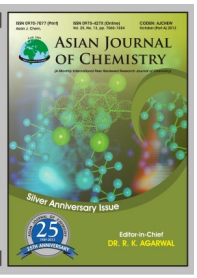
Asian Journal of Chemistry; Vol. 25, No. 15 (2013), 8351-8354

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2013.14738



Synthesis and Biological Evaluation of N'-(2-Hydroxybenzylidene)-1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carbohydrazide and Its Complexes with Cu(II), Ni(II), Zn(II) and Fe(III)

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(*Received*: 25 October 2012; *Accepted*: 21 August 2013) AJC-13948

A new chelating agent, N'-(2-hydroxybenzylidene)-1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carbohydrazide and its complexes with Cu(II), Ni(II), Zn(II) and Fe(III) have been synthesized and characterized on the basis of atomic absorption analysis, IR and UV-visible spectra, elemental analysis and conductance. The antibacterial, antifungal and scavenging activities of the ligands and its metal complexes have also been evaluated. In all the synthesized complexes the metal ligand ratio was found to be 1:1. Metal complexes possess antibacterial/antifungal activities better than the parent ligand while the ligand has better scavenging activity then it complexes.

Key Words: Chelating agent, Characterization, Hydrazone, Metal complexes, Scavenging.

INTRODUCTION

Quinolones constitute a large class of antibacterial agents that are highly effective in treatment of many types of infectious diseases particularly caused by bacteria[1]. The first clinically useful quinolone antibacterial agent *i.e.*, nalidixic acid, was synthesized in early 1960[2]. It has many advantages as a clinical drug and promoted a series of studies that led to the development of new quinolone antibiotics displaying remarkable potency against gram negative as well as gram positive and anaerobic organisms[3]. It has been suggested that transition metals like copper, nickel and others can form DNA interchelating complexes with quinolones, inhibiting metalloenzymes, causing cytotoxicity[4,5] and enhance the activity of drugs[6,7]. Thus metal complexes of nalidixic acid were synthesized in early 1970 and found to be better antibacterial agents[8,9]. Also a number of hydrazones originating from diverse acid hydrazides had been reported to possess, antimicrobial, anticonvulsant, analgesic, antiinflammatory, antiplatelet, antitubercular and antitumoral activities[10,11].

In the present study we have prepared hydrazone of nalidixic acid with salicylaldehyde and its metal complexes.

EXPERIMENTAL

All the chemicals used were of Analytical grade. Ethanol and methanol were however redistilled. Metal Salts were used as obtained from British Drug House.

Measurements: Melting points of all the compounds were taken on a Gallenkamp melting point apparatus and are uncorrected. Infrared (IR) spectra were obtained as KBr disc on a Hitachi FTIR-4300 or Thermo Nicolet FTIR-200 spectrometers. ^{1}H NMR was recorded on Bruker DPX-400 NMR spectrometer in $CDCl_3$ and chemical shifts are given in ppm downfield from tetramethylsilane (TMS) the internal standard. ^{13}C NMR spectra was recorded on Bruker Avance 400 spectrometer in $CDCl_3$ and chemical shifts are given in ppm. Mass spectra (MS) were taken again on a Jeol JMS-HX110 H spectrometer. Elemental analysis (C, H, N) of the synthesized compounds and complexes were carried out on Elemental analyzer (Carlo Erba) The synthesis of metal complexes were confirmed by taking their metal percentages on Atomic Absorption Spectrophotometer, AAnalyst 800 (Perkin- Elmer) by using Flame Technique on air-acetylene flame, (air 17L/min acetylene 2.0 L/min and characteristic wavelength for different metals) by using three standards 0.5, 1.0, 1.5 ppm for each metal. Absorbance λ_{max} was taken on UV-spectrophotometer, Analytikajena Specord 200 and chloride presence was determined by argentometric titration with $AgNO_3$. Conductance was taken on a Jenvey conductivity meter.

Methods

Synthesis of ligand: Methyl ester of nalidixic acid (methyl 7-methyl,1-ethyl, 4-oxo, 1,8-naphthyridine 3-carboxylate) (Fig. 1) and its hydrazide was synthesized following the

methods reported earlier[12-14]. To prepare the hydrazone, a mixture of nalidixic acid hydrazide (10.0 mmol; 2.46 g), salicylaldehyde (11.0 mmol; 1.34 g) and ethyl alcohol (30.0 mL) were heated under reflux for a period of 0.5 h. Then 3-5 drops of orthophosphoric acid was added and further refluxed for 10 min. The hydrazone was instantaneously formed. After completion of reaction as indicated by TLC (chloroform: methanol, 90:10), cold water was added into reaction flask, filtered and washed the precipitate with cold 1:1 ethanol:water mixture and dried in a vacuum oven (Fig. 2). The synthesized ligand has 90 % yield. m.p. 260 ºC, ^{1}H NMR:(400 MHz $CDCl_3$): δ 1.5 (3H, t, *J* = 5.95 Hz,-1CH_3), 2.68 (3H, S-7CH_3), 4.56 (2H, q, *J* = 7.17, 14.33 Hz,- CH_2), 6.99 (1H, d, *J* = 8.19 Hz, Ar-H3'-5'), 7.19 (4H, dd, *J* = 7.7 Hz, Ar-H, 3'-5'), 7.29 (1H, d, *J* = 8.22 Hz, Ar-H-6'), 8.62 (1H, d, *J* = 8.12 Hz, Ar-H-5), 8.33 (1H, s, Ar-H-6'), 8.97 1H, s, Ar-H-2'), 8.97 (1H, s, -N=CH), 11.28 (1H, s, -NH) 13.12 (1H, s, -OH-2'), MS m/z: $C_{19}H_{18}N_4O_3$ 350 [M^+], 215 [M^+-$NO_{22}C_{12}H_{11}$],187.1 [M^+-$ON_2C_{11}H_{11}$], 159.1 [M^+-$ON_2C_9H_6$].

Elemental analysis: m.f. $C_{19}H_{18}N_4O_3$ found (calcd. (%)): C 64.68 (65.14), H 4.96 (5.14), N 15.59 (16). ^{13}C NMR: (100 MHz $CDCl_3$): δ 15.2, 25.2, 47.1, 111.7, 117.1, 117.8, 119.1, 120.1, 121.6, 130.7, 131.3, 136.2, 147.8, 148.5, 150.0, 158.6, 161.0, 163.7, 176.6.

Synthesis of complexes with metal chloride: A mixture of 1.0 mmole of ligand, 30 mL of ethanol/methanol and 2.0 mmol of $CuCl_2{\cdot}2H_2O$, $NiCl_2{\cdot}6H_2O$, $ZnCl_2$ or $FeCl_3{\cdot}6H_2O$ were heated under reflux for a period of 4 h in the case of copper complex while 10-12 h in case of nickel, zinc and iron complexes.

The synthesized complexes, together with the ligand, their characteristics and other data are presented in Tables 1-3.

Determination of scavenging effect on DPPH radical: A stock solution of DPPH, [diphenyl 2-picryl hydrazyl] 0.1 mmol was prepared by dissolving 3.9 μmg in 100 mL of methanol: water (50:50). To test compounds solution one mL of stock solution were added, to achieve a concentration of 250 μg/mL. Then the samples were shaken vigorously and kept in dark

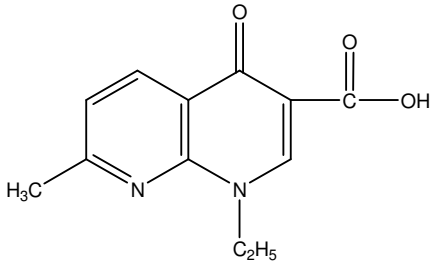


Fig. 1. Nalidixic acid

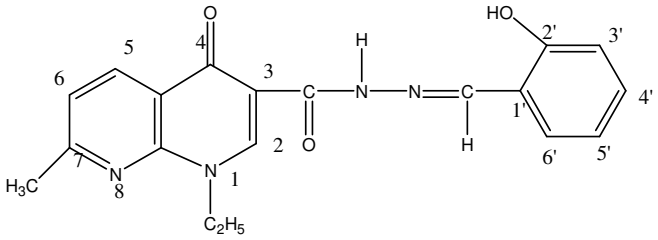


Fig. 2. Structure of ligand. N'-(2-hydroxybenzylidene)-1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carbohydrazide

for 0.5 h. The absorption of samples was measured on a spectrophotometer (Perkin Elmer UV-visible spectrophotometer) at 517 nm. All tests were run in triplicate on Averaged[15]. The results are given in Table-4.

Testing of antibacterial and antifungal activities: Hydrazone ligand and its synthesized complexes were tested for their antibacterial and antifungal activities against at concentration of 1 mg/mL in nutrient agar media employing the cup-plate method[16], having 50 μL in cup. The zone of growth inhibition of bacteria, produced by diffusion of compounds from cup into the surrounding medicine was measured after 24 h for bacteria and for fungal activity it was measured after 72 h and reported in Tables 5 and 6 after subtracting the values from blank (DMSO). Solutions of hydrazone and metal complexes were prepared in DMSO. Two gram negative bacteria (*E. coli, P. auroginosa*), two gram positive (*B. subtilus and S. aureus*) and two fungi (*A. niger, C. albicans*,) were

TABLE-1
PHYSICAL AND ANALYTICAL DATA OF NALIDIXIC ACID, LIGAND AND ITS COMPLEXES*

Compounds/f.w.	Solvent/pH during synthesis	m.f./symbol	Colour/solubility	m.p. (ºC)/λ_{max}	Yield (%)	Conductivity (2.8 × 10^{-6} molar soln.)
Nalidixic acid (NA) 232	–	$C_{12}H_{12}N_2O_2$/NA	Creamy white/$CHCl_3$	230/299	–	–
NSH(L)/350	E/7	$C_{19}H_{18}N_4O_3$/L	Pale white/hot $CHCl_3$	260/267	90	–
$[Cu(L)Cl_2(H_2O)_2]$/520	M/7	$C_{19}H_{18}N_4O_3CuCl_2$/L-Cu	Green/DMSO	>260/330	65	53.01 μs/cm
$[Zn(L)Cl_2]$/486	E/7	$C_{19}H_{18}N_4O_3ZnCl_2$/L-Zn	White/DMSO	>260/343	60	46.20
$[Ni(L)Cl_2(H_2O)_6]$/587	E/7	$C_{19}H_{22}N_4O_9NiCl_2$/L-Ni	Mustard/DMSO	>260/305	61	36.53 mS/cm
$[Fe(L)Cl_2]$/477	E/7	$C_{19}H_{18}N_4O_3FeCl_2$/L-Fe	black/DMSO	>260/302	55	34.13 mS/cm

Where *NSH= ligand nalidixic acid hydrazone of salicylaldehyde, NA = nalidixic acid, M = methanol E = ethanol, DMSO = dimethylsulfoxide, m.f. = molecular formula, m.w. = formula weight L= $C_{19}H_{18}N_4O_3$.

TABLE-2
IR SPECTRAL DATA OF THE NALIDIXIC ACID, LIGAND AND ITS COMPLEXES

Compds. No.	ν(C=O) 4th	ν(C=O) 3rd	ν(C=N)	ν(C-N)	ν(NCO^-)	ν(M-N)	ν(M-O)
NA ($C_{12}H_{12}N_2O_2$)	1710	1615	1517	1444	–	–	–
L ($C_{19}H_{18}N_4O_3$)	1680	1611	1531	1443	1498	–	–
L-Cu ($C_{19}H_{18}N_4O_3CuCl_2$)	1620	1609	1534	1449	1495	456	567
L-Zn ($C_{19}H_{18}N_4O_3ZnCl_2$)	1631	1608	1532	1447	1499	458	593
L-Ni ($C_{19}H_{22}N_4O_9NiCl_2$)	1630	1609	1530	1448	1496	456	590
L-Fe ($C_{19}H_{18}N_4O_3FeCl_2$)	1626	1609	1530	1447	1500	450	580

TABLE-3
INSTRUMENTAL DATA OF LIGAND AND ITS COMPLEXES

Compds. No.	Metal (%)		Elemental analysis (%)		
	Found	Calcd.	C (calcd.)	H (calcd.)	N (calcd.)
L ($C_{19}H_{18}N_4O_3$)	–	–	64.08 (65.14)	4.96 (5.14)	15.59 (16)
L-Cu($C_{19}H_{18}N_4O_3CuCl_2$)	12.43	12.11	42.92 (43.84)	4.16 (4.23)	9.75 (10.76)
L-Zn($C_{19}H_{18}N_4O_3ZnCl_2$)	9.91	9.98	37.99 (38.84)	4.71 (5.11)	10.29 (9.54)
L-Ni($C_{19}H_{22}N_4O_9NiCl_2$)	13.31	13.37	45.56 (46.91)	4.11 (4.52)	12.05 (11.52)
L-Fe($C_{19}H_{18}N_4O_3FeCl_2$)	11.17	11.55	47.82 (46.8)	3.77 (4.1)	11.74 (12.12)

L = Ligand calcd. = calculated Compds.= compounds.

TABLE-4
ANTIOXIDANT ACTIVITY OF COMPOUNDS 250 µg/mL
(BLANK-SAMPLE)/BLANK × 100 = INHIBITION (%)

Compounds	Absorbance at 517nm	Inhibition (%)
Blank	0.2305	–
NA	0.1048	54.53
L	0.1585	31.23
L-Cu	0.2023	12.24
L-Ni	0.1859	19.34
L-Zn	0.1650	28.41
L-Fe	0.1445	37.31

TABLE-5
ANTIBACTERIAL ACTIVITIES OF COMPOUNDS FOR
1 mg/mL ZONE OF INHIBITION IN MM DIAMETER

Comps.	*E. coli*	*P. auroginosa*	*B. subtilus*	*S. aureus*
NA	8.25	11.7	2.2	1.5
L	2.45	1.3	1.1	1.2
L-Cu	3.5	1.3	1.2	1.2
L-Ni	2.9	1.4	1.2	1.3
L-Zn	3.2	1.4	1.3	1.3
L-Fe	2.6	1.3	1.2	1.3

Signifies the Zone of inhibition near blank.

TABLE-6
ANTIFUNGAL ACTIVITIES OF COMPOUNDS FOR
1 mg/mL ZONE OF INHIBITION IN MM DIAMETER

Compounds	*A. niger*	*C. albicans*
NA	3.70	3.30
L	0.60	0.60
L-Cu	0.63	0.61
L-Ni	0.61	0.62
L-Zn	0.65	0.61
L-Fe	0.60	0.61

selected. Nalidixic acid, the starting compound and effective drug for UTI, was used as standard and DMSO as a blank.

RESULTS AND DISCUSSION

The reaction of hydrazone ligand and the metal complexes from different metal salts were found to have a 1:1 metal ligand stoichiometry. The ligand was soluble in hot chloroform but the complexes were insoluble in common organic solvents such as acetone, chloroform and benzene but were fairly soluble in DMF and DMSO, melting point of ligand was 260 ºC but all the prepared complexes were above 260 ºC. Low conductivity values as shown in table, represent non conducting behaviour of complexes. All complexes are intensively coloured.

IR Spectra: The IR spectral data of compounds and their tentative assignments are shown in Table-2. It can be seen that the characteristic absorption peaks in the IR spectra of all of complexes are similar, which indicated that complexes have similar structures and following conclusions may be drawn:

The IR spectra of all the complexes show that ν(C=O) (α-β unsaturated ketone) 4-oxo group in spectra of free ligands had shifted to lower frequency in all metal complexes but frequency for 3rd ν(C=O) remains the same in ligand and in all complexes which showed that it does not take part in coordination with metal. This was further confirmed by band at 1498 in ligand and complexes assigned to NCO^-. The band for C=N, C-N almost constant in ligand and complexes which indicates that the naphthyridine nitrogens did not involve in complex formation. M-N and M-O Coordination bonds were present in all complexes in the range of 470-430 and 593-567, respectively[17]. The bands at *ca.* 3430 cm^{-1} in ligand and all complexes show the presence of -OH group which indicates -OH at position 2', do not coordinate with metal[18].

Atomic absorption spectrophotometric (AAS) studies: Table-3 shows atomic absorption spectrophotometric data of complexes. The synthesis of complexes was confirmed by atomic absorption spectrophotometry. Pre weighed and oven dried samples were digested in nitric acid and then volume was made up to 100 mL then it was subjected to AAS technique for determination of metal percentage in complexes. Then the possibilities of LM, LMCl, $LMCl{\cdot}H_2O$, $LMCl_2$, $LMCl_2{\cdot}2H_2O$, L_2M, $LMCl{\cdot}2H_2O$ was checked. All complexes have ligand: metal ratio of L:M 1:1,in the form of $LMCl_2{\cdot}nH_2O$ in chloride salts and acetate salts were $LM(CH_3COO)_2{\cdot}nH_2O$. This ratio was confirmed by elemental analysis and IR spectra. The AAS studies confirmed that Fe(III) salt was used in complex synthesis but it also gave $LFeCl_2$ complex.

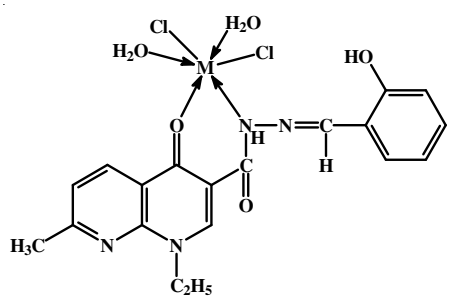
UV-Visible: To 10-20 mg/L solution of ligand and complexes in DMSO, λ_{max} was studied showing that ligand has λ_{max} at 267 nm whereas the complexes have 330 nm for copper, 343 nm for nickel complex 305 nm and 302 for zinc and iron complexes, respectively.

Biological activities: For antibacterial activities two gram negative bacteria, *Escherichia coli* and *Pseudomonas auroginos* and two gram positive bacteria *Bicillus subtillus* and *Stephylocus aureus* were taken (Table-5) while for antifungal activities *Aspergillus niger* and *Candida albican* (Table-6) were selected. It was observed that presently synthesized hydrazone had less antibacterial and antifungal activities than Nalidixic acid. Metal complexes on the other hand had better anti bacterial and antifungal activities than the hydrazone ligand. Antioxidant properties of the ligand and its metal complexes are shown in (Table-6), which indicated that the ligand has better scavenging activity then its metal complexes.

Conclusion

From the preceding discussion following conclusions can be drawn. The salicylaldehyde hydrazone ligand forms stable

complexes with Cu, Ni, Fe and Zn salts. A proposed structure of the complexes is presented in the Fig. 3. Hydrazone was found to be bidentate ligand. The metal ions are coordinated by ligand with one oxygen of 4-oxo group and other nitrogen of hydrazone group at outside of ring systems. The complexes have better antibacterial and antifungal activities as compared to the ligand. The ligand has much better scavenging ability than its metal complexes.



[M=Cu/Zn/Ni/Fe]

Fig. 3. Coordination mode of metal complexes (arrow indicates coordinate covalent bond and straight line indicate covalent bond)

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