Phenoxazinone synthase and antimicrobial activity by a bis(1,3-diamino-2-propanolate) cobalt(III) complex

BISWAJIT CHOWDHURY^a, BIPLAB BHOWMIK^b, AMRITA SAHU^c, MAYANK JOSHI^e,
SUVENDU PAUL^d, ANGSHUMAN ROY CHOUDHURY^e and BHASKAR BISWAS^{a,*}

^aDepartment of Chemistry, University of North Bengal, Darjeeling, West Bengal 734 013, India

^bDepartment of Zoology, Panchakot Mahavidyalaya, Purulia, West Bengal 723 121, India

^cDepartment of Electrical Engineering, Temple University, Philadelphia 741235, USA

^dDepartment of Chemistry, University of Kalyani, Kalyani, West Bengal 741 235, India

^eDepartment of Chemical Sciences, Indian Institute of Science Education and Research Mohali, Mohali, Punjab 140 306, India

E-mail: mr.bbiswas@rediffmail.com; icbbiswas@gmail.com

MS received 7 July 2018; revised 2 September 2018; accepted 24 September 2018; published online 15 November 2018

Abstract. In this work, we have synthesised and structurally characterized a mononuclear cobalt(III) complex, [Co(2-O-pn)₂]Cl·2H₂O (**1**), (2-O-pn = 1,3-diamino-2-propanolate). From the X-ray structure of the cobalt complex, it is revealed that Co(III) ion in **1** adopts an octahedral geometry and crystallizes in the monoclinic system with *C2/c* space group. The lattice aqua molecule in combination with chloride ion in **1** forms a water-chloride cluster, (H₂O)₂^{···}(Cl)₂ through strong H-bonding interaction mediated *via* cobalt(III) complex in a unique binding motif. This cobalt(III) complex has been tested as an effective catalytic system towards the oxidative coupling of 2-aminophenol (2-AP) in the MeCN medium. *In situ* mass spectral analysis confirms that 2-AP forms an adduct with cobalt ion and the course of catalysis proceeds through catalyst-substrate binding followed by oxidative coupling of 2-AP with iminobenzoquinone. This cobalt(III) catalyst affords exclusively aminophenoxazinone species with a significant turnover number, $k_{\text{cat}} : 6.37 \times 10^2 \text{ h}^{-1}$ in the MeCN medium. This cobalt(III) complex is able to screen out the growth of some bacteria and fungi species. Quantum chemical calculations employing density functional theory is used to model structural parameters and spectroscopic behaviour. The theoretical findings corroborate well with the experimental results.

Keywords. Bio-mimicking Chemistry; Cobalt(III); DFT study; Phenoxazinone synthase activity; X-ray structure.

1. Introduction

Cobalt complexes with (N,N)-donor chelators have created a huge appeal to the chemists because of their significant applications in the field of catalysis, drug design, enzymatic bio-functionalities and material science.^{1–3} Among the transition elements, cobalt is a bio-essential element which is found in cobalamin and in some other metalloproteins.⁴ Cobalamin is a necessary part in the formation of myelin that acts as an insulating

layer. It commonly exists around nerves that assist to produce red blood cells including metabolism of fats and carbohydrates, along with protein synthesis.⁴ In general, metal complexes play a pivotal role in the development of antibiotics that is helpful to cure infectious diseases. Metal-based drugs also lead to new antibacterial and antifungal agents which are very effective to resist the growth of microbial infections.^{5,6}

On the other hand, catalytic activity on oxidation from 2-aminophenol (2-AP) to 2-amino-3H-phenoxazine-3-one (2-APX) by coordination compounds has received considerable attraction for providing important information about bioinorganic reactions and mechanistic aspects in the organic transformation which may open

*For correspondence

BHASKAR BISWAS: On lien from: Department of Chemistry, Surendranath College, Kolkata 700 009, West Bengal, India.

a new avenue in molecular science.^{7–9} Actinomycin D, a naturally occurring antineoplastic agent behaves as an inhibitor towards DNA-directed RNA synthesis and is related to questiomycin A which is known as 2-Amino-3H-phenoxazine-3-one.¹⁰ This species is used clinically for the treatment of certain types of cancer.¹¹ It is well-observed that in the final step of the biosynthesis of actinomycin D, the enzyme phenoxazinone synthase catalyzes the oxidative coupling of 2-aminophenol to the phenoxazinone chromophore.^{12–14} A literature survey indicates that Okamoto *et al.*¹⁵ attempted to utilize Co(III) ion as a blocking agent for 1,3-diamino-2-propanol and the stereochemistry of the cobalt complex was determined by ¹³C NMR. At present, we have introduced the synthesis and structural characterization of a cobalt(III) complex containing a diamine. The lattice aqua molecules and chloride ion in **1** interact strongly through H-bonding and help to build a water-chloride cluster, (H₂O)₂⁺ (Cl)₂[−] in the crystalline phase. This cobalt(III) complex behaves as a good catalyst with a significant turn over number, $k_{\text{cat}} : 6.37 \times 10^{-2} \text{ h}^{-1}$ in the oxidative coupling of 2-aminophenol in MeCN medium. Investigation of antimicrobial properties for this cobalt(II) complex reveals that the growth of different bacteria and fungi species is effectively screened by this cobalt(III) complex. Further, detailed theoretical computations on the cobalt(III) complex in vacuum and in methanol are well supported by the experimental observations.

2. Experimental

2.1 Chemicals, solvents and starting materials

Sufficiently pure 1,3-diamine-2-propanol (Alfa-Aesar, UK), cobalt(II) chloride hexahydrate (E-Merck, India), 2-aminophenol (E-Merck, India) and all other materials were purchased from commercial sources. All other chemicals and solvents are of analytical grade and used as received without further purification.

2.2 Preparation for [Co(2-O-pn)₂]Cl·2H₂O (**1**)

An aqueous solution of 1,3-diamino-2-propanol (0.180 g, 2 mM in 20 mL) is added dropwise to an aqueous solution of CoCl₂·6H₂O (0.237 g, 1 mM in 10 mL) and stirred the solution for 30 minutes. The red coloured solution is kept for slow evaporation for 7–10 days and beautiful red coloured single crystal is separated out from the solution,

Yield: ~0.192 g (~81% based on metal salt). Anal. Calc. for C₆H₂₂N₄O₄ClCo (**1**): C, 23.35; H, 7.18; N, 18.15. Found: C, 23.40; H, 7.15; N, 18.21. IR (KBr, cm^{−1}): 3403 (ν_{O–H}), 3311 (ν_{N–H}); UV-Vis (λ_{max}/nm, (Abs) in MeCN 10^{−4} M, 1

cm optical pathlength): 342 (0.86), 379 (0.68), 475 (0.64); ESI-MS (MeCN): m/z, 273.48 [Co(2-O-pn)₂·2H₂O]⁺ (Theoretical m/z, 273.19).

2.3 Physical measurements

Elemental analyses (carbon, hydrogen and nitrogen) were performed on a Perkin-Elmer 2400 CHNS/O elemental analyzer. Fourier Transform Infrared (FT IR) spectra (KBr discs, 4000 – 300 cm^{−1}) were recorded using an FTIR-8400S SHIMADZU spectrophotometer in the range 400–3600 cm^{−1}. Steady-state absorption spectral measurements were operated with a JASCO model V-730 UV-Vis spectrophotometer. The ¹H nuclear magnetic resonance (NMR) spectra were recorded on a Bruker DPX-500 MHz spectrometer. Electrospray ionization (ESI) mass spectrum was recorded using a Q-tof-micro quadrupole mass spectrometer. Molar conductivity measurement was done using a Horiba LAQUA Benchtop DS 70 conductivity meter. A Gouy balance was used to determine the magnetic moments of the powdered samples, employing Hg(II) tetrathiocyanatocobaltate(II) as a calibrant. Diamagnetic corrections were made from Pascal's constants.

2.4 X-ray diffraction study

Single crystal X-ray diffraction data of the cobalt(III) complex are collected using a Rigaku XtaLABmini (Fixed 2Theta and Distance) diffractometer equipped with Mercury375R (2x2 bin mode) CCD detector. The data are collected with graphite monochromated Mo-Kα radiation (λ = 0.71073 Å) at 298 K using ω scans. The data are reduced using CrysAlisPro 1.171.38.46 (Reference Rigaku Corporation: Tokyo, Japan). The space group determination is done using Olex2. The structure is resolved by direct method and refined by full-matrix least-squares procedures using the SHELXL-2014/7¹⁶ software package through OLEX2 suite.¹⁷

2.5 Phenoxazinone Synthase activity by the cobalt(III) complex

To examine phenoxazinone synthase-like activity, 1 × 10^{−4} M solution of **1** in MeCN medium is added to 10 equiv. of 2-aminophenol (2-AP) under aerobic conditions at room temperature. Absorbance vs. wavelength (wavelength scans) of these solutions are recorded at a regular time interval of 8 minutes for 2-AP oxidation in the wavelength range from 300–800 nm.¹⁸

Kinetic experiments were performed spectrophotometrically with complex **1** and 2-AP in MeCN at 298 K for aminophenol oxidation activity.¹⁹ 0.04 mL of the complex solution, with a constant concentration of 1 × 10^{−4} M, is added to 2 mL of 2-AP of a particular concentration (varying its concentration from 1 × 10^{−3} M to 1 × 10^{−2} M) to achieve the ultimate concentration of the complex as 1 × 10^{−4} M. The conversion of 2-aminophenol to 2-aminophenoxazine-3-one was monitored with time at a wavelength 434 nm (time scan)

in MeCN.²⁰ To determine the dependence of rate on substrate concentration, kinetic analyses were performed in triplicate.

Column chromatography technique using neutral alumina as column support and benzene-ethyl acetate as an eluant mixture has been employed to isolate phenoxazinone species in pure form which is obtained through catalytic oxidation of 2-AP. The compound was found in high yield (~73% for **1**). ¹H NMR spectral analysis was used to identify the species. ¹H NMR data for 2-amino-3*H*-phenoxazine-3-one (APX), (CDCl₃, 400 MHz) δ_{H} : 7.62 (m, 1H), 7.44 (m, 3H), 6.47 (s, 1H), 6.39 (s, 1H), 6.28 (s, 1H). The identification of this phenoxazinone product is further consolidated in ESI-MS spectrometry from the appearance of a base peak at *m/z* 213.

2.6 Detection of the presence of hydrogen peroxide in the catalytic oxidation of 2-aminophenol

To ensure the active participation of molecular oxygen in the course of catalysis, the formation of hydrogen peroxide was checked.^{2b, 19} It is well-known that molecular oxygen in oxidative catalytic reactions transforms into hydrogen peroxide or water as a byproduct. In this course of the oxidative catalytic reaction, the solution is acidified with H₂SO₄ to become pH 2. After a certain time, an equal volume of water is added to stop further oxidation. The phenoxazinone species are extracted three times with dichloromethane. 1 mL of 10% solution of KI and three drops of a 3% solution of ammonium molybdate are added to the aqueous layer. The formation of I₃⁻ could be monitored spectrophotometrically because of the development of the characteristic I₃⁻ band (λ_{max} = 353 nm).

2.7 In vitro antimicrobial activity study

Some of the microbial (bacterial and fungal) strains, *Escherichia coli* 8739TM, *Staphylococcus aureus* 6538TM, *Salmonella typhi* 14028TM, *Proteus vulgaris* 8427TM, *Klebsiella pneumoniae* 700721TM and *Cryptococcus neoformans* MYA-565TM, *Fusarium oxysporum* and *Aspergillus niger* 16404TM were sourced from ATCC. This Co(III) complex was tested against these bacterial and fungal species cultured on dextrose agar medium by the well diffusion method.²¹ The growth of bacterial organisms was observed on muller hinton agar while the effect of cobalt complex on fungal species was studied on sabouraud dextrose agar in petri plates. The pathogens were swabbed uniformly onto the individual plates using sterile cotton swabs. Different concentration (10 μ g, 50 μ g and 100 μ g) of the test sample was poured onto each well on all plates. After incubation at 37 °C for 24 h for bacteria and 28 °C for 72 h for fungi, the different levels of the zone of inhibition were measured.

2.8 Computational details

Ignoring symmetrical restrictions, the quantum chemical computations were performed with Gaussian 09W programme suite.²² For all the atoms, B3LYP theoretical model and widely used 6-311G basis set^{23,24} were compiled with

density functional theory (DFT) for the ground state and time-dependent density functional theory (TD-DFT) for excited state computations. Firstly, we optimized the mononuclear cobalt(III) complex, **1** in a vacuum and then in acetonitrile (ϵ = 35.688) employing integral equation formalism polarizable continuum model (IEFPCM).^{25–28} Thereafter, the stability of all the complexes were confirmed through theoretical IR frequency calculations. The images of molecular structure and frontier molecular orbitals (FMOs) were extracted from checkpoint files.

3. Results and Discussion

3.1 Synthesis, spectroscopic behaviour and solution properties of cobalt(III) complex (**1**)

Bis(1,3-diamino-2-propanolate)cobalt(III) chloride dihydrate is prepared (Scheme 1) in the form of single crystal by addition of hydrated cobalt(II) chloride with diamine chelator in an aqueous methanolic medium under stirring condition. The structural formulation is defined through different analytical techniques. The geometry of the cobalt(III) complex (**1**) is determined and confirmed by single crystal X-ray diffraction study.

The IR spectrum for this cobalt(III) complex (Figure S1, Supplementary Information) displays a broad peak at ~3403 cm⁻¹ which is assignable to the O-H stretching frequency for the lattice aqua molecule. Another, strong peaks at ~3311 cm⁻¹ corresponds to the N-H frequency of diimine ligands.²⁹ Room temperature magnetic measurement confirms that cobalt(III) ion in **1** exists in low spin conformation. The magnetic moment (μ_{eff}) is found as ~0.01 BM and consolidates the diamagnetic nature of cobalt centre in the solid state. Room temperature EPR spectrum of Co(III) compound in MeCN remains silent (S=0 in ground state) that authenticates the low spin conformation of Co(III) species in **1**.

The UV-Vis spectrum of **1** for 1×10^{-4} M in MeCN solution shows the main characteristic electronic transitions at 342, 379 and 475 nm. The high energy band at 342 nm is assignable to the ligand to metal charge transfer (LMCT). Another two important characteristics of electronic transitions at 379 and 475 nm arise

Scheme 1. Preparative procedure of Co(III) complex (**1**).

for the existence of low spin cobalt(III) species of octahedral geometry in solution (Figure S2, Supplementary Information).³⁰ Furthermore, these electronic bands at 379 & 475 nm correspond to ${}^1A^{1g} \rightarrow {}^1T^{2g}$ and ${}^1A^{1g} \rightarrow {}^1T^{1g}$ transitions respectively.³⁰ The structural solidity in MeCN for the cobalt(III) complex has been further confirmed by ESI mass spectrum. ESI mass spectral analysis of **1** in MeCN displays the primary zone of coordination for this cobalt(III) complex as the most stable species in solution and exhibits the characteristic peak at m/z 273.43 corresponds to $[Co(2-O-pn)_2 \cdot 2H_2O]^+$ ionic species in solution (Figure S3, Supplementary Information). The optical bands of the cobalt(III) complex in MeCN solution remain unchanged over a period of 72 h. This observation suggests that this mononuclear cobalt(III) is stable in its original form in solution. We have also recorded an EPR spectrum of **1** in the MeCN medium at room temperature. The nature of EPR plot is found as silent and justifies its low spin conformation of cobalt(III) ion in the MeCN medium. The EPR result suggests the diamagnetic nature of the cobalt(III) complex. To investigate the electrolytic nature of the complex in 1.0×10^{-3} M MeCN solution, we have also recorded molar conductance value at room temperature. The value of molar conductivity is found as $95 \mu S/cm$ which suggests about 1:1 electrolytic behaviour in solution.

3.2 Depiction of X-ray structure

Single crystal X-ray diffraction study of the cobalt(III) complex defines the coordination linkages of the primary and secondary zone of coordination around cobalt (III) centre. The ORTEP diagram of the cobalt(III) complex is shown in Figure 1. The molecular structure of **1** reveals that cobalt(III) complex crystallizes in a monoclinic system with $C2/c$ space group. The cobalt(III) ion in the primary zone of coordination satisfies by two tridentate 1,3-diamino-2-propanol ligands in an octahedral geometry. Very interestingly, we are able to isolate the cobalt complex in *trans*-form. This form of complex contains higher symmetry elements like the plane of symmetry and centre of inversion. Furthermore, the coordination motif of this tri-dentate chelator doesn't allow to produce *cis*-form. The existence of *trans*-form is also attested from symmetry perspective.

Though the free amine has an aliphatic-OH group, in reaction with cobalt(III) ion, the ligand acts as a monoanionic system and interestingly, the *trans* form is isolated as red crystals from the solution. The residual cationic charge of the *bis*-chelate cobalt(III) complex is neutralized by counter anionic chloride ion in the secondary zone of coordination. The Co1–O/N bond

Figure 1. An ORTEP diagram of cobalt(III) complex with 30% ellipsoid probability. Inset: Microscopic image of the single crystal for red coloured cobalt(III) complex.

lengths are found in the range from 1.90 to 1.96 Å. A comparison on crystallographic bond distances among this Co(III) complex with previously reported mononuclear cobalt(III) complexes^{30a,b,c, 40} accounts that average Co–N distances of Co(III) compounds with low spin confirmation lie 1.90 to 1.94 Å and helps to predict the existence of Co(III) species in low spin conformation in crystalline state. The single crystal X-ray diffraction refinement parameters for **1** are presented in Table 1. Important bond lengths and angles for **1** are also summarized in Table 2.

3.3 Investigation of crystalline architectures including water-chloride cluster

In order to view the involvement of weak forces in the growth of crystalline architectures in the solid state for this cobalt complex, it is found that lattice water molecules and chloride anions play a significant role through the H-bonded network. It is seen that two chloride ions in association with three lattice water molecules form a water-chloride cluster that extends along *b* axis through another set of H-bonded interactions with metal-ligand backbone and leads to a novel mode of association. As a result, a 3D crystalline architecture is constructed in the solid state (Figure 2). The intermolecular hydrogen bonding association plays a pivotal role in forming a water-chloride cluster along *b*-axis among the lattice aqua molecules and counter anionic chloride ions $[(O2-H2c \cdots Cl1, 2.39; O2-H2d \cdots Cl1, 2.53; C3-H3b \cdots Cl1, 2.82; N2-H2b \cdots Cl1, 2.39; N1-H1a \cdots O2, 2.23 \text{ Å}]$ (Figure 2, Table S1, Supplementary Information). To the best of our knowledge,

Table 1. Crystallographic refinement parameters of [Co(2-O-pn)₂]Cl·2H₂O (**1**).

Crystal parameters	1
Empirical formula	C ₆ H ₂₂ N ₄ O ₄ ClCo
Formula weight	308.43
Temperature	298 K
Wavelength	0.71075 Å
Crystal system	Monoclinic
Space group	C2/c
Unit cell dimensions	a = 15.9657(3) Å, α = 90°; b = 8.0882(3) Å, β = 105.50(5)°; c = 10.7397(5) Å, γ = 90°
Volume	1336.42(11) Å ³
Z	4
Density (calculated)	1.710 g/cm ³
Absorption coefficient	1.693 mm ⁻¹
Reflections collected	8937
Independent reflections	1959
R(int)	0.021
Goodness-of-fit on F ²	1.13
R indices (all data)	R1 = 0.0353, wR2 = 0.0958
Largest diff. peak and hole	1.34 and -0.62 e. Å ⁻³

Table 2. Selected bond lengths (Å) and bond angles (°) for [Co(2-O-pn)₂]Cl·2H₂O (**1**) from the XRD structure (values within the parenthesis denote the corresponding data obtained from DFT study).

Bond lengths (Å)					
Co1-O1	1.9007 (1.886)	Co1-N2	1.9637 (1.992)		
Co1-N1	1.960 (1.993)				
Bond angles (°)					
O1-Co1-N1	86.67 (85.5)	O1-Co1-O1*	180.0 (180.0)	O1-Co1-N2*	95.91 (94.1)
O1-Co1-N2	84.09 (85.5)	O1-Co1-N1*	93.33 (94.7)	N1-Co1-N2	85.75 (85.7)
O1*-Co1-N1	93.33 (94.5)	N1-Co1-N1*	180.0 (180.0)	N1-Co1-N2*	94.25 (94.2)
O1*-Co1-N2	95.91 (94.2)	N2-Co1-N2*	180.0 (179.9)	O1*-Co1-N1*	86.67 (85.5)
O1*-Co1-N2*	84.09 (85.8)	N1*-Co1-N2*	85.75 (85.7)		

this mode of binding between solvent molecules and counter-anionic chloride will be a novel one that wasn't previously observed.

3.4 Theoretical modelling of the structure and frontier molecular orbitals

The structural integrity of the mononuclear Co(III) complex is studied by computational modelling using DFT calculation. The calculated structure of **1** in a vacuum (Figure 3) in accordance with the X-ray structure. In addition, the bond lengths and bond angles are in good agreement with the experimental values (Table 2). The gas phase structure also defines the similar degree of denticity (tridentate) for 2-hydroxy-1,3-diaminopropane ligand towards cobalt ion and consolidates the formation of *bis*-chelate structure in *trans* conformation. The cobalt ion is also octahedrally surrounded by four nitrogen atoms and two oxygen ion

like the mapping of coordination in single crystal X-ray structure.

We have also performed excited state calculation, which is widely used to define the optical properties of the metal complex in MeCN. The energy gap between HOMO → LUMO and HOMO - 1 → LUMO + 1 (Figure 4) portrayed a clear view regarding the electronic activity of **1** in solution. It is well-established that low HOMO-LUMO energy gap provides higher chemical activity and lower thermal stability of a molecule. The experimental and simulated UV-Vis wavelengths along with the major contributing orbitals and oscillator strengths for **1** are reported in Table S4, Supplementary Information.

3.5 Phenoxazinone synthase mimicking the activity of the cobalt(III) complex (**1**)

The 2-aminophenol oxidation activity by the cobalt(III) complex (**1**) is examined using a convenient substrate

Figure 2. Formation of $[(\text{Cl})_2 \cdots (\text{H}_2\text{O})_3] \cdots [\text{Co}(\text{2-O-pn})_2]\text{Cl} \cdot \text{H}_2\text{O}$ crystalline architectures in **1** [Red, green and blue colours in ORTEP indicate O, Cl and N atoms, respectively].

Figure 3. Optimized structure of **1** in vacuum using DFT/B3LYP/6-311G theory.

2-aminophenol (2-AP) under the aerobic condition in MeCN at room temperature (25 °C) (Scheme 2).

To study the catalytic oxidation of 2-aminophenol, 1×10^{-4} M solution of Co(III) complex is added to a 1×10^{-3} M solution of 2-AP in MeCN medium. The course of catalysis is monitored using UV–Vis spectrophotometer. The time-dependent scan is recorded at a time interval of 8 min for 2 h. 2-AP in MeCN solution displays a single band at 267 nm which is an indication for its pure form in solution. It is observed during the spectrophotometric titration, upon addition

Figure 4. Frontier molecular orbitals of **1** along with the corresponding transition energy values calculated using TD-DFT/B3LYP/6-311G theoretical model and IEF-PCM/acetonitrile solvent system.

of cobalt complex to 2-AP in MeCN, absorbance value for 2-AP at 267 nm³¹ is gradually decreased with the progress of time (Figure S4, Supplementary Information). Simultaneously, a new band at 434 nm with incremental absorbance was observed (Figure 5, Figure S4, Supplementary Information). The appearance of the new optical band at 434 nm upon addition of cobalt

Scheme 2. Catalytic oxidation of 2-aminophenol by phenoxazinone synthase.

Figure 5. Increase of absorbance for the production of aminophenoxazinone at 434 nm upon catalytic addition of **1** to 10 equivalents of 2-AP in MeCN. The spectra were recorded after every 8 min at 298 K.

complex is a definite sign for the catalytic production of aminophenoxazinone species in solution.^{32–34} The controlled experiment was also carried out for phenoxazinone synthase activity using 2-AP in MeCN solvent under the aerobic atmosphere at room temperature. We have also performed the controlled experiments in presence of 1,3-diamino-2-propanol to 2-AP under identical reaction conditions (Figure S5, Supplementary Information). However, no significant growth of phenoxazinone species was observed up to 2 h. Close inspection of the spectrophotometric titration on aminophenol oxidation, it is suggested that the titration curves get saturated after 2 h of catalysis by the mononuclear cobalt(III) complex.

The details of kinetic investigations for the catalytic oxidation of 2-AP were performed to realize the efficacy for this cobalt(III) catalyst. The method of initial rates was followed to unveil the nature of kinetic for this catalytic oxidation of 2-AP. The growth of phenoxazinone chromophore was monitored at 434 nm as a function of time (Figure S6, Supplementary Information).^{35,36} The plot of rate constants versus concentration of the substrate displayed the saturation kinetics. The values of kinetics parameters were determined from the Michaelis-Menten approach of enzymatic kinetics **1** and presented in Table 3.

With an aim to check the level of phenoxazinone synthase reactivity between our cobalt complex and few other reported cobalt(III) complexes we have made a comparison in Table 3. The catalytic efficiency for **1**, $k_{\text{cat}}/K_M = 2.02 \times 10^5$ towards catalytic aminophenol oxidation was also found high.

To view the insights of the mechanistic route, we studied important scientific literature about catalytic phenoxazinone activity by the metal complexes. Previously, Chaudhury and co-workers³⁷ modeled a tetracopper complex for the mimicking the bio-functionalities of phenoxazinone synthase enzyme. They proposed an ‘on-off’ mechanism of radicals in association with the electronic participation of the metal centers in favour of 6e oxidative coupling of the substrate in the course of catalysis containing enzyme phenoxazinone synthase. On the other hand, Begley *et al.*,³⁷ recommended the synthesis of 2-aminophenoxazinone through a sequence of three consecutive 2e substrate oxidations. The tautomerization reactions were the controlling unit in the regeneration of the 2-AP during this course of the catalytic oxidation reaction.

We have recorded *in situ* electron spray ionization (ESI) mass spectrum of the reaction mixture in MeCN medium to gain important information to propose the catalytic route by cobalt(III) complex in the oxidative coupling of 2-AP to phenoxazinone species. ESI-MS provides very valuable information about the generation of reactive species and product during the course of catalysis. The ESI mass spectral analysis of the reaction mixture (Figure S7, Supplementary Information) for **1** in MeCN medium exhibited the characteristics peaks at m/z 214.56 and 346.28 with isotope distribution patterns. This ESI mass spectral results strongly recommended the corroboration of [(2-amino-3*H* – phenoxazine-3-ones) + H^+] and [(Co(2-O-pn)₂(2-AP)) + H^+] at m/z 214.56 and 346.28 respectively. Furthermore, ¹H NMR of the cobalt(III) complex in presence of 2-AP in 1:10 ratio in CDCl₃ was recorded (Figure S8, Supplementary Information). The reaction mixture was kept in an open atmosphere for 10 min to allow the participation of molecular oxygen under slow stirring condition. The appearance of proton signals corresponding to aromatic protons (6.0 to 7.8 ppm) of 2-AP and oxidative coupling product, APX respectively attested the generation of substrate–catalyst adduct during the catalytic oxidation of 2-AP. The raising proton signals in the range 2 to 4.2 ppm, however, justified the presence of aliphatic-H and -NH₂ of ligand backbone (Figure S8, Supplementary Information).

ESI-MS spectral analysis made a recommendation that the catalytic oxidations are carried out by these cobalt(III) complexes through catalyst-substrate

Table 3. Kinetic parameters for the catalytic oxidation of 2-AP by **1** in MeCN at 25 °C.

Complex	$V_{\max}$ (M s^{-1})	K_m (M)	k_{cat} (h^{-1})	Ref
1*	1.77×10^{-5}	3.16×10^{-3}	6.37×10^2	This work
1	7.87×10^{-4}	4.31×10^{-4}	2.83×10^4	38
1	4.62×10^{-6}	0.67×10^{-4}	4.62×10^2	39
2	1.74×10^{-5}	3.23×10^{-4}	1.74×10^{-1}	39

Std. Error for **1**, $V_{\max}$ (M s^{-1}) = 8.93×10^{-7} ; Std. Error for K_m (M) = 2.74×10^{-4} .

Scheme 3. Optimized structures of the 2-AP with Co(III) complex in chelating mode (left, A form) & monodentate mode (Right, B-form) of binding for 2-AP.

intermediate in the course of catalytic oxidation. Now, 2-AP can behave as a chelating ligand as well as a monodentate donor system. To explore the mode of binding of the substrate, 2-AP towards catalyst, we made an investigation through computational modeling on substrate-catalyst adducts (Scheme 3). Energy calculations in solvent phase on catalyst-substrate adducts, it was observed that 2-AP adduct with Co(III) centre through chelating mode (A) was more energy efficient than 2-AP adduct with Co(III) ion in monodentate motif (B).

Furthermore, the involvement of molecular oxygen as a participant in the course of catalysis was examined. Hydrogen peroxide was detected and extracted as a byproduct in the course of phenoxazinone synthase activity (Figure S9, Supplementary Information). The cobalt(III) complex in this oxidative catalysis couldn't be regenerated since the structural rearrangement at Co(III) centre in **1** did not allow to return back into its original form. Thus, we were unable to recycle the Co(III) catalyst in its original form during this oxidative catalysis of 2-AP. Now, on the basis of experimental and

theoretical observations, it can be proposed (Scheme 4) that the course of catalytic oxidation of 2-AP primarily proceeds through the formation of substrate-catalyst adduct followed by generation of iminobenzoquinone. The iminobenzoquinone further couples with another molecule of 2-AP to produce aminophenoxazinone species in solution.

3.6 Bio-Assay Investigations

To evaluate the antimicrobial property of the cobalt(III) complex, we applied the Co(III) complex towards some test organisms. 100 μL of each of the microbial suspension was spread onto agar plates which correspond to the broth in which they were maintained. Isolated colonies for each of the organisms those might be playing a pathogenic role should be selected from primary agar plates and tested for susceptibility by well diffusion method. The screened antimicrobial activity by the cobalt(III) complex is displayed in Figure S10 (Supplementary Information) and the results are presented in Tables S2 and S3, Supplementary Information.

Scheme 4. Proposed mechanistic pathway for 2-AP oxidation activity by **1**.

The $[\text{Co}(\text{2-O-pn})_2]\text{Cl}\cdot 2\text{H}_2\text{O}$ (**1**) complex showed the maximum antimicrobial activity against the *Staphylococcus aureus*, *Salmonella typhi*, *Fusarium oxysporum*. This cobalt(III) complex exhibited average or minimum antimicrobial activity against *Escherichia coli*, *Klebsiella pneumonia*, *Proteus vulgaris*, *Cryptococcus neoformans*, *Aspergillus niger* microorganisms.

The enhanced activity of the complex can be explained on the basis of mechanistic aspects of Tweedy's Chelation theory.⁴⁰ It is commonly observed that lipid and polysaccharides which are important constituents of cell walls and membranes show a preference for metal ion interaction. In addition to this, the cell wall also contains amino phosphates, carbonyl and cysteinyl ligands, which maintain the integrity of the membrane acting as a diffusion barrier and also provides a suitable site for bonding. In our case, metal-ligand coordination reduces the polarity of the metal ion significantly which makes this mononuclear Co(III) complex least-polar. The metal-chelate is probably adsorbed on the surface of the cell wall of microorganisms through the interactions with the preferential active sites and disturbs the respiration process of the cell. As a result, synthesis of the proteins is

blocked that restricts further growth of the organisms. So, the Co(III) complex behaves as the growth-inhibitor for the microorganism in an effective way.

4. Conclusions

Herein, we synthesized a mononuclear cobalt(III) complex, $[\text{Co}(\text{2-O-pn})_2]\text{Cl}\cdot 2\text{H}_2\text{O}$ (**1**) (2-O-pn = 1,3-diamino-2-propanolate) and structurally characterized through different analytical techniques. We were able to isolate the compound as red coloured crystals in single phase. X-ray structural analysis revealed that **1** crystallized in the monoclinic system with $C2/c$ space group. The lattice aqua molecules in combination with chloride ions in **1** presents a novel mode of supramolecular association and forms a water-chloride cluster, $(\text{H}_2\text{O})_2^+ (\text{Cl})_2^-$ through strong H-bonding interaction mediated via cobalt(III) complex. Theoretical modeling by Density Functional Theory (DFT) was employed to obtain structural & spectroscopic parameters. The theoretical findings were well-corroborated with the experimental results. This cobalt(III) complex was evaluated as an effective catalytic system towards the oxidative coupling of 2-aminophenol (2-AP) in the MeCN medium. *In situ*

mass spectral analysis confirmed that 2-AP forms an adduct with cobalt ion and the course of catalysis proceeded through catalyst–substrate binding followed by oxidative coupling of 2-AP with iminobenzoquinone. This cobalt(III) catalyst afforded exclusively aminophenoxazinone species with a significant turn over number, k_{cat} : $6.37 \times 10^2 \text{ h}^{-1}$ in the MeCN medium. This cobalt(III) complex was also able to screen the growth of some bacteria and fungi species.

Supplementary Information (SI)

Crystallographic data are available free of charge from The Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44-1223-336033; Email: deposit@ccdc.cam.ac.uk or www: <http://www.ccdc.cam.ac.uk>) upon request, quoting deposition number CCDC 1845554 for the Cobalt(III) complex (1).

Acknowledgements

The work is supported financially by the Department of Science and Technology (DST), New Delhi, India under FAST TRACK SCHEME for YOUNG SCIENTIST (NO. SB/FT/CS-088/2013 dtd. 21/05/2014). The single crystal X-ray facility of the Department of Chemical Sciences, IISER Mohali is acknowledged for the data collection.

References

- Reichmann M E, Rice S A, Thomas C A and Doty P 1954 A Further Examination of the Molecular Weight and Size of Desoxypentose Nucleic Acid *J. Am. Chem. Soc.* **76** 3047
- (a) Lahiri D, Majumdar R, Patra A K and Chakravarty A R 2010 Anaerobic DNA cleavage in red light by dicopper(II) complexes on disulphide bond activation *J. Chem. Sci.* **122** 321; (b) Chowdhury B, Maji M and Biswas B 2017 Catalytic Aspects of a Copper(II) Complex: Biological Oxidase to Oxygenase Activity *J. Chem. Sci.* **129** 1513; (c) De A, Dey D, Das D, Kole N and Biswas B 2018 *J. Chem. Sci.* **130** 1513
- Dey D, De A, Pal S, Mitra P, Ranjani A, Gayathri L, Chandraleka S, Dhanasekaran D, Akbarsha M A, Kole N and Biswas B 2015 Synthesis, crystal structure, catecholase activity, DNA cleavage and anticancer activity of a dinuclear manganese(III)-bipyridine complex *Indian. J. Chem. Sec. A* **54** 170
- Trawick B N, Daniherl A T and Bashkin J K 1998 Inorganic Mimics of Ribonucleases and Ribozymes: From Random Cleavage to Sequence-Specific Chemistry to Catalytic Antisense Drugs *Chem. Rev.* **98** 939
- (a) Protivinsky R 1971 Chemotherapeutics with tuberculostatic action *Antibiot. Chemother.* **17** 101; (b) Joshi K C, Pathak V N and Jain S K 1980 Studies of potential organo-fluorine antibacterial agents. Part 5: Synthesis and antibacterial activity of some new fluorine-containing indole-2,3-dione derivatives *Pharmazie* **35** 677; (c) Biswas B, Patra M, Dutta S, Ganguly M and Kole N 2013 Synthesis, structural characterization and biological activity of a trinuclear zinc (II) complex: DNA interaction study and antimicrobial activity *J. Chem. Sci.* **125** 1445; (d) Shepherd R G 1970 In *Medicinal Chemistry* A Burger (Ed.) (New York: Wiley)
- (a) Sing S P, Shukla S K and Awasthi L P 1983 Synthesis of some 3-(4'-Benzoylhydrazono)-2-Indolinones As Potential Antiviral Agents *Curr. Sci.* **52** 766; (b) Danda A, Kaur V and Singh P 1993 *J. Ind. Pharm. Sci.* **55** 129
- McLain J, Lee J and Groves J T 2000 *Biomimetic Oxidations Catalyzed by Transition Metal Complexes* B Meunier (Ed.) (London: Imperial College Press)
- Benedini F, Galliani G, Nali M, Rindone B and Tollari S 1985 Bis(salicylaldehyde)ethylenedi-iminecobalt(II)-catalysed oxidation of aromatic amines with oxygen *J. Chem. Soc., Perkin Trans. 2* 1963
- Simandi L I, Nemeth S and Rumlis N 1987 Cobalt(II) ion catalyzed oxidation of o-substituted anilines with molecular oxygen *J. Mol. Catal.* **42** 357
- Szeverenyi Z, Mileava E R and Simandi L I 1991 Kinetics of the oxidation of 2-aminophenol by dioxygen in the presence of tetrakis(3,5-di-*t*-butyl-4-hydroxyphenyl)-dodecachlorophthalocyaninatocobalt(II) *J. Mol. Catal.* **67** 251
- Sakaue S, Tsubakino T, Nishiyama Y and Ishii Y 1993 Oxidation of aromatic amines with hydrogen peroxide catalyzed by cetylpyridinium heteropoloxometalates *J. Org. Chem.* **58** 3633
- Kaizer J, Csonka R and Speier G 2002 TEMPO-initiated oxidation of 2-aminophenol to 2-aminophenoxazin-3-one *J. Mol. Catal. A: Chem.* **180** 91
- Horvath T, Kaizer J and Speier G 2004 Functional phenoxazinone synthase models: Kinetic studies on the copper-catalyzed oxygenation of 2-aminophenol *J. Mol. Catal. A: Chem.* **215** 9
- Maurya M R, Sikarwar S, Joseph T and Halligudi S B 2005 Bis(2-[α -hydroxyethyl]benzimidazolato)copper (II) anchored onto chloromethylated polystyrene for the biomimetic oxidative coupling of 2-aminophenol to 2-aminophenoxazine-3-one *J. Mol. Catal. A: Chem.* **236** 132
- Okamoto M S and Barefield E K 1974 Synthesis and stereochemistry of cobalt(III) complexes of 1,3-diamino-2-propanol and related ligands *Inorg. Chem.* **13** 2611
- CrystalClear 2.0; Rigaku Corporation: Tokyo, Japan.
- Sheldrick G M 2008 A short history of SHELX *Acta Cryst.* **A64** 112
- Dey S K and Mukherjee A 2015 Catechol oxidase and phenoxazinone synthase: Biomimetic functional models and mechanistic studies *Coord. Chem. Rev.* **310** 80
- Simandi T M, Simandi L I, Gyor M, Rockenbauer A and Gomory A 2004 Kinetics and mechanism of the ferroxime(II)-catalysed biomimetic oxidation of 2-aminophenol by dioxygen. A functional phenoxazinone synthase model *Dalton Trans.* **15** 1056
- Szigyarto I C, Simandi T M, Simandi L I, Korecz L and Nagy N 2006 A functional phenoxazinone synthase model based on dioximatomanganese(II): Kinetics and

- mechanism of the catalytic oxidation of 2-aminophenols by dioxygen, *J. Mol. Catal. A: Chem.* **251** 270
21. Bauer A W, Kirby W M M, Sherries J C and Truck M 1996 Antibiotic susceptibility testing by a standardized single disk method *Am. J. Clin. Pathol.* **45** 493
 22. Frisch M J, Trucks G W, Schlegel H B, Scuseria G E, Robb M A, Cheeseman J R, Scalmani G, Barone V, Mennucci B, Petersson G A, Nakatsuji H, Caricato M, Li X, Hratchian H P, Izmaylov A F, Bloino J, Zheng G, Sonnenberg J L, Hada M, Ehara M, Toyota K, Fukuda R, Hasegawa J, Ishida M, Nakajima T, Honda Y, Kitao O, Nakai H, Vreven T, Montgomery J A, Peralta J E Jr., Ogliaro F, Bearpark M, Heyd J J, Brothers E, Kudin K N, Staroverov V N, Kobayashi R, Normand J, Raghavachari K, Rendell A, Burant J C, Iyengar S S, Tomasi J, Cossi M, Rega N, Millam J M, Klene M, Knox J E, Cross J B, Bakken V, Adamo C, Jaramillo J, Gomperts R, Stratmann R E, Yazyev O, Austin A J, Cammi R, Pomelli C, Ochterski J W, Martin R L, Morokuma K, Zakrzewski V G, Voth G A, Salvador P, Dannenberg J J, Dapprich S, Daniels A D, Farkas O, Foresman J B, Ortiz J V, Cioslowski J, Fox D J, *Gaussian 09 (Revision A.02)*, Gaussian, Inc., Wallingford, CT, 2009
 23. Zhao Y and Truhlar D G 2006 A new local density functional for main-group thermochemistry, transition metal bonding, thermochemical kinetics, and noncovalent interactions *J. Chem. Phys.* **125** 194101
 24. Paul S, Karar M, Mitra S, Shah S A S, Majumdar T and Mallick A 2016 A Molecular Lock with Hydrogen Sulfate as “Key” and Fluoride as “Hand”: Computing Based Insights on the Functioning Mechanism *ChemistrySelect* **1** 5547
 25. Mennucci B, Cancès E and Tomasi J 1997 Evaluation of Solvent Effects in Isotropic and Anisotropic Dielectrics and in Ionic Solutions with a Unified Integral Equation Method: Theoretical Bases, Computational Implementation, and Numerical Applications *J. Phys. Chem. B* **101** 10506
 26. Cancès E, Mennucci E B and Tomasi J 1997 A new integral equation formalism for the polarizable continuum model: Theoretical background and applications to isotropic and anisotropic dielectrics *J. Chem. Phys.* **107** 3032
 27. Cammi R and Tomasi J 1995 Remarks on the use of the apparent surface charges (ASC) methods in solvation problems: Iterative versus matrix-inversion procedures and the renormalization of the apparent charges *J. Comput. Chem.* **16** 1449
 28. Miertus S, Scrocco E and Tomasi J 1981 Electrostatic interaction of a solute with a continuum. A direct utilization of AB initio molecular potentials for the prevision of solvent effects *J. Chem. Phys.* **55** 117
 29. (a) Nakamoto K 2009 *Infrared and Raman Spectra of Inorganic and Coordination Compounds, Part B: Applications in Coordination, Organometallic, and Bioinorganic Chemistry* 6th edn. (New Jersey: John Wiley & Sons Inc.); (b) Dey D, Kaur G, Patra M, Choudhury A R, Kole N and Biswas B 2014 A perfectly linear trinuclear zinc–Schiff base complex: Synthesis, luminescence property and photocatalytic activity of zinc oxide nanoparticle *Inorg. Chim. Acta* **421** 335
 30. (a) Dey D, Roy A B, Ranjani A, Gayathri L, Chandrleka S, Dhanasekaran D, Akbarsha M A, Shen C Y, Tsai H L, Majhi M, Kole N and Biswas B 2015 Synthesis and bio-catalytic activity of isostructural cobalt(III)-phenanthroline complexes *J. Chem. Sci.* **127** 649; (b) Das S, Ranjani A, Gayathri L, Saha S, Chandrleka S, Dhanasekaran D, Pasan J, Maji M, Akbarsha M A and Biswas B 2016 Recognition of self-assembled water-nitrate cluster in a Co(III)-2,2'-bipyridine host: Synthesis, crystal structure, DNA cleavage, molecular docking and anticancer activity 2016 *J. Chem. Sci.* **128** 1755; (c) Garai M, Dey D, Maji M, Yadav H R, Choudhury A R and Biswas B 2017 Synthesis and phosphatase activity of a cobalt(II)-phenanthroline complex *J. Chem. Sci.* **129** 1513
 31. Bittner M M, Lindeman S V and Fiedler A T 2012 A Synthetic Model of the Putative Fe(II)-Iminobenzosemiquinonate Intermediate in the Catalytic Cycle of o-Aminophenol Dioxygenases *J. Am. Chem. Soc.* **134** 5460
 32. Ely F, Hadler K S, Gahan L R, Guddat L W, Ollisand D L and Schenk G 2010 The organophosphate-degrading enzyme from *Agrobacterium radiobacter* displays mechanistic flexibility for catalysis *J. Biochem.* **432** 565
 33. Mirams R E, Smith S J, Hadler K S, Ollis D L, Schenk G and Gahan L R 2008 Cadmium(II) complexes of the glycerophosphodiester-degrading enzyme GpdQ and a biomimetic N,O ligand *J. Biol. Inorg. Chem.* **13** 1065
 34. Lee M H and Dawson C R 1978 The specific activity value based on the prosthetic copper of ascorbate oxidase *Arch. Biochem. Biophys.* **191** 119
 35. Malkin R, Malmstrom B G and Vanngard T 1969 Spectroscopic Differentiation of the Electron-Accepting Sites in Fungal Laccase *Euro. J. Biochem.* **10** 324
 36. (a) Ramadan A M, Sala J M C and Parac-Vogt T N 2011 Trinuclear rare earth metal complexes based on 1,3,5-triamino-1,3,5-trideoxy-cis inositol as catalysts for the hydrolysis of phosphodiester *Dalton Trans.* **40** 1230; (b) Phale P S, Basu A, Majhi P D, Deveryshetty J, Vamsee-Krishna C and Shrivastava R 2007 Metabolic Diversity in Bacterial Degradation of Aromatic Compounds *OMICS: J. Integr. Biol.* **11** 252
 37. (a) Mukherjee C, Weyhermueller T, Bothe E, Rentschler E and Chaudhuri P 2007 A Tetracopper(II)-Tetraradical Cuboidal Core and Its Reactivity as a Functional Model of Phenoxazinone Synthase *Inorg. Chem.* **46** 9895; (b) Barry C E III, Nayar P G and Begley T P 1989 Phenoxazinone synthase: mechanism for the formation of the phenoxazinone chromophore of actinomycin *Biochemistry* **28** 6323; (c) Barry C E III, Nayar P G and Begley T P 1988 Phenoxazinone synthase: enzymatic catalysis of an aminophenol oxidative cascade *J. Am. Chem. Soc.* **110** 3333; (d) Freeman J C, Nayar P G, Begley T P and J J Villafranca 1993 Stoichiometry and spectroscopic identity of copper centers in phenoxazinone synthase: a new addition to the blue copper oxidase family *Biochemistry* **32** 4826
 38. Mitra M and Ghosh R 2016 Phenoxazinone synthase activity of a mononuclear cobalt(III) complex *Indian J. Chem. Sect. A* **55** 681
 39. Ghosh K, Harms K and Chattopadhyay S 2017 Two Cobalt(III) Schiff Base Complexes of the

- Type [Co(ABC)(DE)X]: Facile Synthesis, Characterization, Catechol Oxidase and Phenoxazinone Synthase Mimicking Activity *ChemistrySelect* **2** 8207
40. Thimmaiah K N, Lloyd W D and Chandrappa G T 1985 Stereochemistry and Fungitoxicity of Complexes of P-Anisaldehydethiosemicarbazone with Mn(II), Fe(II), Co(II) and Ni(II) *Inorg. Chim. Acta* **160** 81