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Research paper

Novel bioinspired acetato-bridged dinuclear nickel(II)-Schiff-base complex: Catechol oxidase and *in vitro* biological activity studies

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ABSTRACT

A versatile bioinspired metallocatalyst $[Ni_2L_2(NCS)(Ac)(H_2O)_{0.5}(MeOH)_{0.5}]\cdot 1.25H_2O$ (HL = 2-((*E*)-(2-(pyridin-2-yl)ethylimino)methyl)-4-chlorophenol) has been synthesized from a Schiff-base ligand and characterized as reported earlier (Sanyal et al., 2016). It portrays catecholase activity as an oxygen dependent enzymatic radical catalysis under completely aerobic conditions (λ_{max} = 375 nm, ε = 1900 M^{-1} cm^{-1}) against the model substrate 3,5-di-*tert*-butylcatechol (3,5-DTBC). Interestingly, Michaelis-Menten analysis of pseudo first-order reaction kinetics establishes that DMF medium provides a better catalytic pathway for catecholase activity ($k_{cat} = 2.8 \times 10^{-3}$ s^{-1}) than acetonitrile (9.11×10^{-4} s^{-1}) under excess substrate conditions. Cell viability study, drug uptake assay, reactive oxygen species (ROS) formation, alteration of mitochondrial membrane potential (MMP), apoptosis study and DNA fragmentation demonstrates a significant dose dependent anti-leukemic activity on KG-1A (AML) and K562 (CML) cell lines. Notably, outstanding anti-bacterial property was also observed on multi-drug resistant *E. coli* and *S. aureus* bacteria.

1. Introduction

Catechol oxidase (CO), often referred as *o*-diphenol oxidase or polyphenol oxidase, catalyzes the two-electron-transfer reaction during the oxidation of *o*-diphenols to their corresponding *o*-quinones by O_2 (Scheme 1), which autopolymerize to form brown polyphenolic catechol melanins, a defence mechanism adopted in damaged plants and crustaceans [1,2]. Notably the sharp selectivity of this reaction is of profound significance in medical diagnoses [3]. Bioinorganic chemists have accepted this as an intellectual challenge to reproduce its structure and function through "artificial models", of course with special emphasis on bimetallic systems comprising Cu^{II} [4–6], Ni^{II} [7–13], Zn^{II} [14,15], Mn^{II} [16,17] and Mn^{IV} [18]. Since it is undoubtedly one of the highly evolved enzymatic systems of the nature in terms of acceleration over the uncatalyzed reaction, there remains many fascinating-cum-untouched arenas in its actual mechanistic pathway despite fine-tuning of crucial factors like metal–metal distance, geometry [19], steric [20], electronic [21] and bridging ligand features [22], redox potentials [23], pH [24] and solvent property [25,26].

Cancer, an umbrella term for numerous distinctive diseases, is characterized by abnormal cell growth resulting from spontaneous, inherited, or environmentally induced genetic mutations [27]. In recent times combating cancer involves multi-targeted or drug-combination regimes which can effectively differentiate between cancer cells, cancerous tumours, normal cells and normal tissues [28]. Although Pt-complexes such as *cis*diamminedichloroplatinum(II) (CDDP), carboplatin, oxaliplatin and regionally approved drugs nedaplatin, heptaplatin and lobaplatin [29], have been considered as the most productive chemotherapeutic agent after the failure of classical organodrugs, recent studies reveal significant tumour resistance [30] as well as nephro-, gastrointestinal and hematological toxicity [31] to a serious extent. Instead, constructive efforts have been directed towards developing alternative non-platinum based metal-complexes that are surprisingly reactive against cancer cells *in vitro* with excellent specificity in action and mechanism. An appreciatively wide spectrum of contrasting bioactivity among complexes of various transition and non-transition metal ions like Zn^{II} [32], Ru [33], Os [34], Au [35], Ni^{II} [36], Cu^{II} [37], Fe^{II} [38], Ga^{III} [39], Rh^{III} [40], V^{V} [41] is an unambiguous depiction of this fact. In due course, exciting data were in hand in cancer therapy and anti-inflammatory, anti-infective and antidiabetic functionalities [42]. Along these lines, our group has currently doc-

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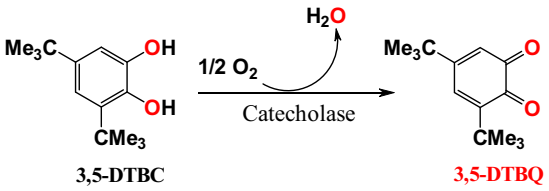

Scheme 1. General schematic representation of catecholase activity.

umented a substantial array of fruitful results exploiting Schiff-base [10,32,43] and Mannich-base ligands [15].

At this juncture, nickel(II), a cofactor of urease and also carbon-monoxide dehydrogenase in *Clostridium pasteurianum*, has emerged as a fundamental alternative owing to several physiological properties favourable for rational anticancer drug design and *in vitro* biological applications [44]. The exciting anti-bacterial properties which are not associated with drug-related side-effects, as compared to analogous complexes of other metal-ions, suggests effective penetration in microbial cells coupled up with killing action via enzyme deactivation [45]. Following these promising results, we had synthesized and characterized a Ni^{II} metallohydrolase from a Schiff-base ligand as described by us [46]. In this work we have intensively explored its biocatalytic promiscuity in terms of catechol oxidase property and bioactivity. The solvent effect perspective of the catecholase-like activity has been discussed against 3,5-di-*tert*-butylcatechol (3,5-DTBC) in acetonitrile and DMF to corroborate with our previous report of phosphatase activity [46]. Cell viability studies highlights potent anti-leukemic activity towards KG-1A (AML) and K562 (CML) cell lines. Finally the anti-bacterial property was investigated upon multi-drug resistant *E. coli* and *S. aureus* bacteria to illuminate the versatility of nickel biology.

2. Experimental section

2.1. Materials and methods

All reagents were of the highest grade commercially available and were used without further purification. 3,5-DTBC, Histopaque 1077, Rhodamine B, RPMI 1640, penicillin, streptomycin, doxorubicin were purchased from Sigma. Fetal bovine serum (FBS) was purchased from GIBCO/Invitrogen. Sodium chloride (NaCl), sodium carbonate (Na_2CO_3), sucrose, Hanks balanced salt solution (HBSS), HEPES–Na^+ buffer, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenil-tetrazolium bromide (MTT) were purchased from Himedia. Tris–HCl, Tris buffer, KH_2PO_4, K_2HPO_4, HCl, formaldehyde, alcohol, Titron X-100, Sodium dodecyl sulphate (SDS), phenol, chloroform, iso-amyl alcohol, ethidium bromide (EtBr), 2-vinylpyridine and other chemicals were procured from Merck and SRL Pvt. Ltd. Ultrapure Milli Q water was used throughout.

Elemental analyses (carbon, hydrogen and nitrogen) were performed using a Perkin-Elmer 240C analyzer. Electronic spectra (200–600 nm) were obtained at 25 °C using a Shimadzu UV-3101 PC. EPR experiments were performed at liquid nitrogen temperature (77 K) in acetonitrile using a JEOL JES-FA200 spectrometer at X band (9.13 GHz). Nikon Eclipse LV100POL was used for fluorescence microscopy and Hitachi F-7000 Fluorescence Spectrophotometer for spectroflurometry. The optical density (OD) was measured on ELISA reader, BIO-RAD, Model 550, Tokyo, Japan.

2.2. Synthesis of ligand and metal-complex

The ligand HL and its Ni^{II} complex was synthesized by procedures reported in literature [46].

Elemental analysis calcd (%) for $C_{31.50}H_{34.50}Cl_2N_5Ni_2O_{7.25}S$: C 46.17, H 4.24, N 8.55, O 14.16, S 3.91, Cl 8.65, Ni 14.32; found C 46.21, H 4.33, N 8.49, O 14.10, S 3.85, Cl 8.60, Ni 14.42.

2.3. Detection of hydrogen peroxide in the catalytic reaction involving catecholase activity

The formation of H_2O_2 during the catalytic reaction was detected by following the development of the characteristic band for I_3^- spectrophotometrically (λ_{max} = 353 nm; ε = 26000 M^{-1} cm^{-1}), upon reaction with I^- [9].

2.4. Cell lines and bacteria culture and maintenance

KG-1A and K562 cell lines were obtained from NCCS, Pune (India). *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) bacteria were previously isolated [47]. The strains (MC 4 – *E. coli* and MMC 20 – *S. aureus*) were multi-drug resistant and were sub-cultured to be used throughout the study.

2.5. Collection of blood and separation of lymphocytes

Blood samples were collected from healthy human volunteers by vein-puncture in 5 ml heparin coated Vacutainers satisfying the method of Hudson and Hay [48a] and approved by Institutional ethics committee (IEC) of Vidyasagar University. The inclusion and exclusion criteria for subject selection was done as per our previous report [48b].

2.6. Drug preparation

A 0.012 (M) stock of complex was prepared. It was then serially diluted with RPMI media to prepare working concentrations. The amount of DMSO for each concentration was never exceeded 0.75%.

2.7. Anti-cancer activity

2.7.1. Experimental design

Each of the 9 group of cells contained 6 petri dishes (2×10^5 cells in each). The cells of each petri dish of control and experimental groups were maintained in RPMI 1640 media supplemented with 10% FBS, 50 μg ml^{-1} gentamycin, 50 μg ml^{-1} penicillin and 50 μg ml^{-1} streptomycin at 37 °C in a 95% air/5% CO_2 atmosphere in CO_2 incubator. KG-1A and K562 cells were treated with various concentrations [0–0.0001 (M)] of complex for 24 h.

The nest treatment protocols were done as reported in literature [49,50]. Intact cells were used for the determination of ROS, mitochondrial membrane potential and different microscopic observations.

2.7.2. In vitro cell proliferation assay

The cytotoxicity of complex was quantitatively estimated by a non-radioactive colorimetric assay system using tetrazolium salt, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenil-tetrazolium bromide (MTT).

2.7.3. In vitro drug uptake assay

Rhodamine B tagged complex at IC_{50} conc. were incubated for 6 h at 37 °C in a 95% air/5% CO_2 atmosphere in CO_2 incubator. Images were acquired at 50× optical zoom and Image J software v.r. 1.43 (NIH) was applied for analysis.

2.7.4. Intracellular ROS measurement

It was performed by following the reported methods [51].