Synthesis and characterization of phenoxo-bridged dinuclear zinc(II) halide complexes: Role of electron withdrawing group on phosphatase activity

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Abstract. Three phenoxo-bridged dinuclear zinc(II) halide complexes $[\text{Zn}(\text{L2})(\text{X})]_2$ ($\text{X} = \text{Cl}, \text{Br}, \text{I}$) were isolated from a reduced Schiff base $[\text{HL2} = 2-(((2-(\text{dimethylamino})\text{ethyl})\text{amino})\text{methyl})-4\text{-nitrophenol}]$. Ligand and complexes were characterized using different physico-chemical and spectroscopic methods. Structural study confirms that $[\text{Zn}(\text{L2})(\text{Cl})]_2$ contains a discrete dinuclear unit where two zinc(II) ions are bridged by the two phenoxo oxygen atoms of the deprotonated ligand (**L2**). The complex adopts a rare *cis*-orientation with respect to chloride ions. The rate of hydrolysis of 4-NPP ((4-nitrophenyl)phosphatehexahydrate) in the presence of zinc(II) complexes was measured by initial rate method giving k_{cat} values in the order $\text{I} > \text{Cl} > \text{Br}$ and mechanistic pathway was monitored using ESI-mass spectroscopy. It is worth mentioning that presence of an electron withdrawing $-\text{NO}_2$ group in **HL2** eventually increases the Lewis acidity of zinc(II) which in turn reduces the nucleophilic character of metal bound water molecule in the intermediate state causing an overall decrease in the rate of hydrolysis of 4-NPP as compared to our previous work with **HL1** (2-((2-(dimethylamino)ethylamino)methyl)phenol).

Keywords. Zinc(II) complex; crystal structure; phosphatase activity.

1. Introduction

Zinc is one of the most biocompatible metals^{1–6} present in living systems.^{7–10} Unlike other first-row transition metals, zinc(II) ion has specific properties such as redox stability, Lewis acidity, capability of undergoing rapid ligand exchange and flat coordination hyper-surface^{11–14} which make zinc(II) as a better catalytic co-factor for many metalloenzymes. Zinc-containing metalloenzyme is the widest category studied till date^{3,6} which includes oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases.^{4–6} Hydrolases constitutes an important class of zinc-containing metalloenzymes. Alkaline phosphatase (AP), is an example of hydrolases which catalyzes the non-specific hydrolysis of phosphate monoesters.^{15,16} Hydrolysis of phosphate

esters catalyzed by metal ion is widely studied because it is involved in many biological functions like energy transduction, biosynthesis, regulation of protein and control of secondary messengers.^{17,18} Various dinuclear zinc(II) complexes^{19–30} have been synthesized and studied as phosphate ester models. We are actively engaged in synthesizing dinuclear zinc(II) compounds with N,N,O-donor ligands and study of their phosphatase activity.³⁰ In this endeavor, we have successfully synthesized and characterized three new dinuclear zinc(II) halide complexes containing a tridentate reduced Schiff base, 2-(((2-(dimethylamino)ethyl)amino)methyl)-4-nitrophenol (**HL2**, Scheme 1) and studied their phosphatase activity. A comparative study of these results is made with our previously (Scheme 1) reported work³⁰ as well as with other similarly reported systems.^{20,26–28}

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Scheme 1. Ligands **HL1**,³⁰ **HL2** (this work) used in isolation of dinuclear zinc(II) complexes.

2. Experimental

2.1 Materials and physical measurements

All the chemicals used in the present study were obtained from different commercial sources. Solvents were distilled before use according to standard procedures. The anhydrous zinc halide salts (ZnCl_2 , ZnBr_2) were obtained from CDH, India. ZnI_2 was synthesized by standard procedure using zinc metal and iodine.³¹ Water used in the physical measurements was Milli-Q grade. (4-nitrophenyl)phosphatehexahydrate (4-NPP) used for catalytic studies was purchased from Spectrochem, India and re-crystallized from ethanol/water. Elemental analyses were performed on a Thermo Scientific Flash 2000 elemental analyzer. Infrared spectra were recorded at room temperature using Perkin Elmer FTIR spectrometer in 4000–400 cm^{-1} range. ESI mass spectra were recorded using G2-S-Q Tof ESI-mass spectrometer. The ^1H NMR spectra were recorded in CDCl_3 and D_2O solvent on Bruker-500 MHz spectrometer. UV-Vis spectra and kinetic studies were performed at room temperature on Agilent Technologies Cary 100 UV-Vis spectrophotometer equipped with multiple cell-holders.

2.2 Synthesis

2.2a 2-(((2-(dimethylamino)ethyl)amino)methyl)-4-nitrophenol (HL2): A solution of 5-nitrosalicylaldehyde (1 g, 5.99 mmol) in methanol was refluxed with *N,N*-dimethylethylenediamine (0.5 g, 5.99 mmol) for 3 h. The resulting yellow solution was cooled to room temperature and then 3 equivalent NaBH_4 (0.67 g, 17.7 mmol) was added in portions at 0 °C to it. The reaction mixture was stirred for 30 min and HCl (11 N) was added to make pH = 1. After that NaOH solution was added to raise the pH to 12. The resulting mixture was extracted with CHCl_3 and dried over anhydrous sodium sulphate. After evaporating the solvent, a yellow solid was obtained which was dried under vacuum (Yield: 1.41 g, 98%). ^1H NMR (500 MHz, CDCl_3 , δ/ppm): δ 6.8–8.0 (3H, aromatic), 4.0 (2H, s, CH_2), 2.7 (2H, t, CH_2),

2.4 (2H, t, CH_2), 2.25 (6H, s, CH_3). ^{13}C NMR (DEPT) (500 MHz, CDCl_3 , δ/ppm): 125.29 (Aromatic –CH), 124.60 (Aromatic –CH), 116.80 (Aromatic –CH), 57.49 (Aliphatic – CH_2), 51.55 (Aliphatic – CH_2), 45.32 (Aliphatic – CH_2), 45.28 (Aliphatic – CH_3), 45.26 (Aliphatic – CH_3). UV-Vis (DMF, $\lambda_{\text{max}}/\text{nm}$ ($\epsilon_{\text{max}}/\text{M}^{-1}\text{cm}^{-1}$)): 407 (7100), 326 (6800).

2.2b $[\text{Zn}_2(\text{L2})_2(\text{Cl})_2]$ (1): A solution of **HL2** (0.5 g, 2.08 mmol) in methanol (5 mL) with NEt_3 (0.21 g, 2.08 mmol) at room temperature was stirred for 15 min. Then ZnCl_2 (0.28 g, 2.08 mmol) dissolved in methanol (5 mL) was added to the above solution with continuous stirring. A yellow solid was formed immediately which was filtered, washed with methanol and dried under vacuum. Yield: 0.58 g (82%). Elemental analysis calculated for $\text{C}_{22}\text{H}_{32}\text{Cl}_2\text{N}_6\text{O}_6\text{Zn}_2$: C 38.96, H 4.76, N 12.39%; found: C 38.65, H 4.56, N 12.04%. ^1H NMR (500 MHz, D_2O , δ/ppm): δ 6.5–8.0 (8H, aromatic), 2.55–4.00 (12H, aliphatic), 2.33 (12H, CH_3). IR (KBr pellets, ν/cm^{-1}): $\nu(\text{N-H})$, 3221, $\nu(\text{C}=\text{C}$, arene), 1604, 1580; $\nu(\text{C-N})$, 1280, $\nu(\text{NO}_2)$, 1504 (asym), 1338 (sym). UV-Vis (DMF, $\lambda_{\text{max}}/\text{nm}$ ($\epsilon_{\text{max}}/\text{M}^{-1}\text{cm}^{-1}$)): 391 (22100).

2.2c $[\text{Zn}_2(\text{L2})_2(\text{Br})_2]$ (2): Complex **2** was isolated by the same procedure as that of **1** by using anhydrous ZnBr_2 (0.46 g, 2.08 mmol) as metal salts instead of ZnCl_2 (0.28 g, 2.08 mmol). The resulting yellow solid formed were filtered and dried under vacuum. Yield: 0.64 g (81%). Elemental analysis calculated for $\text{C}_{22}\text{H}_{32}\text{Br}_2\text{N}_6\text{O}_6\text{Zn}_2$: C 34.44, H 4.20, N 10.95%; found: C 33.68, H 4.22, N 10.58%. ^1H NMR (500 MHz, D_2O , δ/ppm): δ 6.5–8.0 (8H, aromatic), 2.56–4.01 (12H, aliphatic), 2.33 (12H, CH_3). IR (KBr pellets, ν/cm^{-1}): $\nu(\text{N-H})$, 3234, $\nu(\text{C}=\text{C}$, arene), 1604, 1581; $\nu(\text{C-N})$, 1281, $\nu(\text{NO}_2)$, 1500 (asym), 1339 (sym). UV-Vis (DMF, $\lambda_{\text{max}}/\text{nm}$ ($\epsilon_{\text{max}}/\text{M}^{-1}\text{cm}^{-1}$)): 390 (27500).

2.2d $[\text{Zn}_2(\text{L2})_2(\text{I})_2]$ (3): Complex **3** was prepared by the same procedure as that of **1** by using ZnI_2 (0.67 g, 2.08 mmol) as metal salts instead of ZnCl_2 (0.28 g, 2.08 mmol). The resulting yellow solid formed was filtered and dried under vacuum. Yield: 0.68 g (77%). Elemental analysis calculated for $\text{C}_{22}\text{H}_{32}\text{I}_2\text{N}_6\text{O}_6\text{Zn}_2$: C 30.68, H 3.75, N 9.76%; found: C 30.40, H 3.60, N 9.48%. ^1H NMR (500 MHz, D_2O , δ/ppm): δ 6.5–8.0 (8H, aromatic), 2.54–3.99 (12H, aliphatic), 2.31 (12H, CH_3) IR (KBr pellets, ν/cm^{-1}): $\nu(\text{N-H})$, 3242, $\nu(\text{C}=\text{C}$, arene), 1604, 1580; $\nu(\text{C-N})$, 1281, $\nu(\text{NO}_2)$, 1504 (asym), 1338 (sym). UV-Vis (DMF, $\lambda_{\text{max}}/\text{nm}$ ($\epsilon_{\text{max}}/\text{M}^{-1}\text{cm}^{-1}$)): 390 (2.74×10^4).

2.3 X-ray data collection and structure refinement

Single crystals of compound **1** were obtained by slow evaporation of DMF solution. Crystallographic data of **1** was collected on a Bruker Kappa APEX-II CCD diffractometer at 100 K using graphite monochromated $\text{Mo K}\alpha$ radiation ($\lambda = 0.71073 \text{ \AA}$) at Department of Chemistry, IIT Jodhpur. For unit cell determination, the single crystal was exposed to X-rays for 10 s in three sets of frames. The detector

Table 1. Crystal and refinement data for **1**.

Formula	C ₂₂ H ₃₂ Cl ₂ N ₆ O ₆ Zn ₂
Weight	678.17
Crystal system	Orthorhombic
Space group	Pbca
<i>a</i> /Å	14.074(3)
<i>b</i> /Å	18.969(3)
<i>c</i> /Å	20.538(4)
α /°	90
β /°	90
γ /°	90
<i>V</i> /Å ³	5483.2(17)
λ /Å	0.71073
ρ_{calcd} /gm cm ⁻³	1.643
<i>Z</i>	8
<i>T</i> /K	100(2)
μ /mm ⁻¹	1.993
<i>F</i> (000)	2784
θ ranges/°	3.969 to 27.999
<i>h</i> / <i>k</i> / <i>l</i>	−18,18/−25,25/−27,27
Reflections collected	130421
Independent reflections	6590
Data/restraints/parameters	6590/2/355
Goodness-of-fit on <i>F</i> ²	1.061
Final <i>R</i> indices [<i>I</i> > 2σ(<i>I</i>)]	<i>R</i> 1 = 0.0211 and <i>wR</i> 2 = 0.0564
<i>R</i> indices (all data)	<i>R</i> 1 = 0.0250 and <i>wR</i> 2 = 0.0587
Largest peak and hole/eÅ ⁻³	0.469 and -0.335

Weighting scheme: $R = \frac{\sum ||F_o| - |F_c||}{\sum |F_o|}$, $wR = \frac{[\sum w(F_o^2 - F_c^2)^2 / \sum w(F_o^2)^2]^{1/2}}{}$.

frames were integrated by use of the program SAINT³² and the multi-scan absorption corrections were performed using SADABS program.³³ Structure was solved by direct methods, and refined by full-matrix least-squares methods based on *F*² using SHELXS-97³⁴ and SHELXL-97.³⁴ All non-hydrogen atoms were refined with anisotropic displacement parameters whereas hydrogen atoms were placed in calculated positions when possible and given isotropic *U* values 1.2 times that of the atom to which they are bonded. In the final difference Materials for publication were prepared using SHELXTL,³⁴ ORTEP,³⁵ PLATON³⁶ programs. A summary of the crystallographic data and structure determination parameters are given in Table 1.

2.4 Hydrolysis of phosphate ester

Disodium (4-nitrophenyl)phosphatehexahydrate (4-NPP) was used as the substrate to study phosphatase activity and the solvent chosen to study kinetic experiments was 97.5% DMF/H₂O mixture.^{20,26} Freshly prepared solutions of zinc complexes and substrate 4-NPP were used for this study maintaining total volume of 3 mL. The kinetic data was collected after 2 h of the reaction when 2% of the reaction was complete. The hydrolysis of 4-NPP in the presence of zinc complexes (**1–3**) was run on the UV spectrophotometer following the increase in absorption at 428 nm due to the formation of

4-nitrophenolate ion (ϵ , 18500 M⁻¹ cm⁻¹) at 25 °C.²⁶ The kinetic experiments were performed both at variable substrate or complex concentration keeping the either complex or the substrate concentration constant.^{26,27} The study comprised of five sets of experiments having the complex concentration as 0.05 mM and substrate as 0.5 (10 equiv.), 0.7 (14 equiv.), 1.0 (20 equiv.), 1.2 (24 equiv.), 1.5 (30 equiv.) mM. The stock solutions of 2.5 mM of complexes and substrate were prepared in DMF:H₂O mixture. The reactions were performed by injecting 60 μ L of complex and different volumes of substrate (0.6 mL, 0.84 mL, 1.2 mL, 1.44 mL, 1.8 mL) in cuvette and making the final volume as 3 mL with solvent. The visible absorption increase was recorded for a total period of 2 h at an interval of 5 min. All the recordings were performed in triplicate and the average values were taken. The reactions were corrected for the degree of ionization of the 4-nitrophenolate at 25 °C using the molar extinction coefficient for 4-nitrophenolate at 428 nm.²⁶ The final absorbance values for each set were obtained after 72 h.

2.5 Control experiments

Control experiments were performed for zinc(II) salts and the ligand to identify any individual effects on the hydrolysis of 4-NPP. There was no noticeable change in the results.

Scheme 2. Synthesis of dinuclear zinc(II) complexes **1–3**.

3. Results and Discussion

3.1 Synthesis and spectroscopic studies

Dinuclear zinc(II) complexes (**1–3**) were isolated as yellow solids by mixing 1:1 molar ratio of zinc(II) halides and reduced Schiff base (**HL2**) in presence of triethyl amine in MeOH at room temperature. All the reactions were reproducible as were evident from repetitive spectral behaviours. The details of sequential reactions are summarized in Scheme 2.

The compounds were characterized using elemental analyses, FTIR, UV-Vis, ESI-MS and NMR spectroscopy. The elemental analyses (Figures S1–S3 in Supplementary Information) are in good agreement with the formulations **1–3**. All the compounds are stable over a long period of time in powdery or crystalline form. **1–3** are soluble in dimethylformamide and dimethylsulfoxide but partially soluble in water. The ^1H NMR spectrum of **HL2** in CDCl_3 shows four signals in the aliphatic region consisting of two singlet and two triplets. In the aromatic region (δ 6.8–8.0), as expected, three signals are observed (Figure S4, Supplementary Information) for **HL2**. The ligand was further characterized by ^{13}C NMR and ^{13}C (DEPT) NMR spectra in CDCl_3 which showed the presence of aromatic carbon signals at δ 165.48–116.78 in addition to $-\text{CH}_2-$ and $-\text{CH}_3$ signals at δ 57.50–45.27 (Figures S5 and S6 in Supplementary Information). The ^1H NMR of metal complexes (**1–3**) were taken in D_2O have similar type of spectra as **HL2** with slightly shifted chemical shift values³⁰ (Figures S7–S9 in Supplementary Information). In DMF solution, the ligand (**HL2**) exhibits an intra-ligand charge transfer band at 326 nm³⁷ along with another band at 407 nm probably due to the presence of nitro-phenolic group. The corresponding complexes **1–3** in DMF show ligand-to-metal charge transfer band²⁰ at $\sim$ 390 nm which is probably due to $\text{PhO}^- \rightarrow \text{Zn}^{\text{II}}$ (Figure S10 in Supplementary Information). In FTIR spectra, **1–3** show weak to medium stretches in

Figure 1. An ORTEP diagram of **1** (H-atoms are omitted for clarity) with atom labeling schemes and 50% thermal ellipsoid probability for all non-hydrogen atoms.

the 3221–3242 cm^{-1} region corresponding to $\nu(\text{N-H})$, strong band at *ca.* 1338–1504 cm^{-1} attributed to $\nu(\text{NO}_2)$ stretching and strong band at *ca.* 1280 cm^{-1} correspond to $\nu(\text{C-N})$ stretches.³⁸ In mole ratio method, keeping the concentration of metal salt constant, absorbance of various solutions (in DMF) containing different L:M ratio was measured. From the plot, it has been found that each ligand coordinates to zinc(II) ion in 1:1 molar ratio (Figures S11–S13 in Supplementary Information).

3.2 Crystal structure of complex 1

Single crystal X-ray diffraction study shows that compound $[\text{Zn}_2(\text{L2})_2(\text{Cl})_2]$ (**1**) contains a discrete dinuclear unit where two Zn(II) ions [Zn(1) and Zn(2)] are bridged by the two phenoxo oxygen atoms [O(1) and O(2)] of the deprotonated Schiff base ligand (**L2**) (Figure 1). Each five-coordinated Zn(II) center adopts a distorted square pyramidal (SP) geometry³⁹ (Addison parameter, $\tau = 0.21$ for Zn1 and 0.45 for Zn2) with a $\text{ZnO}_2\text{N}_2\text{Cl}$ chromophore. Two amine N atoms (N2, N3 for Zn1 and N5, N6 for Zn2) along with phenoxo oxygen atoms (O1 and O2) form an equatorial plane whereas terminal chloride anions (Cl3 for Zn1 and Cl4 for Zn2) occupy the apical positions. Each Zn(II) center deviates (0.635 Å for Zn1 and 0.667 Å for Zn2) considerably from the mean basal plane towards terminal chloride. Interestingly, **1** adopts an overall *cis*-orientation^{30,40,41} where terminal chlorides are on the same side of the Zn–Zn axis. The *cis*-oriented Zn–Cl distances along with Zn–N(amine) lengths (Table 2) are consistent with the literature.^{20,26–29,40–42} The intranuclear Zn···Zn distance in the dinuclear unit is 3.247 Å.

In **1**, individual dinuclear units are connected to each other *via* weak $\text{N-H}\cdots\text{Cl}$, $\text{C-H}\cdots\text{Cl}$ and $\text{C-H}\cdots\text{O}$ hydrogen bonding interactions (Table 3) involving $-\text{NH}$ -, CH_2 - and NO_2 -groups of **HL2** and Cl atom along three different axes (Figure 2) to form a 3D network.

Table 2. Selected bond distances (Å) and bond angles (°) for **1**.

Bond distances (Å)	
Zn1–O1	2.0932(10)
Zn1–O2	2.0613(10)
Zn1–N2	2.1489(12)
Zn1–N3	2.1282(12)
Zn1–Cl3	2.2814(5)
Zn2–O1	2.0569(10)
Zn2–O2	2.0929(10)
Zn2–N5	2.1757(12)
Zn2–N6	2.1089(12)
Zn2–Cl4	2.2429(5)
Bond angles (°)	
O1–Zn1–O2	77.04(4)
O2–Zn1–N3	138.25(5)
O1–Zn1–N3	92.49(4)
O2–Zn1–N2	87.30(4)
O1–Zn1–N2	150.83(5)
N3–Zn1–N2	82.92(5)
O1–Zn2–O2	77.14(4)
O1–Zn2–N6	128.98(4)
O2–Zn2–N6	92.57(4)
O1–Zn2–N5	88.83(4)
O2–Zn2–N5	155.99(4)
N6–Zn2–N5	81.12(5)

3.3 Phosphatase activity and kinetic study

The efficiency of phosphatase activity of **1–3** was studied using phosphomonoester, (4-nitrophenyl)phosphate (4-NPP).^{26,27} The hydrolytic activity of complexes was determined spectrophotometrically by monitoring the formation of 4-nitrophenolate ($\lambda_{\max} = 428$ nm) through a wavelength scan from 200 to 800 nm in aqueous DMF.^{26,27} The absorption profile of complex-substrate mixture at $\lambda_{\max} = 428$ nm is shown in Figure 3 for complex **1** as a representative one and those of other complexes (**2** and **3**) are given in Supplementary Information (Figures S14 and S15).

The kinetic behaviour of complexes (**1–3**) was monitored by the initial slope method following the increase in absorption of the band corresponding to the formation

Figure 2. Perspective view of 3D network structure in **1** formed via N–H...Cl, C–H...Cl and C–H...O hydrogen bonds. **Figure 3.** Spectral scan for the hydrolysis of 4-NPP in the absence and presence of **1** (substrate: catalyst = 20:1) in 97.5% aqueous-DMF at an interval of 5 min for 2 h. [4-NPP] = 1 mM, [Complex] = 0.05 mM.

of 4-nitrophenolate at 428 nm. The initial rate constant, V ($M \text{ min}^{-1}$) for zinc complexes was obtained using $\log[A_{\infty}/(A_{\infty} - A_t)]$ vs time (t) plot (Figure 4 for **1**).

Table 3. Hydrogen bonds donor/acceptor scheme (Å, °) for **1**.

D–H...A	D–H	H...A	D...A	D–H...A	Symmetry codes
N(2)–H(112)...Cl(3)	0.829	2.662	3.3537(15)	141.9	1–x, 2–y, 1–z
C(21)–H(21)...Cl(4)	0.95	2.69	3.5512(17)	151	
C(8)–H(8)...Cl(3)	0.95	2.81	3.6671(17)	150	
C(14)–H(14A)...O(5)	0.99	2.58	3.297(2)	129	x, 5/2–y, 1/2+z
C(5)–H(5A)...O(3)	0.99	2.48	3.2774(19)	137	1/2–x, 1/2+y, z
C(1)–H(1B)...O(4)	0.98	2.53	3.132(2)	119	1/2+x, 3/2–y, 1–z

Figure 4. Absorption vs time plot for complex **1** at 428 nm (Least-squares fit of second order, $R^2 = 0.999$). Inset shows the plot of $\log[A_\infty/(A_\infty - A_t)]$ vs time ($R=0.998$).

Figure 5. Plot of enzymatic kinetics for **1** (Rate vs [Substrate], $R^2 = 0.957$). Inset shows the Lineweaver-Burk plot ($1/V$ vs $1/[S]$) having intercept = 60.46589, slope = 0.03956 and $R=0.972$.

The dependence of rate constants on five different substrate concentrations gave a first order kinetics at lower concentration and deviates from unity at higher concentration (Figure 5 for **1**). Michaelis–Menten approach of enzymatic kinetics was used to determine the kinetic parameters ($V_{\max}$, K_M , k_{cat}) for the catalyzed reactions of zinc complexes from the Lineweaver–Burk plot of $1/V$ vs $1/[S]$. Similar plots for complexes **2** and **3** are given in Supplementary Information (Figures S16

and S17). Kinetic data of complexes **1–3** is given in Table 4. To determine any role of the ligand (**HL2**) and zinc(II) halide salts on phosphatase activity control experiments were performed, which indicate (Figures S18 and S19, Supplementary Information) no appreciable change. The catalytic activity order of complexes **1–3** follows the trend **3** > **1** > **2** with k_{cat} values 330.82 (for **1**), 300.34 (for **2**) and 373.60 (for **3**) min^{-1} . The k_{cat} values of complexes **1–3** suggest that these complexes have comparable and even ^{20(b)}, ^{26–28} in some cases better ^{20(a)} phosphatase-like activity with similar bis(phenoxo) bridged complexes in literature (Table 5). The effect of electron withdrawing $-\text{NO}_2$ group on **HL2** is clearly noticed as k_{cat} values lowered compared to other reported complexes.³⁰

3.4 Effect of electron withdrawing $-\text{NO}_2$ group on phosphatase activity

It is well-established in literature that hydrolysis of 4-NPP follows a plausible mechanistic pathway.²² As described in Scheme 3, where the complex acts as an effective catalyst by coordinating both the water molecule and 4-NPP at two adjacent zinc(II) centers with an increase in coordination number from 5 to 6 in the intermediate states (**1a/1b** and **1c**). The existence of **1**, **1a** and **1c** was confirmed by ESI-mass spectroscopy (Figures S20–S22 in Supplementary information). It is clearly found that coordinating water molecule plays the key role in hydrolysis of 4-NPP in metal bound state. It is worth noting here that in our previous work,³⁰ the three complexes with ligand **HL1** (without $-\text{NO}_2$ group in *para* position) showed the k_{cat} values as 590 (chloro), 334 (bromo) and 705 (iodo) min^{-1} . In this work, the k_{cat} values of complexes **1–3** are lower compared to the k_{cat} values with previous series of complexes. This decrease in the k_{cat} values is likely due to the electron withdrawing nature^{28,43} of the $-\text{NO}_2$ group in **HL2**. The presence of electron withdrawing group at the *para* position increases the Lewis acidity of zinc(II) ion.^{28,43} This is clearly evident from larger phenoxo-bridged Zn–O distances (2.0569(10)–2.0932(10) Å) compared to our previously reported chloro complex (Zn–O: 2.0298(12)–2.0528(11) Å) with **HL1**. Such an increase in Lewis acidity of zinc(II) ion effectively reduces the

Table 4. Kinetic parameters calculated from Michaelis–Menten plot for **1–3**.

Complex	Concentration (mM)	$V_{\max} \times 10^{-2} (\text{M min}^{-1})$	$K_M \times 10^{-4} (\text{M})$	$k_{\text{cat}} (\text{min}^{-1})$
1	0.05	1.654 ± 0.012	6.53 ± 0.41	330.82
2	0.05	1.501 ± 0.014	4.87 ± 0.31	300.34
3	0.05	1.868 ± 0.017	8.87 ± 0.45	373.60

Table 5. Some selected zinc complexes with phosphatase like activity.

S.No.	Ligand	Comments about structure and catalytic activity of select reported complexes	Ref.
1.	HL	<p>$[\text{Zn}_2\text{L}_2\text{Cl}_2]$ (1), distorted trigonalbipyramid, $k_{\text{cat}} = 17.52 \text{ min}^{-1}$</p> <p>$[\text{Zn}_2\text{L}_2\text{Br}_2]$ (2), distorted trigonalbipyramid, $k_{\text{cat}} = 8.64 \text{ min}^{-1}$</p>	20(a)
2.	HL1	<p>$[\text{Zn}_2(\text{L1})_2\text{Cl}_2]$ (1), $k_{\text{cat}} = 536.4 \text{ min}^{-1}$</p> <p>$[\text{Zn}_2(\text{L1})_2\text{Br}_2]$ (2) distorted trigonalbipyramid, both Br at trans position, $k_{\text{cat}} = 783.6 \text{ min}^{-1}$</p> <p>$[\text{Zn}_2(\text{L1})_2\text{I}_2]$ (3) distorted trigonalbipyramid, both I at trans position, $k_{\text{cat}} = 306 \text{ min}^{-1}$</p>	20(b)
3.	HL ¹	<p>$\text{Zn}_2\text{L}_2^1\text{Cl}_2$ (1), distorted trigonalbipyramid, both Cl at trans position, $k_{\text{cat}} = 561 \text{ min}^{-1}$</p> <p>$[\text{Zn}_2\text{L}_2^1\text{Br}_2]$ (2), distorted trigonalbipyramid, both Br at trans position $k_{\text{cat}} = 528 \text{ min}^{-1}$</p> <p>$[\text{Zn}_2\text{L}_2^1\text{I}_2]$ (3) distorted trigonalbipyramid, both I at trans position $k_{\text{cat}} = 449.8 \text{ min}^{-1}$</p>	26
4.	HL	<p>$[\text{Zn}_2\text{L}_2\text{Cl}_2]$ (1) distorted trigonalbipyramid, both Cl at trans position, $k_{\text{cat}} = 598.2 \text{ min}^{-1}$</p> <p>$[\text{Zn}_2\text{L}_2\text{Br}_2]$ (2) distorted trigonalbipyramid, both Br at trans position, $k_{\text{cat}} = 568.2 \text{ min}^{-1}$</p> <p>$[\text{Zn}_2\text{L}_2\text{I}_2]$ (3) distorted trigonalbipyramid, both I at trans position, $k_{\text{cat}} = 697.2 \text{ min}^{-1}$</p>	27

Table 5. (contd.)

S.No.	Ligand	Comments about structure and catalytic activity of select reported complexes	Ref.
5.	<p> $R' = \text{Br}, R'' = \text{CH}_3 \text{ BrHL2}$ $R' = \text{NO}_2, R'' = \text{CH}_3 \text{ NO}_2\text{HL2}$ </p>	$[\text{Zn}_2(\text{NO}_2\text{L2})(\text{CH}_3\text{COO})_2](\text{PF}_6)$, $k_{\text{cat}} = 0.0456 \text{ min}^{-1}$ $[\text{Zn}_2(\text{BrL2})(\text{CH}_3\text{COO})_2](\text{PF}_6)]$ $k_{\text{cat}} = 0.114 \text{ min}^{-1}$	28(a)
6.	<p>L^{Cl}</p>	$[\text{Zn}_2(\mu - \text{L}^{\text{Cl}}\text{O})(\mu_2\text{-OAc})_2](\text{PF}_6)$, $k_{\text{cat}} = 0.0288 \text{ min}^{-1}$	28(b)

Scheme 3. Plausible mechanistic pathway²² for hydrolysis of 4-NPP in presence of complex **1**.

nucleophilic character of metal bound water molecule which is involved in the hydrolysis of 4-NPP.²² This may cause an overall decrease in rate of hydrolysis for complexes **1–3** with **HL2**. As noted in literature, other factors like inductive effect of halides^{26,27} along with steric effect of the –Me group in –N(Me)₂ and Zn–Zn distance³⁰ in the dinuclear moiety may also play significant roles in the rate of hydrolysis of 4-NPP.

4. Conclusions

In this study, three synthetic analogues of phosphatase enzyme with dinuclear zinc centers have been synthesized using a tridentate N,N,O-donor Schiff base and characterized using analytical and spectroscopic methods. X-ray crystallographic analysis of chloro complex shows phenoxo-bridged dinuclear nature of the complex with rare *cis*-orientation of terminal chlorides. Complexes (**1–3**) show moderate catalytic activity towards hydrolysis of disodium salt of (4-nitrophenyl) phosphate hexahydrate. It is found that the catalytic activity order of complexes (**I** > **Cl** > **Br**) follows a similar

trend as reported in our previous work.³⁰ However, the complexes show a lower value of k_{cat} compared to our earlier reported complexes³⁰ due to the electron withdrawing nature of nitro group^{28,43} in Schiff base (**HL2**). Currently, we are investigating the influence of electron donating group ($-\text{CH}_3$) on phosphatase activity for some dinuclear zinc(II) complexes.

Supplementary Information (SI)

CCDC 1565993 contains the supplementary crystallographic data for **1**. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif. Additionally, the elemental analysis report, NMR, ESI-mass, UV-Vis spectra of ligand (**HL2**) and complexes **1–3**, the graphs of phosphatase activity of complexes **2** and **3** are included in Supplementary Information, available at www.ias.ac.in/chemsci.

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