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Der Pharma Chemica, 2014, 6(2):330-334
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Microwave assisted synthesis and antibacterial study of hydrazone Schiff's base 2-cyano-N'-(1-(4-hydroxy-6-methyl-2-oxo-*2H*-pyran-3-yl) ethylidene)acetohydrazide and its transition metal complexes

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ABSTRACT

In search of some novel antibacterial agents, synthesis of 2-cyano-N'-(1-(4-hydroxy-6-methyl-2-oxo-2H-pyran-3-yl)ethylidene)acetohydrazide Schiff's base and its first transition metal complexes has been reported under microwave assisted conditions. The structure of all the synthesized compounds were established on the bases of various spectroscopic techniques. Ligand and its metal complexes were screened for antibacterial activity against two gram (+) and two gram (-) bacterial strains. Metal complexes were found to possess better antibacterial potential as compared to ligand. Cu(II) Complexes displayed promising in vitro antibacterial activities against the tested strains of bacteria.

Key words: Microwave assisted synthesis, green approach, dehydroacetic acid, Schiff's base, metal complexes, antibacterial activity

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INTRODUCTION

It has been stems from observations that Schiff's bases are one of the most widely used chelating ligands in coordination chemistry [1, 2]. Presence of nitrogen atom of azomethine moiety in Schiff's bases make them of special interest because it can easily chelates with various metal ions by offering its lone pair of electrons to the empty *d*-orbital of metal ions [3]. Schiff's base and their transition metal complexes have wide application in biology [4, 5], medicine, pharmaceutical [6] and in catalysis [7-10]. Dehydroacetic acid (DHA) and its derivatives are known to possess fungicidal and bactericidal activities, hence large number of compounds based on DHA has been synthesized and were evaluated for biological activities [11-14].

Since last few decades chemists were highly interested to do intensive research in the field of green chemistry [15-17]. There main aim is to use non conventional approaches of synthesis because, of less or no solvent requirements, easy isolation, eco-friendly nature, less reaction time with good yield and purity of target molecules. Among them the area of microwave assisted synthesis is of special interest due to simple operational procedure, lesser reaction time and easy workup [18-20]. In view of above mentioned facts, in present investigation we planned to explore the new and efficient methodology for synthesis of bioactive compound by non conventional microwave irradiation technique. The present research work describes the synthesis, spectral and antibacterial studies of 2-cyano-N'-(1-(4-hydroxy-6-methyl-2-oxo-2H-pyran-3-yl)ethylidene)acetohydrazide Schiff's base and its complexes with Cu(II), Ni(II), Co(II), Mn(II) and Zn(II). The complexes have been characterized on the basis of spectroscopic techniques

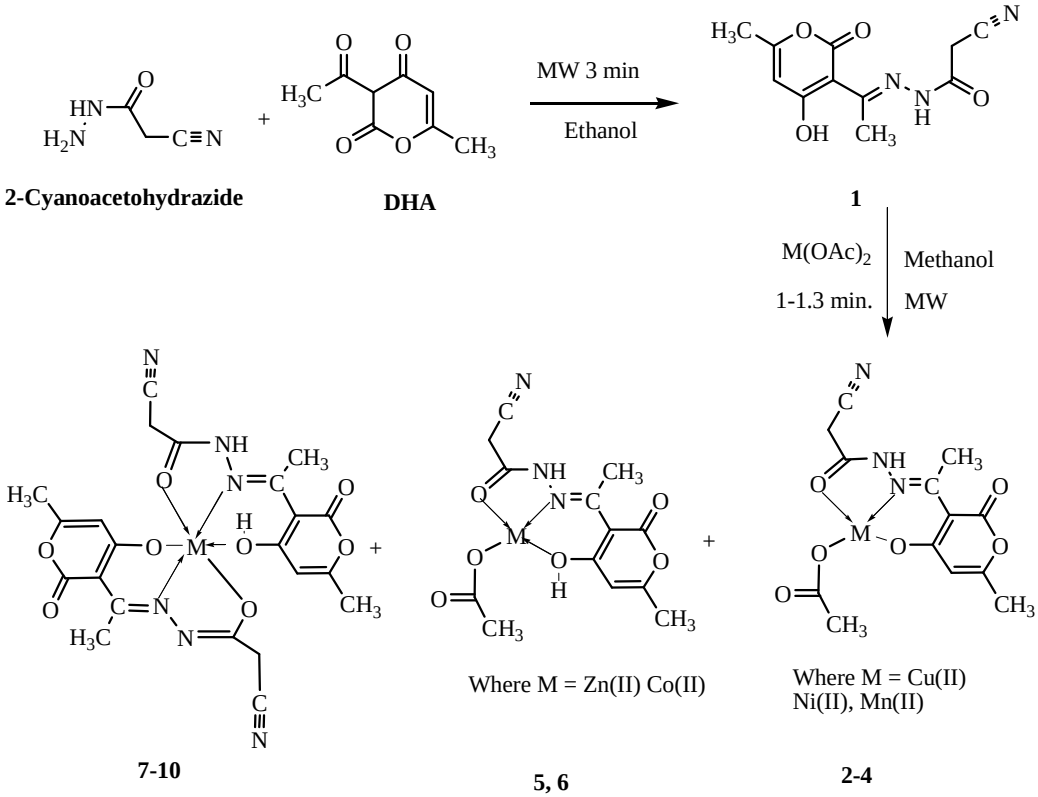
such as elemental analysis, conductance, ^{1}H and ^{13}C NMR, FTIR spectroscopy and Mass spectrometry. Further, all the synthesized compounds were evaluated for *in vitro* antibacterial activities.

MATERIALS AND METHODS

Dehydroacetic acid was purchased from Merck and used as such. All other chemicals including solvents were of LR grade and used as supplied. Microwave assisted synthesis were carried out in open glass vessel on a modified microwave oven model 2001 ETB with rotating tray and a power source 230 V, at output energy of 800W and 2450 MHz frequency. A thermocouple device was used to monitor the temperature inside the vessel of the microwave. The microwave reactions were performed using on/off cycling to control the temperature. ^{1}H and ^{13}C NMR spectra of the ligand and complexes were recorded on Bruker spectrophotometer at 400 MHz and 100 MHz instrument, respectively using TMS as internal reference standard in DMSO-d_6. The electronic spectra were recorded on Shimadzu UV 1800 instrument in DMSO-d_6 as a solvent. Mass spectra were recorded on Agilent Mass Spectrometer. IR spectra were recorded on Shimadzu IR Affinity in the range 4000 to 400 cm^{-1} using KBr pellet.

Synthesis of Ligand

Ligand was prepared by treating cyanoacetic hydrazide (0.01 mol) in 15 ml ethanol with 10 ml (70%) aqueous ethanolic solution of DHA (0.01 mol) under microwave irradiation for 4 min. The colorless crystalline product obtained was filtered and washed twice with aqueous ethanolic solution finally it was dried in hot air oven at 60-65^{o}C for 4 h. Percentage yield 80%, M.p 185-187 ^{o}C.



2-Cyanoacetohydrazide **DHA** **1**

7-10 **5, 6** **2-4**

Scheme 1. Synthesis of ligand and its metal complexes

Synthesis of Metal Complexes

Metal complexes of Co(II), Cu(II), Ni(II), Mn(II) and Zn(II) were prepared by the treatment of 15 ml methanolic solution of the ligand with their metal acetate in different molar ratios (0.5 and 1.0 mmol) in 15 ml methanolic solution under microwave for 2-4 min. The complexes formed were filtered, washed with excess of methanol followed by petroleum ether to remove any traces of unreacted metal salts and dried in hot air oven at 70° C for 6 h. Metal complexes thus obtained (**2-10**) were found to be non hygroscopic and stable at room temperature.

RESULTS AND DISCUSSION

In continuous interest of our group in the development of effective, efficient and greener approaches of synthesis of bioactive compounds [21, 22], in present investigation we report the microwave assisted synthesis of some first transition series metal complexes based on 2-cyano-N'-(1-(4-hydroxy-6-methyl-2-oxo-2H-pyran-3-yl)ethylidene) acetohydrazide Schiff's base. All these compounds have been synthesized by conventional method and were screened for DNA photocleavage activity in our previous research work [24].We were much Inspired from good level of nuclease activity of the complexes. So it was planned to synthesize these metal complexes again *via* non conventional approach *i.e.* microwave assisted synthesis. Ligand and its metal complexes were characterized on the basis of various spectroscopic techniques and were screened for antibacterial properties. In ^{1}H NMR a broad signal at 16.12 ppm (singlet,1H) due to enolic proton of ligand was disappeared in case of Cu(II) metal complex but was intact in proton NMR of LZn and L_2Zn suggesting deprotonation in first case and coordination without deprotonation of enolic proton in later case. IR spectra showed shift in azomethine as well as carbonyl stretching frequency suggested the involvement of these groups in coordination with metal ions. Further, presence of two new non ligand bands in the IR spectra of metal complexes at 400 cm^{-1} and 600 cm^{-1} were assigned due to υ_{M-O} and υ_{M-N} stretching frequencies, respectively; suggested that metal ion is coordinated with oxygen and nitrogen atom of the ligand. The electronic spectra of the ligand in 10^{-4} M DMSO solution displayed two signals centered at 243 nm (π-π*) and 321 nm (n-π* of carbonyl/azomethine moiety), respectively [23]. Mass of all the synthesized compounds were confirmed on the basis of mass spectrometry which are in full agreement with the proposed structures of compounds (**Table 1**)

Table 1. Physio-chemical data of Schiff's base and its metal complexes

Entry	Color	Chemical Formula	Found(Calculated) %				M.P. (°C)
			C	H	N	M	
1	Colorless	$C_{11}H_{11}N_3O_4$	53.01 (52.98)	4.45 (4.47)	16.86 (16.85)	-	185-187
2	Green	$(C_{13}H_{13}N_3O_6)Cu$	42.11 (42.12)	3.53 (3.50)	11.33 (11.32)	17.14 (17.12)	293
3	Blue	$(C_{13}H_{13}N_3O_6)Ni$	42.67 (42.65)	3.58 (3.57)	11.48 (11.46)	16.04 (16.03)	285
4	Yellow	$(C_{13}H_{13}N_3O_6)Mn$	43.11 (43.13)	3.62 (3.62)	11.60 (11.59)	15.17 (15.15)	307
5	Red	$(C_{13}H_{13}N_3O_6)Co$	42.64 (42.65)	3.58 (3.57)	11.47 (11.49)	16.09 (16.06)	283
6	Colorless	$(C_{13}H_{13}N_3O_6)Zn$	41.90 (41.87)	3.52 (3.51)	11.28 (11.26)	17.55 (17.54)	263
7	Blue	$(C_{22}H_{20}N_6O_8)_2Ni$	43.05 (43.01)	3.28 (3.25)	13.69 (13.67)	19.12 (19.05)	291
8	Red	$(C_{22}H_{20}N_6O_8)_2Co$	47.58 (47.55)	3.63 (3.60)	15.13 (15.12)	10.61 (10.55)	294
9	Green	$(C_{22}H_{20}N_6O_8)_2Cu$	47.19 (47.20)	3.60 (3.59)	15.01 (15.03)	11.35 (11.33)	292
10	Colorless	$(C_{22}H_{20}N_6O_8)_2Zn$	47.03 (47.03)	3.59 (3.60)	14.96 (14.99)	11.64 (11.62)	274

Table 2. The yields mentioned in parentheses are according to conventional method

Entry	Compounds	Reaction time (min.)	% Yield
1	Ligand	3.0	80(73)
2	Cu(1:1)	1.3	73(67)
3	Ni(1:1)	1.3	61(58)
4	Mn(1:1)	1.7	73(71)
5	Co(1:1)	1.8	66(62)
6	Zn(1:1)	1.1	76(73)
7	Co(1:2)	1.5	65(58)
8	Ni(1:2)	1.5	66(61)
9	Cu(1:2)	1.2	76(74)
10	Zn(1:2)	1.3	73(71)

Antibacterial Study

All the synthesized compounds were screened for their *in vitro* antibacterial activity against two Gram-positive [*B. subtillis* (2063), *S. aureus* (5021)] and two Gram-negative bacteria [*B. syringae* (5102), *P. aeruginosa* (5029)] (***Table 3***). The antibacterial activity of these compounds were compared with Oxacillin as a standard drug. It has been found that metal complexes have good antibacterial activity than a free ligand under identical experimental conditions for one kind of bacterial strain (**Table 3**). It was evident from the data that *in vitro* antibacterial activity is significantly increased on coordination. Moreover, coordination reduces the polarity [24, 25] of the metal ion mainly because of the partial sharing of its positive charge with the donor groups [26, 27] within the chelate ring system formed. This process, in turn, leads to increase in the lipophilic character of metal chelate so as to make it more permeable through the lipid layer of microorganism [28, 29] thus destroying them more aggressively. Once the compound enters into the microbial cell, it restricts the growth of microorganism by binding at the active site of enzymes which involves in various essential biochemical processes including cell respiration and proteins synthesis of the cell. The mode of action of complexes involves the formation of hydrogen bonding with imino group by the active sites leading to interference with the cell wall synthesis [30]. The hydrogen bond formation damages the cytoplasmic membrane and the cell permeability may also be altered leading to cell death. The antibacterial activity of the ligand and its complexes were found to be in the order $CNDL_2Cu$ > $CNDL_2Zn$ > CNDLCu > CNDLZn > CNDLMn > $CNDL_2Ni$ > $CNDL_2Co$ > CNDLNi > CNDLCo > $CNDLH_1$. The higher activity of Cu(II) complexes can be explained as, on chelation the polarity of Cu(II) ion is found to be reduced to a greater extent due to overlap of the ligand orbital and partial sharing of the positive charge of the copper ion with donor groups. Therefore, Cu(II) ions are easily adsorbed on the surface of the cell wall of microorganisms [31, 32]. The adsorbed Cu(II) ions disturb the respiratory process of the cells, and block the synthesis of proteins. This, in turn, restricts further growth of the organisms. However, all these compounds represent promising new leads for combating the emerging pathogens. Efforts will be made to test these compounds against drug resistant pathogens and their evaluation in human system for their toxicity.

Table 3. Antibacterial activity of Schiff's base and its metal complexes

Entry	Zone of Inhibition in $(mm)^2$			
	B. subtillis **(2063)**	***B. syringae*** **(5102)**	***P. aeruginosa*** **(5029)**	***S. aureus*** **(5021)**
$CNDLH_1$	10	10	10	10
CNDLCo	11	13	10	10
$CNDL_2Co$	14	15	16	16
CNDLNi	12	10	13	13
$CNDL_2Ni$	14	12	11	11
CNDLCu	23	19	20	25
$CNDL_2Cu$	38	20	24	23
CNDLMn	12	11	12	11
CNDLZn	16	19	21	24
$CNDL_2Zn$	27	21	20	27
Oxacillin	38	37	32	33

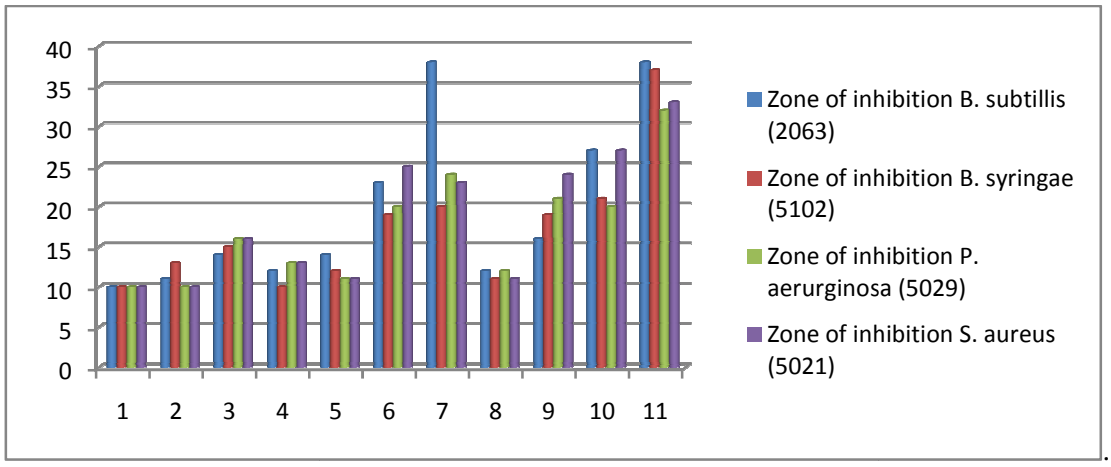


Figure 1. Graphical presentation of antibacterial activity of synthesized compounds

CONCLUSION

In present investigation we reported a non conventional microwave assisted synthesis of some hydrazone Schiff's base metal complexes. *In vitro* antibacterial activity of metal complexes and ligand were studied and it has been observed that metal complexes were found to display higher antibacterial activity than free ligand under identical experimental condition. Some structural modification in ligand can tune the antibacterial activity and may serve as a model compound for the development of some efficient antibacterial drugs in future.

Acknowledgement
We are highly thankful to Maharishi Markandeshwar University for providing lab facility.

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