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Antioxidant and lipoxygenase inhibition studies of 4-(3-bromophenyl)-[2,2'-bipyridine]-6-carboxylic acid and its metal complexes

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Abstract: **Background**: Synthesis of novel enzyme inhibitors and antioxidants is active research in pharmaceuticals sciences. Novel compounds with potential therapeutic value can be synthesized to help the body combat oxidative-stress related diseases. **Objectives**: This study comprised synthesis of 4-(3-bromophenyl)-[2,2'-bipyridine]-6-carboxylic acid, its complexation with Fe(II), Cu(II) and Ni(II), and the evaluation of these compounds as lipoxygenase inhibitors and antioxidant agents. **Methods**: The ligand and its complexes were characterized using NMR, IR, UV–Vis and mass spectroscopies. The synthesized compounds were also screened to evaluate their antioxidant and lipoxygenase properties. Two sample t-test and ANOVA were used to evaluate the significance of biological activity of the complexes. **Results**: The ligand acted as a tridentate donor forming ML_2 complexes. Cu(II) and Ni(II) complexes showed significant lipoxygenase inhibition with the respective IC_{50} values of 50.6 and 59.8 µM, while antioxidant activity was largely attributed to the ligand itself with IC_{50} value of 56.4 µM. **Conclusion**: The study demonstrated that Cu(II) and Ni(II) complexes of the synthesized ligand were promising candidates for lipoxygenase inhibition, while the free ligand exhibited significant antioxidant activity.

Keywords: Antioxidants; Lipoxygenase inhibition; Metal complexes

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INTRODUCTION

Reactive oxygen species (ROS) and free radicals are produced continuously in the human body because of several enzymatic and non-enzymatic biological reactions. ROS may have both beneficial and adverse effects on the human body (Mironczuk-Chodakowska *et al.*, 2018). Within certain limits, ROS are healthy for the human body; however, beyond a threshold, they cause oxidative stress which is relatively detrimental (Iqbal *et al.*, 2019). They can cause oxidative degradation of membrane lipids, monosaccharides, cell membranes, proteins and DNA. ROS can cause numerous diseases including cardiovascular ailments, cancer, atherosclerosis and aging. (Loncaric *et al.*, 2021; Oh and Shahidi, 2018). However, the excessive use of antioxidants is associated with many diseases like dementia, Alzheimer's disease and cancer (Aversa *et al.*, 2016).

Antioxidants are the compounds that resist the formation of ROS and radicals. They do this by donating electrons or hydrogen to radicals or ROS hence slowing down the formation of toxic radicals in the body (Ifeanyi, 2018). Transition metal (II) complexes of halogenated derivatives of quinazoline are reported to have significant antioxidant activity (Kakoulidou *et al.*, 2022). Similarly, pyridine derivatives also possess antioxidant nature (Khan, 2021).

Lipoxygenase (LOX) are iron-containing non-heme dioxygenase enzymes (Thallaj, 2023). They facilitate the insertion of molecular oxygen into cis, cis-1,4-pentadiene moieties of polyunsaturated fatty acids, yielding fatty-acid hydroperoxides. The oxidation state of Fe determines the exact function of LOXs. The active form of the enzyme contains Fe (III). However, metabolic processes reduce it to Fe(II) (Fazal-Ur-Rehman *et al.*, 2019). The enzyme in this state is notorious for developing asthmatic responses, glomerulonephritis, prostate cancer and psoriasis. Hence, the oxidation state of Fe ion plays a critical role in the LOX functioning (Waller *et al.*, 2008).

Lipoxygenase inhibitors play significant role in regulating LOX enzyme activity. They restrict the availability of enzymes by binding themselves to active sites. Halogens containing organic compounds exhibit good lipoxygenase inhibition. This property of the compound increases with the increase in halogens (Makkar and Chakraborty, 2018).

Based on the reported activity of halogenated organic compounds and bipyridine-based ligands, this study was designed to explore the potential of bromophenyl-substituted bipyridine ligand to enhance its biological activity upon complexation with transition metals. It was hypothesized that the synthesized complexes will display improved lipoxygenase inhibition and antioxidant activity compared to the free ligand and corresponding metal ions. The hypothesis was statistically tested using t-test and ANOVA. This research question addressed a gap in current literature, where the influence of halogen substitution on bipyridine–metal coordination and associated biological activities remained underexplored.

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MATERIALS AND METHODS

Materials

The chemicals used in this research were reagent grade and purchased from BDH, Merck or Sigma–Aldrich. TLC plates, silica gel 60 F254 (Merck, Darmstadt, Germany). The characterization studies were performed using Shimadzu 8900 IR spectrophotometer (Shimadzu Corporation, Kyoto, Japan), Bruker Avance 400MHz spectrometer (DMSO-d6) (Bruker BioSpin GmbH, Rheinstetten, Germany). AB Sciex QStar XL MS/MS (ESI-MS) (Applied Biosystems/MDS Sciex, Concord, ON, Canada), JEOL MS Route 600H (EI-MS) (JEOL Ltd., Tokyo, Japan), CHN/ S analyzer Perkin Elmer 2400 series (PerkinElmer Inc., Waltham, MA, USA).

Methodology

4-(3-bromophenyl)-[2,2'-bipyridine]-6-carboxylic acid

The synthesis of 4-(3-bromophenyl)-[2,2'-bipyridine]-6-carboxylic acid ligand was a multistep reaction scheme as shown in fig 1. Synthesis of ligand till step 2 is reported in another study (Basha *et al.*, 2017).

2-bromo-1-(pyridin-2-yl) ethenone (1)

2 g of 2-acetyl pyridine was mixed with 4 mL of 38% hydrobromic acid (HBr). An ice bath was used to cool this mixture. The chilled solution of HBr and 2-acetyl pyridine was mixed with a solution containing 5 g of pyridinium tribromide in 60 mL of acetic acid. This solution was stirred for 6 h at 60°C. Reaction was cooled down after completion and 120 mL diethyl ether was added while stirring the solution. The reaction mixture was refrigerated for 12 h. The precipitates obtained were washed and filtered with acetone; Yield: 99.59%, yellow solid.

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 7.61 (s, 2H, CH_2), 7.54 (td, 1H, J = 4.8 Hz, J = 1.2Hz, Ar-H), 7.89 (td, 1H, J = 7.8 Hz, J = 1.5Hz, Ar-H), 8.18 (d, 1H, J = 7.8 Hz, Ar-H), 8.69 (d, 1H, J = 4.2Hz, Ar-H).
MS (EI): 198.9

1-(2-oxo-2-(pyridin-2-yl) ethyl) pyridin-1-ium bromide (2)

5 g of 2-bromo-1-(pyridin-2-yl) ethenone and 2.5 mL of pyridine were added to 107 mL of tetrahydrofuran (THF). The blend was stirred for 6 h. After the reaction was finished, the precipitates were filtered and washed using THF; Yield 75%, white solid.

^{1}H -NMR ($CDCl_3$, 500 MHz): δ 6.49 (S, 2H, CH_2), 7.84-7.81(m, 1H, Ar-H), 8.15-8.06 (m, 2H, Ar-H), 8.26 (t, 2H, J = 10.5 Hz, Ar-H, 8.72 (t, 1H, J = 5.7 Hz, Ar-H), 8.87 (d, 1H, J = 3.3 Hz, Ar-H), 8.99 (d, 2H, J = 5.6Hz, Ar-H).
MS (EI): 278.8[M^+]

Sodium (E)-4-(3-bromophenyl)-2-oxobut-3-enoate (3)

In 19 mL of water and 83 mL of ethanol, respectively, 2.37 g of sodium pyruvate and 4 g of 3-bromo benzaldehyde were dissolved. The solution of 3-bromo benzaldehyde was mixed with dissolved sodium pyruvate. 43 mL of 10% KOH was added to this aliquot after cooling it in the ice bath. The mixture was stirred for 2 h. During the reaction, the color changed to bright yellow. The resulting precipitates were filtered and washed with ice water; Yield 72%, bright yellow solid.

^{1}H-NMR (DMSO, 500 MHz): δ 7.18 (d, 2H, J = 16.4 Hz, =CH), 7.62 (d, 2H, J = 8 Hz, Ar-H), 7.64 (d, 1H, J = 8 Hz, =CH), 7.72 (d, 2H, J = 8.4Hz, Ar-H).
MS (EI): 275.94[M^+]

4-(3-bromophenyl)-[2,2'-bipyridine]-6-carboxylic acid (4)

1-(2-oxo-2-(pyridin-2-yl)ethyl)pyridin-1-ium bromide (1.81g), sodium (E)-4-(3-bromophenyl)-2-oxobut-3-enoate (2g) and ammonium acetate (4 g) were dissolved in 49 mL of water and refluxed for 6 h at 100°C. The precipitates were filtered and then washed with acetone and water; Yield: 39.5%, pure white.

IR (KBr) υ_{max}: 3600-2500cm^{-1} (broad band –OH), 3055.24 cm^{-1} (sp^2-CH), 2922.0cm^{-1}, 2926.01cm^{-1} (sp^3-CH), 1566.20 cm^{-1} (C=O) and (C=N), 1440.83cm^{-1} (C=C), 1373.32 cm^{-1} (C-C), 1317.38 cm^{-1} (C-N), 1062.78cm^{-1} (C-O).

^{1}H-NMR (MeOD, 500 MHz): δ 7.487 (Merge two triplet, H-5', H-3''), 7.642 (d, 1H, J =8 Hz, H-2''), 7.851 (d, 2H, J = 1.5 Hz, H-4''), 7.968 (t, 1H, J = 1 Hz, H-4'), 8.052 (s, 1H, H-6''), 8.32 (s, 1H, H-5), 8.630 (s, 1H, H-3), 8.648 (d, 1H, J = 8.00 Hz H-3'), 8.753 (d, 1H, J = 8.00 Hz, H-6').
MS (EI): 354.0[M^+]

General procedure for synthesis of Fe(II), Cu(II) and Ni(II) complexes

In 3 mL methanol and water (1:1), 0.15 mmol of ligand and 0.075 mmol of metal salt were dissolved, respectively. For preparation of each metal complex, the ligand solution was mixed with the respective metal solution and stirred for 30 min. The precipitation was instantaneous. The precipitates formed were filtered and washed using methanol. The color of Fe(II), Cu(II) and Ni(II) complexes were blue, light blue and green, respectively.

Biological screening

The synthesized ligand, metal salts and their complexes were assessed for antioxidant and lipoxygenase inhibition activities.

Antioxidant activity (DPPH radical scavenging assay)

The antioxidant potential was studied using the 2,2′-diphenyl-1-picrylhydrazyl (DPPH) assay. DPPH solution (0.3 mM in ethanol) served as a radical source. Test compounds were serially diluted in DMSO to prepare concentrations from 7.8 to 500 µM. For each assay, 90 µL of the DPPH solution was dispensed into wells of a 96-well

plate. Afterwards, 10 µL of test sample was added in each solution to start the reaction. The incubation time was 30 min at 37 °C. Finally, the absorbance was recorded at 517 nm using SpectraMax Plus 384, Molecular Devices, USA. Butylated hydroxyanisole (BHA) was the reference antioxidant, while wells containing DMSO alone served as negative controls. The relative radical scavenging activity and IC_{50} values were obtained using EZ-Fit software (Perrella Scientific Inc., USA) (Ali *et al.*, 2009; Siddiq *et al.*, 2012).

Lipoxygenase inhibition assay

Inhibition of lipoxygenase activity was evaluated by a modified spectrophotometric procedure based on the Tappel method. Each reaction mixture contained 160 µL of 100 mM sodium phosphate buffer (pH 8.0), 20 µL of lipoxygenase solution (~130 U) and 10 µL of test compound prepared in DMSO (final concentration 5–500 µM). Appropriate blanks were prepared to account for enzyme and substrate background absorbance. Following a 10 min pre-incubation at 25°C, the reaction was initiated by adding 10µL of linoleic acid solution (0.5 mM; stabilized with 0.12 % Tween-20 in a 1:2 ratio). To prevent autoxidation, substrate solution was flushed with nitrogen prior to use. The increase in absorbance, corresponding to conjugated diene formation, was monitored at 234 nm. Baicalein was employed as a positive control inhibitor. The extent of inhibition at different concentrations was used to calculate IC_{50} values with EZ-Fit enzyme kinetics software (Perrella Scientific Inc., USA) (Tappel, 1962).

Statistical analysis

The significance of lipoxygenase inhibition activities of the ligand, metal ions and the synthesized complexes was statistically evaluated using ANOVA (Miller and Miller, 2005). Triplicate experimental data was used for statistical analysis. IC_{50} values were reported as mean ± standard deviation. Minitab Statistical Software, version 20 (Minitab LLC, State College, PA, USA) was used to perform statistical tests. The assumptions of normality and homogeneity of variance were verified using the Shapiro–Wilk and Levene's tests, respectively at $p<0.05$. Overall group differences were assessed by one-way ANOVA. To explore specific pairwise comparisons (e.g., ligand vs. complex, metal vs. complex), two-sample t-tests were carried out.

RESULTS

Synthesis and physiochemical properties

The synthesis of ligand followed scheme shown in Fig. 1. The NMR, mass and IR spectra of the synthesized ligand are shown in Fig. 2. All the complexes showed that the metal-to-ligand ratio is 1:2 represented by a general formula shown in Fig. 3. The mole-ratio plots for the synthesized complexes are shown in Fig. 4.

IR spectral studies

IR data of ligand, metals and their complexes are shown in Table 1.

MALDI-MS studies

The molecular masses of Fe, Ni and Cu complexes obtained using mass spectrometry were 764.82, 763.92 and 768.92 amu, respectively.

Visible spectral studies

Visible spectra of complexes and ligands were obtained using DMSO as a solvent and shown in Fig 5. The molar absorptivity of metal, ligand and complexes are listed in Table 2.

UV spectral studies

The wavelength of maximum absorption and molar absorptivity of metal, ligand and complexes are listed in Table 2.

Antioxidant properties

The IC_{50}-values determined to study the antioxidant properties of complexes, ligand and metal salts are presented in fig 6.

Lipoxygenase Inhibition of synthesized complexes

The IC_{50} values for lipoxygenase inhibition by ligand, metal ions and each of the synthesized complexes are shown in fig 7. The Fe-complex was found to be least effective for the enzyme inhibition with IC_{50} value of 99 µM. The Cu-complex was the most effective among all the compounds used in this study with IC50 value of 50.6 µM. The reference drug baicalein, however, had the lowest IC_{50} value, 22.6 µM, indicating it is most effective in the enzyme inhibition.

DISCUSSION

Synthesis and physiochemical properties

Fig. 1 shows the synthesis scheme of the ligand. Synthesized ligand was characterized using UV–Vis, IR, MALDI, EI-Mass, H^1- NMR spectroscopies. Fe(II), Ni(II) and Cu(II) complexes with the ligand were prepared and the mole ratio of the complexes was determined using the mole ratio method. The metal-to-ligand ratio for all the synthesized complexes was 1:2 as shown in fig 4. Synthesized complexes were non-hygroscopic in nature. Complexes of Fe(II), Ni(II) and Cu(II) were soluble in DMSO. The colors of Fe(II), Ni(II) and Cu(II) complexes were blue, green, orange and light blue, respectively. All the complexes were precipitated using methanol as a solvent. It was inferred that complexes were neutral/non-electrolytic as there was a significant drop in conductivity after complexation. $AgNO_3$ was used to detect the presence of Cl^{-1}. The absence of turbidity led to the conclusion that the complex did not contain Cl^{-1}.

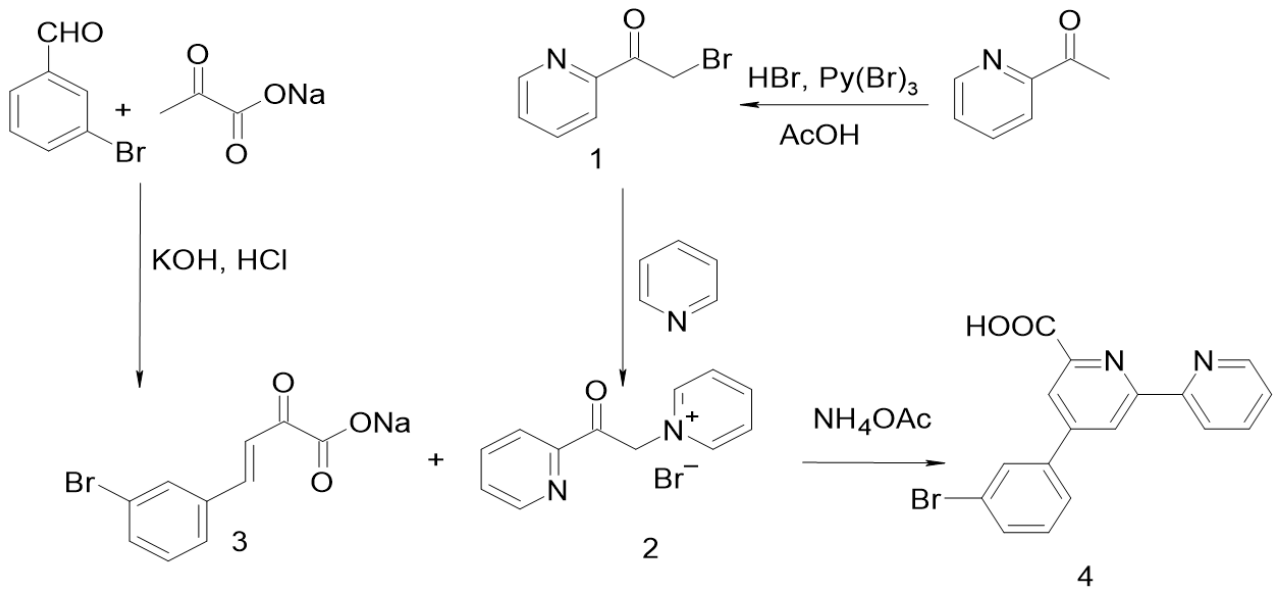

Fig. 1: Scheme for the synthesis of 4-(3-bromophenyl)-[2,2'-bipyridine]-6-carboxylic acid.

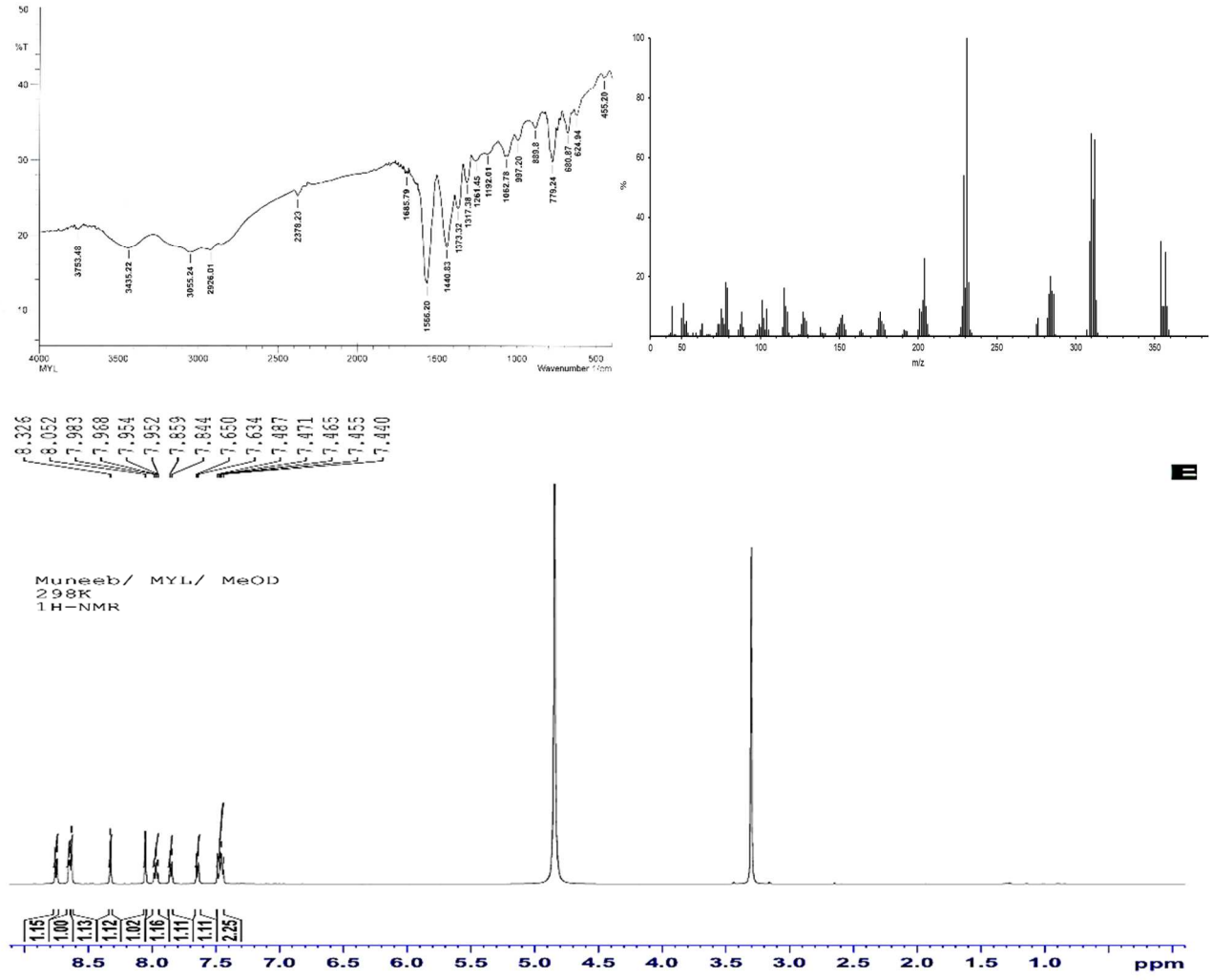

Fig. 2: NMR, IR and mass spectra of the synthesized ligand.

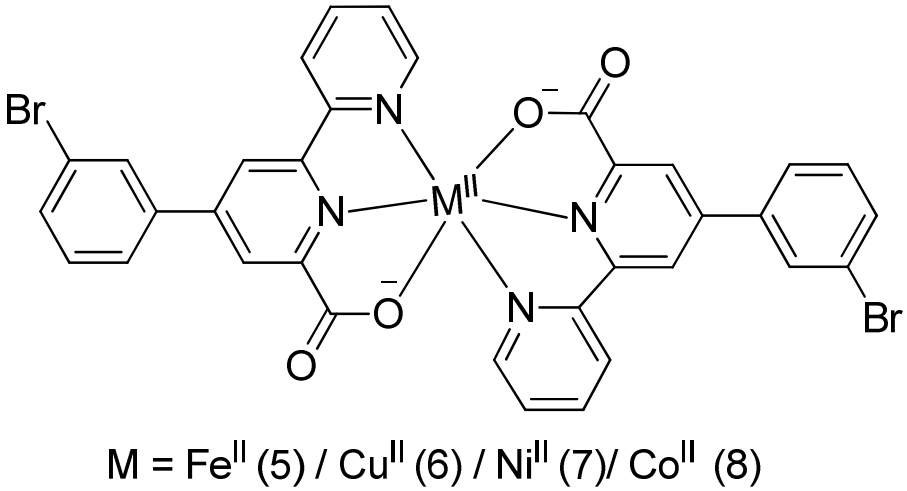

Fig. 3: Proposed structure of metal complexes with 4-(3-bromophenyl)-[2,2'-bipyridine]-6-carboxylic acid.

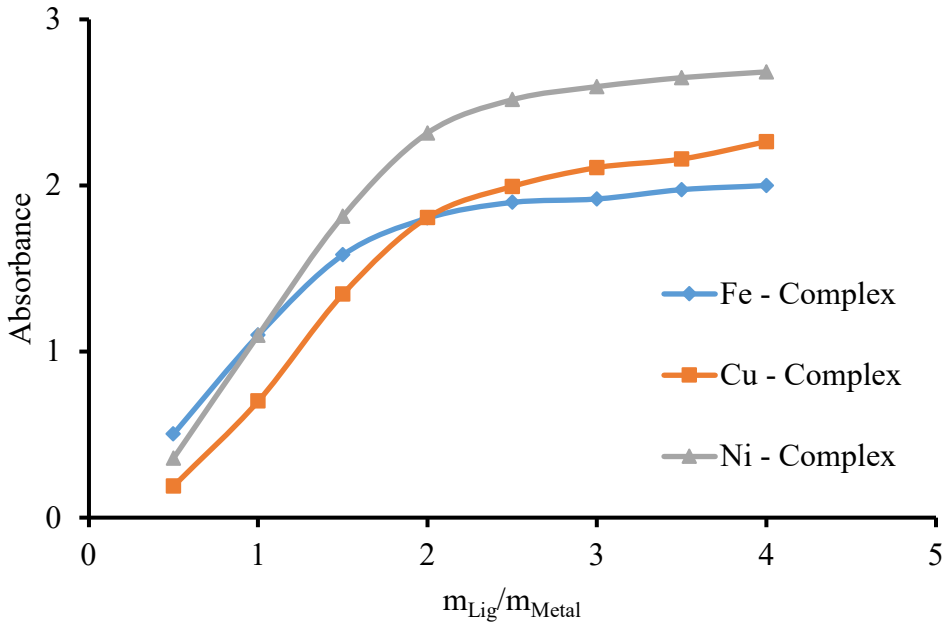

Fig. 4: Mole ratio plots for the stoichiometry of synthesized complexes.

Table 1: IR frequencies (cm^{-1}) of ligand and its complexes

Compound	OH	C=O, C=N	C=C (aromatic)	C-N	C-O
Ligand	3055.24	1566.20 (Merge peak)	1440.83	1317.38	1062.78
Fe-Complex	3441.01	1694.14/1606.70	1431.18	1311.59	1180.15
Ni-Complex	3417.86	1647.21/1606.70	1438.90	1313.52	1261.45
Cu-Complex	3431.36	1651.07/1618.28	1408.04	1369.46	1182.36

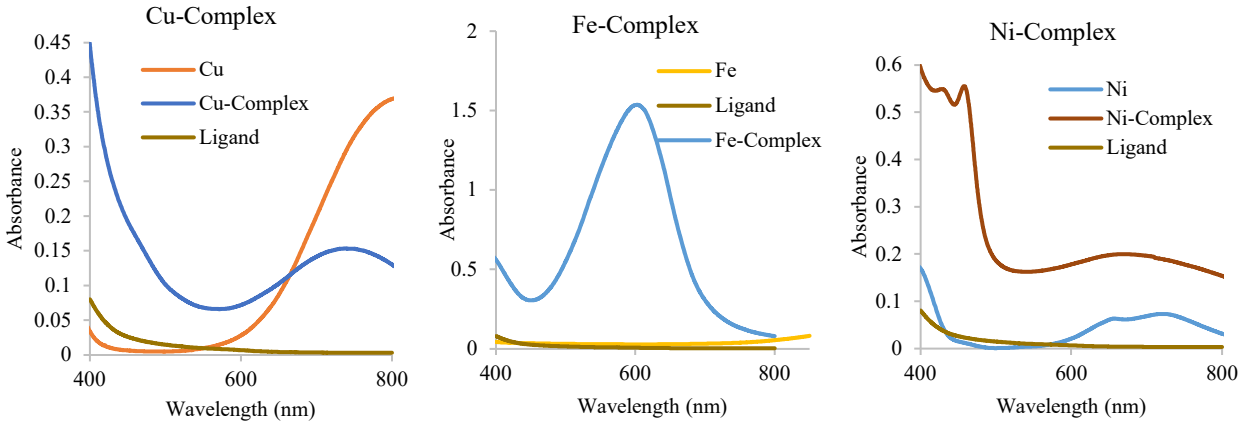

Fig. 5: Visible spectra of Fe(II), Ni(II), and Cu(II) and their corresponding complexes with the synthesized ligand.

Table 2: Molar absorptivities at their λ_{max} in Visible region

Sample	λ_{max} (nm)	ε ($M^{-1}cm^{-1}$)	λ_{max} (nm)	ε ($M^{-1}cm^{-1}$)
Ligand	663.5	5.057×10^{-1}	274.5	6.217×10^{4}
$NiCl_2.6H_2O$	723.5	7.331×10^{-1}	342.5	1.759×10^{0}
Ni complex	458.5	2.054×10^{2}	277.5	5.212×10^{4}
$CuCl_2$ (anhydrous)	754.0	2.871×10^{0}	253.5	1.318×10^{1}
Cu complex	737.0	9.644×10^{1}	275.2	2.713×10^{4}
$(NH_4)_2Fe(SO_4)_2\ 6H_2O$	793.5	5.458×10^{-1}	252.0	2.377×10^{0}
Fe complex	607	1.218×10^{3}	277.0	1.008×10^{4}

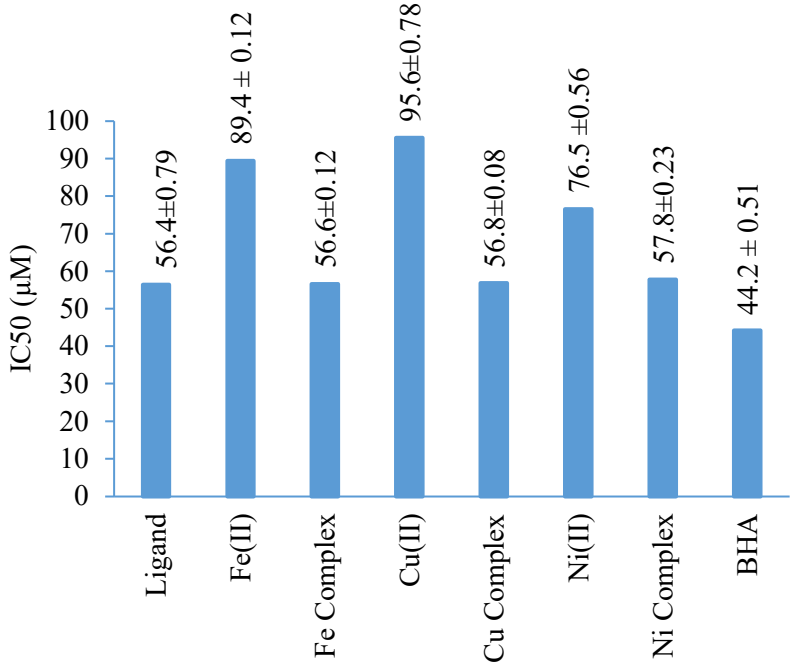

Fig. 6: IC_{50} values for antioxidant activity of metal ions and their complexes with the synthesized ligand.

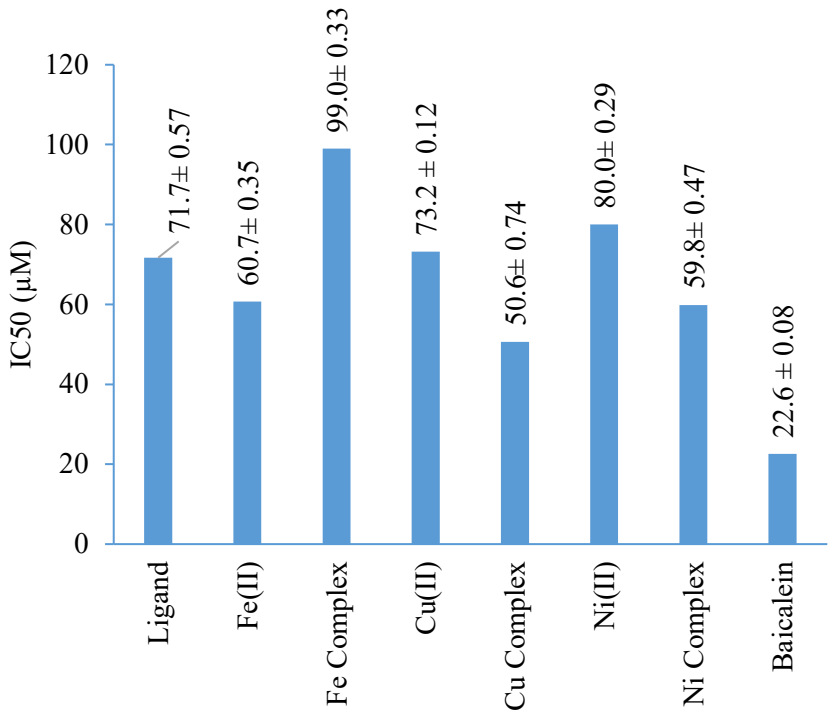

Fig. 7: IC_{50} values lipoxygenase inhibition activity of metal ions and their complexes with the synthesized ligand.

The complexes were heated at 200 to 220°C for 2hrs to check the presence of water in the coordination sphere. No color change was observed that predicts the absence of water in the coordination sphere.

pH drop was observed which showed that the COOH group participated in the complex formation. Furthermore, when the complex was treated with strong acid, the color of the complex disappeared demonstrating the disintegration of the complex (Patel *et al.*, 2024). Synthesized compounds were only soluble in DMSO and were insoluble in water which inferred that the complexes were nonionic in nature or lacked strong molecular lattice.

IR spectral studies

IR spectral data of ligand, metals and their complexes are shown in table 1. It can be observed that the ligand showed a broad OH peak from 3600 – 2600 cm^{-1}. However, this broad peak is absent in the spectra of complexes indicating that OH of the carboxylic group may be coordinated with metal ions (Foley *et al.*, 2023).

The IR spectra of the ligand also showed a merged peak at 1566.2 cm^{-1}, corresponding to C=O, C=N groups. However, the spectra of the complexes had two separate and distinct peaks. The shift in the C=O and C=N wavenumber suggested their participation in complexation. The same spectral shift was observed for C-N peak at 1317.38 cm^{-1} confirming the participation of bipyridine nitrogen in the complexation (Zhang *et al.*, 2024). It is inferred from the above-mentioned studies that the carboxylic group and bipyridine nitrogen participated in the complex formation. Therefore, the ligand should be a tridentate ligand.

MALDI-MS studies

The molecular masses of Fe, Ni and Cu complexes determined using MALDI-MS showed a good agreement with theoretical masses calculated using ML_2 stoichiometry (Haris, 2022).

Visible spectral studies

Spectrum of ligand was obtained using methanol as solvent. The ligand and Fe(II) solution showed no significant absorbance. However, the iron complex had a significant absorbance between 450 and 650 nm with λ_{max} at 564 nm, indicating the presence of charge transfer transitions. The absorbance of copper metal was measured between the range of 650 to 850 nm with λ_{max} at 754 nm and after the formation of the complex, a hyper chromic

shift in absorbance was observed with λ_{max} at 737 nm. The absorbance of nickel metal was negligible between 600 and 800 nm, but after complexation, a change in spectrum was seen between 400 and 500 nm with λ_{max} at 458.5 nm. The molar absorptivity of metal, ligand and complexes are listed in Table 2 and the spectra are shown in Fig 5.

UV spectral studies

The Ligand had a remarkably high value of molar absorptivity at 274.5 nm. Cu(II), Fe(II) and Ni(II) solutions had λ_{max} at 253.5 nm, 252.0 nm and 342.5 nm, respectively. The complex formation resulted in significant wavelength shift. The iron complex had λ_{max} at 277.0 nm, the copper complex had λ_{max} at 275.2 nm and the nickel complex λ_{max} observed at 277.5 nm. The relevant data is listed in table 2.

Antioxidant properties

BHA was selected as the reference antioxidant as it is a widely used phenolic compound with established radical scavenging capacity in DPPH assays (Boulebd, 2020). When compared to conventional medications, butylated hydroxyanisole (BHA), metal salts showed only little antioxidant activity. The IC_{50} values of Fe(II), Ni(II) and Cu(II) were 89.4, 76.5 and 95.6 µM, respectively. The synthesized ligand, on the other hand, showed excellent antioxidant activity with IC_{50} value of 56.4 µM. However, there was no obvious improvement upon complexation with Fe(II), Ni(II) and Cu(II). The respective IC_{50} values of the complexes were 56.5, 57.8 and 56.8 µM, respectively, which were very similar to the IC_{50} value of the ligand as shown in fig 6. The statistical analysis of the IC_{50} values for groups, comprising of ligand, Fe(II), Ni(II) and Cu(II) ions and their respective complexes, was performed using one-way ANOVA. The test suggested significant difference across the groups at $p<0.05$ which means the activities of ligand, metal ions and their respective complexes were statistically different. To identify the source of significant differences, post-hoc analysis was performed using pairwise t-test. The p-value for Fe-complex and ligand pair was 0.707, Cu-complex and ligand pair was 0.475 and Ni-complex and ligand pair was 0.204. The p-value greater than 0.05 suggested that the three pairs had statistically similar activities. To further evaluate the source causing significant differences the Fe-Complex and Fe(II) pair , Cu-Complex and Cu(II) pair, and Ni-Complex and Ni(II) pair were evaluated using t-test. The p-values for these three pairs were 0.000, suggesting that the activities of complexes were statistically different from their respective metal ions. The statistical evaluation confirmed that the antioxidant activity of ligand was statistically like that of the synthesized complexes, whereas the activities of metal ions were the source of statistically significant differences. The activities of synthesized metal complexes and ligand were grouped together with the BHA reference. The test yielded p-value greater than 0.05, concluding that the ligand and synthesized complexes had activities comparable to the reference drug, reinforcing their relevance as antioxidant candidates.

There are three major radical scavenging pathways including proton transfer, electron transfer and chelation. It was observed that ligand possesses superior antioxidant properties compared to the respective metal ions and it had almost same antioxidant properties compared to its complexes with the selected metal ions. It can be inferred that it is the ligand which showed significant antioxidant properties. The ligand structure revealed that it cannot undergo the proton transfer or electron transfer mechanism. It is therefore suggested that the ligand may undergo chelation mechanism to show antioxidant properties. (de la Vega-Hernandez *et al.*, 2017).

Lipoxygenase inhibition of synthesized complexes

Baicalein was chosen as the positive control for lipoxygenase inhibition because of its well-documented potency as a natural LOX inhibitor (Yeo *et al.*, 2025). The study observed that the inhibitory action of metal ions was altered upon complexation with the synthesized ligand. Among the tested complexes, the Cu(II) and Ni(II) complexes showed the strongest LOX inhibition with the respective IC_{50} value of 50.6 and 59.8 µM, while the Fe(II) complex was least active with IC_{50} value of 99.0 µM. The complexes of Cu(II) and Ni(II) are less prone to redox cycling under physiological conditions; hence they act likely through steric or coordination-based blocking of the active sites of enzyme, explaining why the complexes displayed enhanced inhibition relative to the respective free ions. The presence of a bromophenyl substituent may have increased lipophilicity, improving interactions with the enzyme. The Fe(II) and Fe-complex showed anomalous behavior. Fe(II) was more effective than Fe-complex. It may be because Fe(II), in the ionic form, could easily transfer electron to Fe (III) present in the lipoxygenase enzyme, hence effectively inhibiting the enzyme activity whereas, the same is not true for Fe(II) complex, because the ligand present in the complex could have hindered the transfer of electron resulting in poor enzyme inhibition. The same cannot be said for Ni(II) and Cu(II) as they cannot undergo further oxidation under the experimental and physiological conditions. The pictorial representation of inhibition activities is shown in fig 7. The lipoxygenase inhibition activities of the ligand, metal ions and the synthesized complexes were evaluated statistically. One-way ANOVA concluded that lipoxygenase inhibition activity was significantly different among the group as p-value was less than 0.05. The pairwise analysis using student t-test revealed that inhibition activity of Fe-complex was statistically different from the ligand, similarly the inhibition activity of Cu-complex and Ni-complex also differ significantly compared to the ligand – the p-values for the three pairs were 0.000. Pairwise statistical analysis of the metal ions and their respective complexes showed that the inhibition activities of Fe(II), Cu(II) and Ni(II) were significantly different from Fe-complex, Cu-complex and Ni-complex, respectively. The p-values for all these pairs were 0.000. In the present study,

while the synthesized ligand and complexes did not surpass baicalein in LOX inhibition, the Cu(II) and Ni(II) complexes displayed IC_{50} values within the same order of magnitude, highlighting their potential as alternative inhibitor.

CONCLUSION

The study reports the synthesis and characterization of a bromophenyl-substituted bipyridine ligand and its Fe(II), Ni(II) and Cu(II) complexes. The characterization studies revealed that 4-(3-bromophenyl)-[2,2'-bipyridine]-6-carboxylic acid acted as a tridentate ligand. The complexes were found neutral and non-hygroscopic. The synthesized ligand, its complexes and respective metal ions were evaluated for antioxidant and lipoxygenase inhibition properties. The findings demonstrated that antioxidant activities of the ligand and its complexes were comparable to the reference BHA. However, the statistical analysis demonstrated that there was a significant increase in the antioxidant properties of metal ions after complexation. The lipoxygenase inhibition studies concluded that a decent increase was observed in the enzyme inhibition properties of Cu(II) and Ni(II) upon complexation. However, Fe(II) was found more effective for the inhibition when compared to its complex with the ligand. These results highlighted halogenated bipyridines as promising scaffolds for the development of dual-function antioxidant and enzyme-inhibiting agents.

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Authors' contributions
Muneeb Yousuf: Experimental work, data collection, and manuscript drafting. *Agha Arslan Wasim:* Statistical analysis and data interpretation. *Mehreen Latif:* Experimental assistance and data analysis for antioxidant and lipoxygenase inhibition activities. *Mohsin Ali:* Interpretation and revision of the manuscript. *Saba Fazal-ur-Rehman:* Supervisor, project administrator, and final approval of the manuscript.

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Data availability statement
The data for this study are available within the article. Additional data may be obtained from the corresponding author upon reasonable request.

Ethical approval
This study did not involve human participants or animals. Therefore, ethical approval from an institutional review board was not required.

Conflict of interest
The authors declare that there is no conflict of interest regarding the publication of this article.

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