Antioxidant and lipoxygenase inhibition studies of 4-(4-bromophenyl)-2,2'-bipyridine and its metal complexes Synthesis, characterization and biological screening

Saba Fazal-ur-Rehman[1]*, Agha Arslan Wasim[1], Maria Aqeel Khan[2], Mohsin Ali[1] and Shazia Iqbal[2]
[1]Department of Chemistry, University of Karachi, Karachi, Pakistan
[2]HEJ Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Pakistan

Abstract: Synthesis of new antioxidants and enzyme inhibitors is an active area of research in pharmaceutical sciences. This can be used for development of new active product ingredients which can prevent body from different diseases. This study comprises of preparation of transition metal complexes using 4-(4-bromophenyl)-2,2'-bipyridine (BPBP) and their screening for antioxidant and lipoxygenase inhibition properties. 4-(4-bromophenyl)-[2,2'-bipyridine]-6-carboxylic acid was used as starting material and its decarboxylation resulted in BPBP. Decarboxylation by conventional heating method was compared with microwave decarboxylation method. Selected metal complexes of the ligand were synthesized with Ruthenium (II), Iron (II) and Cobalt (II) ions. The complexes were characterized using UV, IR, ^{1}H-NMR, ESI-MS and CHNS techniques. It was observed that BPBP acted as a bidentate ligand. The metal to ligand stoichiometry was 1:3 for all the synthesized complexes. The complexes had octahedral structure with C_3 symmetry. The antioxidant activity was evaluated using free radical scavenging assay. BPBP showed insignificant antioxidant and lipoxygenase activities while its transition metal complexes showed promising activities. Antioxidant activity of Fe and Co-complexes was found significantly higher than the reference drug used in this study.

Keywords: Lipoxygenase inhibition, antioxidant, 4-(4-bromophenyl)-2,2'-bipyridine, synthesis, transition metal complexes.

INTRODUCTION

Human body produces free radicals (NO•, SO•, DPPH•) and other non-radical Reactive-Oxygen-Species (ROS) are formed constantly as byproducts of various enzymatic and non-enzymatic biological reactions (Dickinson and Chang, 2011; Mahmoud *et al.*, 2011; Shrestha *et al.*, 2017). ROS possess dual nature, they can produce beneficial as well as deteriorating effects. ROS are useful within a certain limit in human body because of their involvement in body defense system against engulfed pathogens, and their role in cell signaling (Dickinson and Chang, 2011). However, their overproduction leads to oxidative stress which is quite harmful. It can damage cell membranes, DNA and proteins through oxidation. Oxidative stress has been involved in various diseases including cancer, cardiovascular disorders, rheumatoid arthritis, malaria, atherosclerosis, neurodegenerative diseases (Jagadish *et al.*, 2016; Raziq *et al.*, 2017), cataracts, chronic inflammation, and pre-mature aging (Aruoma, 1998; Fang *et al.*, 2002; Finkel and Holbrook, 2000; Waris and Ahsan, 2006). Higher oxidative stress can be considered as a factor for diabetes or it can increase the complications during diabetes (Ceriello, 2000; Wolff and Dean, 1987).

Antioxidant is a class of compounds that undergoes redox reaction with free radicals and protect the cell from their oxidative damage. Different diseased conditions like Alzheimer's disease, dementia, cancer etc., (Khalaf *et al.*, 2008; Lee *et al.*, 1998) have been significantly controlled by the use of antioxidants.

Lipoxygenases (LOXs) family is non-heme iron-containing dioxygenase enzymes. They perform regulatory functions in plants and animals like metabolism of polyunsaturated fatty acid to hydroperoxy fatty acid (Hadjipavlou-Litina and Pontiki, 2015). Proper functioning of lipoxygenase depends upon oxidation state of its Fe. Active lipoxygenase possesses Fe (III) but during the metabolism process the oxidation state changes to Fe (II). In this state they are involved in development of glomerulonephritis, asthmatic responses, psoriasis, and prostate cancer (Waller *et al.*, 2008). Therefore, functioning of lipoxygenase can be controlled by controlling the oxidation state of iron.

Enzyme inhibitors have great importance in treatment of various diseases. They bind themselves to active sites of enzymes and restricts the availability of enzymes. Organic compounds with halogen as one of its substituents are found to have good lipoxygenase inhibitory properties which is found to increase with the increase in number of halogen substituents per molecule (Ohri *et al.*, 2005).

**Corresponding author:* e-mail: saba@uok.edu.pk

Bipyridines are extensively important heterocycles because of their participation in the development of coordination chemistry(Kaes *et al.*, 2000), supramolecular chemistry (Schubert and Eschbaumer, 2002; Wen *et al.*, 2018) and many others. It has been reported in several studies that bipyridine group can be coupled with organic molecules using Suzuki coupling reactions (Jukes *et al.*, 2004; Zhong *et al.*, 2009). The coupling easily introduces interesting chelating property in adduct. In 1957 a series of 4,4'-Disubstituted 2,2'-Bipyridines was synthesized by Gerhard Maerker and Francis H Case and they also complexed these compounds with iron(Maerker and Case, 1957). The research concluded 4-4' carbon as preferred sites for substitution because of higher value of absorptivity, redox potential, and complex stabilities (Maerker and Case, 1957).

Transition metal bipyridine complexes are used widely due to their biological (Adelaide and James, 2013; Karabasangouda *et al.*, 2009) as well as physicochemical applications (Marin *et al.*, 2004). They are used in chemotherapy and also possess antimicrobial, antifungal, antiviral, anticancer and anti-DNA cleaving properties (Adelaide and James, 2013).

The synthesis of new antioxidants and enzyme inhibitors is a dynamic area of research. In the present work a modified method has been developed for the synthesis of BPBP. This substituted bipyridine was then complexed with ruthenium, iron and cobalt respectively. Resulting complexes were characterized and analyzed for their antioxidant and lipoxygenase inhibition properties.

MATERIALS AND METHODS

General Experimental

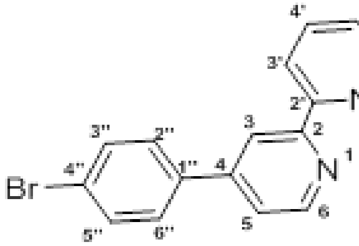
Reagent grade chemicals are used which purchased from BDH, Sigma–Aldrich or Merck. Silica gel pre-coated aluminum plates were used for thin layer chromatography. Shimadzu 8900 IR spectrophotometer was used to obtain FT-IR spectra. ^{1}H NMR were taken from Avane Bruker 400MHz spectrometer in DMSO-d6. JEOL MS Route 600H instrument was used to obtain EI Mass spectra, AB Sciex QStar XL MS/MS was used for ESI-MS studies, CHN was performed on a CHN/ S analyzer Perkin Elmer 2400 series II. For HPLC Agilent technologies 1260 Infinity HPLC is used.

Procedure for the synthesis of 4-(4-bromophenyl)-2,2'-bipyridine (BPBP) Ligand

BPBP was synthesized using multiple steps known scheme (Katoh *et al.*, 2011; Zhong *et al.*, 2009) (fig. 1) with some modification in procedures. Synthesis of ligand till step 4 is reported in another study (Saba *et al.*, 2017).

Synthesis of 4-(4-bromophenyl)-2,2'-bipyridine

Following methods were used for synthesis of BPBP



Following methods were used for synthesis of BPBP

Microwave synthesis of 4-(4-bromophenyl)-[2,2'-bipyridine]

Compound number (4) was suspended in ethylene glycol and heated in microwave at 200℃ for 15 min. TLC was used to ensure reaction completion and then ethylene glycol was removed using freeze drying. After drying gradient column chromatography (Eluent: 5%-20 % ethyl acetate: Hexane) was used to purify crude mixture. Yield: white solid, 51%.

Conventional heating

In conventional method compound (4) was decarboxylated by heating 410mg of compound (4) suspended in 4ml ethylene glycol at 200℃ for 1.5h. TLC and HPLC were used for monitoring reaction progress. Chromatogram of reaction mixture were obtained by taking a fraction of reaction mixture at different time intervals during heating, 10min, 20min, 30min, 45min, 60min, 90min and completion of reaction was observed after 90min. Table 1 compares the conventional and microwave synthesis of compound (5).

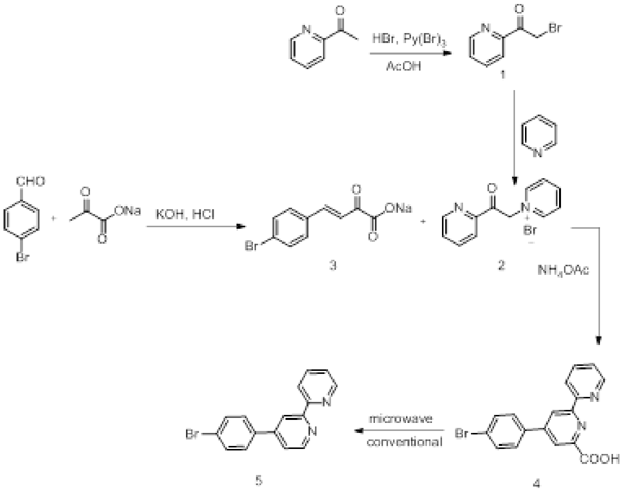


Fig. 1: Stepwise synthesis of BPBP

Synthesis of 4-(4-bromophenyl)-[2,2'-bipyridine] complexes

Synthesis of Ru complex

0.32mmole of ligand (5) and (0.107mmole) of Ru(DMSO)$_4$Cl$_2$ were added in 32.4ml ethylene glycol and allowed to reflux for 6hr under dark in N$_2$ atmosphere. The reaction mixture was cooled down to room temperature and auxiliary ion (ammonium hexafluoro phosphate) was added to the reaction mixture and further stirred for 15 min. Reddish orange colored precipitates of complex obtained were filtered and washed using diethyl ether and water.

Synthesis of Fe and Co complex

A 3ml aliquot of 0.1M ligand solution in dichloromethane was set to stir on a magnetic stirrer. A solution of the selected metal chloride (0.1mmole), prepared using methanol (6ml) as solvent, was added and allowed to further stir for 30 min. Finally, 32 ml of 2% $NH_4F_6PO_4$ (aq) was added to the reaction mixture. The mixture was further stirred for 5min. The solid precipitates obtained were filtered and washed using dichloromethane.

Biological Screening

Ligand, metals and their corresponding complexes were checked for their antioxidant and lipoxygenase inhibition activities.

Antioxidant Activity

DPPH Radical Scavenging Activity: Scavenging ability of synthesized compounds on 2, 2'-diphenyl-1-picryl hydrazyl (DPPH; $C_{18}H_{12}N_5O_6$) free radical was used to determine antioxidant activity. Upon reaction with hydrogen donor DPPH radical reduced to colorless hydrazine. 0.3mM ethanolic solution of DPPH was prepared. Samples were serial diluted in DMSO up to the final concentrations of 500, 250, 150, 125, 62.5, 31.2, 15.6, 7.8 µM. DPPH solution (90 µl) was added in each well of the 96-well plate marked for control and test compounds. 10 µl of each sample was then added to their respective well to start the reaction. After incubating the mixture was stirred well for 30 minutes at 37°C, using microtiter plate reader (Spectra max plus 384 Molecular devices USA) absorbance was measured at 517nm. Standardization of this assay was performed using butylated hydroxyanisole (BHA). DMSO treated control was used to determine Percent radical scavenging activity and EZ fit software (Perrella Software, USA) (Ali *et al.*, 2009; Siddiq *et al.*, 2012) was used to calculate IC_{50}.

$$\text{DPPH radical scavenging effect (\%)} = \text{Ac} - \frac{As}{Ac} x\ 100$$

Lipoxygenase Inhibition

Using the modified spectrophotometric method developed by Tappel, lipoxygenase inhibitory activity was measured.

Lipoxygenase Inhibition Assay

Lipoxygenase enzyme concentration in each sample was about 130U. 160µL of 100mM sodium phosphate buffer (pH 8.0) was added to each well labeled as enzyme blank, substrate blank, control and test. A 10 µL aliquot of each solution of a sample series (5 – 500µM) prepared in DMSO was transferred to wells labeled as test. A 20µL of lipoxygenase (LOX) solution was added to each well except those labelled as substrate blank. After mixing the contents, wells were incubated for 10 min at 25 °C. Autoxidation of substrate solution was avoided by flushing with N_2 before addition of substrate to well. 10 µL substrate solution (linoleic acid, 0.5mM, 0.12 %w/v tween 20 in ratio of 1:2) was added to all wells except the one labeled enzyme blank. After completion of reaction absorbance was measured at 234 nm. Finally, the effect of samples concentrations on the degree of inhibition was used to calculate IC_{50} values using EZ-Fit, Enzyme kinetics Program (Perrella Scientific Inc., Amherset, USA).

RESULTS

Synthesis of 4-(4-bromophenyl)-2,2'-bipyridine

Table 1 shows the results obtained for the synthesis of ligand using conventional and microwave heating of compound (5).

Spectroscopic studies of 4-(4-bromophenyl)-[2,2'-bipyridine]

IR (KBr) cm^{-1}: υ(aromatic-CH) 3290.3, 3055.0, 2923.9, υ(C=N) 1587.2, υ(C=C) 1454.2, υ(C-C) 1377.1, υ(C-N) 1303.8. MS (EI): [M^+] 310.2, [M+2] 312.2, [M^+-Br] 231.2.

^{1}H-NMR (DMSO, 400 MHz) δ(ppm) = 7.49 (1H, td, *J*(Hz) = 7.0, H-5'), 7.76(2H, d, *J* (Hz) = 8.4, H-2", H-6"), 7.79 (1H, dd, *J*(Hz) = 5.0, *J* (Hz) = 1.6, H-5), 7.84 (2H, d, *J* (Hz) = 8.4, H-3", H-5"), 7.99 (1H, td, *J* (Hz) = 7.0, *J*(Hz) = 1.2, H-4'), 8.43 (1H, d, J(Hz) = 8.0, H-3'), 8.65 (1H, s, H-3), 8.68 (1H, d, *J* (Hz) = 4.0 , H-6'), *8.74* (1H, d, H-6).

Spectroscopic and analytical studies of complexes

Ru complex

90% yield; red solid; Molar conductance (MeOH): 148.708 S cm^2 mol^{-1}. Anal. Calcd for $C_{48}H_{33}Br_3F_{12}N_6P_2Ru$: C=43.53%; H=2.51%; N=6.34%. Found: C=43.17%; H=2.80%, N= 6.31%. IR (KBr) cm^{-1}: υ(aromatic-CH) 3084.0, 2923.9, υ(C=N) 1612.4, υ(C=C) 1467.7, υ(C-C) 1386.7, υ(C-N) 1326.9, υ(bpy ring/ PF_6-) 840.9, υ(Ru-N) 557.4. MS (ESI): [M^+-PF_6] 1133.0. ^{1}H-NMR: (300 MHz, MeOD) δ = 7.52 (t, 3H, H-5'), 7.96-7.72 (m, 21H, H-5, H-6, H-6', H-2'', H-3'', H-5'', H-6''), 8.16 (t, 3H, *J*(Hz) = 7.8, H-4'), 8.91 (d, 1H, *J*(Hz) = 7.8, H-3'), *8.98* (s, 3H, H-3).

Fe Complex

99% yield; dark pink solid; Molar conductance (MeOH) =156.867 S cm^2 mol^-1. Anal. Calcd for $C_{48}H_{33}Br_3F_{12}FeN_6P_2$: C=45.07%; H=2.60%; N=6.57%. Found: C=45.17%; H=2.60%, N=6.11%. IR (KBr) cm^{-1}: υ(aromatic-CH) 2923.9, 2856.4, υ(C=N) 1612.4, υ(C=C) 1467.7, υ(C-C) 1386.7, υ(C-N) 1325.0; MS (ESI): 1133.0[M^+-PF_6].

Table 1: Comparison of conventional and microwave decarboxylation of 4-(4-bromophenyl)-[2,2'-bipyridine]-6-carboxylic acid

Variables	Conventional heating	Microwave heating
Temperature	200°C	200°C
Time	1.5h	15min
%yield	47%	51%

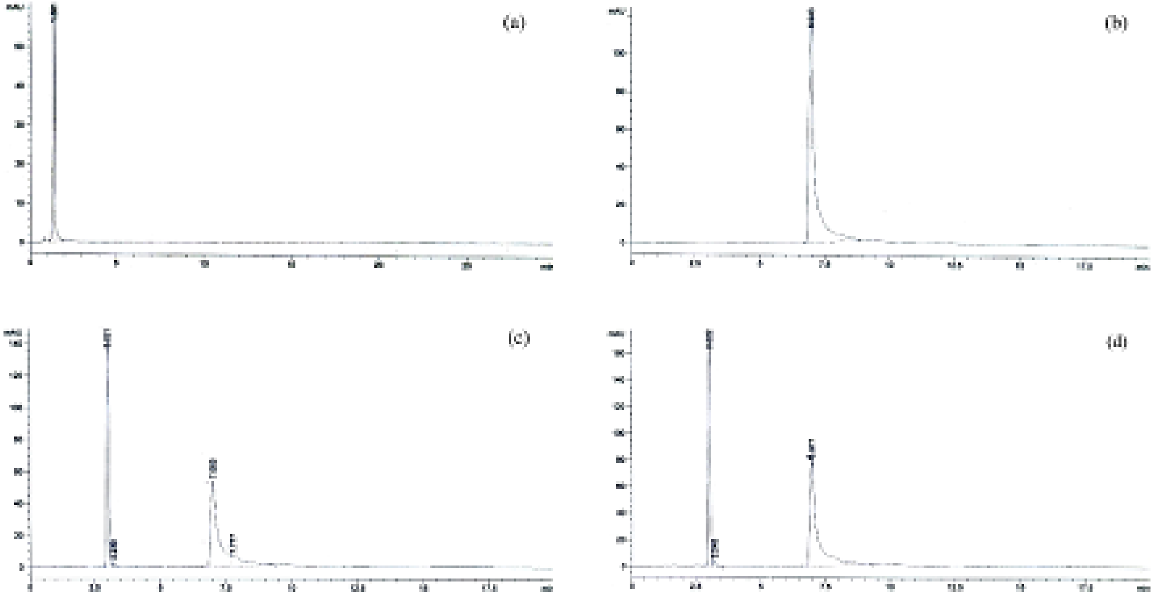


Fig. 2: Chromatograms for synthesis of BPBP a) chromatogram of starting material (4-(4-bromophenyl)-[2,2'-bipyridine]-6-carboxylic acid), b) chromatogram of pure BPBP, c) chromatogram of BPBP for conventional heating method d) chromatogram of BPBP for microwave heating method.

Co complex

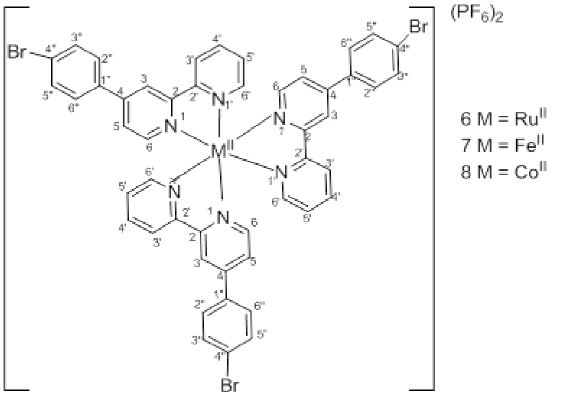
60% Yield; pale yellow solid; Molar conductance (MeOH) = 137.819 S cm^2 mol^{-1}. Anal. Calcd for $C_{48}H_{33}Br_3F_{12}CoN_6P_2$: C=44.96%; H=2.59%; N=6.55%. Found: C=44.15%; H=1.62%, N=6.83%. IR (KBr) cm^{-1}: υ(aromatic-CH) 2926.0, 2856.58, υ(C=N) 1467.7/1462.4, υ(C=C) 1433.0, υ(C-N) 1386.7. MS (ESI): 1136.0[M$^+$-PF$_6$].

Synthesis and physicochemical properties

Ligand (4) was synthesized using scheme shown in fig. 1 and characterized using different spectroscopic and analytical techniques. Later on, its complexes were prepared with Ru(II), Fe(II) and Co(II). Chemical, spectroscopic and analytical data exhibited that BPBP acts as bidentate ligand and hence proposed metal to ligand mole ratio is 1:3, geometry of complexes was octahedral and proposed symmetry is C_3 (Schramm *et al.*, 2013). Proposed reaction was showed in the following reaction.

$$M^{2+} + 3L + NH_4PF_6 \rightarrow [M(L)_3](PF_6)_2$$

All the synthesized complexes (6-8) were colored, amorphous, non-hygroscopic, stable and showed good solubility in solvents like ethanol, methanol etc. Polar nature of complexes was depicted from their solubility in polar solvents. Complexes also showed good conductivity values suggesting that complexes were charged. The proposed structure of complexes is shown below.



Spectroscopy

^{1}H-NMR Spectroscopy

^{1}H-NMR spectra of ligand (5) and its ruthenium complex (6) are shown in fig. 3, whereas, the required data is reported in results section, Iron and cobalt complexes (7,

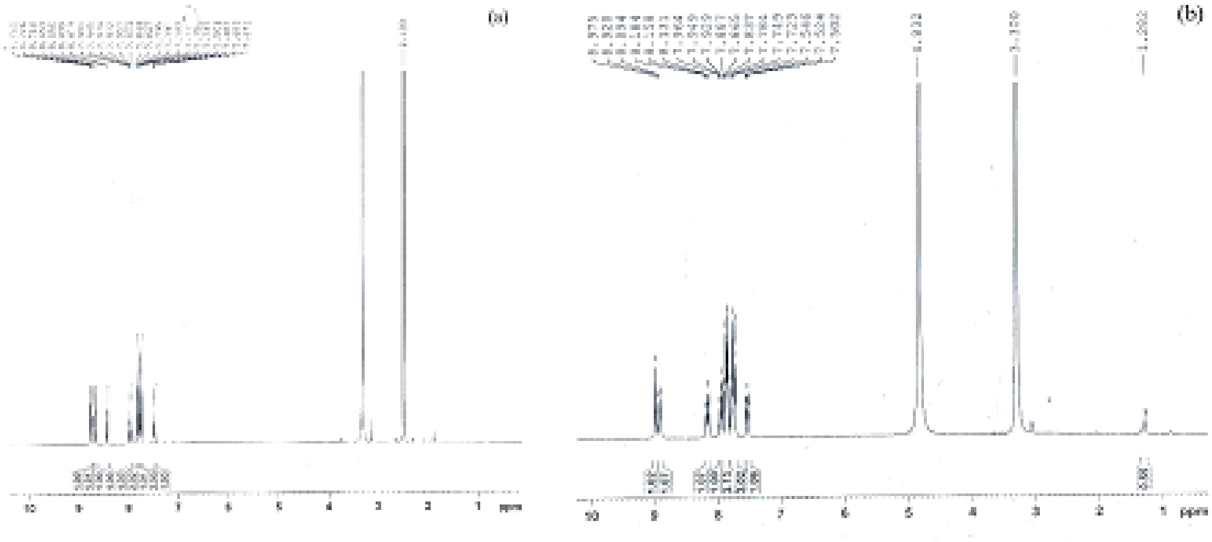


Fig. 3: 1H-NMR Spectrum a) Ligand b) Ru-Ligand Complex

8) were not analyzed from ^{1}H-NMR spectroscopy due to their paramagnetic nature.

Biological screening

Antioxidant activity of metal salts, Ligand and their complexes: Antioxidant activity of synthesized ligand and its complexes are shown in fig. 4.

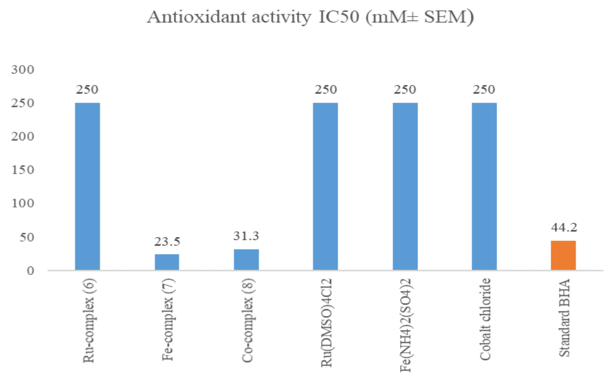


Fig. 4: Antioxidant activity IC50 (mM± SEM)

Lipoxygenase inhibition of compound (5-8)

Lipoxygenase inhibition screening of free ligand and its complexes are shown in fig. 5. Ru-complex is the most active among the three complexes with IC50 value of 32.1µM, which is close to the IC50 value of standard Baicalein.

DISCUSSION

Synthesis and physicochemical properties

The synthesis of BPBP was carried out by conventional heating method and compared with the relatively efficient microwave heating method. The results for the two methods are shown in table 1. The percent yield for microwave heating method was 51 compared to 47% yield for conventional heating method. The time consumed by microwave heating method was 15 min compared to 1.5 hours for conventional heating method. Although they product yield from two methods is comparable however, the time required by microwave heating method was quite low which would result in greener production of the product.

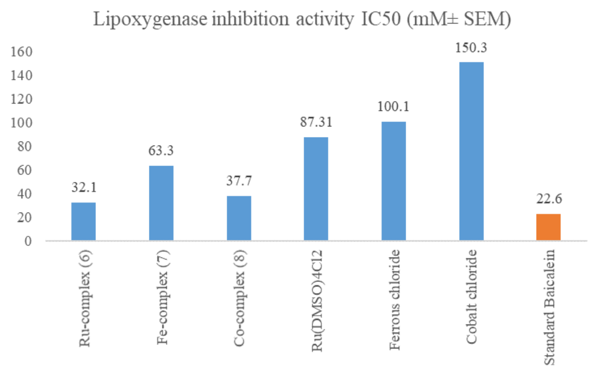
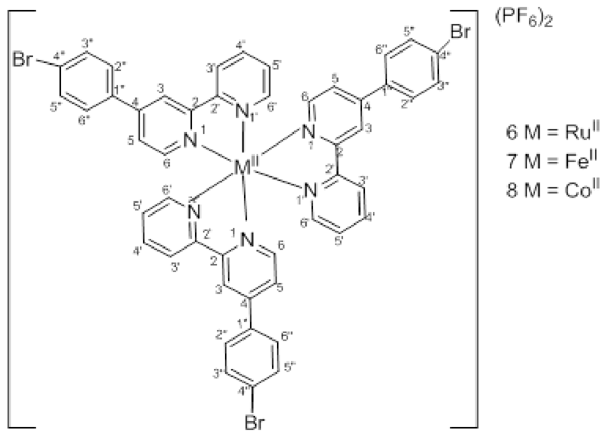


Fig. 5: Lipoxygenase inhibition activity IC50(mM± SEM)

The completion of reaction was monitored using HPLC technique. fig. 2a shows chromatogram of substrate (4-(4-bromophenyl)-[2,2'-bipyridine]-6-carboxylic acid). fig. 2b is a chromatogram of pure BPBP. fig. 2c is a chromatogram obtained for reaction mixture for conventional heating method while fig. 2d shows chromatogram for microwave heating method. The reaction completion for both methods was ascertained by disappearance of substrate peak for the reaction mixture.

Polar nature of complexes was depicted from their solubility in polar solvents. Complexes also showed good conductivity values suggesting that complexes were charged. The proposed structure of complexes is shown below.



Spectroscopy

I.R. Spectroscopy

In case of ligand C-N stretch was observed at 1587.3 cm^{-1} which is significantly shifted to higher wavenumber upon complexation. C=N peak is also shifted towards higher wavenumber after complexation. The wavenumber shifts of C=N and C–N indicates that BPBP formed complexes by donating lone pairs of its N atoms and hence act as bidentate ligand.

^{1}H-NMR Spectroscopy

When ^{1}H-NMR data of ligand and Ru-complex were compared it was observed that hydrogen vicinal to bipyridine nitrogen exhibited significant change in its δ value upon complexation from~ 8.7 (pure ligand) to~ 7.7 (metal-ligand complex). Hence results of ^{1}H-NMR study are in accordance with proposed structure of complexes where ligand chelate metal through its two-nitrogen atom.

Biological screening

Antioxidant activity of metal salts, Ligand and their complexes

When synthesized ligand and its complexes were screened for their antioxidant activity it was observed that the ligand alone was inactive, while metals salts used for complexation had negligible activity. However, complexation of metal and ligand resulted in a drastic improvement in antioxidant activity. Antioxidant activity of Fe and Co complexes were found to be higher than Ru complex this may be due to their smaller size as compare to ruthenium complex. Antioxidant properties of Fe-complex (IC50 = 23.5) and Co complexes (IC50 = 31.3) was even higher than the standard drug as shown in fig. 4.

Lipoxygenase inhibition of compound (5-8)

Lipoxygenase inhibition screening of free ligand and its complexes revealed that complexation was very fruitful. Ligand was inactive, metal salts were moderately active, but their complexation showed significant improvement in activity as shown in fig. 5. Ru-complex is the most active among the three complexes with IC50 value of 32.1μM, which is close to the IC50 value of standard Baicalein.

CONCLUSION

In this study, BPBP (5) was synthesized and microwave method of its synthesis is compared with conventional heating methods. Complexation of Synthesized BPBP was then performed using Fe(II), Co(II) and Ru(II) salts. Complexes (6-8) were then characterized using different spectroscopic and analytical techniques. The characterization studies suggested that compound (5) acted as bidentate ligand and Ligand was coordinated to metal through its bipyridine N. The stoichiometry of the complexes (6-8) was found to be 1:3 :: M:L complexes and proposed symmetry of complexes was octahedral C_3. Biological screening of Ligand, metal salts and their complexes for DPPH Radical Scavenging Activity revealed that unbounded ligand and metals are inactive but complexes (7) and (8) showed promising antioxidant properties. Hence it can be stated that coordination of metal to ligand played a vital role in enhancing their biological applications. When Ligand, metal salts and their complexes were assayed for lipoxygenase inhibition the same trend was observed as in case of antioxidant activity. Complexation has significant effect on the lipoxygenase inhibitory property of free metal salts making the metal more susceptible to bind with active sites of ligand. As synthesized complexes exhibited very good antioxidant properties and lipoxygenase inhibition so their synthesis may prove important for the treatment of different disorders related to oxidative stress.

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