

Synthesis, Characterization and Antimicrobial Activity of Some Heterocyclic Compounds and Its Metal Complexes

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Abstract

New heterocyclic compounds containing quinoline fragment were synthesized. They were characterized by elemental analyses as well as IR and PMR spectroscopic analyses. These compounds were treated with metal (Mn(II), Ni(II) and Cu(II) salts to produce complexes. The complexes were identified and characterized by elemental analyses, IR and electronic spectral studies and magnetic moment studies. The magnetic behaviour and spectroscopic investigation of complexes indicate mononuclear octahedral structure of all the complexes. All heterocyclic compounds and metal complexes were screened for antimicrobial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Escherichia coli* and *Klebsiella pneumonia* using DMF as a solvent. The activity was compared with known antibiotics like Penicillin, Ampicillin, Tetracycline, Chloramphenicol and Norfloxacin.

Key Words: Heterocyclic complex; Antibacterial activity

Introduction

Heterocyclic compounds are comprehensively explored for their antimicrobial activity. Quinolines are also heterocyclic compounds. They contain nitrogen as hetero atom. They are known for their antimicrobial activities (Agui et al.,1977; Amin et al., 2004; Abdel-Moty et al.,2005; Eswaran et al., 2009; Jumade et al., 2009). Formation of quinoline containing complexes is also reported by chemists (Zurowska et al., 2007; Da Silva et al., 2008). Complexes of quinolines and their various derivatives are also assessed for their antimicrobial activities (Thakur et al., 2006; Abou-Melha et al., 2007; Alazawi et al., 2007).

Considering the medicinal significance of quinoline containing compounds, such novel

heterocyclic compounds were synthesized to develop potent bioactive molecules. These compounds were treated with metal salts of Mn(II), Ni(II) and Cu(II) to synthesize complexes. Structures of heterocyclic compounds and metal complexes were confirmed with elemental analyses and spectroscopic studies. All compounds and complexes were screened for antibacterial activity.

Experimental

All the chemicals used were of AR grade and used without further purification.

General method for synthesis of Compounds(1-a to 1-c):

5-Amino-8-quinolinol (0.01 mole),
5-substituted 1,3,4-oxadiazole-2(3H)-thione(0.01

mole) and methanal (0.03 mole) were added to iso-propanol (50 ml). Concentrated hydrochloric acid (4-5 drops) were also added to the reaction mixture. It was heated on the steam bath up to 9-10 hrs. The purity of compounds was monitored by Thin Layer Chromatography (TLC) using Silica Gel-G in the solvent system of n-hexane and ethyl acetate (v/v = 1:3). The spots were observed by exposure to iodine vapour or by UV light. Iso-propanol was distilled out. Water was added to extract product into aqueous layer. Aqueous layer was made alkaline by the addition of 10% aqueous solution of sodium hydroxide. Methylene dichloride (60 ml) was added to the resultant solution. Organic layer was separated, dried over anhydrous sodium sulphate and distilled out atmospherically. In this way, the product 3-[(8-hydroxy quinolin-5-yl)-amino methyl]-amino methyl]-5-(4-nitro phenyl)-1,3,4-oxadiazole-2(3H)-thione was synthesized.

Thus, three such heterocyclic compounds: **1-a**: 3-[(8-hydroxy quinolin-5-yl)-amino methyl]-5-(4-nitro phenyl)-1,3,4-oxadiazole-2(3H)-thione,

1-b: 3-[(8-hydroxy quinolin-5-yl)-amino methyl]-5-(2,4-di chloro phenyl)-1,3,4-oxadiazole-2(3H)-thione and **1-c**: 3-[(8-hydroxy quinolin-5-yl)-amino methyl]-5-naphthyl-1,3,4-oxadiazole-2(3H)-thione were synthesized. The melting points of all these compounds were taken in open capillary glass tubes using paraffin bath and are uncorrected. The % of yield and melting points of such compounds are as under:

1-a: % of yield: 80, Melting Point: 178°C [M.F.:



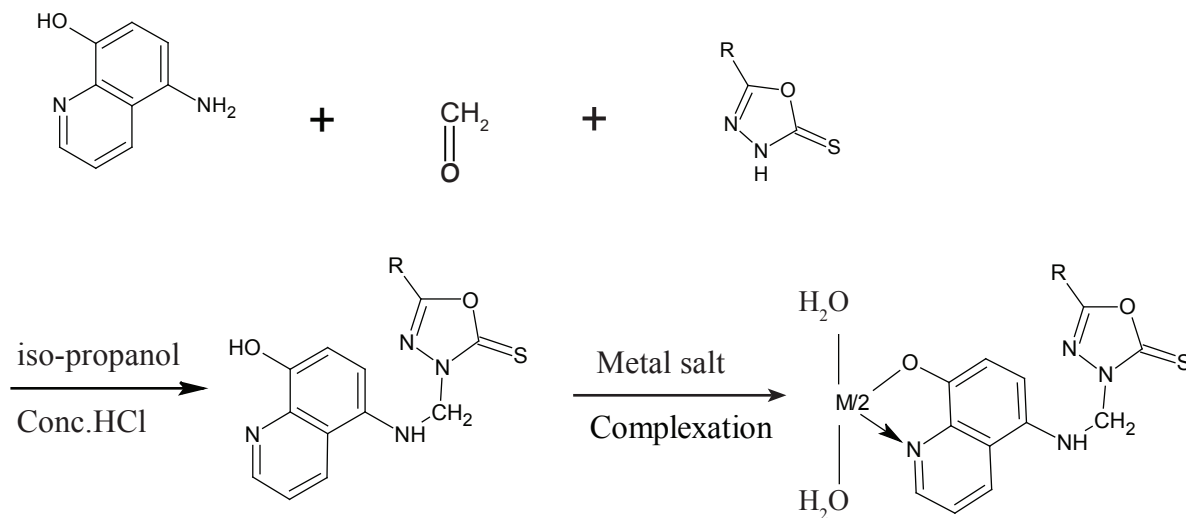
1-b: % of yield: 61, Melting Point: 165°C [M.F.:



1-c: % of yield: 72, Melting Point: 194°C [M.F.:



The elemental analyses were performed using Carlo-Erba 1108 Analyser. The elemental analysis is shown in Table 1. The analyses turn out well with the predicted structure of compounds (1-a to 1-c). General structure of the compounds is shown in Figure 1.



General structure of compound [1]

1-a: R = C₆H₅ (4-NO₂),

1-b: R = C₆H₄ (2,4)(Cl,Cl),

1-c: R = C₁₀H₇ (1-Naphthyl)

General structure of complex [1-a-1] to [1-c-3]

M = bivalent ion: Mn(II), Ni(II), Cu(II)

Figure 1 General outline of preparation of compounds and complexes

Table1 Elemental analysis of heterocyclic compounds

Comp.	C		H		N		S		Cl	
	Found	Calcd.	Found	Calcd.	Found	Calcd.	Found	Calcd.	Found	Calcd.
1-a	54.75	54.68	3.2	3.29	17.7	17.72	8.1	8.10	-	-
1-b	51.56	51.55	2.8	2.86	13.3	13.36	7.6	7.63	16.90	16.94
1-c	65.95	66.00	4.0	4.00	14.0	14.00	8.0	8.00	-	-

Comp.: Compound, Calcd.: Calculated.

IR and PMR spectra

IR spectra were recorded on Nicolet Avator FTIR and Perkin Elmer spectrophotometers using KBr pellets technique. Proton-NMR Spectra were recorded on a Bruker NMR spectrophotometer (400 MHz). PMR chemical shifts were recorded in δ value using DMSO- d_6 as a solvent and TMS as an internal standard.

The important spectral characteristics of compounds were as under:

1-a: 3740(-OH), 3009, 1581, 1492(Aromatic), 1687, 1646, 1551, 1469(hydroxy quinoline), 3391 (-NH-), 2898, 2840, 1443(-CH₂-)cm⁻¹, ¹H NMR: δ = 11.05(s, -NH-), 7.27-7.60(m, quinoline), 5.65(s, phenolic-OH), 3.24(s, -CH₂-).

1-b: 3600(-OH), 3017, 1580, 1501(Aromatic), 1665, 1655, 1560, 1476(hydroxy quinoline), 3365 (-NH-), 2911, 2856, 1444(-CH₂-)cm⁻¹, ¹H NMR: δ = 11.12(s, -NH-), 7.24-7.61(m, quinoline), 5.70(s, phenolic-OH), 3.25(s, -CH₂-).

1-c: 3570(-OH), 3018, 1584, 1495(Aromatic), 1690, 1645, 1570, 1479(hydroxy quinoline), 3384 (-NH-), 2916, 2844, 1440(-CH₂-) cm⁻¹, ¹H NMR: δ = 11.09(s, -NH-), 7.14-7.56(m, quinoline), 5.68(s, phenolic-OH), 3.20(s, -CH₂-).

Synthesis of complexes (1-a-1 to 1-c-3):

For the synthesis of metal complexes of compounds (1-a to 1-c), compound (1-a/1-b/1-c) (0.001 mole) was dissolved in 90 ml of methanoic acid and 2-3 ml of water. Thus, solution-1 is prepared.

Synthesis of Mn (II) complexes

Manganese chloride hexahydrate (0.0005 mole) was dissolved in 100 ml water. Solution-1 was gradually added into it. The pH of the solution was maintained 5.5-5.7. The final product separated as a solid mass digested on water bath at 70°C for 2-3 hrs. The solid product was then filtered, washed with water-ethanol mixture (v/v=1:1) and dried.

Synthesis of Ni (II) complexes:

Nickel nitrate hexahydrate (0.0005 mole) was dissolved in 100 ml water. Solution-1 was gradually added into it. The pH of the solution was maintained 6.0-6.2. The final product separated as a solid mass digested on water bath at 70°C for 2-3 hrs. The solid product was then filtered, washed with water-ethanol mixture (v/v=1:1) and dried.

Synthesis of Cu (II) complexes:

Cupric nitrate hexahydrate (0.0005 mole) was dissolved in 100 ml water. Solution-1 was gradually added into it. The pH of the solution was maintained 4.5-4.6. The final product separated as a solid mass digested on water bath at 70°C for 2-3 hrs. The solid product was then filtered, washed with water-ethanol mixture (v/v=1:1) and dried.

Physicochemical Characterization

The % of yield and melting points of such compounds are given in Table 2.

The elemental analysis of all the complexes was performed using Carlo Erba Analyser 1108.

The elemental analysis data is given in Table 3. This analysis supports the predicted structures of the complexes. General structure of complexes is given in Figure 1.

The metal content(Mn(II), Ni(II) and Cu(II)) of all the complexes was determined by standard methods described in the literature (Vogel,2004). The study indicates that stoichiometry of metal-ligand(M-L) in the complexes is 1:2.

Table 2 Molecular formula, Molecular weight, M.P. and Yield of complexes

Complex	Molecular formula	Molecular weight	M.P.	Yield %
1-a-1	C ₃₆ H ₂₄ N ₁₀ O ₈ S ₂ Mn(II).2H ₂ O	879	214	68
1-a-2	C ₃₆ H ₂₄ N ₁₀ O ₈ S ₂ Ni(II).2H ₂ O	883	235	67
1-a-3	C ₃₆ H ₂₄ N ₁₀ O ₈ S ₂ Cu(II).2H ₂ O	887.5	198	63
1-b-1	C ₃₆ H ₂₂ N ₈ O ₄ S ₂ Cl ₄ Mn(II).2H ₂ O	927	205	66
1-b-2	C ₃₆ H ₂₂ N ₈ O ₄ S ₂ Cl ₄ Ni(II).2H ₂ O	931	185	65
1-b-3	C ₃₆ H ₂₂ N ₈ O ₄ S ₂ Cl ₄ Cu(II).2H ₂ O	935.5	219	65
1-c-1	C ₃₆ H ₂₂ N ₈ O ₄ S ₂ Cl ₄ Mn(II).2H ₂ O	927	158	66
1-c-2	C ₄₄ H ₃₀ N ₈ O ₄ S ₂ Ni(II).2H ₂ O	893	240	68
1-c-3	C ₄₄ H ₃₀ N ₈ O ₄ S ₂ Cu(II).2H ₂ O	897.5	147	70

Table 3 Elemental analysis: % of metal, C, H, N, Cl and S elements of complexes

Com.	Elemental analysis											
	% Metal		%C		%H		%N		% Cl		%S	
	Found	Calcd.	Found	Calcd.	Found	Calcd.	Found	Calcd.	Found	Calcd.	Found	Calcd.
1-a-1	49.70	49.14	3.26	3.19	15.95	15.92	49.18	49.14	-	-	7.29	7.28
1-a-2	48.13	48.92	3.22	3.17	15.84	15.85	48.89	48.92	-	-	7.22	7.24
1-a-3	48.69	48.67	3.10	3.15	15.79	15.77	48.68	48.67	-	-	7.21	7.21
1-b-1	5.90	5.93	46.64	46.60	2.83	2.80	12.13	12.08	15.34	15.31	6.93	6.90
1-b-2	6.28	6.33	46.37	46.40	2.82	2.79	12.01	12.03	15.16	15.25	6.80	6.87
1-b-3	6.82	6.78	46.19	46.17	2.77	2.78	11.96	11.97	15.17	15.17	6.85	6.84
1-c-1	5.89	5.93	46.54	46.60	2.83	2.80	12.14	12.08	-	-	6.93	6.90
1-c-2	6.64	6.60	59.11	59.12	3.86	3.80	12.56	12.54	-	-	7.14	7.16
1-c-3	7.09	7.07	58.90	58.83	3.81	3.78	12.16	12.47	-	-	7.20	7.13

Com.: Complex, Calcd.: Calculated.

The molar conductivity of solution (1×10^{-3} M) of complex in Dimethyl formamide(DMF) was determined by Systronics model-305 direct reading conductivity meter. The values of molar conductivities are shown in Table 4.

Magnetic susceptibility (χ) was determined using a Gouy magnetic balance at room temperature (300K). Mercury (II) tetrathio cyanatocobaltate (II) was used as a standard and diamagnetic corrections were made with Pascal’s constants. The effective magnetic moment was determined by the following equation:

$$\mu_{\text{eff}} = 2.84\sqrt{\chi_M T}$$

where T is the absolute temperature.

The theoretical value of the magnetic moment was calculated on the basis of following equation:

$$\mu_{\text{eff}} = \sqrt{n(n + 2)} \text{ BM}$$

where n=number of unpaired electrons.

The values of magnetic susceptibility and magnetic moment are given in Table-4. The reflectance spectra were recorded on a Beckman DU spectrophotometer using MgO as a reference.

The information regarding characteristic absorption bands is given in Table 5.

The important FTIR spectral features are as below:

1-a-1: 3410(O-H),1412(O-M), 1093(C-O-M), 770(N-M), 522(N-M), 274(S=O)cm⁻¹

1-a-2: 3440(O-H), 1420(O-M), 1096(C-O-M), 766(N-M), 525(N-M), 278(S=O)cm⁻¹

1-a-3: 3430(O-H), 1417(O-M), 1092(C-O-M), 765(N-M), 524(N-M), 272(S=O)cm⁻¹

1-b-1: 3415(O-H),1416(O-M), 1095(C-O-M), 770(N-M), 525(N-M), 270(S=O)cm⁻¹

1-b-2: 3446(O-H), 1420(O-M), 1091(C-O-M), 760(N-M), 523(N-M), 278(S=O)cm⁻¹

1-b-3: 3435(O-H), 1418(O-M), 1088(C-O-M), 766(N-M), 520(N-M), 271(S=O)cm⁻¹

1-c-1: 3433(O-H), 1415(O-M), 1096(C-O-M), 770(N-M), 525(N-M), 274(S=O)cm⁻¹

1-c-2: 3440(O-H), 1409(O-M), 1094(C-O-M), 763(N-M), 522(N-M), 278(S=O)cm⁻¹

1-c-3: 3430(O-H), 1417(O-M), 1095(C-O-M), 766(N-M), 518(N-M), 273(S=O)cm⁻¹

Table 4 Magnetic moment and conductivity of complexes

Complex	χ_g	χ_M	Observed Magnetic Moment μ_{eff} (BM)	Calculated Magnetic Moment μ_{eff} (BM)	Molar conductivity (Mhos cm ² mol ⁻¹)
1-a-1	12.8	0.0146	5.94	5.92	7.10
1-a-2	3.19	0.0036	2.96	2.83	8.20
1-a-3	1.14	0.0013	1.76	1.73	7.98
1-b-1	11.93	0.0129	5.58	5.92	5.94
1-b-2	3.3	0.0036	2.93	2.83	6.20
1-b-3	1.07	0.0011	1.66	1.73	7.10
1-c-1	12.45	0.0134	5.70	5.92	7.92
1-c-2	3.11	0.0035	2.90	2.83	3.8
1-c-3	1.29	0.0014	1.87	1.73	4.5

Table 5 Electronic spectral data of complexes

Complex	Absorption band cm^{-1}	Proposed assignment
1-a-1	20150	${}^6A_{1g} \rightarrow {}^4T_{1g}$ (4G)
	22560	${}^6A_{1g} \rightarrow {}^4T_{2g}$ (4G)
	23485	${}^6A_{1g} \rightarrow {}^4E_g$ (${}^4T_{1g}$)
1-a-2	14240	${}^3A_{2g} \rightarrow {}^3T_{1g}$ (F)
	21650	${}^3A_{2g} \rightarrow {}^3T_{1g}$ (P)
1-a-3	14985	${}^2B_{1g} \rightarrow {}^2A_{1g}$
	20850	Charge transfer transition
1-b-1	20295	${}^6A_{1g} \rightarrow {}^4T_{1g}$ (4G)
	22370	${}^6A_{1g} \rightarrow {}^4T_{2g}$ (4G)
	23265	${}^6A_{1g} \rightarrow {}^4E_g$ (${}^4T_{1g}$)
1-b-2	14630	${}^3A_{2g} \rightarrow {}^3T_{1g}$ (F)
	21855	${}^3A_{2g} \rightarrow {}^3T_{1g}$ (P)
1-b-3	14520	${}^2B_{1g} \rightarrow {}^2A_{1g}$
	20745	Charge transfer transition
1-c-1	20290	${}^6A_{1g} \rightarrow {}^4T_{1g}$ (4G)
	22600	${}^6A_{1g} \rightarrow {}^4T_{2g}$ (4G)
	23140	${}^6A_{1g} \rightarrow {}^4E_g$ (${}^4T_{1g}$)
1-c-2	14880	${}^3A_{2g} \rightarrow {}^3T_{1g}$ (F)
	21610	${}^3A_{2g} \rightarrow {}^3T_{1g}$ (P)
1-c-3	14190	${}^2B_{1g} \rightarrow {}^2A_{1g}$
	20535	Charge transfer transition

Table 6 Antimicrobial activity of standards and solvent

Substance	Zone of inhibition (in mm)				
	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>Bacillus subtilis</i>	<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>
DMF	6	5	7	5	6
Penicillin	22	20	21	24	14
Ampicillin	17	20	25	22	10
Tetracycline	15	24	22	19	9
Chloramphenicol	21	19	19	26	17
Norfloxacin	23	18	25	19	12

Antimicrobial Activity

Heterocyclic compounds and complexes were screened for their antimicrobial activity using agar diffusion technique against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Escherichia coli* and *Klebsiella pneumonia* using DMF as a solvent (Jones et al., 1984; Barry, 1986; Xia et al., 2001, Kurtoglu et al., 2007; Rollas et al., 2007). The culture strains of bacteria were kept on agar slant at 37°C for 24 hrs. Nutrient agar plates were seeded with 0.1 ml of bacterial strain prepared in sterile saline (0.85 %) of 10⁵ CFU/ml dilution. For every bacterial strain, the wells (diameter 6 mm) were filled with 0.1 ml solution at fixed concentration of 25 µg/ml. All the plates were incubated at 37°C for 24 hrs. The inhibition zone was measured in mm. The activity was compared with known antibiotics like Penicillin, Ampicillin,

Tetracycline, Chloramphenicol and Norfloxacin. The activities of antibiotics and DMF are given in Table 6. The results were corrected for DMF. The results of antibacterial studies are shown in Table 7 and Table 8.

Results and Discussion

The Mn(II), Ni(II) and Cu(II) complexes are non-electrolytes as indicated by their molar conductivity values 3.8 to 7.10 Mhos cm² mol⁻¹.

The electronic spectra of Mn(II) complexes demonstrate three bands in the every region of 20150-20295, 22370-22600 and 23140-23485 cm⁻¹ which can be assigned as ⁶A_{1g} → ⁴T_{1g}(⁴G), ⁶A_{1g} → ⁴T_{2g}(⁴G) and ⁶A_{1g} → ⁴E_g(⁴T_{1g}) electronic transitions respectively. It indicates octahedral geometry of Mn(II) complexes. These complexes exhibit magnetic moment (μ_{eff}) from 5.58 to 5.94

Table 7 Antimicrobial activity of compounds

Complex	Zone of inhibition (in mm)				
	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>Bacillus subtilis</i>	<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>
1-a	14	17	12	13	5
1-b	9	13	15	10	8
1-c	11	8	12	13	4

Table 8 Antimicrobial activity of complexes

Complex	Zone of inhibition (in mm)				
	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>Bacillus subtilis</i>	<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>
1-a-1	7	13	11	6	6
1-a-2	10	11	17	10	10
1-a-3	12	16	8	7	8
1-b-1	5	12	8	6	12
1-b-2	11	9	14	8	4
1-b-3	15	10	10	11	5
1-c-1	13	6	12	8	5
1-c-2	14	16	11	5	7
1-c-3	9	8	6	11	6

BM. It is also supporting octahedral geometry of these complexes (Rahmouni, et al., 1999).

The electronic spectra of Ni(II) complexes exhibit two bands in the every region of 14240-14880 and 21610-21855 cm^{-1} which can be attributed to ${}^3A_{2g} \rightarrow {}^3T_{1g}$ (F) and ${}^3A_{2g} \rightarrow {}^3T_{1g}$ (P) electronic transitions respectively for octahedral configuration around central metal ion. These complexes demonstrate magnetic moments from 2.90-2.96 BM. It indicates octahedral geometry of these complexes.

In the electronic spectra, Cu(II) complexes reveal two bands in the every region of 14190-14895 and 20535-20850 cm^{-1} which can be credited to ${}^2B_{1g} \rightarrow {}^2A_{1g}$ and charge transfer transitions respectively signifying octahedral structure around Cu(II) ion. These complexes exhibit magnetic moment (μ_{eff}) from 1.66-1.87 BM. It indicates octahedral geometry of these complexes (Mane, et al., 2001).

Here, compound:1-a showed highest antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Compound 1-b showed highest activity against *Bacillus subtilis* and *Klebsiella pneumonia* while compound 1-c showed highest activity against *Escherichia coli*.

Highest inhibition of bacteria was noted for complex: 1-b-3 against *Staphylococcus aureus*. Both the complexes:1-a-3 and 1-c-2 exhibited highest inhibition against *Pseudomonas aeruginosa*. Here highest inhibition of bacteria was noted for complex: 1-a-2 against *Bacillus subtilis*. Complexes 1-c-3 and 1-b-3 showed highest inhibition against *Escherichia coli* while complex: 1-b-1 was very effective against *Klebsiella pneumoniae*.

Conclusion

The heterocyclic compounds (1-a to a-c) and its metal complexes (1-a-1 to 1-c-3) were synthesized and characterized. On the basis of stoichiometry,

electronic and magnetic data, octahedral geometry can be assigned to all the complexes studied. All the compounds showed moderate antibacterial activity against the pathogenic strains: *S. aureus*, *P. aeruginosa*, *B. subtilis*, *E. coli* and *K. pneumoniae*. The pronounced antimicrobial activity of the compounds may be due to the presence of the heterocyclic ring systems and the metal chelation. Thus, newly synthesized molecules exhibited good antimicrobial activity against bacterial species.

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