International Journal of Advance Research, IJOAR .org
Volume 1, Issue 9, September 2013, Online: ISSN 2320-9178

SYNTHESIS, CHARACTERISATION AND ANTI TUBERCULOSIS ACTIVITY OF NOVEL TRANSITION METAL COMPLEXES OF HETEROCYCLIC SCHIFF BASES

Ramesh S. Yamgar1 Y.Nivid2 and Sudhir S. Sawant2*

1Department of Chemistry, Chikitsak Samuha's Patkar-Varde College of Arts, Science and Commerce, Goregaon (W), Mumbai 400 062, India.
2 P. G. Dept of Chemistry, Govt. of Maharashtra's Ismail Yusuf College of Arts, Science and Commerce, Jogeshwari (East), Mumbai 400 060 INDIA
**Corresponding author: Tel +91 9963476797 e-mail: sawantsudhir@hotmail.com*

ABSTRACT

Novel transition metal Zn(II) complexes of bidentate Schiff base ligands were obtained from 4-Methyl 7-hydroxy 8-formyl coumarin, and Dimethylamino propylene diamine and N-methylamino propylene diamine . Zn(II) complexes were also prepared by condensing with schiffs base of Ethyl 2-(3-formyl-4-hydroxyphenyl)-4-ethyl-1,3-thiazole-5- carboxylate with Dimethylamino propylene diamine .

2-Butyl-5-amino-1-benzofuran, 4-(4-aminobenzyl)-1,3-oxazolidine-2-one and 4-(1H-1,2,4-triazol-1-yl methyl) aniline were synthesized by known litrature procedures and Schiff bases were prepared by condensation with salicyaldehyde and Zn(II) complexes were prepared subsequently by template method. All these Schiff bases and their Zn(II) metal complexes were characterized by physicochemicalal and spectral analytical methods. The synthesized Schiff bases act as bidentate ligand for the complexation reaction with Zn(II) ions. The new compounds, possess general formula $[Zn(L)_2.2H2O]$ and octahedral geometry is proposed. In order to evaluate the effect of metal ions upon chelation, the Schiff base and their metal complexes have been screened for antituberculosis activity against M.Tuberculosis. The transition metal complexes have shown moderate antimycobacterium activities as compared to standard Pyrazinamide and Streptomycin.

Key Words:

Heterocyclic Schiff bases, transition metal complexes, 4-Methyl 7-hydroxy 8-formyl coumarin, Ethyl 2-(3-formyl-4-hydroxyphenyl)-4-ethyl-1,3-thiazole-5- carboxylate , 2-Butyl-5-amino-1-benzofuran, 4-(4-aminobenzyl)-1,3-oxazolidine-2-one, 4-(1H-1,2,4-triazol-1-yl methyl) aniline, M. Tuberculosis.

INTRODUCTION

Tuberculosis, caused by Mycobacterium tuberculosis, is one among the major infectious diseases and leading cause of mortality globally. Two million people die each year worldwide of which half a million are from India. The emergence of multi-drug resistant (MDR)TB, extensively drug-resistant (XDR) TB and HIV co-infection have exacerbated the global scenario of the disease. The development of new drugs that can act against MDR and XDR TB and / or the one that will shorten the chemotherapy are the priority in TB research.

The success story of the clinical uses of cisplatin, cis-[PtII(NH3)2Cl2] and carboplatin has stimulated considerable interest in using other transition metal complexes as new therapeutic agents[1]. This perspective leads us to think and explore further research work on several classes of transition metal complexes for treatment of anti-cancer, anti-HIV treatments and various other therapeutic biological models.

The uses of metal complexes as therapeutic agents can be traced back to 3500 BC.[2] Almost 5000 years ago, copper was used by the Egyptians to sterilize water. Over the past several decades, various antimony complexes were used for treatment of protozoan diseases like leishmaniasis and Trypanosomiasis. Medicinal inorganic chemistry as a discipline, however, started to develop after the serendipitous discovery of the anti-tumor activity of cisplatin.[3] The success of the clinical applications of this platinum compl*uberculosis*ex has stimulated considerable interest in searching for new metal complexes as modern therapeutics, diagnostic and radiopharmaceutical agents, for example, silver(I) complexes commonly used as anti-microbial agents, bismuth(III) complexes for anti-ulcer treatments, gold(I) complexes as anti-arthritic agents, gadolinium(III), manganese(II) and iron(III) complexes as magnetic resonance imaging (MRI) contrast agents, technetium (99Tc) and scandium (47Sc) as radiopharmaceutical agents.[4]

Ever increasing and rapid spread of drug-resistant parasites has reduced the effectiveness of conventional medicines and hence, newer effective drug substances are required by mankind to overcome the ever increasing health challenges. Genetic mutations in micro organisms and drug resistant infections like MDR and XDR TB, pose global threat. Hence there is an urgent need for newer, more effective, less toxic drugs, affordable medicines and drugs against resistant infections and unstoppable cancers.

Transitional metal in combination with organic compounds lead to various possibilities towards developing cost effective and safe medicines for various therapeutic catagories. It is well known that Schiffs bases form coordination complexes with transition metal to get stable compounds having biological activities. Heterocyclic compounds having nitrogen, oxygen or sulphur atom incorporated in carbocylic ring system possesses excellent biological activity due to in built pharmacophores.

New Drug Discovery for treatment of infectious diseases and cancer which could provide ultimate cure is an important area of research. Although cost prohibitive (~USD 500bn) with a meager success rate (~1%), the search for an ideal drug which could revolutionize therapy of infectious diseases and cancer continues unabated.

In order to begin our efforts for such new medicines as effective anti tubercoulosis agents against M.Tuberculosis, we thought of combining heterocyclic aniline scaffold with simple ortho hydroxy benzaldehydes like salicylaldehyde to get a Schiff base and its conversion to Zn (II) metal complex. Recent literature survey for transition metal complexes as anti tuberculosis agents in this field gave few references.[5-8] indicating possibility of getting new lead molecules in this field. Novel anti-TB drugs, which are safe, able to shorten the course of treatment, effective against drug-resistant strains and latent TB infection, are urgently needed, especially in the era of MDR- and XDR-TB.

EXPERIMENTAL

Material and Methods

All chemicals and solvents used were of AR grade. IR spectra were recorded on a Shimadzu FTIR Spectrophotometer. The proton magnetic spectra were recorded on a Varian 300 MHz Spectrometer using DMSO-d6 as solvent and TMS as internal standard. Mass spectrophotometer was API 4000 Triple quadrupole mass spectrometer AB Sciex instruments.

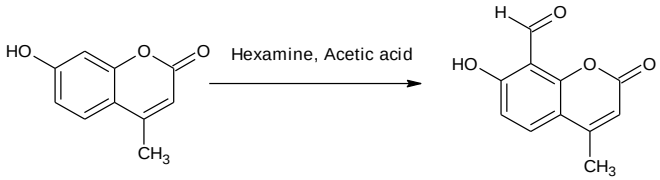
Our approach towards synthesis of transition metal complexes:

We synthesised substituted heterocyclic orthohydroxy benzaldehydes [Scheme 1 and Scheme 2] and substituted heterocyclic aniline compounds [Scheme 3 and Scheme 5]. These anilines were condensed with orthohydroxy benzaldehydes to form "in situ" heterocyclic schiff base ligands. Zinc(II) metal complexes were synthesised by template method and isolated. They were purified by recrystallisation in ethanol.

Synthesis of orthohydroxy heterocyclic aldehydes

Synthesis of 7-hydroxy-4-methyl-2-oxo-2H-chromene-8-carbaldehyde:

Scheme 1



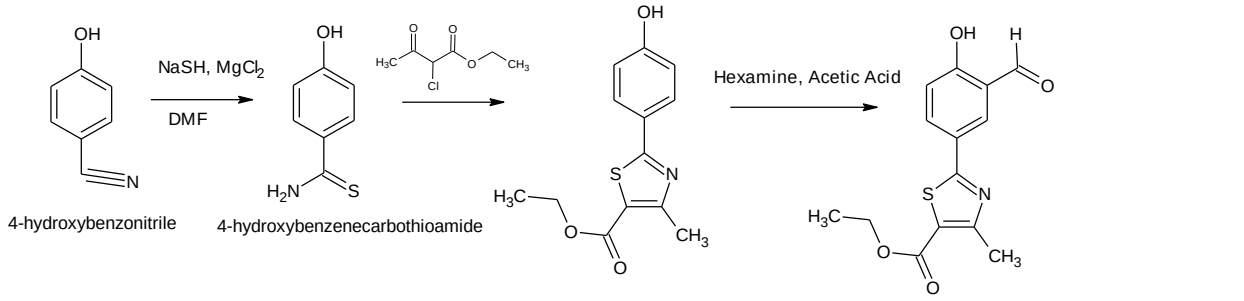
7-hydroxy-4-methyl-2-oxo-2*H*-chromene-8-carbaldehyde

4-Methyl 7-hydroxy coumarin was prepared by the reported method[9] starting with resorcinol. Duff formylation with slight modification, using hexamine and glacial acetic acid gave 7-hydroxy-4-methyl-2-oxo-2H-chromene-8-carbaldehyde .[10,11,12]

4-Methyl-7-hydroxy coumarin (30g, 0.170 moles) was dissolved in 300 ml glacial acetic acid. Hexamine(60 g, 0.428 moles) was added and heated to 85-90oC for 5 hours. Reaction was monitored for its progress by TLC (30% Ethyl acetate in hexane). After reaction was completed as indicated by TLC, reaction mixture was quenched in 20% HCl and heated to 60-80oC for 20 minutes. Reaction mixture was cooled to room temperature and product was extracted in methylene chloride(100 ml x 3 times). Combined MDC extract was washed with distilled water and dried over anhydrous Na2SO4. MDC extract was concentrated and crude product was purified by silica gel column chromatography to get pure 7-hydroxy -4-methyl- 8-formyl coumarin, as pale yellow coloured solid(~7g). Yield: 20%, M. P. 177°C, 1H NMR in DMSO-d6 [300MHz] 2.43(s, 3H), 6.2(s, 1H), 6.87(d, 1H, J=8.1 Hz),6.91(d, 1H, J=8.1 HZ), 10.59(s, 1H), 12.19(s, 1H, exchangeable with D2O).

Synthesis of Ethyl 2-(3-formyl-4-hydroxyphenyl)-4-ethyl-1,3-thiazole-5- carboxylate

[Scheme 2]



Ethyl 2-(3-formyl-4-hydroxyphenyl)-4-ethyl-1,3-thiazole-5- carboxylate [Scheme 2] was obtained by three step synthesis starting with 4-hydroxybenzonitrile as per the methods reported in literature. [13-14]. The starting material 4-hydroxybenzonitrile was converted into 4-hydroxybenzenecarbothioamide and cyclised to form five membered heterocyclic thiazole ring using 2-Chloroethylacetoacetate to obtain ethyl 2-(4-hydroxyphenyl)-4-methyl-1,3-thiazole-5-carboxylate. This was furhter formylated using Duff formylation method [15] to obtain Ethyl 2-(3-formyl-4-hydroxyphenyl)-4-ethyl-1,3-thiazole-5- carboxylate.

Preparation of 4-hydroxybenzenecarbothiomide [16]

4-Hydroxy- benzonitrile (25g, 0.209 moles) was treated with sodium hydrogen sulfide (17.6 g, 0.314 moles) and anhydrous magnesium chloride(30.0g, 0.314 moles) in dimethyl formamide 150ml at 50°C for 4 hours to give 4-hydroxy benezenecarbothioamide precipitate. The product was filtered washed with water to remove inorganic salts and then washed with isopropyl alcohol 10 ml x 2 times and dried in vacuum oven. Wt: 28 g (Yield: 88%)

Preparation of Ethyl 2-(4-hydroxy phenyl)-4-methyl-1,3 thiazole-5-carboxylate[17]

4-Hydroxybenzenecarbothiomide (25 g, 0.16 moles) was suspended in 125 ml isopropyl alcohol and then heated to 50°C for 15 minutes. 2-Chloroethylacetoacetate (30.0 g , 0.18 moles) was added dropwise into above solution at 50°-55°C over 45 minutes. After addition of 2-chloroethylacetoacetate reaction mixture was heated to 80-85°C for 3 hours. Reaction was monitored by TLC (10%Methanol/ ethyl acetate) for absence of starting material. After completion of reaction as indicated by TLC, reaction mixture was cooled to room temperature and then to 10-12°C in ice water mixture. Product precipitate was filtered and washed with cold isopropyl alcohol 10 ml x 2 times, suck dried and then dried in vacuum oven till constant weight. Wt: 39.0 g (Yield:92.5%)

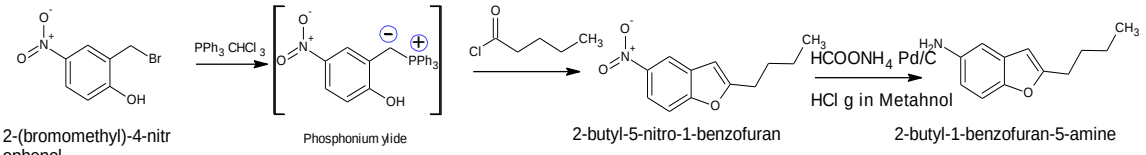
Preparation of Ethyl 2-(3-formyl-4-hydroxyphenyl)-4-ethyl-1,3-thiazole-5- carboxylate [17]

Ethyl 2-(4-hydroxy phenyl)-4-methyl-1,3 thiazole-5-carboxylate (39.0g, 0.148 moles) was suspended in 200ml glacial acetic acid and hexamine (52 g, 0.371 moles) was added neat at room temperature. Reaction mass was then heated to 85-90oC for 7 hours. Reaction was monitored for its progress by TLC (30% Ethyl acetate in hexane). After reaction was completed as indicated by TLC, Reaction mixture was quenched in 20% HCl and heated to 60-80°C for 20 minutes. Reaction mixture was cooled to room temperature and product was extracted in methylene chloride(100 ml x 3 times). Combined MDC extract was washed with distilled water and dried over anhydrous Na2SO4. MDC extract was concentrated and crude product was purified by Silica gel column chromatography to get pure Ethyl 2-(3-formyl-4-hydroxyphenyl)-4-ethyl-1,3-thiazole-5- carboxylate wt: 15g,(Yield: 35%). M.P. 107-108 oC, 1H NMR [DMSO-d6 300 MHz] 1.29-1.31(t, 3H), 2.61(s, 3H), 4.33(q, 2H), 6.743(bs, 1H), 7.79 (dd, 1H, J=8 Hz), 7.84 (bs, 1H), 9.11(s, 1H), 13.5(s, 1H, free OH exchangeable with D2O), MS: [M+2H] : 293

Synthesis of heterocyclic anilines

Synthesis of 2-Butyl-5-amino-1-benzofuran [Scheme 3]

Scheme 3



2-Butyl-5-amino-1-benzofuran [Scheme 3] was synthesized starting with 2-(bromomethyl)-4-nitrophenol in three steps as reported in literature.[18,19] The first step was formation of Phosphonium ylide and the Wittig reaction with Pentanoyl chloride gave 2-butyl-5-nitro-1-benzofuran. Transfer hydrogenation using ammonium formate and Palladium carbon gave desired amino benzofuran compound.

Preparation of 2-Hydroxy-5-Nitro benzyltriphenylphosphonium bromide[20]

2-(bromomethyl)-4-nitro phenol (50 g, 0.215 moles) and triphenylphosphine (56.5 g, 0.215 moles) are heated at 80°C-90°C in Toluene (500 l) for 1 hour. The reaction mixture is cooled and the offwhite precipitate is filtered on Buchner funnel and washed with Toluene (50 ml x 2 times. Product is dried in vacuum oven till constant weight. Wt: 62.5g (Yield:59%)

Preparation of 2-Butyl-5-nitro-1-benzofuran[20]

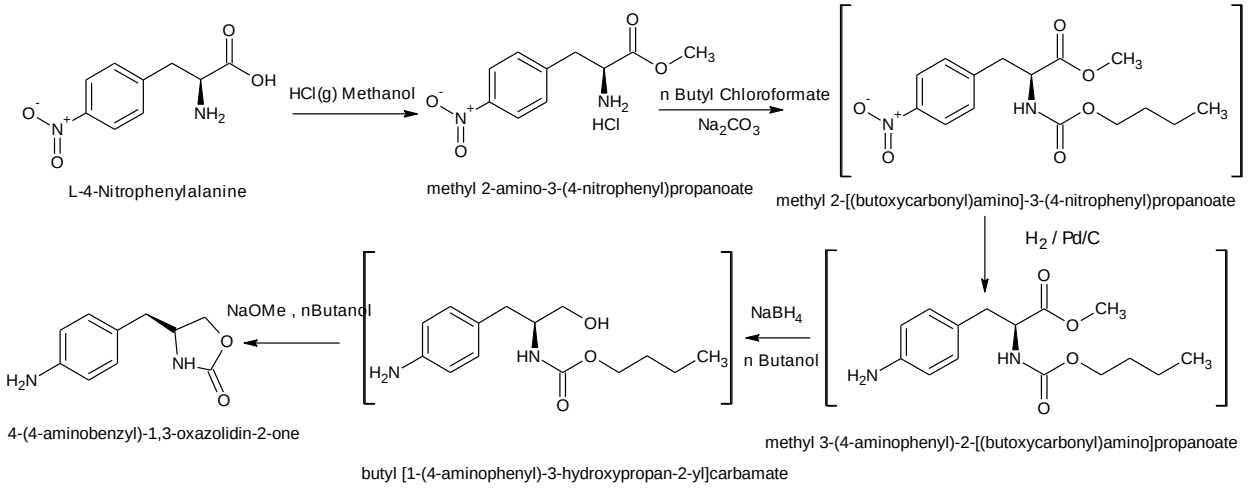
2-Hydroxy 5 nitrobenzylphosphonium bromide (62.0 g, 0.125 mole) dissolved in chloroform (380 ml) and Pyridine (20 ml, 0.253 mole) was added at room temperature. Pentanoyl chloride (19.6 g, 0.163 mole)was added neat over 45 minutes. Reaction mixture is heated at reflux for about 2 hours. Reaction mixture was cooled to room temperature and TLC Toluene (760 ml) was added and about 380 ml of solvents are removed on rotavapor under vaccum. Triethylamine (38.4 g. 0.38 moles) was added to the concentrated mass and reaction mixture is again heated at reflux for 4 hours. It was cooled and the triphenylphosphine oxide is filtered on Buchner funnel, washed with dichloromethane and the filtrate and washings were concentrated on rotavapor. The oily residue is triturated with pentane (100 ml x 2)and then dissolved in Dichloromethane(200 ml) and passed over a Silica bed to remove polar impurities. Silica bed washed with fresh dichloromethane 100 ml x 2 and all eluates were concentrated to get oily liquid. Wt: 16.5 g (Yield:60.0%)

Preparation of 2-Butyl-5-amino-1-benzofuran [21]

2-Butyl-5-nitro-1-benzofuran (16.5 g, 0.075 moles) was dissolved in 70 ml ethanol in reaction flask. Ammonium formate (24.5 g) and 5% Palladium carbon catalyst (1.6 g) was added. The reaction mixture was stirred and heated at 50-55°C for 6 hours and reaction completion was confirmed by TLC (10%Methanol in Chloroform). After complete conversion of starting material into product, reaction mixture was cooled to room temperature and catalyst is filtered over celite bed. Celite bed was washed with ethanol (20 ml x 2 times) and filtrate partially concentrated upto 40 ml volume. It was cooled to 5°C and HCl gas was purged in with stirring till pH of reaction mass was about 1. Grey coloured precipitate was filtered and washed with cold ethanol and dried in vaccum oven at 60°C till constant weight. Wt:16.5 g, Yield: 97%, MS : [M+1]: 190, 1H NMR:[300MHz, DMSO-d6] 0.97(m, 3H), 1.35(m, 2H), 1.73(m, 2H), 2.70(m, 2H), 6.66(s, 1H), 6.94 -6.96(m, 1H), 7.07(d, 1H, J=7.2Hz), 7.39(d 1H, J= 7.2Hz),

Synthesis of 4-(4-aminobenzyl)-1,3-oxazolidine-2-one [Scheme 4]

Scheme 4



As mentioned in literature method [22],L-4-nitrophenylalanine [Scheme 4] was esterified with methanol under anhydrous acidic condition. The methyl ester was then treated with n Butyl chloroformate to get N-BOC protected intermediate, which was catalytically hydrogenated to get corresponding aniline intermediate. Reduction of ester function using sodium borohydride gave alcohol and finally the cyclisation using sodium methoxide gave desired 4-(4-aminobenzyl)-1,3-oxazolidine-2-one .

Preparation of Methyl-2-amino-3(4-nitrophenyl) propanoate hydrochloride:

To the cooled methanol (500 ml) at 5-10°C ,hydrogen chloride gas was bubbled for 1 hr, strength of HCl in methanol by titration was about 15% wt/vol. 4-nitro-(L)-phenylalanine (50 g) was added and refluxed for about 2 hours. Reaction mixture was cooled to about 0°C and product was filtered on Buchner funnel under nitrogen, washed with cold methanol 20 ml x 2 times and dried in vacuum oven at 50°C till constant weight. Wt: 56g (Yield: 90.0%)

Preparation of Methyl 2-[butoxycarbonyl)amino]-3-(4-nitrophenyl) propanoate:

Methyl2-amino-3(4-nitrophenyl) propanoate hydrochloride(56.0g, 0.214 moles) was charged in 125 ml distilled water. Sodium carbonate(25.0g, 0.235 moles) was added, followed by 310 ml of ethylacetate . Reaction mixture was cooled to 20°C under stirring and n-butyl chloroformate (29.62 g, 0.217 moles) was added dropwise over 40 minutes at 20-30°C. Reaction mass was further stirred for 1 hour and monitored by TLC (10% Methanol in Chloroform). After completion of reaction layers were separated and ethyl acetate layer was washed with water 30 ml x 2 times. The ethyl acetate solution of Methyl 2-[butoxycarbonyl)amino]-3-(4-nitrophenyl) propanoate was used directly at the further step.

Preparation of Methyl 3-(4-aminophenyl)-2-[(butoxycarbonyl)amino]propanoate:

To Parr hydrogenation apparatus 5%Pd/C catalyst was charged and above ethyl acetate solution of Methyl 2-[butoxycarbonyl)amino]-3-(4-nitrophenyl) propanoate was charged. Temperature was raised to 30°C and Hydrogen pressure of 20-30 psi was applied. Reaction continued till uptake of hydrogen gas ceased. Completion of reaction was confirmed by TLC (10% Methanol in Chloroform). Catalyst was filtered over celite bed and clear filtrate was concentrated on rotary evaporator. N-Butanol 100 ml was added to the concentrated mass and stripped off. Reaction mass was degassed for 30 minutes and fresh n-Butanol 375 ml was added. The n-Butanol solution of Methyl 3-(4-aminophenyl)-2-[(butoxycarbonyl)amino]propanoate was used directly at the further step.

Preparation of Butyl [1-(4-aminophenyl)-3-Hydroxypropane-2-yl]carbamate

To clean reaction flask, above n-Butanol solution of Methyl 3-(4-aminophenyl)-2-[(butoxycarbonyl)amino]propanoate was charged under nitrogen atmosphere at 25°C.Sodium borohydride (4 g) was added and stirred for 3 hours under nitrogen atmosphere and remaining sodium borohydride was added and further stirred for 6 hours. Reaction mixture was warmed to 35°C and stirred overnight at this temperature. TLC was done to confirm the completion of reaction. Reaction was quenched with concentrated HCl (25 ml) slowly maintaining temperature at 30-35°C. Frothing and effervescence were observed due to decomposition of sodium borohydride with HCl. Distilled water 100 ml was added and pH of reaction mass was adjusted to about 10 using concentrated ammonia. Layers were separated and half the quantity of n- Butanol was recovered under vaccum in ratavapor. Fresh n-Butanol 100 ml was added and stripped off. This azeotropically dried n-Butanol solution of Butyl [1-(4-aminophenyl)-3-Hydroxypropane-2-yl]carbamate was used directly in the next step.

Preparation of 4-(4-aminobenzyl)-1,3-oxazolidine-2-one

Above n-Butanol solution of Butyl [1-(4-aminophenyl)-3-Hydroxypropane-2-yl]carbamate was charged to the clean and dry reaction flask and heated to 80°C with slow addition of sodium methoxide(2.91 g, 0.054 moles). Reaction mixture was stirred for further 30 minutes and activated charcoal(2.0 g) was added at 80°C. It was filtered while hot through celite bed. Filtrate was cooled to 5-8°C with stirring for 10 Hours and product was filtered on Buchner funnel, washed with cold nButanol 15 ml x 2 and dried in vacuum oven at 50°C till constant weight. Wt:24.6 g (Yield:60%), MS [M]+ 189, Melting Point: 110°C, 1H NMR (300 MHz, DMSO-d6): 2.49(m, 2H), 3.82(m, 2H), 4.21(m, 1H), 4.92(s, 2H, exchangeable with D2O), 6.48(d, 2H), 6.85(d, 2H), 7.73(s, 1H, N-H oxazolidine), 13C NMR(75MHz, DMSO-d6): 39.49, 52.72, 68.92, 113.92[2C],123.03, 129.69[2C], 147.03, 158.54 [oxazolidone carbonyl carbon]

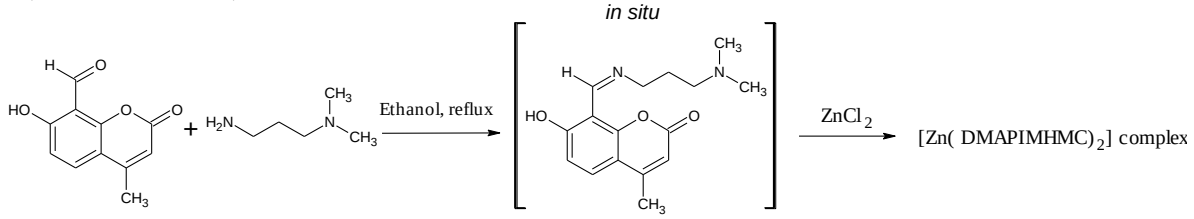
Synthesis of novel heterocyclic Schiff bases *"in situ"* and metal complexes by template method

Schiffs bases were prepared by condensing equimolar quantities of heterocyclic aldehydes and aliphatic amines as shown in Scheme 5. Schiffs bases were not isolated, but were converted "in situ" to Zinc(II) complexes by refluxing with ZnCl2.2H2O by template method [23]

Preparation of *"in situ"* Schiff base Ethyl- 2-{ 4-hydroxy-3-[(Z)-(dimethylamino)propyl]imino}methyl]--4-methyl-1,3-thiazole-5-carboxylate [DMAPIMMTC] and complex $[Zn(DMAPIMMTC)_2].2H_2O$

Ethyl 2-(3-formyl-4-hydroxyphenyl)-4-methyl-1,3-thiazole-5-carboxylate (0.5g, 0.00171 mol) was taken in ethanol (10 ml) and N,N-dimethylpropane-1,3-diamine (0.175g, 0.00171 mol) was added and stirred for an hour at room temperature. A drop of dil. HCl was added and the mixture was refluxed on a water bath for about an hour. The colour of solution was pale yellow. To this hot solution, zinc chloride (0.233g. 0.00171 mol) was added. The solution was refluxed for additional three hours and TLC was checked for completion of reaction. The pale yellow precipitate formed was filtered and washed with ethanol. Product was recrystallised in ethanol at reflux, filtered and dried in oven at 70-80oC till constant weight. (Yield: 0.810 g, 55.86%) , M.P. 220°C, [M]+ 813.7, 1H NMR (DMSO-d6, 300MHz) 1.297-1.317 (t, 3H), 2.046(m, 2H), 2.605-2.65(s, 6H), 2.907(m, 2H), 3.3(s, 3H), 3.66-3.679(t, 2H), 4.273-4.303(q, 2H), 6.743(bs, 1H), 7.843 (bs, 1H), 8.013(bs, 1H), 9.322(s, 1H azomethine)

Preparation of *"in situ"* Schiff base 8-[(*Z*)-{[3-(dimethylamino)propyl]imino}methyl]-7-hydroxy-4-methyl-2*H*-chromen-2-one [DMAPIMHMC] and complex $[Zn(DMAPIMHMC)_2].2H_2O$ [Scheme 5]



7-hydroxy-4-methyl-2-oxo-2*H*-chromene-8-carbaldehyde

8-[(*Z*)-{[3-(dimethylamino)propyl]imino}methyl]-7-hydroxy-4-methyl-2*H*-chromen-2-one [DMAPIMHMC]

Scheme 5 Preparation of $[Zn(DMAPIMHMC)_2].2H_2O$ complex by template method

The preparation of the [Zn(DMAPIMHMC)] complex was carried out by taking 7-hydroxy-4-methyl-2-oxo-2H-chromene-8-carbaldehyde (1.5g, 0.00735 mol) in ethanol (30 ml) and N,N-dimethylpropane-1,3-diamine (0.750g, 0.00735 mol). A drop of dil. HCl was added and the mixture was refluxed on a water bath for about an hour. The colour of solution was pale yellow. To this hot solution, zinc chloride (1.06g. 0.00735 mol) was added. The solution was refluxed for additional three hours and TLC was checked for completion of reaction. The pale yellow precipitate formed was filtered and washed with ethanol. Product was recrystallised in ethanol at reflux, filtered and dried in oven at 70-80oC till constant weight. Yield: 1.8 g, 41% , [M+1] 640, 1H NMR (DMSO-d6, 300MHz) 1.82(t, 2H, J=6.5Hz), 2.40(s, 3H), 2.45(s, 3H), 2.63(s, 3H), 2.93(t, 2H, J=6.4Hz)), 3.84(t, 2H, J =7.8Hz), 6.09(s, 1H), 6.67(d, 2H, J=8.8Hz), 7.67(d,J=9.2Hz) 8.93(s, 1H azomethine).

Preparation of *"in situ"* Schiff base 7-hydroxy-4-methyl-8-[(*Z*)-{[3-(methylamino)propyl]imino}methyl]-2*H*-chromen-2-one[NMAPIMHMC] and complex $[Zn(NMAPIMHMC)_2].2H_2O$

7-hydroxy-4-methyl-2-oxo-2H-chromene-8-carbaldehyde (1.5 g, 0.00735 mol) was dissolved in ethanol (15 ml). N-methylpropane-1,3-diamine (0.646 mol, 0.00735 mol) was added and stirred at room temperature for 15 minutes. Reaction mixture was heated to reflux and zinc chloride (1.0 g, 0.00735 moles) was added and heated for further for 4 hours. Reaction mass was cooled and pale yellow precipitate was filtered and washed with ethanol. Product was recrystalised in ethanol and dried in oven at 70-80oC till constant weight.(Yield: 2.0 g, 42.0%), M.P. >260°C, [M]+ 612, 1H NMR (DMSO-d6, 300MHz) 2.06(m, 2H), 2.34(s,3H), 2.96(t, 2H), 3.73(t, 2H), 5.98(s, 1H), 6.56(d, 2H, J=8.4Hz), 7.56(d, J=8.4Hz) 8.83(s, 1H azomethine).

Preparation of *"in situ"* Schiff base (4*S*)-4-{4-[(*E*)-(2-hydroxybenzylidene)amino]benzyl}-1,3-oxazolidin-2-one [HBABO] and complex $[Zn(HBABO)_2].2H_2O$

(4S)-4-(4-aminobenzyl)-1,3-oxazolidin-2-one(0.5 g, 0.0026 mol) and salicylaldehyde (0.317g, 0.0026 mol) were mixed in ethanol (10 ml). Colour of reaction mass changed to yellow and precipitate of Schiff base was observed after 5-10 minutes. Reaction mass was stirred at room temperature for 45 minutes and then heated to reflux. To this was added zinc chloride (0.354 g, 0.0026 mol) and further stirred for 3 hours. RM cooled to room temperature and greenish yellow precipitate was filtered. It was recrystalised in ethanol and dried in oven at 70-80oC till constant weight.(Yield: 0.800 g, 44.4%), M.P. 240°C, [M+1] 655.3

1H NMR (DMSO-d6, 300MHz) 2.47-2.81(m, 2H), 3.97-4.06(m, 2H), 4.23-4.28(m, 1H), 6.92-6.98(m, 2H), 7.33-7.38(m, 4H), 7.61(d, 2H, J= 7.2Hz), 7.81(s , 1H N-H oxazolidinone), 8.94(bs, 1H azomethine)

Preparation of *"in situ"* Schiff base 2-[(*E*)-({4-[2-(dimethylamino)ethoxy]benzyl}imino)methyl]phenol [DMAEBIMP] and complex $Zn(DMAEBIMP)_2].2H_2O$

2-[4-(aminomethyl)phenoxy]-N,N-dimethylethanamine (0.5g, 0.00257 mol) and Salicylaldehyde (0.313g, 0.00257 mol) was dissolved in ethanol (10 ml) and drop of conc. HCl was added. Reaction mass was heated to reflux for 30 minutes and zinc chloride (0.350 g, 0.00257 mol) was added and reaction mixture was heated at reflux for 3 hours. Reaction mass was cooled pale yellow solid was filtered, washed with ethanol and recrystalised in ethanol. It was dried in overn at 70-80oC till constant weight.(Yield: 0.960 g, 53.70%), M.P. 240°C, [M+1] 659.3,

1H NMR (DMSO-d6, 300MHz) 2.50(s, 6H), 2.66(t, 2H), 3.42(s, 2H), 4.27(t, 2H), 7.09(d,2H, J=8.7Hz), 7.37(m, 4H), 7.82(d 2H J=8.7), 9.12(s, 1H azomethine).

Preparation of *"in situ"* Schiff base 2-{(*E*)-[(2-butyl-1-benzofuran-5-yl)imino]methyl}phenol [BBFIMP] and complex $[Zn(BBFIMP)_2].2H_2O$

2-butyl-1-benzofuran-5-amine hydrochloride (0.5g, 0.00221 mol) was suspended in ethanol (10 ml) and sodium acetate (0.181 g, 0.00221 mol) was added. A clear dark solution was observed. Salicylaldehyde (0.269g, 0.00221 mol) was added. Reaction mass colour changed to brownish yellow. It was heated to reflux and zinc chloride (0.301 g, 0.00221 mol) and further heated at reflux for 3 hours. Dark greening brown precipitate of the product was filtered and washed with water to remove sodium chloride. It was recrystalised in ethanol and dried in oven at 70-80oC till constant weight.(Yield: 0.350 g, 23.08%), M.P. 163-164°C, [M]+ 650, 1H NMR (DMSO-d6, 300MHz) 0.95(m, 3H), 1.33(m, 2H), 1.72(m, 2H), 2.71(m, 2H), 6.67(s, 1H), 6.94 -6.98(m, 2H), 7.08(d, 1H, J=7.2Hz), 7.35-7.39(m , 2H), 7.41(d 1H, J= 7.2Hz), 7.65(s, 1H), 9.17(s, 1H, azomethine)

RESULTS AND DISCUSSION

All of the synthesized metal complexes were air and moisture stable. The complexes are light yellow colored, which may decompose above 2500C. They are insoluble in common organic solvents such as chloroform or acetone, slightly soluble in ethanol and methanol, but completely soluble in DMSO and DMF solvent.

^{1}H and ^{13}C nuclear magnetic resonance spectra

Due to its diamagnetic nature of Zn (II) metal complexes, it was possible to scan 1H NMR spectrum in DMSO-d6 using TMS as internal reference standard.

It was observed that the azomethine proton in Zn(DMAPIMMTC)2.2H2O complex appeared at 9.322 ppm after complexation with Zinc metal. It was shifted significantly downfield due to deshielding effect exerted by Zinc metal atom. The aromatic protons are appearing quiet downfield at 8.48, 8.01 and 7.8 compared to that

appearing in starting material from 6.74 to 7.84 ppm, this is also due to electron withdrawing effect of central metal atom.

In 13C NMR spectrum of Zn(DMAPIMMTC)2.2H2O complex, it is observed that azomethine -HN=C- group carbon atom appeared at 169.25ppm, which is in line with literature values reported so far. Carbonyl carbon observed at 161.487 ppm and the carbon attached to phenolic –OH group appeared at 160.084ppm

In Zn(DMAPIMHMC)2.2H2O complex , the azomethine proton observed at 8.93 ppm after complexation with Zinc metal atom. The aromatic protons were observed in the range of 6.743 - 8.013 ppm. Aromatic protons of coumarin ring were observed at 6.67 ppm and 7.67 ppm due to the electron withdrawing mesomeric effect exerted by Zinc metal atom. Olefinic proton of coumarin ring was shifted down field to 6.09 ppm due to electron withdrawing mesomeric effect operating through the conjugation across the aromatic ring over the α,β-unsaturated double bond of coumarin ring.
In Zn(NMAPIMHMC)2.2H2O complex , the azomethine proton observed at 8.83 ppm after complexation with Zinc metal atom. Aromatic protons of coumarin ring were observed at 6.56 ppm and 7.56 ppm. Olefinic proton of coumarin ring was observed at 5.98 ppm.

In Zn(HBABO)2.2H2O complex , the azomethine proton was observed at 8.94 ppm. In Zn(DMAEBIMP)2.2H2O complex, the azomethine proton appeared at 9.12 ppm and in Zn(BBFIMP)2.2H2O complex the azomethine proton was observed at 9.17 ppm.

Mass Spectra

A rapid, simple and sensitive MS infusion method on tandem mass spectrometry with an electrospray ionization (ESI) source for the simultaneous analysis of Schiffs base metal complexes was employed and detection was performed with a triple-quadrupole mass spectrometer in MS and MSMS Scan mode. A high-resolution mass spectrometer, API 4000 Triple quadrupole mass spectrometer (AB Sciex instruments, Canada) was used for the ionization and fragmentation study of metal complexes. This instrument consists of a quadrupole mass analyzer followed by Qtrap.
High resolution Mass spectrum of Zn(DMAPIMMTC)2.2H2O showed molecular ion peak at [M]+ 813.7, thus confirming formation of metal complex. Similarly mass spectrum of Zn(DMAPIMHMC)2.2H2O showed peak [M+1] + peak at 640 confirming the Zinc metal complexes and 1:2 stiochiometry of Metal to schiff base. Mass spectrum of Zn(NMAPIMHMC)2.2H2O complex showed [M+1]+ peak at 612 confirming the formation of zinc metal complex. Mass spectra of Zn(DMAEBIMP)2.2H2O complex and Zn(BBFIMP)2.2H2O complex showed [M+1]+ peak at 659.3 and [M]+ peak at 650. In summary, the formation of all the metal complexes and their stiochiometry with ligand was confirmed by high resolution mass spectra.

IR spectra

In IR spectra of the metal complexes, appearance of a strong new band in the region 1610 cm-1 to 1644 cm-1 was assigned[24] to the azomethine, γ(C=N) linkage. It suggested that amino and aldehyde moieties of the starting materials are absent and have been converted into the azomethine moiety. It also suggested participation of the azomethine nitrogen in the complexation [Table 1] and indicated that the Schiff base was principally coordinated to the metal atom.

Complex	Lattice water ν (OH) cm^{-1}	ν C=N cm^{-1}	Phenolic C-O cm^{-1}	ν M-N cm^{-1}	ν M-O cm^{-1}
$[Zn(DMAPIMMTC)_2].2H_2O$	3451	1639	1365	549	447
$[Zn(DMAPIMHMC)_2].2H_2O$	3439	1630	1371	554	455
$[Zn(NMAPIMHMC)_2].2H_2O$	3371	1610	1368	545	450
$[Zn(HBABO)_2].2H_2O$	3362	1644	1371	530	453
$[Zn(DMAEBIMP)_2].2H_2O$	3471	1625	1409	545	462
$[Zn(BBFIMP)_2].2H_2O$	3470	1639	1465	503	462

Table 1 Infrared frequencies of Zinc metal complexes

A band appearing at 3362 cm-1 -3474 cm-1 in metal complexes was due to stretching modes of coordinated water molecule. This was significantly different than characteristic γOH stretching vibration.

Further conclusive evidence of the coordination of these Schiff base compounds with the metals, was shown by the appearance of weak low frequency new bands at 503-554 cm-1 and 447-462 cm-1. These were assigned [25-27] to the metal-nitrogen (M-N) stretching vibration and metal-oxygen (M-O) stretching vibration respectively. These new bands were observed in the spectra of the metal complexes and not in the spectra of its Schiff base compounds thus confirming participation of these hetero groups (O or N) in the coordination.

Thermo gravimetric analysis

The thermo gravimetric analysis of the complexes shows that they are thermally stable to a varying degree. The complexes show a gradual but significant loss in weight up to 100-120°C indicating loss of water of crystallization. The thermal analysis clearly indicates the presence of coordinated water molecule with the central metal ion. The metal complexes initially show the loss of coordinated water in the temperature range

150-230°C (Table 2). With further increase in temperature investigated, the complexes show decomposition and thermal degradation of the organic part of the metal complexes.

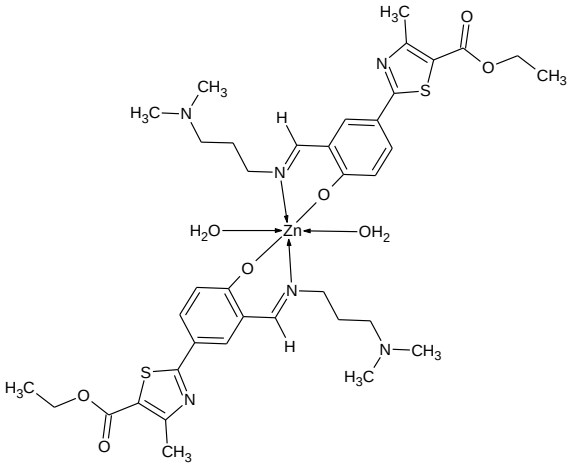
Based on the observations and calculations of TGA graph, two molecules of water are coordinated to metal complex. This corresponds to loss of about 5.3% due to two water molecules.

Table 2 Thermogravimetric Data of the Metal Complexes

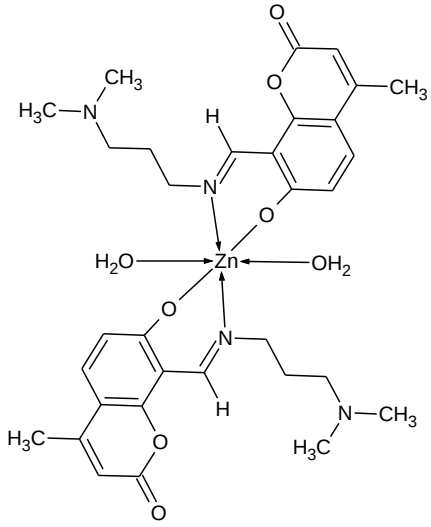
Complexes	**Mass loss due to H_2O**	
	Calculated (%)	Observed (%)
$[Zn(DMAPIMHMC)_2].2H_2O$	5.32	5.0
$[Zn(NMAPIMHMC)2].2H_2O$	5.55	5.1

From the discussion of the results of various spectroscopic details presented above, it may be concluded that transition metal complexes have general formula $[Zn(L)_2].2H_2O$ We are proposing following probable structures (Table 3) with octahedral geometry for these Zn(II) complexes.

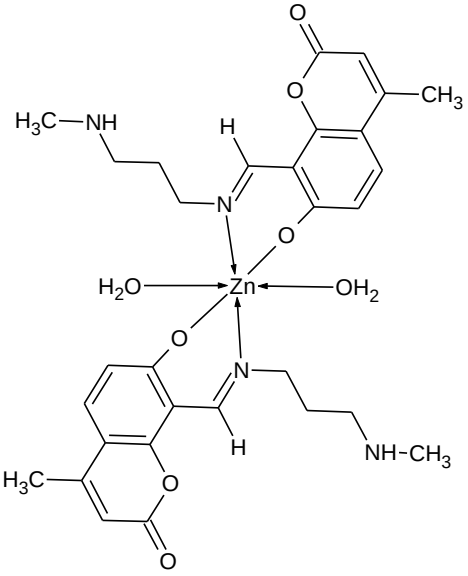
Probable structure of $[Zn(L)_2.2H_2O]$ metal complexes



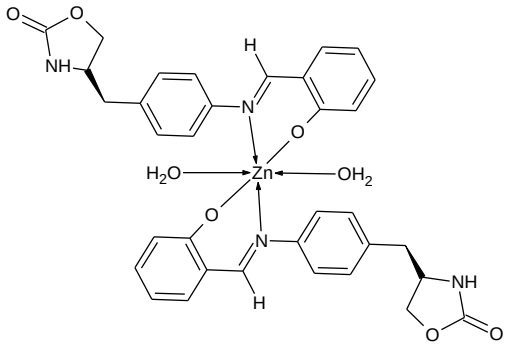
$[Zn(DMAPIMMTC)_2].2H_2O$



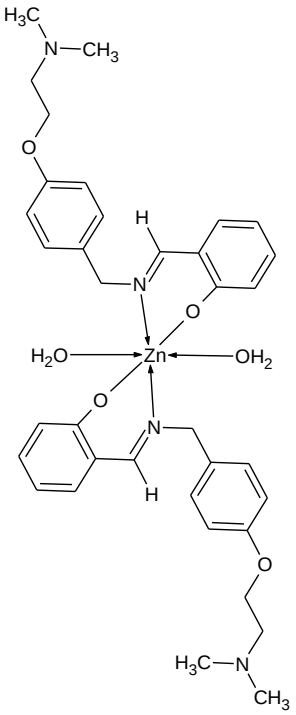
$[Zn(DMAPIMHMC)_2].2H_2O$



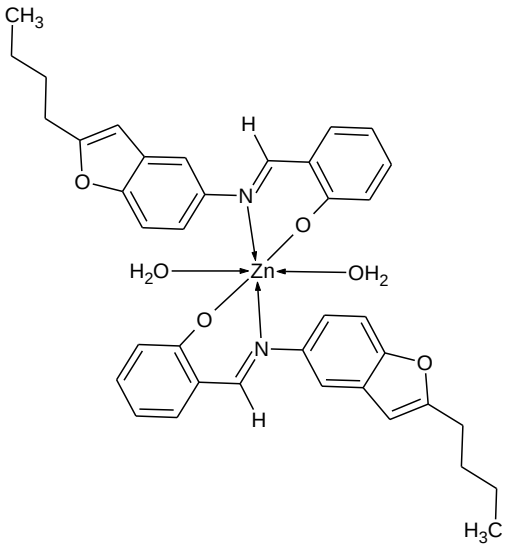
$[Zn(NMAPIMHMC)_2].2H_2O$



$[Zn(HBABO)_2].2H_2O$



$[Zn(DMAEBIMP)_2].2H_2O$



$[Zn(BBFIMP)_2].2H_2O$

Table 3 Proposed structures for [Zn $^{(II)}L_2$]. $2H_2O$ complexes

Anti TB screening

Procedure for Anti-TB activity using Alamar Blue Dye method [28]

The anti mycobacterial activity of compounds were assessed against M. tuberculosis using microplate Alamar Blue assay (MABA).This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method. Briefly, 200µl of sterile deionzed water was added to all outer perimeter wells of sterile 96 wells plate to minimize evaporation of medium in the test wells during incubation. The 96 wells plate received 100 µl of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate. The final drug concentrations tested were 100 to 0.2 µg/ml. Plates were covered and sealed with parafilm and incubated at 37ºC for five days. After this time, 25µl of freshly prepared 1:1 mixture of Almar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. [Refer to Figure 1.] The MIC was defined as lowest drug concentration which prevented the color change from blue to pink.

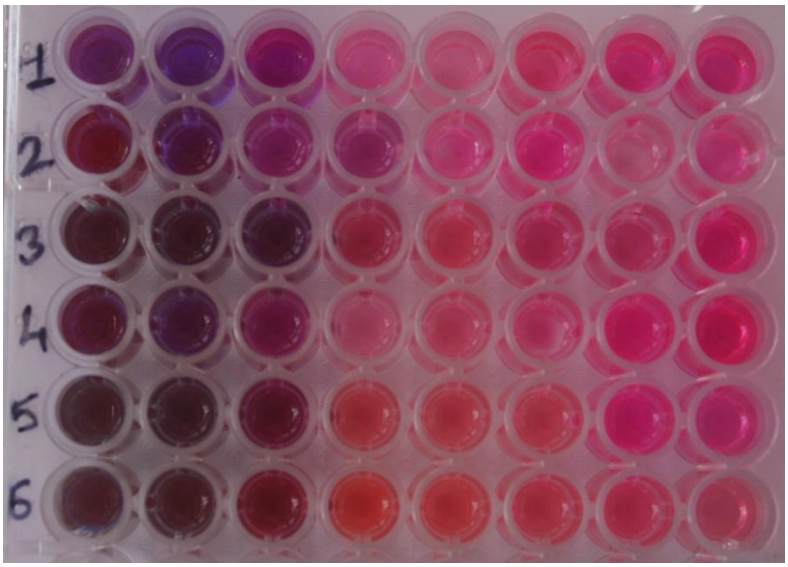


Figure 1 Picture showing Anti-TB activity using Alamar Blue Dye method

Transition metal complexes were evaluated for their antimycobacterium activity against *M. tuberculosis* by the serial dilution technique in MTP. The lowest concentration which showed no visible growth was taken as an end point known as minimum inhibitory concentration (MIC). A comparison of the metal complexes with that of reference Pyrazinamide and Streptomycin showed that the antituberculosis activity of the metal complexes was moderate. This could be due to heterocyclic rings present in the molecular structure of the metal complexes. The results of the studies of minimum inhibitory concentration of the metal complexes are summarized in Table 4.

Sample No.	Zinc Metal complex	MIC values in µg/ml,									
		100	50	25	12.5	6.25	3.125	1.6	0.8	0.4	0.2
1	$[Zn(DMAPIMMTC)_2].2H_2O$	S	S	S	R	R	R	R	R	R	R
2	$[Zn(DMAPIMHMC)_2].2H_2O$	S	S	S	R	R	R	R	R	R	R
3	$[Zn(NMAPIMHMC)_2].2H_2O$	S	S	S	R	R	R	R	R	R	R
4	$[Zn(HBABO)_2].2H_2O$	S	S	S	S	R	R	R	R	R	R
5	$[Zn(DMAEBIMP)_2].2H_2O$	S	S	S	R	R	R	R	R	R	R
6	$[Zn(BBFIMP)_2].2H_2O$	S	S	S	R	R	R	R	R	R	R

Table 4 Anti-TB activity results by MIC method (Note: S-Sensitive, R- Resistant) The standard values for the Anti-TB test which was performed. Pyrazinamide - 3.125µg/ml, Streptomycin - 6.25µg/ml)

Our observation of moderate activity of the test samples could be supported by the fact that low permeability of the mycobacterial cell wall is thought to contribute to the intrinsic drug resistance [29-31]. The molecular shape and size of the molecule is supposed to be an important factor for permeation through the M. *tuberculosis* cell wall. The extent of lateral diffusion relative to transverse diffusion of a molecule within a cell membrane decides the rate of absorption, distribution, metabolism, elimination and toxicity properties of drug candidate. The mycobacterial cell was is extraordinarily thick and tight having mainly two components of characteristic long chain fatty acids i.e. mycolic acids and polysaccharide arabinogalactam. These two constituents are linked together by covalent ester bonds. The lipophilicity of the drug is increased through the formation of chelates and drug action is increased due to effective permeability of the drug into the site of action.

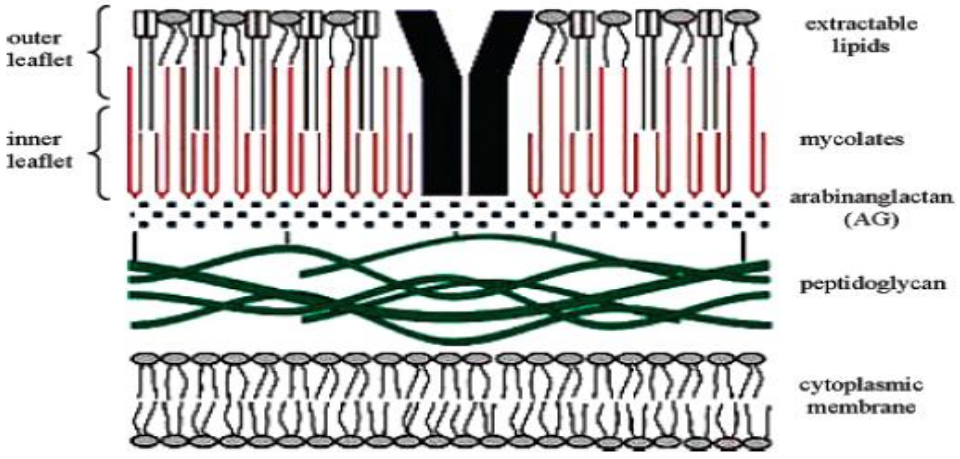


Figure2 : Mycobacterial cell wall model proposed by Minnikin. The funnel shaped structure in the center of the cell wall represents a porin . This figure is taken from reference [32]

However, the low permeability of the mycobacterial cell wall, with its unusual structure as explained above , is now known to be a major factor in this resistance. Mycobacteria show a high degree of intrinsic resistance to most antibiotics and chemotherapeutic agents. Thus hydrophilic agents cross the cell wall slowly because the mycobacterial porin (Figure 2) is inefficient in allowing the permeation of solutes and exists in low concentration. Lipophilic agents are presumably slowed down by the lipid bilayer which is of unusually low fluidity and abnormal thickness. Enzymatic inactivation of drugs, combined with the cell wall barrier can produce significant drug resistance against samples being screened.[33]

CONCLUSION

Novel Zn(II) complexes of bidentate Schiff base ligands were synthesised by condensation of 7-hydroxy-4-methyl-2-oxo-2H-chromene-8-carbaldehyde with Dimethylamino propylene diamine and N-methylamino propylene diamine . Zn(II) complexes were also prepared by condensing with Schiffs base of Ethyl 2-(3-formyl-4-hydroxyphenyl)-4-ethyl-1,3-thiazole-5- carboxylate with Dimethylamino propylene diamine .

2-Butyl-5-amino-1-benzofuran, 4-(4-aminobenzyl)-1,3-oxazolidine-2-one and 4-(1H-1,2,4-triazol-1-yl methyl) aniline were condensed with salicyaldehyde to get in situ heterocyclic schiff bases and Zn(II) complexes were prepared subsequently by template method. These Zn(II) metal complexes were characterized by combination of 1H NMR, 13C NMR, IR and Mass spectroscopy. The stoichiometry of M:L was confirmed by [M+] and [M +1] peaks in mass spectra and presence of two coordination water molecules was confirmed by TGA technique. Based on the analytical data, we are proposing a general formula [Zn(L)2.2H2O] and octahedral geometry for metal complexes.

These Zinc(II) complexes have shown moderate antimycobacterium activity as compared to standard Pyrazinamide and Streptomycin against M.Tuberculosis. This could be attributed to the fact of low permeability of mycobacterial cell wall towards compounds being studied

Note: **Authors have no conflict of interest.**

ACKNOWLEDGEMENT

We thank Dr. Kishor Bhat of Govt. Dental College, Belgaum for facilitating anti tuberculosis assay and providing the procedure for the same. We thank Satish Nalawade and Mustapha Mandewale for having assisted in scanning IR spectra and Dr. Ranjan Das and Ajay Patil of TIFR, Mumbai, Dr. Moneesha Fernandes of NCL, Pune and Dr. Avinash Kumbhar of Dept. of Chemistry, University of Pune for supporting with 1HNMR and 13C NMR spectra, without these supports this work would not have been complete. We also thank Dr. R. Atram, Principal of I. Y College , Jogeshwari, Mumbai and the management of Patkar-Varde College, Goregaon (W), Mumbai for constant encouragement throughout this work.

REFERENCES

[1] Some uses of transition metal complexes as anti-cancer and anti-HIV agents, Raymond Wai-Yin Sun, Dik-Lung Ma, Ella Lai-Ming Wong and Chi-Ming Che, Dalton Trans, 2007, 4884-4892.

[2] P. J. Sadler, Adv. Inorg. Chem., 1991, 36, 1.

[3] B. Rosenberg, L. VanCamp, J. E. Trosko and V. H. Mansour, Nature, 1969, 222, 385.

[4] (a) S. J. Lippard, Nat. Chem. Biol., 2006, 2, 504; (b) K. H. Thompson and C. Orvig, Science, 2003, 300, 936; (c) Chem. Rev., ed. C. Orvig and M. J. Abrams, 1999, 99, issue 9.

[5] Tunali, Yagmur; Canturk, Zerrin; Benkli, Kadriye, Synthesis, characterization, antibacterial, antifungal and antituberculous activities of some macrocyclic coordination compounds of Zn(II), Cd(II) & Hg(II), Asian Journal of Chemistry, 2011, 23(1), 225-228.

[6] Kharadi, G. J.; Patel, J. R.; Dholakiya, B. Z; Antituberculosis, antifungal and thermal activity of mixed ligand transition metal complexes, Applied Organometallic Chemistry, 2010, 24(11), 821-827.

[7] Shashidhara, G. M.; Goudar, T. R., Oxovanadium(IV) and niobium(V) complexes with some new schiff bases, synthesis and reactivity in inorganic and metal-organic chemistry,2000, 30(8), 1581-1599.

[8] Bamigboye M.O, Obaleye J.A, Lawal M. and Yakubu A., Synthesis, characterisation and antimicrobial study of mixed Isoniazidpyridoxine metal drug complexes, Nature and Science 2012;10(8), 1-3-105.

[9] Vogel's text book of Practical Organic Chemistry, 5th Edition, ELBS. Pg.1193.

[10] Bagihalli Gangadhar B., Avaji Prakash Gouda, Badami Prema S.,Patil Sangamesh A., Journal of Coordination Chemistry,2008,61(17),2793-2806.

[11] A. Kulkarni et al. European Journal of Medicinal Chemistry, 44 , 2009, 2904-2912.

[12] Vijaya Kamble, Sangeeta V. Chavan, Ramesh S. Yamgar , R. G. Atram, B. R Thorat, Swati Bisht and Sudhir S. Sawant, Asian J. Research Chem. 2011, 4(8), 1238-1242.

[13](a) Zhang Zhen-xue, Xue Ming-xing, Zhao Yong, Wang Shao-jie, Journal of Shenyang Pharmaceutical University, 2010, 27 (05): 365-368 ISSN: 1006-2858, (b)Chinese patent CN 101928259B. (c) Xie Peng, Zhang Jin, Jin Hua, Li Jian-qi Zhou Bin, Chinese Journal of Medicinal Chemistry, 2012, 22 (5): 369-372 ISSN: 1005-0108.

[14] (a) Luthra Parvenkumar, Khan Rashid, Salunkhe Dadasaheb, Nasir Ali shfakat, Patent No. WO 2012/131590 of Sandoz India Ltd.

(b) Parthasaradhi Reddy Bandi, Rathanakar Reddy Kura, Muralidhara Reddy Dasari, Ramakrishna Reddy Matta, Vamsi Krishna Bandi Patent No. WO 2012 168948 A2 of Hetero Research Foundation.

[15] J. C. Duff, J. Chem. Soc.,1941, 547.

[16] (a) Zhang Zhen-xue, Xue Ming-xing, Zhao Yong, Wang Shao-jie, Journal of Shenyang Pharmaceutical University, **2010**, 27 (05): 365-368 ISSN: 1006-2858; (b)Chinese patent CN 101928259B; (c) Xie Peng, Zhang Jin, Jin Hua, Li Jian-qi Zhou Bin, Chinese Journal of Medicinal Chemistry, 2012 22 (5): 369-372 ISSN: 1005-0108.

[17] Luthra Parvenkumar, Khan Rashid, Salunkhe Dadasaheb, Nasir Ali shfakat, Patent No. WO 2012/131590 of Sandoz India Ltd.

[18] Jean Gubin, Pierre Chatelain, Jean Lucchetti, Henri Inion US Patent No. 5223510 of Sanofi.

[19] Noel Fino, Corinne Leroy US Patent No. 6828448 of Sanofi synthelabo.

[20] Jean Gubin, Pierre Chatelain, Jean Lucchetti, Henri Inion US Patent No. 5223510 of Sanofi.

[21] Noel Fino, Corinne Leroy US Patent No. 6828448 of Sanofi synthelabo.[2004]

[22] Patel Rajnikant Patent No.EP1227095B1 of Astrazeneca.

[23] Otilia Costisor, W. Linert , "Metal mediated template synthesis of ligands" World Scientific Publisher, Singapore, **2004**. ISBN 981-238-813-3.

[24] L. J. Bellamy, " The Infrared Spectra of Complex Molecules". 3rd Ed, Methuen, London, (1966).

[25] Yongxiang Ma, Zhengzhi Zeng, Yun Ma and Gang Zhao,
Chelate complexes of formylferrocene m-nitrobenzoylhydrazone with lanthanide
Inorg. Chim. Acta, 1989, 165, 185

[26] K. Nakamoto, " The Infrared Spectra of Inorganic and Coordination Compounds ", 2nd
Ed, Wiley Interscience, New York, (1970).

[27] Puri, Sharma And Kalia "Principles of Inorganic Chemistry ", Milestone Publications, New Delhi (2008)

[28] Maria C. S. Lourenco, Marcus V. N deSouza, Alessandra C Pinheiro, Marcelle de L. Ferreira, Rasnisb B, Goncalves, Thais Cristina M Nogneira, Monica A Peralta, Evaluation of anti-Tubercular activity of nicotinic and isoniazid analogues. ARKIVOC, 2007 (xv), 181-191.

[29] Xuan Hong, A.J. Hopfinger, Molecular modeling and simulation of mycobacterium tuberculosis cell wall permeability, Biomacromolecules, 2004, 5, 1066-1077.

[30] Dolin, P. J.; Raviglione, M. C.; Kochi, A. Global tuberculosis incidence and mortality during 1990-2000. Bull. W. H. O. 1994, 72, 213-220.

[31] Jarlier, V.; Nikaido, H. Mycobacterial cell wall: structure and role in natural resistance to antibiotics. FEMS Microbiol. Lett. 1994, 123,11-18.

[32] Hong, X.; Hopfinger, A. J. Construction, Molecular Modeling, and Simulation of Mycobacterium tuberculosis Cell Walls. Biomacromolecules, 2004, 5, 1052-1065.

[33] De Rossi E, Aínsa JA, Riccardi G. Role of mycobacterial efflux transporters in drug resistance: an unresolved question. , FEMS Microbiol Rev. 2006 ,30(1), 36-52.