Diamines Derived Transition Metal Complexes of Naproxen: Synthesis, Characterization and Urease Inhibition Studies

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ABSTRACT: *A series of diamines derived transition metal complexes of naproxen with Zn(II), Cu(II), Ni(II), Co(II), Mn(II) have been synthesized and characterized by (FT)-IR, UV-Vis, NMR spectroscopy, magnetic susceptibility, and elemental analysis. Octahedral geometry has been proposed for all synthesized complexes based on magnetic susceptibility and electronic spectra. The crystal structures of Cu(II) complex with 1,2-diaminoethane (4b) is reported in this article. Structural studies provide the confirmation for the formation of* $Cu(H_2O)_2$ $(C_2H_8N_2)_2(np)_2$*, which consists of two ionic naproxen moieties with different OMe orientation having intermolecular hydrogen bonding in solid-state. Jack bean Urease study of synthesized metal complexes was conducted and found that Cu and Ni complexes are more active having lower* IC_{50} *values against urease enzyme as compared to Zn, Co, and Mn complexes. Furthermore, urease inhibition studies showed that 1,2 diaminoethane and 1,3 diaminopropane derived transition metal complexes of naproxen (4a-e) and (5a-e), respectively have more inhibition efficacy as compared to simple metal naproxen complexes (3a-e). Amongst all, the synthesized complexes 4b and 4c have shown significant inhibition activities with* IC_{50} *values of* 17.06 ± 0.05 *and* 18.07 ± 0.17 μM *respectively, as compared to the standard drug thiourea.*

KEYWORDS: *Metal complexes; Diamines; Crystal structure; Enzyme inhibition.*

INTRODUCTION

Urease is an important enzyme which catalyzes the hydrolysis of urea to ammonia and carbamic acid [1] but the byproduct of this reaction are responsible for increasing pH, which ultimately produces negative side effects like stomach cancer and peptic ulcer in humans [2]. These side effects can be suppressed through controlling the enhanced activity of urease by using selected inhibitors which will ultimately reduce infections including gastric and peptic ulcers, proteus related species in the urinary tract, hepatic encephalopathy, hepatic coma, urolithiasis

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and pyelonephritis resulted due to the production of excess ammonia and activity of *Helicobacter pylori in* cirrhotic patients [2-4]. *H pylori,* a gram negative bacteria can survive and grow under elevated pH conditions due to which more than 50 percent of population in the world is convicted by *H. pylori* which reveals its high prevalence in human [5]. Various natural and synthetic origin urease inhibitors comprising metal-organic and organic compounds like heavy metal ions, compounds containing fluorides, boric acids, hydroxyl ketones and thiols have been studied for antiurease activity [6-8].

During last few decades the recommended therapy for *H. pylori* eradication was the combination of antibiotics clarithromycin and amoxicillin with omeprazole. However due to increased resistance developed by *H. pylori* to these antibiotics particularly to clarithromycin, considered this therapy non-attractive treatment in the recent years[8-9]. Some other strategies to fight with *H. pylori* have emerged which include the use of bismuth salts in combination with proton pump cell inhibitors or the combination of new classes of antibiotics e.g, aminopenicillins, fluroquinolones, tetracyclines [10]. However, these therapies against *H. pylori* are not so effective to control its pathogenicity and side effects due to developing resistance against the drugs. Hence, researchers are trying to develop new strategies to cope the catalytic side effects of urease enzymes. It is also observed that enzyme inhibition activities of the ligands containing various donor atoms (O, S, and N) can be increased due to coordination with metal ions [11]. Various metal complexes of transition metals with different ligands have been reported as urease inhibitors [12]. Transition metal complexes (Cu, Ni, and Zn) with Schiff base ligands were evaluated for inhibition activity on jack bean urease and compared with acetohydroxamic acid as reference [13]. Copper Complexes with tridentate aroylhydrazone ligands have been reported as urease inhibitors and also have been found effective against *H. pylori*. It was also observed that complexes with square planar geometry are better model as urease inhibitors [14]. Complexes of N'-(2-hydroxy-5-methoxybenzylidene)-3-methylbenzohydrazide, 2-[(2-di-methyl aminoethyl imino) methyl]-4-methylphenol, N'-(2-hydroxybenzylidene)-3-hydroxyl benzohydr-azide and N,N'-bis(5-methylsalicylidene)-o-phenylenediamine of Schiff bases were evaluated as active compounds against urease [15]. Ni(II) complexes with N,N,N'-trisubstitutedthioureas comprising of square planar geometry and bidentate mode of coordination have also been observed and found very effective against urease enzyme [16].

In the last two decades some clinically used drugs have also been repurposed for new indications because these drugs can directly enter clinical trials. Currently it is needed to slip out of the concept that a single drug can only be used for the treatment of only one disease. Consequently, we have repurposed the naproxen (an NSAID) and its transition metal complexes for the inhibition of urease. NSAIDs are biologically active medicinal drugs having versatile applications in medicinal chemistry. It is obvious that most of the NSAIDs are carboxylic acid derivatives in which carboxylate functional group is available for metal ligand chelation which increases their inhibition capability for selective substrates [17]. Furthermore, the transition metal complexes of these drugs are more potent than their ligands [18]. The interaction of theses NSAIDs with metal ions may lead to better understanding of metal ligand interaction to find distinct biological aspects for new indications [19]. In the last few decades, Naproxen and its metal complexes have been used to counteract anti-inflammatory and in enzyme inhibition studies [20].

No doubt, several potent inhibitors are being used as first line of treatment for infections causes by urease-producing bacteria which are available in current market. But the most effective inhibitors with safe great potency profile for considerable control of urease-related ailments which may have potential of being used as anti-ulcer drugs are still needed. In order to search new potent urease inhibitors, we have introduced relatively new class of Naproxen (a propionic acid carboxylate) and its transition metal complexes with diamines as urease inhibitor.

EXPERIMENTAL SECTION

Analytical grade chemicals and reagents including metal salts (copper acetate, nickel acetate, zinc acetate, cobalt acetate, manganese acetate), ethylene-diamine (1,2diaminoethane), propane-diamine (1,3-diaminopropane) and solvents purchased from Merck Millipore, England were used as received. Naproxen acid was purchased from Sigma Aldrich, England. Reaction progress was monitored by using TLC with aluminum backed plated. ^{1}H-NMR and ^{13}C-NMR spectra of

synthesized complexes were recorded in d6DMSO with Bruker AM 300 and AM 400 spectrometers operating at 300 and 400 MHz, respectively. Infrared spectra's were recorded on Shimadzu (FT)IR-8400S spectrophotometer in the region 4000-400 cm^{-1}using KBr disc. UV-Visible spectra's were recorded on Jenway 6505 UV/Visible double beam spectrophotometer, having accuracy ±0.001% at room temperature in DMSO. Micro analytical data (C, H, N %) of the synthesized complexes was determined using Elemental Analyzer (Perkin Elmer, USA). Molar conductance of the complexes was measured with Inolab Conductivity Bridge 720 at 25 °C. Stanton SM12/S Gouy's balance was used to measure magnetic susceptibility of the metal complexes at room temperature using $Hg[Co(NCS)_4]$ as the calibrant.

General procedure for the synthesis of metal naproxen complexes (3a-e)

Naproxen acid 1 (2.30g, 0.01 mol) was dissolved in 20 mL de-ionized water by adding KOH (0.56 g, 0.01 mol) until the solution became clear; forming potassium salt of naproxen. To this solution, aqueous metal acetate (0.005 mol) was added drop wise with constant stirring in a round bottom flask and stirring was continued for further 15 min. at 40°C. Characteristic precipitates of metal complex with naproxen 3a-e were formed which were filtered, washed with distilled water and ethanol and dried at room temperature.

Complex of $Zn(H_2O)_2(np)_2$ ***(3a)***

The compound (3a) was synthesized by following general procedure 2.1 by using zinc acetate (0.971 g) as white solid, yield 83%, m.p (215-217°C), Anal. Calcd for $C_{28}H_{32}O_8Zn$ (%): C, 59.85; H,5.74, Found: C, 59.87;H, 5.76, Selected FT-IR data (KBr, cm^{-1}): $\bar{\upsilon}(M\leftarrow H_2O)$ 3653 cm^{-1}, $\bar{\upsilon}(M\leftarrow O)$ 484 cm^{-1}**,** $\bar{\upsilon}$(COOH)1650-1730 cm^{-1}, $\bar{\upsilon}$(Ar C=C)1500-1590 cm^{-1}, $\bar{\upsilon}$(C-H) 2700-2900 cm^{-1} and disappearance of broad $\bar{\upsilon}$(O-H) peak at 3200-3400, λmax (cm^{-1}) = 28340, $\Omega^{-1}cm^2mol^{-1}$ = 25, μeff = diamagnetic, ^{1}H NMR (400.13 MHz, [d_6]DMSO, 25 °C): δ = 6.85-6.78(m, 6H, H-3, H-7, H-2, H-3′, H-7′, H-2′), 6.55 (d, ^3J = 7 Hz, 2H, H-8, H-8′), 6.36 (s, 2H, H-6, H-6′), 6.21 (d, ^3J = 7 Hz, 2H, H-4, H-4′), 2.97 (s, 6H, H-11, H-11′), 2.78-2.77 (m, 2H, H-12, H-12′), 0.51 (d, ^3J = 7 Hz, 6H, H-13, H-13′) ppm. ^{13}C NMR (100.61 MHz, [d_6]DMSO, 25 °C): δ = 156.7, 138.9, 132.9, 129.0, 128.5 , 127.1, 126.3, 125.3, 118.3, 105.7, 55.1, 46.4, 19.9.

$Cu(H_2O)_2(np)_2$ ***(3b)***

The compound **(3b)** was synthesized by following general procedure **2.1** using copper acetate 0.908 g (0.005 mol), Light green solid, yield 88%, m.p (203-205°C), Anal. Calcd for $C_{28}H_{32}O_8Cu$ (%): C, 60.04; H, 5.76, Found: C, 60.14;H, 5.70, Selected FT-IR data (KBr, cm^{-1}): $\bar{\upsilon}(M\leftarrow H_2O)$ 3690 cm^{-1}, $\bar{\upsilon}(M\leftarrow O)$453 cm^{-1}, $\bar{\upsilon}$(COOH)1650-1730 cm^{-1}, $\bar{\upsilon}$(Ar C=C)1500-1590 cm^{-1} , $\bar{\upsilon}$(C-H) 2700-2900 cm^{-1} and disappearance of broad $\bar{\upsilon}$(O-H) peak at 3200-3400, λmax (cm^{-1}) = 15280-15350, $\Omega^{-1}cm^2mol^{-1}$ = 23, μeff = 1.7

$Ni(H_2O)_2(np)_2$ ***(3c)***

The compound **(3c)** was synthesized by following general procedure **2.1** using nickel acetate 0.883 g (0.005 mol)**,** light green solid, m.p (211-213°C), yield 83%, Anal. Calcd for $C_{28}H_{32}O_8Ni$ (%): C, 60.57; H, 5.81, Found: C, 60.66;H, 5.76, Selected FT-IR data (KBr, cm^{-1}): $\bar{\upsilon}(M\leftarrow H_2O)$ 3683 cm^{-1}, $\bar{\upsilon}(M\leftarrow O)$ 468cm^{-1}, $\bar{\upsilon}$(COOH)1650-1730 cm^{-1},$\bar{\upsilon}$(Ar C=C)1500-1590 cm^{-1}, $\bar{\upsilon}$(C-H) 2700-2900 cm^{-1} and disappearance of broad $\bar{\upsilon}$(O-H) peak at 3200-3400, λmax (cm^{-1}) =12880, 18330, 27356, $\Omega^{-1}cm^2mol^{-1}$ = 27, μeff = 3.1

$Co(H_2O)_2(np)_2$ ***(3d)***

The compound (3d) was synthesized by following general procedure 2.1 using cobalt acetate 1.245 g (0.005 mol), light pink solid, m.p (198-200°C), yield 82%, Anal. Calcd for $C_{28}H_{32}O_8Co$ (%): C, 60.54; H, 5.81, Found: C, 60.59;H, 5.79, Selected FT-IR data (KBr, cm^{-1}): $\bar{\upsilon}(M\leftarrow H_2O)$ 3620 cm^{-1}, $\bar{\upsilon}(M\leftarrow O)$478 cm^{-1}, $\bar{\upsilon}$(COOH)1650-1730 cm^{-1}, $\bar{\upsilon}$(Ar C=C)1500-1590 cm^{-1}, $\bar{\upsilon}$(C-H) 2700-2900 cm^{-1} and disappearance of broad $\bar{\upsilon}$(O-H) peak at 3200-3400, λmax(cm^{-1}) =14600, 21490, 17580-17650, $\Omega^{-1}cm^2mol^{-1}$ = 25, μeff = 1.8

$Mn(H_2O)_2(np)_2$ ***(3e)***

The compound (3e) was synthesized by following general procedure 2.1 using manganese acetate 1.245 g (0.005 mol)**,** white solid, m.p (188-190°C), yield 87%, Anal. Calcd for $C_{28}H_{32}O_8Mn$ (%): C, 60.98; H, 5.85, Found: C, 60.92;H, 5.82, Selected FT-IR data (KBr, cm^{-1}): $\bar{\upsilon}(M\leftarrow H_2O)$ 3655cm^{-1}, $\bar{\upsilon}(M\leftarrow O)$ 443cm^{-1}, $\bar{\upsilon}$(COOH)1650-1730 cm^{-1}, $\bar{\upsilon}$(Ar C=C)1500-1590 cm^{-1}, $\bar{\upsilon}$(C-H) 2700-2900 cm^{-1} and disappearance of broad $\bar{\upsilon}$(O-H) peak at 3200-

3400, λmax(cm^{-1}) =16648, 20820, 24566, 27330, $\Omega^{-1}cm^2mol^{-1}$ = 28, µeff = 1.8

General procedure for the synthesis of bis and tris (1,2diaminoethane) metal naproxen complexes (4a-e).

To a stirred suspension of synthesized metal complexes (3a-e) in ethanol/water (1:1) solution, 1,2-diaminoethane 1.42 mL (0.02 mol) was added and reaction mixture was stirred for 25-30 minutes at room temperature until the solution became clear. Slow evaporation of this solution produced crystals of Cu (1,2-diaminopropane) metal naproxen complexes while others obtained in powder form (4a-e).

$Zn(H_2O)_2$ $(C_2H_8N_2)_2$ $(np)_2$ ***(4a)***

The compound **(**4a) was synthesized by following general procedure 2.2 using 1,2 diaminoethane, 1.42 mL (0.02 mol), White solid, yield 87 %, m.p (268-270ºC), Anal. Calcd for $C_{32}H_{46}N_4O_8Zn$ (%): C, 56.51;H, 6.82;N,8.24, Found: C, 57.50;H, 6.75;N, 8.26, Selected FT-IR data (KBr, cm^{-1}): $\bar{\upsilon}(M\leftarrow H_2O)$ 3623 cm^{-1}, $\bar{\upsilon}(M\leftarrow N)$ 509cm^{-1}, $\bar{\upsilon}$(COOH)1650-1730 cm^{-1}, $\bar{\upsilon}$(Ar C=C)1500-1590 cm^{-1}, $\bar{\upsilon}$(C-H) 2700-2900 cm^{-1} and disappearance of broad $\bar{\upsilon}$(O-H) peak at 3200-3400, λmax(cm^{-1}) = 29213, $\Omega^{-1}cm^2mol^{-1}$ =188, µeff = diamagnetic, ^{1}H NMR (400.13 MHz, [d_6]DMSO, 25 °C): δ = 7.59(d, ^3J = 8 Hz, 2H, Ar*H*), 7.43 (d, ^3J = 8 Hz, 2H, Ar*H*), 7.39 (s, 2H, Ar*H*), 7.34 (d, ^3J = 8 Hz,2H, Ar*H*), 7.21 (s, 2H, Ar*H*), 7.04 (d,^3J = 8 Hz,2H, Ar*H*), 3.84 (s, 6H, 2×CH_3), 3.24-3.19 (m, 8H, 4×NH_2), 3.12-3.09 (m, 8H, 4×CH_2), 2.98-2.94 (m, 2H, 2×CH), 1.36 (d, ^3J = 7 Hz, 6H, 2×CH_3) ppm. ^{13}C NMR (100.61 MHz, [d_6]DMSO, 25°C): δ = 158.2, 139.4, 133.3, 129.0, 128.5, 127.1, 126.3, 125.3, 118.3, 105.7, 55.1, 46.8, 46.4, 19.9.

$Cu(H_2O)_2$ $(C_2H_8N_2)_2$ $(np)_2.2H_2O$ ***(4b)***

The compound (4b) was synthesized by following general procedure 2.2 using 1,2 diaminoethane, 1.42 mL (0.02 mol), blue crystals, yield 88 %, m.p (254-256ºC), Anal. Calcd for $C_{32}H_{50}CuN_4O_{10}$ (%): C, 53.81;H, 7.06;N,7.84, Found: C, 53.72;H, 6.85;N, 7.76, Selected FT-IR data (KBr, cm^{-1}): $\bar{\upsilon}(M\leftarrow H_2O)$ 3598cm^{-1}**,** $\bar{\upsilon}(M\leftarrow N)$ 516cm^{-1}, $\bar{\upsilon}$(COOH)1650-1730 cm^{-1}, $\bar{\upsilon}$(Ar C=C)1500-1590 cm^{-1}, $\bar{\upsilon}$(CH)2700-2900 cm^{-1} and disappearance of broad $\bar{\upsilon}$(O-H) peak at 3200-3400, λmax (cm^{-1}) = 16891-16918, $\Omega^{-1}cm^2mol^{-1}$ = 182, µeff = 1.9

Ni $(H_2O)_2$ $(C_2H_8N_2)_2$ $(np)_2$ ***(4c)***

The compound (4c) was synthesized by following general procedure 2.2 using1,2 diaminoethane, 2.0 mL (0.03 mol), violet crystalline powder, yield 78 %, m.p (293-295ºC), Anal.Calcd for $C_{34}H_{50}N_6O_6Ni$ (%): C, 58.55;H, 7.23; N,12.05, Found: C, 58.53; H, 7.13; N,12.10, Selected FT-IR data (KBr, cm^{-1}): $\bar{\upsilon}(M\leftarrow N)$ 501 cm^{-1}, $\bar{\upsilon}$(COOH)1650-1730 cm^{-1}, $\bar{\upsilon}$(Ar C=C)1500-1590 cm^{-1}, $\bar{\upsilon}$(CH) 2700-2900 cm^{-1} and disappearance of broad $\bar{\upsilon}$(O-H) peak at 3200-3400, λmax(cm^{-1}) = 18722, 22412, 29543, $\Omega^{-1}cm^2mol^{-1}$ = 194, µeff = 3.3

Co$(H_2O)_2$ $(C_2H_8N_2)_2$ $(np)_2$ ***(4d)***

The compound (4d) was synthesized by following general procedure 2.2 using 1,2 diaminoethane, 2.0 mL (0.03 mol), greenish brown solid, yield 79%, m.p (286-288ºC), Anal. Calcd for $C_{34}H_{50}N_6O_6Co$ (%): C, 58.53;H, 7.22;N;12.04; Found: C, 59.01; H, 7.17; N, 12.00, Selected FT-IR data (KBr, cm^{-1}): $\bar{\upsilon}(M\leftarrow N)$ 569 cm^{-1}**,** $\bar{\upsilon}$(COOH)1650-1730 cm^{-1}, $\bar{\upsilon}$(ArC=C) 1500-1590 cm^{-1}, $\bar{\upsilon}$(CH) 2700-2900 cm^{-1} and disappearance of broad $\bar{\upsilon}$(O-H) peak at 3200-3400, λmax (cm^{-1}) = 14889, 19477, 21765-21815, $\Omega^{-1}cm^2mol^{-1}$=188, µeff = 1.8

Mn$(H_2O)_2$ $(C_2H_8N_2)_2$ $(np)_2$ ***(4e)***

The compound (4e) was synthesized by following general procedure 2.2 using, 1,2 diaminoethane, 2.0 mL (0.03 mol), dark brown solid, yield 77%, m.p (279-281ºC), Anal. Calcd for $C_{34}H_{50}N_6O_6Mn$ (%): C, 58.53;H, 7.22; N, 12.04, Found: C, 58.46; H, 7.20; N, 12.00, Selected FT-IR data (KBr, cm^{-1}): $\bar{\upsilon}(M\leftarrow N)$ 530cm^{-1}, $\bar{\upsilon}$(COOH)1650-1730 cm^{-1}, $\bar{\upsilon}$(Ar C=C)1500-1590 cm^{-1}, $\bar{\upsilon}$(CH)2700-2900 cm^{-1} and disappearance of broad $\bar{\upsilon}$(O-H) peak at 3200-3400, λmax(cm^{-1}) = 18624, 20986, 25731, 29532, $\Omega^{-1}cm^2mol^{-1}$ = 178, µeff = 2.1

General procedure for the synthesis of bis & tris (1,3-diaminopropane) metal naproxen complexes (5a-e).

To a stirred suspension of synthesized metal complexes 3a-e in ethanol/water (1:1) solution, 1,3-diaminopropane 1.68 ml (0.02 mol) was added and reaction mixture was stirred for 25-30 minutes at room temperature until the solution became clear. Slow evaporation of this solution produced solid material of desired bis and tris (1,3-diaminopropane) metal naproxen complexes **(**5a-e).

$Zn(H_2O)_2$ $(C_3H_{10}N_2)_2$ $(np)_2$ **(5a)**

The compound (5a) was synthesized by following general procedure 2.3 using1,3-diaminopropane 1.68 mL (0.02 mol), white solid, yield 82 %, m.p (262-264ºC),Anal. Calcd for $C_{34}H_{50}N_4O_8Zn$ (%): C, 57.67; H, 7.12; N, 7.91; Found: C, 57.61; H,7.15; N, 7.96, Selected FT-IR data (KBr, cm^{-1}): *ῡ(M←H_2O)* 3623 cm^{-1}**,** *ῡ(M←N)* 509cm^{-1}, *ῡ*(COOH)1650-1730 cm^{-1}, *ῡ*(Ar C=C)1500-1590 cm^{-1}, *ῡ*(C-H) 2700-2900 cm^{-1} and disappearance of broad *ῡ*(O-H) peak at 3200-3400, *λ*max (cm^{-1}) =29007, $\Omega^{-1}cm^2mol^{-1}$ = 182, μeff = diamagnetic, ^{1}H NMR (400.13 MHz, [d_6]DMSO, 25 °C): δ = 7.92 (d, ^3J = 7 Hz, 4H, Ar*H*), 7.75 (d, ^3J = 7 Hz, 4H, Ar*H*), 4.16-4.10 (m, 2H, 2×CH), 3.49-3.44 (m, 8H, 4×NH_2), 3.12 (d, ^3J = 8 Hz, 4H, 2×CH_2), 2.69-2.64 (m, 8H, 4×CH_2), 2.57-2.52 (m, 2H, 2×CH), 2.01 (d, ^3J = 7 Hz, 6H, 2×CH_3), 1.60 (d, ^3J = 7 Hz, 12H, 4×CH_3) 1.52-1.46 (m, 4H, 2×CH_2), ppm. ^{13}C NMR (100.61 MHz, [d_6]DMSO, 25 °C): δ = 175.7, 142.2, 138.5, 128.6, 127.8, 44.6, 42.2, 39.2, 38.6, 29.9, 22.5, 20.7.

$Cu(H_2O)_2$ $(C_3H_{10}N_2)_2$ $(np)_2$. $2H_2O$ ***(5b)***

The compound (5b) was synthesized by following general procedure 2.3 using 1,3 diaminopropane,1.68 mL (0.02 mol), blue crystalline powder, m.p (245-247ºC), yield 79 %, Anal. Calcd for $C_{34}H_{54}N_4O_{10}Cu$ (%): C, 56.22; H, 7.71; N, 7.93; Found: C, 56.11; H, 7.78; N, 7.86, Selected FT-IR data (KBr, cm^{-1}): *ῡ(M←H_2O)* 3598 cm^{-1}, *ῡ(M←N)* 516cm^{-1}**,** *ῡ*(COOH)1650-1730 cm^{-1}, *ῡ*(Ar C=C) 1500-1590 cm^{-1} , *ῡ*(CH) 2700-2900 cm^{-1} and disappearance of broad *ῡ*(O-H) peak at 3200-3400, *λ*max(cm^{-1}) = 16840-16860, $\Omega^{-1}cm^2mol^{-1}$ = 178, μeff = 1.9

Ni $(H_2O)_2$ $(C_3H_{10}N_2)_3$ $(np)_2$ ***(5c)***

The compound (5c) was synthesized by following general procedure 2.3 using 1,3diaminopropane, 2.52 mL (0.03 mol), violet crystals, m.p (287-289ºC), yield 78 %, Anal. Calcd for $C_{37}H_{56}N_6O_6Ni$ (%): C, 60.09; H, 7.63; N, 11.36; Found: C, 60.03; H, 7.73; N, 11.30, Selected FT-IR data (KBr, cm^{-1}): *ῡ(M←N)* 501cm^{-1},*ῡ*(COOH) 1650-1730 cm^{-1},*ῡ*(Ar C=C) 1500-1590 cm^{-1}, *ῡ*(CH) 2700-2900 cm^{-1} and disappearance of broad *ῡ*(O-H) peak at 3200-3400, *λ*max(cm^{-1}) = 18654, 22346, 29467, $\Omega^{-1}cm^2mol^{-1}$ = 190, μeff = 3.3

$Co(H_2O)_2$ $(C_3H_{10}N_2)_3$ $(np)_2$ ***(5d)***

The compound (5d) was synthesized by following general procedure 2.3 using 1,3 diaminopropane, 2.52 mL (0.03 mol), greenish brown solid, m.p (281-283ºC), yield 75 %, Anal. Calcd for $C_{37}H_{56}N_6O_6Co$ (%): C, 60.07;H, 7.63; N,11.36; Found: C, 60.01; H, 7.60; N, 11.40, Selected FT-IR data (KBr, cm^{-1}): *ῡ(M←N)* 569cm^{-1}, *ῡ*(COOH)1650-1730 cm^{-1}, *ῡ*(Ar C=C)1500-1590 cm^{-1}, *ῡ*(CH)2700-2900 cm^{-1} and disappearance of broad *ῡ*(O-H) peak at 3200-3400, *λ*max(cm^{-1}) =14843, 19433, 21750-21790, $\Omega^{-1}cm^2mol^{-1}$ = 180, μeff = 1.8.

$Mn(H_2O)_2$ $(C_3H_{10}N_2)_3$ $(np)_2$ ***(5e)***

The compound (5e) was synthesized by following general procedure 2.4 using 1,3 diaminopropane 2.52 mL (0.03 mol), dark brown solid, m.p (269-271), yield 77(%), Anal. Calcd for $C_{37}H_{56}N_6O_6Mn$(%): C, 60.40; H, 7.67; N, 11.42; Found: C, 60.46;H, 7.70;N, 11.40, Selected FT-IR data (KBr, cm^{-1}): *ῡ(M←N)* 530cm^{-1}, *ῡ*(COOH)1650-1730 cm^{-1}, *ῡ*(Ar C=C) 1500-1590 cm^{-1}, *ῡ*(CH) 2700-2900 cm^{-1} and disappearance of broad *ῡ*(O-H) peak at 3200-3400, *λ*max (cm^{-1}) = 18430, 20945, 25670, 29433, $\Omega^{-1}cm^2mol^{-1}$=172, μeff = 2.1

Single crystal X-Rays analysis

For compounds *$Cu(H_2O)_2$ $(C_2H_8N_2)_2$ $(np)_2$.$2H_20$* (4b) X-Ray measurements were made on a Bruker kappa APEXII CCD diffractometer which was equipped with a graphite-mono-chromated Mo-K radiation, λMo = 0.710 73 Å at 100 K. For data collection multi-scan, u scans and absorption correction was applied. The structure of (4b**)** was resolved by using SHELXS97 program. The geometrical considerations were made for hydrogen atoms and methyl groups were defined as rigid groups which can rotate freely. Final structural refinement on F^2 was carried out by least-squares, full-matrix techniques using SHELXL-97[21-22]. Publication material was prepared by Mercury software from Cambridge Crystallographic Resources.

Urease Assay

The enzyme assay is the modified form of the commonly known Berthelot reaction [23]. A total volume of 85 µl assay mixture contained 10 µl of phosphate buffer of pH 7.0 in each well in the 96-well plate followed by the addition of 10 µl of sample solution and 25 µL of enzyme solution (0.1347 units). Contents were pre-incubated at 37ºC for 5 minutes. Then, 40 µl of urea stock solution (20 mM) was added to each well and incubation continued

at 37ºC for further 10 min. After given time, 115 µl phenol hypochlorite reagent was added in each well (freshly prepared by mixing 45 µL phenol reagent with 70 µL of alkali reagent). For color development, incubation was done at 37ºC for another 10 min. Absorbance was measured at 625 nm using the 96-well plate reader Synergy HT. The percentage enzyme inhibition was calculated by the following formula:

Inhibition (%) = 100 - (Absorbance of test sample / Absorbance of control) × 100

IC_{50}values (concentration at which 50% enzyme catalyzed reaction occurs) of compounds were calculated using EZ-Fit Enzyme Kinetics Software (Perrella Scientific Inc. Amherst, USA).

RESULTS AND DISCUSSION

Synthesis

Transition metal complexes of naproxen with 1,2 diaminoethane and 1,3-diaminopropane were prepared according to the (Scheme 1) and characterized by elemental analysis, ^{1}H-NMR, ^{13}C-NMR and FT-IR, UV-VIS spectroscopy, magnetic susceptibility and conductivity measurements. All the transition metal complexes were prepared by the ligand exchange reaction in aqueous solution and then by addition of (1,2diaminoethane) and (1,3-diaminopropane). The synthesized complexes were then evaluated against urease enzyme and most of the complexes have been found active against urease.

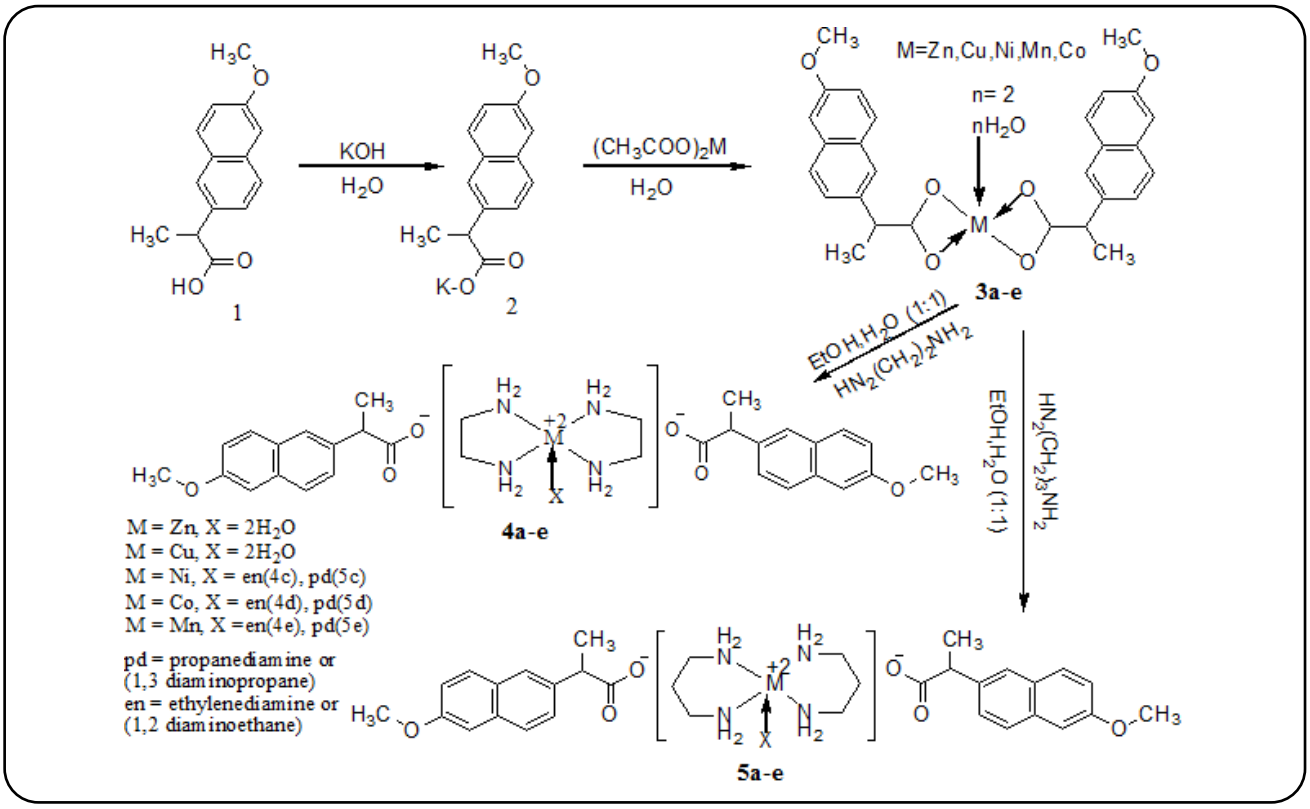
X-ray Crystallographic studies

Attempts to crystallize all the synthesized complexes were made but only one trail yielded (4b) the suitable crystals for XRD analysis. The careful crystallization of $Cu(H_2O)_2$ $(C_2H_8N_2)_2$ $(np)_2$ $.2H_20$ from the water/ethanol (1/1) mixture yielded needle like blue crystals. The crystal was mounted and X-ray data was collected on Bruker Kappa Apex II diffractometer. The crystal data solution and refinement parameters calculated thereafter suggests that the title compound (Fig. 1) consist of 2 types of ionic moieties. The co-ordination sphere around copper cation is octahedral with basal plane A (N1/N2/N3/N4) of four nitrogen atoms from two ethylene-diamines (1,2diaminopropane) and two apical O-atoms from two water molecules. The copper atom Cu1 is at a distance of 0.0204 Å from equatorial plane. The Cu-N bonds are in the range of [2.015(3)-2.024(3) Å] and Cu- O bonds in the range of [2.495(3)-2.536(3) Å]. There are two independent naproxen anions. In first naproxen anion the acetyl moiety B (O3/O4/C5/C6) and the naphthalene ring (C8-C18) are planar with r. m. s deviations of 0.0045 and 0.0167 Å, respectively. The dihedral angle between B/C is 73.27(13)°. Similarly in the second naproxen anion acetyl moiety D (O6/O7/C19/C20) and the naphthalene ring E (C22-C31) are also planar with r. m. s deviations of 0.0123 and 0.0235 Å, respectively. The dihedral angle between D/E is 74.27(13)° which shows a minor difference with the first one. However, the methoxy group in the first molecule is planar whereas in the second it does not. The molecules are stabilized in the form of infinite two dimensional polymeric network with crystallographic base vectors [0 1 0], [1 0 0] and in the plane (0 0 1) due to hydrogen bonding's of N-H….. O and O-H…..O types as shown in Table 1 and reported in Fig. 2.

The H-atoms are drawn as small circles of arbitrary radii (**CCDC 1554506**).

NMR Spectroscopy

Zinc complexes of naproxen were characterized by ^{1}H NMR and ^{13}C NMR in d_6DMSO. The ^{1}H NMR of Zinc complex of naproxen **(3a)** was deduced from their signals of one proton each, chemical shift values and their coupling constants clearly indicate the complex formation. A multiplet and a duplet with a chemical shift value of 6.85-6.78 ppm and 6.36, 6.21 ppm is attributed to aromatic protons. A singlet at 2.97 ppm was assigned to methyl group of (OCH_3). Another multiplet at 2.78-2.77 ppm was observed for tertiary carbon directly attach with aromatic ring. A shielded signal at 0.51 ppm was assigned to methyl group. In ^{13}C-NMR, a characteristic most downfield signal at 157.6 ppm was interpreted for the aromatic carbon directly attached with (OCH_3). At relatively up-field signals at 129, 129.85, 127.1 and 126.3 ppm were assigned to aromatic carbons. Chemical shift values 55.1 pm was attributed for carbon of (OCH_3). It is observed that when 1,2 diaminoethane was added to **(3a)** to synthesize bis (1,2-diaminoethane) zinc flurbiprofen complex **(4a),** their^{1}H NMR and ^{13}C NMR spectra clearly indicate chemical shifts values of ^{1}H NMR at (7.51, 7.45, 7.40-7.35, 7.21-7.17 ppm) for aromatic protons and multiplet 3.59-3.53 ppm for hydrogen of tertiary methyl groups are shifted to downfield region. Appearance of new signals of multiplet



Scheme 1: Synthesis of bis and tris diamines derived metal naproxen complexes

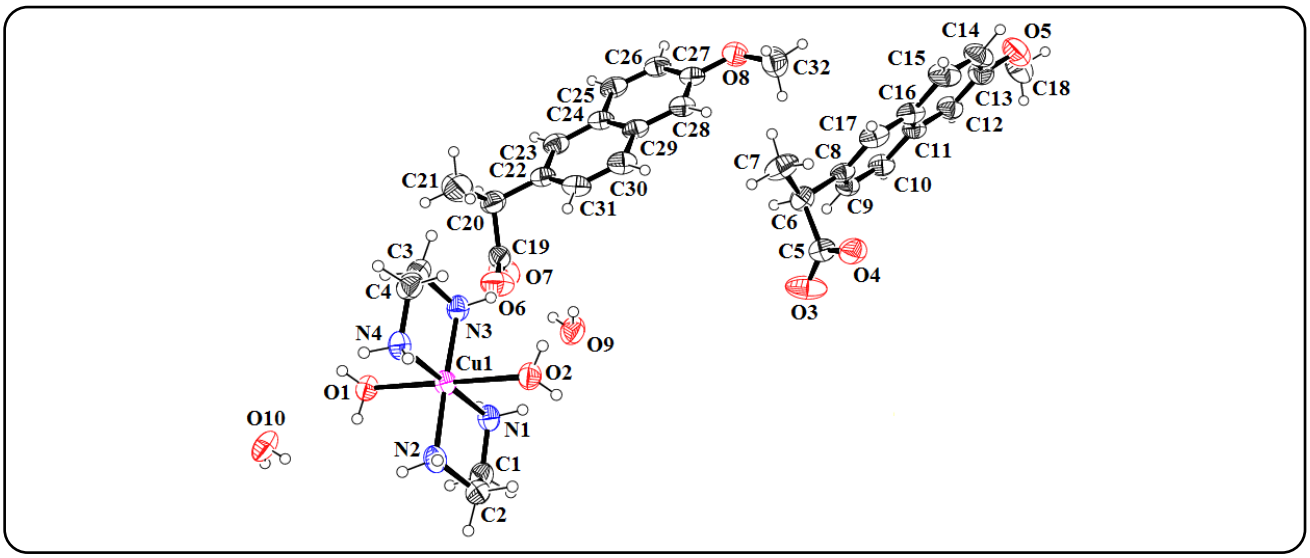


Fig. 1: ORTEP diagram of $Cu(H_2O)_2$ $(C_2H_8N_2)_2$ $(np)_2$.$2H_2O$ with thermal ellipsoids are drawn at 40 % probability level.

at 3.24-3.19 ppm (4×NH_2) and 3.12-3.09 ppm (4×CH_2) are assigned for the formation of (4a). The ^{13}C NMR of (4a) indicates that two new peaks at 46.7 ppm (2×CH_2), 46.2 ppm (2×CH_2) which are attributed to the (4×CH_2) of (1,2-diaminoethane) chelating with zinc metal, that confirms the formation of (4a). When 1,3 diaminopropane was added to (3a) to synthesize bis (1,3-diaminopropane) zinc naproxen complex (5a), their ^1H NMR and ^{13}C NMR spectra clearly indicate that chemical shifts values of ^1H NMR at (7.72, 7.67, 7.64, 7.44, 7.23, 7.09 ppm)

Table 1: Crystallographic and structural refinement parameters for 4b.

	CUNAPED
Crystal data	
Chemical formula	$C_{32}H_{50}CuN_4O_{10}$
M_r	714.30
Crystal system, space group	Monoclinic, $P2_1$
Temperature (K)	296
a, b, c (Å)	14.9121 (4), 5.9231 (2), 20.8791 (6)
β (°)	105.561 (2)
V (Å^3)	1776.57 (9)
Z	2
Radiation type	Mo Kα
μ (mm^{-1})	0.67
Crystal size (mm)	0.40 × 0.20 × 0.18
Data collection	
Diffractometer	Bruker Kappa APEXII CCD
Absorption correction	Multi-scan (SADABS; Bruker, 2005)
T_{min}, T_{max}	0.780, 0.880
No. of measured, independent and observed [I> 2σ(I)] reflections	15901, 7489, 5289
R_{int}	0.028
$(\sin \theta/\lambda)_{max}$ (Å^{-1})	0.650
Refinement	
R[F^2> 2σ(F^2)], wR(F^2), S	0.042, 0.101, 0.96
No. of reflections	7489
No. of parameters	452
No. of restraints	13
H-atom treatment	H atoms treated by a mixture of independent and constrained refinement
$\Delta\rangle_{max}$, $\Delta\rangle_{min}$ (e Å^{-3})	0.35, -0.29
Absolute structure	Flack x determined using 1705 quotients [(I+)-(I-)]/[(I+)+(I-)] (Parsons, Flack and Wagner, ActaCryst. B69 (2013) 249-259).
Absolute structure parameter	0.004 (11)

Table 2: Hydrogen bonds for 4b [Å and °].

D-H...A	d(D-H)	d(H. ..A)	d(D...A)	<(DHA)
O(1)-H(1A)...O(10)^{i}	0.816(13)	1.896(19)	2.694(4)	165(6)
O(1)-H(1B)...O(3)ii	0.818(13)	1.87(2)	2.663(4)	165(6)
O(2)-H(2A)...O(9)iii	0.828(13)	1.899(13)	2.726(4)	177(4)
O(2)-H(2B)...O(6)iv	0.821(13)	1.917(13)	2.733(4)	173(4)
N(1)-H(1C)...O(2)v	0.89	2.17	3.050(6)	170.9
N(1)-H(1D)...O(9)	0.89	2.55	3.199(5)	130.6
N(2)-H(2C)...O(3)iii	0.89	2.45	3.204(6)	142.7
N(2)-H(2D)...O(4)ii	0.89	2.18	3.057(5)	166.3
N(3)-H(3A)...O(7)iv	0.89	2.20	3.088(5)	171.9
N(3)-H(3B)...O(6)	0.89	2.46	3.205(6)	141.9
N(4)-H(4B)...O(1)iv	0.89	2.16	3.047(5)	174.3
C(18)-H(18B)...O(8)vi	0.96	2.55	3.368(6)	143.1
O(9)-H(9A)...O(6)	0.809(13)	1.998(18)	2.796(6)	169(6)
O(9)-H(9B)...O(7)iv	0.815(13)	1.885(17)	2.693(6)	171(5)
O(10)-H(10A)...O(4)vii	0.816(13)	1.95(2)	2.724(5)	158(5)
O(10)-H(10B)...O(3)ii	0.814(13)	1.924(14)	2.736(6)	175(6)

Symmetry transformations used to generate equivalent atoms:

i = -x,y+1/2,-z+1 ii = -x+1,y-1/2,-z+1 iii= -x+1,y+1/2,-z+1 iv= x,y+1,z v= x,y-1,z vi= -x+2,y+1/2,-z vii = -x+1,y-3/2,-z+1

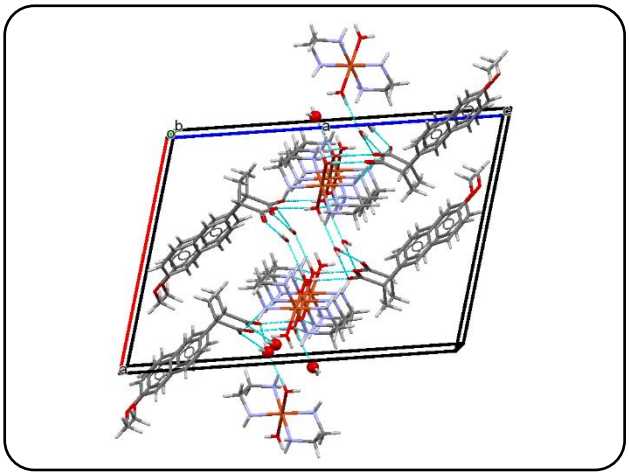


Fig. 2: Packing diagram of 4b showing H-bonding

for aromatic protons and 3.84 ppm for methyl groups are shifted to downfield region. Appearance of new signals of multiplet at 3.49-3.44 ppm (4×NH_2), 2.79-2.59 ppm (4×CH_2) and a multiplet at 1.52-1.46 ppm (2×CH_2) are due to formation of (5a). The ^{13}C NMR of (5a) indicates that two new peaks at 39.8 ppm (4×CH_2), 38.5 (2×CH_2) ppm are attributed to the (6×CH_2) of (1,3-diaminopropane) chelating with zinc metal, which confirms the formation of (5a).

FT-IR Analysis

The FT-IR analysis of compounds is in agreement with the literature which reveals the complex formation. The appearance of bands in the range 1554-1695 cm^{-1} is attributed to (–COOH) functional group. IR peaks appearing in the range of 1440-1498 cm^{-1} belongs to (aromatic –CH) functionality. The bands within 443-484 cm^{-1} range are assigned to (M←O) metal oxygen bonds and bands ranging from 501-569 cm^{-1} are attributed to the (M←N) metal nitrogen bonds. It can be observed from the IR analysis of the complexes that (M-N) metal nitrogen peaks are not observed for the (3a-e) which are synthesized from only transition metals and ligands but when (1,2 diaminoethane) and (1,3-diaminopropane) are added to the these complexes to form bis and tris (1,2 diaminoethane) and bis and tris (1,3-diaminopropane) metal naproxen complexes (4a-e and 5a-e), the (M-N) metal nitrogen peaks

appear in the range of 501-569 cm^{-1}which confirms the formation of chelating bond between metal and two nitrogen's of (1,2 diaminoethane and 1,3-diaminopropane).

Uv/Vis and magnetic susceptibility studies

UV-Visible spectra give us very import and supportive information about the complex formation and symmetry of complexes inconsistent with the other spectroscopic measurements. Peak position, as well as the number of peaks are observed to find the symmetry of transition metal complexes. Each metal complex has specific allowed transitions after the splitting of d-d orbitals of metal when ligand approaches it. The electronic spectra of 3d-transition metals were recorded in 10^{-3} to 10^{-5} M solutions of each metal complex in DMSO in the range of 200-800 nm. The electronic spectra of Zn(II) exhibited only one peak at 28340 cm^{-1} in (3a), 29213cm^{-1}in (4a) and 29007 cm^{-1} in (5a) which were due to the ligand to metal charge transfer. The effective magnetic moment (B.M.) value of Zn was found to be zero which shows the diamagnetic nature of the complex. The electronic spectra of Cu(II) complexes showed only a single low-intensity broad-band in the range of 15280-15350 cm^{-1} for (3b),16891-16918 $cm^{-1}$for (4b) and 16840-16860 cm^{-1} for (5b). This low-intensity broad-band is expected due to $^2E_g \rightarrow {}^2T_{2g}$ transition which shows octahedral symmetry of complex. The difference in the position of peaks of (3b), (4b) and (5b) of copper complexes is due to different ligands because different ligands cause different magnitude of splitting in d-orbitals of metal. The B.M. values of the complexes are 1.7, 1.9 and 1.9 respectively which are in agreement with the octahedral geometry of copper complexes. The electronic spectra of Ni(II) complex showed three absorption peaks in the range of 12880, 18330 and 27356 cm^{-1} in case of (3c), 18722, 22412, 29543cm^{-1} for (4c), and 18654, 22346 and 29467 cm^{-1} in case of (5c). These peaks are expected due to $^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$, $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$ and $^3A_{2g}(F) \rightarrow {}^3T_{2g}(P)$ transitions which are consistent with the octahedral symmetry of complex. The B.M. values of nickel complexes are calculated 3.1, 3.3 and 3.3 which are in agreement with the octahedral geometry of nickel complexes. The electronic spectra of Mn(II) complex showed different sharp peaks in the range of 16648,20820,24566 and 27330 cm^{-1} in case of (3e) and 18430, 20945, 25670 and 29433 cm^{-1} for (4e). These peaks are due to $^4T_{1g}(G) \rightarrow {}^4T_{2g}(G)$, $^4T_{2g}(G) \rightarrow {}^4E_g(G)$, $^4T_{1g}(G) \rightarrow {}^4A_{1g}$, $^4E_g(D) \rightarrow {}^4T1_g(P)$ transitions which are expected in octahedral symmetry of complexes. The B.M. values of manganese complexes are calculated 1.8, 2.1 and 2.1 which are in agreement with the octahedral geometry of manganese complexes. The electronic spectra of Co(II) complexes in DMSO exhibited well-resolved bands around 14600, 17580-17650 cm^{-1} and 21490 cm^{-1} in the case of (3d) and a strong high-energy band at 21750-21790 cm^{-1}, 14843 cm^{-1} along with high energy band at 19433 cm^{-1} in case of (4d) and 14657, 17712-17724 cm^{-1} and 21823 cm^{-1} in the case of (5d) which were assigned to the transitions $^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$, $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$, respectively for a low-spin octahedral geometry. The B.M. values of both cobalt complexes are calculated 1.8 and 1.8 which are in agreement with the octahedral geometry of cobalt complexes.

Elemental analysis

The obtained C, H, N, and metals (Zn, Cu, Ni, Co, Mn) %age of all the synthesized transition metal (II) complexes were compared with the calculated values of their suggested molecular structures. The calculated and found values of all the synthesized metal (II) complexes are reported in the experimental section. The calculated and found %age values were all in the acceptable range (± 0.03-0.95) which provided an evidence for the formation of proposed transition metal (II) complexes.

Conductivity measurements

Molar conductivities of synthesized metal complexes were evaluated at room temperature. These conductivities values are providing very useful information regarding formation of metal complexes as well as their nonionic and ionic nature. All the solutions of metal complexes were prepared having a concentration of ($1x10^{-3}$M). It is found that molar conductivities of synthesized transition metal complexes (3a-e) are 25, 23, 27, 25 and 28 $\Omega^{-1}cm^2mol^{-1}$ indicating their non-ionic nature but after the addition of (1,2 diaminoethane) and (1,3-diaminopropane) to these transition metal complexes to form bis and tris (1,2 diaminoethane) metal naproxen complexes (4a-e) and bis and tris (1,3-diaminopropane) metal naproxen complexes (5a-e), the conductivities of these metal complexes increase to many folds which are 188, 182, 194, 190, 178 $\Omega^{-1}cm^2mol^{-1}$ for (4a-e) and 182, 178, 190, 180 and 172 $\Omega^{-1}cm^2mol^{-1}$ for (5a-e).

Table 3: In vitro urease inhibition study of transition metal complexes of naproxen.

Sample Code	Compounds	Anti-urease activity(μM ±SEM)[a]	
		Inhibition (%) at 0.5 Mm	IC_{50} μM
3a	$C_{28}H_{32}O_8Zn$	76.06±0.21	41.09±0.07
4a	$C_{32}H_{46}N_4O_8Zn$	103.03± 0.62	23.04 ± 0.02
5a	$C_{34}H_{50}N_4O_8Zn$	68.03±0.39	34.09±0.11
3b	$C_{28}H_{32}O_8Cu$	89.06±0.29	36.03±0.09
4b	$C_{32}H_{50}N_4O_{10}Cu$	43.02±0.31	[b]17.06 ± 0.05
5b	$C_{34}H_{50}N_4O_8Cu$	70.01±0.12	23.03±0.24
3c	$C_{28}H_{32}O_8Ni$	86.14±0.73	45.12±0.23
4c	$C_{34}H_{54}N_6O_8Ni$	121.23 ±0.91	18.07 ± 0.17
5c	$C_{37}H_{56}N_6O_6Ni$	110.32 ± 0.76	23.12±0.11
3d	$C_{28}H_{32}O_8Co$	45.04 ±0.07	85.07±0.15
4d	$C_{34}H_{50}N_6O_6Co$	76.61 ± 0.47	54.04± 0.31
5d	$C_{37}H_{56}N_6O_6Co$	37.51 ± 0.36	76.63±0.02
3e	$C_{28}H_{32}O_8Mn$	31.04±0.52	72.17±0.16
4e	$C_{34}H_{50}N_6O_6Mn$	77.05 ± 0.42	61.05± 0.43
5e	$C_{37}H_{56}N_6O_6Mn$	66.61 ± 0.47	67.09±0.05
A*	Thiourea	99.15±0.13	21.25±0.17

[a]*Values are expressed as mean (standard error of the mean of at least three experiments.* [b]*Bold values are for highly active compounds than standard drug thiourea.*

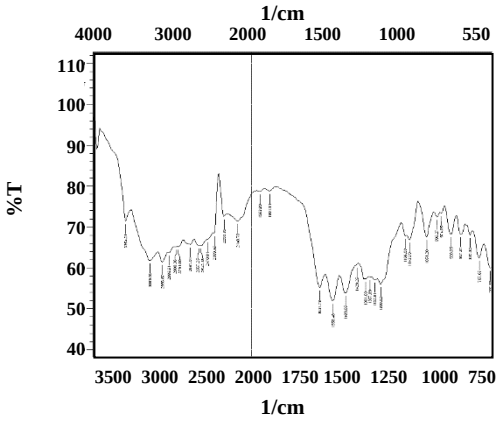


Fig. 3: FT-IR pectra of compound 4b.

This massive increase in conductivity of these diamines derived bis and tris transition metal complexes indicates that these complexes are electrolytic or ionic in nature.

Enzyme inhibition studies

Development of resistance against selective inhibitors by *H. pylori* is so effective that it is difficult to control its pathogenicity and side effects. Hence researchers are trying to develop new strategies to cope the catalytic side effects of urease enzymes. Anti-urease activities of bis and tris (1,2-diaminoethane) metal naproxen complexes and bis and tris (1,3-diaminopropane) metal naproxen complexes have been compared (Table 3). It is observed that metal complexes (4a-e) are the most potent as antiurease, processing lower IC_{50} values as compare to (3a-e) and (5a-e). Copper and nickel complexes are found most active with lower IC_{50} values and are inconsistent with the earlier work [19-20]. Zinc complexes have been found moderate inhibition activities but cobalt and manganese complexes are lower active among all the synthesized complexes. Amongst all, the synthesized complexes 4b and 4c have shown significant inhibition activities which have IC_{50} 17.06 ± 0.05 and 18.07 ± 0.17 μM as compared to the Standard-Thiourea.

CONCLUSIONS

Naproxen and its diamines derived transition metal complexes with Mn(II), Co(II), Ni(II), Cu(II) and Zn(II) have successfully been synthesized and characterized by, ^{1}H-NMR, ^{13}C-NMR, (FT)-IR, UV-Vis spectroscopy, magnetic susceptibility and conductivities measurements. All the metal complexes were proposed octahedral geometry on the basis of electronic spectra and B.M values. The synthesized compound (4b) was also studied by single crystal X-Ray analysis in the solid phase which showed planar structure

having 2 types of ionic moieties in naproxen with different dihedral angles between acetyl moiety and the naphthalene ring.Most of the synthesized transition metal complexes exhibited significant urease inhibition activity. Copper and nickel complexes were found most active against urease enzyme as compared to other metal complexes. It is also observed that bis and tris (1,2diaminoethane) metal naproxen complexes are most effective for urease inhibition as compare to bis and tris (1,3-diaminopropane) metal naproxen complexes. So in future these synthesized diamines derived transition metal complexes of naproxen can be recommended as better therapeutic agents for urease inhibition.

Conflict of interests

Authors of this paper declare no conflict of interests.

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