Synthesis, Spectroscopic Characterizations, and Biological Evalution of Some Substituted 1-Ethyl-1,4-Dihydro-7-Methyl-4-Oxo-1,8-Naphthyridine-3-Carbohydrazide and their Copper Complexes

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Summary: Different analogs of 1-ethyl-1, 4-dihydro-7-methyH-4-oxo-1, 8-naphthyridine-3-carbohydrazide **(**carbonyl hydrazone derivatives of Nalidixic acid, H 1-10) and their copper complexes have been synthesized from reaction of Nalidixic acid hydrazide with substituted benzaldehyde. These are characterized on the basis of IR spectra, ^{1}H-NMR spectra, Mass spectra, elemental and AAS studies for metals. Copper complexes were prepared from $CuCl_2.2H_2O$ and these ligands, the metal ligand ratio was found to be 1:1 and found to be bidentate. The antibacterial, antifungal and antioxidant activities of these hydrazone ligands and their copper complexes have also been evaluated. Copper complexes possess antibacterial and antifungal activities better than the parent hydrazone ligands while they have better antioxidant activity with DPPH radical than copper complexes. Nalidixic acid have maximum of all biological activities, but H-6 showed slightly better antioxidant activity than Nalidixic acid and other activities were close to it.

Keywords: Synthesis, Biologic evaluation, Complexes, IR Spectra, ^{1}H-NMR Spectra.

Introduction

Substituted heterocyclic compounds having N, S, O in ring systems, offer a high degree of structural diversity that broadly proved its therapeutic uses [1]. Further fused heterocyclic system containing one or more nitrogen in ring systems are marked among the most versatile bioactive compounds such as fungicidal, insecticidal [2] herbicidal [3] and virucidal [4-5]. One of such system of compounds having two fused pyridine rings is commonly known as naphthyridines. Derivatives of 1, 8-naphthyridine have been investigated for a long period of time due to their interesting complexation properties and medicinal uses. These compounds are known to possess antibacterial, anti-inflammatory, anti-hypertensive, and anti-platelet activities. They have also been widely utilized as molecular recognition receptors for urea, carboxylic acids and guanine. Few 1, 8-naphthyridine derivatives have been reported to be excellent fluorescent markers of nucleic acids and probe molecules.

Among derivatives of carbonyl compounds (carboxylic acids, ketones, aldehydes, ester etc) hydrazones are important class, which often is found to be a constituent of biologically active compounds. They are obtained by the reaction of aromatic and heterocyclic hydrazide with mono and dialdehydes or ketones [6]. It was investigated that hydrazones have discovered to possess, antimicrobial, anticonvulsant, analgesic, anti-inflammatory, antiplatelet, antitubercular and antitumoral activities [7-10]. Quinolones constitute a large class of antibacterial agents that is highly effective in the treatment of many types of infectious diseases particularly caused by bacteria [11]. Nalidixic acid was the first developed compound of this family of 1,8-naphthyridines. It was described by Lesher and his coworkers in [12] and since then it has been used extensively in therapy [13-15]. After the discovery of first quinolone, Nalidixic acid, medicinal and industrial chemists have made numerous structural modification and new quinolones [16-17] have continually been made, in the hope of incremental microbiological advantages over Nalidixic acid, displaying remarkable potency against gram negative as well as gram positive and anaerobic organisms [18].

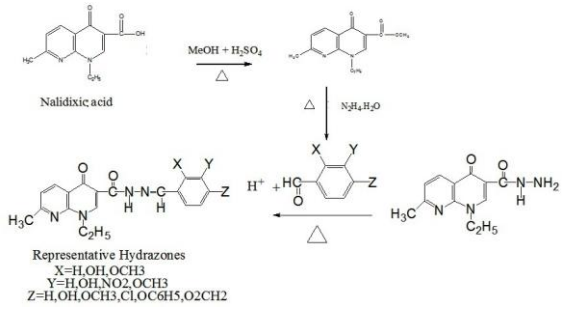
We have synthesized various derivatives of Nalidixic acid hydrazide which have two fused pyridine rings (1,8-naphthyridines) ring nitrogen, hydrazone group - N-N=C- and ketone group in the system. As literature reveals that these groups must have various biological activities due to lone pairs on nitrogen and oxygen atoms. Some of substituted 1, 8-naphthyridines along with their copper complexes and their biological activities are reported in this research paper.

Experimental

All the chemicals used in these studies were purchased from E. Merck, BDH or Fluka and employed without purification. However, solvents were purified through drying with appropriate drying agents (CaO for ethanol /methanol and $CaCl_2$ for chloroform) followed by distillation just before use. Melting points of all the compounds were taken on a Gallenkamp melting point apparatus and are uncorrected. Infrared (IR) spectra were obtained on a Thermo Nicolet FTIR-200 spectrometer, in the 4000—400 cm^{-1} regions. ^{1}H-NMR spectra were recorded on a Bruker DPX-400 NMR spectrometer in DMSO-d6 or $CDCl_3$ and chemical shifts are given in ppm downfield from tetramethyl silane (TMS) as the internal standard. Mass spectra (MS) were taken on a Jeol JMS-HX110 H spectrometer. Elemental Analysis (C ,H, N) of the synthesized compounds was carried out on an Elementar microanalyzer.The copper analysis was carried out on Atomic Absorption Spectrophotometers Perkin Elmer Aanalyst 800. The completion and progress of all the reactions were monitored by thin layer chromatography (TLC) which was performed on glass plates and aluminum sheets pre-coated with silica gel (Merck, Kiselgel 60 F-254 0.25mm). Spots were detected with a model UVGH-25 Mineralight multi-band UV-254/366 nm lamp.

Synthesis of Ten Hydrazone Ligands

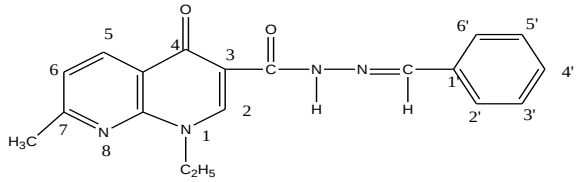
Firstly Nalidixic acid hydrazide was prepared by reaction of Nalidixic acid,hydrazinium hydroxide and methanol. Then equimolar quantities of Nalidixic acid hydrazide and ten substituted benzaldehyde were refluxed in the presence of methyl alcohol for a period of 30 minutes. Then 3-5 drops of concentrated HCl was added and further refluxed for 4 h. After completion of the reaction as indicated by TLC (Chloroform: methanol, 90:10) three fourth of the solvent was evaporated and contents were cooled down to room temperature. The synthesized Hydrazone Ligand was filtered and washed 2-3 times with hot 1:1 ethanol: water quantity and dried in a vacuum oven.



Scheme For all Hydrazone Ligands

Hydrazone Ligand H-1

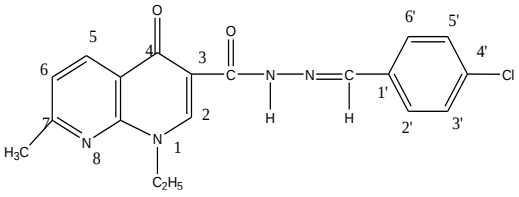
N'-benzylidene-1- ethyH-1,4-dihydro-7-methyH-4-oxo-1,8-naphthyridine-3-carbohydrazide.



An equimolar quantity of Nalidixic acid hydrazide (10.0 mmol; 2.46 g), benzaldehyde (11.0mmol; 1.17 g) and ethyl alcohol (30. mL) was refluxed with 3-5 drops of conc.HCl for 4 h. Yield:1.63g,(45%),M.P:258°C,Color:off-white,Solubility:$CHCl_3$,Mass:MS m/z :334.1 [M^+], 215.0 [M^+ -C_7H_8 N_2 -100%]188.1[M^+ - C_7H_8 N_2CO-60 %]159.0[M^+ - C_7H_8 N_2C OC_2H_5 -13.9%],^{1}H-NMR:(400M*Hz*$CDCl_3$)δ1.49(3H,t,J=5.72*Hz*,-1CH_3)2.69(3H,S,-7CH_3)4.56 (2H,q,J=5.69,11.45 *Hz*, -1CH_2)7.79 (1H,s, Ar-H) 7.31(4H,dd,J=5.49 *Hz*,Ar-H-2'-5')7.80(1H,d, J=2.24*Hz*,Ar-H,6),8.21(1H,s,Ar-H,2)8.63(1H,d,J=6.49*Hz*,Ar-H,5)9.01(1H,s,-N=CH)13.10(1H,s,-NH)ppm .

Hydrazone Ligand H-2

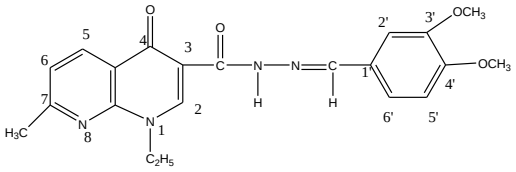
N'-(4-chlorobenzylidene)-1-ethyH-1,4-dihydro-7-methyH-4-oxo-1,8-naphthyridine-3-carbohydrazide.



An equimolar quantity of Nalidixic acid hydrazide (10.0 mmol; 2.46 g), p -chloro benzlaldehyde (10.0 mmol; 1.40 g) and ethyl alcohol (30 mL) was refluxed with 3-5 drops of ortho phosphoric acid for 4 h. Yield 1.85g (48%) M.P:>270°C, Color: white, Solubility: $CHCl_3$, MassMSm/z:368.2[M^+]215.[M^+-$C_7H_7N_2Cl$]188.1[M^+-$C_7H_7N_2ClCO$]159[M^+-$C_7H_7N_2ClCOC2H5$], ^{1}H-NMR:(400 M *Hz* $CDCl_3$) δ1.50(3H,t, J=7.16 *Hz*,-1CH3) 2.70 (3H,S ,-7CH3)4.56(2H,q,J=7.268,13.258*Hz*,-1CH_2)8.63(1H,d,J=8.108*Hz*,Ar-H-5)8.18(1H,s,Ar-H-2)7.310(4H,dd,J=8.29*Hz*,Ar-H2',3',5',6')7.23(1H,d,J=8.388*Hz*,Ar-H-6)9.00(1H,s,-N=CH),13.14 (1H,s,-NH)ppm.

Hydrazone Ligand H-3

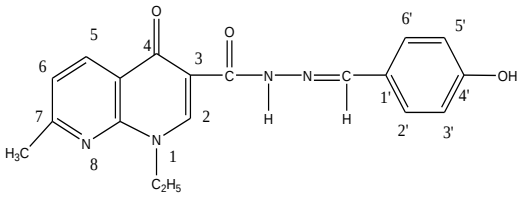
N'-(3,4-dimethoxybenzylidene)-1-ethyH-1,4-dihydro-7-methyH-4-oxo-1,8-naphthyridine-3-carbohydrazide



An equimolar quantity of Nalidixic acid hydrazide (10.0 mmol; 2.46 g), 3,4-dimethoxy benzaldehyde (10.0 mmol; 1.66g) and ethyl alcohol (30mL) was refluxed with3-5 drops of orthophospheric acid for 4 h. Yield .2.55g(62%) M.P.: 258ºC, Colour: offwhite, Solubility$CHCl_3$, Mass: MSm/z:394.2[M^+]215[$M^+C_9H_{12}O_2N_2$] 188[$M^+COC_9H_{12}O_2N_2$]159[$M^+C_9H_{12}O_2N_2COC2H5$],[1] H-NMR(400 M *Hz* $CDCl_3$):δ:1.49(3H,t, J=7.13 *Hz*,-1CH3) ,2.69(3H,S ,-7CH3) 3.82 (3H,s,-OCH_3,)3.83(3H,s,-OCH_3) 4.5 (2H,q, J=7.13,14.31 *Hz* ,-1CH_2) 6.83 (1H,d, J=8.05 *Hz*,Ar-H-6),7.29 (1H,d, J=8.18*Hz*,, Ar-H-5')7.53(1H,d,J=1.64*Hz*,,Ar-H-6')8.13(1H,s,Ar-H-2') 8.62(1H,d, J=11.74 Hz,Ar-H-5) 8.99(1H,s, Ar-H-2)9.01 (1H,s,-N=CH) 12.98 (1H,s,-NH) ppm.

Hydrazone Ligand H-4

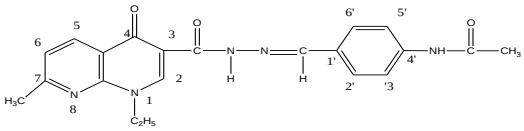
N'-(4-hydroxybenzylidene)-1-ethyH-1,4-dihydro-7-methyH-4-oxo-1,8-naphthyridine-3-carbohydrazide



An equimolar quantity of Nalidixic acid hydrazide (10.0 mmol; 2.46 g), 4-hydroxy benzaldehyde (11.0 mmol; 1.34 g) and ethyl alcohol (30 mL) was refluxed with3-5 drops of orthophospheric acid for two h. Yield 1.44 g(38 %)M P: >270ºC,Colour:off-whiteSolubility:$CHCl_3$, Mass: MS m/z:350[M^+]215.1[M^+-$C_7H_8N_2O$]188 [M^+ -C_7H_8 N_2OCO] 160[$M^+C_7H_8N_2OCOC2H5$], [1]H-NMR :(400 M *Hz* $CDCl_3$): δ :1.38(3H,t,J=9.2 *Hz*,-1CH3)2.6 (3H,S,-7CH3)4.57(2H,q,J1=9.2*Hz*,J2=13.26*Hz*,-1CH_2) 8.55(1H,d,J=10.8*Hz*,Ar-H-5) 8.27(1H,s,Ar-H-2)7.48(4H,dd,J=11.2*Hz*,Ar-H2',3',5',6') 6.81(1H,d, J=11.2*Hz*,Ar-H-6)s9.07 (1H,s,-N=CH) 9.93(1H,s,-NH)12.91(1H,s,-OH-4') ppm.

Hydrazone Ligand H-5

N'-(4-acetamidobenzylidene)-1-ethyH-1,4-dihydro-7-methyH-4-oxo-1,8 naphthyridine-3-carbohydrazide

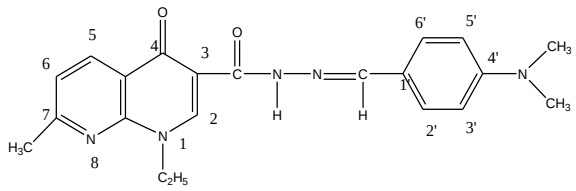


(6)

An equimolar quantity of Nalidixic acid hydrazide (10.0 mmol; 2.46 g), 4-acetamido benzaldehyde (10.0 mmol; 1.47 g) and ethyl alcohol (30 mL) was refluxed with3-5 drops of orthophospheriacidfor6-8h. Yield:1.57g (40%)M.P:>270ºC,Colour: offwhite, Solubility: $CHCl_3$, Mass: MSm/z:391.2[M^+]215[M^+-$C_8H_{11}N_3O$]187[M^+-$C_8H_{11}N_3OCO$] 160[$M^+C_8H_{11}N_3OCOC2H5$], [1]H-NMR: (400 M *Hz*$CDCl_3$):δ:1.38(3H,t, J=9.6 *Hz*,-1CH3)2.49 (3H,S ,-7CH3) 3.32 (3H,s,NCO-CH_3)4.58 (2H,q,J1=9.2*Hz*, J2=18.8*Hz*,-1CH_2)8.58(1H,d,J=10.8*Hz*,Ar-H-5)9.09(1H,s,Ar-H-2)7.310(4H,dd,J=8.3*Hz*,Ar-H2',3',5',6')7.5(1H,d,J=10.8*Hz*,Ar-H-6),10.13(1H,s,-N=CH),13.012 (1H,s,-NH)ppm .

Hydrazone Ligand H-6

N'-(4-(dimethylamino) benzylidene)-1-ethyH-1,4-dihydro-7-methyH-4-oxo-1,8-naphthyridine-3-carbohydrazide

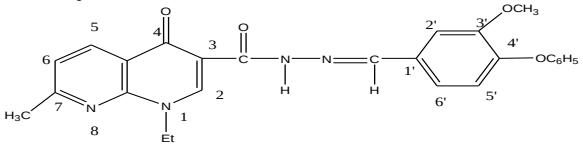


(7)

An equimolar quantity of Nalidixic acid hydrazide (0.01 mmol ; 2.46 g), 4 -N,N-dimethyl benzaldehyde (10.0 mmol; 1.49 g) and ethyl alcohol (30 mL)was refluxed with3-5 drops of orthophospheric acid for 6-8 h. Yield2.17g(55%)M.P:>300ºC,Colour:orange, Solubility:$CHCl_3$,Mass:MSm/z:377[M^+]215[M^+-$C_9H_{13}N_3$]187[M^+-$C_9H_{13}N_3OC$]158[M^+-$COC2H5C_9H_{13}N_3$], [1]H-NMR(400 M *Hz* $CDCl_3$)δ :1.49(3H,t, J=5.72 *Hz*,-1CH3)1.67(3H,S ,-7CH3)2.68(3H-S-4'-NCH_3) 2.99 (3H-S-4'-NCH_3)4.54 (2H,q,J1=5.7*Hz*, J2=11.43*Hz* ,-1CH_2) 8.63(1H,d,J=8.11*Hz*,Ar-H-5)8.11(1H,s,Ar-H-2)6.66(4H,dd,J=6.74*Hz*,Ar-H2',3',5',6')7.66 (1H,d, J=8.39 *Hz*,Ar-H-6)9.00(1H,s,-N=CH) 12.90 (1H,s,-NH)ppm.

Hydrazone Ligand H-7

N'-(3-methoxy-4-phenoxybenzylidene)-1-ethyH-1,4-dihydro-7-methyH-4-oxo-1,8-naphthyridine-3-carbohydrazide

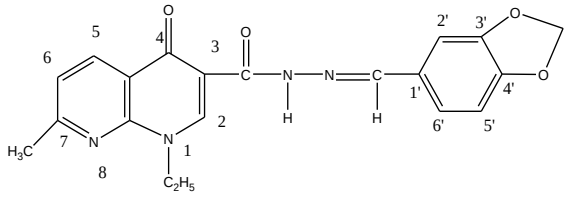


(08)

An equimolar quantity of Nalidixic acid hydrazide (10.0 mmol; 2.46 g), 3-methoxy 4-benzoxy benzaldehyde (10.0 mmol; 2.28 g) and ethyl alcohol (30 mL)was refluxed with3-5 drops of orthophospheric acid for 6-8 h. Yield 2.65g (56 %) M P: 240 ^{o}C Colour: yellow Solubility: $CHCl_3$, Mass:MS m/z :456[M^+],379[M^+-C_6H_5] 215 [M^+-$C_{12}H_{14}$ N $_2$ O_2]. 187 [M^+ --$C_{12}H_{14}$ N $_2$ O_2CO]. 160[M^+ -$C_{12}H_{14}$ COC2H5N $_2$ O_2], ^{1}H-NMR (400 M *Hz* $CDCl_3$):δ : 1.49(3H,t, J=5.7 *Hz*,-1CH3) 2.69(3H,s,-7CH3)3.96(3H,s,-OCH_3) 4.56 (2H,q, J=5.68 ,11.44 *Hz* ,-1CH_2) 7.24-7.42(5 H,-OC6H5)7.56 (1H,d, J=1.12 *Hz*,,Ar-H-6)6.84 (1H,d, J=6.58 *Hz*,,Ar-H-5')7.03 (1H,d, J=1.04 *Hz*,,Ar-H-6')5.17(1H,s,Ar-H-2') 8.63 (1H,d, J=6.48 *Hz*,Ar-H-5) 8.13(1H,S ,Ar-H-2) 9.00 (1H,s,-N=CH) 13.03(1H,s,-NH)ppm.

Hydrazone Ligand H-8

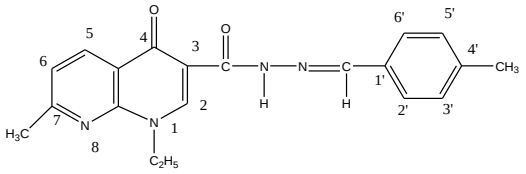
N'-((benzo[d][1,3]dioxoH-5-yl)methylene)-1-ethyH-1,4-dihydro-7-methyH-4-oxo-1,8-naphthyridine-3-carbohydrazide



An equimolar quantity of Nalidixic acid hydrazide (10.0 mole ; 2.46 g), 3-4 methylene dioxy benzaldehyde (10.0mmol; 1.49 g) and ethyl alcohol (30 mL)was refluxed with3-5 drops of orthophosphoric acid for 6-8 h. Yield 2.29 g (58 %), M P: >270^{o}C , Colour : off- white, Solubility:DMF /DMSO, Mass: MS m/z :378 [M^+]215[M^+-$C_{18}H_8$ N $_2$ O_2]187 [M^+-$C_{18}H_8$ N $_2$ O_2CO]159[M^+ -COC2H5$C_{18}H_8$ N $_2$ O_2], ^{1}H-NMR (400 M *Hz* $CDCl_3$): δ 1.03(3H,t, J=7.316 *Hz*,-1CH3) 2.35(3H,S ,-7CH3)3.32(2H,s,O_2-CH_2)4.59 (2H,q, J=5.88*Hz*, -1CH_2)7.51 (1H,d, J=6.57 *Hz*,Ar-H-6)7.21(1H,d, J=6.0 *Hz*,Ar-H-6') 8.38(1H,s,Ar-H-2')8.58 (1H,d, J=6.52 *Hz*,Ar-H-5)6.99 (1H,d, J=6.23 *Hz*,Ar-H-5') 9.08 (1H,S ,Ar-H-2) 10.51 (1H,s,-N=CH) 12.99(1H,s,-NH)ppm.

Hydrazone Ligand H-9

N'-(4-methylbenzylidene)-1-ethyH-1, 4dihydro-7-methyH-4-oxo-1, 8-naphthyridine-3carbohydrazide

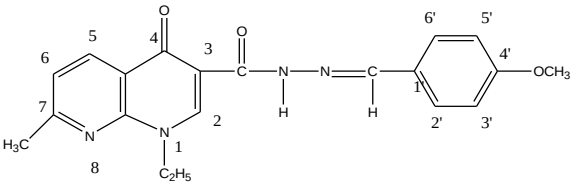


An equimolar quantity Nalidixic acid hydrazide (10.0 mmol ; 2.46 g), toulo-aldehyde (10.0 mmol; 1.20g) and ethyl alcohol (30 mL)was refluxed with 3-5 drops of orthophospheric acid for 6-8 h.Yield:2.45g(67%)MP:>300^{o}C,Color:deepyellow,SolubilityCHCl$_3$,Mass:MS m/z:348[M^+]215[M^+-$N_2C_8H_{10}$]187[M^+-CO N $_2C_8H_{10}$]159[M^+ N $_2C_8H_{10}$C OC_2H_5], ^{1}H-NMR(400 M *Hz* $CDCl_3$): δ:1.49(3H,t,J=5.72*Hz*,-1CH3)2.36(3H,s,Ar-CH3,4')2.69(3H,S,-7CH3)4.56 (2H,q,J1=5.7*Hz*,J2=11.42*Hz*,-1CH_2)8.63

(1H,d,J=6.51*Hz*,Ar-H-5)8.18(1H,s,ArH_2)7.18(4H,dd,J=6.4*Hz*,Ar-H2',3',5',6')7.68(1H,d,J=6.36*Hz*,Ar-H-6) 9.00(1H,s,-N=CH) 13.04 (1H,s,-NH)ppm .

Hydrazone Ligand H-10

N'-(4-methoxybenzylidene)-1-ethyH-1,4-dihydro-7-methyH-4-oxo-1,8-naphthyridine-3-carbohydrazide

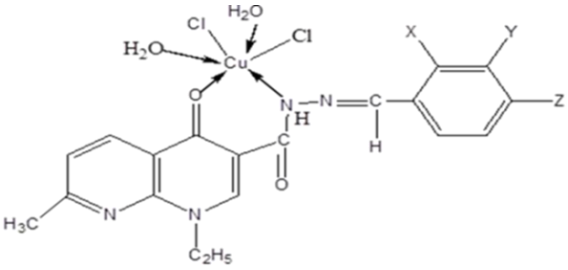


An equimolar quantity of Nalidixic acid hydrazide (10.0 mmol; 2.46 g), P- anisaldehyde (10.0mmol; 1.36 g) and ethyl alcohol (30 mL) was refluxed with 3-5drops of orthophospheric acid for 6-8 h. Yield:2.29 g (60%), M P: > 290^{o}C, Color :white, Solubility: $CHCl_3$, Mass: MS m/z: 365.14 [M^+]215[M^+-ON $_2C_8H_{10}$]187 [M^+-CO_2N $_2C_8H_{10}$]159[M^+ - ON $_2C_{10}H_{15}$], ^{1}H-NMR:(400 M *Hz*

$CDCl_3$): δ :1.48(3H,t, J=9.6 *Hz*,-1CH3) 2.69 (3H,S ,-7CH3) 3.82(3H,s, -OCH3,4') 4.54 (2H,q, J=9.6 ,18.8 *Hz* ,-1CH_2) 8.62 (1H,d, J=10.8 *Hz*,Ar-H-5) 8.16(1H,s,Ar-H-2) 6.88 (4H,dd,J=11.2 *Hz* ,Ar-H 2',3',5',6')7.72(1H,d, J=11.6 *Hz*,Ar-H-6)9.00(1H,s,-N=CH)13.01 (1H,s,-NH)ppm.

Synthesis of Copper Complexes of Hydrazone Ligands With Copper Chloride

1 mmol of all ligands H-1-H-10 and 2 mmole of hot ethanolic/ methanolic/THF solution of $CuCl_2.2H_2O$ was refluxed for a period of 3-6 h. After completion of the reaction, cooled down and reduced the volume and then added ice into the reaction flask. The complexes were settled down after 24 h in the form of precipitates. Washed the ppt. of complex 3-4 times with 1:1 hot ethanol: water quantity, kept the ppt. in desicator. Copper complexes of ligands H 1 - 10 were successfully prepared by adopting above procedure.



(X, Y =H,OH,OCH_3 Z= H,OH,OCH_3,Cl)

This is the proposed structure of copper complexes of all hydrazone ligands formed by the reaction of ligands and copper chloride having 1:1 metal: ligand stoichiometry and also depicts the coordination mode of metal complexes. Arrows indicate coordinate covalent bond and straight line indicate covalent bond. This structural formula is supported by IR spectra of hydrazone and copper complexes obtained.

Biological Activities

Synthesized Hydrazone ligands and their copper complexes were tested for their antibacterial and antifungal activities by *E. coli* and *Espergillus niger* respectively, and antioxidant activity was measured through scavenging of DPPH radical. All tests were run in triplicate and reported on average.

Testing of Antibacterial Activities by Turbidimetric Method

Escherichia coli were selected for testing of antibacterial activity of hydrazones and their copper complexes. 1mg/5mL of hydrazones and copper complexes were first prepared in DMSO then added to nutrient broth, After autoclaving of test tubes having test solution, an inocculum of E. Coli (20 ul) prepared in saline solution was added to each test tube. Initial absorbance of each tested solution was taken at 600 nm [19-21] afterwards placed them in shaking incubator at 37 ^oC. After 24 h other absorbance was taken at 600 nm and then % age inhibition of ligands and their copper complexes were calculated from removal of turbidity. Nalidixic acid was taken as reference to measure the comparative inhibition.

Testing of Antifungal Activities by Turbidimetric Method

Aspergillus niger was selected for the antifungal activity of hydrazone ligands and their copper complexes at concentration of 1mg/5mL in nutrient broth. 1mg/mL solutions of all hydrazones and complexes were first prepared in DMSO, then added 4mL nutrient broth in each test tube with concentration of 200ug/mL, after autoclaving of test tubes having test solution, an inocculum of *Espergillus niger* (20 ul) prepared on slant of agar was added to each test tube. Take initial absorbance at 550 nm,then keep test tube in shaking incubator at 26 +_ 2 ^0C. After 72 h readings were taken at 550 nm [21] and noted the % inhibition of ligands and their copper complexes from removal of turbidity. Nalidixic acid was taken as a reference to measure the comparative inhibition.

Determination of antioxidant activity by Scavenging DPPH radical

A stock solution of DPPH, [diphenyl 2-picryl hydrazyl] 0.1mmol was prepared by dissolving 3.9 µg in 100 mL of methanol: water (50:50). To all tested compound solution 1mg/ mL in DMSO then 3mL of stock solution was added, to achieve a concentration of 250 µg/mL. Then the samples were shaken vigorously and kept in the dark for 0.5 h. The absorption of the samples was measured on a spectrophotometer (Perkin Elmer UV-visible spectrophotometer) at 517 nm and noted the change in purple color of DPPH radical [22-23]. The percentage inhibition was determine by using following equation,

$$\%\ inhibition = \frac{(Blank - Sample) \times 100}{Blank}$$

Results and Discussion

The structure of hydrazone ligands and their copper complexes were found to have a 1:1 metal ligand stoichiometry. The hydrazone ligands were

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. All complexes are intensively colored mostly green and blue. Melting points of hydrazone ligands were mostly >270 and copper complexes >300 ^{0}C.

IR Spectra

The characteristic absorption peaks (Table 1) for all hydrazones are similar, which indicate that they have similar structures. The IR spectral data showed that two very strong, distinct peaks were found in all ligands and Nalidixic acid as well, one at ~1680cm^{-1}, and other at ~ 1610 cm^{-1} attributed to carbonyl at 3rd position and ketone at 4th position respectively, in all hydrazone ligands. A strong IR absorption peak is also observed at ~ 1498cm^{-1} in all Hydrazone Ligands and their complexes, assigned to (NCO^-) [24] but not observed in Nalidixic acid, which suggested the enolic coordination mode of ligands for complexation with metal atoms. Comparing the IR bands obtained from Nalidixic acid and our synthesized ligands, two other distinct peaks are obtained at ~1444- and ~1517 cm^{-1}, which are assigned to -C =N and –C-N respectively, for naphthyridine ring system in Nalidixic acid and hydrazones in the same manner. These peaks have shifted to higher frequencies~4 and ~ 13 cm^{-1} respectively, after hydrazones formation from Nalidixic acid. The bands at ~3430 cm-1 in some ligands and showed the presence of hydroxyl group [24].

On observing table-1for IR of copper complexes, it was revealed that the complexation involves the oxygen of oxo -group at 4th position and N- of hydrazone group .During these complex formation, it seems that the ligands react through their enolic form because a strong peak appeared in all ligands, at ~1498 cm^{-1}, due to –NCO^- shifted towards lower frequency 1452 cm^{-1} after complex formation, this C=O was not involved in metal complexation. The bands for -C =N and –C-N in naphthyridine ring were almost same at ~1444- and ~1517 cm^{-1} respectively in spectral data of Ligands and their copper complexes.

Furthermore the IR spectra of the all complexes showed two new absorption bands assigned to the vibration of the metaH-ligand bonds, M-N at430-470 and M-O at 550-595cm^{-1} respectively [24]. These peaks are absent in Ligands. The absorption bands at ~3430-3600 cm^{-1} in almost all complexes, can be assigned to the vibration of coordination water molecules.

^{1}H-NMR Spectra

The ^{1}H-NM Spectra of Hydrazone Ligands (H-1-H-10) showed protons of ethyl –CH_3 as triplet and as quartet for –CH_2 at1st position in all ligands.The ^{1}H-NMR Spectra exhibited Ar- CH_3 protons of 7th position of naphthyridines as singlet for all hydrazones. The rest of the naphthyridine protons in all hydrazones, a very small difference in δ values was observed and showed two doublet (5,6 position) and one singlet (2nd position) at values between δ9.09-7.22ppm.The ^{1}H-NMR Spectra showed the –OH proton in one hydrazone at δ ~ 13.12ppm.The two protons of -HN-N=CH in all hydrazones appeared as singlet at δ~ 9.00 and δ ~ 8.9ppm respectively. The methoxy protons in case of H-3 appeared as singlet at δ 3.82 and 3.83 ppm respectively.

Table-1: IR spectral data of Hydrazone ligands and their copper complexes.

Compounds no.	υ(C=O)4th	υ(C=O) 3rd	υ(C=N)	υ(C-N)	υ(NCO^-)	υ(M-N)	υ(M-O)
NA	1710	1615	1517	1444	---	----	-----
H-I	1673	1618	1524	1442	1495	----	----
H-1-$CuCl_2$	1650	1619	1524	1442	1494	459	592
H-2	1680	1608	1526	1440	1489	----	
H-2-Cu	1621	1576	1524	1445	1456	449	594
H-3	1680	1607	1532	1440.4	1504	----	-----
H-3-Cu	1620	1576	1530	1433.7	1453	457	576
H-4	1680	1615	1537	1440	1498	---	---
H-4-Cu	1620	1582	1530	1440	1456	450	590
H-5	1680	1610	1531	1443	1489	---	
H-5-Cu	1620	1582	1530	1441	1452	452	596
H- 6	1680	1610	1530	1442	1494	---	
H-6-Cu	1623	1580	1529	1441	1451	477	556
H-7	1680	1604	1537	1443	1498	----	---
H-7-Cu	1620	1575	1534	1441	1455	455	532
H-8	1680	1610	1537	1444	1498	---	---
H-8-CuCl2	1620	1575	1524	1416	1453	457	576
H-9	1680	1608	1530	1440	1495	----	---
H-9-CuCl2	1620	1566	1525	1441	1451	476	540
H-I0	1680	1610	1532	1440	1498	---	---
H-10-CuCl2	1620	1580	1534	1440	1452	466	570

-----* signifies turbidity near to blank.

Table-2: Instrumental data of Hydrazone ligands and their copper complexes.

Compounds no.	Metal %		Elemental Analysis %		
	Found	(cald.)	C (cald.)	H(cald.)	N(cald.)
H-I ($C_{19}H_{18}N_4O_2$/334)	----	---	68.13 (68.26)	5.69 (5.39)	16.62 (16.76)
H-1-$CuCl_2$	12.33	12.59	44.84 (45.23)	4.23 (4.36)	10.77 (11.11)
H-2 ($C_{19}H_{16}N_4O_2$/ 368)	----	---	61.77 (61.87)	4.492 (4.34)	15.01 (15.19)
H-2-Cu	11.75	11.71	41.322 (42.37)	4.01 (3.71)	10.94 (10.40)
H-3 ($C_{21}H_{23}N_4O_4$/ 394)	----	---	64.31 (63.96)	6.35 (5.83)	14.46 (14.21
H-3-Cu	11.25	11.17	43.55 (44.68)	4.92 (4.78)	9.33 (9.92)
H-4 ($C_{19}H_{18}N_4O_3$/ 350)	----	---	64.99 (65.14)	4.95 (5.14)	14.45 (16.0)
H-4-Cu	13.2	13.01	46.45 (47.11)	3.12 (3.719)	10.66 (11.57)
H-5 ($C_{21}H_{20}N_5O_3$/ 390)	----	---	63.912 (64.61)	5.28 (5.13)	16.58 (17.94)
H-5-CuCl2	11.17	11.34	44.03 (45.0)	4.06 (3.57)	13.01 (12.5)
H-6 ($C_{21}H_{23}N_5O_2$/ 377)	----	---	63.10 (66.84)	4.13 (6.10)	15.03 (18.56)
H-6-Cu	12.0	12.32	48.7 (49.31)	3.99 (4.5)	12.88 (13.69)
H-7 ($C_{26}H_{24}N_4O_4$/ 456)	----	---	69.24 (68.42)	5.95 (5.26)	12.1 (12.28)
H-7-$CuCl_2$	10.02	10.14	48.88 (49.84)	4.41 (4.47)	9.4 (8.95)
H-8 ($C_{20}H_{18}N_4O_4$/ 378)	----	---	62.95 (63.49)	4.66 (4.76)	14.52 (4.81)
H-8-$CuCl_2$	11.75	11.58	42.11 (43.79)	3.98(4.01)	10.11 (10.21)
H-9 ($C_{20}H_{26}N_4O_2$/ 348)	---	----	68.73 (8.96)	6.16(5.74)	16.02 (16.09)
H-9-$CuCl_2$	12.75	12.25	45.66 (46.33)	6.2(5.79)	11.2 (10.81)
H-I0 ($C_{20}H_{20}N_4O_3$/ 364)	----	---	66.7 (65.75)	5.44(5.48)	15.59 (15.34)
H-10-$CuCl_2$	12.6	12.62	47.7 (48.09)	3.9(4.01)	10.77 (11.22)

-----* signifies turbidity near to blank

Mass Spectra

The mass spectra exhibit the expected molecular ion peaks [M^+]. The general pattern of fragmentation was observed as [M^+] 215[M^+ - C_7H_4 N $_2$XYZ]188 [M^+ - C_8H_4 N $_2$OXYZ]159[M^+ - $C_{10}H_9$ N $_2$OXYZ]. Where **X**=H, OH, OCH_3 **Y**=H, OH, OCH_3 **Z**=H, OH, OCH_3, OC_6H_5, O_2CH_2

Elemental Analysis

Elemental analytical data gave carbon, hydrogen and nitrogen percentage of hydrazone ligands and their copper complexes. The C, H and N percentages for synthesized metal complexes were close to expected ratios as proposed by different characterizations techniques. Ligands and their copper complexes which are closed to calculated values.

Atomic Absorption Spectrophotometric (AAS) Studies

Table-2 shows Atomic Absorption spectrophotometric data of copper complexes of hydrazone ligands. The synthesis of copper 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 copper percentage in complexes. All copper complexes have ligand:metal (L:Cu) ratio of 1:1 in the form of $LCuCl_2 \cdot nH_2O$. This ratio was confirmed by elemental analysis and IR spectra.

X-Rays Crystallogaphy

Two Hydrazone Ligands H-I and H-4 were evaluated from x- rays crystallographic analysis which confirmed theoretical molecular structure having inter and intra-molecular hydrogen bonding and enolic mode for copper complexes. Fig. 1-3 represent the x-rays structure of ligands.

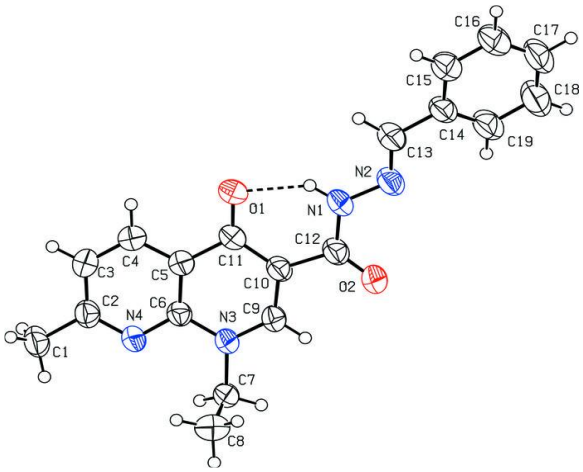


Fig. 1: H-1 ($C_{19}H_{18}N_4O_3$)

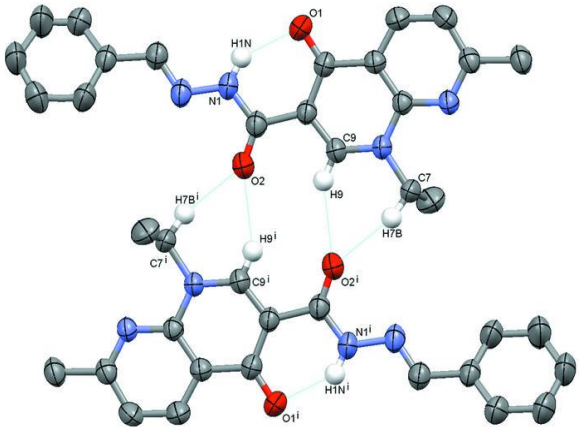


Fig. 2: H-1 ($C_{19}H_{18}N_4O_3$).

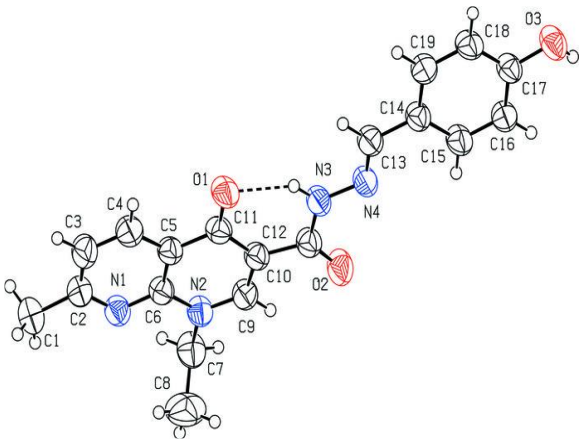


Fig. 3: H-4 ($C_{19}H_{18}N_4O_4$)

Biological Activities

For three biological activities colorimetric methods were adopted. Antibacterial, Antifungal and Antioxidant properties of the Nalidixic acid, ligands and copper complexes are shown in Table-3 to 5. For microbial activity turbidity method and readings at specific wavelength was adopted. *Escherichia coli* were selected for testing of antibacterial activity of hydrazone ligands and their copper complexes, at concentration of 1 mg/5mL in nutrient broth. After 24 h other readings were taken at 600 nm and % age inhibition of ligands and their complexes were calculated from removal of turbidity. *Aspergillus niger* was selected for the antifungal activity at concentration of 1 mg/ 5mL in nutrient broth. Initial absorbance at 550 nm and after 72 h readings at 550 nm showed the % inhibition of ligands and their complexes from removal of turbidity. Antioxidant activity was measured from scavenging DPPH [diphenyl 2-picryl hydrazyl] radical.

Table-3: Antibacterial activity of ligands and their copper complexes at 600nm.

Compounds	Initial Reading	Reading after 24 H	% Inhibition
Blank	**0.0073**	**0.0207**	**----**
Nalidixic acid	**0.869**	**0.208**	**76.06**
H-1	**0.097**	**0.084**	**13.05**
H-I-Cu	**0.288**	**0.225**	**21.58**
H-2	**0.3736**	**0.4145**	**10.22**
H-2-Cu	**0.672**	**0.732**	**11.10**
H-3	**1.513**	**1.252**	**17.24**
H-3-Cu	**0.072**	**0.057**	**19.54**
H-4	**2.295**	**2.3808**	**10.10**
H-4-Cu	**0.145**	**0.129**	**10.48**
H-5	**0.4957**	**0.5331**	**10.22**
H-5-Cu	**0.091**	**0.105**	**11.32**
H-6	**1.948**	**0.715**	**63.70**
H-6-Cu	**0.184**	**0.063**	**65.88**
H-7	**1.2945**	**1.6044**	**9.35**
H-7- Cu	**0.664**	**0.986**	**10.15**
H-8	**1.811**	**1.636**	**9.63**
H-8-Cu	**0.885**	**0.79**	**10.11**
H-9	**2.708**	**2.296**	**15.21**
H-9-Cu	**2.807**	**2.459**	**12.39**
H-10	**0.505**	**0.452**	**10.38**
H-10-Cu	**0.323**	**0.285**	**11.55**

* signifies turbidity near to blank

Table-4: Antifungal (aspergillus niger) activity of ligands series-1 and their metal complexes at 550 nm.

Compounds no.	Initial reading	Reading after 24 h	% Inhibition
Blank	**0.0073**	**0.0207**	**--- ***
Nalidixic acid	**0.869**	**0.208**	**60.12**
H-1	**0.097**	**0.084**	**13.05**
H-I-Cu	**0.288**	**0.225**	**21.58**
H-2	**0.3736**	**0.4145**	**---**
H-2-Cu	**0.672**	**0.732**	**---**
H-3	**1.513**	**1.252**	**17.24**
H-3-Cu	**0.072**	**0.057**	**19.54**
H-4	**2.295**	**2.3808**	**----**
H-4-Cu	**0.145**	**0.129**	**10.48**
H-5	**0.4957**	**0.5331**	**----**
H-5-Cu	**0.091**	**0.105**	**----**
H-6	**1.758**	**0.815**	**53.64**
H-6-Cu	**0.174**	**0.083**	**55.29**
H- 7	**1.2945**	**1.6044**	**--**
H-7- Cu	**0.664**	**0.986**	**--**
H-8	**1.129**	**1.0253**	**---**
H-8-Cu	**0.456**	**0.9716**	**---**
H-9	**2.708**	**2.796**	**---**
H-9-Cu	**1.322**	**1.262**	**4.53**
H-10	**0.505**	**0.452**	**10.17**
H-10-Cu	**0.323**	**0.285**	**11.23**

-----* signifies turbidity, near to blank

Table-5: Scavenging effect with DPPH radical on ligands and their metal complexes at 517 nm.

Compounds	Readings after ½ hour	Antioxidant %age
Blank	0.2305	----*
Nalidixic acid	0.1048	54.53
H-1	0.2105	8.67
H-I-Cu	------	------
H-2	0.2069	10.2
H-2-Cu	0.216	6.28
H-3	0.135	41.4
H-3-Cu	0.1567	32.01
H-4	0.1362	40.9
H-4-Cu	0.1682	27.03
H-5	0.1106	52.01
H-5-Cu	0.1404	39.08
H-6	0.1013	56.04
H-6-Cu	0.1801	21.84
H-7	0.1448	37.17
H-7- Cu	0.2257	2.05
H-8	0.1343	41.71
H-8-Cu	0.2405	------
H-9	0.122	47.05
H-9-Cu	0.1827	20.71
H-10	0.1775	22.97
H-10-Cu	0.1822	20.95

* signifies results near blank.

It was observed that synthesized hydrazone have less antibacterial and antifungal activities than Nalidixic acid from which compound they were prepared. Copper complexes have better antibacterial and antifungal activities than the hydrazone ligands and the ligands have better antioxidant activity than their copper complexes. Nalidixic acid has greatest all biological activities. Fig. 4 reveals percentage of three activities of Nalidixic acid at first point, H-1 at second, H-1-Cu at third etc. showing clear comparison of three biological activities. It was cleared that Hydrazone ligands H-6 has maximum all biological activities i.e. antibacterial, antifungal and anti-oxidant activities than other synthesized compounds, may be due to N-$(CH_3)_2$ group present in its molecules.

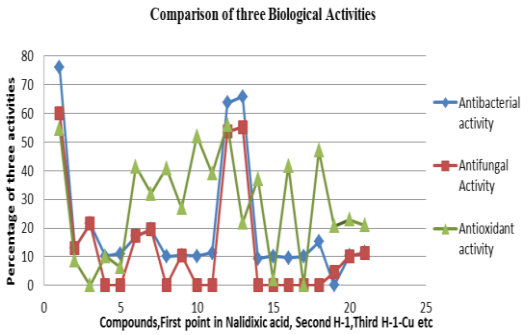


Fig. 4: Represent the comparison of three biological activates

Conclusions

From the preceding discussion following conclusions can be drawn,

- These hydrazone ligands can easily make stable metal coordination complexes with different heavy metals (Cu, Ni and Zn) .They are bidentate and form complexes in enolic mode as derived from IR spectra and x-rays crystallographic data.
- These hydrazone ligands are coordinated with metal ions by one oxygen of 4-oxo group of naphthyridines and nitrogen of hydrazone group (-N-N=C-) at outside of ring systems.
- The copper complexes are intensively colored (mostly green) and have better antibacterial and antifungal activities as compared to their respective hydrazone ligands.
- The ligands have much better DPPH scavenging activities than their copper complexes.
- Hydrazone ligands H-6 has maximum of all biological activities than other synthesized compounds as shown in Figure 4, may be due to N-$(CH_3)_2$ group present in its molecules.
- Nalidixic acid our starting compound has better biological activities than synthesized hydrazone ligands and their copper complexes but H-6 has slightly higher antioxidant activity with DPPH radical along with other activities very close to Nalidixic acid which is potent antibiotic for UTI. If further activities will be explored in pharmaceutical field, this compound can be as good antibiotic as others.

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