Copper complexes with a flexible piperazinyl arm: nuclearity driven catecholase activity and interactions with biomolecules

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ABSTRACT
Three new Cu(II) complexes, [Cu(HL1)(pyridine)(H2O)][ClO4]2·2MeOH (1), [Cu2(HL1)2(NO3)2]·3H2O (2) and [Cu(HL2)(NO3)2]·MeCN (3), have been synthesized from two Schiff base ligands [HL1 = 1-phenyl-3-((2-(piperazin-4-yl)ethyl)imino)but-1-en-1-ol and HL2 = 4-((2-(piperazin-1-yl)ethyl)imino)pent-2-en-2-ol] using the chair conformer of a flexible piperazinyl moiety. Structural analysis reveals that 1 and 3 are monomeric Cu(II) complexes consisting of five- and six-coordinate Cu(II), respectively, whereas 2 is a dinuclear Cu(II) complex consisting of two different Cu(II) centers, one square planar with the other distorted octahedral. Screening tests were conducted to quantify the binding of 1–3 towards DNA and BSA as well as the DNA cleavage activity of these complexes using gel electrophoresis. Enzyme kinetic studies were also performed for the complexes mimicking catecholase-like activities. Antibacterial activities of these complexes were also examined towards Methicillin-Resistant Staphylococcus aureus bacteria. The results reflect that 2 is more active than the monomeric complexes, which is further corroborated by density functional theory study.

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1. Introduction

Copper is a bio-essential element [1] but its biological importance is only explored with development of bioinorganic chemistry and successful studies of interaction of model complexes with various bio-macromolecules [2–5]. Copper-based enzymes capable of binding molecular oxygen at ambient conditions have received attention to develop biologically active model systems which are able to oxidize catechol to its corresponding diquinones following an enzyme catalysis pathway [6]. These oxidation reactions also have a role in determination of the hormonally active catecholamines: adrenaline, noradrenaline, and L-dopa [7, 8]. Apart from studying biological processes induced by copper, many new molecules have been developed showing antibacterial, antifungal, antimicrobial and anticancer/antiproliferative activity where the copper center performs a pivotal role in terms of structural organization and overall functionality [9–12]. Interactions of copper complexes with DNA and proteins are key research areas for development of new therapeutic agents particularly showing anti-tumor properties and the possibility of transporting these molecules through the physiological system via protein binding [13–15]. As copper complexes are capable of binding and cleaving DNA selectively under physiological conditions without an external reagent, metal-based pseudonucleases generate a new era in nucleic acid chemistry for footprinting, sequence-specific binding to nucleic acids, and as new structural probes and therapeutic agents [16, 17].

We reported two new Schiff bases, HL1 and HL2 [HL1 = 1-phenyl-3-((2-(piperazin-4-yl)ethyl)imino)but-1-en-1-ol and HL2 = 4-((2-(piperazin-1-yl)ethyl)imino)pent-2-en-2-ol] with a flexible piperazinyl ring which can take either chair or boat form during complexation [18]. During complexation with nickel ion, both ligands prefer the boat form of flexible piperazinyl moiety to act as a tetradentate ligand to fulfill the square planar geometry surrounding nickel. The complexes showed interesting DNA and protein binding activities and quite high catalytic activities towards catechol oxidation. However, we were unable to synthesize any nickel complex of the same ligands where the piperazinyl ring had the chair form. To explore the probable influence of the ligand on similar properties when the piperazinyl ring is preferentially chair conformation (where the ligand backbone can have profound influence on catecholase-like activity) [19], we have reacted those Schiff base ligands with copper salts to obtain two mononuclear and one dinuclear copper complexes, [Cu(HL1)(pyridine)(H2O)](ClO4)2·2MeOH (1), [Cu2(HL1)2(NO3)2](NO3)2·3H2O (2), and [Cu(HL2)(NO3)2]·MeCN (3). Interaction of 1–3 with DNA applying both binding and cleavage experiments are carried out. Affinity of complexes towards BSA protein is also determined. Moreover, complexes were also investigated for possible catecholase-like activity. The Schiff bases and copper(II) complexes were screened for their antibacterial activity against Methicillin-Resistant nosocomial Gram-positive bacteria Staphylococcus aureus (MRSA). The experimental results indicate that dinuclear 2 is more active than 1 and 3 towards DNA binding and cleavage, BSA binding and catechol oxidation. Furthermore 2 has considerable activity towards S. aureus as a promising new compound, exhibiting higher antimicrobial activity than reported copper complexes [20, 21]. Computational studies for DNA binding and catechol oxidation also reflect the similar trend of activity order among the Cu(II) complexes as we found experimentally.

2. Experimental

2.1. Materials and methods

Chemical reagents were purchased from Sigma and used without purification. Infrared spectra (4000–500 cm⁻¹) were recorded with a BRUKER TENSOR 27 instrument in KBr pellets. NMR spectra were recorded on an AVANCE III 400 Ascend Bruker BioSpin machine at ambient temperature. Mass spectrometric analyses were done on a Bruker-Daltonics, microTOF-Q II mass spectrometer and elemental analyses were carried out with a ThermoFlash 2000 elemental analyzer. Spectrophotometric measurements were performed on a Varian UV–vis spectrophotometer (Model: Cary 100) (for absorption) and Fluoromax-4p Spectrofluorometer from Horiba Jobin Yvon (Model: FM-100) (for emission) using a quartz cuvette with path length of 1 cm. HL1 and HL2 were prepared following reported methods [18].
Caution! Perchlorate compounds are potentially explosive. Only a small amount of material should be prepared and handled with care.

2.2. X-ray crystallography

Single crystal X-ray structural studies of 1–3 were performed on a CCD Agilent Technologies (Oxford Diffraction) SUPER NOVA diffractometer. Data for 1–3 were collected at 150(2) K using graphite-monochromated Mo Kα radiation (λ = 0.71073 Å). The strategy for the data collection was evaluated by CrysAlisPro CCD software. The data were collected by the standard ‘phi-omega scan techniques and were scaled and reduced using CrysAlisPro RED software. The structures were solved by direct methods using SHELXS-97 and refined by full matrix least-squares with SHELXL-97, refining on F² [22]. The positions of all atoms were obtained by direct methods. All non-hydrogen atoms were refined anisotropically. The remaining hydrogens were placed in geometrically constrained positions and refined with isotropic temperature factors, generally 1.2 Ueq of their parent atoms. The crystal and refinement data are summarized in table 1.

2.3. Synthesis of [Cu(HL1)(pyridine)(H2O)](ClO4)2·2MeOH (1)

Methanolic solution (15 mL) containing HL1 (0.136 g, 0.5 mmol) and Cu(ClO4)2·6H2O (0.185 g, 0.5 mmol) was stirred at room temp for 1 h after adding one drop of pyridine; the resulting sky blue solution was concentrated by evaporating the solvent. Finally after two or three days, red needle-shaped crystals were obtained from the reaction mixture after layering the mother liquor with diethyl ether. Yield: 78%. Anal. Calcd (%): C23H38Cl2CuN4O12 C, 39.69; H, 5.50; N, 8.05. Found (%): C, 39.71; H, 5.11; N, 8.32.

Table 1. Crystallographic data and structure refinement parameters for 1–3.

| Complex | 1        | 2        | 3        |
|---------|----------|----------|----------|
| Empirical formula | C23H38Cl2CuN4O12 | C32H44Cu2N10O16 | C13H23CuN6O7 |
| Formula weight | 687.94 | 951.85 | 438.91 |
| Crystal system | Monoclinic | Monoclinic | Monoclinic |
| Space group | P 21/c | 2/a | P 21/n |
| a (Å) | 12.8829(4) | 9.9050(2) | 10.5658(2) |
| b (Å) | 9.4603(2) | 16.6715(3) | 14.6046(2) |
| c (Å) | 25.0205(5) | 52.6457(10) | 12.5972(2) |
| α (°) | 90 | 90 | 90 |
| β (°) | 90.087(3) | 94.498(2) | 94.980(2) |
| γ (°) | 90 | 90 | 90 |
| V (Å³) | 3011.13(13) | 8666.7(3) | 1936.53(5) |
| μ (mm⁻¹) | 0.71073 | 1.5418 | 0.71073 |
| ρ (mg m⁻³) | 1.518 | 1.459 | 1.505 |
| Z | 4 | 8 | 4 |
| T (K) | 150(2) | 150(2) | 150(2) |
| F (0 0 0) | 1416 | 3936 | 912 |
| Crystal size (mm³) | 0.23 × 0.16 × 0.13 | 0.23 × 0.18 × 0.13 | 0.33 × 0.26 × 0.21 |
| θ ranges (°) | 3.01–25.00 | 3.37–50.00 | 2.98–25.00 |
| h/k/l | −1515/−1011/−2929 | −98/−1616/−5252 | −1212/−1717/−1414 |
| Reflections collected | 23,960 | 19,630 | 15,337 |
| Independent reflections | 5289 | 4457 | 3406 |
| T min and T max | 0.8845 and 0.8080 | 0.7915 and 0.6708 | 0.7904 and 0.6978 |
| Data/restraints/parameters | 5289/2/388 | 4457/0/556 | 3406/0/251 |
| Goodness-of-fit (GOF) on F² | 1.088 | 1.056 | 1.038 |
| Final R indices | R1 = 0.0537 | R1 = 0.0782 | R1 = 0.0633 |
| Final R indices | wR2 = 0.1441 | wR2 = 0.2254 | wR2 = 0.1751 |
| R indices (all data) | R1 = 0.0628 | R1 = 0.0831 | R1 = 0.0685 |
| R indices (all data) | wR2 = 0.1517 | wR2 = 0.2329 | wR2 = 0.1805 |
| Largest peak and hole (e Å⁻³) | 0.714 and −0.517 | 1.601 and −0.599 | 2.870 and −1.378 |


\[ \text{[C}_{16}\text{H}_{22}\text{CuN}_{3}\text{O}]^{+} \ (m/z) \text{ calculated} = 335.11 \ (m)^{+}; \text{ obtained} = 335.11 \ (m)^{+} \]. Selected IR on KBr \((\nu/\text{cm}^{-1})\): 1597 \((–\text{C=N})\), 1097 \((\text{ClO}^-)\).

### 2.4. Synthesis of \([\text{Cu}_2(\text{HL}^1)_2(\text{NO}_3)_2]_2\text{H}_2\text{O} \ (2)\)

Methanolic solution (5 mL) containing HL\(^1\) (0.136 g, 0.5 mmol) was added dropwise to a 10 mL solution of Cu(NO\(_3\))\(_2\)_3H\(_2\)O (0.120 g, 0.5 mmol) and the resultant mixture was stirred at room temperature for 1 h; the resulting solution was concentrated by evaporating the solvent. Layering of the reaction mixture with diethyl ether furnished suitable crystal after a few days. Yield: 82\%. Anal. Calcd (%): C\(_{32}\)H\(_{46}\)Cu\(_2\)N\(_{10}\)O\(_{14}\) C, 41.69; H, 5.03; N, 15.19. Found (%): C, 41.71; H, 5.12; N, 15.15. \([\text{C}_{32}\text{H}_{44}\text{Cu}_{2}\text{N}_{7}\text{O}_{5}]^{+} \ (m/z) \text{ calculated} = 732.20 \ (m)^{+}; \text{ obtained} = 732.17 \ (m)^{+} \]. Selected IR on KBr \((\nu/\text{cm}^{-1})\): 1605 \((–\text{C=N})\), 1342 \((\text{NO}_3^-)\).

### 2.5. Synthesis of \([\text{Cu}(\text{HL}^2)(\text{NO}_3)_2]\cdot\text{MeCN} \ (3)\)

At first, 15 mL of methanolic solution containing HL\(^2\) (0.105 g, 0.5 mmol) and Cu(NO\(_3\))\(_2\)_3H\(_2\)O (0.120 g, 0.5 mmol) was stirred at room temperature for 1 h. After evaporating the solvent, the sticky compound obtained was dissolved in MeCN. After six or seven days, green block-shaped crystals of 3 were obtained from this solution after layering with diethyl ether. Yield: 55\%. Anal. Calcd (%): C\(_{13}\)H\(_{24}\)CuN\(_6\)O\(_7\) C, 35.49; H, 5.50; N, 19.10. Found (%): C, 35.12; H, 5.59; N, 18.91. \([\text{C}_{11}\text{H}_{20}\text{CuN}_{3}\text{O}]^{+} \ (m/z) \text{ calculated} = 273.09 \ (m)^{+}; \text{ obtained} = 273.08 \ (m)^{+} \]. Selected IR on KBr \((\nu/\text{cm}^{-1})\): 1605 \((–\text{C=N})\), 1342 \((\text{NO}_3^-)\).

### 2.6. DNA binding study

DNA binding for 1–3 is measured by a fluorescence spectral technique using ETBr displacement assay from ETBr bound CT-DNA in Tris-HCl buffer at pH 7.4. The changes in fluorescence intensities at 605 nm (520 nm excitation) of ETBr (20 \(μM\)) bound to DNA were measured with respect to concentration of the complex (0–100 \(μM\)). ETBr was non-emissive in Tris-HCl buffer solution (pH 7.4) due to fluorescence quenching of the free ETBr by solvent.

### 2.7. DNA cleavage experiments

In each experiment, the extent of DNA cleavage was monitored by agarose gel electrophoresis. A solution of 25 \(μL\) containing pBR322 DNA (0.25 \(μg \mu L^{-1}\)), the metal complexes (50–400 \(μM\)), and H\(_2\)O\(_2\) (60 \(μM\)) was incubated for 1 h at 40 °C. Subsequently, 2 \(μL\) of 6X DNA loading buffer containing 0.25% bromophenol blue, 0.25% xylene cyanol, and 60% glycerol was added to the reaction mixture and loaded onto a 1% agarose gel containing 1.0 mg mL\(^{-1}\) of ethidium bromide. The electrophoresis was performed at 65 V for 1.5 h in a TAE buffer. The bands were visualized under UV light and photographed. The cleavage efficiencies were measured by determination of the ability of each complex to convert the supercoiled DNA (SC) to the nicked circular form (NC). After electrophoresis, the proportion of DNA in each fraction was estimated quantitatively on the basis of the band intensities using the BIORAD Gel Documentation System. The intensity of each band relative to that of the plasmid SC form was multiplied by 1.43 to take account of the reduced affinity for ethidium bromide [23].

### 2.8. Protein binding study

The binding interactions of 1–3 with BSA protein were carried out using standard Trp fluorescence with excitation at 295 nm and the corresponding emission at 340 nm using a Fluoromax-4p Spectrofluorometer [from Horiba Jobin Yvon (Model: FM-100)] with a rectangular quartz cuvette of 1 cm path length. A stock solution of BSA protein was prepared in TRIS-HCl buffer (pH ~7.4). Concentrated stock solutions of these complexes were prepared by dissolving them separately in TRIS-HCl buffer and
diluted suitably with TRIS-HCl buffer to get the required concentrations. An aqueous solution (2 mL) of BSA (10 μM) was titrated by successive additions of the respective complexes (0–100 μM).

2.9. Catecholase activity study

35-Di-tertbutylcatechol (35-DTBC) (100 equivalent) in methanol were added to 10⁻⁴ M solutions of 1–3 in methanol under aerobic conditions. Absorbances of the resultant reaction mixtures were plotted with respect to wavelength at 10 min intervals in a spectrophotometer from 300 to 500 nm. The dependence of the rate on various concentrations and kinetic parameters were obtained by treatment of a 10⁻⁴ M solution of different complexes with 20–500 equivalents of substrate and monitoring the increase in absorbance at 402 nm (the peak corresponding to the quinone band maximum) as a function of time.

2.10. Detection of hydrogen peroxide in the catalytic reactions

Modification of the iodometric method is employed to detect H₂O₂ during the catalytic reaction. Reaction mixtures were prepared as in the kinetic experiments. After 1 h of reaction, an equal volume of water was added to extract the formed quinone using dichloromethane. The aqueous layer was acidified with H₂SO₄ to pH ≈ 2 to stop further oxidation, and 1 mL of a 10% solution of KI and three drops of 3% solution of ammonium molybdate were added. In the presence of hydrogen peroxide I⁻ is oxidized to I₂⁻, H₂O₂ + 2I⁻ + 2H⁺ → 2H₂O + I₂, and with an excess of iodide ions, the tri-iodide ion is formed according to the reaction I₂(aq) + 2I⁻ → I₃⁻. The reaction rate is slow but increases with increasing concentrations of acid, and the addition of an ammonium molybdate solution condenses the reaction almost immediately. The formation of I₃⁻ could be monitored by UV–vis spectroscopy due to the development of the characteristic I₃⁻ band (λ = 353 nm, ε = 26,000 M⁻¹ cm⁻¹).

2.11. Antimicrobial study

2.11.1. Bacterial strains

The reference strain MRSA ATCC® 43300™ was used in this study.

2.11.2. Growth conditions

The bacterial isolates were streaked from −80 °C stocks into Mueller-Hinton agar (MHA) (Oxoid Ltd, Basingstoke, UK) plates and incubated overnight at 37 °C. From this streaking, one isolated colony was inoculated into 5 mL of Mueller-Hinton broth and incubated for a further 18 h at 37 °C with shaking (approx. 220 rpm).

Ligands and complexes were incorporated in the wells and incubated at 37 °C for 18 h. After the incubation period, we measured the diameter of the inhibition zone generated by each compound using an antibiogram zone measuring scale (figure S1). From the results obtained, HL₁ and 2 were selected for further experiments.

2.12. Antibacterial activity

The antibacterial activity was evaluated by determining the minimum inhibitory concentration (MIC) of the compounds using the broth microdilution method by following the CLSI guidelines [24]. The MIC, calculated by the dilution factor of the starting concentration, was considered at the concentration of compound where no visible growth was observable. Wells having no growth were plated (5 μL) into MHA-plates and incubated overnight at 37 °C. On the following day, the results were recorded and the minimum concentration of compound with no growth obtained was considered as the minimum bactericidal concentration (MBC). All experiments were carried out in triplicate in three separate occasions.
3. Results and discussion

3.1. Syntheses of the complexes

HL₁ and HL₂ are obtained by simple condensation using our previously reported methodology [18] with sufficient purity and yield for use without further purification in the synthesis of 1–3, according to scheme 1. Both the ligands have been characterized by ¹H and ¹³C NMR and ESI-MS spectroscopy. All the complexes were characterized by IR and ESI-MS spectroscopy, elemental analysis and single crystal X-ray crystallography. The characteristic band around 1600 cm⁻¹ in IR spectra of all three complexes are assigned for v(C=N) stretch [25]. A strong band around 1100 cm⁻¹ in 1 indicates the presence of perchlorate [25]. Nitrate stretching is observed at 1350–1385 cm⁻¹ in IR spectra of both 2 and 3. ESI-MS spectra of 1 and 2 show molecular ion peak at 335 for [Cu(L₁)]⁺ and 732 for [Cu₂(L₁)₂(NO₃)]⁺ (figure S2). Thus solution state ESI-MS spectra of 2 clearly indicate dimeric species in solution. Similarly 3 shows molecular ion peak at 273 corresponding to [Cu(L₂)]⁺ (figure S2). In all three cases, mono positive molecular ion is generated during ESI-MS experiment due to formation of mono anionic Schiff base ligands.

The electronic spectra of 1–3 (figure S2A) have been studied in CH₃OH showing absorptions in 250–682 nm ranges [1: 256, 682 nm; 2: 250, 291, 336, 617 nm; 3: 250, 298, 633 nm]. The transition around 600–700 nm corresponds to a d–d transition of copper(II) and the band around 300 nm is intramolecular LMCT transition [26]. In 1 and 3, d–d transitions are observed at 680 and 630 nm, respectively, which indicate five-coordinate and six-coordinate structures [27, 28] but in 2, there was a broad band from 530 to 720 nm which may be due to two different types of Cu(II) centers (four-coordinate and six-coordinate) present in solution [29].

Scheme 1. Formation of mononuclear and dinuclear metal complexes.
3.2. Crystal structure of 1–3

Crystal structures of these three complexes portray Cu(II) centered coordination geometries of four (2), five (1), and six (2 and 3) formed via almost similar reactions. The major coordination bond lengths between Cu and N/O-donors are 1.900(5)–2.423(3) Å (table S1), matching earlier report [30]. Exceptions are found in Cu(1)–O(8) and Cu(1)–O(4) in 2 and Cu(1)–O(3) in 3, which are mainly caused by Jahn–Teller distortion as mentioned in previous reports [31–33].

1 crystallizes in the space group P 21/C with copper coordinated to the tridentate HL¹, one pyridine and one water to give five-coordinate square-pyramidal geometry (figure 1). N, N, O donors of HL¹ and pyridine are on the equatorial face of the square pyramidal geometry while water lies on the axial position. The piperazinyl ring takes the chair conformation where secondary nitrogen has an extra proton and does not coordinate. Distortion of the coordination geometry of five-coordinate systems can be calculated by the $\tau_5$ value, to describe the degree of distortion for square-pyramid and trigonal-bipyramid [square pyramid, $\tau_5 = 0$; trigonal-bipyramid, $\tau_5 = 1$; $\tau = (\beta - \alpha)/60^\circ$, $\alpha$ and $\beta$ are the two largest angles around the central atom] [34]. The $\tau_5$ value for 1 is 0.101, indicating a distorted square-pyramidal geometry adopted by copper. In 1, the average coordination bond angle around the Cu center of square pyramidal geometry is 92° whereas the least bond angle was observed for N(1)–Cu(1)–N(2) (~83°) (table S1) due to the formation of a chelated five-membered ring. Molecules are connected into a 1-D supramolecular chain (figure 1) via formation of hydrogen bonds (viz. C4–H4⋯O666 and N3–H3⋯O888) with perchlorate. Each 1-D chain is further connected via C10–H10B⋯O111 and C14–H14B⋯O444 hydrogen bonds to build a 2-D sheet-like structure (figure 1). These parallel 2-D sheets are also interconnected through C10–H10B⋯O111 and C14–H14B⋯O444 to build a 3-D structure (figure 1).

Complex 2 is binuclear with space group I 2/a with both copper centers having different coordination number and geometry. Cu(1) is six-coordinate distorted octahedral geometry while Cu(2)
is four coordinate with square planar geometry (figure 2). This geometry of Cu(II) is further proved by $\tau_4$ index which defines the distortion between a perfect tetrahedron ($\tau_4 = 1$) and a perfect square planar geometry ($\tau_4 = 0$) using the formula: $\tau_4 = ([360° - (\alpha + \beta)]/141°$, with $\alpha$ and $\beta$ (in °) being the two largest angles around the central metal in the complex [35]. The $\tau_4$ value for Cu(1) is 0.190, satisfying a somewhat distorted square planar geometry for this metal center. This is an unusual example of metal complex where two different Cu(II) centers exist, but similar reports are available in the literature [31]. The average coordination bond angle around Cu(1) of distorted octahedral geometry is 93°. Two trans angles O(4)–Cu(1)–O(8) (129.27°) and O(4)–Cu(1)–N(2) (134.57°) at Cu(1) deviate from the linearity of a perfect octahedron. As a consequence, Cu(1) has a considerably distorted octahedral geometry, which can be attributed to the chelating influence of nitrate [36]. For 2, individual molecules connect by hydrogen bonding (viz. C6–H6B⋯O4, C1–H1B⋯O4, C21–H21B⋯O3, C19–H19A⋯O2, C22–H22A⋯O2, C22–H22A⋯O8, C20–H20B⋯O8, C20–H20B⋯O7, C17–H17B⋯O6 and C18–H18A⋯O6) to build a 1-D chain (figure 2). Each 1-D chain is further connected by more hydrogen bonding (viz. N6–H6⋯O222, C17–H17A⋯O111, C19–H19B⋯O111, C4–H4A⋯O333, C3–H3B⋯O333, and N3–H3⋯O333) with non-coordinated nitrate to build a railway track-like 2-D supramolecular network (figure 2). Now 2-D network is further expanded through C6–H6A⋯O3 hydrogen bonding to build a 3-D supramolecular system (figure 2).

The structure determination of 3, crystallized in space group P 21/n, reveals that it has six-coordinate copper with distorted octahedral geometry. The average coordination bond angles are similar to Cu(1) of 2. Similarly one trans angle O(3)–Cu(1)–O(5) (137.06°) deviates from linearity to generate distorted octahedral geometry (figure S3). Hydrogen bonding networks, N3–N1H⋯O1, N3–N1H⋯O7, C2–H2A⋯O4, and C3–H3B⋯O2, play an important role in creating a 1-D zig-zag supramolecular network (figure S3).

Figure 2. Dinuclear unit of 2 and its 1-D, 2-D, and 3-D supramolecular polymeric networks.
1-D chains are joined via C6–H6A⋯O4 and C7–H7A⋯O3 hydrogen bonds to generate a 2-D sheet-like structure (figure S3).

### 3.3. Complex-DNA interaction studies

Copper-Schiff base complexes are well known for their interaction with DNA [37–40]. The fluorescence quenching spectra of DNA-bound ETBr by 1–3 are shown in figure S4. Reduction in fluorescence intensity at 607 nm in all the spectra indicates that ETBr molecules get displaced from DNA by the addition of copper complexes. Though the displacement is not large, it lies in the range of earlier report [41]. Furthermore, $K_{SV}$ values for 1–3 are $6.2 \times 10^2$, $1.1 \times 10^3$, and $7.7 \times 10^2$ M$^{-1}$, respectively, which were obtained from the classical Stern–Volmer equation [14],

$$\frac{F_0}{F} = 1 + K_{SV}[Q]$$

where $F_0$ and $F$ are the fluorescence intensity of the ETBr – CT DNA adduct before and after addition of complexes, $K_{SV}$ is the Stern–Volmer constant, and $[Q]$ is the concentration of the complex added. The binding constant ($K_b$) values obtained from the plot of log($F_0 – F$/$F$) versus log($[Q]$) (from Scatchard equation) [42] (figure S4) were $1.2 \times 10^3$, $5.5 \times 10^4$, and $8.5 \times 10^2$ M$^{-1}$ for 1–3, respectively, which signifies moderate to weak binding affinity but comparable to other mono- and dinuclear Schiff base copper systems [43–48]. The order of binding affinity of complexes towards DNA (or, ETBr displacement ability) is 2 > 1 > 3. Probable reason for this order may be charge density. Being neutral and for less conjugative Schiff base moiety in 3, complex 3 is less active towards DNA while positively charged binuclear 2 interacts more with DNA. We have also performed the control experiment using only HL1 and did not observe activity. Furthermore we repeated the experiment for 2 taking 0.5eqv of 2 compared to 1 and 3 (with same number of copper ions for all the complexes). Still we found higher activity for 2.

### 3.4. DNA cleavage studies

The efficiencies by which the metal complexes sensitize DNA cleavage are determined by the interaction of plasmid pBR322 DNA with 1–3. Gel electrophoresis of the plasmid is employed to monitor the transition from naturally occurring, covalently closed circular form (Form I) to the NC relaxed form (Form II). The cleavage of SC DNA (Form I) to the NC DNA (Form II) with variation in the concentrations (50–400 μM) of these complexes in presence or absence of external oxidizing agents are represented in figures 3 and S5, comparable with previous report [49]. Analysis of these results revealed no significant cleavage in the control while increasing metal complex concentrations enhanced DNA cleavage. Cleavage is due to enhanced reaction of copper ions with H$_2$O$_2$, thereby producing diffusible hydroxyl radicals or molecular oxygen, both of which are capable of damaging DNA by Fenton-type chemistry [50]. To elucidate the mechanism involved in DNA cleavage by 2, experiments are carried out in the presence of hydroxyl radical scavengers (DMSO and EtOH), singlet oxygen quencher (NaN$_3$), and superoxide radical scavenger. Figure S6 shows that NaN$_3$ inhibits more than the other three scavengers to cleave DNA from SC form to NC form, indicative that Cu(II) complexes cleave SC form of pBR322 DNA by producing singlet molecular oxygen. The groove binding preference of 2 was verified using the minor groove binder DAPI and the major groove binder methyl green [51]. Figure S6 indicates that minor groove binders produced a slight inhibition of the DNA damage mediated by 2 (Lane 6), suggesting that 2 preferentially interacts through minor groove of DNA helix. We have also performed the control experiment using only copper nitrate (various concentrations) and H$_2$O$_2$. We found that at 400 μM concentration of copper nitrate it shows 28.5% of NC DNA formation, whereas at similar concentration with respect to copper ion i.e. 200 μM of 2 produces above 40% of NC DNA.
3.5. Protein binding studies

Interaction of transition metal complexes with BSA protein are generally monitored by intrinsic fluorescence intensity. Generally tryptophan, tyrosine, and phenylalanine residues are the main components for showing fluorescence intensity of a protein [52]. Fluorescence quenching refers to any process, which decreases the fluorescence intensity of a fluorophore due to molecular interactions including excited-state reactions, molecular rearrangements, energy transfer, ground-state complex formation, and collision quenching. The fluorescence spectrum of 1–3 (figures 4 and S7) with BSA indicates that there is a progressive decrease in the fluorescence intensity along with a significant red shift for 2. This shifting of the emission maxima towards lower energy indicates probable energy transfer from the indole unit of the tryptophan to the protein bound compound [18]. The fluorescence quenching data were further analyzed by the Stern–Volmer relation which again can be expressed in terms of bimolecular quenching rate constant and average lifetime of the fluorophore as shown in the following equation [18],

\[
\frac{F_0}{F} = 1 + k_q \tau_0 [Q] = 1 + K_{SV}[Q]
\]

where \(F_0\) and \(F\) are the fluorescence intensities in the absence and presence of a quencher, \(k_q\) is the bimolecular quenching rate constant, \(\tau_0\) is the average lifetime of fluorophore in the absence of a quencher and \([Q]\) is the concentration of a quencher (metal complexes). \(K_{SV}\) is the Stern–Volmer quenching constant in \(\text{M}^{-1}\). However, for 2, a plot with upward curvature concave in the direction of the y-axis was found. This positive deviation clearly indicated that there must be a two-way quenching by collision as well as by complex formation with the same quencher. In a Stern–Volmer plot, lower concentration of 2 shows linearity, however at higher concentrations it shows a high positive deviation from linearity.
This indicates formation of more than one ground state complex BSA-2 system, so it can be predicted that at lower concentration of 2 the quenching could be started by a stable ground-state complex formation (1 : 1 type), however at higher concentration an upward bending in the direction of the $F_0/F$ axis specifies the formation of the second (1 : 2 type) BSA-2 complex. To determine the binding constant and number of binding sites, the Scatchard equation was employed, where $K_a$ and $n$ are the binding constant and number of binding sites, respectively, and $F_0$ and $F$ are the fluorescence intensities in the absence and presence of the quencher, respectively. Thus, a plot of $\log\left(\frac{F_0 - F}{F}\right) = \log(K_a) + n \log(Q)$ (figure 4) can be used to determine the value of binding constant (from intercept) and number of binding sites (from slope). All the data from Stern–Volmer plots and Scatchard plots are tabulated in table 2. The results indicate that 2 has more binding affinity towards BSA than 1 and 3. Protein binding capacity of all three complexes are more than reported nickel complexes with same Schiff base ligand [18]. We have also repeated the experiment in case of 2 taking 0.5eqv of 2 compared to 1 and 3 (with same number of copper ions for all the complexes). Moreover in this case also we found 2 is more active with respect to 1 or 3, indicating a possible role of nuclearity.

### Table 2. Various parameters obtained from bio-macromolecular interaction study.

| System   | DNA interaction with | BSA interaction with |
|----------|---------------------|----------------------|
| Complex  | 1       | 2       | 3       | 1       | 2       | 3       |
| $K_{W}^{0}$ (M$^{-1}$) | $6.2 \times 10^{2}$ | $1.1 \times 10^{3}$ | $7.7 \times 10^{2}$ | $4.5 \times 10^{3}$ | $7.0 \times 10^{4}$ | $1.0 \times 10^{4}$ |
| $K_{W}^{1}$ (M$^{-1}$ S$^{-1}$) | –       | –       | –       | $7.2 \times 10^{11}$ | $1.1 \times 10^{13}$ | $1.6 \times 10^{12}$ |
| $K_{W}^{2}$ (M$^{-1}$) | $1.2 \times 10^{1}$ | $5.5 \times 10^{1}$ | $8.5 \times 10^{2}$ | $1.3 \times 10^{4}$ | $4.0 \times 10^{7}$ | $6.4 \times 10^{4}$ |
| $n$      | 1.06    | 1.43    | 1.01    | 1.11    | 1.63    | 1.2     |

### 3.6. Catecholase activity study

Catecholase-like activities of 1–3 were studied taking 35-DTBC as the substrate in the presence of two bulky t-butyl substituents in the ring for showing a low quinone-catechol reduction potential [6, 53].
Figure 5. Catecholase activity by change in time-dependent spectral pattern of 1–3 after addition of 35-DTBC. Corresponding Michael–Menten plot (inset). Lineweaver–Burk plot (bottom right).
The reactions were carried out at 25 °C in aerobic conditions and monitored by UV–vis spectroscopy. The oxidation product 35-di-tert-butylquinone (35-DTBQ) is stable and shows a maximum absorption at 400 nm in methanol. To monitor the reaction 10−4 M methanolic solutions of 1–3 were treated with 100 equivalent of 35-BTDC; upon addition of catecholic substrate a new band starts to gradually appear at 402 nm due to the formation of the oxidized product 35-DTBQ (figure 5).

To understand the kinetic aspects of catalysis for 1–3, the rate constant for a catalyst complex was determined by traditional initial rate method (detailed description in the Experimental Section). The observed rate versus substrate concentration data were then analyzed on the basis of the Michaelis–Menten approach of enzymatic kinetics to determine the Michaelis–Menten constant ($K_M$) and maximum initial rate ($V_{max}$) using Michaelis–Menten plots and Lineweaver–Burk plots [54]. The turnover

| Complex/Catalyst | Fixed complex/ Catalyst conc. (M) | $V_{max}$ (M min$^{-1}$) | Std. error | $K_M$ (M) | Std. error | $k_{cat}$/T.O.N (h$^{-1}$) |
|-----------------|-----------------------------------|---------------------------|------------|-----------|------------|--------------------------|
| 1               | 0.0001                            | 0.01724                   | 0.00365    | 3.9 × 10$^{-4}$ | 2.5 × 10$^{-4}$ | 10.3 × 10$^3$ |
| 2               | 0.0001                            | 0.04763                   | 0.0092     | 0.04271   | 0.03571    | 28.5 × 10$^3$ |
| 3               | 0.0001                            | 0.02175                   | 0.00135    | 2.6 × 10$^{-4}$ | 8.9 × 10$^{-5}$ | 13.0 × 10$^3$ |

![Complex 1](image1.png)
![Complex 2](image2.png)
![Complex 3](image3.png)

**Figure 6.** Probable complex–substrate aggregate of 1–3 during catechol oxidation.

![Scheme 2](image4.png)

**Scheme 2.** Probable catechol oxidation mechanism by 2.

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frequency values \( (k_{\text{cat}}) \) were obtained by dividing the \( V_{\text{max}} \) values by the concentration of the corresponding complexes. All data (table 3) unambiguously demonstrate that 1–3 are active, due to the positive charge on the piperazinyl moiety may facilitate catalyst–substrate interaction by forming a positive channel which might be a prerequisite for showing better catalytic activities. A similar mechanism has been proposed to explain the activity of copper/zinc superoxide dismutase where positively charged arginine and lysine residues play a role to attract the anion, guiding it towards the catalytic center [55]. The turnover frequency \( (k_{\text{cat}}) \) 28.5 × 10³ h⁻¹ obtained for 2 is high with respect to other dimeric copper complexes [56–58].

We have investigated the probable complex–substrate aggregate (figure 6) through ESI-MS spectroscopy (figure S8). The molecular ion peaks \( (m/z \text{ value}) \) are observed at 362.13 for \( \{\text{Cu(HL1)}(\text{Py})–\text{DTBC}–(\text{Py})(\text{HL}^1)\text{Cu + K}\}^{3+} \) (Structure A in scheme S1) for 1, at 1043.51 for \( \{\text{Cu}_2(\text{HL}^1)_2(\text{NO}_3)_2–\text{DTBC} + \text{Na}\}^+ \) (Structure B in scheme 2) for 2, and at 384.13 for \( \{\text{Cu(HL}^2)–\text{DTBC}–(\text{HL}^2)\text{Cu}\}^{2+} \) (Structure C in scheme S1) for 3 with some deviation which is in the range of earlier report [59]. This result suggests that for mononuclear copper complexes two mononuclear units are involved to catalyze the catechol oxidation reaction for one substrate (i.e. one 35-DTBC) whereas for 2, one dimer is attached with one 35-DTBC to form a complex–substrate aggregate. The probable reaction mechanism for 1 and 3 are represented in scheme S1 and for 2 in scheme 2. Similar mechanisms were reported earlier [60]. As 2 has shown best catalytic activities, we focused our attention on the mechanism for 2. In this case, one molecule of 35-DTBC is attached with two metal centers in a bridging fashion by removal of one nitrate to form complex–substrate aggregate B (scheme 2). Then simultaneous or stepwise reduction of Cu centers takes place with subsequent removal of oxidized catechol moiety. After that a second catechol moiety gets inserted into the copper centers to complete the cycle.

Formation of \( \text{H}_2\text{O}_2 \) during catalytic oxidation procedure gives some insight into the plausible mechanism for catechol oxidation. Taking 2 as representative, we have obtained positive results for quantitative detection of \( I^- \) (~353 nm) (figure S9) by UV–vis spectroscopy (applying reported methodology [61]) which is indicative for formation of \( \text{H}_2\text{O}_2 \) during the process. According to previous report, either water or dihydrogen peroxide can be formed as a side product in the catalytic oxidation of catechol by copper(II) complexes [62]. Though there are few reports of formation of \( \text{H}_2\text{O}_2 \) in the reaction mixture [63–66], studies aimed to definitely establish the mode of the dioxygen reduction to either water or dihydrogen peroxide are scarce. In some cases, formation of dihydrogen peroxide is correlated with detection of the semiquinone intermediate in the catalytic reaction [67, 68]. Dihydrogen peroxide may get formed as a product of the oxidation of the copper(I)-semiquinone intermediate, as proposed by Kodera et al. [67]. The mode of oxidation can be rationalized as follows. For dicopper(II) complexes, the simultaneous reduction in two copper(II) centers to copper(I) results in oxidation of one equivalent of catechol, leading to the release of one quinone molecule. For mononuclear copper(II) complexes (or dinuclear intermediate species, formed by self-assembly of two mononuclear units via DTBC bridge), only one electron transfer may occur, resulting in the formation of copper(I)-semiquinonate intermediates. The reaction of such species with dioxygen may result in the two-electrons reduction of the latter, leading to reoxidation of copper(I), a release of the quinone molecule, and dihydrogen peroxide formation.

We have performed all the above experiments keeping the number of metal centers constant for all three complexes to compare their activities. The results indicate that 2 shows higher activity towards DNA binding \( (K_a = 3.1 \times 10^4 \text{ M}^{-1}) \), BSA binding \( (K_a = 2.5 \times 10^7 \text{ M}^{-1}) \), and catecholase–like activity \( (K_{\text{cat}} = 17.3 \times 10^3 \text{ h}^{-1}) \) implying that the nuclearity of the metal complex plays a role in greater activity.

| Compounds | MW (g mol⁻¹) | Solvent | Concentration (M) | MRSA MIC (mg L⁻¹) | MRSA MBC (mg L⁻¹) |
|-----------|--------------|---------|------------------|-------------------|-------------------|
| HL¹ | 273.18 | Methanol | 0.461 | 491.7 | 491.7 |
| 2 | 335.11 | Methanol | 0.010 | 10.7 | 103.8 |

Table 4. MIC and MBC MRSA results for HL¹ and 2 against MRSA strain.
3.7. Antibacterial activity

Nosocomial infections (more commonly hospital-acquired infections) are defined as an infection in a patient or health care professional not evident prior to accessing a hospital or health care facility [69]. These infections cause problems for patient health and have a health-economic impact (1.4 million patients admitted to a hospital will experience nosocomial derived difficulties at any given time) [70]. The most frequent nosocomial infections reported are caused by bacteria of the Gram-positive MRSA. This bacteria, a non-motile, non-spore-forming coccus and a significant human pathogenic bacterium, is one that currently causes the majority of nosocomial infections [69, 71]. In this study, we evaluated the antibacterial activity of Schiff base ligands and copper complexes. The free Schiff bases did not show significant antibacterial activity. Complex 2 exhibits moderate activity against S. aureus (MRSA) with a MIC value of 10.7 mg L⁻¹ (10 μg mL⁻¹) compared to commercially available antibiotics including vancomycin (MIC of 1 μg mL⁻¹) and linezolid (MIC ranged from 2 to 4 μg mL⁻¹) [72]. Complex 2 is effective against S. aureus (MRSA) and is a promising compound showing activity against this multi-drug resistant strain (table 4). The antibacterial activity of 2 opens the possibility to further explore this compound against clinical multi-drug resistant strains.

3.8. Computational study

We performed density functional theory (DFT) calculations to understand the DNA binding and catechol oxidation catalyzed by 1–3. We have modeled the DNA structure as a deoxyguanosine monophosphate (dGMP) [73] unit where one phosphate oxygen is methylated to satisfy the valency; the dGMP unit is negatively charged. All the calculations are carried out using UB3LYP level of theory as implemented in the Gaussian 09 package [74]. The 6–311++G** basis set is used for main group elements (C, N, O, P and H) and LANL2DZ ECP for Cu [75, 76]. We have optimized these complexes in two different solvents using the polarizable continuum model [77] as the experiments are performed in different solvents.
(water for DNA cleavage and methanol for catechol oxidation). The vibrational frequency calculations are performed for all the structures to confirm the nature of the stationary points.

3.9. Theoretical comparison of DNA binding study

The optimized structures of Cu(II) complexes and dGMP are presented in figure S10(a)–(d). The frontier orbitals of dGMP and 1–3 are investigated to understand their role towards DNA binding. In figure 7, we have shown the HOMO energy of the dGMP structure with respect to the LUMO energies of 1–3 to understand the binding of DNA to the Cu-complexes [78, 79]. The HOMO energy of the dGMP fragment is −3.66 eV and the LUMO energies of 1–3 are −4.90, −5.28, and −3.42, respectively. Therefore, the LUMO is highly stabilized for 2 (−5.28 eV) in comparison to 1 (−4.90 eV) and 3 (−3.42 eV). Electron transfer (figure 7) will be highly favorable from HOMO of dGMP to 2. On the other hand, the LUMO (−3.42 eV) of 3 is higher in energy than the HOMO (−3.66 eV) of dGMP. So, the electron transfer will not be favorable from dGMP to 3. The dGMP-bonded Cu(II) complexes are modeled where the phosphate group of the dGMP fragment binds to the Cu-center of the metal complexes (figure S11(a)–(c)). All three dGMP-bonded complexes are fully relaxed and found to be minima in their potential energy surfaces. The binding energy ($E_B$) of the dGMP fragment with the Cu(II) complex is calculated using the following equation:

$$E_B = E_{Cu-dGMP} - (E_{dGMP} + E_{Cu-complex})$$

where $E_{Cu-dGMP}$ is the total energy of the Cu–dGMP complex. $E_{Cu-complex}$ and $E_{dGMP}$ are single point energies of the Cu-complex and the dGMP fragment within the geometry of the Cu–dGMP complex. The calculated dGMP binding energies are −6.60, −10.66, and −3.14 kcal mol$^{-1}$ for 1–3, respectively, consistent with the experimental DNA binding constants of 1–3.

3.10. Theoretical comparison of catechol oxidation study

The ground state structures of all three Cu(II) complexes are presented in figure S10(a)–(c). Mononuclear Cu(II) complexes (1 and 3) have open-shell doublet (S = 1/2) configuration whereas binuclear 2 has open-shell triplet (S = 1) configuration. During the oxidation reaction, catechol first binds to the metal center [19, 80]. In case of 2, one catechol binds to one metal complex but for 1 and 3 two metal complexes bind to one catechol molecule. Thus, during catechol oxidation, the binuclear metal complex will be reduced by two electrons (2e$^-$) whereas mononuclear complexes will be reduced by one electron (e$^-$). Thus, during the catechol oxidation, Cu(II) complexes are reduced to Cu(I). All three reduced Cu(I) complexes are calculated to be most stable with an open-shell singlet structure (S = 0). We have plotted the spin density difference (SDD) of the Cu(II) and Cu(I) complexes to understand their role towards catechol oxidation. We found a drastic change in the spin multiplicity of all the three Cu(II) complexes, which can be clearly seen from their spin density difference plots (figure S12(a)–(c)). Initial spin density is concentrated on Cu of the Cu(II) complexes whereas the spin density vanishes after reduction. We have also calculated the SOMO-LUMO gap of Cu(II) (before reduction) and HOMO-LUMO gap of the Cu(I) complexes (after reduction). The HOMO-LUMO gap is higher in the Cu(I) systems compared to the SOMO-LUMO gap of the Cu(II) systems. We have also investigated the SOMO-LUMO gap of 1–3 to find out their reactivity towards catechol oxidation. We found that dinuclear 2 (1.36 eV) has lower SOMO-LUMO gap compared to 1 (4.08 eV) and 3 (2.44 eV) (figure S13 and table S2); 2 is more reactive towards catechol oxidation compared to 1 and 3. Thus, the binuclear complex shows superior catalytic activity, related with their SOMO energies. The calculated SOMO energies are −6.08, −7.19, and −5.88 eV for 1–3, respectively, showing the SOMO is highly stabilized in 2 and thus ready to accept an electron, which triggers the catechol oxidation reaction. The catechol oxidation trend (2 > 3 > 1) is in agreement with our experimental findings.
The catechol oxidation trend \( (2 > 3 > 1) \) could also be influenced by geometry around copper. More asymmetric nature of the geometry around metal ion increases the activity towards catechol oxidation [81]. Taking the crystallographic data for \( 1 \text{–} 3 \) and following the mentioned correlation, the higher/lower distances of the Cu first coordination sphere are \( 2.265/1.934 = 1.17 \) for \( 1 \), \( 2.75/1.985 = 1.385 \) for \( 2 \) and \( 2.423/1.921 = 1.26 \) for \( 3 \). That means the distortion of the symmetry follows the trend \( 2 > 3 > 1 \), which is manifested in terms of catalytic activity of the complexes.

4. Conclusion

Three new Cu(II) complexes, \([\text{Cu(HL}1\text{)(Pyridine)(H}_2\text{O})(\text{ClO}_4\text{)}_2\text{2MeOH]}(1), [\text{Cu}_2(\text{HL}1\text{)}_2(\text{NO}_3\text{)}_2\text{2(NO}_3\text{)}_2\text{3H}_2\text{O}(2), and [\text{Cu(HL}2\text{)(NO}_3\text{)}_2\text{MeCN]}(3)\), have been synthesized and characterized. Cu(II) geometries have four, five, and six coordination. Complex 2 has two Cu(II) centers in different geometrical environments. ETBr displacement assay, gel electrophoresis experiment, and fluorescence quenching for BSA clearly indicate that \( 1 \text{–} 3 \) have DNA binding and cleavage as well as protein binding activities. These complexes are quite active towards catechol oxidation and have antibacterial properties. All the data reflect that dinuclear 2 is the most promising molecule towards all kinds of these activities. The nuclearity driven activity of 2 towards DNA binding and catechol oxidations are further explained by DFT.

**Supplementary materials**

CCDC 1016329, 1016328 and 1016327 contain the supplementary crystallographic data for \( 1 \text{–} 3 \), respectively. These data can be obtained free of charge via [http://www.ccdc.cam.ac.uk/conts/retrieving.html](http://www.ccdc.cam.ac.uk/conts/retrieving.html), or from the Cambridge Crystallographic Data Center, 12 Union Road, Cambridge CB2 1EZ, UK; Fax: (+44) 1223-336-033; or E-mail: deposit@ccdc.cam.ac.uk.

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**Disclosure statement**

No potential conflict of interest was reported by the authors.

**References**

[1] M.C. Linder, C.A. Goode. *Biochem. Copper*, Plenum, New York (1991).
[2] W. Kaim, J. Rall. *Angew. Chem. Int. Ed. Engl.*, 35, 43 (1996).
[3] T.N. Sorrell. *Tetrahedron*, 45, 3 (1989).
[4] N. Kitajima, Y. Moro-oka. *Chem. Rev.*, 94, 737 (1994).
[5] S.S. Bhat, A.A. Kumbhar, H. Heptullah, A.A. Khan, V.V. Gobre, S.P. Gejji, V.G. Puranik. *Inorg. Chem.*, 50, 545 (2011).
[6] S.K. Dey, A. Mukherjee. *ChemCatChem*, 5, 3533 (2013).
[7] C. Fernandes, A. Neves, A.J. Bortoluzzi, A.S. Mangrich, E. Rentschler, B. Szpoganicz, E. Schwingel. *Inorg. Chim. Acta*, 320, 12 (2001).
[8] E. Moura, J. Afonso, L. Hein, M.A. Vieira-Coelho. *Br. J. Pharmacol.*, 149, 1049 (2006).
[9] R. del Campo, J.J. Criado, E. García, M.R. Hermosa, A. Jiménez-Sánchez, J.L. Manzano, E. Monte, E. Rodríguez-Fernández, F. Sanz. *J. Inorg. Biochem.*, 89, 74 (2002).
[10] N.C. Kasuga, K. Sekino, C. Koum, N. Shimada, M. Ishikawa, K. Nomiya. *J. Inorg. Biochem.*, 84, 55 (2001).
[11] A. Vessieres, S. Top, W. Beck, E. Hillard, G. Jaouen. *Dalton Trans.*, 4, 529 (2006).
[12] I. Bhat, S. Tabassum. *Spectrochim. Acta, Part A*, 72, 1026 (2009).
[13] V.C. Silveira, M.P. Abbott, M. Cavicchioli, M.B. Gonçalves, H.M. Petrilli, L. de Rezende, A.T. Amaral, D.E.P. Fonseca, G.F. Caramori, A.M. da Costa Ferreira. *Dalton Trans.*, 42, 6386 (2013).
[14] A. Patra, T.K. Sen, A. Ghorai, G.T. Musie, S.K. Mandal, U. Ghosh, M. Bera. *Inorg. Chem.*, 52, 2880 (2013).
[15] X.-W. Li, L. Tao, Y.-T. Li, Z.-Y. Wu, C.-W. Yan. *Eur. J. Med. Chem.*, 54, 697 (2012).
[16] G. Prattivel, J. Bernadou, B. Meunier. *Angew. Chem. Int. Ed. Engl.*, 34, 746 (1995).
[17] A.K. Patra, T. Bhowmick, S. Roy, S. Ramakumar, A.R. Chakravarty. *Inorg. Chem.*, 48, 2932 (2009).
[18] M. Das, R. Nasani, M. Saha, S.M. Mobin, S. Mukhopadhyay. *Dalton Trans.*, 44, 2299 (2015).
[69] J.V. Bennett, W.R. Jarvis, P.S. Brachman. In Bennett & Brachman's Hospital Infections, pp. 5–7, Lippincott Williams & Wilkins, Philadelphia, PA (2007).
[70] G. Ducel, J. Fabry, L. Nicolle. Prevention of Hospital-acquired Infections, World Health Organization (2002).
[71] J.L. Brusch. In Endocarditis Essentials, pp. 7–10, Jones and Barlett, Sudbury (2011).
[72] S. Thangamani, H. Mohammad, M.F.N. Abushahba, T.J.P. Sobreira, V.E. Hedrick, L.N. Paul, M.N. Seleem. Sci. Rep., 6, 22571 (2016).
[73] T.-F. Miao, J. Li, S. Li, N.-L. Wang. J. Phys. Chem. A, 118, 5692 (2014).
[74] A.D. Becke. Phys. Rev. A, 38, 3098 (1988).
[75] C. Lee, W. Yang, R.G. Parr. Phys. Rev. B, 37, 785 (1988).
[76] M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G.A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H.P. Hratchian, A.F. Izmaylov, J. Bloino, G. Zheng, J.L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J.A. Montgomery Jr., J.E. Peralta, F. Ogliaro, M.J. Bearpark, J. Heyd, E.N. Brothers, K.N. Kudin, V.N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A.P. Rendell, J.C. Burant, S.S. Iyengar, J. Tomasi, M. Cossi, N. Rega, N.J. Millam, M. Klene, J.E. Knox, J.B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A.J. Austin, R. Cammi, C. Pomelli, J.W. Ochterski, R.L. Martin, K. Morokuma, V.G. Zakrzewski, G.A. Voth, P. Salvador, J.J. Dannenberg, S. Dapprich, A.D. Daniels, Ö. Farkas, J.B. Foresman, J.V. Ortiz, J. Cioslowski, D.J. Fox. Gaussian 09, Gaussian Inc., Wallingford, CT, USA (2009).
[77] J. Tomasi, B. Mennucci, R. Cammi. Chem. Rev., 105, 2999 (2005).
[78] X.W. Liu, J. Li, H. Deng, K.C. Zheng, Z.W. Mao, L.N. Ji. Inorg. Chim. Acta, 358, 3311 (2005).
[79] W.J. Mei, J. Liu, K.C. Zheng, L.J. Lin, H. Chao, A.X. Li, F.C. Yun, L.N. Ji. Dalton Trans., 7, 1352 (2003).
[80] P. Chakraborty, J. Adhikary, B. Ghosh, R. Sanyal, S.K. Chattopadhyay, A. Bauzá, A. Frontera, E. Zangrando, D. Das. Inorg. Chem., 53, 8257 (2014).
[81] M.A. Vázquez-Fernández, M.R. Bermejo, M.I. Fernández-García, G. González-Riopedre, M.J. Rodríguez-Doutón, M. Maneiro. J. Inorg. Biochem., 105, 1538 (2011).