leads to the formation of the croton condensation product**.** In our reaction conditions the proton attack is directed to the nitrogen atom of the cyano group (intermediates **9** and **9a**)**,** . After leaving the proton from hydroxy group of protonated intermediate **9a-c** intramolecular cyclization process takes place as the result of the closure of the bond between negative charged oxygen atom and positive charged carbon atom of cyano group in **8a**. The heat of this reaction stage is equal -202.9 kcal/mol.

Table 1. Reaction of pyridone **1** with aldehydes in the presence of catalytic amounts of NaOH in EtOH at 25^{o}C

Product	Molar ratio: pyridone **1** : HetCHO: NaOH	Reaction time, h	Yield, %	Melting point, ^{o}C
 **2**	1.0 : 1.5 : 0.25	2	72	263-265
 **3**	1.0 : 1.5: 0.25	15	50	226-228 (dec)
 **4**	1.0 : 1.0 : 0.25	1	68	221-223 (dec)
 **5**	1 : 1.5 : 0.25	2	97	223-225 (dec)
 **6**	1.0 : 1.5 : 0.25 1.0 : 1.0 : 0.075	16 20	22 35	244-246 (dec)
 **7**	1.0 : 1.5 : 0.25	2	90	276-278 (dec)

 **8**	1.0 : 1.5 : 0.25 1.0 : 1.5 : 0.25 1.0 : 1.0 : 0.25 1.0 : 1.0 : 0.075	17 0.25 2 17	45 * 44 * 65 0	283-285 (dec)
 **8a**	1.0 : 1.25 : 0.075	2	71	204-206 (dec)

* Product contain polymeric impurities

Structure of compound **7** was confirmed by X-Ray structural data (see Experimental section). Fig. 1 shows a perspective view of the molecule of **7** with thermal ellipsoids and the atom-numbering scheme followed in the text. The molecule of **7** is characterized by *E*-conformation: the torsion angle of C(4)–C(10)–C(11)–C(12) is equal 176.1(5)º. The envelope conformation occurs for the 5,6-dihydropyridone system: the deviation of C(6) from the plane of N(1)−C(2)−C(3)−C(4)−C(5) is 0.556(4) Å. In crystal structure the dimers of molecules **7** form by means of intermolecular hydrogen bonds of NH···O type. The length of these bonds is 2.907(4) Å (H···O = 2.00 Å, N–H···O = 176º).

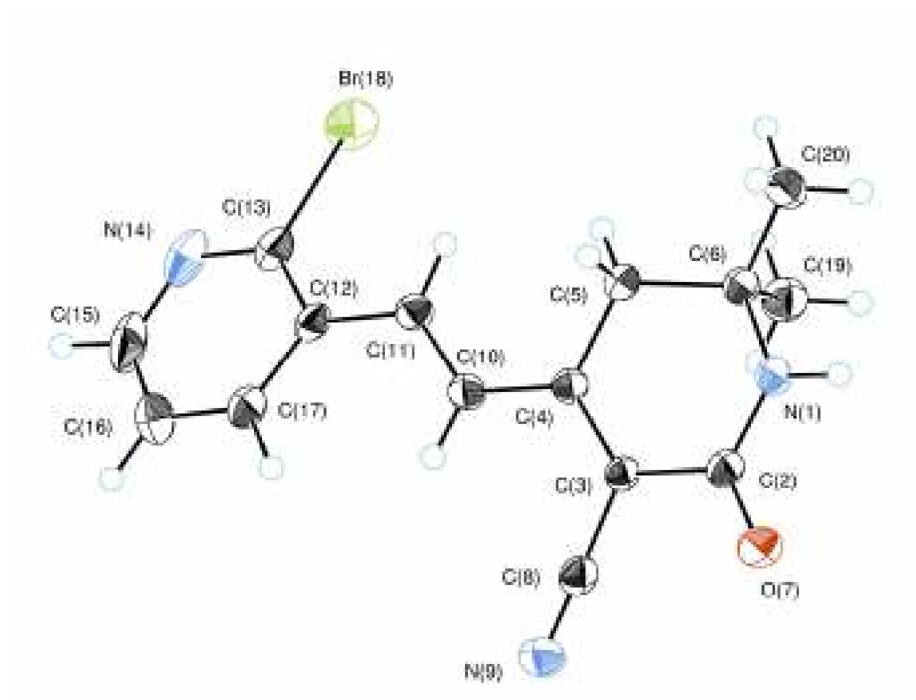


Figure 1. ORTEP molecular structure of the compound **7**.

Cytotoxic activity of 4-[(E)-hetaryl-vinyl]-6,6-dimethyl-2-oxo-1,2,5,6-tetrahydro-pyridine-3-nitriles **2-8** was tested *in vitro* on the monolayer tumor cell lines: MG-22A (mouse hepatoma) and HT-1080 (human fibrosarcoma) (Table 2). Concentrations providing 50% of tumor death effect (IC_{50}) were calculated according to the known procedure using 96 well plates. A preliminary analysis of the structure-activity relationship for the cytotoxic action clearly indicate the strong influence of substituent (Br or H) in 2-pyridyl substituted products **3-5** on toxic effects *in vitro*. Among 2-pyridyl substituted compounds **3-5** compound **4** exhibits the high cytotoxicity on the MG-22A cell line (IC_{50} 4 μg/mL). However, on the HT–1080 cell line this compound was not so active. Among 3-pyridyl substituted products **6** and **7** compound **7** exhibit high cytotoxicity on the HT–1080 (IC_{50} 8 μg/mL) and MG-22A (IC_{50} 10 μg/mL) cell lines in the comparison with cytotoxicity of compound **6** (IC_{50} 27 and 18 μg/mL, correspondingly). 4-[(*E*)-2-(4-Pyridyl)-vinyl]-6,6-dimethyl-2-oxo-1,2,5,6-tetrahydro-pyridine-3-nitrile **(8)** exhibit middle cytotoxicity on the HT–1080 and MG-22A cancer cell lines.

Acute toxicity of synthesized compounds was tested on 3T3- Swiss Albino mice embrio fibroblasts**.** In general, the compounds **2-8** exhibit middle to high toxicity in the range LD_{50} 166-1955 mg/kg (Table 2).

Table 2. Cytotoxicity of 4-[(*E*)-hetaryl-vinyl]-6,6-dimethyl-2-oxo-1,2,5,6-tetrahydro-pyridine-3-nitriles 2-8 IC_{50} (μg/ml)

Compound	HT-1080, IC_{50}	MG-22A, IC_{50}	3T3, LD_{50}
2	34	33	1955
3	22	11	342
4	42	4	299
5	48	23	811
6	27	18	166
7	8	10	199
8	23	13	152

EXPERIMENTAL SECTION

^1H and ^{13}C NMR spectra were recorded on a spectrometer Varian 400MR (400 MHz) in DMSO-D_6 using TMS as internal standard. ^1H and ^{13}C chemical assignment were supported by 2D ^1H—^{13}C correlations (HSQC and HMBC). LC-MS spectra were recorded on Alliance Waters 2695 instrument and Waters 3100 mass detector. 4,6,6-Trimethyl-2-oxo-1,2,3,4-tetrahydropyridine-3-nitrile was prepared as described in article [7]. Heterocyclic aldehydes (Acros and Aldrich) were used without additional purification.

Typical procedure for the preparation of 4-[(E)-hetaryl-vinyl]-6,6-dimethyl-2-oxo-1,2,5,6-tetrahydro-pyridine-3-nitriles 2-8 and 1-imino-6,6-dimethyl-3-(pyridin-4-yl)-1,3,4,5,6,7-hexahydro-pyrano[3,4-*c*]pyridin-8-one (8a). A mixture of 4,6,6-trimethyl-2-oxo-1,2,3,4-tetrahydropyridine-3-nitrile (**1**), aldehyde and solid NaOH in EtOH was stirred at 25^{o}C for 0.25-20 h The precipitated product was filtered off, washed with ethanol and then recrystallized from ethanol. For molar ratio of reactants see Table 1.

4-[(*E*)-2-(2,3-Dihydro-benzo[1,4]dioxin-6-yl)-vinyl]-6,6-dimethyl-2-oxo-1,2,5,6-tetrahydro-pyridine-3-nitrile (2). LC-MS, 311 (M^++1). ^1H NMR δ (ppm): 1.22 (s, 6H, CH_3), 2.80 (s, 2H, CH_2), 4.25-4.3 (m, 4H, OCH_2CH_2O), 6.94 (d, 1H, J = 8.5 Hz, 8-H), 7.06 and 7.28

(both d, 2H, J = 15.8 Hz, H-α and H-β), 7.17 (d, 1H, J = 1.9 Hz, H-2′), 7.19 (dd, 1H, J = 8.5 and 1.9 Hz), 8.08 (s, 1H, NH). ^{13}C NMR δ (ppm): 28.23 (CH_3), 36.85 (CH_2), 50.51 (C-6), 63.96 and 64.40 (OCH_2CH_2O), 103.91 (C-3), 115.12 (CN), 116.59 (C-2′), 117.85 (C-8′), 121.70 (C-7′), 122.05 (C-α), 128.43 (C-1′), 141.26 (C-β), 143.67 (C-6′a), 145.77 (C-2′a), 159.47 (C-4), 160.77 (C=O).

4-[(*E*)-2-(2-Pyridyl)-vinyl]-6,6-dimethyl-2-oxo-1,2,5,6-tetrahydro-pyridine-3-nitrile (3). LC-MS, 254 (M^++1). ^1H NMR δ (ppm): 1.24 (s, 6H, CH_3), 2.86 (s, 2H, CH_2), 7.41 (ddd, 1H, J = 7.6, 4.8 and 1.1 Hz, H-5′), 7.57 and 7.84 (both d, 2H, J = 15.9 Hz, H-α and H-β), 7.60 (dd, 1H, J = 7.9 and 1.1 Hz, H-3′), 7.88 (ddd, 1H, J = 7.6, 7.9 and 1.7 Hz, H-4′), 8.21 (s, 1H, NH), 8.69 (dd, 1H, J = 4.8 and 1.7 Hz, H-6′). ^{13}C NMR δ (ppm): 28.45 (CH_3), 36.86 (CH_2), 50.63 (C-6), 106.72 (C-3), 114.84 (CN), 124.65 (C-5′), 125.62 (C-3′), 127.04 (C-α), 137.41 (C-4′), 140.09 (C-β), 150.24 (C-6′), 152.51 (C-2′), 158.85 (C-4), 160.35 (C=O).

4-[(*E*)-2-(6-Bromo-2-pyridyl)-vinyl]-6,6-dimethyl-2-oxo-1,2,5,6-tetrahydro-pyridine-3-nitrile (4). LC-MS, 332 (M^+). ^1H NMR δ (ppm): 1.25 (s, 6H, CH_3), 2.85 (s, 2H, CH_2), 7.53 and 7.72 (both d, 2H, J = 15.5 Hz, H-α and H-β), 7.66 (d, 2H, J = 7.8 Hz, H-3′ and 5′), 7.74 (t, 1H, J = 7.8 Hz, H-4′), 8.25 (s, 1H, NH). ^{13}C NMR δ (ppm): 28.24 (CH_3), 36.86 (CH_2), 50.65 (C-6), 107.47 (C-3), 114.71 (CN), 124.73 (C-3′), 128.28 (C-α), 128.78 (C-5′), 138.24 (C-β), 140.67 (C-4′), 141.91 (C-6′), 154.01 (C-2′), 158.33 (C-4), 160.19 (C=O).

4-[(*E*)-2-(5-Bromo-2-pyridyl)-vinyl]-6,6-dimethyl-2-oxo-1,2,5,6-tetrahydro-pyridine-3-nitrile (5). LC-MS, 324 (M^++2). ^1H NMR δ (ppm): 1.25 (s, 6H, CH_3), 2.85 (s, 2H, CH_2), 7.57 and 7.82 (both d, 2H, J = 15.5 Hz, H-α and H-β), 7.56 (d, 1H, J =8.2 Hz, H-3′), 8.15 (dd, 1H, J = 8.2 and 2.2 Hz, H-4′), 8.24 (s, 1H, NH), 8.82 (d, 1H, J = 2.2 Hz, H-6′). ^{13}C NMR) δ (ppm): 28.26 (CH_3), 36.84 (CH_2), 50.64 (C-6), 107.11 (C-3), 114.78 (CN), 121.02 (C-5′), 126.92 (C-3′), 127.66 (C-α), 138.81 (C-β), 139.96 (C-4′), 151.04 (C-6′), 151.36 (C-2′), 158.56 (C-4), 160.26 (C=O).

4-[(*E*)-2-(3-Pyridyl)-vinyl]-6,6-dimethyl-2-oxo-1,2,5,6-tetrahydro-pyridine-3-nitrile (6). LC-MS, 255 (M^++2). ^1H NMR: 1.24 (s, 6H, CH_3), 2.85 (s, 2H,CH_2), 7.33 and 7.59 (both d, 2H, J = 16.2 Hz, H-α and H-β), 7.48 (dd, 1H, J = 8.0 and 4.8 Hz, H-5′), 8.15 (ddd, 1H, J= 8.1, 2.2 and 1.6 Hz, H-4′), 8.20 (s, 1H, NH), 8.59 (dd, 1H, J = 4.7 and 1.6 Hz, H-6′), 8.81 (d, 1H, J = 2.2 Hz, H-2′). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 28.26 (CH_3), 36.81 (CH_2), 50.58 (C-6), 105.93 (C-3), 114.70 (CN), 124.17 (C-5′), 125.66 (C-α), 130.77 (C-3′), 133.99 (C-4′), 138.04 (C-β), 149.86 (C-2′), 150.85 (C-6′), 158.79 (C-4), 160.40 (C=O).

4-[(*E*)-2-(2-Brom-3-pyridyl)-vinyl]-6,6-dimethyl-2-oxo-1,2,5,6-tetrahydro-pyridine-3-nitrile (7). LC-MS, 334 (M^++2). ^1H NMR δ (ppm): 1.25 (s, 6H, CH_3), 2.86 (s, 2H, CH_2), 7.28 and 7.49 (both d, 2H, J = 16.0 Hz, H-α and H-β), 7.55 (dd, 1H, J = 7.8 and 4.7 Hz, H-5′), 8.25 (s, 1H, NH), 8.29 (dd, 1H, J = 7.8 and 1.9 Hz, H-4′), 8.41 (dd, 1H, J = 4.6 and 1.9 Hz, H-6′). ^{13}C NMR δ (ppm): 28.21 (CH_3), 36.75 (CH_2), 50.68 (C-6), 107.22 (C-3), 114.51 (CN), 124.17 (C-5′), 128.97 (C-α), 132.08 (C-2′), 136.64 (C-β), 136.79 (C-4′), 143.25 (C-3′), 151.01 (C-6′), 158.02 (C-4), 160.12 (C=O).

4-[(*E*)-2-(4-Pyridyl)-vinyl]-6,6-dimethyl-2-oxo-1,2,5,6-tetrahydro-pyridine-3-nitrile (8). LC-MS, 254 (M^++1). ^1H NMR δ (ppm): 1.25 (s, 6H, CH_3), 2.85 (s, 2H, CH_2), 7.42 and 7.53 (both d, 2H, J = 16.2 Hz, H-α and H-β), 7.63 and 8.67 (A_2B_2 type m, 4H, J = 6.1 Hz, H-3′, 5′ and H-2′, 6′). ^{13}C NMR δ (ppm): 28.26 (CH_3), 36.82 (CH_2), 50.62 (C-6), 107.17 (C-3), 114.51 (CN), 121.71 (C-3′, 5′), 127.96 (C-α), 138.64 (C-β), 141.92 (C-4′), 150.55 (C-2′, 6′), 158.39 (C-4), 160.17 (C=O).

1-Imino-6,6-dimethyl-3-(pyridin-4-yl)-1,3,4,5,6,7-hexahydro-pyrano[3,4-*c*]pyridin-8-one (8a). LC-MS, 272 (M^{+}+1). ^{1}H NMR δ (ppm): 1.18 (s, 6H, CH_3), 2.64 (s, 2H, H-5), 2.78 and 2.83 (dd and dd, 2H, J = 13.1 and 8.8 Hz, 13.1 and 4.5 Hz CH_2), 4.94 (ddd, 1H,J = 8.8, 4.7 and 4.5 Hz, H-3), 5.88 (d, 1H, J = 4.7 Hz, =NH), 7.41 (d, 2H, J = 5.1 Hz, H-3′ and H-5′), 8.02 (d, 2H, J = 5.1 Hz, H-2′ and H-6′). ^{13}C NMR δ (ppm): 28.55 (CH_3), 41.65 (C-5), 45.66 (C-4), 50.77 (C-6), 69.04 (C-3), 108.57 (C-8a), 114.91 (C-4a), 121.10 (C-3′,5′), 149.74 (C-2′,6′), 153.48 (C-4′), 160.10 (C=O), 168.08 (C-1).

In vitro cytotoxicity assay. Monolayer tumor cell lines –HT-1080 (human fibrosarcoma), MG-22A (mouse hepatoma), 3T3 (mouse Swiss Albino embryo fibroblasts), - were cultured in standard medium (Dulbecco`s modified Eagle`s medium; "Sigma") supplemented with 10% fetal bovine serum ("Sigma"). Tumor cell lines were obtained from the "ATCC". After the ampoule had thawed, cells from one to four passages were used in three concentrations test compound: 1, 10 and 100 μg ml^{-1}. About 10 x10^4 cells ml^{-1} were placed in 96-well plates immediately after compounds were added to the wells; the volume of each plate was 200 μl. The control cells without test compounds were cultured on separate plate. The plates were incubated for 72h, 37°C, 5% CO_2. The number of surviving cells was determined using tri(4-dimethylaminophenyl)methyl chloride (crystal violet: CV) or 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolinium bromide (MTT) [8, 9]. The quantity on the control plate was taken in calculations for 100%. LD_{50} was tested according „Alternative Toxicological Methods" [10]. The program Graph Pad Prism® 3.0 was used for calculations ($r < 0.05$.).

X-Ray crystallographic study of compound 7. Diffraction data were collected at –80°C on a Bruker-Nonius KappaCCD diffractometer using graphite monochromated Mo-Kα radiation (λ = 0.71073 Å). The crystal structure of **7** was solved by direct methods [11] and refined by full-matrix least squares [12]. All nonhydrogen atoms were refined in anisotropical approximation, all H-atoms were refined by riding model. Crystal data for **7**: triclinic; a = 7.0308(2), b = 9.5194(3), c = 11.0298(4) Å, α = 98.278(1), β = 92.209(1), γ = 101.458(1)°; V = 714.25(4) Å^3, Z = 2, μ = 2.876 mm^{-1}; space group is $P\bar{1}$. A total of 4702 reflection intensities were collected up to $2\theta_{max}$ = 57°; for structure refinement 2389 independent reflections with $I > 3\sigma(I)$ were used. The final R-factor is 0.043. For further details, see crystallographic data for **7** deposited with the Cambridge Crystallographic Data Centre as Supplementary Publication Number CCDC 862918. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK.

Quantum-chemical calculations. Quantum-chemical calculations were carried out by the AM1 method [13] using the MOPAC2009 set of programs [14]. The optimized structures are minimum points on the potential energy surface of the molecular systems.

ACKNOWLEDGEMENTS

The authors wish to acknowledge financial support from the European Social Fund (project No. 2009/0203/1DP/1.1.1.2.0/09/APIA/VIAA/023).

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Received on March 8, 2012.