Research paper

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Catalytic promiscuity of two novel cobalt(III) complexes derived from redox non-innocent Schiff base ligands: unraveling the role of methyl groups in the ligand backbone on catalytic efficiency

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ABSTRACT

(E)-4-bromo-2-(((1-hydroxy-2-methylpropan-2-Two Schiff base ligands namely yl)imino)methyl)-6-methoxyphenol(H_2L1) and (*E*)-4-bromo-2-(((2hydroxyethyl)imino)methyl)-6-methoxyphenol (H_2L2) were designed and synthesized by varving methyl groups to prepare cobalt(III) complexes [Co(HL1)(L1)](1) and [Co(HL2)(L2)](2) using cobalt(II) acetate as metal precursor. Complexes 1 and 2 were characterized by usual physicochemical techniques and solid state structure of complex 2 was determined by X-Ray crystal structure analysis. The electrospray ionization-mass spectrometry (ESI-MS) analysis proved that complexes 1 and 2 were present as mononuclear entity in solution phase. The newly synthesized complexes were used to exploit their catalytic promiscuity. Catecholase like activity of both complexes 1 and 2 was carried out in N, Ndimethylformamide (DMF) medium using 3,5-di-tert-butyl catechol (3,5-DTBC) as model substrate (substrate : complex = 100:1) and the rate of the activity followed the trend, 1>2. Phosphatase like activity was studied by using disodium salt of 4-nitrophenylphosphate (4-NPP) as model substrate in DMF-H₂O (substrate: complex = 20:1) mixture, indicating that the complexes were enormously accelerated the rate of phosphate monoester hydrolysis and the rate of the activity followed the reverse trend of catecholase like activity *i.e.* 2>1. The role of methyl groups in the ligand backbone of H_2L1 is supposed to be the determining factor to differentiate the catalytic efficiency of complexes 1 and 2 and the experimental observation has been supported by DFT calculations.

Keywords: cobalt(III) complex; methyl groups; catalytic promiscuity; catecholase like activity; Phosphatase like activity; DFT study

1. Introduction

An important objective of bioinorganic chemistry is the development of coordination complexes that display catalytic activity analogous to the activity of enzymes. Over the course of time, designing of model catalysts exhibiting catalytic promiscuity i.e. biomimicking activity of different enzymes, draws emerging attention of bio-inorganic chemists.[1-7] A current approach for the development of bio-inspired catalysts by utilizing first row transition metals is highly prevalent because of its low cost, global availability and reduced toxicity.[3] Cobalt, although being a trace element in humans, has a predominant role in ruminant nutrition and a key function in Vitamin B₁₂, whose deficiency causes anemia in humans indicating the significant role of cobalt.[8,9] The substitution of cobalt(II) in active site(s) bio-mimetic models help to elucidate the structural feature, spectroscopic properties and mechanism of native enzymes especially hydrolytic enzymes.[10] At this stage two redox active Schiff base ligands namely H_2L1 and H_2L2 have been designed by varying methyl groups to prepare cobalt(III) complexes namely [Co(HL1)(L1)](1) and [Co(HL2)(L2)](2) with the aim to study catalytic promiscuity (Scheme 1). The oxidative property of complexes 1-2 was checked in terms of catecholase like activity using 3,5-di-tert-butyl catechol (3,5-DTBC) as model substrate. Catechol Oxidase (CO) is a type-3 copper protein which contains antiferromagnetically coupled copper(II) centers in its active site and catalyzes oxidation of catechol to quinone.[11-14] There are several eminent groups around the world involved themselves in devising models of CO and there are two possible mechanistic pathways which are accepted widely, *i.e.* metal centered redox participation and ligand centered radical formation.[15-23] There are numerous factors dictating the efficiency of model complexes. Among them solvents, pH of reaction medium, nature of the substrates are the external contributors whereas the distance between the metals, substitution by other metals, nature of ligands, coordination environment around metal center(s) are the internal deciding factors. [24-29] On the other hand hydrolytic property of the complexes was tested in terms of phosphatase like activity using 4-nitrophenyl phosphate dianion (4-NPP) as model substrate. Phosphatase, a class of hydrolytic enzymes which hydrolyze P-O bonds of mono-, di- and triesters and majorly contains dinuclear zinc(II) centre(s) in their active site.[30-33] Apart from that iron(III) is also present in the active site(s) of the purple acid phosphatase.[34] Moreover substitution of Co^{II} in place of Zn^{II} in *Escherichia coli* alkaline phosphatase results in an

enzymatically active protein with the incorporation of striking visible absorption spectrum.[10,35-36] The availability of nucleophile (H_2O/OH^-) in the close proximity of active site is the key deciding factor of phosphatase activity.[37] It has been already reported that reaction conditions, nature of metal centers, stereochemistry of model complexes, coordination environment of metal ions, presence of anions also affects the rate of activity indirectly.[38-42] In this present work the incorporation of methyl groups to ligand backbone helps us to unveil their role in catecholase like activity and phosphatase activity by using cobalt(III) complexes with help of experimental tools and further supported by DFT study.



Scheme 1. Schematic representation of complexes 1 and 2.

2. Experimental Section.

2.1. Physical methods and materials

A Perkin-Elmer 240C elemental analyzer was utilized for elemental analyses of complexes (carbon, hydrogen and nitrogen). Fourier transform infrared spectra were recorded on KBr disks (400-4000 cm⁻¹) with the help of Perkin Elmer RXI FT-IR spectrophotometer. Electronic spectra and kinetic studies of both catecholase like activity and phosphatase like activity (200-900 nm) were performed at room temperature on a Shimadzu UV-3101PC Spectrophotometer using dry *N*,*N*-dimethylformamide (DMF) as the solvent. WATERS Xevo G2-S Q-TOF mass spectrometer was utilized for electrospray ionization mass spectra using HRMS grade acetonitrile as solvent. The electron paramagnetic resonance (EPR) experiment was recorded in pure DMF medium at room temperature using a Bruker EMX-X band spectrometer and a JEOL JES-FA200 spectrometer.

5-Bromo-2-hydroxy-3-methoxybenzaldehyde, 2-amino-2-methylpropanol, 2-aminoethanol were purchased from commercial sources (Alfa Aesar, Sigma Aldrich) and used as received.

Solvents are distilled by following standard procedures. All other chemicals used were of AR grade. 3,5-di-*tert*-butylcatechol (3,5-DTBC) and Di sodium salt of (4-nitrophenyl)phosphate(4-NPP) were purchased from Sigma-Aldrich. Reaction solutions for 3,5-DTBC and 4-NPP were prepared according to the standard sterile techniques.

2.2. Synthesis of [Co(HL1)(L1)](1)

A methanolic solution (5ml) of cobalt(II) acetate tetrahydrate (0.249 g,1 mmol) was added *in situ* to Schiff base ligand **H**₂**L1** formed by condensation between 5-Bromo-2-hydroxy-3methoxybenzaldehyde (0.115 g, 0.5 mmol) and 2-amino-2-methylpropanol (0.044 g, 0.5 mmol) and the resulting solution was refluxed for 6 h. The reddish brown solution was filtered and kept in a CaCl₂ desiccator. Reddish brown coloured crystals suitable for X-ray data collection were obtained after three days (yield: 78 %).Elemental analysis calcd (%) for C₂₄H₂₈Br₂N₂CoO₆: C 43.58 ; H 4.23 ; N 4.53; found: C 43.65;H 4.73; N 4.88; FT-IR (KBr): v(C-N) 1634 cm⁻¹; v(skeletal vibration) 1453 cm⁻¹;UV-Vis (DMF): $\lambda_{max}(\varepsilon) = 402$ nm (2189 L mol⁻¹ cm⁻¹).

2.3. Synthesis of [Co(HL2)(L2)](2)

Complex 2 was prepared by following the similar procedure as obtained in case of complex 1. 2-aminoethanol was used instead of 2-amino-2-methylpropanol during the preparation of ligand H₂L2. The reddish brown solution was filtered and kept in a CaCl₂ desiccator. Reddish brown coloured crystals suitable for X-ray data collection were obtained after three days(yield: 84%). Elemental analysis calcd (%) for C₄₀H_{41.05}Br₄Cl_{0.95}Co₂N₄O₁₃: C 38.22; H 3.29; N 4.46; found: C 38.26;H 3.23; N 4.48; FT-IR (KBr): v(C=N) 1651 cm⁻¹; v(skeletal vibration) 1453 cm⁻¹; UV-Vis (DMF): $\lambda_{max}(\varepsilon) = 397$ nm (1543 L mol⁻¹ cm⁻¹).

2.4. X-Ray crystallography

Intensity data collection of complex 2 was performed at 100 K at the Elettra Synchrotron (Trieste, Italy), with a monochromatic wavelength of 0.700 Å. The diffraction data were indexed, integrated and scaled using XDS.[43] Both the structures were solved by direct methods and subsequent Fourier analyses [44] and refined by the full-matrix least-squares method based on F^2 with all observed reflections.[44] Hydrogen atoms were included at calculated positions except those of the OH groups which were located on Fourier map. Diffraction data were treated with SQUEEZE tool of PLATON [45] to take into account the small void in the unit cell filled by disordered solvent molecules.All calculations were

performed using the WinGX System, Ver 2013.03,[46] implemented with program Cameron used to prepare molecular pictures.

Crystal data: CCDC Number: 1938379, complex **2**, $C_{40}H_{41.05}Br_4Cl_{0.95}Co_2N_4O_{13}$, M = 1257.89, monoclinic, space group *C* 2/c, *a* = 23.249(5), *b* = 30.222(6), *c* = 14.769(3) Å, β = 109.87(3) °, *V* = 9760(4) Å³, *Z* = 8, *D_c* = 1.712 g/cm³, μ (Mo-K α) = 3.824 mm⁻¹, *F*(000) = 4997, θ range = 1.38 - 27.82 °. Final *R*1 = 0.0658, *wR*2 = 0.1730, *S* = 1.177 for 69 parameters and 22405 reflections, 11357 unique [R(int) = 0.0161], of which 9397 with *I*> 2 σ (*I*), max positive and negative peaks in ΔF map 4.519, -2.083e. Å⁻³.

2.5. Catecholase like activity and kinetics study of complexes 1 and 2

The catecholase like activity of complexes **1** and **2** were performed using 3,5-di-*tert*butylcatechol (3,5-DTBC) as model substrate, since the presence of two bulky *tert*-butyl groups lower the catechol-quinone redox potential and they also reduce polymerization of oxidized product. The catalytic reaction was monitored by addition of 1×10^{-4} (M) complex solution to 1×10^{-2} (M) 3,5-di-*tert*-butylcatechol (3,5-DTBC) solution under aerobic condition at 25 °C in dioxygen saturated *N*,*N*-dimethylformamide (DMF) medium. The progress of the reaction was monitored by time dependent wavelength scan for 1 hour at 5 min interval of time.

The kinetic study of the oxidation of 3,5-DTBC by complex **1** were performed by initial rate method at 25 °C. Solutions of concentration varying from 0.001 to 0.05 (M) were prepared from a freshly prepared 1×10^{-2} (M) 3,5-DTBC solution. The dependence of the initial rate on the concentration of the substrate was monitored by UV-Vis spectroscopy at the respective wavelength by maintaining the fixed catalyst concentration $[1 \times 10^{-4}(M)]$ for each set. The complexes showed saturation kinetics, and a treatment based on the Michaelis-Menten found to be the most appropriate. The binding constant (K_M), maximum velocity (V_{max}), and rate constant for dissociation of the substrates (*i.e.* turn over number, k_{cat}) were calculated for the complexes using the Lineweaver-Burk graph of $\frac{1}{V}$ versus $\frac{1}{|S|}$, with the equation,

$$\frac{1}{V} = \left(\frac{K^{M}}{V^{max}}\right) \times \frac{1}{[S]} + \frac{1}{V^{max}}$$

Kinetic experiments were carried out thrice and average values are reported.

2.6. Detection of Hydrogen Peroxide in the Catalytic Reaction

In order to detect the formation of hydrogen peroxide during the catalytic cycle reaction mixtures were prepared as in kinetic experiments. After 1 hour of reaction equal volume of water was added and 3,5-DTBQ formed was extracted with dichloromethane. pH of the aqueous layer was fixed at 2 with H₂SO₄. 1 mL of a 10% solution of KI and three drops of 3% solution of ammonium molybdate were added. In the presence of hydrogen peroxide, the reaction, H₂O₂ + 2I⁻ + 2H⁺ \rightarrow 2H₂O + I₂ occurs, and with an excess of iodide ions, the triiodide ion is formed according to the reaction I₂(aq) + I⁻ = I₃⁻. The reaction rate is slow but increases with increasing concentrations of acid, and the addition of an ammonium molybdate solution renders the reaction almost instantaneous. The formation of I₃⁻ could be monitored spectrophotometrically due to the development of the characteristic I₃⁻ band (λ_{max} = 353 nm, ε = 26000 L mol⁻¹ cm⁻¹).

2.7. Computational details

We opted quantum mechanical calculation to understand the difference in rate of catecholase activity of **1** and **2**. The calculations were performed on Gaussian09 program utilizing Minnesota functional M06-2X, in conjunction with 6-31G(d) basis set to optimize plausible intermediates of the catalytic pathway [47,48]. LANL2DZ was employed as basis set and effective core potential (ECP) for cobalt. Grimme's d3 dispersion was utilized in computations to tackle dispersive interactions [49]. The implicit solvent effect of DMF was considered by polarizable continuum model (PCM). All the optimized structures are minima on potential energy surface as verified by the absence of imaginary frequency (NIMAG = 0) in analytical frequency analysis. Thermally corrected Gibbs free energies are presented at 25 °C in kcal mol⁻¹.

2.8. Phosphatase like activity and kinetics study of complexes 1 and 2

The phosphatase like activity of complexes **1** and **2** was studied by monitoring hydrolysis of phosphate-ester bond of model substrate disodium salt of (4-nitrophenyl)phosphate (4-NPP) hexahydrate. The hydrolytic property of complexes is studied by monitoring the hydrolysis of a solution in 97.5% (v/v) DMF-H₂O containing 1×10^{-3} (M) 4-NPP and 5×10^{-5} (M) complex for 1 hour with the help of UV-Vis spectroscopy at 25 °C. The course of the reaction was observed by increment of a peak at $\lambda_{max} \sim 425$ nm characteristics of 4-nitrophenolate anion which is hydrolyzed product of 4-NPP.

The kinetics study was performed in five sets having a catalyst of 5×10^{-5} (M) concentration and substrate concentrations of 0.5 (10 equiv), 0.7 (14 equiv), 1.0 (20 equiv), 1.2 (24 equiv), 1.5 mmol (30 equiv). The reactions were initiated by addition of 0.03 mL of metal complex (5×10^{-3} M) into 2.94 mL of a 4-NPP solution, and the spectrum was recorded only after complete mixing at 25 °C. The rates of reaction for various substrate concentrations were interpreted with the help of Michaelis-Menten approach and the kinetic parameters were calculated from Lineweaver-Burk plot.

Kinetic experiments were carried out thrice and average values are reported.

3. Results and Discussions

3.1. Synthesis of complexes

The ligands were prepared by Schiff base condensation between an activated aldehyde and a primary amine. The Schiff-base ligands(*E*)-4-bromo-2-(((1-hydroxy-2-methylpropan-2-yl)imino)methyl)-6-methoxyphenol (H_2L1) and (*E*)-4-bromo-2-(((2-hydroxyethyl) imino)methyl)-6-methoxyphenol (H_2L2) were prepared by refluxing 5-Bromo-2-hydroxy-3-methoxybenzaldehyde with 2-amino-2-methylpropanol and 2-aminoethanol,respectively in methanol medium.

The ligands H_2L1 and H_2L2 were treated with methanolic solution of cobalt(II) acetate tetrahydrate *in situ* separately and refluxed for 6 h to prepare complexes 1 and 2, respectively. Reddish brown coloured crystals were obtained for both the complexes after few days that were characterized by routine physicochemical techniques and solid state structures were characterized by X-ray single crystal structure analyses.

3.2. FT-IR and UV-Vis spectra of complexes

Both the complexes were initially characterized by FT-IR and UV-Vis spectroscopy. The FT-IR spectra of complex 1 and complex 2 consist of a sharp band at ~1634 cm⁻¹ and 1651cm⁻¹, respectively due to C=N stretching. The sharp bands at ~1430 and 1440 cm⁻¹ are due to skeletal vibration for complexes 1 and 2 respectively (**Fig. S1** and **Fig. S2**).[50]

UV-Vis spectra of complexes 1 and 2 were recorded in DMF medium. In case of both the complexes 1 and 2 the absorption band at $\lambda_{max} \sim 400$ nm was observed due to ligand to metal charge transfer (Fig. S3). [51]

3.3. Structural description

Complex 2 crystallizes in monoclinic system, space group C2/c with two crystallographic independent complexes. An Ortep view of one complex is depicted in **Fig. 1** (complex B in **Fig. S4**) and a selection of bond distances is reported in Table 1. The structural analysis indicates a slightly distorted hexa-coordination about the cobalt centre where the two ligands act as tridentate through the phenoxo O, imino N, and alcoholic O donors, with the nitrogen atoms located in trans positions. The two independent complexes are comparable with Co-N/O bond lengths that vary from 1.882(5) to 1.936(4) Å, being the longer values those of alcoholic OH groups. These bond distances, as confirmed by BVS calculation, indicate that in solid state the metal underwent oxidation to Cobalt(III), and the electroneutrality is guaranteed by deprotonation of the alcoholic group in one ligand leading to a complex formulation of [Co(HL2)(L2)].

The crystallographic analysis of **1** unambiguously indicated the structure of the complex (see Fig. 2), similar to that of **2** described above. Any attempt to obtain better crystals was unsuccessful. Since the refinement of this was very poor due to a heavy crystal disorder, the crystallographic results are not reported.



Fig. 1. ORTEP view (ellipsoid probability at 35%) of one of the two complexes of **2** of formulation [Co(HL2)(L2)].



Fig. 2. Molecular structure of complex 1.

Table 1. A selection of coordination bond distar	ices (Å) an	n angles (°) of the two	independent
complexes of 2				

Complex A		Complex B		
Co(1)-N(1)	1.883(5)	Co(2)-N(3)	1.890(5)	
Co(1)-N(2)	1.897(5)	Co(2)-N(4)	1.895(5)	
Co(1)-O(1)	1.892(4)	Co(2)-O(7)	1.891(4)	
Co(1)-O(4)	1.883(4)	Co(2)-O(10)	1.895(4)	
Co(1)-O(3)	1.918(4)	Co(2)-O(9)	1.916(4)	
Co(1)-O(6)	1.902(4)	Co(2)-O(12)	1.937(4)	
N(1)-Co(1)-N(2)	176.3(2)	N(3)-Co(2)-N(4)	174.5(2)	
O(4)-Co(1)-O(6)	177.29(19)	O(7)-Co(2)-O(9)	175.17(17)	
O(1)-Co(1)-O(3)	178.77(17)	O(10)-Co(2)-O(12)	175.97(16)	

3.4. EPR Study of complexes

To confirm the oxidation state of complexes 1 and 2, X-band EPR spectra of polycrystalline solid samples were recorded at 25 °C. Both the complexes are EPR silent. Generally cobalt(III) in octahedral geometry exhibits d^6 low spin state and odd electron is not available

in the orbital. Hence it is confirmed that oxidation state of cobalt in both complexes is +3 in solid states. [52,53]



Fig. 3. X-band EPR spectra of complex 1 and 2 in solid state at 25 °C.

3.5. ESI-MS study of complexes

In order to have an insight about the solution phase structure of complexes 1 and 2, ESI-MS study were performed in acetonitrile medium. The ESI-MS spectrum of complex 1 consisted of a base peak at m/z = 661.0499 amu which matches with the mononuclear species $C_{24}H_{29}Br_2N_2CoO_6 + H^+$ (calc. m/z = 660.9802 amu). Complex 2 in solution phase showed base peak at m/z = 604.8436 amu which was corroborated with the mononuclear entity $C_{20}H_{21}Br_2N_2CoO_6 + H^+$ (calc. m/z = 604.9156 amu). Hence it is confirmed that both the complexes retained their solid state structural entity in solution phase also (**Fig. S5** and **S6**).

3.6. Catecholase like activity

The active site of catechol oxidase contains dinuclear copper(II) centers and plausible mechanistic pathways of bio-mimicking models of catechol oxidase using 3d transition metal ions have been already explored. In this present work we are willing to establish the role of methyl groups in ligand backbone towards the rate of catecholase like activity of cobalt(III) based model systems. The activity of complexes 1 and 2 was studied using 3,5-DTBC as model substrate in DMF medium at 25 °C under aerobic condition and the progress of catalytic reaction was monitored by time dependent UV-Vis spectroscopy at 5 min interval of time for 1 hour. The gradual increment of peak at $\lambda_{max} \sim 400$ nm (**Fig. 4**) is indicative of formation of oxidized product 3,5-di-*tert*-butylbenzoquinone (3,5-DTBQ) in case of both the complexes 1 and 2.



Fig. 4. Oxidation of 3,5-DTBC catalyzed by (a) complex 1 and (b) complex 2 in DMF medium at 25 °C (Substatre : catalyst = 100:1).

Before explaining the role of methyl groups, the detection of the species formed due to reduction of di-oxygen during the oxidation of 3,5-DTBC is very important. There occurs the oxidation of I to I₂ followed by the formation of I₃ as was detected in UV-Vis spectra of the reaction mixture of 3,5-DTBC, complex and KI. The characteristic bands of I₃ were observed at 352.5 nm for both complexes 1 and 2 (Fig. S7). The spectroscopic data indicated that hydrogen peroxide was formed during oxidation and hinted that radical pathway may be followed during catalysis.

3.7. ESI-MS Study of reaction mixture of complexes and 3,5-DTBC

ESI-MS study of the reaction mixture of each complex was carried out to detect the composition of intermediate formed during catalytic cycle. A very small amount of reaction mixture was diluted with acetonitrile and spectrum was recorded in positive mode. In case of complex **1** the base peak was observed at m/z = 243.1362 amu which corroborated with sodium adduct of 3,5-DTBQ. The peak at m/z = 639.4069 amu corresponds to molecular formula $C_{26}H_{36}BrCoNO_5 + 2H_2O + Na^+$ (calcd. m/z = 639.1218 amu) (**Fig. S8**). Hence it can be said that 1:1 adduct was formed between complex **1** and 3,5-DTBC after liberation of one ligand from coordination site. In case of complex **2** 1:1 adduct was also formed between substrate and complex **2**. The base peak was observed at m/z = 243.1362 amu and the peak at m/z = 591.9860 amu corresponds to molecular formula $C_{24}H_{32}BrCoNO_5 + 2H_2O + H^+$ (calcd. m/z = 591.1065 amu) (**Fig. S9**).

3.8. EPR Study of reaction mixture of complexes and 3,5-DTBC

To confirm the formation of organic radical during the catalytic oxidation of 3,5-DTBC, X-Band EPR spectra of reaction mixture was recorded at room temperature. In case of reaction mixtures of both the complexes **1** and **2** an isotropic signal at $g \sim 2$ was observed during catalytic cycle. Hence it can be concluded that organic radical was surely formed during catalytic cycle. Since the complexes were derived from phenol based Schiff base ligand, there was a probability of phenoxy radical and benzene anion radical generation. Previous reports suggested that formation of imine radical was energetically more favourable than phenoxy radical and benzene anion radical.[18,54] Therefore the position of organic radical was located at imine bond of ligand backbone.



Fig. 5. X-band EPR spectra of complexes 1 and 2 in presence of 3,5-DTBC in DMF medium.

3.9. Kinetic Studies of catecholase like activity

Kinetics parameters of catecholase like activity was monitored to quantify higher activity of complex 1 than complex 2 as was observed from time dependent UV-Vis spectroscopy. The two complexes showed saturation kinetics and Michaelis Menten approach seemed to be appropriate. From Lineweaver-Burk plot (**Fig. S10** and **Fig. S11**) kinetic parameters were calculated and presented in table 2. From k_{cat} values of the complexes it was observed that complex 1 is more active catalyst than complex 2. Since both the complexes exhibited similar solution phase structure and the reaction proceeded via radical formation there must be role of the methyl groups in the ligand backbone of complex 1 in stabilizing the radical intermediate. DFT calculation was carried out to support experimental observations.

	Complex	V _{max} (M s ⁻¹)	$K_{M}(M)$	$k_{\rm cat}$ (h ⁻¹)	R ²	
	Complex 1	(8.42±0.07)×10 ⁻⁷	(5.35±0.3)×10 ⁻³	30.3	0.9832	
	Complex 2	(5.77±0.3)×10 ⁻⁷	$(4.44\pm0.5)\times10^{-3}$	20.7	0.9970	

Table 2. First order kinetic parameters of catecholase like activity for complexes 1 and 2 obtained from Lineweaver-Burk plot

3.10. DFT Study to rationalize the role of methyl groups

The mechanism of catecholase like activity of a Ni^{II} - complex was previously reported by our group with the aid of experimental observations and DFT computations [18]. The mechanism demonstrates that the catechol binds to the metal center via displacement of a labile ligand. In the next step, the coordinated catechol transfers one electron to the Ni^{II} coordinated imine functionality which belongs to the organic ligand. The electron transfer associates a concomitant proton transfer from the catechol unit to the ligand. A dioxygen molecule in its triplet state binds with the generated radical cation (catechol⁺⁻) and eventually performed catechol oxidation by releasing hydrogen peroxide. It has been observed that the rate-limiting step of metal-organic complex catalyzed catecholase like activity is the electron transfer coupled proton transfer process from metal ion coordinated catechol to the metalorganic complex. Therefore, we investigated the rate-limiting step of catecholase pathway by D3-M06-2X/6-31G(d) [LANL2DZ : basis set and ECP for Co; solvent = DMF (PCM)] associated with 1 and 2. In both cases, the cobalt ion coordinated to catechol (1-INT1 and 2-INT1) possesses highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) which is located over Schiff base ligand (Fig.6). This implies a feasible electron transfer process from catechol to the Schiff base ligand. This electron transfer process is endergonic in both the complexes and it demands only 2.4 kcal mol⁻¹ of free energy in 1-INT1 whereas it demands 12.4 kcal mol⁻¹ of energy in 2-INT1 (Fig.7). Therefore, the catecholase reaction rate is faster with complex 1 than that of 2. The significant difference arises possibly due to the additional stabilization of electron-transfer generated organic radical by the methyl groups present in Schiff base ligand of complex 1.



Fig.6. HOMO and LUMO diagrams of catechol coordinated Co-complexes, 1-INT1 and 2-INT1.



Fig.7. M06-2X/6-31G(d) calculated Gibbs free energy (25 °C) changes for electron transfer associated with **1-INT1** and **2-INT1**.

3.11. Phosphatase like activity

Phosphatase enzymes mostly contain zinc(II) centre(s) in its active site because of its highest Lewis acidity among 3d transition metal ions. In this project we employed our complexes 1 and 2 to study hydrolysis property. At first the hydrolytic property was checked by monitoring the cleavage of P-O bond of phosphate monoester dianion using 4-NPP as model substrate in DMF-water (97.5% v/v) mixture. The course of the reaction was followed by time dependent UV-Vis spectroscopy at 5 min interval of time as shown in **Fig. 8**. The gradual increment of band at $\lambda_{max} \sim 426$ nm, characteristics of 4-nitrophenolate ion, was observed with time. Hence it can be said that both the complexes are capable of hydrolyzing P-O bond.



Fig.8. Hydrolysis of phosphate-ester bond in aqueous DMF medium catalysed by (a) complex **1** and (b) complex **2** at 25 °C (Substatre: catalyst = 20:1).

3.12. ESI-MS Study of reaction mixture of complexes and 4-NPP

In order to identify the composition of intermediate formed during hydrolysis of P-O bond ESI-MS study was performed in positive mode. A minute amount of reaction mixture was diluted with acetonitrile and spectrum was recorded. In case of complex 1 the base peak was observed at m/z = 613.8039 amu which corroborated with molecular formula $C_{18}H_{20}BrCoN_2O_9P + 2H_2O + H^+$ (calcd. m/z = 613.9706 amu), thereby suggesting that 1:1 adduct was formed between complex 1 and 4-NPP. In this case also, dissociation of ligand occurred as observed in case of catecholase like activity. (Fig. S12) In case of complex 2, 1:1 adduct was formed between substrate and catalyst. The base peak was observed at m/z = 573.9686 amu which corroborated with molecular formula $C_{16}H_{16}BrCoN_2O_9P + Na^+$ (calcd. m/z = 573.8986 amu). (Fig. S13)

3.13. Kinetic studies of phosphatase like activity

The kinetics study of phosphatase like activity for both the complexes 1 and 2 were performed using initial rates method which involves the increase of the product concentration, *i.e.* p-nitrophenolate ion (4-NP), at 426 nm in aqueous DMF medium. All the kinetic parameters were evaluated from Lineweaver-Burk plot (V_{max} , k_M , k_{cat}) (Fig. S14, S15)

and were presented in table 3. The kinetic parameters (V_{max} , k_M , k_{cat}) for the catalyzed reactions were derived from Lineweaver Burk plot. The k_{cat} value of complex 1 is 25.8 s⁻¹ and that of complex 2 is 29.3 s⁻¹. These values suggested that complex 2 exhibited a little higher hydrolytic efficiency than complex 1 towards hydrolysis of P-O bond of phosphate monoester dianion. Hence it can be concluded that hydrolytic property of the complexes majorly depends on Lewis acidity of metal ions but methyl groups on the ligand backbone have indirect influence on phosphatase like activity of complexes 1 and 2.

Table 3. First order kinetic parameters of phosphatase like activity for complexes 1 and 2 obtained from Lineweaver-Burk plot

Complex	V _{max} (M s ⁻¹)	K _M (M)	$k_{\rm cat}({\rm s}^{-1})$	R ²
Complex 1	(12.8±0.14)×10 ⁻⁴	(1.96±0.55)×10 ⁻³	25.8	0.9703
Complex 2	(14.6±0.15)×10 ⁻⁴	(2.00±0.06)×10 ⁻³	29.3	0.9918

To further rationalize the role of methyl groups on phosphatase activity Mulliken charge on cobalt(III) centre of intermediate species (1-INT1' and 2-INT2') derived from complexes 1 and 2 were calculated (Fig. 9). It was observed that charge on cobalt in 1-INT1' was 0.653 whereas that for 2-INT2' is 0.664 which indicates that cobalt(II) in intermediate of complex 2 possess slightly higher positive charge density than that of complex 1. Since formation of nucleophile *i.e.* hydroxo species in close proximity of the metal centre is one of the key steps of phosphatase activity, which in turn depends on Lewis acidity of metal centre. Therefore formation of hydroxo species is more feasible in case of complex 2. On the other hand, the rate limiting step of hydrolysis is attack of nucleophile to phosphorus centre according to previous reports. [37] On considering the Mulliken charges over P centre of 4-NPP it can be claimed that ease of OH⁻ attack is more viable in case of 2-INT2' leading to a greater hydrolytic efficiency of complex 2 over complex 1. [55]



Fig. 9. Mulliken charges on cobalt(II) metal ion in intermediate 1-INT1' and 2-INT2'.

4. Conclusions

The thoughtful design of Schiff base ligands by insertion of methyl groups on the amino alcohol moiety helps us to reveal their role in determining the rate of catecholase- and phosphatase-like activity of cobalt(III) based models. Experimental results and theoretical study support a higher catecholase like activity of complex 1 than 2. Therefore it is concluded that the presence of methyl groups accelerate the rate determining step involved in catecholase like activity, thereby enhances the rate of complex 1 over 2. But a reverse trend is observed in case of phosphatase like activity. Mulliken charge calculation supports that positive charge density over cobalt(III) is lower in intermediate of complex 1 as compare to that observed in 2, thus reflecting the trend of their hydrolytic property. Complex 2 showed a little higher phosphatase like activity than that of complex 1.The present work unveils the contributing factors involved in catecholase as well as in phosphatase like activity using cobalt(II) based models.

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Highlights of the Research:

- Investigation of catecholase and phosphatase like activity of Co^{III} complexes.
- Redox non-innocent Schiff-base ligands on catalytic promiscuity of Co^{III} complexes.
- Combined experimental and theoretical study on catalytic activity of Co^{III}-complexes.

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