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New Benzothiazole-Derived Schiff base and its Co(III)/Ni(II) Ternary and Binary Complexes as Biologically Active Compounds: Synthesis, Structure and Biological Potency Investigation

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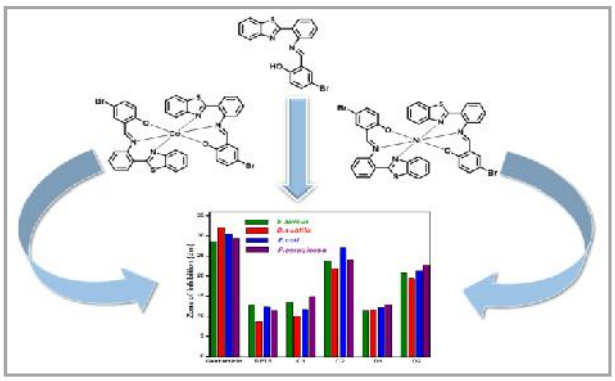
ABSTRACT

A series of new binary and ternary transition metal complexes bearing Ni(II) and Co(III) metal ions have been designed and synthesized using 2-((E)-(2-(benzo[d]thiazol-2-yl)phenylimino)methyl)-4-bromophenolligand (BPTB) derived from 2-(benzo[d]thiazol-2-yl)benzenamine and 5-bromo salicylaldehyde along with 1,10 -Phenanthroline as co-ligand. The ligand BPTB and its metal complexes were characterized using elemental analyses, UV, NMR, FT-IR, mass spectroscopy and thermogravimetric analyses. It is interesting to note that BPTB forms octahedral geometry with metal ions through azomethine nitrogen, benzothiazole nitrogen, and phenolic oxygen which yields ternary complexes whereas the inclusion of 1,10-Phenanthroline results binary complexes as evident from the magnetic measurement and electronic spectral studies. The ligand and its complexes were tested for antimicrobial, antioxidant, anti-haemolytic, and antidiabetic studies. The studies indicate that the binary complexes are associated with enhanced biological activity as compared to ternary complexes and ligand.

Graphical abstract:



Antibacterial screening data of the ligand and its complexes.

Keywords: Benzothiazole, Binary complex, Ternary complex, Biological activity.

INTRODUCTION

The heterocyclic Schiff base metal complexes form one of the interesting areas of research in the field of inorganic biology as it is associated with potential antioxidant and cytotoxic properties which governs fundamental importance in designing metal based anticancer drugs [**1**]. Designing and fabricating metal-based chemotherapeutics comprising heterocyclic Schiff base motif with inbuild biocompatibility, target specificity, and less harmful features are the challenging task in the field of drug discovery. Benzothiazole comprising Schiff base ligands are the most privileged compounds in coordination chemistry and promising candidate for constructing drug molecule due to their diversified pharmacological properties including antioxidant, antibacterial, antifungal, anti-inflammatory, anti-convulsant, anti-tuberculosis, anticancer, and anthelmintic [**2-6**]. The presence azomethine functional group is essential for the biological activity of Schiff base ligands and they execute by coordinating with active metal centres of enzymes and hampering cellular metabolism [**7**]. In addition to that, the cellular constituents of microbes may be involved in the H-bond formation with azomethine nitrogen of Schiff base which halts normal cellular functions and thereby executing pharmacological actions [**8**]. Recent studies indicates that the antimicrobial activity of some Schiff bases might be due to obstructing the synthetic pathway of aminoacyl-tRNA which is essential for the protein synthesis [**9**].

The malfunctioning of endocrine glands causes chronic hyperglycaemia due to the impaired functioning of insulin which disturbs metabolism of glucose, proteins, and lipids leads to diabetes mellitus [**10**]. There will be increasing trend of mortality and morbidity due to diabetes all over the world as reported by World Health Organisation [**11, 12**]. The cellular metabolism requires Insulin for the conversion of starch into glucose and then into glycogen. New transition metal complexes bearing heterocyclic imine base ligands garnered special interest for the treatment of diabetes by mimicking the action of insulin thereby restricting the activity of α-glucosidase and α-amylase [**12, 13**]. Now a day, metal-based drugs govern fundamental importance for the treatment of diabetes collaborated with coordination chemistry after discovering insulin mimicking activity of some vanadium complexes in 1980 [**14**]. The coordination admittance of metal ion into imine base ligands can modify mechanistic pathways of pharmacological action with enhanced biocompatibility and specificity. Furthermore, the ligation of some active drug molecules into metal ion can enhance their biological properties with reduced side effects [**15, 16**].

Cellular metabolism generates free radical species which can cause oxidative damage to tissues and DNA. The antioxidant which can destroy free radical and prevent oxidative damage are significant class of compounds, studies infers that the Schiff base motifs are excellent sources of antioxidants. Erythrocytes are also known as red blood cells plays fundamental role in carrying oxygen and carbon dioxide within the biological system, owing to this they experience continuous stress and generates ROS (reactive oxygen species) during their whole life span. On top of that, erythrocytes are very sensitive to oxidative stress which causes an irreversible damage to lipid layer of red blood cells. The oxidative damage of erythrocytes is characterized by protein oxidation, liposomal peroxidation, and reduced glutathione level which in turn induce morphological changes in the erythrocytes and results in various conditions like sickle cell anaemia, Alzheimer disease, renal malfunctioning, β-thalassemia, and random cellular aging [**17**].

Therefore, the primary investigation begins with structural fabrication of transition metal complexes comprising benzothiazole based Schiff base ligands with excellent antioxidant property, antibacterial, antifungal, antidiabetic properties. In this view, herein we designed and tailored some Ni(II) and Co(III) complexes derived from 2-((E)-(2-(benzo[d]thiazol-2-yl)phenylimino)methyl)-4-bromophenol as primary ligand BPTB to construct ternary complexes and 1,10-Phenanthroline as secondary ligand to construct binary complexes as extension of the previous work. The afresh molecules were screened for antioxidant, antibacterial, antifungal, anti-haemolytic and *in vitro* antidiabetic assay using reference protocols.

MATERIALS AND METHODS

Materials and methods: The 2-(benzo[d]thiazol-2-yl)benzenamine, 5-bromo salicylaldehyde, 1,10-Phenanthroline, $CoCl_2.6H_2O$, $NiCl_2.6H_2O$, and triple distilled water were procured from Sigma Aldrich Chemicals, Bangalore and used as received. The FT-IR spectrum of ligand and its complexes were scrutinized within 4000-400 cm^{-1} using Shimadzu 8300 Spectrometer following KBr pellet method. The chemical environment around proton and carbon can be identified using NMR spectroscopic method using Bruker-HC400 MHz spectrometer in which the samples were dissolved in deuterated DMSO solvent. The MS-ESI method is followed in order to elucidate structural fragments and molecular mass. The millimolar solution of ligands and complexes were utilized to record electronic absorption spectrum in which DMSO solvent used as blank. The magnetic susceptibility measurement of complexes were done by using Guoy's balance against to a reference compound $Hg[Co(CN)_4]$.

Chemical synthesis

Synthesis of 2-((E)-(2-(benzo[d]thiazol-2-yl)phenylimino)methyl)-4-bromophenol (BTPB): As per the protocol described in the literature, we prepared 2-((E)-(2-(benzo[d]thiazol-2-yl)phenylimino)methyl)-4-bromophenol ligand by mixing an equimolar alcoholic solution of 2-(benzo[d]thiazol-2-yl)benzenamine (2 mmol, 0.45g in 10 mL) and 5-bromo salicylaldehyde (2mmol, 0.40 g in 10 mL), the reaction takes place under reflux condition for 6 h as shown in Scheme 1. A brown solid product was precipitated which is filtered, dried, and collected for further analysis.

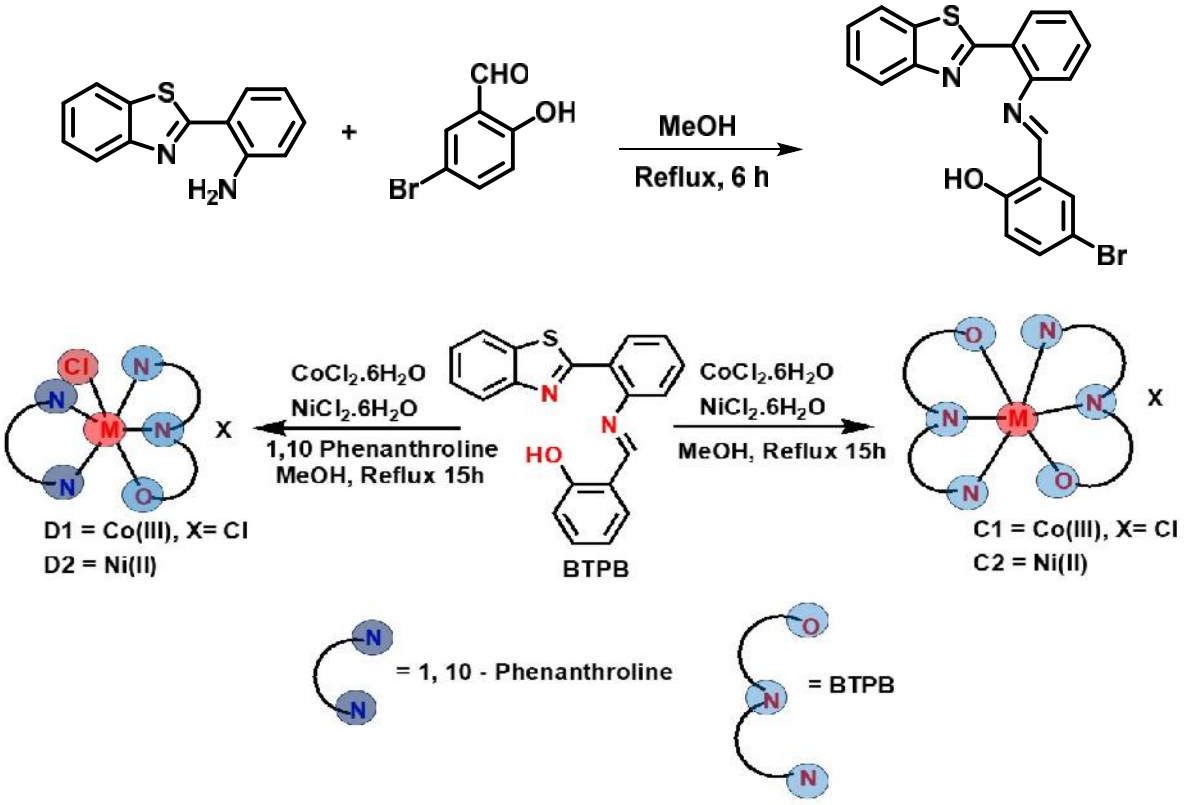
Synthesis of Co(III) and Ni(II) metal complexes (C1, C2, D1 and D2): The coordination of synthesized ligand can be affected by mixing 1mmol methanolic solution of metal salt ($CoCl_2.6H_2O$ (0.475g in 10 mL CH_3OH) and $NiCl_2.6H_2O$ (0.475 g in 10 mL CH_3OH) with 2mmol (0.818g in 20 mL methanol) methanolic solution of BTPB under reflux condition for about 15-20 h. The colour of the reaction mixture changes in the initial stages of the reaction and solid product was precipitated after completion of the reaction. The product was dried at room temperature for 7-8 days and collected for further analysis.

The binary metal complexes can also be prepared by using 1, 10-Phenanthroline as coligand which is added after 6 h of the reaction. An equimolar solution of ligand BTPB (1 mmol, 0.4 g), metal salt ($CoCl_2.6H_2O$ and $NiCl_2.6H_2O$ (1mmol, 0.475g), and 1,10-Phenanthroline (1mmol, 0.18 g) taken in 10 mL methanol were mixed at the suitable interval of time and the reaction is progressed under reflux condition for about 15 h, as displayed in Scheme 1. The obtained product was dried and collected for further analytical investigation.

Biological assay

In vitro antibacterial and antifungal studies: The binary and ternary transition metal complexes and its ligand counterpart BTPB were examined for their efficacy to inhibit bacterial and fungal growth against a panel of two positive, two gram negative bacterial and two fungal strains such as *Staphylococcus aureus* MTCC-7443, Bacillus subtilis MTCC 121, *Escherichia coli* MTCC-7410, and Pseudomonas aeruginosa MTCC 7093; fungal strains *Aspergillus flavus* MTCC-9606 and *Pichia anomala* MTCC-237 using low cost, efficient, less tedious Nutrient agar disc diffusion method [**18**]. The stock solution (1mg mL^{-1}) of the ligand BPTB and its metal complexes were prepared by dissolving 10 mg in 10 mL of DMSO solvent. A series of sample solution was made from the stock solution with a concentration ranging from 100 µg to 400 µg by dilution method in which DMSO can be used as control for antibacterial assay. All the microorganisms were grown and cultivated in nutrient agar medium (Muller Hinton agar for bacterial strain and Czapek's-Dox agar media for fungal strains) and the bacterial efficacy of the ligand and its metal can be measured in comparison with reference drug for antibacterial assay is Doxycycline and Amoxycillin and for fungal studies Fluconazole is used as positive control. The disc containing samples were loaded aseptically into nutrient agar media containing microorganism such as *E.coli*, *P. aeruginosa*, *B. subtilis*, and *S.*

aureus. The samples are seeded using 8 mm discs into agar media in which the fungal strains are cultivated separately in another petri dish. After seeded with test solution, the agar media is incubated at 37°C for 24 h for antibacterial assay and 72 h at 28°C for fungal inhibition assay. The antibacterial activity is measured using a Vernier caliper in mm and expressed in terms of zone inhibition in diameter.



Scheme 1. Synthetic route for ligand and its metal complexes.

In vitro antioxidant assay (DPPH radical scavenging assay): The radical scavenging ability of the ligand and its complexes were assessed using DPPH method [19]. Diphenyl picryl hydrazyl (DPPH) is stable free radical which accepts electron or H from antioxidant molecule and make a visible transition from purple to yellow which can be captured by measuring absorbance spectrophotometrically at 517 nm. A 0.2 mL of sample aliquots with a concentration ranging from 0 to 50 µg which is made using DMSO solvent were mixed with 2 mL of DPPH solution (0.1 mM) and the solution is made upto 3 mL using methanol. The reaction mixture is used to record absorbance at 517 nm against blank after the incubation period at 28°C for 45 min. DPPH radical scavenging ability of the test compounds were assessed in comparison with reference antioxidant BHT using the below equation and expressed in IC50 value.

$$\text{DPPH radical scavenging capacity (\%)} = \frac{A \quad o\ t\ \ t(\ \ cc \quad a\ 5\ \ n}{A \quad o\ r\cdot \quad cc \quad a\ 5\ \ n} \times 100$$

In vitroAAPH stimulated anti-haemolytic assay: The potential Anti-haemolytic activity of ligand BPTB and its complexes can be assessed spectrophotometrically at 540 nm using 0.25 mL heparinized red blood samples collected from healthy person. The blood samples are washed with saline water and centrifuged for 10 min to remove plasma at 3000 rpm. The red blood stock solution (40% v/v) was made by using PBS (phosphate buffer saline) and set pH to 7.4. In the reaction system, 100 mM AAPH containing 0.05mg mL^{-1} sample induces 10 % erythrocytes hemolysis, without test compounds can be taken as negative control and without AAPH serves as blank. The obtained data

were analysed in comparison with positive control L-ascorbic acid and 600 mM AAPH solution in PBS is referenced for 100 % haemolysis. The results were compared with standard L-ascorbic acid as positive control. The reaction mixture was incubated for 2 h at 37 °C and then the aliquots is made by diluting with PBS (1:8), after centrifugation for 10 min at 2000 rpm the supernatant solution is utilized to measure absorbance at 540 nm and the potential anti-haemolytic activity is expressed in percentage.

α-amylase inhibition assay: An enzymatic assay (DNS method) was implemented to perform α-amylase inhibition activity of ligand PBTB and its metal complexes. A 0.5 mL of sample aliquots is mixed with 0.5 mL of 1%starch solution made using 20 mM sodium phosphate buffer (pH 6.9). This solution is now mixed with an equal volume of enzymatic solution and the reaction mixture is incubated at 37°C for 10 min. Further, the solution is seeded with colouring reagent 3, 5–dinitro salicylic acid (DNS) to all sample aliquots and incubated on water bath for 5 min at 85°C. Finally, the aliquots were removed from water bath and kept aside until it reaches room temperature. Then the aliquots were diluted with 10 mL triple distilled water and the absorbance was monitored spectrophotometrically at 540 nm [**20**]. A blank solution is also taken without the test sample and the antidiabetic activity is measured in comparison reference compound α-acarbose. The antidiabetic inhibition was calculated as per the equation below and also expressed in terms of IC50 values in which the test sample shows more than 50% α-amylase inhibition activity.

$$\text{α-amylase inhibition index (\%)} = A(\text{Blank}) - A(\text{sample})/\ A(\text{Blank}) \times 100$$

α-glucosidase inhibition assay: An *in vitro* α-glucosidase inhibition assay was performed at 37^{o}C using 100 mM phosphate buffer saline (PBS) in which the experiment is carried out at pH 6.8 [**21**]. The phosphate buffer containing sample aliquots with variable concentrations was incubated for 20 min at 37°C. The enzymatic substrate p-nitrophenyl-α-D-glucopyranoside is loaded into buffer solution and then the absorbance is monitored spectrophotometrically at 400 nm. Acarbose is used as reference α-glucosidase inhibitor and then the % enzyme inhibition is calculated as per the equation below

$$\text{α-glucosidase inhibition index (\%)} = A(\text{Control}) - A(\text{sample})/\ A(\text{Control}) \times 100$$

RESULTS AND DISCUSSION

Physico-chemical studies: The ligand BTPB and its transition metal complexes exhibits extensive air stability, non-hygroscopic, immiscible with water but forms complete miscibility with DMSO and DMF. Cobalt and Nickel complexes are coloured with fine amorphous powder and exhibits photostability for long time. The physico-analytical data of the ligands and complexes were taken in the solution of DMSO and are summarized in table 1. The analytical data indicates that the Co(III) and Ni(II) complexes shows ternary and binary coordination behaviour in accordance with the ligand nature 1:2 (M:BPTB) (C1 and D1) and 1:1:1 (C2 and D2) (M:BTPB:Phen) stoichiometric ratio. The molar conductance measurement predicts the electrolytic nature, a 0.1 mM solution of metal complexes in DMSO was used to record molar conductance at room temperature. The complex C1 and C2 exhibits molar conductance at 79.8 and 86.2 indicates uni-uni valent electrolytic nature whereas the complex D1 and D2 displays molar conductance at 5.3 and 6.1 $\text{Ʊmol}^{-1}\text{cm}^{-1}$, respectively infers non-electrolytic nature. Spectroscopic investigation on structural elucidation indicates that the Cobalt and Nickel centre is coordinated with tridentate imine base ligand BTPB and bidentate 1,10-phenanthroline ligand to complete octahedral geometry.

Elemental analysis: The elemental composition of the ligand and its complexes have been estimated by using PerkinElmer 2400 elemental analyzer. The metal content in the metal complexes was determined by employing EDTA complexometric titration after digesting the samples with hot concentrated nitric acid. The microanalytical data suggests that the stoichiometric ratio between

ligand and metal ion for the complex C1 and D1 is 1:2 and C2 and D2 complex is 1:1:1. The experimental elemental composition is in good agreement with the theoretical data as displayed in table 1.

Table 1. Physical and Elemental data for the ligand BTPB and its complexes (C1, C2, D1, and D2).

Compounds	Mol.wt	Yield (%)	Colour	M.P (^{0}C)	C	H	N	S	M
BPTB $C_{20}H_{13}BrN_2OS$	410.97	78	Pale brown	186	58.69 (58.66)	3.20 (3.23)	6.84 (6.82)	7.83 (7.81)	--
C1 $C_{40}H_{28}Br_2ClCoN_4O_4S_2$	941.22	65	Dark brown	>350	50.73 (50.71)	2.98 (2.97)	5.92 (5.90)	6.77 (6.75)	6.22 (6.19)
C2 $C_{32}H_{20}BrCl_2CoN_4OS$	715.92	70	Reddish brown	334	53.50 (53.48)	2.81 (2.79)	7.80 (7.78)	4.46 (4.42)	8.20 (8.16)
D1 $C_{40}H_{26}Br_2N_4NiO_3S_2$	889.80	72	Pale green	>350	53.78 (53.74)	2.93 (2.90)	6.27 (6.24)	7.18 (7.16)	6.57 (6.52)
D2 $C_{32}H_{20}BrClN_4NiOS$	679.28	73	Reddish green	321	56.30 (56.27)	2.95 (2.93)	8.21 (8.19)	4.70 (4.67)	8.60 (8.56)

Electronic absorption studies: In order to assess the electronic absorption of the ligand BPTB and metal complexes using 0.01 mM solution with DMSO solvent. The measurement is made against DMSO as blank, and readings were taken at room temperature. The ligand BPTB shows three absorption bands at 350 nm – 362 nm, and 385 nm – 420 nm and at 327 nm which is correlated to intra ligand transition such n - π* and π - π* located on aromatic chromophore, azomethine nucleus, and thiazole ring as illustrated in figure 1. Upon coordination with Co(III) and Ni(II) metal ion, the π – π* transition at 327 nm experience bathochromic shift along with slight hypochromism indicates the involvement of benzothiazole N towards coordination. Similarly, the intra ligand n - π* transitions from 352 – 420 nm experience slight blue shift accompanying hypochromism indicates coordination involvement of azomethine N. There is an additional band surfaced at 690 nm due to d – d transition for the complex **C1** and the inclusion of 1,10-Phenanthroline removes d – d transition as it induces more planarity to metal chelate. The LMCT characteristic bands of metal complexes surfaced in the region of 435 – 508 nm for C1, 440 – 498 nm for C2, 465 – 480 nm for D1 and 467 – 492 nm. The UV spectral data of the ligand and its complexes have been summarized in table 2.

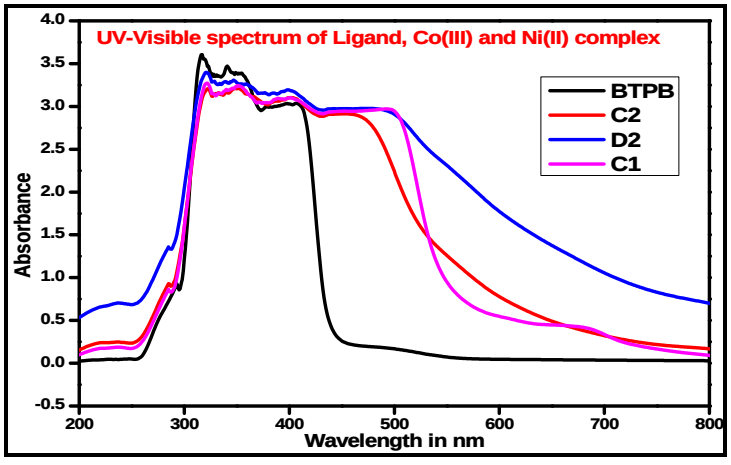


Figure 1. UV-Visible spectra of BTPB ligand and its complexes.

FT-IR spectral analysis: The coordination interaction between the imine base ligand with metal ion (Co(III) and Ni(II)) can be assessed by studying FT-IR spectrum of ligand BTPB and its complexes

within 4000 cm^{-1} to 400 cm^{-1}. There will be a broad band at 3600–2500 cm^{-1} due to hydroxyl group of the ligand which will disappear in the complex upon complexation as outlined in the figure 2. The presence of imine bond can be identified from a band at 1597 cm^{-1} which is due to stretching frequency of C=NH bond. Upon coordination, this bond can be stretched due to coordination admittance through imine N and hence the stretching frequency shifts to lower wave number. The azomethine stretching frequency is observed for the complex C1 at 1562 cm^{-1}, C2 at 1580 cm^{-1}, D1 at 1593 cm^{-1}, and for D2 at 1588 cm^{-1} (Figs. S1 and S2). The ternary Co(III) complex shows broad band in the region of 3600–2600 cm^{-1} due to the presence of lattice water molecule. In addition to that, there are weak bands surfaced in the region 419 cm^{-1} – 460 cm^{-1} and 512 cm^{-1} – 530 cm^{-1} correlated to stretching frequency of M-O and M-N as it confirms the subsequent involvement of ligand's N and O in the formation of coordination framework. In the free ligand BTPB, the involvement of phenolic O can be identified with a band at 1271 cm^{-1} which experiences shifted to lower region of stretching frequency by an amount of 60 cm^{-1} – 70 cm^{-1}. Further, the band at 1482 cm^{-1} due to stretching frequency of ring C=N which shifted to lower wavenumber in the metal complexes by an amount of 30 cm^{-1} – 40 cm^{-1} indicates the involvement ring C=N for coordination [22]. As the ring N donates electron to metal d-orbital, the double bond character decreases partially and hence lower stretching frequency for C=N group. From the IR spectrum of ligand and its complexes, it can be concluded that the coordination skeleton formed through azomethine N, benzothiazole N and phenolic O atom of imine base ligand.

Table 2. UV-Visible and FT-IR spectra of ligand BTPB and its complexes.

UV-Visible spectral data (nm)					FT-IR data (ν in cm^{-1})					
Sample	**n – π***	**π – π***	**LMCT**	**d-d**	**-CH=N**	**Ph-OH**	**C-O**	**C-Br**	**M-O**	**M-N**
BPTB	350-362 385-420	327	--	--	--	3600-2800	1482	813	--	--
C1	360, 415	330	435-508	690	1597	3338	1404	696	512	447
C2	365, 410	333	440-498	--	1562	--	1442	760	530	452
D1	360, 397	341	465-480	--	1580	--	1478	734	518	419
D2	362, 414	335	467-492	--	1588	--	1404	709	520	460

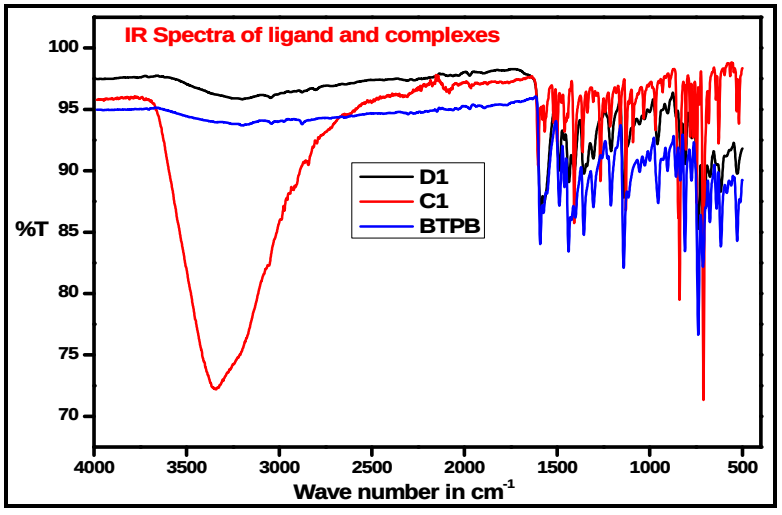


Figure 2. FT-IR Spectrum of ligand BTPB and its Co(III) complex C1 and Ni(II) complex D1.

NMR spectral analysis: The d_6-DMSO solvent is employed for the ^{1}H and ^{13}C NMR spectral investigation of ligand BPTB (Figure 3). The NMR spectrum of the ligand shows a characteristic peak at 8.840 ppm which is attribute to azomethine group. The phenolic -OH resonates as singlet at 12.049 ppm with respect to TMS and aromatic protons appears in the region of 8.377–6.533 ppm. In the ^{13}C-NMR spectrum (Figure 4), there are 20 peaks corresponding to 20 different carbon environments in the ligand skeleton. The peak with the highest chemical shift at 162.003 ppm is correlated to C planked between S and N in thiazole ring and characteristic of imine-C resonates at 156.983 ppm. The phenolic carbon containing hydroxyl group resonates at 154.648 ppm with respect to TMS as internal standard. The aromatic carbon nucleus in the ligand skeleton resonates in the region of 154.055 to 125.354 ppm.

Mass spectral analysis: Mass spectroscopy is an important analytical tool in order to elucidate the structural fragments with their mass and to predict molecular mass of the newly synthesized ligand BPTB and its complexes (C1, C2, D1, and D2). The electron spray ionization technique was employed in order to record the molecular ion peaks and their relative intensity of the ligand as shown in the FigureS5. The mass spectrum of the ligand BPTB shows sharp m/z peak surfaced at 410.9758 and 411.9793 attributed to M+ and M+1 peak. The molecular is consistent with the molecular formula of the ligand $C_{20}H_{13}BrN_2OS$. The ternary complex of Co(III) ion C1 exhibits M+ peak at 941.2278, M+1 peak at 942.2327, and M+2 peak at 943.2319 corresponding to the molecular formula $C_{40}H_{28}Br_2ClCoN_4O_4S_2$ (Fig. S6). The complex D1 with molecular formula $C_{40}H_{26}Br_2N_4NiO_3S_2$ exhibits M+ peak at 891.8085, M+2 peak at 893.8033, and M+4 895.8004 due to the isotopic peaks of bromine atom (Fig. S7). The binary complex with the molecular formula $C_{32}H_{20}BrClN_4NiOS$ exhibits M+ peak at 679.2833. In conclusion, the mass spectral investigation data is in good agreement with experimental data.

Biological investigations

In vitro antimicrobial studies: The microbial growth inhibition assay of ligand and its binary and ternary imine base metal complexes were studied against two positive gram bacterial strain *Staphylococcus aureus* MTCC-7443, *Bacillus subtilis* MTCC 121, two negative bacterial strain *Escherichia coli* MTCC-7410, and *Pseudomonas aeruginosa* MTCC 7093; and two fungal strains *Aspergillus flavus* MTCC-9606 and *Pichia anomala* MTCC-237. Gentamicin is used as reference drug for antibacterial study and fluconazole is used for antifungal examinations. The low cost and most efficient agar disc diffusion method were followed to assess the antimicrobial investigations. The antimicrobial data were analyzed, interpreted, and summarized in table 3and 4 along with corresponding MIC values. All the complexes show moderate to significant antimicrobial activity as compared ligand counterpart which is due to reduction in metal ion polarity which enables permeability factor to induce cell death [**23, 24**]. The mixed ligand binary complex C2 and D2 shows significant bacterial and fungal growth inhibition activity as compared to ternary complexes which might be due to the enhanced π-electron delocalization over phenanthroline nucleus and increased planarity of the metal chelate drastically change permeability, polarity, solubility, biocompatibility, and enhanced biomolecular (proteins and enzymes) interactions. As per the table 2, C2 and D2 shows potential antibacterial activity against gram negative bacterial strains *Escherichia coli* MTCC-7410, and *Pseudomonas aeruginosa* MTCC 7093 with MIC values 190 µg mL^{-1} to 210 µg mL^{-1} and moderate activity against positive gram bacterial strain with MIC value ranging from 270 µg mL^{-1} to 370 µg mL^{-1}. The binary complexes also execute potential activity against fungal species *Aspergillus flavus* MTCC-9606 with MIC value 160 µg mL^{-1} as illustrated in the figure 3. The Co(III) binary complex emerged as efficient antimicrobial agent against *E.coli* bacterial strain and *A.flavus* fungal strain with highest zone inhibition.

Table 3. Antimicrobial investigation data (MIC values in µg/mL) of BPTB and its metal complexes

Microorganism	MIC values (µg mL^{-1})						
	Doxycycline	Amoxycillin	Fluconazole	BPTB	C1	C2	D1
S. aureus	115	110	--	360	220	190	270
B. subtilis	100	105	--	330	210	210	290
E. coli	85	90	--	305	240	160	320
P. aeruginosa	70	80	--	270	300	145	310
A. flavus	--	--	110	330	260	140	280
P. anomala	--	--	120	360	290	155	310

Table 4. Antimicrobial data of ligand and its complexes

Microorganism	Con (µg mL^{-1})	Microbial zone inhibition in diameter							
		Doxycycline	Amoxycillin	Fluconazole	BTPB	C1	C2	D1	D2
S. aureus	100	13.89	14.60	--	--	4.30	9.13	4.13	6.22
	200	21.24	22.13	--	--	6.23	12.13	5.12	11.31
	300	29.45	26.30	--	4.10	11.24	19.11	10.77	16.42
	400	32.98	30.50	--	11.86	13.46	24.16	12.26	19.76
B. subtilis	100	15.22	16.20	--	--	4.55	8.22	3.45	6.22
	200	19.12	19.18	--	--	6.83	11.95	5.20	9.84
	300	23.78	25.12	--	3.82	9.20	16.88	9.24	14.26
	400	29.78	32.62	--	7.44	12.41	22.77	11.22	19.16
E. coli	100	16.22	14.33	--	--	4.44	9.13	3.78	7.45
	200	22.45	19.23	--	--	6.24	14.55	7.33	11.66
	300	28.74	24.44	--	5.70	7.25	21.22	9.13	17.23
	400	34.78	31.33	--	13.23	12.57	28.19	14.10	23.13
P. aeruginosa	100	13.89	16.99	--	--	4.11	8.66	3.98	7.44
	200	19.26	18.92	--	--	7.24	13.18	5.19	11.11
	300	24.89	23.88	--	6.20	10.10	17.85	10.85	16.12
	400	30.55	29.94	--	12.14	15.66	25.88	13.11	21.15
A. flavus	100	--	--	11.20	--	1.97	8.44	2.12	3.33
	200	--	--	15.56	--	3.31	11.44	4.06	8.12
	300	--	--	20.81	5.77	6.63	16.47	6.88	12.49
	400	--	--	24.76	10.69	8.22	20.87	8.11	16.55
P. anomala	100	--	--	10.78	--	2.22	3.11	2.46	2.37
	200	--	--	13.78	--	4.15	4.32	4.14	4.28
	300	--	--	18.22	3.56	5.17	5.98	4.93	6.66
	400	--	--	22.82	5.97	6.12	8.11	5.77	7.09

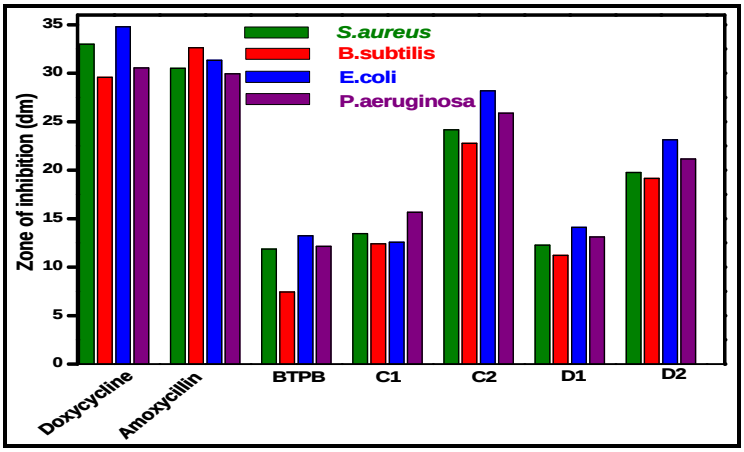


Figure 3. Antibacterial screening data of the ligand and its complexes.

In vitro DPPH scavenging assay: The antioxidant ability of a newly synthesized ligand BTPB can be assessed by using a stable DPPH free radical in which it accepts electron or H from antioxidant and accompanies with visible color change from purple to yellow [25, 26]. These changes can be captured by measuring absorbance at 517 nm using UV-Visible spectrophotometer. Hence, DPPH is classic material to study *in vitro* antioxidant properties. As evident from the Figure4, the absorbance at 517 nm decreases drastically at higher concentration of test compounds which is due to radical scavenging effect by the sample molecules through H-donation and paramagnetic DPPH becomes diamagnetic. The newly designed imine base ligand shows potential scavenging activity, and the absorbance ceases completely at higher concentration of the ligand molecule. The classical antioxidant butylated hydroxy toluene (BHT) is used as reference compound and all the compounds show lesser activity as compared standard molecule. The potential antioxidant activity of different compounds found in the following order BHT > BPTB > C2 > D2 > C1 > D1. The binary mixed ligand complex C2 shows greater scavenging activity with IC50 value 32.89 µg mL^{-1} after ligand BPTB with IC50 value 26.98 µg mL^{-1} whereas standard BHT exhibits IC50 value at 23.93 µg mL^{-1}.

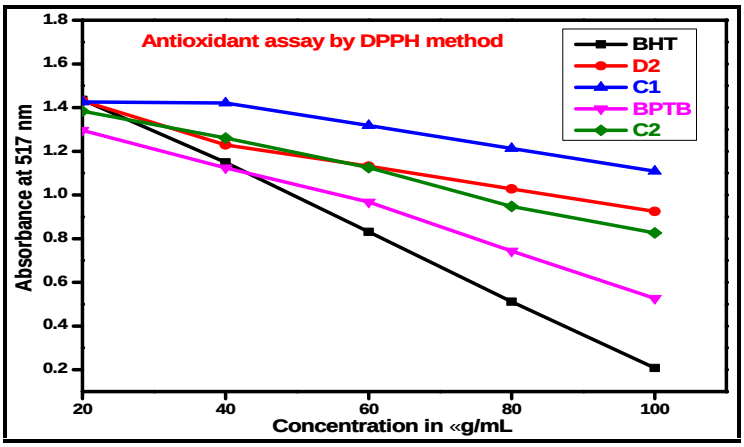


Figure 4. *In vitro* DPPH radical assay of the ligand and its complexes.

Antihemolytic assay-Membrane stabilization: In plenty of pathological conditions such as sickle cell anaemia, malaria, and thalassemia, the membranes of red blood cells are ruptured and makes leaky towards hemoglobin [27]. The RBC are very sensitive to the level of oxygen and at high concentration they are vulnerable to oxidative hemolysis which is correlated to high amount of polyunsaturated fatty acid content [28]. Therefore, any substrate undergoing oxidative damage will protect hemolysis of RBC membrane which are summarized as antioxidants. The fundamental importance of imine base complexes is governed by its antioxidant properties with wide pharmacological behaviour. Therefore, the supplementation of heterocyclic imine base and its complexes can drastically enhance radical defence mechanism of erythrocyte cell by suppressing oxidative stress and combat hemolysis. There are several internal and external factors which triggers inflammation to cause oxidative damage to cell and hemolysis of RBC. The binary mixed ligand complex C2 shows potential antihemolytic activity by reducing oxidative stress at RBC with percentage inhibition of 56.31. The antihemolytic data is summarized in Table 4 and displayed in the Figure 5. The ligand and ternary complexes show poor hemolytic inhibition as compared binary mixed ligand complexes. The highest hemolytic inhibition property is associated with standard compound vitamin C (85.26%). The antihemolytic activity of newly synthesized compounds are depends on concentration and can stabilize the cell membrane of RBC. The imine scaffolds induced membrane stability can be correlated to antioxidant properties which hampers cytotoxicity.

Antidiabetic properties: Diabetes Mellitus (DM) is associated with impaired functioning of insulin and hunting public health on long-term causing financial crisis. The drugs which slow down carbohydrate metabolism by hampering digestive enzymes such as α-amylase and α-glucosidase

garnered fundamental importance due to painless therapy, enhanced absorption, and effective therapeutic action.

Table 4. Antihemolytic screening data of ligand and complexes.

Compounds	Antihemolytic assay Inhibition index (%)
Vitamin C	85.26 ± 1.83
BPTB	23.12 ± 1.32
C1	30.27 ± 2.49
C2	56.31 ± 1.65
D1	28.35 ± 1.54
D2	38.12 ± 1.87

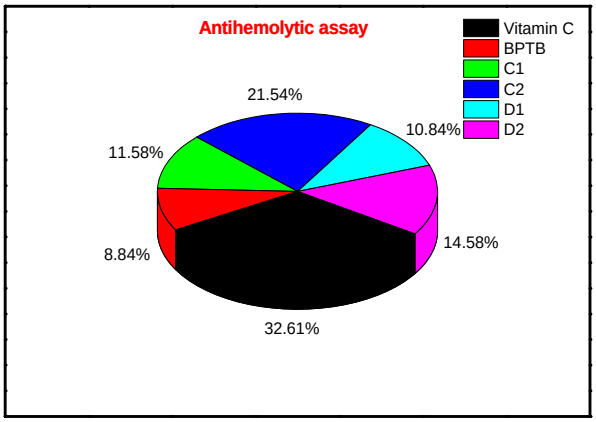


Figure 5. AAPH induced antihemolytic assay of ligand and complexes.

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α-amylase inhibition assay: The standard α-amylase inhibitor acarbose is used in order to assess the antidiabetic properties of ligand BPTB and its ternary and binary metal complexes. The mixed ligand C2 complex shows significant antidiabetic properties by inhibiting more than 50 % with an IC50 value at 37.17 µg mL^{-1} whereas standard acarbose exhibits IC50 at 32.14 µg mL^{-1}. The ternary Co(III) complex shows moderate activity and Ni(II) complexes as well as ligand exhibits poor activity. The greater activity of mixed ligand complex might be due to synergistic effects BPTB and 1,10-Phenanthroline with Co(III) metal ion makes enhanced gastrointestinal absorption leads to better enzyme inhibition. The inhibition data is summarized in the table 5 and outlined in the figure 6. All the compounds show enhanced activity at higher concentration and showing concentration dependent antidiabetic activity.

α-glucosidase inhibition assay: The newly synthesized molecules were exploited for their antidiabetic efficacy by carrying *in vitro* α-glucosidase inhibition assay. Among the compounds, binary complexes show promising inhibition activity as compared to its ternary complexes and ligand counterpart. All the compounds show poor to significant antidiabetic activity with IC50 values ranging from 28.75 µg mL^{-1} to 54.11 µg mL^{-1} whereas IC50 value for classical inhibitor acarbose found to be at 23.28 µg mL^{-1}. The greater activity C2 mixed complex might be due the presence of 1,10-phenanthroine makes the metal chelate more planar and allows extensive π-electron delocalization enhances cellular absorption by reducing polarity. In addition to that the presence N-atom in the metal chelate might execute different types of molecular interactions such as electrostatic, polar, and hydrogen bonding interaction on enzymatic surface which perturb the conformation and hence reduced activity. The inhibitory action of synthesized metal complexes is displayed in the Table 6 and illustrated in the figure 6.

Table 5. Anti- α-amylase activity of the ligand and complexes

Compounds	Con in μg mL^{-1}	Absorbance at 540 nm	Inhibition index (%)
Blank	**0**	**0.492**	**0**
BPTB	10	0.481	2.23577
	20	0.454	7.72357
	30	0.397	19.30894
	40	0.353	28.25203
	50	0.310	36.99186
Cl	10	0.423	14.02439
	20	0.368	25.20325
	30	0.315	35.97561
	40	0.252	48.78049
	50	0.201	59.14634
C2	10	0.406	17.47967
	20	0.327	33.53659
	30	0.281	42.88618
	40	0.176	64.22764
	50	0.103	79.06504
D1	10	0.461	6.300813
	20	0.405	17.68293
	30	0.362	26.42276
	40	0.318	35.36585
	50	0.264	46.34146
D2	10	0.423	14.02439
	20	0.368	25.20325
	30	0.315	35.97561
	40	0.252	48.78049
	50	0.201	59.14634
Acarbose	10	0.373	24.18699
	20	0.264	46.34146
	30	0.197	59.95935
	40	0.082	83.33333
	50	0.008	98.37398

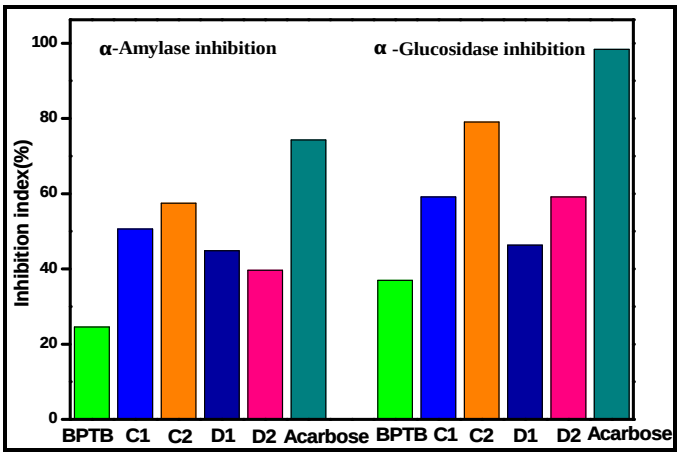

Figure 6. Antidiabetic properties of the ligand BTPB and its complexes.

Table 6. Anti- α-Glucosidase activity of the ligand and complexes

Compounds	Con in µg mL^{-1}	Absorbance at 507 nm	Inhibition index (%)
Blank	0	1.824	0
	10	1.732	5.04385
	20	1.693	7.18201
BPTB	30	1.632	10.52631
	40	1.498	17.87280
	50	1.376	24.56140
C1	10	1.632	10.52632
	20	1.453	20.33991
	30	1.247	31.63377
	40	1.019	44.13377
	50	0.901	50.60307
C2	10	1.606	11.95175
	20	1.393	23.62939
	30	1.161	36.34868
	40	0.986	45.94298
	50	0.776	57.45614
D1	10	1.743	4.440789
	20	1.615	11.45833
	30	1.392	23.68421
	40	1.204	33.99123
	50	1.006	44.84649
D2	10	1.682	7.78508
	20	1.553	14.85745
	30	1.447	20.66885
	40	1.219	33.16885
	50	1.101	39.63815
Acarbose	10	1.451	20.44956
	20	1.224	32.89474
	30	0.948	48.02632
	40	0.732	59.86842
	50	0.469	74.28728

CONCLUSION

Benzothiazole motif containing imine base have been designed and synthesized, along with the ternary and binary complexes of Co(III) and Ni(II) ion coordinated with primary ligand 2-((E)-(2-(benzo[d]thiazol-2-yl)phenylimino)methyl)-4-bromophenol BTPB and 1,10-phenanthroline as secondary ligand was synthesized. The afresh ligand and complexes have screened for their elemental composition, coordination site, molecular weight, structure, geometry, and conducting behaviour using wide array of Spectro-analytical techniques. The spectral investigation is in good alignment with the proposed structural formula and geometry. The synthesized compounds were screened for different biological and pharmacological properties. The ligand BTPB shows prominent in vitro DPPH antioxidant activity with IC50 value 26.98 µg mL^{-1} whereas the complexes show moderate to poor antioxidant properties. The binary complexes C2 and D2 shows good antimicrobial activity with IC50 value ranging from 185 µg mL^{-1} to 210 µg mL^{-1} against gram negative bacterial strain as compared to gram positive bacterial strain. The binary complexes are more specific towards gram negative bacterial strain which might be correlated to biocompatibility, reduced polarity, and enhanced delocalization over planar aromatic chromophore. The complex C2 shows significant antihemolytic activity with inhibition index of 56.31% and the complex D2 with 38.12%. In the antidiabetic assay, the mixed ligand complex emerged as potent α-amylase and α-glucosidase inhibitor with IC50 value 37.17 µg mL^{-1} and 31.66 µg mL^{-1}, respectively.

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Conflict of interest: it is declare no conflict of interest.

Supplementary information:

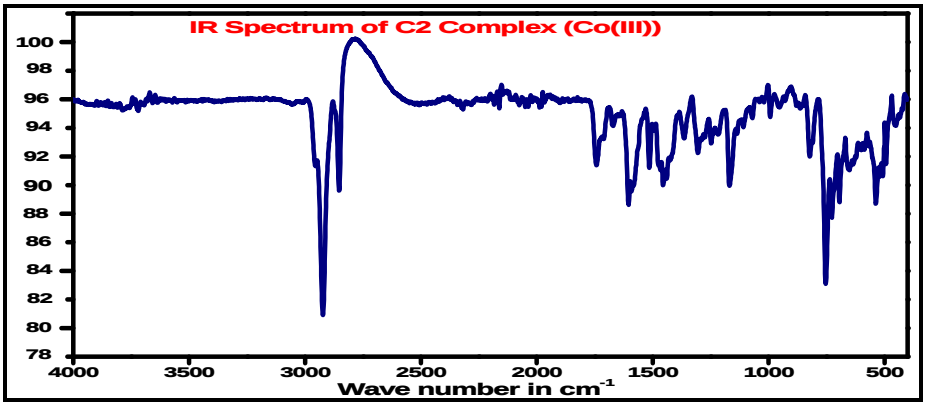

Figure S1. FT-IR spectrum of Co(III) complex C2.

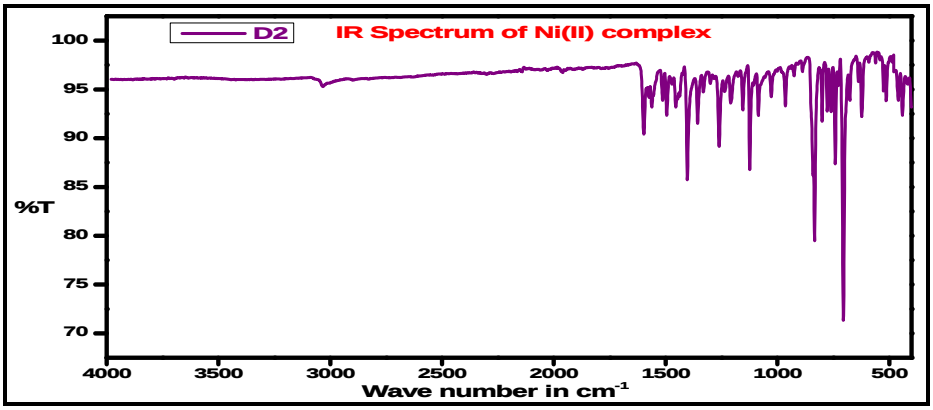

Figure S2. FT-IR spectrum of Ni(II) complex D2.

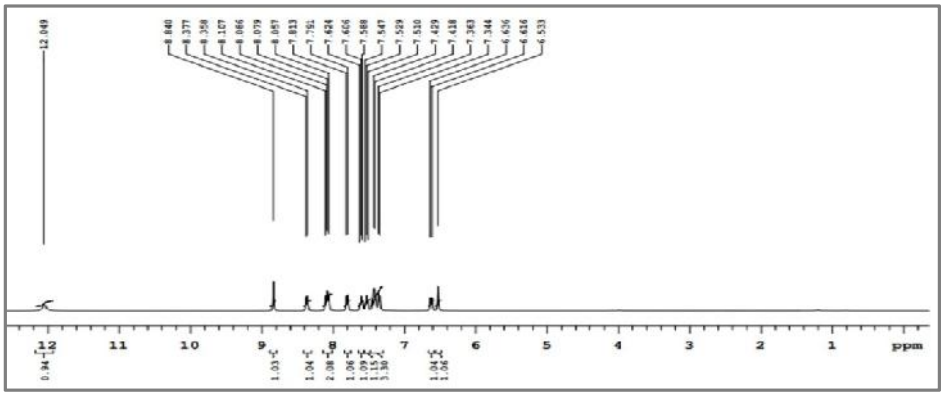

Figure S3. ^{1}H-NMR Spectrum of ligand BTPB.

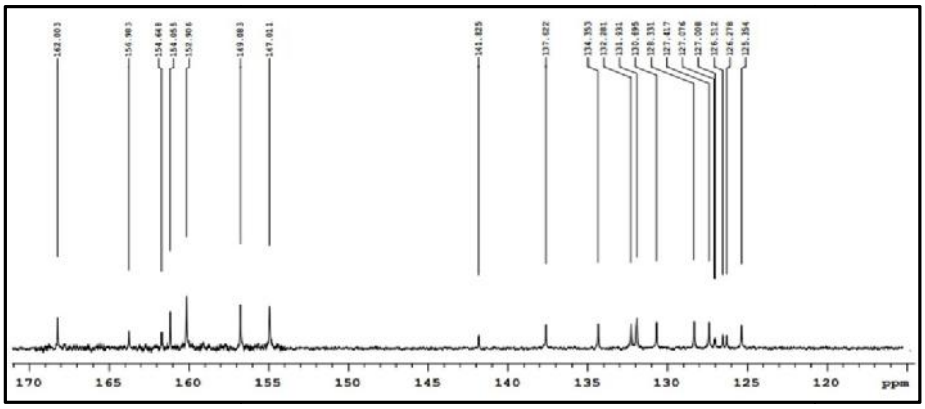

Figure S4. ^{13}C-NMR Spectrum of ligand BTPB.

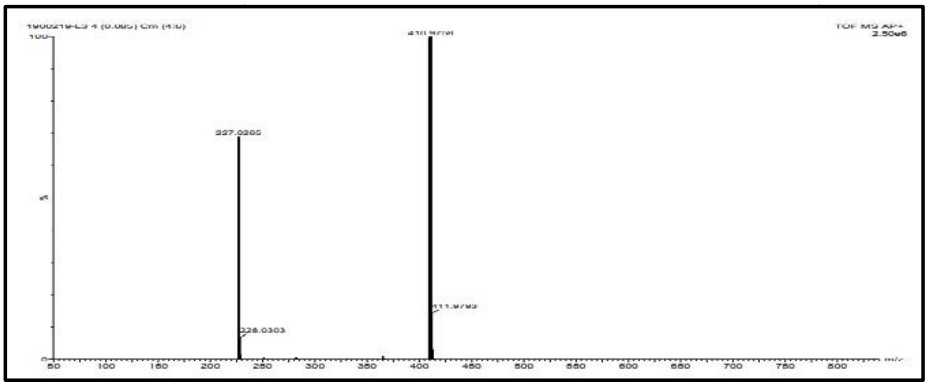

Figure S5: Mass spectrum of the ligand BTPB..

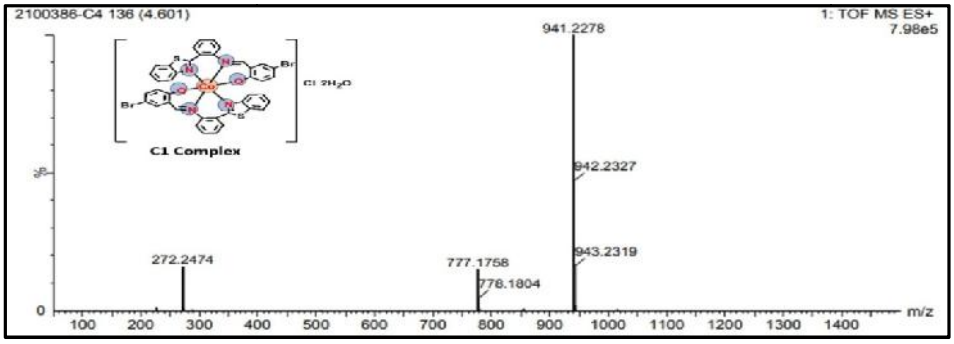

Figure S6. Mass spectrum of Co(III) complex C1.

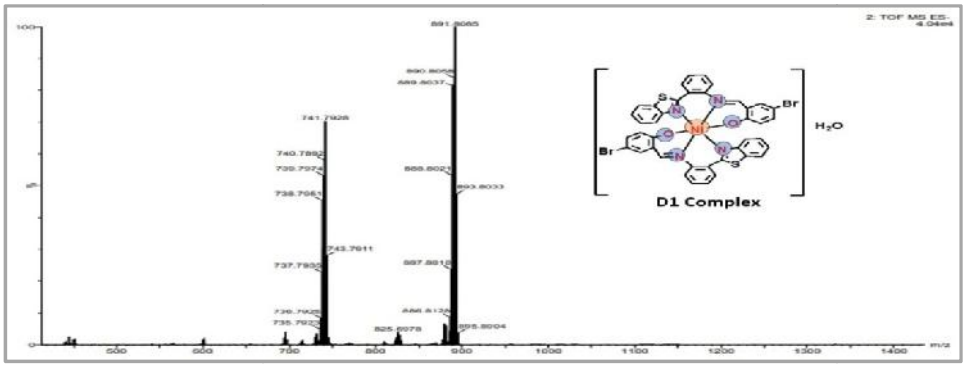

Figure S7. Mass spectrum of Ni(II) complex D1.

REFERENCES

[1]. A. Fetoh, O. A. El-Gammal, G. M. Abu El-Reash, Antioxidant and Antitumor activities of Cr(III), Mn(II), Fe(III), Cd(II), Zn(II), and Hg(II) complexes containing a carbohydrazone ligand ending by 4-pyridyl ring, *J. Mol. Struct.*, **2018**, 1173, 100 -110. http://doi.org/10.1016/j.molstruc.2018.06.087.

[2]. K. Gopichand, V. Mahipal, N. Nageswara Rao, A. Majeed Ganai, P. Venkateswar Rao, Co(II), Ni(II), Cu(II), and Zn(II) complexes with Benzothiazole Schiff base ligand: Preparation, Spectral, Characterization, DNA binding, and *in vitro* Cytotoxic Activities, *Results in Chemistry*, **2023**, 5, 100868.

[3]. P. C. Sharma, A. Sinhmar, A. Sharma, Medicinal significance of benzothiazole scaffold: an insight view, *J. Enzyme Inhib. Med. Chem*., **2013**, 28(2), 240-266. http://doi.org/10.3109/14756366.2012.720572.

[4]. B. Soni, M. S. Ranawat, R. Sharma, A. Bhandari, S. Sharma, Synthesis, and Evaluation of some new Benzothiazole derivatives as Potential Antimicrobial agents, *Eur. J. Med. Chem*., **2010**, 45, 2938 -2942.

[5]. D. Bhavsar, J. Trivedi, S. Parekh, M. Savant, S. Thakrar, A. Bavishi, Synthesis and *in vitro* anti-HIV activity of N-1,3-benzo[d]thiazol-2-yl-2-(2-oxo-2H-chromen-4-yl)acetamide derivatives using MTT method, *Bioorg. Med. Chem. Lett*., **2011**, 21, 3443-3446.

[6]. R. Alizadeh, I. Yousuf, M. Afzal, S. Srivastav, S. Srikrishna, Enantiomeric Fluoro-substituted Benzothiazole Schiff base- valine Cu(II)/Zn(II) Complexes as Chemotherapeutic agents: DNA binding profile, Cleavage activity, MTT assay and Cell Imaging Studies, *J. Photochem. Photobiol. B*, **2014**, 1344(14), 1-40, http://doi.org/10.1016/j.jphotobiol.2014.12.027.

[7]. A. T. Odularu, Ease to Challenges in Achieving Successful Synthesized Schiff Base, Chirality, and Application as Antibacterial Agent. *BioMed Res. Int*., **2023**, 1626488.

[8]. K. Vashi, H. B. Naik, Synthesis of Novel Schiff Base and Azetidinone Derivatives and Their Antibacterial Activity, *J. Chem*., **2004**, 1, 272-275.

[9]. A. K. Jain, R. K. Singla, B. Shrivastav, Thiazole: A Remarkable Antimicrobial and Antioxidant Agents. *Pharmavologyonline*., **2011**, 2, 1072-1084.

[10]. A. K. Neelu Patel, R. N. Prajapati, R. N. Jadeja, S. Patel, Dioxidovanadium (V) complexes of a tridentate ONO Schiff base ligand: Structural characterization, quantum chemical calculations and *in vitro* antidiabetic activity, *Polyhedron*, **2020**, 180, 114434. http://doi.org/10.1016/j.poly.2020.114334.

[11]. A. Nihath Nazleen, M. Umadevi, Insight of anti-diabetes, anti-microbial, DFT and Molecular Docking Analysis of Imine Group Palladium (II) Complexes, *Chem. Inorg. Mater*, **2023**, 1, 10009.

[12]. L. Sundaramurthy Santa, G. Kannappan, M. Gayathri, Tridentate Schiff Base (ONO) Transition Metal Complexes: Synthesis, Crystal Structure, Spectroscopic Characterization, and *in vitro* Antidiabetic Studies of New Schiff Base Copper (II) Complexes, *J. Chem. Sci*., **2016**, 128(7), 1095-102. http://doi.org/10.1007/s12039-016-1099-8.

[13]. S. Torabi, M. Mohammadi, M. Shirvani, Antidiabetic, Antioxidant, Antibacterial, and Antifungal of Vanadyl Schiff Base Complexes, *Tren. Pharma. Sci*., **2018**, 4(2), 87-94.

[14]. I. P. Tripathi, M. Mahendra Kumar, K. Arti, M. Chinmayi, T. Ruchita, Synthesis, Characterization of Some Antidiabetic Copper Complexes with Ethylenediamine, *Res. j. Chem*., **2013**, 3(12), 54-59.

[15]. J. E. Weder, C. T. Dillon, T. W. Hambley, B. J. Kennedy, Copper Complexes of Non-steroidal Anti-inflammatory Drugs: An Opportunity yet to be Realized, *Coord. Chem. Rev*., **2002**, 232, 95-126.

[16]. K. B. Pandey, I. P. Tripathi, M. K. Mishra, N. Dwivedi, Y. Pardhi, A Critical Review on Traditional Herbal Drugs: An Emerging Alternative Drugs for Diabetes, *Int. J. Org. Chem*., **2013**, 3(1), 1-22.

[17]. H. L. Yang, M. Korivi, M. Kuem Lin, H. Chun-Wei Chang, Antihemolytic and Antioxidant Properties of Pearl Powder against 2, 2-azobis(2-amidinopropane) dihydrochloride-induced Hemolysis and Oxidative Damage to Erythrocytes Membrane Lipids and Proteins, *J. Food. Drug. Anal*., **2017**, 25(4), 898-907.
[18]. F. K. Ommenya, E. A. Nyawade, D. M. Andala, J. Kinyua, Synthesis, Characterization and Antibacterial Activity of Schiff Base, 4-Chloro-2-{E)-[(4-Fluorophenyl)imino]methyl}phenol Metal (II) Complexes, *J. Chem*., **2020**, 1-8. http://doi.org/10.1155/2020/1745236
[19]. N. Karali, O. Guzel, N. Ozsoy, S. Ozbey, Synthesis of New Spiroindolinones Incorporating a Benzothiazole Moiety as Antioxidant Agents, *Eur. J. Med. Chem*., **2010**, 45, 1068-1077.
[20]. S. Ali, H. Zheng, A. Zaidi, F. Ahmed Khan, S. Majid Bukhari, R. Muhammad, Moxifloxacin Metal Complexes: Synthesis, Characterization, Antimicrobial and Antidiabetic Activities with Docking Studies, *Bioinorg. Chem. Appl*., **2023**, 1-15. http://doi.org/10.1155/2023/374561.
[21]. N. U. Rehman, R. Maqsood, S. Ullah, S. A. Halim, M. U. Anwar, A. Khan, Eupholaricanone, A Potent α-glucosidase anthracene derivative from Euphorbia larica. *Boiss. S. Afr. J. Bot*., **2022**, 148, 88-92.
[22]. T. Rosu, E. Pahontu, C. Maxim, R. Georgescu, Some New Cu(II) complexes containing ON donor Schiff Base: Synthesis, Characterization and Antibacterial Activity, *Polyhedron*., **2011**, 30, 154-162.
[23]. K. Savithri, H. D. Revanasiddappa, Synthesis and Characterization of Oxovanadium (IV) Complexes of 2-((E)-(6-Fluorobenzo[d]thiazol-2-ylimino) methyl)-6-methoxyphenol and Their Antimicrobial, Antioxidant, and DNA-Binding Studies, *Bioinorg. Chem. Appl*., **2018**, 1-12. http://doi.org/10.1155/2018/2452869.
[24]. N. Vamsikrishna, M. Pradeep Kumar, S. Tejaswi, A. Rambabu, DNA Binding, Cleavage and Antibacterial Activity of Mononuclear Cu(II), Ni(II), and Co(II) Complexes Derived from Novel Benzothiazole Schiff Bases, *J Fluoresc*., **2016**, 1-13. http://doi.org/10.1007/s10895-016-1818-z.
[25]. I. Kostova, L. Saso, Advances in Research of Schiff Metal Complexes as Potent Antioxidants, *Curr. Med. Chem*., **2013**, 20, 4609-4632.
[26]. N. U. Rehman, M, Shah, S. Ullah, M. Khan, O. Ullah, Enzymes Inhibition and Antioxidant Potential of Medicinal Plants Growing in Oman, *BioMed Res. Int*., **2022**, 1-10.
[27]. A. K. Pandey, P. P. Kashyap, C. D. Kaur, Anti-inflammatory Activity of Novel Schiff bases by *in vitro* Models, *Bangladesh. J. Pharmacol*., **2017**, 12(1), 41-43. http://doi.org/10.3329/bjp.v12i1.29675.
[28]. H. A. Hammed, M. Hassan, H. M. Hussein, A. F. Elhusseiny, Green Synthesis of Nanosized N, N'-bis(1-naphthylidene)-4,4'-diaminophenylmethane and its Metal (II) Complexes and Evaluation of Their Biological activity, *Sci. Rep*., **2022**, 12, 21142. http://doi.org/10.1038/s41598-022-25650-z..