# Synthesis, Characterization and *in vitro* Antibacterial Studies of Ternary Complexes using Quinolone Antibiotics as Primary Ligand

[1]MUHAMMAD IMRAN*, [1]TABINDA KOKAB, [1]SHOOMAILA LATIF,
[2]MITU LIVIU AND [1]ZAID MAHMOOD
[1]*Institute of Chemistry, University of the Punjab Lahore-Pakistan.*
[2]*Department of Physics and Chemistry, Faculty of Science, University of Pitesti, Pitesti, 110040, Romania.*

(Received on 9th April 2009 accepted in revised form 16th December 2009)

**Summary**: Some new first row transition metal ternary complexes of quinolone antibiotics (Levofloxacin, Moxifloxacin) and 1,10-phenenthroline were prepared and characterized on the basis of UV-Vis spectroscopy, IR spectral data, magnetic moment, elemental analysis and conductometric measurements. Ligands were found to be bidentate in nature and elemental analysis data revealed [MLL'] configuration for all metal complexes. (Where L is respective quinolone antibiotic and L' is 1,10-phenenthroline). Conductometric measurements suggested electrolytic nature of all metal complexes. All the synthesized metal complexes were evaluated for their *in vitro* antibacterial and antifungal activity against three bacterial species, *Escherichia Coli* (Gram -ve), *Mycobacterium smegmatis* (Gram +ve), *Pseudomonas aeuroginosa* (Gram -ve) and three fungal species, *Nigrospora oryzae*, *Aspergillus niger and Candida albicans* respectively. All the metal complexes were found to have appreciable antimicrobial activity.

## Introduction

Antibiotic is a class of natural or synthetic compounds that inhibit the growth or kill microorganism in dilution. This inhibitory effect can be *in vivo* or *in vitro*. These are usually small molecules with a molecular weight less than 2000 Da. A large number of antibiotics produced by microorganisms have been reported in the scientific literature. Practically few (~200) have become commercial products for human and veterinary uses [1].

Quinolones represent a group of antibiotics that have been extensively used in treatment against numerous bacterial diseases [2]. The first compound of this series was discovered in 1962 and then the field has grown exponentially [3,4]. Currently the mechanism of action of these compounds is not entirely understood, although there are some evidences signifying that these drugs interact directly with DNA, blocking the activity of DNA-gyrase [5-6].

Metal complexes performed a wide range of tasks such as carrying oxygen throughout the body and scuttling electrons. Hemoglobin, an iron containing complex that binds to oxygen through its iron atom, carries this vital molecule to body tissues. It has been exposed that biological activities of quinolone compounds are influenced significantly by metal coordination [7]. The previous literature also reveals that the quinolone antibiotics act as a monoanionic bidentate ligand through their carboxylate and carbonyl groups [8-11].

In the present paper, we describe the synthesis and characterization of ternary complexes with quinolone antibiotics and nitrogen donor ligand (1,10-phenanthroline). The examination of their antimicrobial activities against three bacterial and three fungal species revealed the advantages of ternary metal complexes. The main objective of this work is to understand the coordination chemistry of these novel compounds and the search of target antibiotics instead of broad-spectrum antibiotics.

## Results and Discussion

### *Physical Analysis*

The ternary metal complexes were prepared by reacting an appropriate amount of quinolone antibiotic (Moxifloxacin, Levofloxacin) and 1,10-phenanthrolone with Cu(II), Ni(II) and Co(II) in 1:1:1 ratios, respectively. The complexes are stable solids, which decompose above 180°C (Table-1). The solubility data of the metal complexes has been

*To whom all correspondence should be addressed.

Table-1: Physical Properties of ternary metal complexes.

| S. No. | Compound/complex | Color | Physical State | $\mu_{eff}$(B.M) | Conductance µs/cm |
|---|---|---|---|---|---|
| 1. | Levofloxacin | Light Yellow | Powdered | - | - |
| 2. | Moxifloxacin | Dark Yellow | Granular | - | - |
| 3. | 1, 10-Phenanthroline | Colorless | Crystalline | - | - |
| 4. | Cu (II)-complex (levo) | Dark Green | Crystalline | 1.74 | 62.0 |
| 5. | Ni (II)-complex (levo) | Sea Green | Crystalline | Diamagnetic | 52.1 |
| 6. | Co (II)-complex (levo) | Dark Brown | Crystalline | 2.34 | 71.7 |
| 7. | Cu (II)-complex (moxi) | Yellowish Green | Powdered | 1.76 | 79.5 |
| 8. | Ni (II)-complex (moxi) | Yellow | Powdered | Diamagnetic | 62.3 |
| 9. | Co (II)-complex (moxi) | Light Brown | Powdered | 2.37 | 55.2 |

Levo = Levofloxacin , Moxi = Moxifloxacin

Table-2: Solubility data of ternary metal complexes.

| S. No. | Compound | Water | Methanol | Ethanol | Acetone | DMSO | DMF | Chloroform |
|---|---|---|---|---|---|---|---|---|
| 1. | Levofloxacin | - | + | + | + | + | + | + |
| 2. | Moxifloxacin | - | + | + | + | + | + | + |
| 3. | 1,10-phenanthroline | - | + | + | + | + | + | + |
| 4. | Cu (II)-complex (levo) | - | - | - | - | + | + | - |
| 5. | Ni (II)-complex (levo) | - | - | - | - | + | + | - |
| 6. | Co (II)-complex (levo) | - | - | - | - | + | + | - |
| 7. | Cu (II)-complex (moxi) | + | + | + | + | + | + | + |
| 8. | Ni (II)-complex (moxi) | + | + | + | + | + | + | + |
| 9. | Co (II)-complex (moxi) | + | + | + | + | + | + | + |

+ = Soluble - = insoluble

DMSO = Dimethlyl sulphoxide , DMF = Dimethlyl formamide

Levo = Levofloxacin , Moxi = Moxifloxacin

recorded in Table-2, which indicates that these complexes have different solubilities in different solvents. However DMF and DMSO dissolve all the complexes. All the complexes were dissolved in DMSO and molar conductivities of $10^{-3}$ M solutions at 25 ± 2 °C were measured. It is concluded that the complexes are found to have molar conductance values in the 55-80 µs/cm, which shows that they are electrolytes. Elemental analysis data revealed [MLL'] configuration for all metal complexes (Table-3).

*Electronic Spectra*

The electronic absorption spectra are often very helpful in the evaluation of result furnished by other methods of structural investigation. The electronic spectral measurements were used for assigning the stereochemistries of metal ions in the complexes based on the position and number of transition peaks. The electronic spectrum of ligand levofloxacin showed $\lambda_{max}$ at 300 nm with absorption at 9.999. This transition can be assigned to $n$-$\pi^*$ transition. The electronic spectrum of second ligand 1,10-phenanthroline showed $\lambda_{max}$ at 265nm and 230nm with absorption of 1.001 and 1.566 respectively, which can be assigned to $\pi$-$\pi^*$ transitions. In the ternary complexes of levofoxacin and 1,10-phenanthroline, the peak of $\lambda_{max}$ has been shifted toward 295nm-300nm respectively. So there occurs a blue shift for Cu, Ni and Co complexes. The shifting of band together with color change confirms complex formation. The electronic spectrum of moxifloxacin showed two peaks at 230nm and 285nm (Table-4). In the ternary complexes of moxifloxacin and 1,10-phenanthroline, the peak of $\lambda_{max}$ has been shifted towards 300-340nm. So there occurs a red shift for Cu, Ni and Co complexes. This shifting of band together with color change also confirms the formation of metal complexes [12]. The electronic spectra of Cu (II) complexes of both the ligands further exhibit two bands at 31055-31020 $cm^{-1}$ and 19820-19765 $cm^{-1}$ and are assigned to Charge transfer and $Eg \leftarrow {}^2T_2g$ transitions. These two bands are in agreement with those usually observed for square planar complexes of Cu (II) thus supporting proposed square planar geometry of these complexes.

Table-3: Elemental analysis data of ternary metal complexes.

| S. No | Compound | C % Obs. (Cal.) | H % Obs. (Cal.) | N % Obs. (Cal.) | M. % Obs. (Cal.) |
|---|---|---|---|---|---|
| 1 | Cu (II)-complex (levo) | 69.43 (69.63) | 3.97 (4.64) | 12.63 (13.54) | 10.67 (11.34) |
| 2 | Ni (II)-complex (levo) | 69.87 (70.31) | 3.96 (4.69) | 12.79 (13.67) | 9.90 (10.58) |
| 3 | Co (II)-complex (levo) | 69.70 (70.45) | 4.57 (4.70) | 13.30 (13.45) | 10.02 (10.61) |
| 4 | Cu (II)-complex (moxi) | 70.43 (70.97) | 4.67 (5.20) | 11.95 (12.55) | 9.87 (10.59) |
| 5 | Ni (II)-complex (moxi) | 71.34(71.61) | 4.54 (5.24) | 11.69 (12.66) | 8.56 (9.87) |
| 6 | Co (II)-complex (moxi) | 71.20 (71.74) | 4.97 (5.25) | 11.98 (12.68) | 9.10 (9.90) |

Levo = Levofloxacin , Moxi = Moxifloxacin

Table-4: UV/VIS data of ternary metal complexes.

| S. No. | Compound | $\pi - \pi^*$ | | $n - \pi^*$ | |
|---|---|---|---|---|---|
| | | $\lambda_{max\ (nm)}$ | Abs | $\lambda_{max\ (nm)}$ | Abs |
| 1 | Levofloxacin | 300 | 9.999 | - | - |
| 2 | Maxifloxacin | 230 | 0.934 | 285 | 9.999 |
| 3 | 1,10-phenanthroline | 230 | 1.566 | 265 | 1.001 |
| 4 | Cu (II)-complex (levo) | - | - | 300 | 3.757 |
| 5 | Ni (II)-complex (levo) | - | - | 300 | 1.204 |
| 6 | Co (II)-complex (levo) | - | - | 295 | 1.805 |
| 7 | Cu (II)-complex (moxi) | - | - | 335 | 1.288 |
| 8 | Ni (II)-complex (moxi) | - | - | 330 | 1.442 |
| 9 | Co (II)-complex (moxi) | - | - | 325 | 1.253 |

Levo = Levofloxacin , Moxi = Moxifloxacin

*Magnetic Moment*

The magnetic moment value for Cu(II) complexes is 1.74 and 1.76 B.M (Table-1) that favors their square planar geometry. The magnetic moment value of both Ni(II) complexes is zero and the complexes are diamagnetic in nature. The zero magnetic moment as well as $d^8$ configuration of Ni(II) ions is also an indication for square planar geometry [13]. The Co(II) complexes shows magnetic moment value in the range 2.34 - 2.37 B.M (Table-1) which is supportive for the assignment of square planar geometry for cobalt complexes The proposed square planar geometry for these cobalt complexes is also in agreement with the previous literature where it has been quoted that monoanionic bidentate ligand usually form square planar complexes with Co(II) ions [13 ].

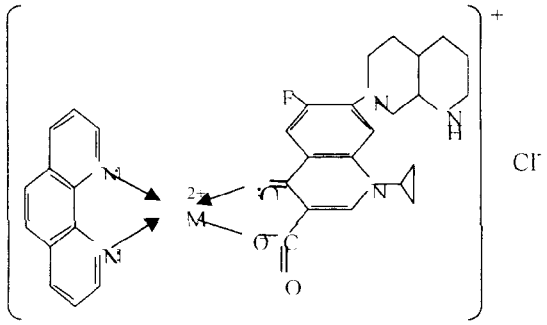

Fig. 1: Proposed Structures of Ternary Complexes of Levofloxacin.
where $M^{2+}$ = Co (II), Ni(II) and Cu(II)

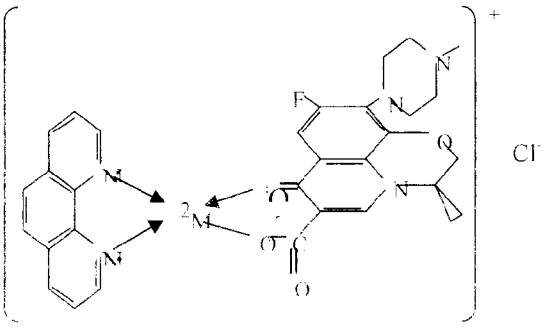

Fig. 2: Proposed Structures of Ternary Complexes of Moxifloxacin.
where $M^{2+}$ = Co(II), Ni(II) and Cu(II).

*IR Spectral Analysis*

IR spectra are very useful in the evaluation of results obtained by other methods of structural investigation. Most important region of IR spectra was interpreted (Table-5). The IR spectra of the complexes are compared with those of the free ligand in order to determine the coordination sites that may involve in bonding. The most important region in IR spectra of flourequinolone is 1800-1300$cm^{-1}$. The peaks of (C=O) in the carboxylic group ($COO^{-1}$) of both levofloxacin and moxifloxacin appeared at 1723.6 $cm^{-1}$ and 1710.0 $cm^{-1}$ respectively., which shift in the metal complexes towards lower wavelength due to coordination of carbonyl oxygen with metal. The peaks of (C-O) in the carboxylic group ($COO^{-1}$) of both levofloxacin and moxifloxacin appeared at 1239.2 $cm^{-1}$ and 1184.5 $cm^{-1}$, respectively, this peak is disappeared in the metal complexes, which indicates that carboxylic group is

Table-5: IR data of ternary metal complexes.

| S. No | Compound | COC (C=O) ($cm^{-1}$) | COC Asym. (C=O) ($cm^{-1}$) | COC Sym. (C=O) ($cm^{-1}$) | Av ($cm^{-1}$) | C-N ($cm^{-1}$) | C-X ($cm^{-1}$) | C-H ($cm^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| 1 | Levofloxacin | 1723.6 | 1619.4 | 1539.6 | 79.8 | 1239.2 | 650.9 | 2922.8 |
| 2 | Maxifloxacin | 1720.0 | 1624.4 | 1518.0 | 106.4 | 1184.5 | 649.4 | 2917.9 |
| 3 | 1,10-phenanthroline | - | - | - | - | 1344.1 | - | 2918.5 |
| 4 | Cu (II)-complex (levo) | 11709.2 | 1661.8 | 1582.6 | 79.2 | 1149.0 | 651.9 | 2921.5 |
| 5 | Ni (II)-complex (levo) | 1703.5 | 1621.5 | 1516.1 | 105.4 | 1047.8 | 724.0 | 2918.9 |
| 6 | Co (II)-complex (levo) | 1710.0 | 1652.8 | 1552.3 | 100.5 | 1098.3 | 740.8 | 2916.5 |
| 7 | Cu (II)-complex (moxi) | 1711.9 | 1623.0 | 1516.2 | 106.8 | 1048.4 | 722.0 | 2853.0 |
| 8 | Ni (II)-complex (moxi) | 1708.3 | 1622.7 | 1515.1 | 107.6 | 1088.8 | 721.6 | 2853.2 |
| 9 | Co (II)-complex (moxi) | 1707.3 | 1621.3 | 1518.2 | 103.1 | 1076.1 | 27.3 | 2863.0 |

Levo = Levofloxacin , Moxi = Moxifloxacin
Asym= Asymmetric, Sym= Symmetric

definitely taking part in complex formation after deprotonation. A carboxylate ligand can bind to the metal as either a monodentate or bidentate, resulting in changes in the relative positions of the asymmetric and symmetric stretching vibrations [14]. The IR spectra of the complexes give a separation value ($\Delta\nu$) less than 200 $cm^{-1}$ which confirms the bidentate nature of carboxylate group [15-17]. Metal complexes of both antibiotics have shown similar types of shifts. The peaks of (C-X) and (C-H) at 650-750 $cm^{-1}$ and 2900-3000 $cm^{-1}$ respectively are almost same in ligands and metal complexes. Another peak of (C-N) has appeared at 1000-1200 $cm^{-1}$ in the metal complexes, which is not present in both levofloxacin and moxifloxacin, this peak shows the coordinate bond formation between nitrogen atom of 1,10-phenanthroline and metal ions[18].

*Biological Studies*

*Antibacterial Studies*

Metal complexes were screened against three bacterial species like *Escherichia coli*, *Mycobacterium smegmatis* and *Pseudomonas aeuroginosa* by using paper disc diffusion method [19]. Study of *in vitro* antibacterial activity of metal complexes exhibit appreciable antibacterial activity that is in accordance with the previous studies [20-22]. This enhanced antibacterial activity can be explained on the basis of Overtone concept and chelation theory [23]. Such enhancement may be due to an increase in cell permeability of the lipophilic metal conjugates, which allow for the intracellular drug accumulation. It is also likely that the intracellular reduction of these metal compounds may lead to higher cytoplasmic concentration of metal species, which prove lethal for bacteria.

*Antifungal Studies*

The antifungal activity of all the synthesized metal complexes was determined against three fungal strains namely; *Nigrospora oryzae*, *Aspergillus niger and Candida albicans by* the agar plate technique. It was observed that metal complexes are more active than the corresponding ligand and toxicity increases with increasing concentration, which can also be explained on the basis of chelation theory [23].

## Experimental

All chemicals used were of analytical grade and obtained from E-Merck/ BDH/ Fluka. Levofloxacin and Moxifloxacin were obtained from a local pharmaceutical company. I.R. spectra were recorded on a Philips analytical PU 9800 FTIR (USA). U.V.-visible spectra were obtained in DMSO by SPECORD-200 spectrophotometer using software ACTUA 710 (Analytik Jena, Germany). Conductances of the metal complexes were determined in DMF on a Hitachi YSI-32 model conductometer. Magnetic measurement was done on the solid complexes using the Gouy method. Melting points were determined by Gallenkamp apparatus (UK). C, H and N analyses were performed with CHN Elemental analyzer (UK). All the apparatus used throughout the experimental work had standard quick fit joints and were dried at 110 °C.

*Synthesis of Ternary Complexes of Levofloxacin*

Equimolar solutions of Levofloxacin (0.1 M, 1.80 g) and 1, 10-phenanthroline (0.1 M, 0.90 g) were prepared in 50 mL distilled ethanol and stirred for 1h. Then 0.1 M solutions of Cu(II), Ni(II) and Co(II) salts in distilled water were added in the above solution gradually with constant stirring. The color of the solutions changed immediately due to formation of complexes. The above solutions were concentrated to one third in water bath followed by slow evaporation of solvent at room temperature. The crystals obtained were washed several times with distilled water, ethanol and recrystallized.

*Synthesis of Ternary Complexes of Moxifloxacin*

Ternary complexes of Cu(II), Ni(II) and Co(II) were prepared by reacting together equimolar solutions of Moxifloxacin and 1,10-phenanthroline according to the same procedure as described above.

*Antibacterial Studies*

Table-6: Antibacterial activity data of ternary metal complexes.

| S. No. | Complexes | Bacterial strain inhibition zone (mm) | | |
|---|---|---|---|---|
| | | a | b | C |
| 1 | Levofloxacin | ++++ | ++++ | ++++ |
| 2 | Moxifloxacin | +++ | +++ | ++++ |
| 3 | Cu (II)-complex (levo) | +++ | ++ | +++ |
| 4 | Ni (II)-complex (levo) | ++ | ++ | ++ |
| 5 | Co (II)-complex (levo) | +++ | ++++ | +++ |
| 6 | Cu (II)-complex (moxi) | +++ | ++ | ++ |
| 7 | Ni (II)-complex (moxi) | ++ | + | ++ |
| 8 | Co (II)-complex (moxi) | ++++ | ++++ | ++ |

a = *E.coli*
b = *Mycobacterium smegmatis*
c = *Pseudomonas aeuroginosa*
Levo = Levofloxacin, Moxi = Moxifloxacin
\+ = 0-5mm ++ = 6-10mm
+++ = 11-15mm ++++ = 16-20mm

Table-7: Antifungal activity data of ternary metal complexes.

| S. No. | Compound | % Inhibition of Growth | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | A | | | B | | | C | | |
| | | 100 µg/mL | 500 µg/mL | 1000 µg/mL | 100 µg/mL | 500 µg/mL | 1000 µg/mL | 100 µg/mL | 500 µg/mL | 1000 µg/mL |
| 1 | Levofloxacin | 6.8 | 10.2 | 13.8 | 4.5 | 7.5 | 10.1 | 6.5 | 10.3 | 15.4 |
| 2 | Moxifloxacin | 10.2 | 15.1 | 19.4 | 10.3 | 12.4 | 23.4 | 12.2 | 20.6 | 26.5 |
| 3 | Cu (II)-complex (levo) | 19.0 | 33.5 | 57.8 | 23.4 | 39.5 | 61.2 | 6.4 | 10.11 | 15.9 |
| 4 | Ni (II)-complex (levo) | 25.2 | 43.5 | 64.4 | 17.5 | 36.2 | 60.3 | 14.4 | 29.2 | 47.5 |
| 5 | Co (II)-complex (levo) | 29.6 | 48.5 | 63.0 | 21.4 | 34.3 | 50.2 | 20.3 | 40.2 | 55.4 |
| 6 | Cu (II)-complex (moxi) | 11.0 | 23.5 | 37.8 | 21.4 | 33.5 | 56.2 | 8.4 | 10.01 | 16.9 |
| 7 | Ni (II)-complex (moxi) | 22.2 | 33.5 | 64.4 | 13.5 | 26.2 | 50.3 | 11.4 | 21.2 | 40.5 |
| 8 | Co (II)-complex (moxi) | 23.6 | 41.5 | 56.0 | 22.4 | 34.3 | 45.2 | 20.4 | 30.2 | 47.4 |

Levo Levo = Levofloxacin , Moxi = Moxifloxacin

where, a. *Nigrospora oryzae* b. *Aspergillus niger* c. *Candida albicans*

Antibacterial activity of metal complexes was investigated by formerly reported disc diffusion method [15] against three bacterial strains such *as Escherichia Coli, Mycobacterium smegmatis and Pseudomonas aeuroginosa.*

The nutrient paper discs (Whatman No.1) were used. The compounds were dissolved (30ug) in DMF (0.01mL). The filter paper disc was soaked in solution of ligand as well as complexes, dried and then placed in petri plates previously sealed with the test organism. The plates were incubated for 24 hours at 37°C and the inhibition zone around each disc was measured in millimeter.

*Antifungal Studies*

The fungicidal activity of the ligand and the complexes were evaluated against *Nigrospora oryzae, Aspergillus niger and candida albicans* by the agar plate technique [24] at concentration of 100, 500 and 1000ppm with triplicate determination in each case. The average percentage inhibition after 96 h was calculated from the expression:

$$\text{Inhibition (\%)} = 100(C-T)/C$$

where C and T are the diameters of the fungus colony in the control and test plates, respectively. The obtained results are recoded in Table-6.

## Conclusion

It can be concluded from this study that in all the ternary metal complexes the respective antibiotic ligand has monoanionic bidentate nature while the second ligand 1,10-phenanthroline possess only bidentate nature and the resulting ternary metal complexes were found to have coordination number 4. All the metal complexes were found to have appreciable antimicrobial activity against some bacterial and fungal strains.

## Dedication

This paper is dedicated to our beloved teacher Prof. Dr. Javed Iqbal (late).

## References


1. R. M. Evans, "The chemistry of antibiotics used in medicine", Ballantyne & Co Ltd, p 78 (1965).
2. D. C. Hooper and J. S. Wolfson, "Quinolone antimicrobial agents", 2nd Edit., American Society for Microbiology, Washington, D.C., p 1 (1993).
3. L.A. Mitscher, P. Devasthale and R. Zavod, "Structure-activity relationships in quinolone antimicrobial agents", 2nd Edit., American Society for Microbiology, Washington, D.C., p 3 (1993).
4. K. Grohe, *Chemistry in Britain*, **28**, 34 (1992).
5. L. Shen and A. G. Pernet, *Proceeding of the National Academy of Sciences*, **82**, 307 (1985).
6. L. L. Shen, "Quinolone antimicrobial agents", 2nd Edit., American Society for Microbiology, Washington, D.C., p 77 (1993).
7. K. Timmers and R. Sternglanz, *Bioinorganic Chemistry*, **9**, 145 (1978).
8. G. Mendoza, L .M. R. Martinez, R. P. Alonso, X. Solans and R. M. Esparza, *Inorganic Chimica Acta*, **138**, 41 (1987).
9. G. M. Diaz, L. M. R. M. Auguilera, R. M. Esparza and K. H. Pannell., *Journal of Inorganic Biochemistry*, **50**, 65 (1993).
10. S. C. Wallis, L. R. Gahan, B. G. Charles, T. W. Hambley and P. A. Duckworth, *Journal of Inorganic Biochemistry*,**62**, 1 (1996).
11. G. M. Diaz, R. P. Alonso and R. M. Esparza, *Journal of Inorganic Biochemistry*, **64**, 207 (1996).

12. P. S Kalsri; "Spectroscopy of organic compounds", New Age International (P) Ltd, 6th Ed, p 415 (2004).
13. F. A. Cotton, G. Wilkinson, C. A. Murillo and M. Bochmann, "Advanced inorganic chemistry", John Wiley and Sons, INC.,6th Ed, p.821 (1999)
14. F. Gao, P. Yang, J. Xie and H. Wang, *Journal of Inorganic Biochemistry*, **60**, 61 (1995).
15. K. Nakamoto, Infrared and Raman Spectra of Inorganic and Coordination Compounds, Wiley, New York (1986).
16. G. B. Deacon and R. J. Phillips, *Coordination Chemistry Review*, **33**, 227 (1980).
17. M. Imran, J. Iqbal, S. Gul, S. Iqbal and M. Z. Haq, *Journal of the Chemical Society of Pakistan*, **31**, 69 (2009).
18. P. Crews, J. Rodriguez and M. Jaspals; "Organic structure analysis", Theory Design & Applications. Elsevier, Amsterdam, p. 317 (2004).
19. S. Iqbal, S. A. Tirmizi, F. H. Watoo, M. Imran, H. S. Watoo, S. Sharfudin and S. Latif, *Turkish Journal of Biology*, **30**, 1 (2006).
20. M. Imran, J. Iqbal, T. Mehmood and S. Latif., *Journal of Biological Sciences*, **6**, 946 (2006).
21. M. Imran, J. Iqbal, S. Iqbal and S.Latif, *Journal of the Chemical Society of Pakistan*, **29**, 151 (2006).
22. M. Imran, J. Iqbal, S.Iqbal and N.Ijaz, *Turkish Journal of Biology*, **31**, 67 (2007)
23. L. Mishra and V. K. Singh, *Indian Journal of Chemistry*, **32 A**, 446 (1997).
24. M. U. Hassan, Z. H. Chohan and C.T. Supuran, *Main Group Metal Chemistry*, **25**, 291 (2002).