Synthesis, spectroscopic properties, crystal structures, DFT studies, and the antibacterial and enzyme inhibitory properties of a complex of Co(II) 3,5-difluorobenzoate with 3-pyridinol

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Abstract
A new complex, [Co(DFB)₂(3-Pyr)₂(H₂O)₂] (where DFB = 3,5-difluorobenzoate, 3-Pyr = 3-pyridinol), is synthesized and characterized using different techniques (elemental analysis, Fourier transform infrared spectroscopy, and single-crystal X-ray diffraction). Looking at the crystal structure of the complexes, the cobalt atom is coordinated by two nitrogen atoms from two 3-Pyr ligands, two carboxylate oxygen atoms from two DFB anions, and two oxygen atoms from two water molecules. The complex has distorted octahedral geometry around the cobalt atom center complex and crystallizes in the P2₁/n space group (monoclinic system). Geometry optimization, frequency analysis, and energy quantum chemical calculations on the complex are performed by Density Functional Theory [B3LYP/6-31G (d,p) basis set] to predict the molecular properties. The novel complex is tested against the metabolic isoenzymes human carbonic anhydrases I and II. The novel complex shows Kᵢ values of 317.26 ± 23.25 µM against hCA I and 255.41 ± 48.05 µM against hCA II; the IC₅₀ values for these isoenzymes are 274.37 and 204.33 µM.

Keywords
Cobalt complex, 3,5-difluorobenzoate, 3-pyridinol, Density Functional Theory studies, antibacterial, enzyme inhibition

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lowering of the intracellular blood pressure has been deemed beneficial. Acetazolamide is also used for the treatment and prevention of acute mountain sickness (also known as altitude sickness) and in some types of epilepsy.  

In this study, a new complex of Co(II) 3,5-difluorobenzoate (DFB) with 3-pyridinol, [Co(C,H,F,O,),(C,H,NO),(H,O)], was synthesized and its structure was characterized by elemental analysis, Fourier-transform infrared (FT-IR) spectroscopy, and single-crystal X-ray diffraction methods. In addition, the antibacterial properties of the complex were investigated by the agar-well diffusion method against Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, and Staphylococcus aureus. Geometry optimization and frequency analysis of the difluorobenzoate complex was performed using the DFT/B3LYP/6-31G(d,p) method. The TD-DFT/B3LYP/6-31G(d,p) method was used to obtain the molecular orbital energies of the complex.

**Results and discussions**

**X-ray structural determination**

The experimental details are given in Table 1. The asymmetric unit of the centrosymmetric title compound contains one half of the complex molecule (Figure 1). The metal atom is located on a center of symmetry (Figure 2). The molecule contains two DFB and two 3-Pyr ligands and two coordinated water molecules, all ligands being monodentate. The four O atoms (O1, O4, and the symmetry-related atoms, O1′, O4′) in the equatorial plane around the Co atom form a slightly distorted square-planar arrangement, while the slightly distorted octahedral coordination is completed by the two N atoms (N1, N1′) of the 3-Pyr ligands in the axial positions (Figure 2). The near equality of the C1–O1 [1.250 (4) Å] and C1–O2 [1.256 (4) Å] bonds in the carboxylate group indicates a delocalized bonding arrangement, rather than localized single and double bonds, and may be compared with the corresponding distances: 1.256 (6) and 1.245 (4) Å in [Mn(diethylnicotinamide),4(4-chlorobenzoato),2(benzoato)4]2− (VII).  

![Figure 1](image_url)  

**Table 1. Experimental details.**

| Crystal data |  |
| --- | --- |
| Chemical formula | Co3H2CoF4N2O6 |
| M, g/mol | 599.35 |
| Crystal system, space group | Monoclinic, P21/n |
| Temperature (K) | 296 |
| No. of measured, independent, and observed reflections | 15,871, 2469, 2278 |
| No. of parameters | 191 |
| SHELXS97, SHELXL97, ORTEP-3 for Windows, WinGX publication routines, and PLATON | |

Co1 and O3 are −0.0891 (1) and −0.003 (3) Å away from the adjacent A and B rings, respectively. Hence, they are nearly co-planar with the corresponding rings. The dihedral angle between the planar carboxylate group (O1/O2/C1) and the adjacent benzene ring A (C2–C7) is 15.9 (2)°, while that between benzene ring A and pyridine ring B (N1/C8–C12) is A/B = 74.4 (1)°. In the crystal structure, the O–H···O hydrogen bonds (Table 3) link the molecules into a network.
of the structure. The π–π contacts between the benzene rings, Cg1–Cg1 [symmetry codes: (i) −x + 1, −y + 1, −z + 2; (ii) −x + 3/2, y + 1/2, −z + 3/2; (iii) x−1, y, z; (iv) −x−1, −y + 1, −z + 1.] may further stabilize the structure, with a centroid–centroid distance of 3.804 Å.

Infrared spectra

In the IR spectra of the complex, the ν(O–H) vibration of the coordinated water molecules is seen at 3504 cm⁻¹. When we examine the synthesized complex, the carbonyl group COO⁻ asymmetric and symmetrical vibrations are observed at 1541–1390 cm⁻¹, respectively. The vibration of the coordinated water molecules is seen at 1052 cm⁻¹. In the IR spectra of the complex, the ν(O–H) vibration of water. In general, the calculated and theoretical FT-IR spectra were compared with the experimental data. The calculated bond lengths and angles are in agreement with the experimental data. The experimental value for the Co1–O2 distance is 2.049 Å and the calculated value is 1.942 Å. The Co1–N1 distance was 2.146 Å, while the calculated value is 1.962 Å. The Co1–O4 distance was found experimentally to be 2.149 Å and calculated theoretically as 2.274 Å, while the O1–C1 distance is the same experimentally and theoretically as 1.250 Å. The O2–C1 bond distance was reported experimentally as 1.256 Å and calculated theoretically as 1.285 Å. Co1–O2 and O2–C1 bonds are rotatable; however, the Co1–N1, Co1–O4, and O1–C1 bonds are not rotatable.

The experimental angle value of O2–Co1–N1 is 89.48° and the computational value is 89.77°. The experimental and theoretically calculated values for O2–Co1–O4 are 88.99° and 85.07°, respectively. The experimental and the theoretically calculated values for N1–Co1–O4 are 90.88° and 86.38°, respectively, while O1–C1–O2 is 124.4° experimentally and 126.69° theoretically.

All the molecular orbital energies (highest occupied molecular orbital (HOMO), lowest unoccupied molecular orbital (LUMO), and energy gaps (E_{GAP} = E_{HOMO} − E_{LUMO}) of the complex were studied in dimethyl sulfoxide (DMSO). The energy gap value (eV) of the complex was calculated as 3.998 eV. This value is 3.763 eV in the gas phase. The energy gap, ionization potential, polarizability, dipole moment, electronegativity, electrophilicity index, electron affinity, and softness and hardness values of the complex are given in Table 5.

The electron density of the synthesized complex was investigated in DMSO. The blue region is electropositive, the reddish region is electronegative, and the green region is neutral. The carboxylate group (O2–C1–O1 and opposite) that interacts with water is electronegative. The hydrogen atom in the hydroxy group attached at the third position of the pyridine ring is electropositive and its oxygen atom is slightly electronegative. The other regions in the molecule are neutral (green).

Vibrational frequency analysis was studied for the complex in the gas phase and the calculated FT-IR spectra were compared with the experimental FT-IR spectra. The peak at about 3800 cm⁻¹ in the calculated spectrum is related to the hydroxy group on the pyridine. The large peak for the complex at 3143 cm⁻¹ shows an interaction of the carboxylate group with water. In general, the calculated and theoretical FT-IR spectra values are in agreement with each other (Figure 6).
Table 4. Calculated thermochemical values of the complex with B3LYP/6-31G(d,p) level of theory in gas phase.

|                  | $\varepsilon_0$ (Hartree) | $\varepsilon_0 + \varepsilon_{ZPE}$ (Hartree) | $\varepsilon_0 + \varepsilon_{corr}$ (Hartree) | $\varepsilon_0 + H_{corr}$ (Hartree) | $\varepsilon_0 + G_{corr}$ (Hartree) |
|------------------|---------------------------|-------------------------------------------|-------------------------------------------|-------------------------------------------|-------------------------------------------|
| Complex          | −3420.077                 | −3419.659                                 | −3419.620                                 | −3419.619                                 | −3419.735                                 |
| H$_2$O           | −76.420                   | −76.398                                   | −76.396                                   | −76.395                                   | −76.417                                   |

$\varepsilon_{corr}$ is the total internal thermal energy, $C_{tot}$ is the total constant volume heat capacity and $S_{tot}$ is the total entropy. $G_{corr}$ is the correction for the Gibbs free energy. $H_{corr}$ is the correction for the enthalpy.

Table 5. The calculated parameters of the complex using B3LYP/6-31G(d,p) basis set at DFT/TD-DFT.

|                  | Value     |
|------------------|-----------|
| Polarizability ($\alpha$) | 327.056   |
| LUMO energy ($E_{LUMO}$)   | −1.274    |
| HOMO energy ($E_{HOMO}$)   | −5.272    |
| Energy gap ($E_{\text{Gap}}$) | 3.998     |
| Dipole moment ($\mu$)      | 0.000252  |
| Ionization potential ($I$) | 5.272     |
| Electron affinity ($A$)    | 1.274     |
| Electronegativity ($\chi$) | 3.273     |
| Chemical hardness ($\eta$) | 1.999     |
| Global softness ($\sigma$) | 0.250     |
| Electrophilicity index ($\omega$) | 0.003 |

$E_{\text{Gap}} = E_{\text{HOMO}} - E_{\text{LUMO}}$  
$I = -E_{\text{HOMO}}$  
$A = -E_{\text{LUMO}}$  
$\chi = (I + A)/2$  
$\eta = (I - A)/2$  
$\sigma = 1/(2\eta)$  
$\omega = \chi^2/(2\eta)$

Dipole moment unit is Debye (D). Polarizability unit is Hartree (a.u.). HOMO and LUMO energy, energy gap, electron affinity, ionization potential, chemical hardness, global softness, electronegativity, and electrophilicity index unit is electron volt (eV).

LUMO: lowest unoccupied molecular orbital; HOMO: highest occupied molecular orbital.
The complex significantly inhibited hCA II with $K_i$ in the low micromolar range. $K_i$ value was determined to be 317.26 ± 84.90 µmol/L against hCA II (Table 7). In comparison, the $K_i$ for the standard CA inhibitor acetazolamide (AZA), a definitive hCA II inhibitor, was 433.22 ± 84.90 µmol/L against hCA II.

**Conclusion**

In this study, a new complex, [Co(DFB)$_2$(3-Pyr)$_2$(H$_2$O)$_2$], has been synthesized. The Co$^{II}$ atom is coordinated in a monodentate fashion with the oxygen atoms of the DFB anions, the nitrogen atoms of the 3-Pyr ligands, and the oxygen atoms of coordinated water molecules. The ratio of cobalt/DFB/3-Pyr ligands was determined as 1:2:2. The N2–O4 bonding sets around the metal center formed a distorted octahedral geometry. Geometry optimization, vibrational frequency analyses, and the molecular energy values (molecular orbitals) of the complex were studied using the B3LYP/6-31G(d,p) basis set in DFT/TD-DFT calculations. The experimental and theoretical values were compared with each other. The HOMO and LUMO energy values are negative, indicating that the structure is stable. The theoretical results obtained in this study are useful to obtain new complex derivatives as antimicrobial agents. The antimicrobial activity of the complex was investigated against *P. aeruginosa* (ATCC 27853), *K. pneumoniae* (ATCC 4352), *E. coli* (ATCC 25922), and Gram-positive *S. aureus* (ATCC) 6538. In addition, the complex had similar effects to those of commercially available neomycin X3385 and streptomycin X3385, but was less effective than ampicillin X3261. This novel complex would further studied for potential benefit from therapy of diseases such as epilepsy, gastric and duodenal ulcers, mountain sickness, glaucoma, osteoporosis, and neurological disorders.

**Experimental**

3,5-Difluorobenzoic acid (Alfa Aesar™), 3-pyridinol (Merck), sodium bicarbonate (Merck), and cobalt(II) sulphate heptahydrate (Merck) were used without any further purification. The C, H, and N percentages were measured using a LECO CHNS-932 elemental analyzer. FT-IR spectra were recorded on a Perkin Elmer Frontier™ FT-IR spectrometer from solid samples using a Diamond ATR accessory in the range of 4000–600 cm$^{-1}$. The crystal structure of the complex was determined on a Bruker SMART BREEZE CCD diffractometer.

**Synthesis of the complex**

To obtain sodium DFB, 3,5-difluorobenzoic acid (1.58 g, 10 mmol) and sodium bicarbonate (0.84 g, 10 mmol) were stirred at 60 °C in 100 mL of distilled water until complete removal of CO$_2$ gas. Next, CoSO$_4$.7H$_2$O (1.42 g, 5 mmol) and 3-pyridinol (1.22 g, 10 mmol) were dissolved in water (50 mL), and the obtained solution was added to the cobalt sulfate solution. The resulting mixture was allowed to crystallize. After 6–7 days, pink single crystals were obtained; crystals were filtered and washed with distilled water and then dried at room temperature. Anal. Calcd (%) for $C_{24}H_{20}CoF_4N_2O_8$ (molecular weight (MW) = 599.35): C, 48.09; H, 3.36; N, 4.72; Selected IR bands (cm$^{-1}$): $\delta$(OH)$_{H_2O}$ 3504, $\nu$(C–N)$_{py}$ 1052, $\nu$(COO$^\cdot$)$_{as}$ 1541, $\nu$(COO$^\cdot$)$_{s}$ 1390, ($\Delta$ν 151), $\nu$(Co–O) 643.
Computational details

The Gaussian 09 program \(^42\) was used for theoretical calculations and GaussView 6.0 \(^43\) and Avogadro \(^44\) were used to visualize the calculated values. The 6-31G(d,p) basis set of the B3LYP (Becke-3-Lee-Yang-Parr) \(^45,46\) functional correlation in Density Functional Theory (DFT) was used for geometry optimization and frequency analyses of the complex. In addition to the calculations in the gas phase, all stages were repeated in the DMSO as the solvent in order to investigate the solvent effects. TD-DFT (Time Dependent–Density Functional Theory) calculations were also calculated using the B3LYP/6-31G(d,p) basis set. The excited state properties were calculated as 50 single excited states via TD-DFT. Electron-density surfaces were displayed according to the self-consistent field (SCF) density matrix. All calculations were compared with experimental data.

Antibacterial properties

The antibacterial properties of the synthesized complex were investigated against Gram-positive \(S.\) aureus (ATCC 6538), Gram-negative \(E.\) coli (ATCC 25922), \(P.\) aeruginosa (ATCC 27853), and \(K.\) pneumoniae (ATCC 4352). Microorganisms obtained from microbiological environmental protection laboratories were cultivated in the research laboratories of Kafkas University Faculty of Engineering and Architecture and the obtained bacteria were used in experiments. The antimicrobial effects of the resulting molecules were investigated as biological applications in Mueller–Hinton agar (MHA) medium. First, the Mueller–Hinton broth (MHB) for activation of the bacterial stock was carried out for 24 h with incubation at 37°C. Bacteria which were standardized with 0.5 McFarland standard were seeded in sterile prepared petri dishes; 0.05 g of the synthesized complex was dissolved in 5 mL of DMSO and homogeneous solutions were prepared. Samples (50 \(\mu\)L) from the stock solutions were transferred into wells drilled 4 mm in diameter using an automated pipette. The inhibition zone was incubated at 37°C ± 1°C for 18–24 ± 2 h to determine the diameters.\(^{47-50}\) All inhibition zones were measured in millimeter.

Author’s Note

Crystallographic data for complex reported in this article have been deposited with the Cambridge Crystallographic Data Center as Supplementary Publication No. CCDC 1972765. Copies of these data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223 336033, or online via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk. The numerical calculations reported in this paper were fully performed at TUBITAK ULAKBIM, High Performance and Grid Computing Center (TRUBA resources).

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Supplemental material

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Table 6. Antibacterial zone diameters of complexes (mm).

| Complex                  | \(P.\) aeruginosa | \(K.\) pneumonia | \(E.\) coli | \(S.\) aureus |
|--------------------------|-------------------|------------------|-------------|--------------|
| Ampicillin X3261         | 26                | 16               | 15          | 20           |
| Neomycin X3385           | 36                | 35               | 34          | 37           |
| Streptomycin X3385       | 17                | 16               | 16          | 13           |
|                           |                   |                  |             |              |

Table 7. The enzyme inhibition results of novel complex against human carbonic anhydrase isoenzymes I and II (hCA I and II).

| Compounds          | \(IC_{50}\) (\(\mu\)M) | \(K_i\) (\(\mu\)M) |
|--------------------|------------------------|-------------------|
|                    | hCA I   | hCA II  | hCA I   | hCA II   |
| Novel Co (II) Complex | 274.37 | 0.9736 | 204.33 | 0.9904 | 317.26 ± 23.25 | 255.41 ± 48.05 |
| AZA\(^a\)          | 394.30 | 0.9683 | 337.86 | 0.9693 | 433.22 ± 55.30 | 384.14 ± 84.90 |

\(^a\)Acetazolamide (AZA) was used as a control for hCA I and II.
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