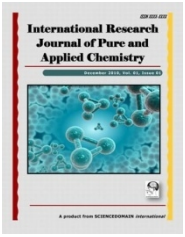

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Azamacrocycle Complexes: Synthesis and Xanthine Oxidase and Antioxidant Activity

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Authors' contributions

This work was carried out in collaboration between all authors. Author ABB performed experiments, managed the literature searches and wrote the first draft of the manuscript. Author SAK designed the study and revised the manuscript. Authors FZ and RH performed biological assays and statistical analysis. Authors VLR, TP and MSA participated in group discussions and helped in preparation of final manuscript. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: Gout is caused by high uric acid in the blood that leads to excess uric acid crystallizing in the joints causing swelling and pain. Uric acid is produced from the breakdown of the purine which is released when cells die or introduced from the food we eat. The enzyme that helps in breakdown of purine to uric acid is xanthine oxidase (XO). Since XO makes the conversion of purine into uric acid happen, preventing its activity results to slow down of uric acid production. Such is the role of xanthine oxidase inhibitors (XOI). In this context we aimed to synthesize new complex which can be

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potent towards XOI and antioxidant properties.
Study Design: Based on the literature we have designed azamacrocyclic complexes for advanced biological applications.
Place and Duration of Study: Department of Chemistry, Research Laboratory, Yuvaraj's College, University of Mysore, Mysore for synthesis and for biological activities Mahajana Life Science Research Laboratory, Department of Biotechnology, Microbiology and Biochemistry. June 2012-may 2013.
Methodology: The macrocyclic metal complexes were synthesized by the template condensation of diamine and formaldehyde in MeOH. After stirring for 10 min a solution of 1, 4-diaminobutane, metallic salt and 2, 4-pentanedione in MeOH was added and the resulting mixture was refluxed.
Results: Macrocylic metal complexes containing phenylene bridges have been synthesized and subjected to biological activities. Among the four synthesized complexes (3a-3d), 3d exhibited 83.2% of XO inhibition and also showed potent antioxidant activity. The same was also evident from structure activity relationship with atomic contact energy values of -285.78 compared to allopurinol with -200.02.
Conclusion: From the present study, we infer that, aza macrocyclic metal complexes could lead to the development of newer therapeutics for gout and other inflammatory diseases which are caused by oxidative stress.

Keywords: Antioxidant activity; azamacrocyclic complexes; molecular docking; xanthine oxidase.

1. INTRODUCTION

Xanthine oxidase (XO), a molybdoflavo protein, is a key enzyme in purine metabolism that has been isolated from a wide range of organisms, including bacteria and humans [1,2]. The enzyme is responsible for the medical condition known as gout which is caused by the deposition of uric acid in the joints leading to painful inflammation [3]. It has attracted lots of attention because of its potential role in tissue and vascular injuries, as well as in inflammatory diseases and chronic heart failure [4,5]. The production of reactive oxygen species (ROS) by XO and its damaging consequences has prompted investigations into the ability of some compounds to control and/or inhibit the enzyme activity or to scavenge the free radicals produced [6,7]. Allopurinol (1, 5-dihydro-4Hpyrazolo [3, 4-d] pyrimidin-4-one), a purine analogue was the first XO inhibitor approved by the FDA in 1966 and has been the cornerstone of the clinical management of gout and conditions associated with hyperuricemia for several decades [4]. However due to lack of free radical scavenging activity against superoxide anions produced, use of allopurinol is associated with some side effects that include allergy, hypersensitivity reactions, gastrointestinal upset, skin rashes and acute interstitial nephritis [5]. Therefore there is a need for the development of novel compounds with better safety profiles that could be used to relieve associated side effects.

Alterations of XO activity by various metals have also been probed with mixed results of either stimulation or inhibition, depending on the metal [8]. As mentioned above, because of its ubiquity and its ability to bind to proteins, copper would be one of the metals to probe in priority. However, although partial inhibition of XO activity by Cu^{2+} has been reported [9] there is no thorough investigation on the effect of the metal on the enzyme activity and structure, nor on the potential attachment sites for the metal.

Damage to cells caused by free radicals is believed to play a central role in the aging process and in disease progression. Antioxidants are our first line of defense against free radical damage, and are critical for maintaining optimum health and wellbeing. Some of the more well-known antioxidants include ascorbic acid (Vitamin C), alpha-tocopherol (Vitamin E), beta-carotene and enzymes such as catalase, superoxide dismutase (SOD) and glutathione peroxidase. The need for antioxidants becomes even more critical with increased exposure to free radicals [10].

Transition metal complexes have notably shown important antioxidant properties, *viz* SOD mimetic activity [11]. The macrocyclic nature of the ligand seems important for the SOD mimetic activity of the corresponding complexes as well as for their stability in the presence of proteins, even if the metal ion does not lie inside the cavity [12]. Copper (II) complexes can enclose a SOD mimetic activity hindering increased levels of reactive oxygen species [13]. Several macrocyclic copper complexes have been reported to scavenge the superoxide anion [14-17].

Among the non-platinum complexes for metal based chemotherapy, copper and zinc complexes have been much explored due to the fact that both copper and zinc are bioessential elements responsible for numerous bioactivities in living organism [18]. A number of nitrogen donor macrocyclic derivatives have long been used in analytical, industrial and medical applications [19-21].

Considering the importance of macrocyclic metal complex as XOI and antioxidant, the objective of the present study was to synthesize aza macrocyclic ligand and its metal complexes by using simple condensation method. The synthesized compounds were subjected to biological study and among these complexes, 3d exhibited potent XOI and antioxidant property.

2. EXPERIMENTAL SECTION

2.1 Materials and Methods

All the reagents used in the preparation of macrocyclic Ligands and their metal complexes were of reagent grade(Merck).The solvents used for the synthesis of macrocyclic ligands and metal complexes were distilled before use. All other chemicals were of AR grade and used without further purification. The elemental analysis of the compounds was performed on a Perkin Elmer 2400 Elemental Analyser. The FT-IR spectra were recorded using KBr discs on FT-IR Jasco 4100 infrared spectrophotometer. The ^{1}H NMR spectra were recorded using Bruker DRX 400 spectrometer at 400 MHz with TMS as the internal standard. The magnetic moments were measured out using gouy balance. Purity of the compound checked by TLC.

2.2. Chemistry

2.2.1 Preparation of the Macrocyclic Ligand and Its Metal Complexes (3a-d)

The macrocyclic ligand and its metal complex **3a-d** were synthesized by the template condensation of 2, 3, 5, 6-tetra methyl, 1, 4-diaminobenzene in MeOH, a solution of 36% formaldehyde in MeOH was added. After stirring for 10 min a solution of 1, 4-diaminobutane in MeOH was added. Finally a solution of metallic salt and 2, 4-pentanedione in MeOH was

added and the resulting mixture was refluxed for ca. 7 hrs. The brown solid product was filtered off, washed with MeOH and dried over fused $CaCl_2$ in desiccators. The product was recrystallized from hot MeOH.

2.2.2 Synthesis of copper (II) complex (3a)

The macrocyclic ligand and its metal copper complex were synthesized by the template condensation of 2, 3, 5, 6-tetra methyl, 1, 4-diaminobenzene (1.00g, 6.10 mmol) in MeOH (20 mL), a solution of 36% formaldehyde (6.10 mmol, 0.7 mL) in MeOH (20 mL) was added. After stirring for 10 min a solution of 1, 4-diaminobutane (6.10 mmol, 2.447 mL) in MeOH (20 mL) was added. Finally a solution of $CuSO_4.5H_2O$ (6.10 mmol, 1.00g) and 2, 4-pentanedione (6.10m mol, 1.2428 mL) in MeOH (40 mL) was added and the resulting mixture was refluxed for ca. 7 hrs. The brown solid product was filtered off, washed with MeOH and finally washed with water. The solid obtained was dried over fused $CaCl_2$ in desiccators.

Yield: 70%. Brown solid; IR (Nujol): 3271 (N-H), 1605 cm^{-1} (C=N), 1182 (C-N), 456 (Cu-N), 332 cm^{1} (Cu-SO_4); ^{1}H NMR ($CDCl_3$): 0.9 (s, 12H, $4CH_3$), 1.3 (m, 8H, $4CH_2$-N=C), 1.3 (t, $J = 7$ Hz, 8H, $4CH_2$-N=C=), 1.41 (m, 8H, $4CH_2$), 2.35 (s, 12H, 4Ar-CH_3), 2.55 (t, $J = 7$ Hz, 8H, $4CH_2$), 3.31 (s, 4H, $2CH_2$), 4.61 (s, 8H, $4CH_2$) (δ, ppm) ; EI-MS: m/z 1011 ($[M]^+$). Anal. Calcd. for $C_{40}H_{72}N_{10}Cu_2S_2O_8$(1011.1): C, 47.47 ; H,7.12 ; N, 13.85; Cu, 12.57; S,6.32; O, 12.66%. Found: C, 47. 3; H, 7.15; N, 13.67; Cu, 12.48; S, 6.4; O, 12.4%.

2.2.3 Synthesis of nickel (II) complex (3b)

The macrocylic nickel (II) complex was prepared by a method similar to that for copper complex except that $NiCl_2.6H_2O$. was used instead of $CuSO_4.5H_2O$.

Yield: 85%. Brown solid; IR (Nujol): 3260 (N-H), 1601 cm^{-1} (C=N), 1172 (C-N), 453 (Ni-N), 320 cm^{1} (Ni-Cl); ^{1}H NMR ($CDCl_3$): 0.9 (s, 12H, 4CH3), 1.3 (m, 8H, $4CH_2$-N=C), 1.3 (t, $J = 7$ Hz, 8H, $4CH_2$-N=C=), 1.41 (m, 8H, $4CH_2$), 2.35 (s, 12H, 4Ar-CH_3), 2.55 (t, $J = 7$ Hz, 8H, $4CH_2$), 3.31 (s, 4H, $2CH_2$), 4.61 (s, 8H, $4CH_2$) (δ, ppm); EI-MS: m/z 951 ($[M]^+$). Anal. Calcd. for $C_{40}H_{72}N_{10}Ni_2Cl_4$ (951.2): C, 50.46 ; H,7.57 ; N, 14.72; Ni, 12.34; Cl, 14.9 Found: C, 50.34 ; H,7.62 ; N, 14.48; Ni, 12.23; Cl, 14.88%.

2.2.4 Synthesis of zinc (II) complex (3c)

The macrocylic zinc complex was prepared by a method similar to that for copper complex except that $ZnCl_2$ was used instead of $CuSO_4.5H_2O$.
Yield: 90%. Pale yellow solid; IR (Nujol): 3250 (N-H), 1615 cm^{-1} (C=N), 1175 (C-N), 446 (Zn-N), 325 cm^{1} (Zn-Cl); ^{1}H NMR ($CDCl_3$): 0.9 (s, 12H, 4CH3), 1.3 (m, 8H, $4CH_2$-N=C), 1.3 (t, $J = 7$ Hz, 8H, $4CH_2$-N=C=), 1.41 (m, 8H, $4CH_2$), 2.35 (s, 12H, 4Ar-CH_3), 2.55 (t, $J = 7$ Hz, 8H, $4CH_2$), 3.31 (s, 4H, $2CH_2$), 4.61 (s, 8H, $4CH_2$) (δ, ppm); EI-MS: m/z 964 ($[M]^+$). Anal. Calcd. for $C_{40}H_{72}N_{10}Zn_2Cl_4$ (964.56): C, 49.76 ; H,7.47 ; N, 14.51; Zn, 13.56; Cl, 14.7 Found: C, 49.45 ; H, 7.44 ; N, 14.73; Zn, 13.48; Cl, 14.59%

2.2.5 Synthesis of Silver (I) complex (3d)

The macrocylic silver complex were prepared by a method similar to that for copper complex except that Silver nitrate was used instead of $CuSO_4.5H_2O$.

Yield: 65%. Dark brown solid; IR (Nujol): 3269 (N-H), 1598 cm^{-1} (C=N), 1201 (C-N), 461 (Ag-N), 343 cm^{1}(Ag-NO_3); ^{1}H NMR ($CDCl_3$): 0.9(s, 12H, 4CH3), 1.3 (m, 8H, 4CH_2-N=C), 1.3 (t, $J = 7$ Hz, 8H, 4CH_2-N=C=), 1.41 (m, 8H, 4CH_2), 2.35 (s, 12H, 4Ar-CH_3), 2.55 (t, $J = 7$ Hz, 8H, 4CH_2), 3.31 (s, 4H, 2CH_2), 4.61 (s, 8H, 4CH_2) (δ, ppm); EI-MS: m/z 1031 ([M]$^+$). Anal. Calcd. for $C_{40}H_{72}N_{12}Ag_2O_6$ (1031.72): C, 46.52 ; H, 6.99 ; N, 16.28; Ag, 20.91; O, 9.30%. Found: C, 46.48 ; H,7.12 ; N, 16.12; Ag, 20.89; O, 9.21%

3. BIOLOGY

3.1 Xanthine Oxidase Inhibition Studies:

The XO inhibitory activity was monitored spectrophotometrically (UV-1800, Japan) following the absorbance of uric acid at 292 nm [22,23]. Briefly, rat liver was excised, perfused and homogenized in 0.01 M Tris-HCl pH (8.0) containing 1 mM EDTA. The homogenate was centrifuged and the supernatant was used as a source of enzyme. It was stored at -80^o C until use and the protein content was determined by the Lowry's method [24], using bovine serum albumin (BSA) as standard.

The enzyme assay mixture consisted of 20 mM potassium phosphate buffer (pH 7.4) containing 0.3 mM EDTA and the enzyme source in a total volume of 2 mL. In dose dependent inhibition studies, the reaction was initiated by the addition of xanthine (50 µM) as the substrate to the above assay mixture and the test compounds. The absorption rate at a wavelength of 292 nm indicates the formation of uric acid at 10 min intervals at ambient temperature. Duplicate assays were repeated thrice. Allopurinol was used as positive control and DMSO was used as blank. The inhibitory activity of each test compound against XO was indicated by their IC_{50} values. The percentage inhibition of XO activity was calculated using the following formula.

$$\text{Xanthine oxidase Inhibition (\%)} = \frac{\text{Abs control - Abs sample}}{\text{Abs control}} \times 100$$

Abs control = Absorbance of the control reaction (containing all reagents except the test compound). Abs sample = Absorbance of the test compound.

3.2 ANTIOXIDANT ACTIVITY

CHEMICALS

Butylated hydroxy anisole(BHA), 1,1-diphenyl-2-picrylhydrazyl (DPPH), thiobarbituric acid (TBA), ethylenediaminetetra acetic acid (EDTA), trichloroacetic acid (TCA), deoxyribose, and ascorbic acid were obtained from M/s (Sisco Research laboratories, Mumbai, India). All reagents were of analytical grade.

BIOLOGY

3.2.1 DPPH radical scavenging assay

DPPH radical-scavenging assay was done according to Yamaguchi, Takamura, Matoba, and Treao (1998) [25]. Briefly, 1 mL of DPPH solution (0.1 mM, in 95% ethanol (v/v)) was incubated with different concentrations of the compounds. The reaction mixture was shaken

and incubated for 20 min at room temperature and the absorbance was read at 517 nm against a blank. The antioxidant BHA was used as a positive control in all the assays. The radical scavenging activity was measured as a decrease in the absorbance of DPPH and calculated using the following equation:

$$\text{Scavenging effect (\%)} = [(A_{control} - A_{sample})/A_{control}] \times 100$$

Where A= absorbance.

3.2.2 Lipid per oxidation assay

Lipid peroxidation (LPO) inhibitory activity was measured according to Kulkarni, Aradhya, and Divakar (2004) [26]. Egg lecithin (3 mg/mL in phosphate buffer, pH 7.4) was sonicated (Hielscher GmbH UP 50H ultra-challprocessor sonicator) for 30 min to obtain small membrane liposome vesicles. Different concentrations of the compounds were added to 0.5 mL of liposome mixture. Lipid peroxidation was induced by adding 10µL of 400 mM $FeCl_3$ and 10 µL of 200 mM L- ascorbic acid. After 60 min of reaction at 37 °C, the reaction was stopped by the addition of 1 mL of 0.25 N HCl containing 15% TCA and 0.375% TBA and incubation in a boiling water bath for 15 min. After centrifugation at 10,000 rpm, absorbance of the supernatant was measured at 532 nm. The scavenging effect was calculated using the equation as described for DPPH.

3.2.3 Reducing power assay

The reducing power was measured by incubating the reaction mixture (1mL) containing the samples in the phosphate buffer (0.2 M, pH 6.6) with potassium ferricynaide (1 g/100 mL water) at 50 °C for 20 min. The reaction was terminated by adding TCA (10g/100mL water), the mixture was centrifuged at 3000 rpm for 10 min and the supernatant was mixed with ferric chloride (0.1 g/100 mL of water); the absorbance was measured at 700 nm [27]. Increased absorbance of the reaction mixture indicated increased reducing power.

3.2.4 Hydroxyl radical-scavenging activity

The reaction mixture, containing compound(at different concentration) was incubated with deoxyribose (15 mM), H_2O_2 (10 mM), $FeCl_3$ (500 µM), EDTA (1 mM) and ascorbic acid (1mM) in potassium phosphate buffer (100 mM) pH 7.4 for 60 min at 37°C. The reaction was terminated by adding 1 ml TBA (1% w/v) and then heating tubes in a boiling water bath for 15 min. The contents were cooled and the absorbance of the mixture was measured at 535 nm against the reagent blank.

3.2.5 Metal ion chelating assay

The Fe^{2+} chelating ability of the extract was measured by the ferrous iron-ferrozine complex at 562nm (Decker & Welch, 1990) [28]. The reaction mixture containing $FeCl_2$ (2 mM/L) and ferrozine (5 mM/L) along with extracts was adjusted to a total volume of 0.8 mL with methanol, mixed and incubated for 10 min at room temperature. The absorbance of the mixture was read at 562nm against a blank. EDTA was used as positive control. The ability of the extract to chelate ferrous ion was calculated using the equation described for DPPH.

3.3 STATISTICAL ANALYSIS

All experiments were carried out in triplicates and repeated in three independent sets of experiments. Data are shown as means± standard deviation (SD). The SPSS10.0.5 version for windows (SPSS software Inc., USA) computer programme was used for statistical analysis. The significance of the study was assessed by one way ANOVA, followed by hoc comparison test. Correlations between quantitative properties were evaluated by calculating the Duncan and Dunnett's co-efficient. Statistical significance value was set at $p< 0.05$.

4. MOLECULAR DOCKING

The human milk xanthine oxidase (HMXO) (EC: 1.17.3.2) is mainly associated with gout disorder and subsequently for other diseases. The crystal structure of HMXO was studied with PDB: 2CKJ was built using CPH Models server 3.0. Energy computations were performed on the molecule using GROMOS 96 implementation of Swiss-PDB Viewer. Electrostatic point charges on the molecules were calculated. The structures of XOI of the current study were constructed using a public domain web server Dundee PRODRG Server [29] which optimizes the conformation of the side chains and minimizes the energy. The ligand was saved in PDB format. The minimum energy conformers of ligands were interactively docked into close proximity with the enzyme active site pocket. The possibility of binding, precise location of binding sites and mode of ligand binding was carried out using an automated docking software, Molegro Virtual Docker 2008, version 3.2.1 (Molegro ApS, Aarhus, Denmark, http://molegro.com), that is based on guided differential evolution and a force field based screening function [30]. Possible binding conformation and orientations were analyzed by clustering methods, embedded in Molegro Molecular Viewer 2008, version 1.2.0. Docking studies were carried out using the HMXO model. The enzyme was visualized using sequence option, A and B chain, salicylic acid, water, co-factors except molybdenum were deleted. The C chain as well as molybdenum co-factor was retained and subjected to docking studies. Molecular structures of the imported ligands were manually checked and corrected before docking. The initial geometry and topologies of the protein and ligand were imported to Molegro Virtual Docker. The binding site was computed within spacing such that the binding site was well sampled with a grid resolution of 0.3 Å. The ligand was docked into this grid using the MolDock Optimizer algorithm and its interactions monitored using detailed energy estimates. A maximum population of 100 and maximum interactions of 10,000 were used for each run and the 5 best poses were retained. The software Molegro Molecular Viewer 2008, version 1.2.0 (http://molegro.com) was utilized to identify hydrogen bonds and hydrophobic interactions between residues at the active site and the ligand.

5. RESULTS AND DISCUSSION

5.1 Chemistry

Aza macrocylic metal complexes were prepared by reacting 1 equivalent of tetra methyl pheneylene diamine with 4 equivalent of formaldehyde, 4 equivalents of 1, 4-diaminobutane and 2 equivalent of 2, 4-pentanedione in the presence of metallic salt. Aza macrocyclic products obtained are air stable but during the preparation of complex the reaction was affected by the molar ratio of the reactant. All the complexes were synthesized by similar method except metallic salt. The elemental analysis data of the complexes are in good agreement with theoretical values. The molar conductance of all the complexes in DMSO show that they are non-electrolytes.

The infrared spectra gave some important information regarding to the skeleton of the complexes. The important IR spectral bands of all the four complexes (3a-d) exhibit an intense band around 3270 cm^{-1} region which may be assigned to stretching v (N–H) of the coordinated secondary amino group. All the complexes show bands in the 1600 cm^{-1} region, attributed to coordinated v (C=N). A band in the 1180 cm^{-1} region is assigned to the v (C–N) vibration in all the complexes, and bands in the region 2800~2850 cm^{-1} and 1445~1455 cm^{-1} region, to v (C–H) and v (C–H) respectively. On the other hand, the complexes have no bands assignable to carbonyl group stretching modes, which indicates that the carbonyl groups are not involved in coordination bond. The coordination of nitrogen to the metal atom is supported by the appearance of a new band in the region 430~478 cm^{-1} assignable to (M-N) vibration. It confirms the involvement of nitrogen in coordination. The M-X vibrations were identified through the bands appeared in the region 320~350 cm^{-1} for all the complexes.

The magnetic moments of Ni (II) complex are a little higher than the calculated values, possibly due to ferromagnetic interactions, in accordance with other octahedral. The magnetic moment value of this complex was found 3.16 BM which indicates the octahedral environment around the metal ion. The magnetic moment of Cu (II) complex was found 1.83 BM which confirmed the distorted octahedral geometry. The Zn (II) complex is found to be diamagnetic.On the basis of elemental analyses, IR, and magnetic moment values the probable structure of complexes have been given in Fig. 1.

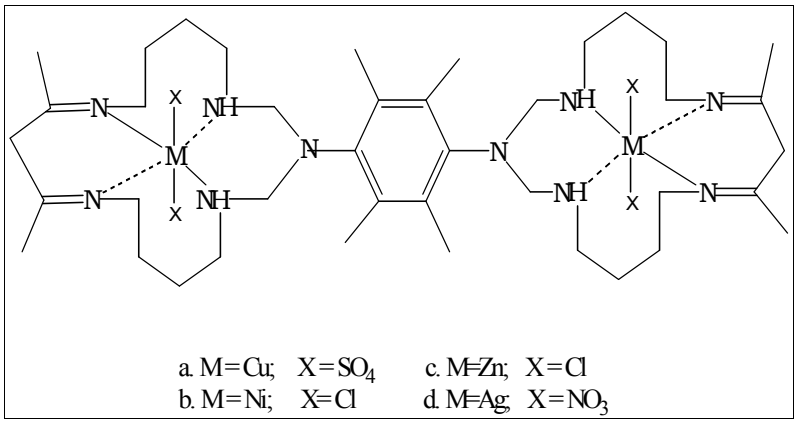
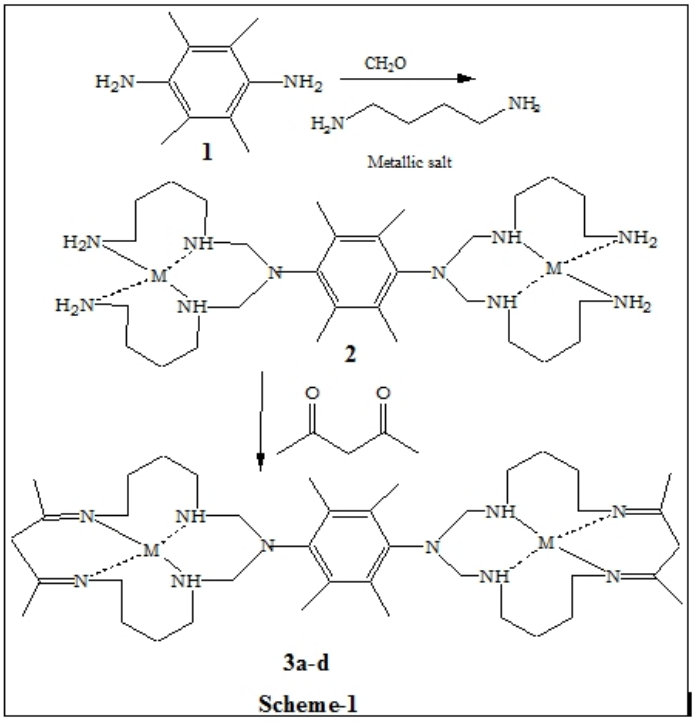


Fig. 1. Proposed structure of the synthesized complexes.

5.2 Biology

The aza macrocyclic complexes were synthesized as depicted in Scheme 1. Complex compounds were screened for the XO inhibition and antioxidant activity. The effect of different doses of metal complexes on XO in rat liver is shown in Table 1. The significant inhibition was shown by complex 3d with silver as central metal ion, followed by complex 3c with zinc, complex 3b with nickel and complex 3a with copper. These compounds showed potent inhibitory activity near to allopurinol. All the four complex compounds showed good inhibitory activity (Table 1). Complex 3d with silver as central metal ion is the most potent of all the inhibitory compounds tested, may be this is due to high soluble property of the complex in all solvents including water whereas other complexes are poorly soluble in other organic solvents and soluble in DMSO (3d > 3c > 3b >3a). Further, the inhibitory activity of the newly synthesized complex compounds (3a-d) against XO was studied using the method

of Kadam and Iyer [22] and Litwack et al. [23]. Rate of formation of uric acid from the oxidation of xanthine in presence of these inhibitors against different sources of XO was studied. The inhibitory activity of synthesized complex compounds was compared with the inhibitory activity shown by the standard inhibitor allopurinol. All the four complex compounds were tested for their ability to block the XO activity for the substrate xanthine. Present study identifies a new class of XO inhibitor. The inhibitory property of synthesized complex compounds was investigated in this work and we found that it effectively inhibited the enzymatic formation of uric acid from xanthine. The known X-ray structure of xanthine oxidoreductase bound molecule shows highly specific binding pocket presenting a long narrow cavity leading towards the Mo (IV) complex. Molybdenum protein site of both XO and xanthine dehydrogenase are structurally equivalent. Further, to understand the binding mode of newly synthesized complex compounds with XO, molecular docking studies of the compounds (3a-d) and the structure of XO (PDB entry code 2CKJ) was obtained from protein data bank. The protein was prepared by removing the ligands and substrate, however, active site of amino acids was retained [31]. It is possible that diaminobenzene nucleus bind to active site and pentanedione may bind to the peripheral site of enzyme and transfer electrons to molybdenum center (a2) electron acceptor from no site of XO. All the complex compounds were tested for their ability to block the XO activity for the substrate xanthine. The present study helps to understand structural activity relationship mode of interaction and extent of inhibition of complex compounds against rat liver (*in vitro*) and human milk XO (*in silico*).



Scheme-1: Steps for the synthesis of metal complexes with Cu (II), Ni (II), Zn (II) and Ag (I) ions.

Table 1. Comparative inhibitory activities of compounds 1-4 against Rat liver xanthine oxidase in percentage inhibition

Compounds	Rat liver
3a	15.3 %
3b	24.2 %
3c	61 %
3d	83.2 %
Allopurinol	100 %

The mode of interaction was analyzed by docking using human milk XO (PDB Code -2CKJ) on selected active site of amino acids of chain C and molybdenum. Complexes were subjected for structure activity relationship and their structure permitted various modes of interaction with active site of amino acid. Compound **3d** produced relatively better activity against HMXO (83.2% inhibition) which was also supported by docking studies (Fig. 2. and Table 2). The level of XO in the serum is significantly increased in several pathological statuses. Thus inhibition of this enzyme by complex compounds may have properties that are of therapeutic interest.

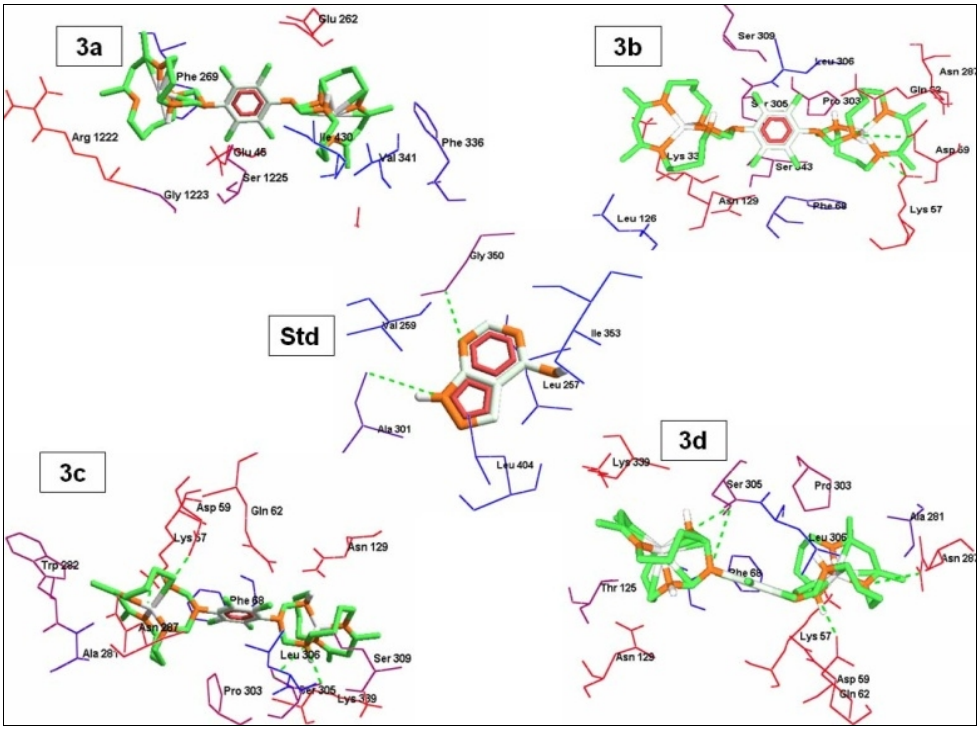


Fig. 2. Docking results of compound 3a, 3b, 3c and 3d with standard allopurinol

Table 2. Hydrogen bonding between the docked top ranked pose of potent compounds and standard against human milk XO

Sl. No	Description of the compound	Residues in H bonding	No. of H bond interaction and energy	Atomic contact energy values
1	3a	Gly 45, Gly 262	0	- 221.04
2	3b	Asp 69, Gln 60, Asn 129, Ser 343)	3 (-0.71, -0.13, -2.37)	- 241.21
3	3c	Lys 57, Asp 59, Ser 305, Leu 306)	5 (1.30, 1.33, 2.25, -2.5, -1.57)	- 265.10
4	3d	Lys 57, Asn 287, Ser 305,	4 (2.57, -0.13, -2.46, -0.90)	- 285.78
5	Std (allopurinol)	Ala 301, Gly 350	2 (-2.8, -2,2)	- 200.02

In order to evaluate the radical–scavenging ability of the complex compounds 3a-d DPPH radical scavenging assay, lipid peroxidation assay, reducing power assay, hydroxyl radical-scavenging and metal chelating activities was employed. Componds 3a-c shown very poor activity for all the above assays. Compound 3d showed to be a moderately potent DPPH radical scavenger that is known to abstract the labile hydrogen atom. It also inhibited the peroxidation of lipids. The antioxidant activity of compound 3d with IC_{50} value of 15.21 µg/mL, and synthetic antioxidant BHA was 13.81µg/mL (Fig. 3). Proton radical-scavenging action is an important attribute of antioxidants; however, hydrogen donating ability of the antioxidant molecule contributes to its free radical-scavenging nature [32].

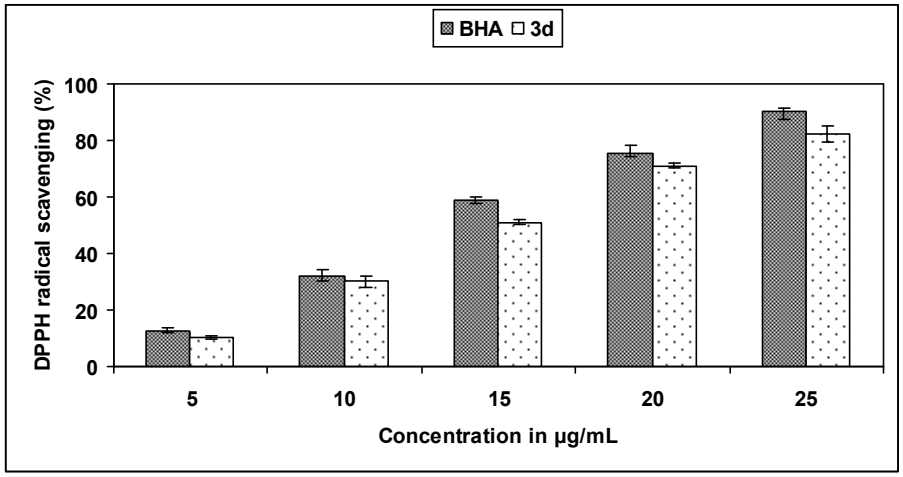


Fig. 3. DPPH scavenging assay of potent compound 3d

Initiation of peroxidation sequence in a membrane is due to abstraction of a hydrogen atom from a double bond in the fatty acid. The antioxidant activities of complex 3d and BHA with the IC_{50} value of 15.38 µg/mL and 13.72 µg/mL respectively (Fig. 4). The free radical tends to stabilize by a molecular rearrangement to produce a conjugated diene, which then readily reacts with an oxygen molecule to give a peroxy radical [33].

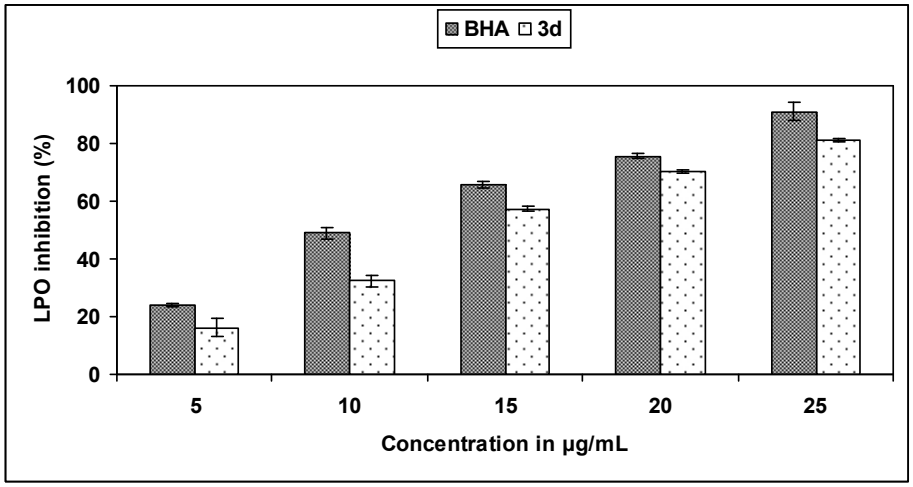


Fig. 4. Inhibition of Lipid per oxidation by potent compound 3d

It is believed that antioxidant activity and reducing power are related [34]. Reductones inhibit lipid peroxidation by donating a hydrogen atom and thereby terminating the free radical chain reaction [27]. All the compounds showed the concentration dependent activity (Fig. 5).

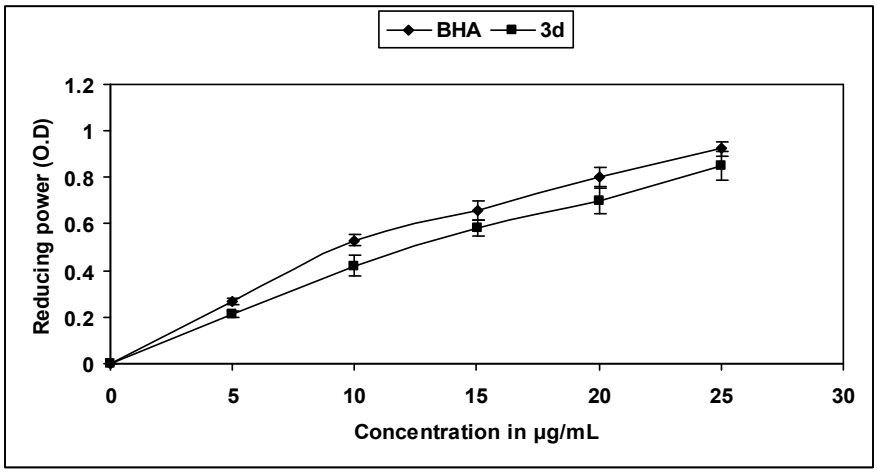


Fig. 5. Reducing power assay of potent compound 3d

Complex 3d and BHA displayed hydroxyl radical scavenging activity with IC_{50} values of 16.37µg/mL, 14.59 µg/mL respectively (Fig. 6). The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging biomolecules of the living cells [35]. Hydroxyl radical has the capacity to cause DNA strand breakage, which contributes to carcinogenesis, mutagenesis and cytotoxicity. In addition, this radical species is considered as one of the quick initiators of the LPO process, abstracting hydrogen atoms from

unsaturated fatty acids. Further, Metal chelating ability was also accessed for complex 3d and EDTA with IC_{50} values of 16.1 µg/mL, 13.97 µg/mL respectively (Fig.7).

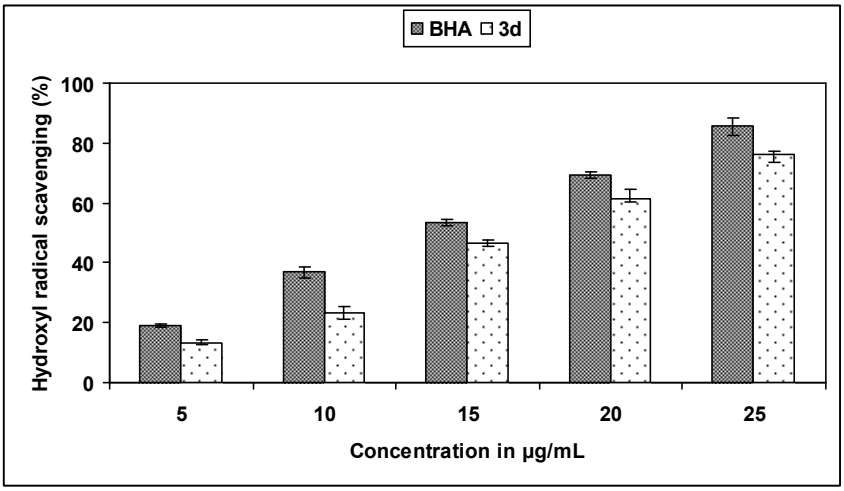


Fig. 6. Hydroxyl radical scavenging assay of potent compound 3d

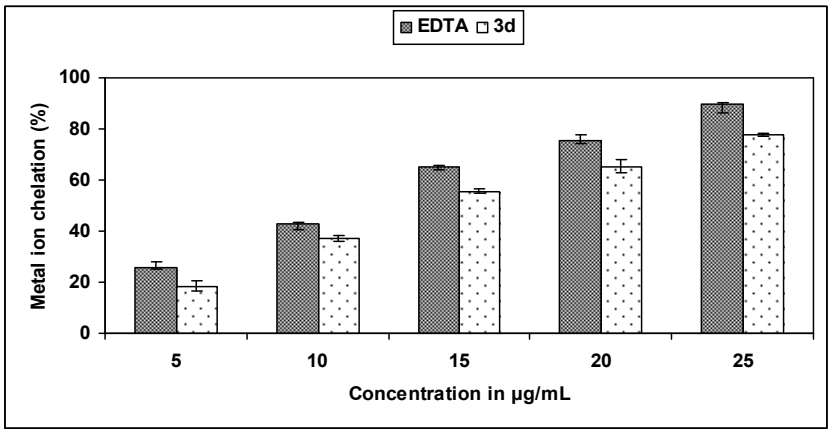


Fig. 7. Metal ion chelation assay of potent compound

6. CONCLUSION

From the results of the present study, it is concluded that, aza macrocyclic metal complexes 3a-d were synthesized and their XO inhibitory and antioxidant activities have been evaluated. Compounds 3d demonstrated potent inhibitory activities against XO and a good antioxidant. Further research in this area is in progress in our laboratory.

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COMPETING INTEREST

Authors have declared that no competing interests exist.

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