Research Paper Medical Science

# Spectral,Thermal Study and Antimicrobial Assay of Six Co-Ordinate Complex And Heterocyclic Base Adducts of Zn (II) Derived From N (4) Thiosemicarbazone Moiety

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**ABSTRACT**

Adducts of zinc (II) complex have been synthesized by the reaction of zinc (II) chloride with 5-chloro-2-hydroxy acetophenone N(4) methyl thiosemicarbazone in presence of heterocyclic base like pyridine (py), α/β/γ-picoline in the moe ratio 1:1:1. Thiosemcarbazone has been characterized by 13C-NMR, 1H NMR,ESI-MS as well as IR, electronic spectra.Spectral and magnetic measurements indicated octahedral geometry for the six coordinate complexes. The thiosemicarbazone and its Zn (II) complexes have been found antifungal,antibacterial and showed growth inhibitory activity against Citrobactor, Acinetobactor Aspergillus Niger and Candida Albicans.

**KEYWORDS** Thiosemicarbazone, metal salts,Bioactive metal complexes, antimicrobial activity.

**Introduction**

The biological activity of certain thiosemicarbazones is because of their ability to form complexes with transition metal ions bonding through oxygen ,nitrogen and sulfur atoms ONS. But in some cases, decrease of activity is due to lowering of the thiosemicarbazone's denticity[1].Thiosemicarbazones are the important compounds constituting N,S donor atoms.They show heterodentate nature on complexation with metal ion [2-7]. Thiosemicarbazones are good ligands with high biological activity.Their biological activity is due to ability to coordinate to metal centres.Thiosemicarbazone derivatives also have additional functional groups which can coordinate to metal ion.This suggests the biological activity may also depend on the non coordinating groups. Zinc pyrithione an agent of antibacterial and antifungal activity.The drug inhibits the membrane transport processes by blocking the protone pump in fungi. However, at low concentration of the agent fungi are able to inactivate pyrithione [8].

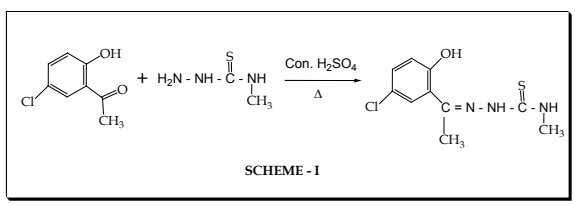
Zinc is a trace element. It is found in the human body in a variety of tissues, such as skin, bone, liver, muscle or brain. Zinc is the most abundant transition metal in the brain after iron [9]. It is important for their biological activity as a constituent of proteins and enzymes that belong to cellular signaling pathways. It is not only essential for the folding of DNA-binding domains of transcription factors [10] but also has a variety of effects on the nervous system.It plays an important role in regulating the aspects of cellular metabolism, including protein,hormone, transcription and replication functions [11]. Overabundant levels of zinc can lead to apoptosis and neuronal death [12].

In this research work the synthesis, spectral characterisation and biological studies of six coordinate complexes of zinc (II) with 5-chloro 2-hydroxy acetophenone N (4) methyl thisemicarbazone have been reported.

**Experimental**

**Synthesis of thiosemicarbazone :**

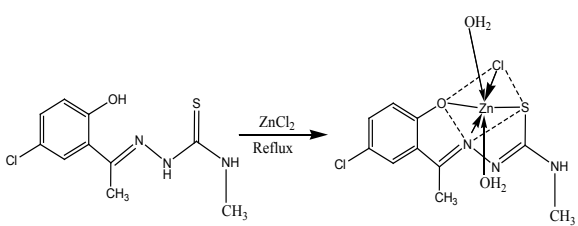
The thiosemicarbazone was synthesized by refluxing ethanolic solutions of 5-chloro 2-hydroxy acetophenone and N (4) methyl thiosemicarbazide in the mole ratio 1:1 for 3 hours. The pale yellow product obtained after cooing was filtered and washed with hot water then cold ethanol and finally with diethyl ether. It was recrystalized from hot ethanol and dried over $P_2O_5$ in vacuum.




SCHEME - I

**Synthesis of complex:**

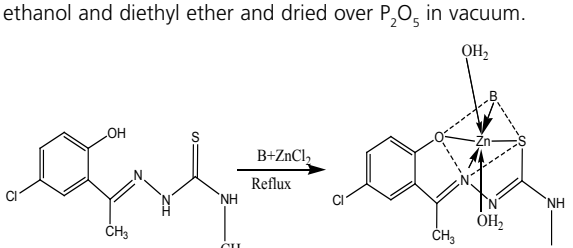
The complex was synthesized by refluxing ethanolic solutions of thiosemicarbazone, zinc chloride in ratio 1:1 for an hour on a hot water bath.The light yellow product thus obtained was fitered and washed well with hot water,cold absolute ethanol and then diethyl ether and dried over $P_2O_5$ in vacuum.




Scheme II

**Synthesis of adducts:**

The complex of the type Zn.L.B (B is heterocyclic base like pyridine, α-picoline, β-β –picoline,γ-picoline) was synthesized by adding slowly ehanolic solution of $ZnCl_2$ ,heterocyclic base to the hot ehanolic solution of thiosemicarbazone in the ratio 1:1:1 and refluxing reaction mixture for one hour. The yellow adduct obtained was filtered and washed with hot water, cold ethanol and diethyl ether and dried over $P_2O_5$ in vacuum.




Scheme III

(B= pyridine,α-picoline,β-picoline,γ-picoline)

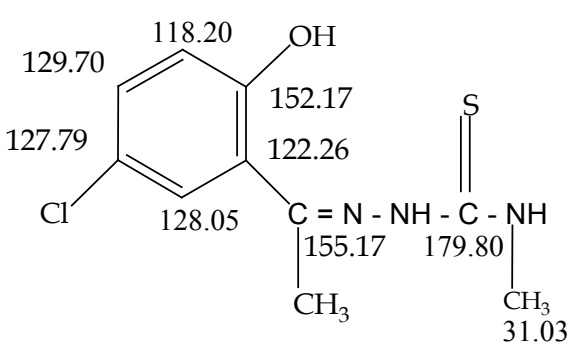
**Physical measurements-**
Faraday method was used to measure magnetic moments. IR spectra were recorded in the range 4000-200 $cm^{-1}$ range. NMR spectra were recorded in the mixture of $CDCl_3$ and DMSO-$d_6$ (1:1 v/v) with a Bruker AC-300F 300MHz spectrometer. Thermo gravimetric analysis was carried out in the temp rnge 30-800°C. UV-Visible spectra were measured on Jasco UV-visible double beam spectrophotometer.Metal in the complex and adducts was estimated by standerdized E.D.T.A using Erichrome black-T as an indicator and PH-10 buffer solution. Chloride in the complex was determined by Mohr's method.

**Table 1 Physical properties:**

| Compounds | Colour | Empirical Formula | Molar conductance Ohm$^{-1}$cm$^2$mole$^{-1}$ | Magnetic Moment B.M. |
|---|---|---|---|---|
| L | Yellow | $C_{10}H_{12}N_3ClOS$ | - | - |
| Zn-L.Cl.$(H_2O)_2$ | Yellow | $C_{10}H_{14}N_3O_3Cl_2SZn$ | 20.4 | Diamagnetic |
| Zn.L.Py.$(H_2O)_2$ | Yellow | $C_{15}H_{19}N_4O_3ClSZn$ | 21.9 | Diamagnetic |
| Zn.α-Pico.$(H_2O)_2$ | Yellow | $C_{16}H_{21}N_4O_3ClSZn$ | 22.6 | Diamagnetic |
| Zn.L β-Pico.$(H_2O)_2$ | Yellow | $C_{16}H_{21}N_4O_3ClSZn$ | 21.3 | Diamagnetic |
| Zn.L.γ-Pico.$(H_2O)_2$ | Yellow | $C_{16}H_{21}N_4O_3ClSZn$ | 22.2 | Diamagnetic |

**$^1$H-NMR**
NMR signals at 13.00 and 2.2 ppm are assigned to – OH and – $CH_3$ protons respectively. The signals at 2.40, 3.00 correspond to $^4$NH and H$^4$N-$CH_3$ respectively. Signal at 10.3 ppm corresponds to $^2$NH. Aromatic protons show multiplets at 6.9, 7.25, 7.45, ppm.

**$^{13}$C-NMR (DMSO-$D_6$): δppm** 118.20 (C = C), 129.70 (C = C), 127.79 (C = C – Cl), 128.05 (C = C), 122.26 (C = C), 152.17 (C = C – OH), 155.39 (C = N), 179.80 (C = S), 31.03 (NH – $CH_3$)




(Calcd) found ESI-MS m/z, ion $M^+$ : $C_9H_{10}ClN_3OS$ (243.70) 243.02, $C_{10}H_{14}N_3O_3Cl_2SZn$ (392.59) 392.11, $C_{15}H_{19}N_4O_3ClSZn$ (436.24) 436.92, $C_{16}H_{21}N_4O_3ClSZn$ (450.26) 450.88, $C_{16}H_{21}N_4O_3ClSZn$ (450.26) 450.89, $C_{16}H_{21}N_4O_3ClSZn$ (450.26) 450.91.

**Analytical data**
1.L: % C 46.07 (46.80),% H 4.87 (4.69),% N 16.72 (16.30),% S 12.89 (12.44)

2. Zn-L.Cl.$(H_2O)_2$: % Zn 18.11 (16.66), %Cl 9.87 (9.03), %C 29.02 (30.59), %H 3.12 (3.59), %N 10.30 (10.70), %S 8.81 (8.17).

3. Zn.L.Py.$(H_2O)_2$ : % Zn 14.32 (14.99), %C 41.72 (41.30), %H 4.80 (4.39), %N 12.18 (12.84), %S 7.98 (7.35).

4.Zn.α-Pico.$(H_2O)_2$: % Zn 14.06 (14.52), %C 42.12 (42.68), %H 4.16 (4.70), %N 12.79 (12.44), %S 7.73 (7.12).

5. Zn.L β-Pico.$(H_2O)_2$: % Zn 14.21 (14.52), %C 42.84 (42.68), %H 4.20 (4.70), %N 12.84 (12.44), %S 7.80 (7.12).

6. Zn.L.γ-Pico.$(H_2O)_2$: % Zn 14.30 (14.52), %C 42.25 (42.68), %H 4.02 (4.70), %N 12.10 (12.44), %S 7.90 (7.12).

**Table 2 .Electronic spectral data ($cm^{-1}$)**

| Compound | Mode | MLCT | n→π* | π→π* |
|---|---|---|---|---|
| L | DMF | - | 30892 | 35866 |
| Zn-L.Cl.$(H_2O)_2$ | DMF | 25245 | 32406 | 34652 |
| Zn.L.Py.$(H_2O)_2$ | DMF | 25352 | 32436 | 34635 |
| Zn.α-Pico.$(H_2O)_2$ | DMF | 25435 | 32459 | 34563 |
| Zn.L β-Pico.$(H_2O)_2$ | DMF | 25250 | 32350 | 34500 |
| Zn.L.γ-Pico.$(H_2O)_2$ | DMF | 25326 | 32560 | 34660 |

**Infrared Spectroscopic data ($cm^{-1}$)**
1.L: ν (- OH) 3325; ν (C = N) 1635; ν (- C = S) 785, 1360 ; ν (N – N) 1060; ν ($^2$N-H) 3245; ν (C – O) 1290.

2 [Zn-L.Cl.$(H_2O)_2$]: ν (C = N) 1590; ν (C = N-N=C) 1550, ν (C-S) 710, 1312,ν (N-N) 1125, ν(M - N) 455, ν (M-O) 527, ν (M-S) 320, ν (C - O) 1225, ν($H_2O$) 3540,3555.

3.[Zn.L.Py.$(H_2O)_2$]: ν (C = N) 1592; ν (C = N-N=C) 1530, ν (C-S) 715, 1315; ν (N-N) 1127, ν (M - N) Base 275, ν (M - N) 457, ν (M - O) 530, ν (M-S) 317, ν (C - O) 1230, Band due to HB 1460, ν($H_2O$) 3545,3560.

4.[Zn.α-Pico.$(H_2O)_2$]: ν (C = N) 1593; ν (C = N-N=C) 1552, ν (C-S) 710, 1312, ν (N-N) 1122, ν (M - N) Base 278, ν (M - N) 460, ν (M - O) 529, ν (M-S) 318, ν (C - O) 1235, Band due to HB 1465, ν($H_2O$) 3550,3575.

5.[Zn.L β-Pico.$(H_2O)_2$]: ν (C = N) 1590; ν (C = N-N=C) 1551, ν (C-S) 712, 1308,ν (N-N) 1122, ν (M - N) Base 279, ν (M - N) 455, ν (M - O) 535, ν (M-S) 320, ν (C - O) 1230, Band due to HB 1472, ν($H_2O$) 3556,3578.

6.[Zn.L.γ-Pico.$(H_2O)_2$]: ν (C = N) 1598; ν (C = N-N=C) 1553, ν (C-S) 711, 1310,ν (N-N) 1125, ν (M - N) Base 276, ν (M - N) 459, ν (M - O) 539, ν (M-S) 317, ν (C - O) 1237, Bands due to HB 1470, ν($H_2O$) 3557,3578.

**TGA analysis data:**
1.[Zn-L.Cl.$(H_2O)_2$]: First step, 112 °C, Mass loss 9.20 % second step, 200 °C, Mass loss, 28.23 % Third Step 350.40 °C, Mass loss, 38.22 % Fourth Step, 650.36 °C, Mass loss 61.5 %, Residue 778 °C, % of ZnO, 22.08 (22.73).

2.[Zn.L.Py.$(H_2O)_2$]:First step, 113 °C, Mass loss 8.28 % second step,220 °C, Mass loss, 28.32 % Third Step 355 °C, Mass loss, 37.02 % Fourth Step, 669.30 °C, Mass loss, 62.01 %, Residue, 779 °C, % of ZnO, 18.12 (18.66).

3.[Zn.α-Pico.$(H_2O)_2$]: First step, 114 °C, Mass loss 8.04 % second step,230 °C, Mass loss, 27.20 % Third Step 366 °C, Mass loss, 38.40 % Fourth Step, 665.15 °C, Mass loss 60.5 %, Residue 778 °C, % of ZnO, 18.70 (18.08).

4.[Zn.L β-Pico.$(H_2O)_2$]:First step, 115 °C, Mass loss 8.07 % second step, 235 °C, Mass loss, 27.25 % Third Step 360 °C, Mass loss, 39.02 % Fourth Step, 666 °C, Mass loss, 63.27 %, Residue, 779 °C, % of ZnO, 18.78 (18.08).

5.[Zn.L.γ-Pico.$(H_2O)_2$]:First step, 114 °C, Mass loss 8.06 % second step, 205.20 °C, Mass loss, 28.25 % Third Step 365 °C, Mass loss, 38.28 % Fourth Step, 669 °C, Mass loss 61.78 %, Residue 800 °C, % of ZnO, 18.88 (18.08).

**Antimicrobial Assay ( Well diffusion method)**
**Table.3 % Activity index of L , Zn (II) complexes and standered**

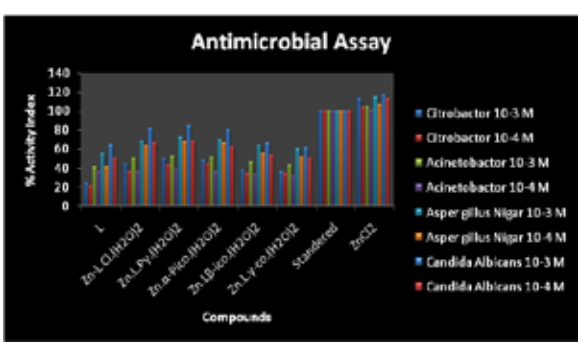
| Compound % Activity Index | Citrobactor | | Acinetobactor | | Asper gillus Nigar | | Candida Albicans | |
|---|---|---|---|---|---|---|---|---|
| | $10^{-3}$ M | $10^{-4}$ M | $10^{-3}$ M | $10^{-4}$ M | $10^{-3}$ M | $10^{-4}$ M | $10^{-3}$ M | $10^{-4}$ M |
| L | 24.10 | 21.30 | 41.12 | 36.30 | 55.62 | 41.31 | 64.67 | 50.03 |
| Zn-L. Cl.$(H_2O)_2$ | 45.10 | 36.17 | 51.10 | 36.62 | 68.23 | 64.22 | 82.32 | 66.11 |
| Zn.L.Py. $(H_2O)_2$ | 49.89 | 42.87 | 53.02 | 38.70 | 72.21 | 67.19 | 84.35 | 68.12 |
| Zn.α-Pi-co.$(H_2O)_2$ | 48.06 | 44.68 | 51.58 | 36.40 | 70.24 | 66.74 | 80.10 | 62.10 |
| Zn.L β-ico. $(H_2O)_2$ | 38.21 | 34.38 | 46.24 | 33.42 | 63.61 | 54.91 | 66.35 | 53.34 |
| Zn.L.γ-co.$(H_2O)_2$ | 36.15 | 34.11 | 43.14 | 32.12 | 60.12 | 52.12 | 61.41 | 51.00 |
| Standered | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| $ZnCl_2$ | 113.5 | 103.57 | 104.35 | 101.32 | 115.22 | 107.08 | 117.33 | 113.23 |

(Standered -Cefodoxine,Fluconazole)

% activity index was calculated by the formula

$$\% \text{ Activity Index} = \frac{\text{Zone of inhibition of test compound}}{\text{Zone of inhibition of standard (diameter)}} x100$$

**Fig 1 Antimicrobial Assay**




**Results and discussion**

Physical data of ligand and its complexes is presented in Table 1. The complex and all adducts are soluble in DMF in which conductivity measurements were made (30$^0$C), showing all complexes are non electrolyte [13]. Elemental analysis data showed that the calculated and experimental percentages are matched and hence confirmed 1:1 mole ratio of metal ion, thiosemicarbazone for complex and 1:1:1 mole ratio for metal, thiosemicarbazone and heterocyclic base for all adducts. The magnetic moments were measured at room temperature (27$^0$C) by Faraday method showed diamagnetic behavior expected for $d^{10}$ configuration. Mass spectral data confirmed the structure of the thiosemicarbazone and complexes as indicated by molecular ion peak (M + 1) corresponding to their molecular weights.UV-visible spectra were measured in DMF (Table 2). The thiosemicarbazone showed π- π* transition band [14-16] at 35,866 $cm^{-1}$ and n- π*transition band[17,18] at 30,892 $cm^{-1}$. The d-d bands are slightly shifted to higher energy side in complexes. The π- π*absorption bands are shifted to higher energy side in complexes due to the weakening of the C=S bond and conjugation system gets enhanced on complexation [19,20].On complexation,there is transfer of lone pair of electrons to the metal.This results in reduction in intensity of n- π* transition and indicate coordination of azomethine nitrogen. MLCT bands in the complexes are observed in the region 25000-26000 $cm^{-1}$ which are assignable to Zn→S transitions.

Coordination of the azomethine nitrogen shifts the ν($^7$C=N$^1$) to the lower side [21]. The apperence of new band in the range 450-460 $cm^{-1}$ confirms the coordination of azomethine nitrogen [22,23]. There is increase in ν($^1$N-$^2$N).This may be due to the increase in double bond character off-sets the loss of electron density via donation to the metal. This confirms the coordination of the ligand through the azomethine nitrogen atom. The band $^2$N-H of thiosemicarbazone disappears in the complexes indicating the deprotonation of the $^2$N-H proton. ν(C=S) band is found at 1360 and 785$cm^{-1}$ in uncomplexed thiosemicarbazone, shifted to lower side in complexes indicating coordination through thiolate sulfur [24]. A new band in the range 315-320 is due to Zn-S.This confirms the coordination through sulfur.The phenolic oxygen occupies the third coordination on loss of OH proton.Hence ν(C-O) band 1290 $cm^{-1}$in uncomplexed thiosemicarbazone is shifted to lower side in the complexes. The apperence of new bands due to Zn-O in complexes in the range 525-540 $cm^{-1}$ confirm coordination through oxygen. The heterocyclic base nitrogen atom occupies fourth coordination site in adducts. The band is assigned for ν (Zn-N) due to heterocyclic base in 275-280 $cm^{-1}$ range in the spectra of all complexes. The characteristics bands of coordinated heterocyclic bases are also observed in IR spectra of all complexes [25-27].

The TGA curves of Zn (II) complex and adducts recorded in the temperature range 30-800$^0$C show the decomposition of complex proceed in four steps. Hydrations of water molecules were lost in between 30-110$^0$C. The two coordinated water molecules were lost in the temperature range 112-115$^0$C.At this stage corresponding mass loss is about 8-9 %.This shows the loss of two water molecules. Up to ~ 200$^0$C no change is observed. There is break in the curves due to evaporation of a molecule of organic ligand, the remaining ligand is removed from the coordination sphere at ~ 600-700$^0$C. Finally the metal oxides were formed above 700$^0$C. The decomposition was complete and metal oxide is formed at ~ 800$^0$C.

Thus Zn (II) complex and adducts are stable up to 200$^0$C and decomposition starts at this temperature. The second step temperatures are in the range of 200-250$^0$C. The third step temperatures are in the range of 300-400$^0$C. The fourth step temperatures are in the range of 600-700$^0$C. The solid residue is ZnO . The complexes prepared with different metals may decompose in steps. It is evaluated that the coordination of metal ion to ligand is responsible for the thermal stabilities of metal complexes [28].

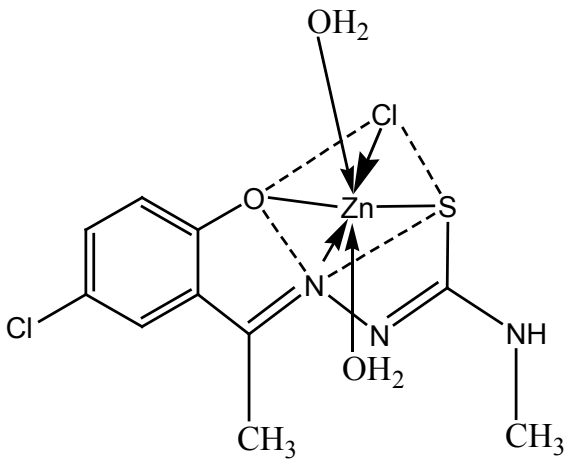
The antibacterial activity was determined using the well diffusion method. The activity was determined by measuring the diameter of the inhibition zone in mm. Biological activity was measured in two different molar concentrations ,$10^{-3}$M, $10^{-4}$ M. The chelate Zn.L.py $(H2O)_2$ showed maximum activity against bacterial and fungal species than free ligand and other complexes. The results of antimicrobial assay are given in Table 3.The thiosemicarbazone was found less active than its zinc complex and adducts. This may be due to chelation which reduces the polarity of the central metal atom because of the partial sharing of its positive charge with donor groups and possible π-electron delocalisation within the whole chelating ring.In Zn.L.α/β/γ-pico $(H_2O)_2$ the avaibility of electron density on nitrogen atom of heterocyclic base is more due to electron donating effect of $–CH_3$ group. As a result of all these factors, the lipophilic nature of the central metal atom increases, which favours the permeation of the complexes through the lipid layer of the cell membrane [29].Thus increase in coordination number in complexes increases antimicrobial activity. Acinetobactor and Citrobactor are gram –ve bacterial species. In gram negative bacteria the outer membrane is thin and they possess outer membrane. So, it might not be easy for the complexes to diffuse inside the bacterial cell. A very good inhibitory activity was also found against the yeast Candida al-

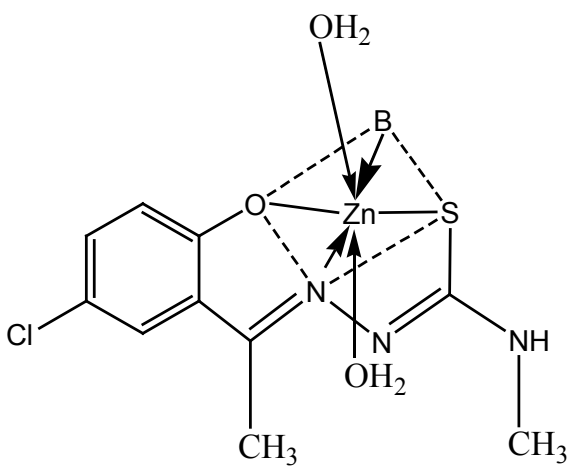
bicans [30]. The zinc chloride salts were found more effective than complexes. The $Zn^{2+}$ ions have small size.This increases the permeability to cell membrane..But use of metal ion solution to treat bacterial and fungal diseases is toxic,so it can be used in coordinated form with ligand. The general sequence of % activity index can be represented as -

Standard > metal salt > complexes > ligand.

The % activity index decreases on dilution ie it is more in concentrated solution.

**Expected structure**




(B = pyridine, α-picoline, β-picoline,γ-picoline)

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