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Research Article
Synthesis, Characterization and Antimicrobial Activities of Copper Derivatives of NHC-II Complexes

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Abstract

Background: Caffeine, 1, 3, 7-trimethylxanthine is one of the xanthine derivatives that are for the most part utilized as a part of solutions as diuretics. The Cu (II) complexes have been synthesized from the N-heterocyclic carbene ligands. **Materials and Methods:** The Cu (II) NHC complexes were characterized using analytical and spectral techniques. Antibacterial and antifungal activities of the Cu (II) NHC complexes were determined using the reported techniques. The SOD activity was assayed using nitrobluetetrazolium as O_2 scavenger. **Results:** The X-band ESR spectra of the copper complexes in DMSO solution at 300 and 77 K were recorded and their salient features are reported. The *in vitro* biological screening effects of the investigated compounds were tested against the bacterial species, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Pseudomonas aeruginosa* and fungal species, *Aspergillus niger*, *Rhizopus stolonifer*, *Aspergillus flavus*, *Rhizoctonia bataicola* and *Candida albicans* by serial dilution method. **Conclusion:** The Cu (II) complexes exhibit square planar geometry. A comparative study of inhibition values of the individual metals and their complexes indicate that the complexes exhibit higher antimicrobial activity than the individual metals. Superoxide dismutase and reducing power activities of the copper complexes have also been studied. Depending on the molecular structure, the Cu (II) NHC complex possess promising SOD mimetic activities. Further we are trying to explore more biological properties of Cu (II) NHC complexes *in vitro* and *in vivo*.

Key words: NHC complexes, biological screening, disc diffusion, inhibition, superoxide dismutase

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Caffeine 1, 3, 7-trimethylxanthine is one of the xanthine derivatives that are for the most part utilized as a part of solutions as diuretics, focal sensory system stimulants and inhibitors of cyclic adenosine monophosphate (c-AMP) phosphodiesterase[1]. It is the most mainstream methylxanthine because of its business accessibility, minimal effort and low danger. The vicinity of the methylimidazole moiety in the structure of caffeine makes it an important possibility for the blend of a N-heterocyclic carbene. The initial step is to acquire an imidazolium cation, for example, 1, 3, 7, 9-tetramethylxanthinium or methylated caffeine from caffeine. Already, arrangement of methylated caffeine with hydroxide[2], methylsulfate[1,3], tosylate[3] and iodide[4] anions were accounted for. The physiological activity of 1, 3, 7, 9-tetramethylxanthinium hydroxide in frogs was explored[2]. After presentation of the accompanying work, another NHC antecedent of methylated caffeine with tetraflouroborate anion was additionally reported by Herrmann and coworkers[5].

Several metal complexes of xanthines incorporating caffeine in which xanthines are connected to metal by means of N-9 iota were synthesized[6]. However, to the best of our insight development of a copper (II) NHC complex from caffeine has not been accounted for. We have reported in this the amalgamation and portrayal of two bis (NHC) copper (II) edifices 2a, 2b from methylated caffeine 1a, 1b and a dinuclear copper (II) bis (NHC) complex, 2d with the crossing over nitrate units from nitrate salt of the methylated caffeine 1d (Fig. 1)[7].

MATERIALS AND METHODS

General conditions: All the reactions were carried out in air. The compounds dimethyl sulfate and Cu_2O were purchased from Aldrich and used without further purification. Caffeine was purchased from Sigma and used without further purification. The 1H and 13C-NMR spectra were measured on a Varian XL-400 MHz spectrometer with DMSO-d6 as solvent at room temperature and tetramethylsilane (TMS) as the internal standard. The LC-MS were recorded on a 2010A LQC system (Finnigan MAT) with MeCN as mobile phase. The UV-visible spectra were recorded on an Elico Bio-spectrophotometer model BL198. Emission spectra were carried out by using an Elico Bio-spectrofluorimeter model SL174 at room temperature.

Synthesis of 1, 3, 7, 9-tetramethylxanthinium methyl sulfate[1] (1a): Caffeine (10.0 g, 51.5 mmol) was dissolved in nitrobenzene (150 mL) at 100°C for 1 h. Dimethyl sulfate (10.5 mL) was added to the solution and the mixture was refluxed at 100°C for 24 h. The reaction mixture was cooled to room temperature, excess diethyl ether was added and the solvent was decanted. The residue was washed with diethyl ether several times and a white solid 1a (2.38 g, 7.43 mmol, 72%) was obtained. Mp: 175°C. 1H NMR (300 MHz, D_2O): δ 8.93 (s, 1H, NCHN), 4.17 (s, 3H, CH_3), 4.05 (s, 3H, CH_3), 3.73 (s, 3H, CH_3), 3.35 (s, 3H, O_3SOCH_3), 3.27 (s, 3H, CH_3). 13C {1H} NMR (75 MHz, D_2O): δ 154.9 (C=O), 151.8 (C=O), 140.1 (C=C), 139.8 (NCHN), 109.0 (C=C), 55.3 (O_3SOCH_3), 37.2, 36.0, 31.9, 28.8 (NCH_3). ESI-MS: m/z [M^+] calcd for $C_{10}H_{16}N_4O_6S$ 209.2, found 209.1.

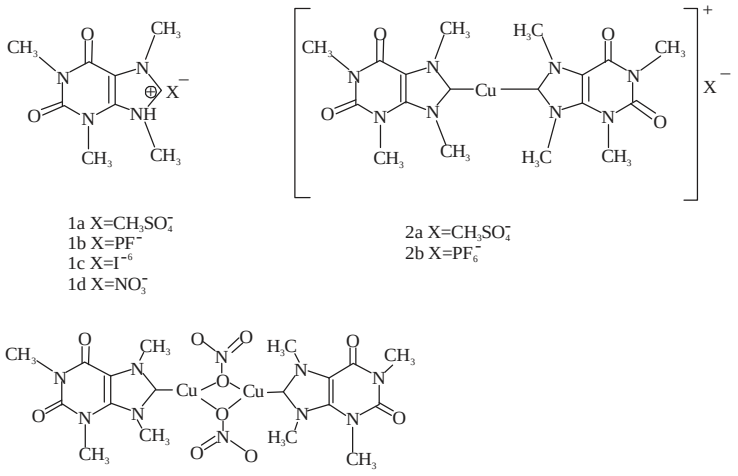

Fig. 1: Methylated caffeine salts and (II) complexes

Synthesis of 1, 3, 7, 9-tetramethylxanthinium hexafluorophosphate (1b): Compound 1a (1.44 g, 4.50 mmol) was dissolved in water and NH_4PF_6 (0.75 g, 4.6 mmol) was added. The white crystalline product 1b (1.20 g, 3.39 mmol, 76%) was obtained by filtration. Mp: 240°C. Anal. Calcd for $C_9H_{13}F_6N_4O_2P$: C, 30.52; H, 3.70; N, 15.82. Found: C, 30.40; H, 3.71; N, 15.71. 1H NMR (300 MHz, d6-DMSO): δ 9.25 (s, 1H, NCHN), 4.13 (s, 3H, CH_3), 4.05 (s, 3H, CH_3), 3.73 (s, 3H, CH_3), 3.27 (s, 3H, CH_3). 13C {1H} NMR (75 MHz, d6-DMSO): δ 153.4 (C=O), 150.3 (C=O), 139.7 (C=C), 139.4 (NCHN), 107.9 (C=C), 36.8, 35.7, 31.4, 28.5 (NCH_3). FAB-MS (m/z): [M+] calcd for C9H13F6N4O2P, 209.2; found 209.0.

X-ray crystal structure analysis of 1b: Formula $C_9H_{13}F_6N_4O_2P$, Mw = 354.20, colorless crystal 0.20×0.10×0.02 mm, a = 12.776 (1) Å, b = 6.4242 (7) Å, c = 17.118 (2) Å, α = 90°, β = 111.836 (2)°, γ = 90°, V = 1304.1 (3) Å3, Dcalc = 1.804 Mg cm^3, μ = 0.299 mm^{-1}, Z = 4, monoclinic, space group P21/c (No. 14), λ = 0.71073Å, T = 100 K, ω and Ψ scans, 8899 reflections collected, 2290 independent (Rint = 0.0236), 252 refined parameters, R1/wR2 ($I \geq 2\sigma(I)$) = 0.0528/0.1249 and R1/wR2 (all data) = 0.0584/0.1285, maximum (minimum) residual electron density 0.779 (-0.366) e Å-3, hydrogen atoms were found from the difference map and their positions refined.

Synthesis of NHC copper (II) complex (2a): Compound 1a (0.64 g, 2.0 mmol) was dissolved in water (72 mL) and Cu_2O (0.285 g, 2.0 mmol) was added. The mixture was stirred at room temperature for 2.5 h. The bluish brown suspension was filtered to give a bright blue solution. The volatiles were removed in vacuo. Compound 2a (0.52 g, 0.82 mmol, 92%) was obtained as an bluish solid. Mp: 125°C. The 1H NMR (300 MHz, D_2O): δ 4.21 (s, 6H, CH_3), 4.13 (s, 6H, CH_3), 3.82 (s, 6H, CH_3), 3.36 (s, 3H, O_3SOCH_3), 3.27 (s, 6H, CH_3). The 13C {1H} NMR (75 MHz, D_2O): δ 187.1 (C-Cu), 155.0 (C=O), 152.1 (C=O), 141.1 (C=C), 110.1 (C=C), 55.6 (O_3SOCH_3), 39.5, 38.2, 37.0, 35.9, 32.2, 31.9, 28.8 (d, CH_3). The ESI-MS (m/z): [M+] calcd for $C_{19}H_{27}CuN_8O_8S$, 523.1; found 523.1.

Synthesis of NHC copper (II) complex (2b): 1b (1.4 g, 4.0 mmol) was dissolved in DMSO (144 mL) and Cu_2O (0.571 g, 4.0 mmol) added. The mixture was stirred at 60°C for 2.5 h to form a brown suspension. After filtration a clear, pale blue solution was obtained. The volatile compounds were removed in vacuo to yield the brick red solid 2b (2.59 g, 3.87 mmol, 97%). Mp: 205°C. 1H NMR (300 MHz, d6-DMSO): δ 4.15 (s, 6H, CH_3), 4.01 (s, 6H, CH_3), 3.72 (s, 6H, CH_3), 3.38 (s, 2H, H_2O), 3.21 (s, 6H, CH_3). The 13C {1H} NMR (75 MHz, d6-DMSO): δ 186.6 (C-Cu), 153.0 (C=O), 150.3 (C=O), 140.2 (C=C), 108.6 (C=C), 40.1, 37.5, 31.2, 27.9 (N-CH_3). FAB-MS (m/z): [M$^+$] calcd for $C_{18}H_{24}CuF_6N_8O_4P$, 523; found 523.

X-ray crystal structure analysis of 2b: Formula $C_{32}H_{40}CuF_6N_8O_4P$, Mw = 853.56, light blue colour crystal 0.33×0.18×0.10 mm, a = 32.090 (12) Å, b = 6.590 (2) Å, c = 8.354 (3) Å, α = 90°, β = 90°, γ = 90°, V = 1766.6 (11) Å3, Dcalc = 1.605 Mg cm^3, μ = 0.697 mm^{-1}, Z = 2, orthorhombic, space group 1 mm^2 (No. 44), λ = 0.71073Å, T = 100 K, ω and Ψ scans, 7814 reflections collected, 2332 independent (Rint = 0.0273), 174 refined parameters, R1/wR2 ($I \geq 2\sigma(I)$) = 0.0294/0.0762 and R1/wR2 (all data) = 0.0307/0.0857, maximum (minimum) residual electron density 0.559 (-0.494) e Å-3, all hydrogen atoms were calculated and refined as riding atoms.

Synthesis of 1, 3, 7, 9-tetramethylxanthinium nitrate (1d): 1, 3, 7, 9- tetramethylxanthinium iodide (3.27 g, 9.72 mmol) was dissolved in 25 mL acetonitrile and $AgNO_3$ (1.65 g, 9.72 mmol) was added. The mixture was stirred and the yellow precipitate was filtered. After removal of the solvent, a white crystalline product (1.56 g, 5.74 mmol, 85%) was obtained. MP: 184-186°C. Anal. Calcd for $C_9H_{13}N_4O_2$: C, 39.85; H, 4.83; N, 25.82. Found: C, 39.75; H, 4.70; N, 26.14. 1H NMR (300 MHz, d6-DMSO): δ 9.33 (s, H, CH), 4.15 (s, 3H, CH_3), 4.05 (s, 3H, CH_3), 3.73 (s, 3H, CH_3), 3.26 (s, 3H, CH_3). The 13C {1H} NMR (75 MHz, d6-DMSO): δ 153.3 (C=O), 150.2 (C=O), 139.7 (NCHN), 139.3 (C=C), 107.7 (C=C), 36.7, 35.5, 31.2, 28.3 (CH_3). The ESI- MS (m/z): [M$^+$] calcd for $C_9H_{13}N_4O_2$ 209.1; found 209.1.

Synthesis of bis (1, 3, 7, 9-tetramethylxanthine-8-ylidene) copper (II) complex with bridging nitrates (2d) 1, 3, 7, 9-tetramethylxanthinium nitrate (1.56 g, 5.74 mmol) was dissolved in acetonitrile (100 mL) by stirring at 50-60°C and cooled to room temperature. Copper oxide (0.079 g, 5.74 mmol) was added. The mixture was stirred at room temperature for 2.5 h and filtered. The volatiles were removed in vacuo. The light blue color solid was recrystallized in methanol/acetonitrile mixture (1:4 by volume) (0.70 g, 0.93 mmol, 32%). MP: 239-241°C. Anal. Calcd for $C_{18}H_{24}CuN_{10}O_{10}Cu_2$: C, 28.59; H, 3.20; N, 18.52. Found: C, 28.58; H, 3.09; N, 18.74. 1H NMR (300 MHz, d6-DMSO): δ 4.17 (s, 3H, CH_3), 4.02 (s, 3H, CH_3), 3.74 (s, 3H, CH_3), 3.24 (s, 3H, CH_3). The 13C {1H} NMR (75 MHz, d6-DMSO): δ 187.1 (C-Ag), 153.2 (C=O), 150.6 (C=O), 140.5 (C=C), 108.9 (C=C), 39.2, 37.8, 31.5, 28.2 (CH_3). The ESI-MS (m/z): 525.2 $[C_{18}H_{24}CuN_8O_4]^+$, 315.1 $[C_9H_{12}CuN_4O_2]^+$.

X-ray crystal structure analysis of 2d: Formula $C_{18}H_{24}Cu_2N_{10}O_{10}$, Mw = 756.21, blue color crystal 0.38×0.11×0.09 mm, a = 8.4940 (11) Å, b = 8.8197 (12) Å, c = 9.1395 (12) Å, α = 73.089 (2)°, β = 66.525 (2)°, γ = 76.990 (2)°, V = 596.18 (14) Å3, Dcalc =2.106 Mg cm^3, μ = 1.721 mm^{-1}, Z = 1, triclinic, space group P-1, λ = 0.71073Å, T = 100 K, ω and Ψ scans, 5329 reflections collected, 2775 independent (Rint = 0.0274), 185 refined parameters, R1/wR2 (I≥2σ(I)) = 0.0390/0.0982 and R1/wR2 (all data) = 0.0433/0.1012, maximum (minimum) residual electron density 1.370 (-1.738) e Å-3.

Maintenance of bacterial cultures: All the bacterial cultures were maintained in nutrient broth at 37°C. For short-term storage they were maintained on nutrient agar slants. Glycerol stocks were prepared for long-term storage. The cultures were revived for experiments. A loopful of culture was inoculated into nutrient broth and incubated for 18 h at 37°C. The culture was then given three passages before it was used to determine Minimum Bactericidal Concentration (MBC). Only log phase culture was used for experiments[8].

Maintenance of fungal cultures: The fungal cultures were maintained on PDA slants. A loopful of fungal spores was inoculated into the broth and incubated for 48 h on a gyratory shaker at 30°C. The cultures were sub cultured three times before they were used for antifungal assays.

Antibacterial study by the zone of inhibition assay: All complexes were tested for their antibacterial activity by the zone of inhibition assay. For this purpose, filter paper discs of 5 mm diameter were prepared from Whatman No. 1 filter paper 5, 10 and 20 μmol L^{-1} complexes were loaded onto the disc. The discs were placed on PDA plates spread with bacterial culture. The plates were incubated at 30°C for 2 days after which the zone of inhibition was measured.

Determination of MBC of the copper (II) NHC complexes using nonpathogenic bacteria: Nonpathogenic bacterial strains were used for measuring the MBC of the Cu (II) NHC complexes by serial dilution method[9]. The essential steps are mentioned. A twofold serial dilution of the complex was carried out in the nutrient broth and (or) sucrose peptone broth. Each test tube was inoculated with 10^4-10^5 cells mL^{-1} of actively growing bacterial culture in log phase. The cultures (tubes) were incubated at 37°C for 24 h. After incubation, a loopful of these treated cultures was streaked on the nutrient agar and (or) sucrose peptone agar plates. The plates were incubated and were checked for the growth of bacteria and the MBC of each test complex was determined. The MBC was expressed in microlitres per millilitre (v/v).

Control: Control cultures (in absence of complexes) were maintained to check the growth of the organism. The MBC of commonly used antibiotics, viz., ciprofloxacin, gentamicin, ampicillin, streptomycin and chloramphenicol was determined to compare the effectiveness of complexes.

Determination of time course of lethal action of complexes: Initially, the MBC of the complexes and ampicillin was determined. Then the bacteria (*E. coli*, *Xanthomonas oryzae* and *Staphylococcus aureus*) were treated with test compounds at MBC levels. At regular time intervals, aliquots of treated culture were drawn, diluted appropriately and plated on incubated for colony development. The number of colonies was counted and the number of viable cells was estimated.

Antifungal assay by disc diffusion method: The antifungal testing was conducted using standard disc diffusion assay, according to the procedures of Kumar *et al.*[10] and Elgayyar *et al.*[11] on PDA medium. After sterilization, plates were prepared at room temperature. Filter paper discs of 5 mm diameter and 5, 10 and 20 mol L^{-1} complexes were loaded onto the agar plates seeded with fungal spores. The plates were incubated at 30°C for 4 days and the zone of inhibition was measured.

SOD activity: *In vitro* SOD activity was measured using alkaline DMSO as a source of superoxide radical O_2^- and nitrobluetetrazolium (NBT) as O_2^- scavenger. Four hundred microliters sample to be assayed was added to a solution containing 2.1 mL of 0.2 M potassium phosphate buffer (pH 8.6) and 1 mL of 56 μM NBT. The tubes were kept in ice for 15 min and then 1.5 mL of alkaline DMSO solution was added while stirring. The absorbance was then monitored at 540 nm against a sample prepared under similar condition except NaOH which was absent in DMSO. A unit of superoxide dismutase (SOD) activity is the concentration of complex or enzyme, which causes 50% inhibition of alkaline dimethylsulphoxide (DMSO) mediated reduction of nitrobluetetrazolium chloride (NBT)[12,13].

RESULTS AND DISCUSSION

N-heterocyclic carbene ligands have proven very popular in the last 20 years. The electronic and steric modularity

associated with the resulting complexes have made NHCs obvious candidates when designing new metal complexes for catalysis. Conjugate reduction of α, ß-unsaturated ketones and esters, the hydrosilylation of ketones, the cyclopropanation of terminal alkenes, as well as olefinations, carbene transfer reactions, aziridination of olefins and methylenation of aldehydes are among some examples of the uses of Cu-NHC complexes (specifically (IPr) CuCl) in modern catalysis. Finally, these catalysts are air and moisture-stable and they can be used as precursors to synthesize more air-sensitive complexes[14].

Methylated caffeine 1, 3, 7, 9-tetramethylxanthinium 1a, was synthesized by the reaction of caffine with dimethyl sulphate in a 1:2 M ratio[1] and converted to 1b by ion exchange with NH_4PF_6. The 1H and 13C NMR spectra of 1a and 1b are similar and consistent with their molecular structures. In the 1H NMR spectra of 1a and 1b, the imidazolium protons appear at 8.93 and 9.25 ppm, respectively. This is consistent with the general C-H acidic proton shift of imidazolium salts (δ = 8-10)[12]. The 13C NMR shift of the N-C-N sp^2 carbons, that later become the carbene centers, appear at 139.8 and 139.4 ppm for 1a and 1b, respectively.

Reaction of water soluble ligand 1a with Cu_2O in water to form 2a: Complex 2a is stable in water and dark for 9 days. Similarly, ligand 1b readily reacted with Cu_2O in DMSO at 60°C to yield the copper NHC complex 2b in high yield. Complex 2b is stable in air and light up to its melting point and is only soluble in DMSO. It is stable in wet DMSO for months in the light. The formation of 2a and 2b can be monitored by changes in the 1H NMR and 13C NMR spectra (Fig. 2). The disappearance of the resonance for the imidazolium protons of 1a and 1b and the appearance of the resonance for the carbene carbon atoms at 187.1 and 186.6 ppm respectively, shows the formation of expected NHC copper carbene complexes. The lack of C-^{107}Cu and C-^{109}Cu couplings suggests fluxional behavior on the 13C NMR time scale as observed with many copper (II) complexes[15-18]. It has been reported that copper-carbene complexes without Cu-carbene couplings are useful as carbene transfer reagents due to their dynamic behaviour in solution[19-23].

Methylation of caffeine was also performed with methyl iodide in boiling DMF in order to obtain an NHC precursor, 1c with a biologically relevant anion. The procedure for the synthesis of 1c, its reaction with copper (II) precursors and antimicrobial applications are given. Compound 1c was dissolved in acetonitrile and one equivalent of $AgNO_3$ was added. The resulting copper (II) iodide precipitate was filtered off and solvent removed in vacuom to obtain 1d. The 1H NMR and 13C NMR spectra of 1d are very similar to spectra of 1a and 1b. The imidazolium proton resonance for 1d appears at 9.33 ppm. The ESI-MS analysis shows the $[M\text{-}NO_3]^+$ cation by a m/z peak at 209.1. Elemental analysis of crystals of 1d obtained from the slow evaporation of concentrated methanol solution confirms the presence of nitrate anion. The reaction of 1d with copper (I) oxide in 2:1 ratio in acetonitrile did not result in formation of a copper (I) complex even by heating. Complex 2d was formed by the reaction of 1d with excess copper (I) oxide (1:1 ratio) in acetonitrile at room temperature. The 2d is a light stable solid and was observed to be stable in water for 45 days. The 1H NMR and 13C NMR spectra of 2d are also very similar to spectra of 2a and 2b. Carbene carbon appearing at 187.1 ppm and ESI-MS spectrum show the existence of the $[Cu(NHC)_2]^+$ cation as well as $[Cu(NHC)]^+$. The molecular structure of complex 2d was revealed by x-ray diffraction studies[24,25].

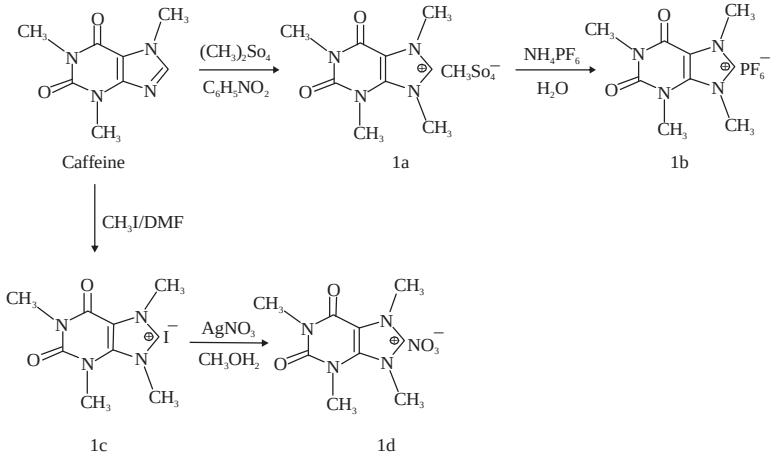

Fig. 2: Synthesis of 1a, 1b, 1c and 1d

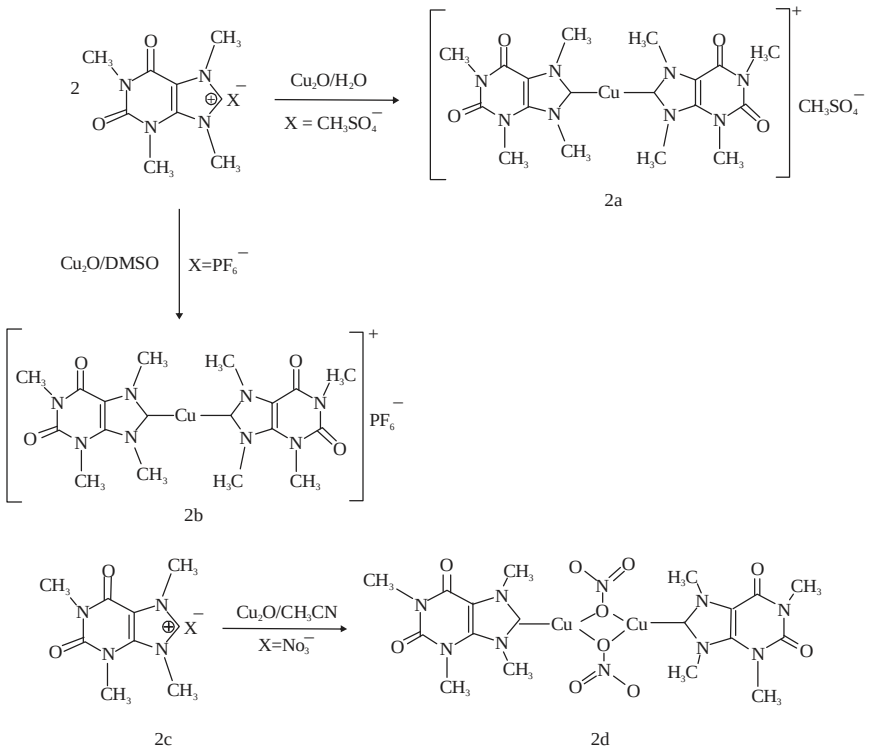

Fig. 3: Synthesis of 2a, 2b, 2c and 2d

Table 1: Selective bond lengths and angles for 1b, 2b and 2d

Bond lengths and angles	1b	2b	2d
N1-C1	1.325 (4) Å	1.327 (6) Å	1.361 (4) Å
N2-C1	1.297 (4) Å	1.434 (6) Å	1.372 (5) Å
N1-C1-N2	109.11 (3)°	106.5 (3)°	103.5 (3)°
C1-Cu	-	2.086 (4) Å	2.107 (4) Å
Cu1-O3	-	-	2.292 (2) Å
Cu1A-O3	-	-	2.347 (2) Å
C1-Cu-C1A	-	174.1 (3)°	-
C1-Cu1-O3	-	-	150.04 (12)°
C1-Cu1-O3A	-	-	141.91 (11)°
O3A-Cu1-O3A	-	-	65.09 (10)°

Single crystals of 1b were obtained by the anion exchange of 1a in water. The asymmetric unit of this molecule consists of one methylated caffeine cation and one hexafluorophosphate counteranion. The ligand 1b has an N1-C1-N2 angle of 110.9(3)°. The N1-C1 and N2-C1 bond lengths are 1.341 (4) Å and 1.311 (4) Å, respectively (Fig. 3, Table 1)[26].

Crystals of 2b suitable for single crystal x-ray diffraction studies were grown from a concentrated solution of DMSO and toluene (Fig. 4). The asymmetric unit contains one quarter of both the methylated caffeine copper complex and the disordered hexafluorophosphate anion together with toluene as a noncoordinating solvent molecule located on the (x, 0, z) mirror plane. The copper and phosphorus atoms are on special positions (0, 0, z) and (0, 1/2, z), respectively, with both having mm^2 symmetry. Complex 2b is a planar structure, crystallographically imposed with a copper carbene bond distance of 2.068 (4) Å. The C1-Cu-C1A bond angle is 171.4 (3)° that slightly deviates from the linear geometry expected for the complex[27].

Single crystals of 2d were grown from the concentrated 1:4 methanol/acetonitrile solution (Fig. 5). The two copper atoms are in distorted trigonal planar geometry with the bond angles of 150.04 (12)°, 141.91 (11)° and 65.09 (10)°. Copper carbene bond length is 2.086 (4) Å. Nitrate molecules act bridging ligands between two copper atoms. The copper-oxygen bond lengths of 2.292 (2) Å and 2.347 (2) Å are consistent with those reported for copper (II) nitrate complexes in the literature (Table 1). The distance between two copper atoms is too long to have a non-bonding interaction (Cu···Cu) distance of ~3.9 Å vs van der waals radii 3.44 Å. Although several copper (II) NHC complexes with bridging halides have been characterized, to the best of our knowledge copper (II) NHC complexes with bridging nitrate anions are rare[28,29].

Antimicrobial activity: The complexes were dissolved in water and tested for their antimicrobial activity. Complex concentrations ranging from 0-100 μmol L^{-1} were used in

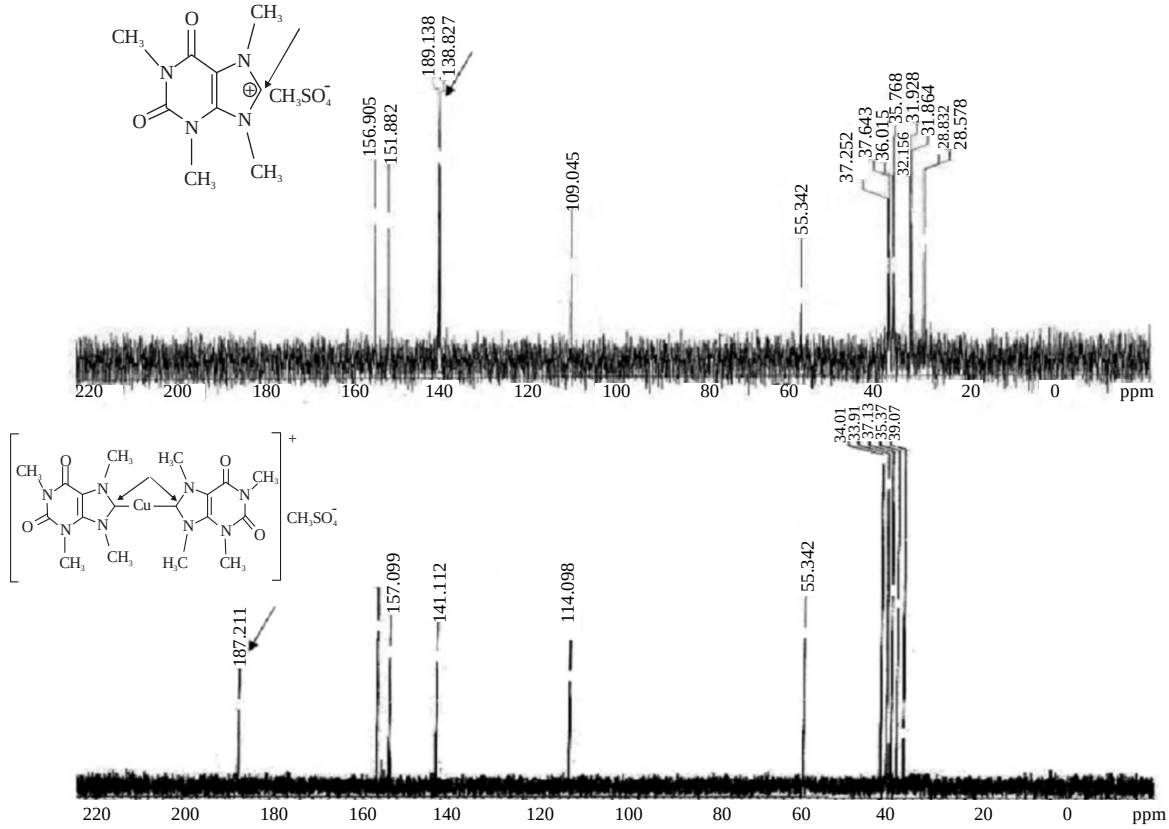

Fig. 4: Formation of 2a and 1a confirmed by 13C-NMR

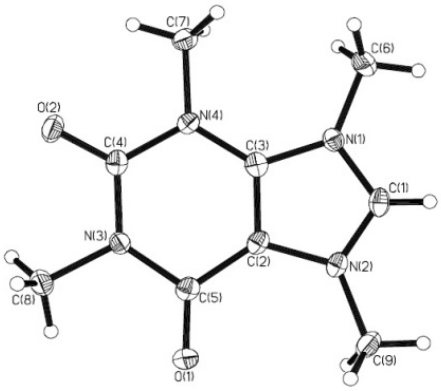

Fig. 5: Molecular structure of the cationic part of 1b (Thermal ellipsoids are drawn at 50% probability)

the initial antibacterial assay. Based on the results of this preliminary test, three different concentrations were chosen (5, 10 and 20 µmol L^{-1}) to check their efficiency in inhibiting microbial growth. The results presented in Table 2 indicate that complexes 1b, 2b and 2c had high antibacterial activity. Since copper alone has antimicrobial activity towards many microbes (bacteria and fungi), it was observed that Cu-NHC complexes showed their antimicrobial activity at a millimolar range, which was much larger than the complex concentrations used. However, they were found to have no effect on the growth of the bacteria. Similar antimicrobial results were reported by Tarafder *et al.*[30] and also by Patel *et al.*[31] on simple nickel (II) binary and ternary complexes.

Table 2: Antimicrobial activity of copper (II) NHC complexes on *Escherichia coli*

	Zone of inhibition (mm)		
Complex name	5 µmol	10 µmol	25 µmol
Copper	0.3	0.7	0.8
Complex-1b	0.9	1.4	2.6
Complex-2b	1.4	2.0	3.1
Complex-2d	2.0	2.9	4.7
Ampicillin	1.9	2.8	4.2
Strptomycin	2.0	2.7	4.6

MBCs of Cu (II) NHC complexes: The results in Table 3 indicate that the phen complex possesses the highest MBC. Very little of the complex (0.098–0.78 µL) is needed to inhibit the bacterial growth completely. Similar to the zone of inhibition results, Cu (II) NHC complexes showed higher MBCs, next to the phen complex. The remaining complexes also possessed a MBC but were little higher than that of the mentioned complexes. For comparision purpose, the MBC (in micrograms per millilitre) (m/v) of some commonly used antibiotics, viz.,

Table 3: Minimum Bactericidal Concentration (MBC) of copper (II) NHC complexes against nonpathogenic bacteria

	Minimum inhibitory concentration (μL mL^{-1})		
Bacterial species	Complex-1b	Complex-2b	Complex-2d
Escherichia coli	198.2	49.2	12.4
Salmonella typhimurium	49.6	99.2	99.2
Proteus vulgaris	6.2	3.1	1.56
Pseudomonas aeruginosa	99.2	49.6	24.8
Staphylococcus aureus	24.8	24.8	12.4
Bacillus subtilis	12.4	6.2	6.2
Xanthomonas oryzae	6.2	6.2	3.1

Table 4: Antifungal activity of copper (II) NHC complexes

	Zone of inhibition (mm)		
Complex name	5 μmol	10 μmol	25 μmol
Copper	1.7	2.8	3.2
Complex-1b	1.2	2.8	3.2
Complex-2b	1.2	2.3	4.1
Complex-2d	2.4	4.3	5.7
Ampicillin	2.1	3.9	4.9
Streptomycin	1.6	4.1	4.6

Table 5: Superoxide dismutase activity of Cu (II) NHC complexes

Complex	SOD activity (mol mL^{-1} min^{-1})
1b	54
2b	66
2d	80

ciprofloxacin, gentamicin, ampicillin, streptomycin and chloramphenicol was determined and the results were presented in Table 3. These results were in correlation to the data reported by Nagababu *et al.*[3].

Antifungal activity of Cu (II) NHC complexes: Preliminary experiments were conducted on the antifungal activity of Cu (II) NHC complexes against *A. niger*, *N. crassa* and *F. oxysporum* by the disc diffusion method as described by Kumar *et al.*[10] and Elgayyar *et al.*[11]. The effective concentration and the diameters of zones of inhibition were shown in Table 4. Of the 3 complexes (1b, 2b and 2c) that showed antifungal activity against all the three fungi tested, the activity was observed in terms of inhibition of fungal growth around the discs loaded with complexes (Table 4). All the three complexes were the most potent in terms of inhibiting growth of the fungi around the disc loaded with these complexes.

SOD activity: A great deal of interest has been shown in the development of therapeutic SOD mimetic for the scavenging of superoxide ðO$_2$-Þ which is a precursor to reactive oxygen and nitrogen species (RONS) known to contribute to oxidative stress.

The SOD mimetic activities of the copper (II) NHC complexes were determined and compiled in Table 5. In the present complexes, the higher SOD activity is due to electron withdrawing substituent compared to other complexes. A greater interaction between superoxide ion and Cu (II) complex is induced due to the stronger axial bond, which results in an increased catalytic activity. In addition, ligands containing electron withdrawing substituent stabilizes the Cu (II) complex formed during superoxide dismutation reaction which further reacts with superoxide ion to give hydrogen peroxide. The distorted geometry of these complexes may favor the geometrical change, which is essential for the catalysis as the geometry of copper in the SOD enzyme also changes from distorted square planar geometry[12,13].

CONCLUSION

The stability in aerobic conditions and relatively easy synthesis of methylated caffeine make it a very useful N-heterocyclic carbene precursor. We have synthesized bis (NHC) copper (II) complexes 1b, 2b and a dinuclear copper (II) bis (NHC) complex, 2d from different salts of methylated caffeine. These are the first examples of copper (II) NHC complexes derived from caffeine. The formation 2d in water suggests that the deprotonation of the imidazolium salt and coordination to the metal centre is a concerted process. The synthesized NHC copper (II) complexes show fluxional behaviour in solution and can be used as carbene transfer reagents for the synthesis of other transition metal complexes. Further, the intermediate complexes and the final NHC copper (II) complex showed good antibacterial and antifungal activities to their level best in comparison to the standard antibiotics used in the study. As for cationic derivative complexes, homoleptic and heteroleptic bis-NHC edifices have been accounted for and have been productively utilized as a part of catalysis permitting imperative changes.

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