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Interaction of a new copper(II) complex by bovine serum albumin and dipeptidyl peptidase-IV

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ABSTRACT

Dipeptidyl peptidase-IV (DPP-IV) is one of the mammalian serine proteases participated in the pathogenesis of diseases and DPP-IV inhibitors are now widely used as antidiabetic drugs. A new water soluble ternary copper (II) complex,-[Cu(py-phen) (phe) (H_2O)]$NO_3 \cdot H_2O$-(py-phen:pyrazino[2,3f][1,10]phenanthroline, phe:phenylalanine), has been synthesized and characterized by CHN analysis, ESI-MS, FTIR and single-crystal X-ray diffraction techniques. Fluorescence spectroscopy was researched to study the interaction between the complex and bovine serum albumin (BSA) and dipeptidyl peptidase-IV (DPP-IV). Chromophore of BSA and DPP-IV enzyme is changed upon addition of the complex. Additionally, the complex was shown to have promising inhibitory activities against DPP-IV with lower IC_{50} value. This study may provide new insights into the development of effective agents against diabetes.

1. Introduction

Dipeptidyl peptidase-IV (EC 3.4.14.5) is an ectopeptidase belonging to the prolyloligopeptidase family. The enzyme truncates the N-terminal dipeptide from peptides with proline or alanine in the second position [1]. Dipeptidyl peptidase-IV is responsible for the degradation many bioactive peptides of medical importance such as glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) associated with diabetes disease. That's why; Dipeptidyl peptidase-IV inhibition is accepted as a new approach to the treatment and prevention of some diseases as type 2 diabetes.

The uses in the medicinal field of transition metals and its complexes are increasing. Research has shown significant progress in utilization of transition metal complexes as drugs to treat several human diseases like carcinomas, lymphomas, infection control, anti-inflammatory, diabetes, and neurological disorders [2].

Cu is an essential trace metal that exists in oxidised (Cu^{2+}) and reduced (Cu^{+}) states within the body. Due to the physiological importance of Cu, many different Cu complexes have been synthesized and investigated for their therapeutic and diagnostic potential in human disease [3]. Among various copper complexes, containing 1,10-phenanthroline derivatives and amino acids have attracted much attention is due to their potential use as enzyme inhibitors, antimicrobial or anticancer agents [4–7]. 1,10-phenanthroline derivatives ligands such as pyrazino[2,3-f][1,10] phenanthroline with an extended planar pyrazine moiety, can act as bidentate ligands as well as good binders to DNA and proteins. Additionally, phenylalanine is an aromatic and hydrophobic amino acid. It prefers to be buried in protein hydrophobic cores and it could play a role in substrate recognition [8].

Quite recently, we have reported the synthesis, characterization, and biological activities ofternary copper (II) complexes [6,7,9–13]. In this report, our group has focused on the synthesis and property studies (CHN analysis, ESI-MS, FTIR and single-crystal X-ray diffraction techniques) of a new water soluble ternary copper (II) complex with pyrazino[2,3-f][1,10]phenanthroline (py-phen) and phenylalanine (phe) ligands. The detailed structure of a new ternary copper (II) complex has also been determined by single-crystal X-ray analysis. The biological activity of the complex has been performed to evaluate the BSA and DPP-IV interaction.

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Recently, different DPP-IV inhibitors have been reported [14,15]. Furthermore, we found that the complex as a potent DPP-IV inhibitor with high inhibitory activity.

2. Experimental section

2.1. Materials and measurements

All chemicals were of reagent grade, purchased from different sources, and were used without further purification. Pyrazino [2,3-f] [1,10]phenanthroline, Copper (II) nitrate trihydrate, methanol, KOH, NaCl, tris-(hydroxymethyl)aminomethane-HCl, BSA were provided from Sigma-Aldrich. All solutions were prepared with analytical grade water ($R = 18\ M\Omega$) using grade A glassware. Gly-Pro-*p*-nitroanilide (pNA) (Bachem), Porcine kidney (Merck), Diprotin A (Bachem) and Trizma® base (Sigma) were used in the DPP-IV inhibitory activity measurements. C, H, and N elemental analysis were performed using a Costech elemental analyzer at the Technical and Scientific Research Council of Turkey, TUBITAK Bursa Test, and Analysis Laboratory. Infrared spectra were recorded in the 4000-400 cm^{-1} region with a Thermo-Nicolet 6700 Fourier-Transform Infrared Spectrometer by using KBr pellets. The ESI mass spectra were recorded using a Bruker Daltonics Microtof II-ESI-TOF mass spectrometer. The spectrophotometric studies were performed using a GBC Cintra 303 UV–Visible spectrophotometer connected with a Peltier thermocell. Emission intensity measurements were carried out using a Jasco FP-750 spectrofluorometer. The DPP-IV inhibitory activity measurements carried out with microplate reader (Thermo Multiskan Go, USA).

2.2. General procedure for the synthesis of the complex

The complex was prepared by a general synthetic method in which a mixture of py-phen (0.5 mmol) and copper (II) nitrate trihydrate (0.5 mmol) in 20 mL methanol was added drop wise to an aqueous solution (5 mL) of L-phenylalanine (0.5 mmol) and KOH (0.5 mmol) with stirring for about 20 min. The resulting solution was left to evaporate slowly at room temperature. After three days, the blue crystals were obtained.

[Cu(py-phen) (phe) (H_2O)]NO_3·H_2O Yield was 88%. Melting point: 228 °C. Anal. calcd. for C_{23} H_{22} Cu N_6 O_7 (558.0 g mol^{-1}) (%): C, 49.51; H, 3.97; N, 15.06. Found: C, 49.53; H, 4.00; N, 15.16. ESI-MS (*m/z*): 459.07 for [Cu(py-phen) (phe)]$^+$. IR (KBr phase, cm^{-1}; w, weak; m, medium, s, strong): $\delta(C{=}N)_{py\text{-}phen}$ 815 m, $\delta(C{=}C)_{py\text{-}phen}$ 704 m, $\nu(COO_{asym})_{phe}$1601s, $\nu(COO_{sym})_{phe}$ 1482 m, $\nu(M\text{-}N)_{py\text{-}phen}$ 557w, $\nu(M\text{-}O)_{phe}$ 421w, (NO_3^-) 1336 m. UV–Vis [λ_{nm}; Tris-HCl buffer (5 mM Tris-HCl/50 mM NaCl buffer at pH 7.2)]: 257, 608.

2.3. X-ray data collection and structure refinement

Data were obtained with Bruker APEX II QUAZAR three-circle diffractometer. Indexing was performed using APEX2 [16]. Data integration and reduction were carried out with SAINT [17]. Absorption correction was performed by multi-scan method implemented in SADABS [18]. The structure was solved using SHELXT [19] and then refined by full-matrix least-squares refinements on F^2using the SHELXL [20] in SHELXTL Software Package [21]. All non-hydrogen atoms were refined anisotropically using all reflections with $I > 2\sigma(I)$. Aromatic and aliphatic C-bound H atoms were positioned geometrically and refined using a riding mode. The positions of the N-bound H atoms were located from the difference Fourier map and restrained to be 0.89 Å from N atom using DFIX command and their position was constrained to refine on their parent N atoms with *U*iso(H) = 1.2*U*eq(N). The H atoms of water molecules were located in a difference Fourier map and the O–H distances restrained to be 0.84 Å from O atom using DFIX command and their positions were constrained to refine on their parent O atoms with *U*iso(H) = 1.5*U*eq(O). Crystallographic data and refinement details of the data collection for the complex are given in Table 1. The selected bond lengths and bond angles are given in Table 2. Crystal structure validations and geometrical calculations were performed using Platon software [22]. Mercury software [23] was used for visualization of the cif file. Additional crystallographic data with CCDC reference number 1414872 have been deposited within the Cambridge Crystallographic Data Center via www.ccdc.cam.ac.uk/deposit.

2.4. BSA interaction studies

All experiments involving BSA were performed in Tris-HCl buffer solution (5 mM Tris-HCl/50 mM NaCl buffer at pH 7.2). Final solutions of BSA and the complex were prepared by dissolving them in the Tris-HCl buffer solution to required concentrations, respectively. In the tryptophan fluorescence quenching experiment, quenching of the tryptophan residues of BSA was done by keeping the constant concentration of BSA while varying the complex concentration. The fluorescence measurements were performed at room temperature. The fluorescence spectra were recorded at an excitation wavelength of 280 nm and an emission wavelength of tryptophan residues of BSA at 342 nm after addition of the complex.

2.5. DPP-IV interaction studies

All experiments involving DPP-IV were performed as miu (milli-international units) which is one-thousandth of an international unit. One unit corresponds to the hydrolysis of 1 mmol of substrate per minute at 37 °C and pH 8.5 (Dipeptidyl Peptidase-IV (CD26), Porcine Kidney, 250 miu). Stock solutions of 10 μM the complex in Tris-HCl buffer solution (5 mM Tris-HCl/50 mM NaCl buffer at pH 7.2) were prepared at room temperature. The DPP-IV concentration

Table 1
Crystal data and refinement parameters.

CCDC	1414872
Empirical Formula	$C_{23}H_{22}CuN_6O_7$
Formula weight (g. mol^{-1})	558.00
Temperature (K)	173 (2)
Wavelength (Å)	0.71073
Crystal system	Monoclinic
Space group	$P2_1$
a (Å)	5.8257 (7)
b (Å)	19.906 (2)
c (Å)	10.2597 (12)
α(°)	90
β(°)	98.111 (2)
γ(°)	90
Crystal size (mm)	0.22 × 0.27 x 0.48
V (Å^3)	1177.9 (2)
Z	2
ρ_{calcd} (g. cm^{-3})	1.573
μ (mm^{-1})	0.985
F (000)	574
θ range for data collection (°)	3.53–25.68
h/k/l	$-6 \le h <= 7$, $-24 \le k <= 24$, $-12 \le l <= 12$
Reflections collected	12908
Independent reflections	4439 [R (int) = 0.0340]
Absorption correction	Multi-scan
Data/restraints/parameters	4439/9/352
Goodness-of-fit on F^2	1.031
Final R indices [$I > 2\sigma(I)$]	$R_1 = 0.0266$, $wR_2 = 0.0601$
R indices (all data)	$R_1 = 0.0288$, $wR_2 = 0.0609$
Largest diff. peak and hole (e.Å^{-3})	0.307 and −0.207