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# Antitumour and Antimicrobial Studies of a Series of Mn(II) and Ni(II) Complexes derived from N-Amidino-N[1]-Naphthylthiourea

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**Abstract:** A series of Mn(II) and Ni(II) complexes with N-amidino-N[1]-naphthylthiourea (L) have been synthesized and the nature of bonding and structure of complexes have been deduced from elemental analysis, IR, $^1$H NMR, electronic spectra, magnetic susceptibility and conductivity measurements. The physico chemical studies and spectral data reveals that the ligand acts as neutral bidentate and coordinates through S and N. An octahedral geometry has been suggested for all the complexes and are formulated as $[ML_2X_2]$ where M = Mn(II), Ni(II), X = $Cl^-$, $NO_3^-$, $NCS^-$. The complexes $[MnL_2Cl_2]$ and $[NiL_2Cl_2]$ have been subjected to thermal decomposition study. The *in-vitro* antimicrobial and antitumour properties of the ligand in comparison to their metal complexes have been evaluated and it is observed that the complexes show more potent activity than the ligand.

**Keywords :** Mn(II) complexes, Ni(II) complexes, N-amidino-N[1]-naphthylthiourea , thermal study, antimicrobial, antitumour.

## INTRODUCTION :

The chemistry of transition metal complexes has received much attention in recent years on account of their rational design and synthesis in coordination chemistry, potential applications as functional materials, enzymatic reaction mechanism and in bioinorganic chemistry[1]. Transition metals exhibit different oxidation states and can interact with a number of negatively charged molecules. This activity of transition metals has started the development of metal based drugs with promising pharmacological application and may offer unique therapeutic opportunities.

Amidinothiourea has several potential coordinating modes since it can act as an NN or SN donor ligand due to thiol-thioketo tautomerism [2,3]. Amidinothiourea and its derivatives are extremely important industrial and biological molecules. Recently research work on safe accelerators has gained interest worldwide and amidinothiourea derivatives are reported to be non-toxic and it is used in many pharmaceutical applications [4].

With the growing interest of amidinothiourea the present work was undertaken in order to investigate the ligational behaviour of amidinothiourea derivative viz. N-amidino-N[1]-naphthylthiourea towards the metal ions Mn(II) and Ni(II). In addition, the complexes are characterized and screened for their *in vitro* antimicrobial and antitumor activities.

## MATERIALS AND METHODS:

All the reagents and solvents used were of analytical grade quality obtained from commercial suppliers Fluka/Sisco Research Laboratories (India).

**Synthesis of N-amidino-N$^1$-naphthylthiourea :**

The synthesis of ligand **c**onsists of 2 stages. First stage is the preparation of naphthyl- isothiocyanate, which was prepared by standard procedure [5]. Liquor ammonia (115 ml) was taken in a round bottom flask (500 ml) and $CS_2$ (55 ml) was added slowly. Naphthylamine was added slowly by shaking the mixture throughout. The crystalline product formed was filtered, dissolved in water (100 ml) and steam distilled with lead nitrate (200 g) dissolved in water (400 ml) for 3 h. The yellow oily liquid formed was extracted with ether.

In the second stage guanidine hydrochloride (0.1 mol) and NaOH (0.1 mol) were dissolved in $CH_3CN$ (25 ml) to which naphthylisothiocyanate (0.1 mol) was added drop wise with constant stirring for about 2 h. It was diluted with water and precipitated solid was collected, recrystallised from hot water, dried and kept over anhydrous $CaCl_2$. The yield was about 75%, m.p. = 143 °C.

For $C_{12}H_{12}N_4S$

anal. calcd, %: C, 58.90;H, 4.82;N, 23.01;S, 13.08.

Found, %;C, 59.01;H, 4.90;N, 22.95;S, 13.11.

The ligand was characterized by elemental analysis IR and NMR spectra. The structure of the ligand is shown in **Figure. 1.**

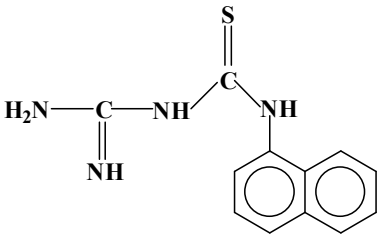



**Fig. 1. Structure of the N-amidino-N$^1$-naphthylthiourea**

**Synthesis of metal complexes**

A hot ethanolic solution (20 ml) of the required metal salts (0.001 mol) was mixed with a hot ethanolic solution (40 ml) of the ligand (0.002 mol). Immediate colour change with commencement of the separation of solid was observed in most cases. The contents were refluxed in a water bath for ~ 4 h, cooled, filtered, washed with ethanol and dried over anhydrous $CaCl_2$. The thiocyanate complexes were prepared by mixing metal salt (0.001 mol) and ligand (0.002 mol) with ~0.5 g of $NH_4CNS$ and were refluxed for ~ 1h.

**Analysis and physicochemical studies**

The elemental analysis were performed using a LECO-CHN 600 Elemental Analyser at Central Drug Research Institute, Lucknow, India. Sulphur was estimated gravimetrically after decomposing the complex with nitric acid and a few drops of bromine water . The halogen and thiocyanate were estimated by Volhard's method [6]. The metal content of the complexes were analysed using an atomic absorption spectrometer (GBC Avanta). IR spectra were recorded on a Schimadzu 8000 FT-IR spectrophotometer. The far IR spectra were recorded on a Polytec FIR 30 Fourier spectrometer using CsI disc. The electronic spectra were recorded on a Hitachi 320 UV-vis spectrophotometer. $^1$H NMR spectrum was recorded on a Bruker-Avence 400 MHz Spectrometer employing TMS as internal reference and DMSO-$d_6$ as solvent. The room temperature magnetic susceptibility measurements were made using a Guoy magnetic balance and the diamagnetic corrections for various atoms and structural units were computed from Pascal's constants [7,8]. Molar conductance measurements were carried out using $10^{-3}$ M solutions of the complexes in DMF at room temperature using a Thoshniwal conductivity meter with a dip type conductivity cell. The thermogravimetric analysis was carried out using a thermobalance of type Mettler Toledo STARe system.

**Antimicrobial experiments**

The antimicrobial activities of the ligand and the metal complexes against *E.coli*, *S.aureus*, *L.leishmanni* and *M.tuberculosis* were screened by resazurin assay method [9,10]. The bacterial culture was grown till their mid-log phase in the appropriate media (LB broth for *E.coli*, Nutrient broth for *S.aureus*, *L.leishmanni* and Middle brook 7H9 broth for *M.tuberculosis* H37 Rv). From this, the culture (50 µl) were added to fresh medium (450 µl) in 2 ml microcentrifuge tubes. The drugs (complexes) were prepared at a stock concentration of 2 mg/ml in DMF. The drugs were tested at 10 µg/ml and 100 µg/ml concentrations. Control tubes had the same volumes of medium, bacterial culture and DMF without any drug. All the samples were taken in duplicates. The tubes were then kept for incubation at 37 °C for 7 days. After incubation 0.01% resazurin (20 µl) (Sigma, St. Louis, MO.USA) in water was added to each tube. The control tubes showed change of colour from blue to pink after 24 h at 37 °C. The complexes which prevented the change of colour of the dye were considered to be inhibitory to the microbes.

**Antitumor experiments**

In order to analyse the potential of the complexes as antitumor agents, the cytotoxicity of each of the complexes and the ligand were evaluated towards Human Cervical cancer cell line HeLa by MTT assay method [11,12]. HeLa (obtained from National centre for cell science, Pune, India) were seeded into T 25 cm$^2$ tissue culture flask and allowed to become 80% confluent. All the cells were routinely maintained in DMEM (Dulbecco's Modified Eagle's Medium)

containing 10% FBS (Fetal Bovine Serium), streptomycin (100 μg/ml), penicillin (100 units/ml) and amphotericin B (2.5 μg/ml). The cells were incubated at 37 °C in a 5% $CO_2$ incubator in humid condition. When the cells were grown to confluency, the medium was removed and washed with phosphate buffered saline (PBS). Then 0.25% Trypsin-EDTA solution in PBS was added and incubated for 3-5 minutes at 37 °C. After incubation fresh medium with serum was added and cells were dispersed gently by pipette.

For the drug treatment 5000 cells per well were seeded into a 96 well plate and incubated for 24 h at 37 °C in a $CO_2$ incubator. Complexes dissolved in DMSO were added in different concentration to the cells. Six wells were kept as control. The cells were then incubated for 72 h at 37 °C. Then the medium was removed and fresh medium was added along with MTT (5 mg/ml) to each well. The cells were incubated for 2 h. The yellowish coloured MTT was reduced to dark blue coloured formazan by the viable cells only. The cells were solubilised with 0.1 ml of lysis buffer (20% SDS in 50 % DMF).

The plates were then kept for 4 h incubation at 37 °C. The optical densities at 570 nm were measured using an ELISA reader. The relative cell viability in percentage was calculated by comparing the viability of the treated cells with that of the control. The cell survival was expressed as

$$\text{Cell Survival (CS)} = \frac{\text{Mean optical density}\left[\text{Drug exposed cell}\right]}{\text{Mean optical density (control)}} \times 100$$

**Table 1: Analytical data of the complexes**

| Complexes | Contents (found /calcd), % | | | | | Molar conductance $Scm^{-2}\ mol^{-1}$ | $\mu_{eff}$ |
|---|---|---|---|---|---|---|---|
| | M | C | H | N | S | | |
| $[MnL_2Cl_2]$ | 9.41/9.52 | 46.48/46.66 | 3.87/3.91 | 18.24/18.12 | 10.44/10.36 | 32 | 5.93 |
| $[MnL_2(NO_3)_2]$ | 7.14/7.08 | 34.63/34.65 | 2.93/2.91 | 16.88/16.84 | 7.74/7.71 | 37 | 5.86 |
| $[MnL_2(NCS)_2]$ | 8.91/8.87 | 47.09/47.04 | 3.51/3.65 | 21.18/21.10 | 19.26/19.32 | 36 | 5.89 |
| $[NiL_2Cl_2]$ | 10.19/10.21 | 46.17/46.22 | 3.72/3.88 | 17.78/17.97 | 10.32/10.28 | 38 | 2.85 |
| $[NiL_2(NO_3)_2]$ | 7.64/7.56 | 34.51/34.46 | 2.73/2.89 | 16.78/16.75 | 7.59/7.67 | 33 | 2.98 |
| $[NiL_2(NCS)_2]$ | 9.51/9.50 | 46.79/46.71 | 3.68/3.60 | 20.88/20.95 | 19.14/19.19 | 34 | 2.82 |

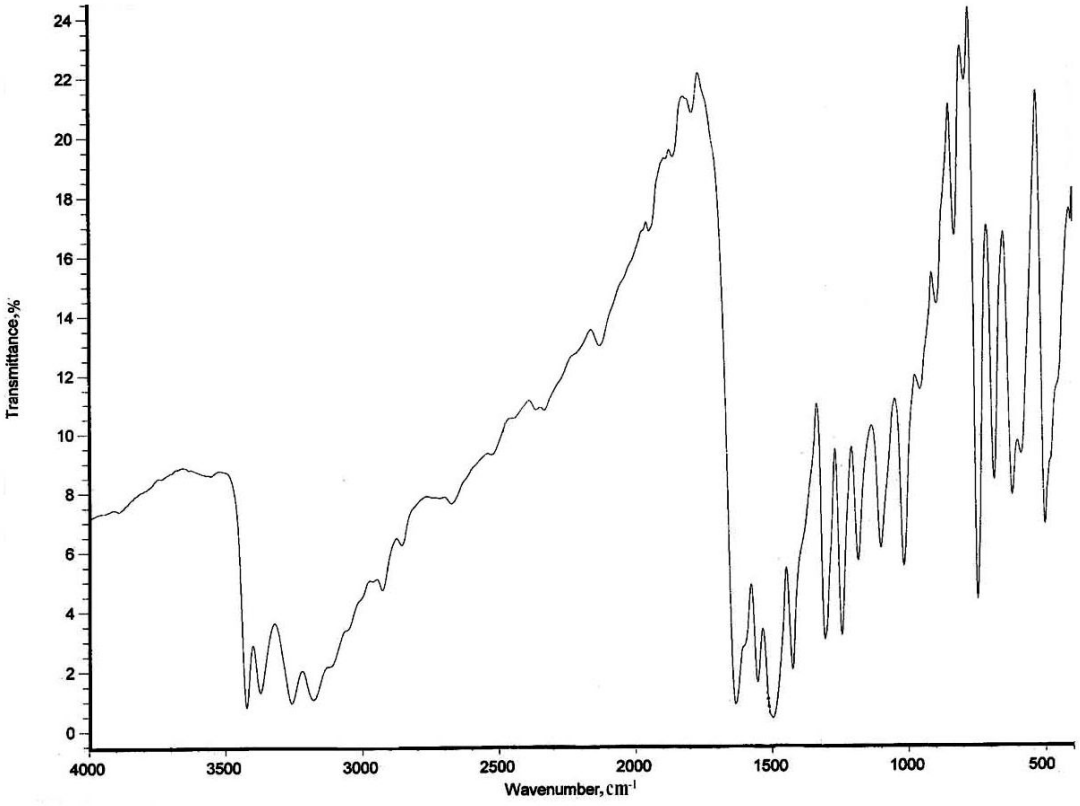



**Fig. 2 : IR spectrum of N-amidino-$N^1$-naphthylthiourea**

**Table 2: IR spectral data of ligand and complexes**

| Ligand /complex | ν(N-H) | ν(C=N) | $\nu_1$(NCS) | $\nu_2$(NCS) | $\nu_3$(NCS) | ν(C=S) | ν(M-N) | ν(M-Cl) | ν(M-NCS) | ν($NO_3$) |
|---|---|---|---|---|---|---|---|---|---|---|
| L | 3360 | 1638 | 1492 | 1325 | 955 | 895 | - | - | - | |
| $[MnL_2Cl_2]$ | 3330 | 1612 | 1480 | 1295 | 960 | 859 | 535 | 346 | - | |
| $[MnL_2(NO_3)_2]$ | 3325 | 1618 | 1484 | 1288 | 958 | 860 | 520 | - | - | 1522 ($\nu_4$) 1435 ($\nu_1$) |
| $[MnL_2(NCS)_2]$ | 3335 | 1620 | 1478 | 1294 | 962 | 862 | 525 | - | 482 | |
| $[NiL_2Cl_2]$ | 3328 | 1621 | 1486 | 1290 | 961 | 864 | 522 | 325 | - | |
| $[NiL_2(NO_3)_2]$ | 3322 | 1615 | 1483 | 1289 | 966 | 861 | 529 | - | - | 1528 ($\nu_4$) 1432 ($\nu_1$) |
| $[NiL_2(NCS)_2]$ | 3332 | 1619 | 1485 | 1285 | 969 | 864 | 530 | - | 480 | |

## RESULTS AND DISCUSSION

The ligand is expected to act as a potent bidentate due to thiol-thioketo tautomerism. Though various donor sites are present in the ligand it predominantly functions as SN donor ligand in the thioketo form.

Formation of the metal complexes can be represented by the following general equations.

$$MX_2 + 2L \rightarrow [ML_2X_2]$$

M = Mn(II) or Ni(II)

X= $Cl^-$, $NO_3^-$, $NCS^-$

All the complexes are stable at room temperature and possess good keeping qualities. They are non-hygroscopic solids and are insoluble in common organic solvents such as ethanol, benzene, chloroform and carbon tetrachloride. Formulation of these complexes has been done on the basis of elemental analysis, molar conductance and magnetic susceptibility measurements. The chemical analyses data are given **in Table 1.**

**Molar conductance and magnetic susceptibility measurement** :

The molar conductance values in DMF ($10^{-3}$ M solution) for the complexes fall ~10-50 $Scm^2mol^{-1}$ showing their non-electrolyte nature[13].

The observed magnetic moment of Mn(II) complexes in the range (5.82-5.94 $\mu_B$) is consistent with highspin octahedral geometry, but much higher than the spin-only value due to large orbital contribution[14]. The observed magnetic moment values in the range (2.78-3.30 $\mu_B$) measured for Ni (II) complexes lie in the range expected for a $d^9$ system and there is not expected appreciable spin-spin interaction indicating a distorted octahedral geometry for the complexes[15,16].

**IR spectra :**

The binding mode of the ligand to metal ions was further elucidated by analysis of the IR spectra (Table. 2) of the ligand and the metal complexes. The spectrum of the ligand (**Figure. 2)** shows a number of strong and weak bands in the region 3400-3100 $cm^{-1}$ and these bands are assigned to N -H stretching vibrations [17,18]. A strong band ~ 1625 $cm^{-1}$ is assigned to ν(C=N) and characteristic bands at 1490, 1300 and 940 $cm^{-1}$ are due to NCS frequencies. The band at 890 $cm^{-1}$ is attributed to ν(C=S). In the complexes the absorption band due to ν(C=N) of the ligand is shifted to lower frequencies.

The $\nu_3$(NCS) band at 940 $cm^{-1}$ in the ligand is considerably shifted to higher frequencies and ν(C=S) band at 890 $cm^{-1}$ in the ligand is observed at lower frequencies in the complexes. These observations show that ligand behaves as bidentate and the metal ion is coordinated through the S and N atoms of the ligand in the thiol form. Moreover an additional band around 450 $cm^{-1}$ is observed in the complexes which is due to metal-nitrogen coordination [19,20].

The occurrence of two strong bands in the nitrate complex of Mn(II) and Ni(II) ~ 1520 $cm^{-1}$ and 1430 $cm^{-1}$ are attributed to $\nu_4$ and $\nu_1$ modes of vibrations of nitrate ions. The absence of $\nu_2$ band ~1000 $cm^{-1}$ shows that no ionic nitrate is present. Also the frequency separation $\nu_4$-$\nu_1$ ~ 100-200 $cm^{-1}$ is reasonable to infer that nitrate group is coordinated monodentately [21,22]. The thiocyanate complexes of Mn(II) and Ni(II) show a very strong band around 2070 $cm^{-1}$ due to C-N stretching [23]. Bands with medium intensity ~ 830 $cm^{-1}$ and δ (NCS) band near 490 $cm^{-1}$ clearly indicate the N-coordinated nature of the thiocyanate group. Far infrared spectra of the metal complexes showed several absorption bands which were not observed in the ligand spectrum. The bands appearing around 320 $cm^{-1}$ for the chloro complexes of Mn(II) and Ni(II) are assignable to ν(M-Cl) stretching vibration [24].

**$^1$H NMR spectra :**

The $^1$H NMR spectrum of the free ligand recorded in DMSO-$d_6$ gave signals ~ 6.96–7.53 ppm which are due to aromatic ring protons. A singlet of 2H at 3.2 ppm reveals the presence of $NH_2$ group [25] and the signal at 10.2 ppm shows the presence of N-H protons [26] and the signal at 8.46 ppm is due to NH-Ar.

**Electronic spectra :**

The ground state term for the Mn(II) ion is $^6$S and it is not split in presence of any ligand field but transforms into $^6A_{1g}$ state in an octahedral field. This is the only sextet state possible. Therefore, all the transitions to higher states are spin forbidden in an octahedral field. Due to spin orbit coupling many spin forbidden transitions are being shown by Mn(II) complexes. Some of the spin forbidden electronic transitions are $^6A_{1g} \rightarrow {}^4T_{1g}$, $^6A_{1g} \rightarrow {}^4T_{2g}$ , $^6A_{1g} \rightarrow {}^4E_g$ and $^6A_{1g} \rightarrow {}^4A_{1g}$. These transitions are responsible for the pale pink colour of Mn(II) complexes [27,28]. The newly synthesized Mn(II) complexes show a very intense band around 420 nm and a very faint shoulder around 500 nm. This intense peak may be attribute to the ligand absorption and the weak shoulder may be due to the overlapping of one spin forbidden d-d transition with the ligand. These transitions support an octahedral geometry around the Mn(II) ion.

The electronic spectra of Ni(II) complexes showed d-d bands around 950nm, 630nm and 370nm . These are assigned to the transitions $^3A_{2g}$ (F) $\rightarrow {}^3$ T $_{2g}$ (F), $^3A_{2g}$ (F) $\rightarrow {}^3$ T $_{1g}$ (F) and $^3A_{2g}$ (F) $\rightarrow {}^3$ T $_{2g}$ (P) respectively, consistent with their well-defined octahedral configuration. The band around 950nm was assigned to metal $\rightarrow$ ligand charge transfer transition [29,30].

**Antimicrobial activity :**

The ligand and the metal complexes were screened for their antimicrobial activity by resazurin assay method. Resazurin is an oxidation-reduction indicator used for the evaluation of cell growth **(Figure.3).** It is a blue non-fluorescent and non-toxic dye that becomes pink and fluorescent when reduced to resorufin (**Figure. 4)** by oxidoreductases with in viable cells [31].

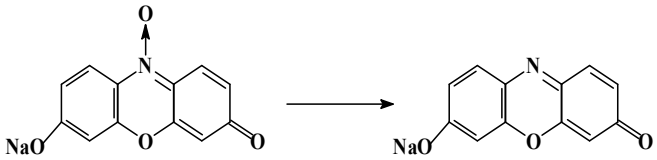



**Fig. 3 Reduction of Resazurin to Resorufin**

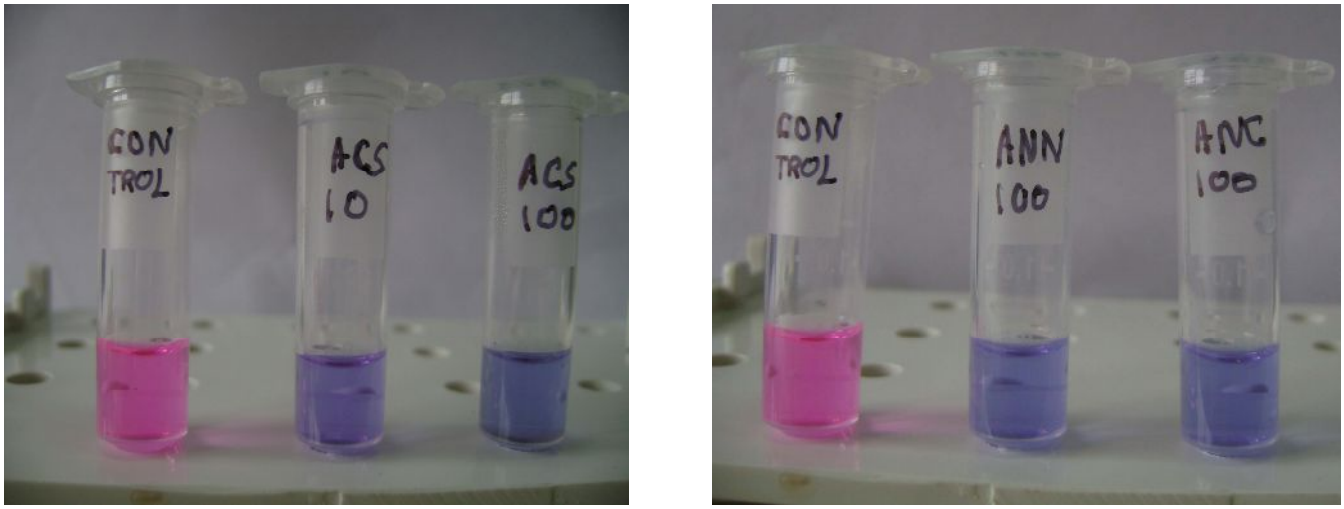



**Fig. 4: Antibacterial and antitubercular activity of $[MnL_2(NCS)_2]$, and $[NiL_2Cl_2]$**

**Table 3: Antimicrobial activity of ligand and its complexes (Resazurin assay method)**

| Ligand/Complex | Conc.μg/ml | Activity against | | | |
|---|---|---|---|---|---|
| | | *E.coli* | *S.aureus* | *L.leishmanni* | *M.tuberculosis* |
| L | 10 | - | - | - | - |
| | 100 | - | - | - | - |
| $[MnL_2Cl_2]$ | 100 | + | + | - | + |
| $[MnL_2(NO_3)_2]$ | 100 | - | + | - | + |
| $[MnL_2(NCS)_2]$ | 100 | + | + | + | + |
| $[NiL_2Cl_2]$ | 10 | + | - | + | - |
| | 100 | + | + | + | + |
| $[NiL_2(NO_3)_2]$ | 100 | - | + | - | + |
| $[NiL_2(NCS)_2]$ | 10 | - | - | + | - |
| | 100 | + | + | + | + |

The results obtained are presented in table 3. The increased activity of the metal chelates than the ligand can be explained on the basis of Overtone's concept and Tweedy's chelation theory [32-33]. The lipid membrane that surrounds the cell favours the passage of only lipid soluble materials. Hence lipophilicity is an important factor which controls the antimicrobial activity. On chelation the polarity of the metal ion is reduced to a greater extent due to the overlap of the positive charge of the metal ion with the donor groups. Further, it increases the delocalisation of π-electrons over the whole chelate ring and hence enhance the liposolubility of the complexes. This increased liposolubility enhances the penetration of the complexes into the lipid membrane. Some important factors other than chelation which determine the activity are nature of the metal ion, nature of the ligand, coordinating sites, geometry of the complex, concentrations, hydrophilicity, steric and pharmacokinetic effects.

From the result it is clear that ligand shows no activity against the microbes. All the complexes are active against M-tuberculosis in 100 μg concentration. The chlorido and thiocyanato complexes are more active than nitrato complexes. This is because the bonding capacity of nitrate ion towards the central metal ion is greater than that of the chloride ion and the extent of metal ion available is decreased for the display of antibacterial activity.

**Antitumour activity**

The cell viability over untreated control was determined using MTT assay[34]. MTT assay is a laboratory test and standard colourimeteric assay for measuring cellular proliferation, cell viability and activation. It is used to determine cytotoxicity of potential medicinal agents and other toxic material. Yellow MTT [3-(4,5-dimethyl thiozol-2-yl)-2,5-diphenyltetrazolium bromide] is reduced to purple formazan in the mitochondria of living cells.

In general the antiproliferative activities of the metal complexes were observed at lower concentration than the parent ligand indicating that complexation clearly offers an advantage in terms of lowering the therapeutic dose table 4. Also it is clear that as the concentration of the drug increases relative cell viability decreases .

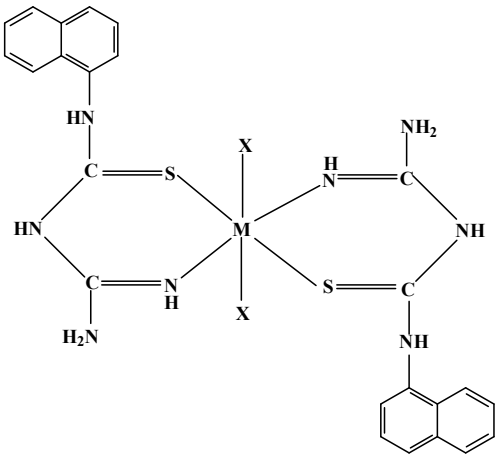
On the basis of the above spectral data and physicochemical studies, a distorted octahedral geometry (Figure. 5) has been tentatively proposed for all the complexes.

**CONCLUSION :**

The ligand N-amidino-$N^1$-naphthylthiourea acts as a neutral bidentate ligand, coordinating through nitrogen and sulphur in its complexes. From analytical data it is concluded that all the complexes are neutral and associated with two molecules of ligand in the coordination sphere. The non-electrolytic behaviour of the complexes were confirmed using IR and conductance data. A coordination number of 6 is proposed to Mn(II) and Ni(II) in these complexes. Based on several physico-chemical studies the following molecular formulae are assigned to the complexes, $[MnL_2X_2]$ and $[NiL_2X_2]$ where X represents monovalent anions $NO_3^-$, $NCS^-$, $Cl^-$. Biological studies reveal that both ligand and complex possess antitumor and antibacterial activity. It can be concluded that complexation with metal ion lead to enhanced activity as compared to the parent ligand. Since these complexes exhibit antitumor and antibacterial activity further studies in these fields may find fruitful.

**Table 4: The percentage viability of cells as determined by MTT assay method**

| Compound | Conc. μg/ml | Relative cell viability, % |
|---|---|---|
| L | 10 | 89.15 |
| | 25 | 85.06 |
| | 50 | 83.50 |
| | 100 | 73.58 |
| | 200 | 53.58 |
| $[MnL_2Cl_2]$ | 10 | 86.39 |
| | 25 | 62.87 |
| | 50 | 43.96 |
| | 100 | 24.01 |
| | 200 | 19.81 |
| $[MnL_2(NO_3)_2]$ | 10 | 80.16 |
| | 25 | 68.87 |
| | 50 | 53.14 |
| | 100 | 34.18 |
| | 200 | 20.11 |
| $[MnL_2(NCS)_2]$ | 10 | 74.43 |
| | 25 | 42.84 |
| | 50 | 30.18 |
| | 100 | 26.34 |
| | 200 | 18.19 |
| $[NiL_2Cl_2]$ | 10 | 74.14 |
| | 25 | 39.18 |
| | 50 | 29.89 |
| | 100 | 18.18 |
| | 200 | 15.71 |
| $[NiL_2(NO_3)_2]$ | 10 | 78.16 |
| | 25 | 49.14 |
| | 50 | 38.14 |
| | 100 | 22.42 |
| | 200 | 18.16 |
| $[NiL_2(NCS)_2]$ | 10 | 73.14 |
| | 25 | 44.16 |
| | 50 | 31.13 |
| | 100 | 18.14 |
| | 200 | 16.24 |




M = Mn(II), Ni(II); X = $Cl^-, NO_3^-, NCS^-$

**Figure. 5 Proposed 2D structure of metal complexes**

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**REFERENCES:**

1. Nagar, R. Structural and microbial studies of some transition metal complexes *J. Inorg. Biochem.*, **37(3),** 1989, 193-200.
2. Saha C R and Roy N K, Syntesis and structural investigation of metal complexes of 1-amidino-2-thiourea.*J. Coord. Chem.,* 12, 163, (1983).
3. Vilar R, Mingos D M P, White A J P, et al., Aufbau síntesis of a mixed metal anion receptor cage. *Chem. Commun.,* 229, (1999).
4. Susamma A P, Kurian M and Kuriakose A. P,A new binary Accelerator System for filled Natural Rubber Vulcanization. *Iran. Polym. J.,* 11, 311, (2002).
4. Chikwana E. and Simoyi R H,Oxyhalogen-Sulphur chemistry: Kinetics and Mechanism of oxidation f Amidinothiourea by acidified Iodate., *J. Phys. Chem. A.,* 108, 1024, (2004).
5. Finar I L, *Organic Chemistry,* Vol I, Addison – Wesley Longman Inc., 1998.
6. Vogel A I, *A Text Book of Quantitative Chemical Analysis,* 6th Ed., Pearson Education, (1998).
7. Figgis B N, Lewis J and Wilkin R G, *Modern Coordination Chemistry,* New York, Interscience, (1960).
8. Dutta R L and Syamal A, *Elements of Magneto Chemistry,* New Delhi: East West Press, 8, (1992).
9. Martin A, Camacho M, Portaels F, et al., Resazurin microtiter assay plate testing of mycobacterium tuberculosis susceptibilities of second-line drug: rapid, simple and inexpensive method., *Antimicrob. Agents Chemother,* 47, 3616. (2003).
10. Banfi E, Scialino G and Bragadin C, M.,Development of microdilution method to evaluate Mycobacteriumtuberculosis drug susceptibility. *J. Antimicrob. Chemotherapy,* 52, 796, (2003).
11. Alley M C, Scudiero D A, Monks A, et al., Feasibility of drug screening with panels of human tumour cell lines using a microculture tetrzolium assay, *Cancer Res.,* 48, 509, (1988).
12. Saha D K, Padhye S, Sinn E, et al., Synthesis, structure, Spectroscopy and antitumour activity of hydroxynaphthaquinone thiosemicarbazone and its metal complexes against MCF-7 human breast cancer cell line. *Indian J. Chem.,* 41, 279, ( 2002).
13. Geary W J, Use of conductivity measurements in organic solvents for the characterization of coordination compounds. *Coord. Chem. Rev.,* 7, 81,( 1971).
14. Schlapp R and Denney W G, *Phys. Rev.,* 42, 666,( 1932).
15. Figgis, B N. *Introduction to Ligand Field,* New York, Interscience, (1969).
16. Bailer J C, Emeleus H J, Nyholm R, et al., *Comprehensive Inorganic Chemistry,* Oxford: Vol. 3, Pergamon Press, (1975).
17. Upadhyay, R K, Chemistry of mixed ligand complexes of copper(II) involving Ketoanil and Thiourea, Ammonia or Anionic Ligands.with *J. Indian Chem. Soc.,* , 74, 535, (1997).
18. Sbirna L S, Muresan V, Sbirna S, et al., Complex compounds of nickel(II) with bidentate heterocyclic ligands using both S and N donor atoms., *J. Indian Chem., Soc.*, 389,( 2005).
19. Ferrero J R, *Low Frequency Vibrations of Inorganic and Coordination compounds,* New York: John Wileys, (1971).
20. Nakamato K, *Infrared and Raman Spectra of Inorganic and Coordination Compounds,* New York: John Wiley, (1997).
21. Addison C C, Logan N, Wall work S C, et al., Structural aspects of coordinated nitrate groups., *Quat. Rev. Chem. Soc.,* 25, 289, (1971).
22. Ferraro J R, The nitrate symmetry in metalic nitrates.*J. Mol. Spectrosc.* 4, 99, (1960)
23. Bailey R A, Michelsen T W and Mills W N, Observations on the i.r intensity criterion for the bonding mode in thiocyanate complexes. *J. Inorg. Nucl. Chem*., 33, 3206, (1971).
24. Kharodawala M J and Rana A K, Synthesis, characterization and biological activity of some transition metal chalates of 4-acyloximo-2-pyrazolin-5-one. *Synth. React. Inorg. Met. Org. Chem.,* 33, 1483, (2003).
25. Pouchert C J, *The Aldrich Library of NMR Spectra,* Ed. II., Vol. II, (1983).
26. Sancheti A, Swarnkar N, Soni M D, Microwave assisted synthesis and characterisation of some new annulated pyrimidinone derivatives. et al., *J. Indian Chem. Soc.*, 84, 234,(2007).
27. Ueno K and Martet A E, Ultraviolet and visible absorption spectra of metal chelates of bis-

acetylacetone ethylenediimine and related compounds. *J. Phys. Chem.,* 61, 257,(1957)

28. Lever A B P, *Inorganic Electronic Spectroscopy,* 2nd Ed., Amsterdam : Elsevier, (1984).
29. Balhausen C J, *An Introduction to Ligand Field*, New York: McGrow Hill, (1962).
30. Dyer J R, *Applications of Absorption Spectroscopic of Organic Compounds,* London: Prentice- Hall, (1965).
31. Palomino, J C, Portaels, F, Simple procedure for drug susceptibility testing of Mycobacterium tuberculosis using a commercial colorimetic assay. *Eur. J. Clin. Microbiol. Infect. Dis*., 18,380-383, (1999)
32. Patel N H, Parekh H M and Patel M N, Synthesis, physicochemical characeristics and biocidal activity of some transition metal mixed-ligand complexes with bidentate(NO and NN) Schiff bases.*Phar. Chem. J.,* , 41, 78, (2007).
33. Chohan Z H, Shaikh A U, Naseer M M, et al., *In-vitro* antibacterial, antifungal and cytotoxic prpperties of metal- based furanyl derived sulphonamides.*J. Enz. Inhibit. Med. Chem.,* 21, 771, (2006).
34. William H and Stephen V, *Theory and Application of Microbiological Assay,*San Diego: Academic Press, (1989).

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