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Synthesis, Characterization and Preliminary Antifungal Activity of Some Co(II) and Zn(II) Complexes Derived from Oximes of 2-Acetyl Aromatic Heterocycles

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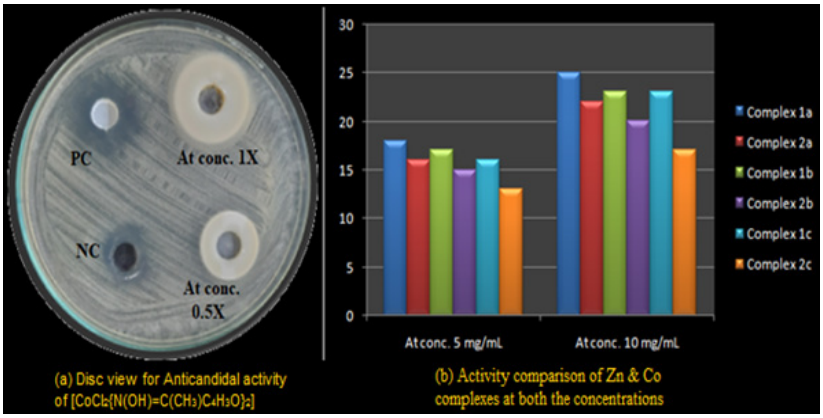
Present work focuses on syntheses, characterization, preliminary antifungal screening of some transition metal complexes derived from oximes of 2-acetylpyridine, 2-acetylthiophene and 2-acetylfuran; using Zn(II) and Co(II) chlorides. These newly synthesized complexes have been analyzed, characterized and compared with parent ligands on the basis of spectral techniques such as ^{1}H-NMR, ^{13}C-NMR, IR and elemental analysis. The antifungal screening performed on derived complexes proved that they show appreciable activity against *Candida albicans*.

Graphical abstract



(a) Disc view for Anticandidal activity of [$CoCl_2${N(OH)=C(CH_3)C_4H_3O}$_2$]

(b) Activity comparison of Zn & Co complexes at both the concentrations

Keywords

Zn(II) complexes
Co(II) complexes
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Antifungal activity

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1. Introduction

Researchers have often argued and proven that metal complexes show higher, better biological activities as compared to their corresponding free ligands; since, addition of active metal ions boosts the stability of the complexes and makes them quite important probes for biological system [1-6]. Metal complexes incorporating imine (>C=N-) group have been very fascinating due to their facile synthesis, catalytic utility, redox properties, and bioactivities [7, 8]. Varied metal complexes have come out to be significant, showing antitumor, antitubercular, antimalarial, antiviral, antilepral activities [9-11].

Zinc has a vital role to play in the metabolism of cells [12] and it provides advantageous effects to human health, while alteration in its metabolic mechanism can lead to various diseases [13, 14]. Zinc complexes have been extensively studied for their antifungal [15], antibacterial [16], pharmacological [17], antiproliferative [18], catecholase [19], phenoxazinone synthase-like [20] activities and in addition; they have played advantageous role in chemotherapeutic process [21]. On the other hand, Cobalt is found in vitamin B12 and its deficiency results in pernicious anemia. Cobalt complexes have also been investigated for their important and versatile biological activities [2, 16, 20, 22-24].

In a sequence to our earlier research work [15, 25, 26] and keeping in view the aforementioned applications, herein; we report synthesis, characterization, preliminary antifungal screening of some Zn(II), Co(II) complexes derived from 2-acetylpyridine oxime, 2-acetylthiophene oxime and 2-

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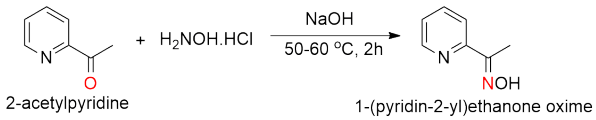
acetylfuran oxime.

2. Material and Methods

Reagents and chemicals (from Merck/Sigma) were used as procured, without further purification. C, H and N were estimated using Perkin-Elmer C, H, N and S II series 2400 analyzer. Cl, Zn and Co were estimated using standard literature methods [27]. KBr was used for registering IR spectra on a Perkin Elmer (Spectrum Version: 10.4.00) spectrophotometer in the region 4000-400cm^{-1}. ^{1}H-NMR spectra were recorded using TMS as an internal reference in d_6-DMSO solvent, on a Bruker Ascend 300 MHz system. The IR & ^{1}H-NMR studies were carried out at *MNIT, Jaipur*. Antifungal activity was performed at *Dr. B. Lal Clinical Laboratory Pvt. Ltd. - CIRD, Jaipur.*

2.1. Green synthesis of 2-Acetylpyridine oxime HON=C(CH$_3$)C$_5$H$_4$N [25]

A mixture of 2-acetylpyridine (12.3 g, 101.54 mmol) and equimolar amount of hydroxylamine hydrochloride (7.06 g, 101.54mmol) in 20 mL water was warmed, stirred at 50-60 °C for half an hour. NaOH (4.06 g, 101.54 mmol) was then added portion wise and stirring was continued for another 1-1.5 hours at room temperature (Scheme 1). The precipitate was filtered off under reduced pressure and washed twice with water, dried to obtain the desired solid product 1-(pyridin-2-yl)ethanone oxime **A**, HON=C(CH$_3$)C$_5$H$_4$N.

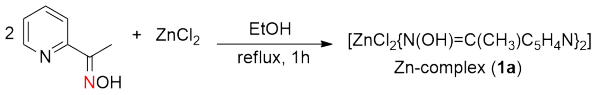


Scheme 1. Green synthesis of HON=C(CH$_3$)C$_5$H$_4$N.

A similar procedure resulted in synthesis of 2-acetylthiophene oxime **B**, HON=C(CH$_3$)C$_4$H$_3$S and 2-acetylfuran oxime **C**, HON=C(CH$_3$)C$_4$H$_3$O.

2.2. Synthesis of [ZnCl$_2${N(OH)=C(CH$_3$)C$_5$H$_4$N}$_2$]

Ethanolic solution of ZnCl$_2$ (2.92 g, 21.425 mmol) and HON=C(CH$_3$)C$_5$H$_4$N (5.83 g, 42.850 mmol) was refluxed for 1 hour (Scheme 2). Contents were cooled; precipitate was filtered off under reduced pressure and washed twice with ethanol to obtain the corresponding Zn-complex [ZnCl$_2${N(OH)=C(CH$_3$)C$_5$H$_4$N}$_2$] **(1a)** (Yield: 74%; M.p.: 174-176 °C).



Scheme 2. Synthesis of [ZnCl$_2${N(OH)=C(CH$_3$)C$_5$H$_4$N}$_2$].

A similar procedure resulted in synthesis of [CoCl$_2${N(OH)=C(CH$_3$)C$_5$H$_4$N}$_2$] **(2a)**, [ZnCl$_2${N(OH)=C(CH$_3$)C$_4$H$_3$S}$_2$] **(1b)**, [CoCl$_2${N(OH)=C(CH$_3$)C$_4$H$_3$S}$_2$] **(2b)**, [ZnCl$_2${N(OH)=C(CH$_3$)C$_4$H$_3$O}$_2$] **(1c)** and [CoCl$_2${N(OH)=C(CH$_3$)C$_4$H$_3$O}$_2$] **(2c)**. The newly synthesized complexes were obtained in 71-87% yield.

3. Results and Discussion

Interaction of Zn(II), Co(II)chlorides with oximes of 2-acetylpyridine, 2-acetylthiophene and 2-acetylfuran in 1:2 molar ratio yielded Zn(II) and Co(II) complexes of the type [MCl$_2$L$_2$], where M = Zn/Co and L = corresponding ligand. All these complexes are soluble in coordinating solvents and have been characterized by elemental analysis and spectral studies. Physical and analytical results are given in Table 1.

Table 1. Physical and analytical results of the synthesized complexes.

Metal Complex	Color	Yield (%)	M.p. (°C)	Elemental Analysis % Found (% estimated.)				
				C	H	Cl	N	Metal
[ZnCl$_2${N(OH)=C(CH$_3$)C$_5$H$_4$N}$_2$] **(1a)**	White	74	174-176	40.98 (41.15)	4.01 (3.95)	17.50 (17.35)	13.49 (13.71)	15.84 (16.00)
[CoCl$_2${N(OH)=C(CH$_3$)C$_5$H$_4$N}$_2$] **(2a)**	Pink	71	192-194	41.69 (41.81)	4.15 (4.01)	17.81 (17.63)	13.88 (13.93)	14.70 (14.65)
[ZnCl$_2${N(OH)=C(CH$_3$)C$_4$H$_3$S}$_2$] **(1b)**	White	84	164-166	34.56 (34.43)	3.23 (3.37)	16.79 (16.94)	6.82 (6.69)	15.83 (15.62)
[CoCl$_2${N(OH)=C(CH$_3$)C$_4$H$_3$S}$_2$] **(2b)**	Pink	77	150-152	34.87 (34.96)	3.49 (3.42)	17.11 (17.20)	6.58 (6.80)	14.13 (14.30)
[ZnCl$_2${N(OH)=C(CH$_3$)C$_4$H$_3$O}$_2$] **(1c)**	Creamy White	83	146-148	37.40 (37.29)	3.51 (3.65)	18.46 (18.34)	7.33 (7.25)	16.76 (16.91)
[CoCl$_2${N(OH)=C(CH$_3$)C$_4$H$_3$O}$_2$] **(2c)**	Dark Pink	87	158-160	38.07 (37.92)	3.45 (3.71)	18.74 (18.66)	7.45 (7.37)	15.38 (15.51)

3.1. IR Spectra

Signals in the range 3355-3265 cm^{-1} were observed in the IR spectra of all the complexes. These are assigned to υ(OH) of the ligands and suggests that during metal-ligand interaction, M-O covalent bond has not formed via deprotonation of oxime (=N-O-H) group (Table 2). In spectra of complexes, the bands observed in the range 1610-1550 cm^{-1} are assigned to υ(>C=N) of azomethine group; and these bands are lower in values as compared to similar bands in the range 1690-1640 cm^{-1} in the spectra of free oximes. This suggests a N→M (M = metal) coordinate bond formation [25]. Furthermore, in spectra of complexes, the υ(C-X) (where; X = N, S or O of aromatic ring) have been observed at lower values in the range 1460–1375 cm^{-1} compared to free oximes (1490–1405 cm^{-1}) [28]. This suggests Ar→M coordinate bond formation (where; Ar = C$_5$H$_4$N, C$_4$H$_3$S or C$_4$H$_3$O). Signals in the range 470-455 cm^{-1} and 485-440 cm^{-1} can be accounted to υ(Zn-N) and υ(Co-N), respectively.

Table 2. IR spectral results of the synthesized complexes (values are in cm^{-1}).

Metal Complex	υ(O-H)	υ(C=N)	υ(C-X; oxime aromatic ring)	υ(Zn-N) or υ(Co-N)
$[ZnCl_2\{N(OH)=C(CH_3)C_5H_4N\}_2]$	3355	1585	1460	470
$[CoCl_2\{N(OH)=C(CH_3)C_5H_4N\}_2]$	3335	1590	1435	485
$[ZnCl_2\{N(OH)=C(CH_3)C_4H_3S\}_2]$	3315	1610	1385	460
$[CoCl_2\{N(OH)=C(CH_3)C_4H_3S\}_2]$	3275	1550	1395	470
$[ZnCl_2\{N(OH)=C(CH_3)C_4H_3O\}_2]$	3330	1600	1420	455
$[CoCl_2\{N(OH)=C(CH_3)C_4H_3O\}_2]$	3265	1575	1375	440

3.2. ^{1}H-NMR Spectra

Signals in the range 11.77-11.09 ppm assigned to OH, were observed in the proton spectra of complexes; this suggested that during metal-ligand interaction, M-O covalent bond was not formed via deprotonation of oxime (=N-O-H) group (Table 3). An appreciable shifting of signals, observed in spectra of complexes as compared to that of free ligands [28, 29]; suggests the L→M (L = ligand) coordinate bonds formation.

Table 3. ^{1}H-NMR spectral results of the synthesized ligands and their metal complexes

Ligand/ Metal Complex	^{1}H-NMR δ in ppm
$HON=C(CH_3)C_5H_4N$	10. 88 (s, 1H), 8.43 (s, 1H), 8.21 (dd, *J* = 16.3, 7.7 Hz, 2H), 7.78 (s, 1H), 2.87 (s, 3H)
$[ZnCl_2\{N(OH)=C(CH_3)C_5H_4N\}_2]$	11.77 (s, 1H), 8.58 (s, 1H), 8.02 (dd, *J* = 16.3, 7.7 Hz, 2H), 7.63 (s, 1H), 2.82 (s, 3H)
$[CoCl_2\{N(OH)=C(CH_3)C_5H_4N\}_2]$	11.45 (s, 1H), 8.49 (s, 1H), 7.75 (dd, *J* = 16.3, 7.7 Hz, 2H), 7.39 (s, 1H), 2.25 (s, 3H)
$HON=C(CH_3)C_4H_3S$	10.47 (s, 1H), 8.09 (s, 1H), 7.92 (d, *J* = 3.8 Hz, 1H), 7.46 - 7.41 (m, 1H), 2.81 (s, 3H)
$[ZnCl_2\{N(OH)=C(CH_3)C_4H_3S\}_2]$	11.44 (s, 1H), 7.90 (s, 1H), 7.66 (d, *J* = 3.8 Hz, 1H), 7.37 - 7.30 (m, 1H), 2.70 (s, 3H)
$[CoCl_2\{N(OH)=C(CH_3)C_4H_3S\}_2]$	11.09 (s, 1H), 7.61 (s, 1H), 7.24 (d, *J* = 3.8 Hz, 1H), 7.08 - 7.03 (m, 1H), 2.18 (s, 3H)
$HON=C(CH_3)C_4H_3O$	10.59 (s, 1H), 8.04 (s, 1H), 7.22 (d, *J* = 3.4 Hz, 1H), 6.98 (s, 1H), 2.66 (s, 3H)
$[ZnCl_2\{N(OH)=C(CH_3)C_4H_3O\}_2]$	11.39 (s, 1H), 7.87 (s, 1H), 7.05 (d, *J* = 3.4 Hz, 1H), 6.87 (s, 1H), 2.58 (s, 3H)
$[CoCl_2\{N(OH)=C(CH_3)C_4H_3O\}_2]$	11.13 (s, 1H), 7.59 (s, 1H), 6.67 (d, *J* = 3.4 Hz, 1H), 6.57 (s, 1H), 2.10 (s, 3H)

3.3. ^{13}C-NMR Spectra

The ^{13}C-NMR spectra of complexes resemble characteristic peaks for C-atoms in ligand systems (Table 4). Shifting of C=N and aryl carbon signals in ^{13}C-NMR Spectra of the complexes as compared to that of free ligands [28-30], again suggests the L→M (L = ligand) coordinate bonds formation.

^{13}C-NMR and ^{1}H-NMR spectra of the synthesized compounds are provided as **Supplementary data (Fig. S1, S2, ...S6).**

Table 4. ^{13}C-NMR spectral results of the synthesized ligands and their metal complexes

Ligand/ Metal Complex	^{13}C-NMR δ in ppm
$HON=C(CH_3)C_5H_4N$	11.1 (C- methyl); 120.2 (C5 - pyridine); 124.2 (C3 - pyridine); 135.5 (C4 - pyridine); 150.4 (C6 - pyridine); 153.0 (C2 - pyridine); 154.9 (C - imine)
$[ZnCl_2\{N(OH)=C(CH_3)C_5H_4N\}_2]$	10.6 (C- methyl); 119.4 (C5 - pyridine); 123.3 (C3 - pyridine); 134.8 (C4 - pyridine); 149.3 (C6 - pyridine); 154.6 (C2 - pyridine); 155.7 (C - imine)
$[CoCl_2\{N(OH)=C(CH_3)C_5H_4N\}_2]$	10.3 (C- methyl); 119.9 (C5 - pyridine); 123.7 C3 - pyridine); 135.4 (C4 - pyridine); 150.1 (C6 - pyridine); 153.9 (C2 - pyridine); 155.1 (C - imine)
$HON=C(CH_3)C_4H_3S$	12.6 (C - methyl); 126.0 (C4 – thiophene); 127.3 (C3 - thiophene); 127.6 (C5 - thiophene); 141.2 (C2 - thiophene); 150.6 (C - imine)
$[ZnCl_2\{N(OH)=C(CH_3)C_4H_3S\}_2]$	12.0 (C - methyl); 125.6 (C4 - thiophene); 126.4 (C3 - thiophene); 127.0 (C5 - thiophene); 144.1 (C2 - thiophene); 152.7 (C - imine)
$[CoCl_2\{N(OH)=C(CH_3)C_4H_3S\}_2]$	11.8 (C - methyl); 125.0 (C4 - thiophene); 126.8 (C3 - thiophene); 127.3 (C5 - thiophene); 142.7 (C2 - thiophene); 151.2 (C - imine)
$HON=C(CH_3)C_4H_3O$	11.5 (C - methyl); 109.1 (C4 - furan); 112.2 (C3 - furan); 142.5 (C5 - furan); 149.1 (C2 - furan); 152.8 (C - imine)
$[ZnCl_2\{N(OH)=C(CH_3)C_4H_3O\}_2]$	10.3 (C - methyl); 105.4 (C4 - furan); 110.8 (C3 - furan); 142.1 (C5 - furan); 150.7 (C2 - furan); 153.6 (C - imine)
$[CoCl_2\{N(OH)=C(CH_3)C_4H_3O\}_2]$	10.9 (C - methyl); 105.9 (C4 - furan); 111.4 (C3 - furan); 139.5 (C5 - furan); 152.2 (C2 - furan); 154.2 (C - imine)

3.4. Antifungal Activity

A preliminary antifungal screening of complexes proved that all the complexes are appreciably active against *Candida albicans*, whereas the corresponding ligands show very slight activity at similar concentrations (Table 5).

Kirby-Bauer well diffusion method [31] was used for

carrying out *in vitro* screening on *Candida albicans* (ATCC 14053), swabbed upon Sabouraud's Dextrose Agar as Muller Hinton (MH) Agar medium. Antifungal plates were incubated for 2 days at 28°C (Fig. S7 in Supplementary data for 24h grown culture of the strain). Test compounds were examined at two different concentrations viz., 10 mg/mL and 5 mg/ml (prepared from stock solution); using 100µL in each well in solvent DMSO (Fig. 1). Itraconazole as PC (positive control) at concentration 50 µg/mL and DMSO as NC (negative control), were taken into consideration.

Table 5. Anticandidal activity results of the synthesized ligands and their metal complexes.

Ligand/Metal Complex	Organism	PC	NC	at conc. 5 mg/mL	at conc. 10 mg/mL
$HON=C(CH_3)C_5H_4N$	*Candida albicans*	18mm	10mm	NZI*	7mm
$[ZnCl_2\{N(OH)=C(CH_3)C_5H_4N\}_2]$				18mm	25mm
$[CoCl_2\{N(OH)=C(CH_3)C_5H_4N\}_2]$				16mm	22mm
$HON=C(CH_3)C_4H_3S$				3mm	12mm
$[ZnCl_2\{N(OH)=C(CH_3)C_4H_3S\}_2]$				17mm	23mm
$[CoCl_2\{N(OH)=C(CH_3)C_4H_3S\}_2]$				15mm	20mm
$HON=C(CH_3)C_4H_3O$				2mm	10mm
$[ZnCl_2\{N(OH)=C(CH_3)C_4H_3O\}_2]$				16mm	23mm
$[CoCl_2\{N(OH)=C(CH_3)C_4H_3O\}_2]$				13mm	17mm

*NZI: No Zone of Inhibition.

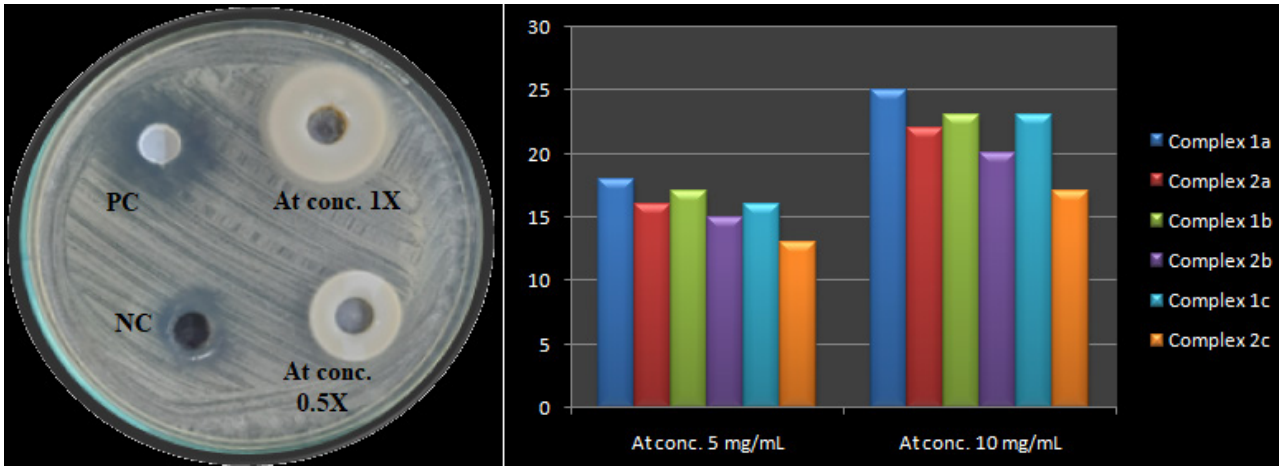
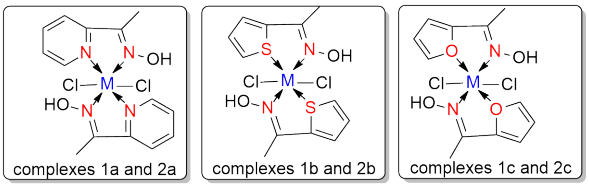


Fig. 1. (a) Disc view for anticandidal activity of $[CoCl_2\{N(OH)=C(CH_3)C_4H_3O\}_2]$. (b) Activity comparison of Zn and Co complexes at both the concentrations.



Proposed strcutures for the synthesized complexes
1a, 1b and 1c: M = Zn
2a, 2b and 2c: M = Co

Fig. 2. Proposed coordination for synthesized Zn(II) and Co(II) complexes.

4. Conclusions

In view of the aforementioned findings, the following tentative coordination (Fig. 2) can be proposed, and the complexes can be represented as $[MCl_2L_2]$ (where M = Zn/Co, L = corresponding oxime ligand).

The preliminary *in-vitro* antifungal screening of all these complexes confirms that they are appreciably active against *Candida albicans*. It is also evident that the ligands are very slightly active in contrast to their metal complexes; where Zn-complexes exhibited better activities compared to corresponding Co-Complexes at similar concentrations against the fungal strain.

Supporting Information

^{13}C-NMR and ^{1}H-NMR spectra of the synthesized compounds are provided as Supplementary data (Fig. S1, S2, ...S6).

Author Contributions

Deepankar Sharma: Performed the experiments; Analyzed and interpreted the data; Wrote the paper. Purnima Nag: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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