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Synthesis, characterization, DFT calculations and biological activity of derivatives of 3-acetylpyridine and the zinc(II) complex with the condensation product of 3-acetylpyridine and semicarbazide

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ABSTRACT

A Schiff base of 3-acetylpyridine with semicarbazide as well as the corresponding tetrahedral Zn(II) complex were synthesized and characterized by X-ray crystal structure analysis and spectroscopic methods. It is interesting to note that the ligand coordinated as a monodentate although there are several donor atoms in it. Computational studies showed that such structure is more stable than the hypothetical structure with one ligand bound as a bidentate. The complex exibited moderate antibacterial, antifungal and cytotoxic activities while the ligand was mostly inactive. The complex strongly induced formation of reactive oxygen species in tumor cell lines. It also influenced cell cycle progression in tumor cell lines, and induced autophagy. The latter effect is, at least in part, a protective one.

1. Introduction

Condensation derivatives of 2-acetylpyridine with hydrazines, hydrazides and dihydrazides and their transition metal compexes have been extensively studied by our group [1–3]. These ligands have a large number of possible donor atoms and therefore display a diverse behavior in metal coordination. The central metal atom and the nature of the ligand determine the way of coordination.

There is a small number of papers concerning derivates of 3-acetylpyridine and their complexes in none of which biological activity was studied [4–8]. Bis hydrazone of hydrazine and 3-acetylpyridine was coordinated to Zn(II) via pyridine nitrogen and not via hydrazone nitrogen [4].

In the complexes of Pt(II), Co(II), Ni(II) and Cu(I) with condensation derivative of 3-acetylpyridine and thiosemicarbazide the ligand is coordinated as a bidentate via thione sulfur and azomethine nitrogen while the heteroaromatic nitrogen is not involved in coordination [5–7].

There is only one publication concerning complexes of the condensation derivative of 3-acetylpyridine with semicarbazide. Based on similarity of spectral properties of oxovanadium(IV) complexes with 3-acetylpyridine semicarbazone and 2-acetylpyridine semicarbazone and X-ray structure of the latter complex, it was assumed that the 3-acetylpyridine derivative was coordinated as a monodentate in neutral form via the carbonyl oxygen of the side chain [8].

Zinc is considered the most abundant trace intracellular element and a substantial amount of this element is incorporated in the nucleus [9]. As some other metal ions zinc has many positive physiological effects but changes in its metabolism or trafficking may be related to some diseases [10,11].

Zinc(II) ions are present in zinc finger proteins that recognize and bind to DNA [12,13]. In zinc fingers the zinc ion is usually

Abbreviations: HL1, (2*E*)-2-[1-(pyridin-3-yl)ethylidene]hydrazinecarboxamide hydrochloride dihydrate; HL2, 2′-[1-(3-pyridinyl)ethylidene]oxamohydrazide; HL3, (2*E*)-2-[1-(pyridin-3-yl)ethylidene]hydrazinecarboxylate hydrochloride hydrate; TMS, tetramethylsilane; COSY, correlation spectroscop; HSQC, heteronuclear single quantum coherence; HMBC, heteronuclear multiple bond correlation; HL-60, human promyelocytic leukemia; U251, human glioma; C6, rat glioma; L929, mouse fibrosarcoma; B16, mouse melanoma; PI, propidium iodide; ROS, reactive oxygen species.

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tetrahedrally coordinated by cysteine and histidine residues [14]. Lippert has stressed that the role of zinc in nucleic acid chemistry is unique in many aspects [15]. For example, from all metal ions only zinc(II) ions are able to facilitate the rewinding of molten DNA [15].

In the present paper condensation products of 3-acetylpyridine with different hydrazide type compounds were studied for their coordination behavior and antitumor and antimicrobial activities. The present work describes synthesis, characterization and biological activity of derivatives of 3-acetylpyridine with semicarbazide, oxamic hydrazide, ethyl carbazate and zinc(II) complex with 3-acetylpyridine semicarbazone. Coordination behaviour of the ligands was investigated by DFT calculations.

2. Experimental

2.1. Materials and methods

All solvents (reagent grade) were obtained from commercial suppliers and used without further purification. The elemental analyses (C, H, N) were performed by the standard micromethods using the ELEMENTAR Vario ELIII C, H, N analyser. IR spectra were recorded on Perkin-Elmer FT-IR 1725X spectrophotometer by the KBr technique in the region 4000–400 cm^{-1}. Abbreviations used for IR spectra: vs very strong; s, strong; m, medium; w, weak. NMR spectral assignments and structural parameters were obtained by combined use of ^1H homonuclear spectroscopy (2D NOESY) and multinuclear proton-detected spectroscopy (2D HSQC, 2D HMBC). The NMR spectra were performed on Bruker Avance 500 equipped with broad-band direct probe. All spectra were measured at 298 K. 2D NOESY spectra were collected with 4 scans per t1-increment and mixing time of 1 s. Chemical shifts are given on δ scale relative to tetramethylsilane (TMS) as internal standard for ^1H and ^{13}C. Abbreviations used for NMR spectra: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; q, quartet; m, multiplet. All microorganism tests were performed in Műller Hinton broth for the bacterial strains and in Sabouraud dextrose broth for the yeast. Human promyelocytic leukemia (HL-60), human glioma (U251), rat glioma (C6), mouse fibrosarcoma (L929) and mouse melanoma (B16) cell lines were obtained from the European Collection of Animal Cell Cultures (Salisbury, UK). Flow cytometry analysis was performed on a FACSCalibur flow cytometer (BD Biosciences, Heidelberg, Germany), using CellQuest Pro software for acquisition and analysis. All statistical data were analyzed by *t*-test or ANOVA followed by Student–Newman–Keuls test. Statistical significance of the differences of means was analyzed at the significance level of $p < 0.05$.

2.2. Synthesis of (2E)-2-[1-(pyridin-3-yl)ethylidene]hydrazine carboxamide hydrochloride dihydrate (**HL1**, $C_8H_{15}ClN_4O_3$)

3-Acetylpyridine (0.11 cm^{-3}, 1 mmol) was added to a solution of semicarbazide hydrochloride (111 mg, 1 mmol) in water (25 cm^{-3}) and the mixture was refluxed for 3 h. After 7 days colorless crystals were obtained (180 mg, 72%), mp 214–230 °C. *Anal.* Calc. for $C_8H_{15}ClN_4O_3$: C, 38.33; H, 6.03; N, 22.35. Found: C, 38.53; H, 6.08; N, 22.49%. ^1H NMR (500 MHz; DMSO-d_6; Me_4Si): δ = 9.84 (s, 1 H, N(3)H), 9.39 (d, 1 H, ^4J = 1.7, H-1), 8.96 (m, 1 H, ^3J = 8.9, ^4J = 1.8, H-5), 8.81 (dd, 1 H, ^3J = 6.3, ^4J = 0.8, H-3), 8.02 (dd, 1 H, ^3J = 8.2, ^4J = 2.8, H-4), 6.75 (s, 2 H, N(4)H_2), 2.26 (s, 3 H, C(7)H_3) ppm; ^{13}C NMR (125 MHz, DMSO-d_6; Me_4Si): δ = 156.90 (C-8), 141.72 (C-5), 140.32 (C-1), 139.43 (C-6), 138.73 (C-3), 137.28 (C-2), 126.64 (C-4), 12.92 (C-7) ppm. IR (KBr; ν/cm^{-1}): $\bar{\nu}$ = 3424vs, 3289vs, 2358w, 2167w, 1691s, 1573m, 1533m, 1455m, 1411m, 1313w, 1247w, 1157m, 1088w, 892w, 668m.

2.3. Synthesis of 2′-[1-(3-pyridinyl)ethylidene]oxamohydrazide (**HL2**, $C_9H_{10}N_4O_2$)

To a solution of oxamic hydrazide (103 mg, 1 mmol) in water (25 cm^{-3}) 3-acetylpyridine (0.11 cm^{-3}, 1 mmol) was added and the mixture was refluxed for 3 h. After 4–5 days colorless crystals were obtained (130 mg, 65%), m.p.: 240 °C. *Anal.* Calc. for $C_9H_{10}N_4O_2$: C, 52.42; H, 4.89; N, 27.17. Found: C, 52.17; H, 4.86; N, 26.92%. ^1H NMR (500 MHz; DMSO-d_6; Me_4Si): δ = 10.88 (s, 1 H, N(3)H), 9.00 (d, 1 H, ^4J = 2.0, H-1), 8.65 (m, 1 H, ^3J = 6.0, ^4J = 1.5, H-5), 8.35 (s, 1 H, N(4)H_2), 8.22 (dd, 1 H, ^3J = 7.5, ^4J = 1.5, H-3), 8.05 (s, 1 H, N(4)H_2), 7.50 (dd, 1 H, ^3J = 5.5, ^5J = 3.0, H-4), 2.36 (s, 3 H, C(7)H_3) ppm. ^{13}C NMR (125 MHz, DMSO-d_6; Me_4Si): δ = 161.92 (C-9), 156.68 (C-8), 155.47 (C-6), 150.20 (C-5), 147.32 (C-1), 134.39 (C-3), 133.28 (C-2), 123.69 (C-4), 14.03 (C-7) ppm. IR (KBr; ν/cm^{-1}): $\bar{\nu}$ = : 3392m, 3268s, 3059vs, 2931m, 2651m, 2118w, 1725w, 1692vs, 1605s, 1511m, 1366s, 1175m, 974w, 816m, 646w.

2.4. Synthesis of ethyl (2E)-2-[1-(pyridin-3-yl)ethylidene]hydrazinecarboxylate hydrochloride hydrate (**HL3**, $C_{10}H_{16}ClN_3O_3$)

To a solution of ethyl carbazate (104 mg, 1 mmol) in water (25 cm^{-3}) was added 3-acetylpyridine (0.11 cm^{-3}, 1 mmol) followed by a few drops of conc. HCl and the mixture was refluxed for 3 h. After 10 days colorless crystals were formed (200 mg, 76%), m.p.: 215 °C. *Anal.* Calc. for $C_{10}H_{16}ClN_3O_3$: C, 45.89; H, 6.16; N, 16.06. Found: C, 45.73; H, 6.16; N, 15.87%. ^1H NMR (500 MHz; DMSO-d_6; Me_4Si): δ = 10.59 (s, 1 H, N(3)H), 9.05 (d, 1 H, ^4J = 2.0, H-1), 8.87 (dd, 1 H, ^3J = 5.6, ^4J = 1.0, H-5), 8.77 (m, 1 H, ^3J = 8.5, ^4J = 1.5, H-3), 8.04 (dd, 1 H, ^3J = 6.0, ^5J = 2.0, H-4), 4.18 (q, 2 H, ^3J = 7.0 C(9)H_2), 2.30 (s, 3 H, C(7)H_3), 1.24 (s, 3 H, C(10)H_3) ppm. ^{13}C NMR (125 MHz, DMSO-d_6; Me_4Si): δ = 153.99 (C-8), 143.74 (C-6), 141.52 (C-5), 141.41 (C-1), 139.42 (C-3), 137.15 (C-2), 126.79 (C-4), 61.02 (C-9), 14.55 (C-7), 13.64 (C-10) ppm. IR (KBr; ν/cm^{-1}): $\bar{\nu}$ = : 3407s, 3361s, 2997vs, 2361w, 2026w, 1738vs, 1644m, 1550m, 1530s, 1462s, 1395m, 1229vs, 1041m, 890w, 727w.

2.5. Synthesis of the complex $[ZnCl_2(HL1)_2]$ (**1**, $C_{16}H_{20}Cl_2N_8O_2Zn$)

To a solution containing (2*E*)-2-[1-(pyridin-3-yl)ethylidene]hydrazinecarboxamide hydrochloride (**HL1**) (25 mg, 0.1 mmol) in methanol (15 cm^{-3}) was added $Zn(CH_3COO)_2{\cdot}2H_2O$ (21 mg, 0.1 mmol) dissolved in 5 cm^{-3} of methanol. The mixture was refluxed for 4 h forming a colorless solution. After ten days white crystals suitable for X-ray diffraction were formed (18 mg, 73 %), m.p.: 240 °C. *Anal.* Calc. for $C_{16}H_{20}Cl_2N_8O_2Zn$: C, 39.01; H, 4.09; N, 22.74. Found: C, 38.91; H, 4.15; N, 22.44%. ^1H NMR (500 MHz; DMSO-d_6; Me_4Si): δ = 9.17 (s, 2 H, N(3)H), 8.96 (s, 2 H, H-1), 8.55 (d, 2 H, ^3J = 7.5, H-5), 8.08 (d, 2 H, ^3J = 7.8, H-3), 7.36 (dd, 2 H, ^1J = 7.8, ^3J = 5.0, H-4), 6.25 (s, 4 H, N(4)H_2), 2.27 (s, 6 H, C(7)H_3) ppm. ^{13}C NMR (125 MHz, DMSO-d_6; Me_4Si): δ = 157.29 (C-8), 148.84 (C-5), 146.91 (C-1), 141.63 (C-6), 134.09 (C-3), 133.67 (C-2), 123.70 (C-4), 13.10 (C-7) ppm. IR (KBr; ν/cm^{-1}): $\bar{\nu}$ = : 3430m, 3196m, 1697s, 1630w, 1466m, 1314w, 1098m, 1058m, 824w, 768w, 695w, 537w.

2.6. Crystal structure determination

Crystal data and refinement parameters of compounds **HL1**, **HL3** and **1** are listed in Table S1. The X-ray intensity data were collected at room temperature with a Nonius Kappa CCD diffractometer equipped with graphite-monochromated Mo Kα radiation (λ = 0.71073 Å) for **HL1** and **1** and with an Agilent SuperNova dual