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Original article

Copper complexes of pyridine derivatives with superoxide scavenging and antimicrobial activities

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ABSTRACT

Superoxide anions are reactive oxygen species that can attack biomolecules such as DNA, lipids and proteins to cause many serious diseases. This study reports the synthesis of copper complexes of nicotinic acid with related pyridine derivatives. The copper complexes were shown to possess superoxide dismutase (SOD) and antimicrobial activities. The copper complexes exerted SOD activity in range of 49.07–130.23 μM. Particularly, copper complex of nicotinic acid with 2-hydroxypyridine was the most potent SOD mimic with an IC_{50} of 49.07 μM. In addition, the complexes exhibited antimicrobial activity against *Bacillus subtilis* ATCC 6633 and *Candida albicans* ATCC 90028 with MIC range of 128–256 μg/mL. The SOD activities were well correlated with the theoretical parameters as calculated by density functional theory at the B3LYP/LANL2DZ level of theory. Interestingly, the SOD activity of the copper complexes was demonstrated to be inversely correlated with the electron affinity, but was well correlated with both HOMO and LUMO energies. The vitamin–metal complexes described in this report are great examples of the value-added benefits of vitamins for medicinal applications.

1. Introduction

Free radicals such as superoxide anions ($O_2^{-\bullet}$) are very reactive oxygen species (ROS), which are primarily produced in the human body and has been involved in many cellular processes, which causes various diseases [1]. The deleterious effects of ROS are counterbalanced by antioxidant enzymes such as superoxide dismutase (SOD), catalase and glutathione peroxidase as well as by small molecular antioxidants. Under oxidative stress conditions, ROS can induce molecular and cellular damages to DNA, lipids and proteins. Such events lead to the development of many serious diseases [2,3], e.g. cancer [4], atherosclerosis [5], asthma [6], and neurodegenerative disorders [7]. SOD, in particular, is the antioxidant enzyme, which can convert superoxide anion to a less-reactive form, e.g. H_2O_2 [8]. The utilization of SOD as a therapeutic agent has been reported, however there are many limitations, which hinders its application, such as its high molecular weight [9]. Therefore, small molecular weight SOD mimics as afforded by transition metal complexes are more lucrative alternatives. Some examples include metalloporphyrins [10], metal–drug complexes [11] and metallopyridine complexes [12].

Pyridine derivatives possess a diverse array of bioactivities as well as playing crucial roles for physiological functions. They have been extensively used as ligands in the formation of coordination compounds as medicinal agents. The most striking one to our interest is nicotinic acid (NA) or pyridine-3-carboxylic acid, also known as vitamin B3 (niacin), which acts as an antihyperlipidemic drug and as effective HDL cholesterol raising agent for reduction of cardiovascular risks [13,14]. Complexation of NA with various metals, e.g. manganese, cobalt, nickel, copper and zinc, had previously been reported [15], where nicotinic acid–copper complex has particularly been shown to stimulate blood flow and prevent gastric congestion [16], reduce total lipids in sera hepatic tissue as well as reducing the levels of ALT, AST, ALP, GGT and oxidative markers, e.g. nitric oxide (NO) and lipid peroxidation. Such complex was also demonstrated to exhibit SOD activity for treatment of patients with hepatocellular carcinoma [17]. Recently, we have reported promising SOD mimic activity for copper complexes of nicotinic-carboxylic acids [12]. Such copper complexes were formed by coordination of NA via the ring N-atom with bidentate carbonyl and oxy/amino groups of carboxylic acids as electron donors. Aside from NA, other interesting ligands used in this study bearing suitable N- and/or O-electron donors are bioactive pyridine derivatives and related compounds such as hydroxypyridines,

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aminopyridines, and picolinic acid (pyridine-2-carboxylic acid). Picolinic acid, an isomeric form of NA, is a metabolite derived from tryptophan of humans and animals. The picolinic acid plays an important role in zinc transport [18,19]. Its complexes have been demonstrated to possess antiviral [20], antifungal [21] and antibacterial [22] activities as well as being involved in the induction of apoptosis [23] and immune responses [24]. 2-Hydroxypyridines (2-pyridone) have been reported to form complex with metals [25]. The complex of hydroxypyridine bases, such as 2-, 3- and 4-hydroxypyridines, with platinum or palladium was shown to possess promising anticancer activity against human ovarian cancer cells [26–28]. Moreover, 4-aminopyridines and their derivatives were reported to be involved in the inactivation of voltage-dependent K^+ channels [29,30] as well as implicated in reversing anesthesia and relaxing muscles [31,32]. The copper ion is an essential element, which possesses interesting anti-inflammatory and anti-ulcer activities [33]. As a metal cofactor in the copper–zinc superoxide dismutase, copper exerts important catalytic function in the dismutation of superoxide anions [34,35]. It is known that copper deficiency is responsible for hematological disorders, hypopigmentation, defective connective tissue cross-linking and ataxia [36,37].

The aforementioned statements have motivated us, as part of a continuous study, to synthesize low molecular weight copper complexes of biologically important pyridines as SOD mimics. The nicotinic acid was used as a primary ligand for such synthesis. Therefore, novel copper complexes of nicotinic acid with 2-substituted pyridines containing bidentate N, O and/or N, N electron donors were synthesized as SOD mimic activity. The 2-substituted pyridines are 2-hydroxypyridine, 2-aminopyridine and pyridine-2-carboxylic acid. To give insight into the cardinal mechanisms of SOD activities, quantum chemical parameters calculated at density functional theory (DFT) level were used as physicochemical description of the compounds. DFT has wide applications in the life sciences particularly in the development of quantitative structure–activity/property relationship (QSAR/QSPR) models, for the elucidation of enzymatic reactions as well as in clarification of the superoxide radical scavenging activity. Results indicated that the calculated physicochemical parameters were well correlated with the experimental SOD activities [12]. The theoretical parameters used are comprised of energies of the highest occupied molecular orbital (HOMO), energies of the lowest unoccupied molecular orbital (LUMO), and electron affinity (EA). Moreover, the antimicrobial activity of the novel complexes was also explored.

2. Materials and methods

2.1. Materials

All chemicals were of analytical reagent grade and were purchased from Sigma–Aldrich. Bovine erythrocyte superoxide dismutase was supplied from the same source. Melting points of the complexes were determined on the Griffin capillary melting point apparatus and are reported without correction. Infrared (IR) spectra were obtained on Perkin Elmer System 2000 FTIR as a potassium bromide (KBr) pellet. Magnetic moment was performed by Magnetic Susceptibility Balance, Mark 1, Serial 15257, Sherwood Scientific, Cambridge, UK.

2.2. Synthesis of copper complexes of pyridine derivatives

2.2.1. Copper complex of nicotinic acid-2-hydroxypyridine (**1**)

Nicotinic acid (NA, 0.123 g, 1 mmol) dissolved in methanol (30 mL) was heated (70 °C) and stirred under reflux until the solution was clear. Cupric chloride dihydrate (0.170 g, 1 mmol) was dissolved in methanol (2 mL) and added dropwise to the prepared NA solution. After heating for 45 min, a solution of 2-hydroxypyridine (2Hy, 1 mmol, 0.138 g) in methanol (2 mL) was added dropwise to the reaction mixture. The mixture was then heated for 1 h at the same condition. The precipitated solid was collected by filtration, washed with cold methanol and dried *in vacuo* over silica gel at room temperature to yield cyan powder of complex **1**, m.p. >300 °C; IR (KBr, cm^{-1}): 1389 (m, C–N), 1375 (m, C–N), 1191 (w, C–O), 1093 (w, C–O), 1053 (w, C–O); μ_{eff}: 1.5545 BM.

2.2.2. Copper complex of nicotinic acid-2-aminopyridine (**2**)

Complex **2** was prepared in the same method as complex **1** using NA (0.123 g, 1 mmol), cupric chloride (0.170 g, 1 mmol) and 1 mmol (0.137 g) of 2-aminopyridine (2Am). As aquamarine powder, product **2** was obtained by filtration, washed with cold methanol and dried over silica gel, m.p. >300 °C; IR (KBr, cm^{-1}): 1386 (s, C–N), 1375 (s, C–N), 1191 (s, C–O), 1155 (w, C–O), 1093 (m, C–O), 1050 (m, C–O); μ_{eff}: 1.9381 BM.

2.2.3. Copper complex of nicotinic acid–picolinic acid (**3**)

Copper complex of the nicotinic–picolinic acids (**3**) was prepared in an analogous fashion using 1 mmol of NA, cupric chloride and picolinic acid (Pi; 1 mmol, 0.166 g). The precipitated complex was obtained as blue powder after filtration, washing with cold methanol, and dried over silica gel. m.p. >300 °C; IR (KBr, cm^{-1}): 1475 (w, O–H), 1348 (vs, C–N), 1286 (s, C–O), 1152 (w, C–O), 1049 (s, C–O); μ_{eff}: 1.7014 BM. Structures of pyridine derivatives are shown in Fig. 1.

2.3. Determination of superoxide dismutase activity

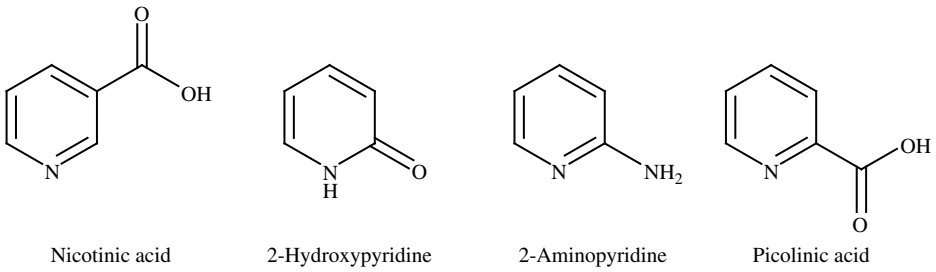
The complexes were tested for SOD activity using the previously described method [11,38]. The SOD activity of copper complexes was assayed in the pH range of 6.9–7.4 by measuring the inhibition of the photoreduction of nitro blue tetrazolium (NBT). This indirect assay is comprised of several reactions: the photochemically excited riboflavin was first reduced by methionine into a semiquinone, which donated an electron to oxygen to form a superoxide source. The superoxide readily converted NBT into a purple formazan product, being spectrophotometrically detected at 550 nm. In this regard, the SOD activity was inversely related to the amount of formazan formed, and expressed in terms of 50% inhibition concentration (IC_{50}) of NBT reduction.

2.4. Antimicrobial activity

The antimicrobial activity of the complexes was investigated against representative microorganisms using the method previously reported [38,39]. Briefly, the tested complexes dissolved in dimethyl sulfoxide (DMSO) were mixed with Müller Hinton (MH) broth to receive a final volume of 1 mL. Two-fold dilution was prepared and the MH broth of the tested sample then was transferred to MH agar solution to yield the final concentrations ranging from 64 to 256 μg/mL. The solutions were placed onto the plates. Twenty-seven strains of microorganisms (Table 1), cultured in the MH broth at 37 °C for 24 h, were diluted with 0.9% normal saline solution to 3×10^8 cells/mL. The organisms were inoculated onto each plate and incubated at 37 °C for 18–48 h. The complexes found to be effective against the tested strains were selected. The inhibition of microbial cell growth was determined.

2.5. Molecular model of superoxide dismutase mimics

The copper complexes were drawn with GaussView 3.09 [40] and calculated by Gaussian 03 [41]. Fullyoptimized geometry without symmetry constraints of the copper complexes was



Nicotinic acid 2-Hydroxypyridine 2-Aminopyridine Picolinic acid

Fig. 1. Chemical structures of the pyridine derivatives.

performed *in vacuo* using density functional theory (DFT) at B3LYP/LANL2DZ level [42].

3. Results and discussions

Copper-based pyridine complexes **1**–**3** were synthesized by the reaction of cupric chloride with nicotinic acid and pyridine derivatives (2-hydroxypyridine, 2-aminopyridine and picolinic acid) in a molar ratio of 1:1:1. The complexes were obtained in good yields (75–87%) as cyan, aquamarine and blue powder, respectively. They are highly polar compounds, insoluble in methanol and water, but soluble in DMSO. Their melting points and magnetic moment are shown in Table 2. IR spectral data are outlined in Table 3.

3.1. Infrared spectra

From the IR spectral data, it can be seen that the C–N stretching vibration of complex **1** showed at 1389 and 1375 cm^{-1} whereas the C–N absorption of free NA and free 2Hy appeared at 1324 and 1243 cm^{-1}, respectively. It was observed that the hydroxyl absorption of NA at 1418 cm^{-1} δ(O–H) disappeared. In addition, strong absorptions at 1575 and 781 cm^{-1} δ(N–H) for 2Hy were not observed. The C–O stretching vibration of complex **1** displayed weak bands at 1191, 1093 and 1053 cm^{-1}, whereas the C–O absorptions of free NA appeared at 1299 cm^{-1} and for 2Hy at 1156 and 1098 cm^{-1}. Based on the IR spectra, the carbonyl absorption of complex **1** could not be observed. It is suggested that complex **1** was formed by coordination of copper atom with the two carbonyl groups (carboxylic of NA and 2-pyridone) and the two nitrogen atoms of pyridine ring of both NA and 2Hy.

Table 1
Microorganisms for antimicrobial assays.

Microorganism	Reference strain	Clinical isolates
Gram-positive bacteria	*Staphylococcus aureus* ATCC 29213	*Streptococcus pyrogenes* II
	Staphylococcus aureus ATCC 25923	*Bacillus cereus*
	Staphylococcus epidermidis ATCC 12228	*Listeria monocytogenes*
	Enterococcus faecalis ATCC 29212	
	Enterococcus faecalis ATCC 33186	
	Micrococcus lutens ATCC 10240	
	Bacillus subtilis ATCC 6633	
	Corynebacterium diphtheriae NCTC 10356	
Gram-negative bacteria	*Escherichia coli* ATCC 25922	*Shigella dysenteriae*
	Klebsiella pneumoniae ATCC 700603	*Salmonella enteritidis* type C
	Serratia marcescens ATCC 8100	*Morganella morganii*
	Salmonella typhimurium ATCC 13311	*Aeromonas hydrophila*
	Shewanella putrefaciens ATCC 8671	*Citrobacter freundii*
	Achromobacter xylosoxidans ATCC 2706	*Plesiomonas shigelloides*
	Pseudomonas aeruginosa ATCC 15442	
	Pseudomonas stutzeri ATCC 17587	
Yeasts	*Saccharomyces cerevisiae* ATCC 2601	
	Candida albicans ATCC 90028	

The IR spectra of complex **2** pointed out the disappearance of carbonyl absorption, while the free NA exhibited absorption at 1718 and 1700 cm^{-1}. The amino (NH_2) absorption of 2Am appeared at 3448 and 3315 cm^{-1}, while the NH absorption of **2** was shown at 3116 cm^{-1}. Strong C–N stretching vibration of complex **2** was observed at 1386 and 1375 cm^{-1}. Such C–N absorption of NA appeared at 1324 cm^{-1} and at 1492, 1442 and 1326 cm^{-1} for 2Am. The strong C–O stretching of free NA was observed at 1299 cm^{-1}, weak hydroxyl (O–H) stretching was noted at 3072–2449 cm^{-1} and strong OH bending displayed at 1418 cm^{-1}. The C–O absorption of complex **2** was observed at 1191, 1155, 1093 and 1050 cm^{-1}. As evidence by the IR spectra, complex **2** was formed by coordination of the carbonyl group and the pyridine ring nitrogen atom of NA with the amino group and nitrogen ring atom of 2Am.

Similarly, the IR spectra of complex **3** were determined by comparing with the free ligands. It can be seen that the frequency of C–O stretching vibration was presented at 1286 cm^{-1} υ(C–O), while the absorption of free NA and Pi appeared at 1299 and 1294 cm^{-1}, respectively. Furthermore, disappearances of the stretching vibration at 1718 and 1700 cm^{-1} of the carbonyl groups υ(C=O) of both NA and Pi were observed. The weak band at 1475 cm^{-1} could be attributed to the O–H bending vibration of complex **3** when compared with the spectra of both NA and Pi, which showed strong bands at 1418 and 1454 cm^{-1}, respectively. The C–N stretching vibration of complex **3** exhibited very strong band at 1348 cm^{-1}, while the absorption of free NA and Pi showed υ(C–N) at 1324 and 1342 cm^{-1}, respectively. Hence, complex **3** was formed using carbonyl groups and nitrogen atoms of pyridine ring of NA and Pi as bidentate ligands to coordinate with copper atom.

It is observed that the copper complexes were formed by the coordination of NA as a primary bidentate ligand with 2-substituted pyridines (2Hy, 2Am and Pi). The bidentate NA used carbonyl moiety and ring nitrogen atom as electron donating group, which caused the C–N shift from 1324 cm^{-1} to higher frequency range of 1348–1389 cm^{-1}. Apparently, the carbonyl absorptions of complexes **1**–**3** were not observed. Likewise, the 2-substituted pyridine was coordinated to the central copper atom as bidentate ligands. In this regard N–H bending of **1** at 781 and 1575 cm^{-1} disappeared with a shift of C–O stretching from 1156 cm^{-1} to higher frequency. This suggested that the copper

Table 2
Physicochemical parameters of pyridine–copper complexes and its ligands.

Compound	Chemical formulae	Formula weight (g/mol)	Color	Melting point (°C)	Yield (%)	μ_{eff} (BM)
NA	$C_6H_5NO_2$	123.11	White	236–239	–	–
2Hy	C_5H_5NO	95.10	White	105–107	–	–
1	$C_{11}H_{10}CuN_2O_4$	297.75	Cyan	>300	84	1.5545
2Am	$C_5H_6N_2$	94.11	White	54–58	–	–
2	$C_{11}H_{11}CuN_3O_3$	279.76	Aquamarine	>300	87	1.9381
Pi	$C_6H_5NO_2$	123.11	White	139–142	–	–
3	$C_{12}H_{10}CuN_2O_4$	309.76	Blue	>300	75	1.7014

μ_{eff}, Magnetic moment; B.M., Bohr magnetron.

Table 3
IR spectra of pyridine-copper complexes and its ligands.

Compound	υC=O	υC–O	υC–N	υO–H	δO–H	υNH	δNH
NA	1718(s), 1700 (s)	1299(s)	1324(s)	3072–2449(w)	1418(s)	–	–
2Hy	1690(s), 1652(vs)	1156(s), 1098(m)	1243(s)	–	–	–	1575(br, s), 781(vs)
1	–	1191(w), 1093(w), 1053(w)	1389(m), 1375(m)	3400(br, w)	1458(m)	–	–
2Am	–	–	1492(s), 1442(s), 1326(m)	–	–	3448(sh, s), 3315(sh, w)	772.84(s)
2	–	1191(s), 1155(w), 1093(m), 1050(m)	1386(s), 1375(s)	3400(br, w)	1426(m)	3116(sh, w)	760.72(s)
Pi	1718(w), 1700(w)	1294(s), 1165(w), 1086(w)	1342(m)	2609(br, w), 2362(m), 2341(m)	1454(s)	–	–
3	–	1286(s), 1152(w), 1049(s)	1348(vs)	3400(br, w)	1475(w)	–	–

Note: vs = Very strong, s = strong, m = medium, w = weak, br = broad, sh = shape.

atom of complex **1** formed a complex by means of the bidentate amino ketone (2-pyridone). Similarly, the coordination of the copper atom from complex **2** is made possible via the amino-pyridine (2Am) ligand, which is evident by the disappearance of NH_2 stretching absorptions and the shift of C–N stretching. As for complex **3**, Pi is also a bidentate ligand which uses the ring nitrogen atom and the carbonyl group to coordinate with the copper atom.

Magnetic susceptibility determination showed that complexes **1**–**3** were paramagnetic with a magnetic moment (μ_{eff}) of 1.55, 1.94 and 1.70 BM, respectively. The μ_{eff} value indicates that the complexes were of tetrahedral geometry with a copper center. Based on the IR spectra and μ_{eff}, we can confirm that complexes **1**–**3** were of copper tetrahedral geometry.

3.2. Superoxide scavenging activity

Complexes **1**–**3** were assayed for SOD activity using the modified approach [11,38] which involved measuring the inhibition of NBT reduction. The results (Table 4) revealed that all of the tested complexes exhibited SOD activity with IC_{50} in the range of 49.07–130.23 μM. Complex **1** was the most powerful superoxide scavenger with an IC_{50} value of 49.07 μM. In addition, the SOD activity of complex **2** was higher than that of complex **3** with IC_{50} values of 50.32 and 130.23 μM, respectively. The SOD activity of these complexes are shown to be in the order of **1** > **2** > **3**. It should be noted that the activity of complex **2** was comparable to that of **1** as observed from the IC_{50} values of 49.07 and 50.32, respectively. Furthermore, the SOD activity of the free ligands NA, 2Hy, 2Am and Pi were elucidated and was found to be very low to the point that the IC_{50} value could not be obtained. The result strongly suggests that the copper atom is of prime importance for the SOD activity as its coordination to the ligands profoundly increased the SOD activity. Such phenomenon can be attributed to the change in oxidation state of the copper atom upon coordination with the ligands. Fascinatingly, these copper complexes were significantly small in molecular weight than the native enzyme. Moreover, these copper coordination compounds are easily formed from bioactive molecules such as nicotinic acid.

Table 4
Superoxide dismutase activity of copper complexes.

Compound[c]	IC_{50} (μM)[a]
1	49.07
2	50.32
3	130.23
SOD[b]	0.0026

[a] IC_{50} was defined as fifty percent inhibition concentration of NBT reduction.
[b] Superoxide dismutase from bovine erythrocytes was a homodimeric protein.
[c] Substituted pyridines (ligands) mimic very low activity that the IC_{50} cannot be obtained.

3.3. Antimicrobial activities

Nicotinic acid has been widely used for hyperlipidemic treatment. The biological activities of 2-hydroxypyridine, 2-amino-pyridine and picolinic acid have previously been reported [19,27,30]. The exploration of antimicrobial activities for the complexes of nicotinic acid and its derivatives is a relatively new endeavor. Results (Table 5) demonstrated that complexes **1**–**3** could completely inhibit the growth of *Bacillus subtilis* ATCC 6633 with MIC of 256 μg/mL. In addition, the tested complexes **1**–**3** could partially inhibit the growth of *Candida albicans* ATCC 90028 with MIC in the range of 128–256 μg/mL. Such augmentation in the observed antimicrobial activity of the metal complexes over those of the free ligands could be attributed to the chelation effect [43,44]. As a result, this alters the lipophilic property of the central metal ion thereby allowing its permeation through the cell membrane to exert its antimicrobial activity [45]. In parallel with

Table 5
Antimicrobial activity of pyridine–copper complexes.

Compound	Concentration (μg/mL)	Activity
Ampicillin	25	Active[a]
NA	256	Not active
$CuCl_2$	64	Not active[h]
	32	Not active[h]
2Hy	256	Not active
1	256	Active[b,e]
	128	Active[c, f]
	64	Not active
2Am	256	Not active
2	256	Active[b, f]
	128	Active[d, g]
	64	Not active
Pi	256	Not active
3	256	Active[b, f]
	128	Active[d, g]
	64	Not active

[a] Active (100% antigrowth) against *P. aeruginosa* ATCC 15442, *S. putrefaciens* ATCC 8671, *A. xylosoxidans* ATCC 2706, *S. aureus* ATCC 25923, *S. epidermidis* ATCC 12228, *E. faecalis* ATCC 29212, *B. subtilis* ATCC 6633, *S. cerevisiae* ATCC 2601, *S. pyrogenes* II, *S. enteridis* type C, *P. shigelloides*, *L. monocytogenes*.
[b] Active against *B. subtilis* ATCC 6633 with 100% antimicrobial activity.
[c] Active against *B. subtilis* ATCC 6633 with 75% antimicrobial activity.
[d] Active against *B. subtilis* ATCC 6633 with 50% antimicrobial activity.
[e] Active against *C. albicans* ATCC 90028 with 75% antimicrobial activity.
[f] Active against *C. albicans* ATCC 90028 with 50% antimicrobial activity.
[g] Active against *C. albicans* ATCC 90028 with 25% antimicrobial activity.
[h] $CuCl_2$ was tested against *B. subtilis* ATCC 6633 and *C. albicans* ATCC 90028.

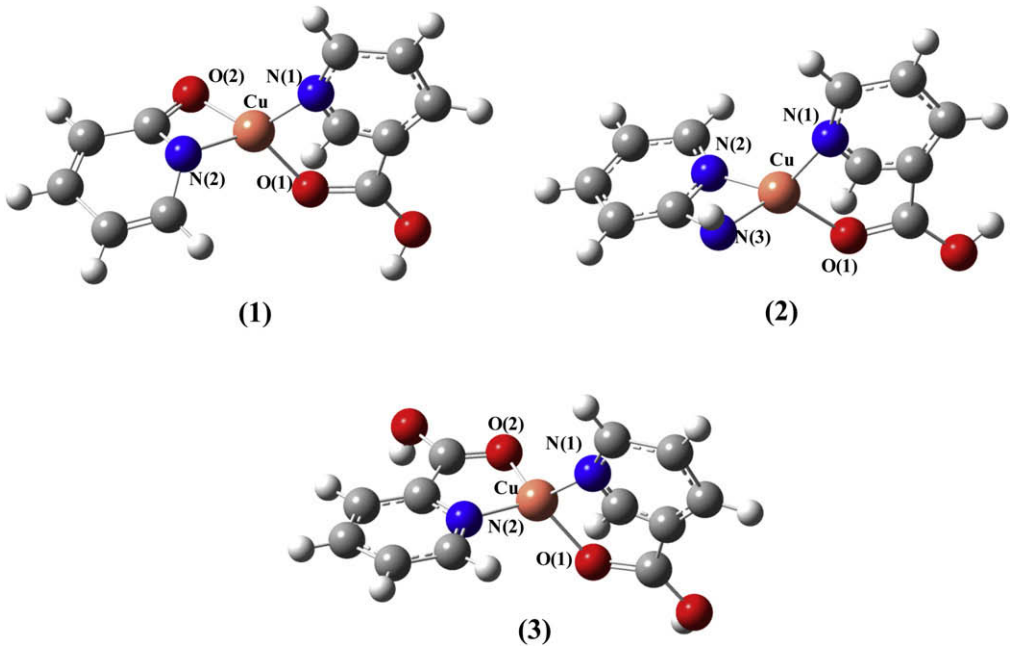


Fig. 2. Geometrically optimized structures of complexes **1**–**3** calculated at B3LYP/LANL2DZ level.

our results, previous studies have found that the free ligands used in this study, comprising of 2-hydroxypyridine [46], 2-aminopyridine [47], and picolinic acid [24], possessed antibacterial, antifungal, and antiviral activities. To discern whether the observed antimicrobial activities arose from the ligand themselves or as a result of coordinating with the metal ions, the biological activities of the uncomplexed ligands were examined. Antimicrobial activities were observable only in the metal complexes and not in the uncomplexed ligands, therefore confirming the participating role of the metal ion in the biological activities.

3.4. Theoretical studies of SOD mimics by computational chemistry

Quantum chemical parameters of the metal complexes were calculated using the minimum energy conformers in order to deduce quantitative relationships of the theoretical descriptors with the superoxide scavenging activity by performing Density Functional Theory calculations at B3LYP/LANL2DZ level. The fully optimized structures of complexes **1**–**3** were of tetragonally distorted geometry as shown in Fig. 2. The calculated theoretical parameters, e.g. binding energy (BE) and electron affinity (EA), has previously been reported to be good descriptors for characterizing superoxide radical scavenging activity [42,48–50]. It is well established that EA is a suitable parameter in accounting for the rate of electron transfer from superoxide anion to copper ion. Ji and Zhang previously reported that compounds with the lowest EA possessed the highest electron transfer rate, thereby conferring the highest SOD activity [50]. The calculated results for EA as shown in Table 5 were in the following order **1** > **2** > **3** and their respective values are −100.40, −109.39, and −162.71 kcal/mol, respectively. The observed trend is positively correlated with the SOD activity where complex **1** had the highest SOD activity as well as the highest calculated EA. Such findings contradict with those previously reported by Branco et al. [51,52] where an inverse relationship was observed for the calculated EA and the SOD activity. A plausible explanation for such discrepancy can be made on the basis of differences in the coordination geometry of the copper coordination complexes used in this study (tetrahedral) and those previously reported by Ji et al. (square planar and square pyramidal) [49,50]. Such observation is further corroborated by previous studies on active site distortion of CuZnSOD [51,52].

Examination of the energy level of HOMO revealed that complex **3** possessed the highest HOMO energy with a value of −3.32 eV while coordination compounds **2** and **1** gave HOMO energies of −4.65 and −5.09 eV, respectively (Table 6). Higher HOMO energy as deduced from the frontier molecular orbital indicates that the molecule can readily transfer electron to the unoccupied orbital of the metal ion thereby indicating strong binding capacity [53]. Such molecules displaying high binding capacity was demonstrated to also exhibit lower SOD activity [54,55]. This coincides with our findings that complex **1** possessed the highest SOD activity as well as having the lowest HOMO energy and vice versa for complex **3** which exhibited the lowest SOD activity while having the highest HOMO energy. An analysis of the calculated energy level for the LUMO indicates the order of **3** > **2** > **1** for the coordination compounds bearing values of −2.61, −2.96, and −2.99, respectively. It was previously reported by Schepetkin et al. [55] that low LUMO energy was important for superoxide dismutation. Moreover, the calculated energies of HOMO and LUMO were significantly correlated with the SOD activity which was represented as $-\log(1/IC_{50})$, yielding r of 0.97 and 0.99, respectively. The results clearly suggest that the energies of HOMO and LUMO are potentially useful theoretical parameters for elucidating superoxide scavenging activity, a phenomenon involving charge- or electron-transfer.

In characterizing the physicochemical properties of the metal complexes, it is worthy to consider the tendency for nicotinic acids to dissociate a proton from the carboxylic acid moiety to form a carboxylate anion. Such notion is valid when nicotinic

Table 6
Quantum chemical parameters of copper complexes calculated at B3LYP/LANL2DZ level.

Complex	TE_{CuII} (hartree)	TE_{CuI} (hartree)	EA[a] (kcal/mol)	HOMO (eV)	LUMO (eV)
1	−955.7254	−955.8854	−100.40	−5.09	−2.99
2	−935.8071	−935.9814	−109.39	−4.65	−2.96
3	−1069.5318	−1069.7911	−162.71	−3.32	−2.61

[a] $EA = TE_{Cu(I)} - TE_{Cu(II)}$.

Table 7
Selected bond lengths and angles of complexes **1**–**3**.

Complex	Bond length (Å)		Angle (°)	
1	Cu–N(1)	1.8013	N(1)–Cu–N(2)	124.420
	Cu–O(1)	2.0100	N(1)–Cu–O(1)	90.624
	Cu–N(2)	1.9076	N(1)–Cu–O(2)	124.119
	Cu–O(2)	1.8561	N(2)–Cu–O(1)	123.331
			N(2)–Cu–O(2)	74.389
			O(1)–Cu–O(2)	124.407
2	Cu–N(1)	1.8014	N(1)–Cu–N(2)	124.390
	Cu–O(1)	2.0105	N(1)–Cu–N(3)	126.729
	Cu–N(2)	1.8336	N(1)–Cu–O(1)	89.214
	Cu–N(3)	1.8542	N(2)–Cu–N(3)	73.359
			N(2)–Cu–O(1)	125.380
			N(3)–Cu–O(1)	122.785
3	Cu–N(1)	1.7990	N(1)–Cu–N(2)	116.156
	Cu–O(1)	2.0031	N(1)–Cu–O(1)	92.119
	Cu–N(2)	1.8255	N(1)–Cu–O(2)	122.541
	Cu–O(2)	1.8221	N(2)–Cu–O(1)	122.623
			N(2)–Cu–O(2)	91.883
			O(1)–Cu–O(2)	114.330

acids are present as free ligands. However such situation may not apply in our case since the ligand is coordinated to a metal ion. Such finding coincides with previous observation that metal coordination exerts direct effect on the ligand's acidity [56]. Correspondingly, Bóka et al. had observed in their efforts to mimic the SOD activity that strong electron donating ability of ligands is detrimental towards SOD activity [57]. The coordination complexes was shown to possess good SOD activity which implied that the coordinated nicotinic acids should be in the neutral form whereas the anionic form are expected to possess greater electron donating properties, which would consequently hinder its reactivity with superoxide anions. Similar findings were also observed in our previous investigation on mixed ligand complexes of copper with nicotinic and other selective carboxylic acids (phthalic, salicylic and anthranilic acids) [12]. It was shown that the complex constituting stronger electron withdrawing group exhibited better SOD activity.

The contributions of metal–ligand binding affinity on the observed SOD activity was investigated using information such as bond lengths and angles as derived from geometrically optimized structures at B3LYP/LANL2DZ level (Table 7). It was observed that the mean bond distances at the axial position for Cu–N(1) and Cu–O(1) of complexes **1**–**3** were 1.801 and 2.008 Å, respectively. The longest axial distance for Cu–N(2) and Cu–O(2) bonds corresponds to that of complex **1** with 1.908 and 1.856 Å, respectively. The bond lengths for Cu–N(2) and Cu–N(3) at axial position for complex 2 were observed to be 1.834 and 1.854 Å, respectively, which was shorter than that of complex **1**. This is attributed to the fact that the amino group of 2-aminopyridine from complex **2** possessed greater electron donating capability than the hydroxyl group of 2-hydroxypyridine from complex **1** as indicated by the higher HOMO energy of **2** (Table 6). The shortest axial bond lengths for Cu–N(2) and Cu–O(2) were observed in complex **3** which gave values of 1.826 and 1.822 Å, respectively. This result coincides with the fact that complex **3** possessed the lowest SOD activity which could be attributed to the higher metal binding affinity. Previous study by Li et al. has reported that long axial bond lengths were critical for the dismutation of superoxide anion [58]. Interestingly, complex **1** which had the longest axial bond length was also shown to possess the highest SOD activity. The calculated bond angles for the three coordination complexes further corroborate a distorted tetragonal geometry with bond angles in the range of 73.35°–126.73°.

4. Conclusion

We report the synthesis of novel copper complexes **1**–**3** with nicotinic acid as the primary ligand along with 2-substituted pyridines: 2-hydroxypyridine, 2-aminopyridine and picolinic acid. The experimentally derived magnetic moments and the calculated bond angle suggest a tetragonally distorted geometry for the coordination complexes. Nicotinic acid is a bidentate ligand, which utilizes the ring N-atom of pyridine and the carbonyl group of carboxylic acid. Likewise, the 2-substituted pyridines are also bidentate ligands making use of the following functional groups: amino ketone of 2-pyridone (2Hy), aminopyridine (2Am) and carbonyl pyridine (Pi), respectively. All of the coordination compounds used in this study displayed promising superoxide radical scavenging properties as well as antimicrobial activities against *B. subtilis* ATCC 6633 and *C. albicans* ATCC 90028. Of these compounds, complex **1** displayed the greatest superoxide scavenging activity with IC_{50} of 49.07 μM, while complex **1** possessed the highest antimicrobial activity with IC_{50} of 256 μg/mL. The physicochemical parameters as derived from low energy conformer calculated at B3LYP/LANL2DZ level were well correlated with the observed superoxide scavenging properties. In particular, it has been shown that HOMO and LUMO energies are useful theoretical parameters for elucidating the superoxide scavenging activity as observed from the correlation coefficient of 0.97 and 0.99, respectively. Such coordination complexes described herein demonstrate great potential for the development of value-added metallovitamins for therapeutic applications.

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