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Cu(II) complexes with heterocyclic Schiff bases of 2-amino benzoxazole and acetophenone derivatives: Synthesis, characterization and biological activity

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Two heterocyclic Schiff bases (named- CO, HO) have been developed from 2-amino benzoxazole and 2-chloro or 2-hydroxyacetophenone. Then, these have been used for complexation with $CuCl_2.2H_2O$ salt to prepare Schiff base metal complexes *via* thermal reflux method. Molar conductivity measurement, magnetic moment determination, spectral techniques including FTIR, ^{1}H NMR and mass spectrometry have been utilized for detailed characterization of the synthesized compounds. Biological assessment has been carried out employing agar well diffusion process to check the bio-efficacy of the synthesized compounds against selected microbes, *E. coli* and *B. subtilis* (bacteria) and, *C. albicans* (fungus). It is concluded from the findings that ligand CO has higher potential amongst all the synthesized compounds.

Keywords: 2-Aminobenzoxazole, Heterocyclic Schiff bases, Metal complexes, Antifungal potential, Antibacterial potential

In wide spectrum natural and synthetic biologically active compounds having benzoxazole[1,2] scaffold to be known for their key role in biological field[3-8], for instance, antimicrobial[9], antiviral, antimalarial and antileishmanial, also accompanying anti-cancer activity against MDR cancer[10,11] cells. Various marketed drugs (Fig. 1) such as; Boxazomycin B (antibacterial), Benoxaprofen and Flunoxaprofen (anti-inflammatory), Chloroxazone (muscle relaxant), Calcimycin (antibiotic) are accessible that are also containing benzoxazole moiety. Thus, the wide diversity of benzoxazole[12] analogues prompted researchers to innovate numerous synthetic derivatives (Schiff bases) with innate chemotherapeutic[13,14] potentials. In this manner, we have developed two Schiff bases of benzoxazole containing *ortho*-substituted acetophenones.

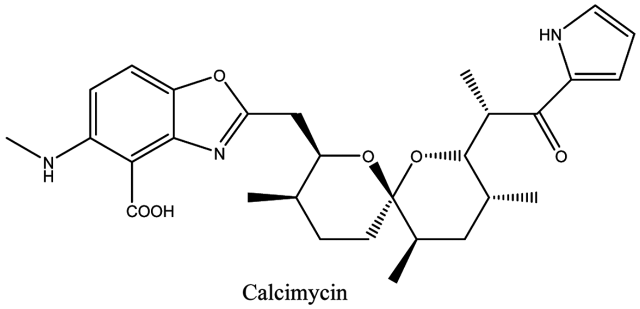
Moreover, it has been noted that these types of Schiff bases of benzoxazole derivatives[15] works as good complexing agent for a number of transition metal ions by using its N-atom as donor site. Pharmaceutical industries find metal complexes to be very useful, as various molecules with antimalarial, anticancer and anti-neurodegenerative efficiency have been found in clinical studies. Our findings suggest that coordination complexescontaining Schiff bases of 2-aminobenzoxazole[16-20] ligand and acetophenone[21] derivatives may enhance their antimicrobial potential or antibacterial and antifungal effect.

In this consideration, we have developed two Schiff bases containing 2-amino benzoxazole moiety and 2-chloro acetophenone, 2-hydroxyacetophenone. Further, their complexation done with cupric chloride salt to synthesize metal complex and made a comparative biological study to check most efficient Schiff base or metal complex against selected bacterial and fungal pathogens, *E. coli* and *B. subtilis* (bacteria), *C. albicans* (fungus) employing Agar well diffusion method (Chart 1).

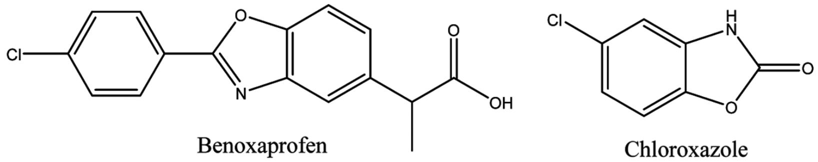
Experimental Section

Materials and Characterization Methods

2-Aminobenzoxazole and both acetophenone derivatives received from Sigma Aldrich, used as they were gained. Solvents utilized in the synthesis were of high level of purity and used without subsequent purification. Fourier-Transform Infrared spectroscopy (FTIR) technique was utilized to identify the functional groups present in particular compound and FTIR spectra recorded in a range of wavelength 400-4000 cm^{-1} on Perkin-Elmer FTIR spectrophotometer model in presence of KBr pellets (as carrier). Further, ^{1}H NMR technique using Jeol-Resonance ECS-400 Spectrometer at fixed value of frequency (400 MHz) has been used to find out chemically different protons in these synthesized compounds in DMSO-d_6 solvent.



Calcimycin



Benoxaprofen

Chloroxazole

Fig. 1 — Structure of some marketed drugs of benzoxazole

A volume of the microbial inoculum was applied to the whole surface of the agar plate to inoculate it.

↓

A sterilised metal tip was used to punch an aseptic hole measuring 6 mm in diameter.

↓

A volume of 20, 40, 60, 80 μL of each synthesized compound prepared in 100% DMSO and added to the well, agar plates were incubated overnight at 37°C temperature.

↓

The antimicrobial ingredient diffuses throughout the agar media, preventing the tested microbial strain from growing.

↓

Zone sizes encompassing each well were used to determine the extract's antibacterial or antifungal spectrum in terms of MIC (Minimum Inhibitory Concentration).

Chart 1 — Flow chart for antimicrobial assessment (Agar well diffusion method)

Mass spectrometry was also employed for metal complexes using TOF MS ES+ mass spectrometer at ionization potential 70 eV.

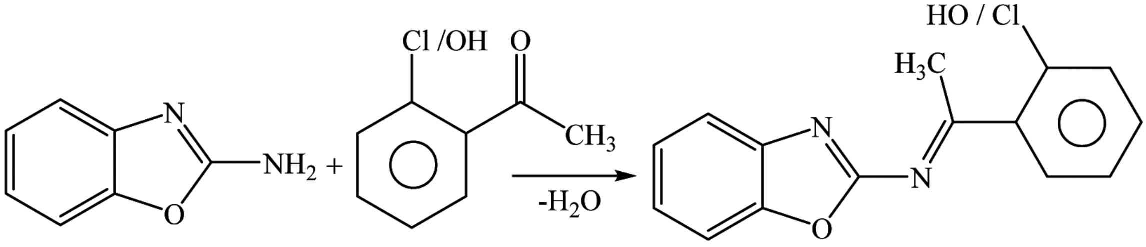
Synthetic Procedures

Synthesis of Schiff bases

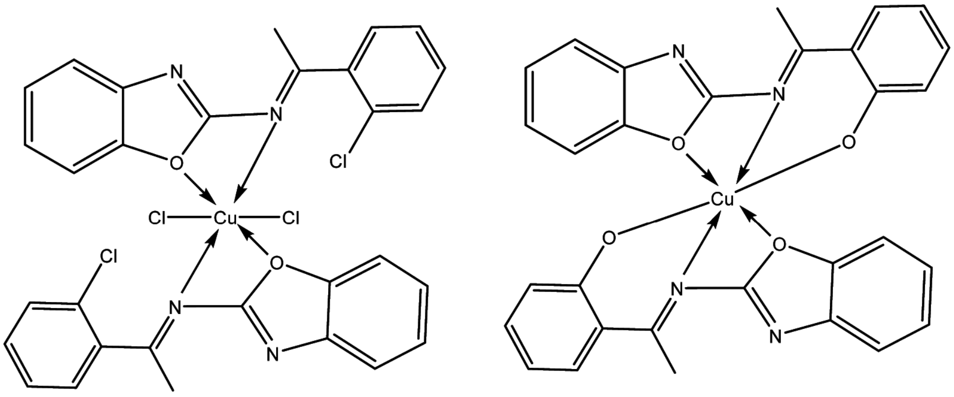
Similar reflux method was adopted for the development of both Schiff bases. The comprehended discussion involve proper mixing of ethanolic solution of amine moiety (2-amino-benzoxazole, 10mmol) and equimolar ethanolic solution of keto group containing moiety *i.e.* 2-chloro acetophenone / 2-hydroxyacetophenone in 1:1 ratio (Scheme 1) with continuously stirring on magnetic stirrer for ~15-20 min in a round bottom flask. No chemical change was observed, the reaction mixture put for thermal condensation (approx. 4-5 h) in presence of condensing agent (few drops of mild glacial acetic acid and a pinch sodium acetate powder). The progress of reaction and purity of prepared compounds checked through Gel-g TLC measurements. Afterwards, the reaction mixture put on watch glass overnight and formed precipitate was dried in oven and recrystallized using hot ethanolic solution.

Mechanistic route for tailoring new Cu(II) complexes

5 mL solution of $CuCl_2.2H_2O$ metal salt dissolved in absolute ethyl alcohol blended in equimolar 10 mL



Scheme 1 — Synthetic mechanism for benzoxazole derived Schiff bases



CBO-I **CBO-II**

Fig. 2 — Intended structure of Cu(II) metal complexes designed from benzoxazole based Schiff bases

Table 1 — Physicochemical measurements of synthesized compounds

S. No.	Compd/ complex notation	Mol. Wt. Found (Calcd)	Colour	Yield (%)	m.p. (°C)	Magnetic moment (μ_{eff})	Molar conductivity Ω^{-1} cm^2 mol^{-1}
1	CO	271.06 (270.73)	Dark Green	62.1	153.5 (Decomp)	–	–
2	HO	252.88 (252.29)	Light Brown	70.5	103.4	–	–
3	CBO-I / $[Cu(CO)_2Cl_2]$	675.32 (675.91)	Green	58.1	203.5	1.78	10.92
4	CBO-II / $[Cu(HO)_2]$	566.82 (566.30)	Light Brown	63.5	151.3	1.75	12.93

ethanolic solution of one of the Schiff bases in a stoichiometric ratio of 1:2 (Fig. 2) in continuous stirring condition on the magnetic stirrer. Afterwards, when precipitate was not developed even ~15-20 min stirring then this reaction solution put up for complexation under heating reflux method at set temperature (50°C) for ~3-4 h. Same procedure was followed as discussed above.

Results and Discussion

Elemental analytical measurements

CHNX method employed for the determination of the mass percentage of carbon, hydrogen, nitrogen and heteroatoms oxygen and chlorine. Some physical and chemical analysis of all synthesized compounds were made which are tabulated in Table 1 and Table 2.

Molar conductivity

Molar conductance of the metal complexes determined through direct reading Systronics conductivity meter-304 in DMSO solvent. For both metal complexes conductivity found in a range 10-13 Ω^{-1} cm^2 mol^{-1}showing non-electrolytic nature.

Magnetic moment

The magnetic moment were measured on Gouy balance of model no: HO-ED-EM-08 of all

Table 2 — Elemental analysis data of synthesized compounds

S. No.	Compd/ Empirical formula	Elemental Data Found % (Calcd)			
		C	H	O	Cu
1	CO $C_{15}H_{11}ON_2Cl$	66.57 (66.54)	4.11 (4.09)	5.92 (5.90)	–
2	HO $C_{15}H_{12}O_2N_2$	71.44 (71.41)	4.80 (4.79)	12.70 (12.68)	–
3	CBO-I $C_{30}H_{22}O_2N_4Cl_4Cu$	53.34 (53.31)	3.30 (3.28)	4.75 (4.73)	9.43 (9.40)
4	CBO-II $C_{30}H_{22}O_4N_4Cu$	63.57 (63.54)	3.86 (3.89)	11.29 (11.32)	11.30 (11.23)

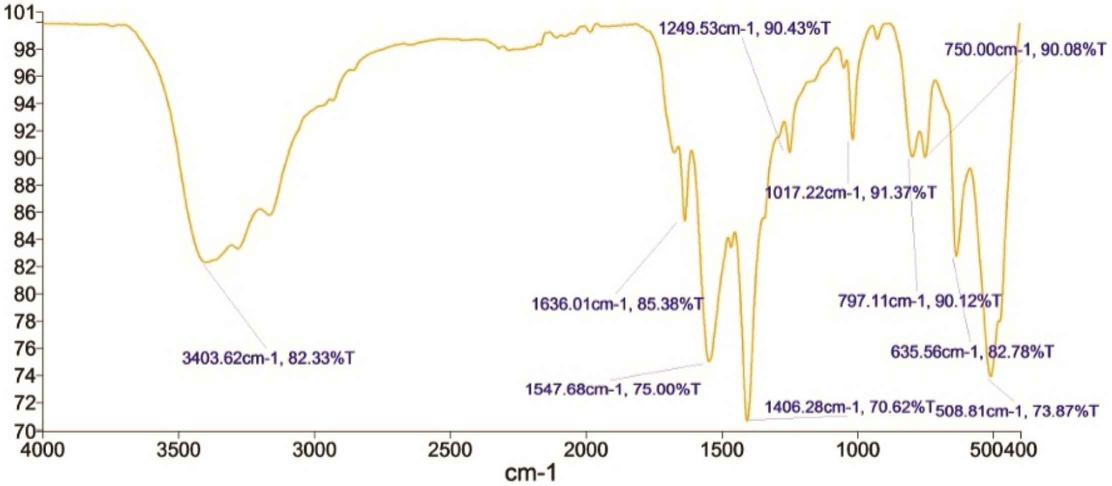


Fig. 3 — FTIR spectrum of complex CBO-I

synthesized metal complexes and the μ_{eff} values 1.78 and 1.75 for each complexes denoting presence of one unpaired electron.

Spectral Studies

Schiff base CO and complex CBO-I

FTIR spectra recorded for the complex (Fig. 3) involving various stretching bands C=N, C=C, C-H at 1547 cm^{-1}, 1406 cm^{-1}, 3140 cm^{-1} respectively, in addition some metal-ligand bands were also present that were Cu-N, Cu-O, Cu-Cl at 506 cm^{-1}, 635 cm^{-1}, 797 cm^{-1}. A vibrational band at 3403 cm^{-1} ensured the presence of water molecule with its coordination sphere. NMR spectra for both ligand and complex were also developed in DMSO-d_6 solvent and some change was noticed in chemical shift values in the spectra of ligand and complex. As per observation ^{1}H NMR data for ligand CO (Fig. 4) was defined as δ 6.8-7.2 (m, Ar-H), δ 1.1 (s, CH_3) and for complex (Fig. 5) δ 6.9-7.3 (m, Ar-H), δ 1.5 (s, CH_3) and δ 3.3-3.5 (broad, s, H_2O). All chemical shift values are reported in δ (ppm). Mass spectrum (HR-MS) was measured at a fix potential 70 eV and the parent molecular ion peak was found at m/z 674.8 $(M-1)^+$ that was corresponding to its molecular weight 675.9. So, the formation of this complex ensured on the basis of mass data (Fig. 6).

Schiff base HO and complex CBO-II

The Infrared spectral study executed for complex (Fig. 7) and the values assigned as C=N, C=C, C-H at 1560 cm^{-1}, 1459 cm^{-1}, 3148 cm^{-1} respectively and the metal-ligand vibrational bands were also found that were Cu-N, Cu-O at 636 cm^{-1}, 729 cm^{-1}. Next, the ^{1}H NMR spectra for both ligand and complex (Fig. 8 and Fig. 9) were comprising these types of proton signals δ 6.4-7.5 (m, Ar-H), δ 3.2 (s, O-H) and for complex δ 6.9-7.5 (m, Ar-H), δ 2.2 (s, CH_3) and δ 3.3-3.5 (broad, s, H_2O) [chemical shift values assigned as δ (ppm)]. The recorded mass spectrum (Fig. 10) for the complex validated its molecular weight as its molecular ion peak found at m/z 606.3 and its coordination sphere involved two water molecules as $[Cu(L)_2]$. $2H_2O$.

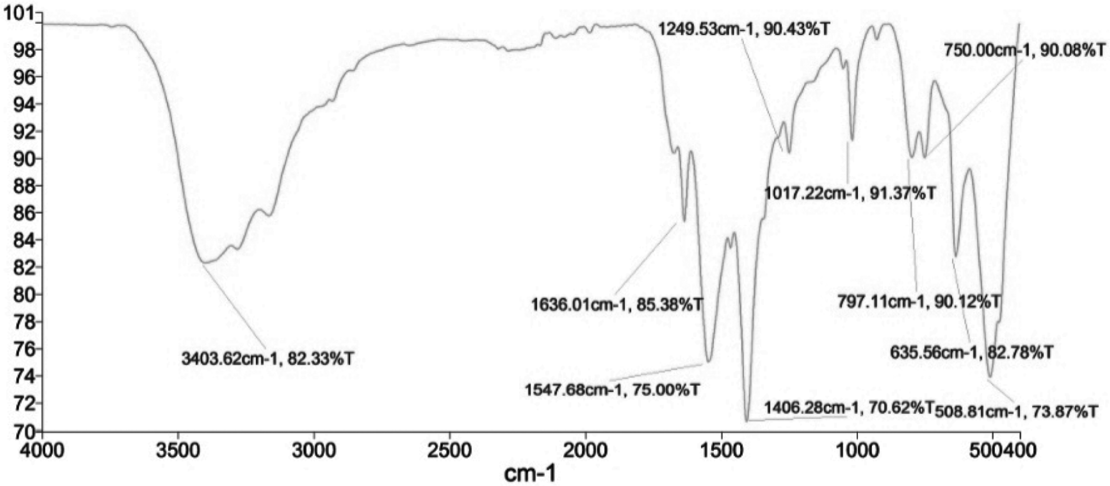


Fig. 4 — ^{1}H NMR spectrum of ligand CO

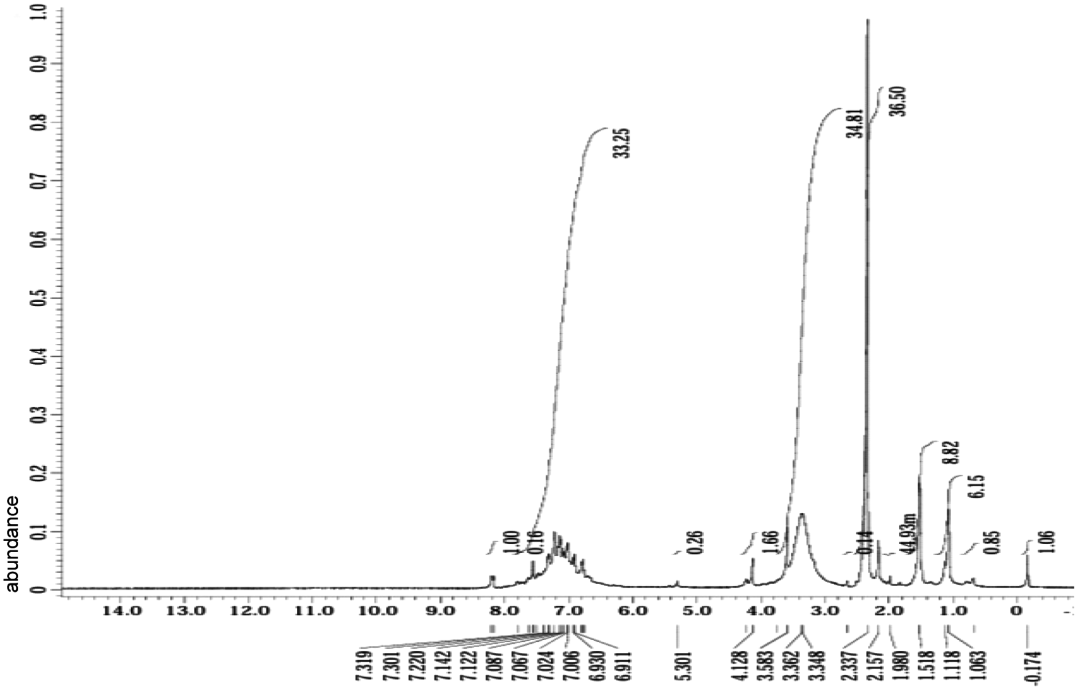


Fig. 5 — ^{1}H NMR spectrum of complex CBO-I

Biological results

Biological assessment was carried out against two selected bacterial strains *E. coli*, *B. subtilis* and one fungal strain *C. albicans via* agar well diffusion procedure employed on petri dishes. The inhibition potential (Fig. 11) was measured by the difference calculation of diameter of well of control and samples taking different concentrations of test sample as 20, 40, 60 and 80 µg/mL, respectively. The findings were tabulated in Table 3 at sample concentration 80 µg/mL.

The comprehensive studies of antimicrobial tests clearly demonstrated that the ligands and metal complexes (Fig. 12 and Fig. 13) all were significantly active and ligand CO showed highest bioactivity (21 mm) against *E. coli*, comparable to standard (30 mm). In same manner, complex CBO-I comprising ligand CO revealed highest potential for all microbes but there was a lessening in case of *E. coli* and *C.albicans*. Furthermore, it was also noted the antibacterial activity against *B. subtilis* increased (approx. doubled).

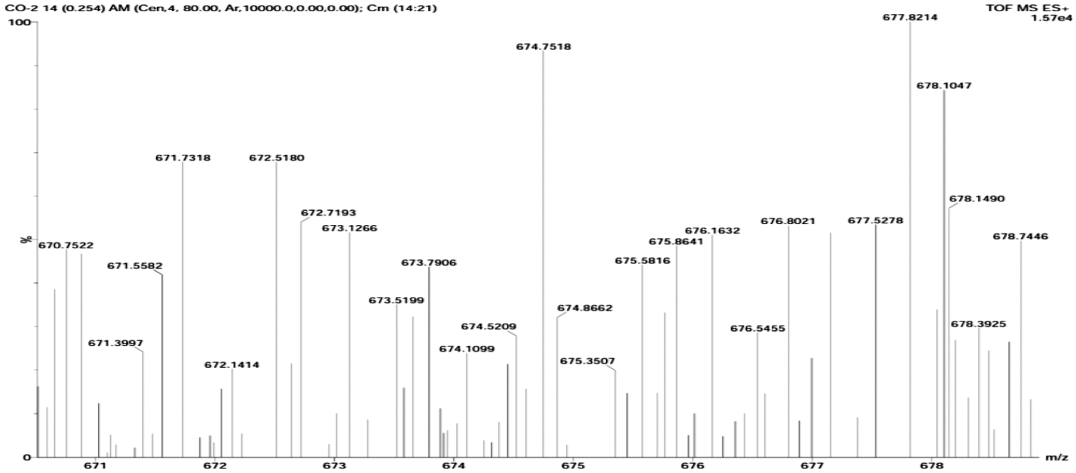


Fig. 6 — Mass spectrum of complex CBO-I

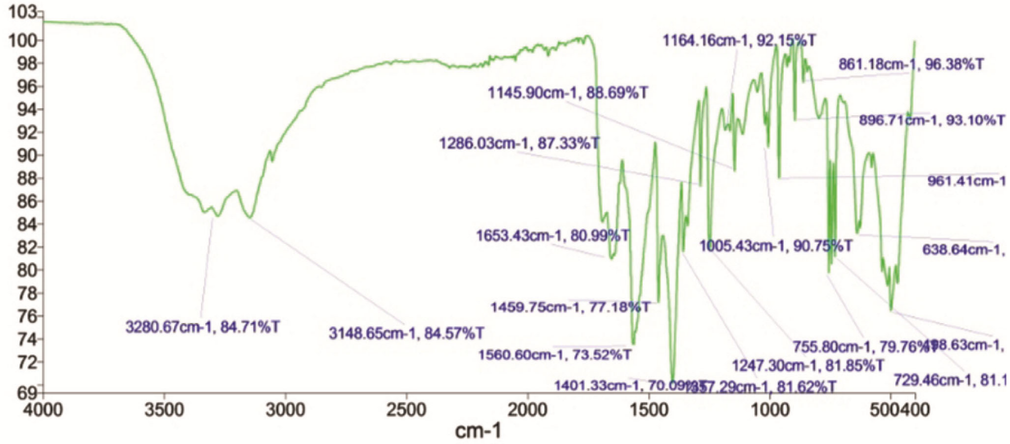


Fig. 7 — FTIR spectrum of complex CBO-II

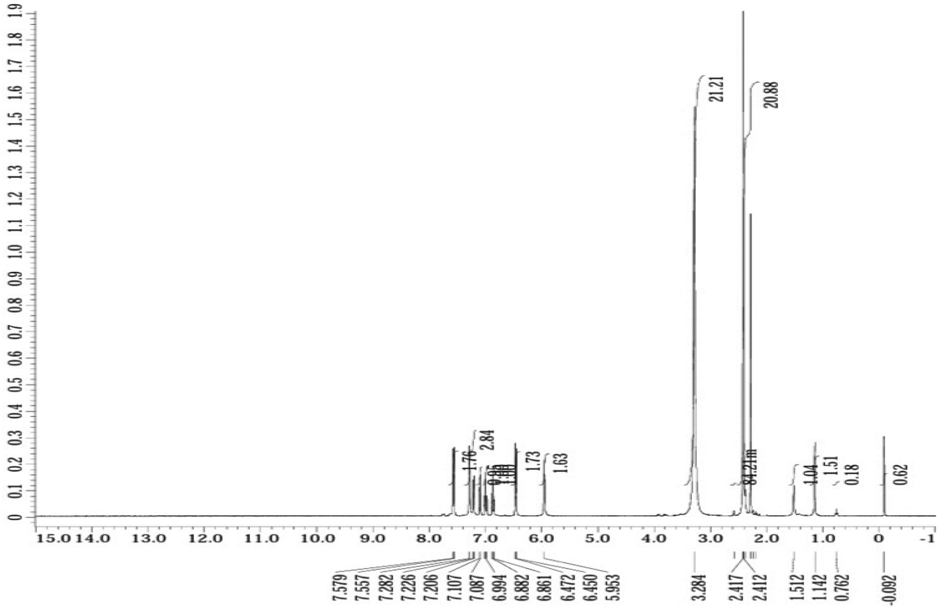


Fig. 8 — ^{1}H NMR spectrum of ligand HO

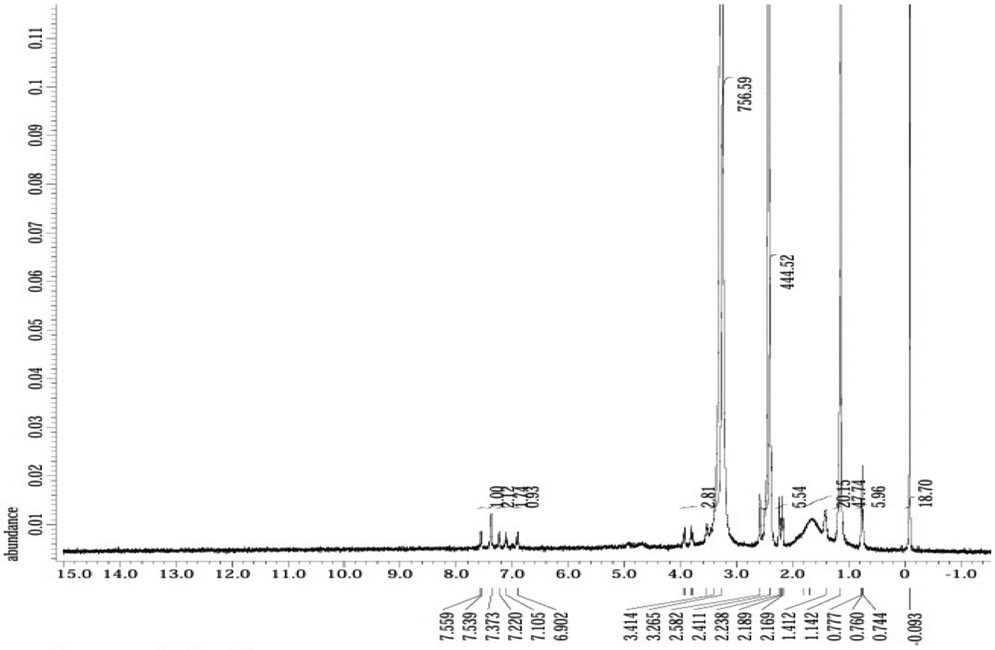


Fig. 9 — ^{1}H NMR spectrum of complex CBO-II

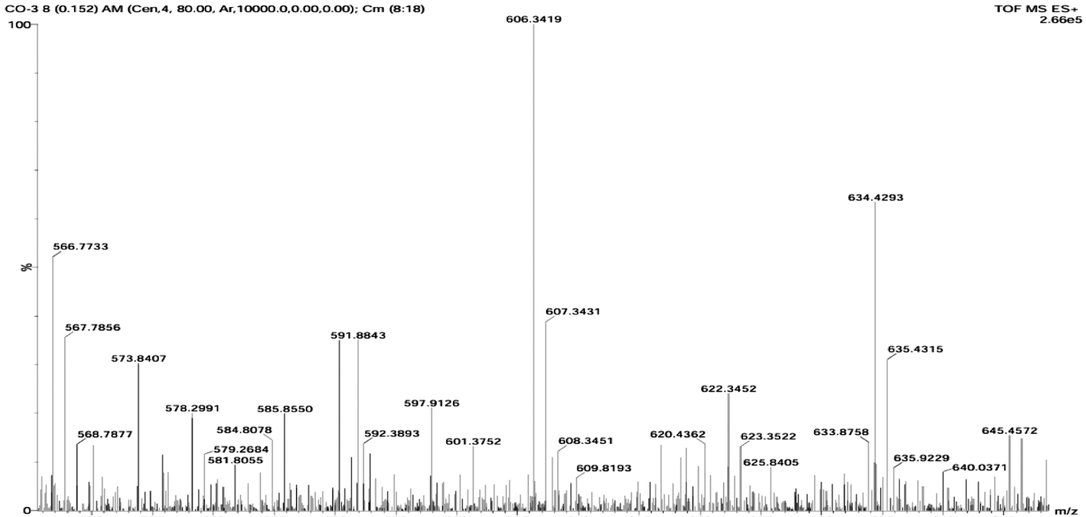


Fig. 10 — Mass spectrum of complex CBO-II

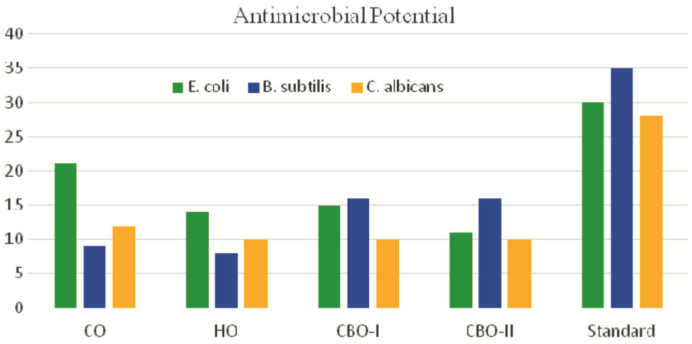


Fig. 11 — Bar diagram of antibacterial and antifungal results

Table 3 — Antimicrobial data of test ligands and complexes

S. No	Compd	Zone of inhibition (mm)		
		E. coli	*B. subtilis*	*C. albicans*
1	CO	21	9	12
2	HO	14	8	10
3	CBO-I	15	16	10
4	CBO-II	11	16	10
5	Standard (40 μg/mL)	30	35	28

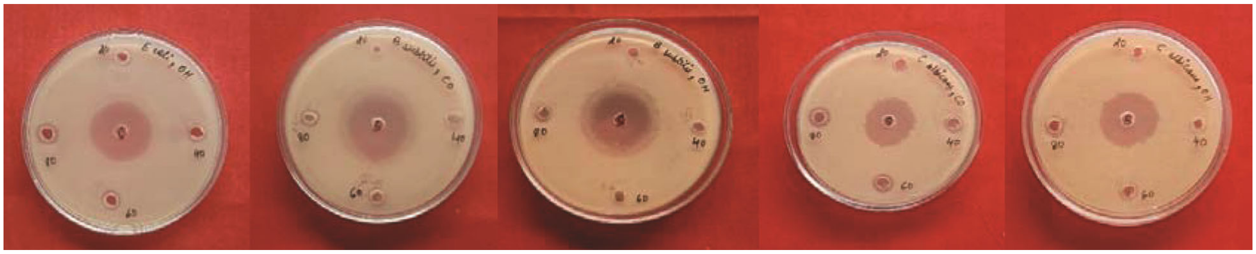


Fig. 12 — Antimicrobial activity against some selected microbes; *E. coli*, *B. subtilis* and *C. albicans* for ligands

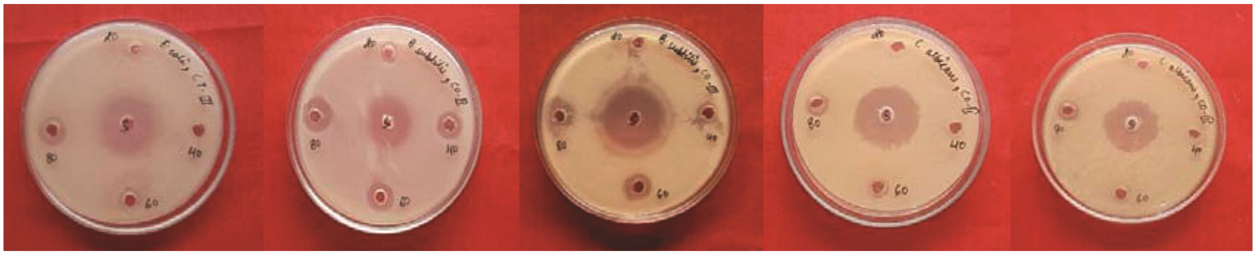


Fig. 13 — Antimicrobial results of some selected microbes; *E. coli*, *B. subtilis* and *C. albicans* for metal complexes

Further, the biological activity of ligands follow the order; *E. coli* > *C. albicans* > *B. subtilis* and for metal complexes the above order found as *B. subtilis* > *E. coli* > *C.albicans*.

Conclusions

On the basis of investigations made through characterization techniques, spectral assessment, it was proposed that both complexes exist in octahedral geometry surrounded by N (azomethine), O-heteroatoms (benzoxazole moiety) of bidentate benzoxazole based Schiff bases. In complex CBO-I remaining two coordination sites were fulfilled by chloride ions and in complex CBO-II these two valances fulfilled by deprotonated oxygen ion (O^-) of hydroxyacetophenone group. Hence the ligand HO behaved as tridentate Schiff base ligand. Observed mass spectra of both complexes conform to the value of molecular weight and ensured the formation of Cu(II) complexes from benzoxazole carrying Schiff bases and also assured about the presence of n numbers of water molecules (where; n = 2-3) within coordination sphere. Next, the observations made through the biological assessment of all compounds against *B. subtilis*, *E. coli*, *C.albicans* microbes, the results laid emphasise on comparable antimicrobial potential together with highest activity of ligand CO and corresponding complex CBO-II. Further, we have planned to explore anti-cancerous, anti-proliferative effects of these specimen so that these synthesized compounds can be purposed as multifarious drug agents.

Conflict of Interest

There is no conflict of interest to be declared.

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