Asian Journal of Chemistry; Vol. 25, No. 2 (2013), 633-636

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2013.11696

Antimicrobial Activity of Some Derivatives of Triacetic Lactone and Their Cd(II) and Hg(II) Complexes

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(*Received*: 6 February 2011; *Accepted*: 9 August 2012) AJC-11944

Triacetic lactone derived compounds and Cd(II) and Hg(II) complexes have been synthesized, characterized and screened for biological activities. Antibacterial activity was carried out against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, *Bacillus subtilis*, *Streptococcus mutans* and *Sarcina lutae*. *In vitro* antifungal activities were carried out against *Trichoderma viridis*, *Aspergillus flavus*, *Fusarium laterifum*, *Aspergillus fumigatus*, *Candida albicans*, *Trichophyton mentogrophytes* and *Microsporum canis*. The results of these studies showed that these metal complexes to be have more potential for antimicrobial activates as compared to the ligand. Cd(II) complex is more active against the all tested bacterial and fungal strains.

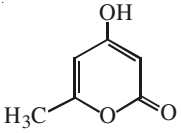
Key Words: Triacetic lactone derivatives, Metal complexes, Antimicrobial.

INTRODUCTION

Many complexes by coordination with various metal ions become more active than uncoordinated form. This is due to the facts that these metals are essential for life .These metals cause several diseases occur only when an excess or deficiency of *in vivo* metals appears[1-4].

2-Pyrones and their derivatives are natural product and their chemistry very interesting. Triacetic lactone (4-hydroxy-6-methyl-2*H*-pyran-2-one) was originally prepared by previously reported reactions of triacetic lactone with electrophilic reagents[5] include bromination[6] nitration acylation[7] at the 3-position.

Triacetic lactone (**1**) and its derivatives not only have important structural variety but also significant biological[8-10] and pharmacological[11,12] properties. The oxygen containing hetrocyclic compounds especially the pyrones and their derivatives were used for the preparation of transition metal complexes with hetrocyclic system are studied extensively because of their biological activates.

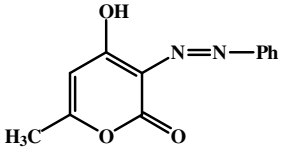


(**I**)

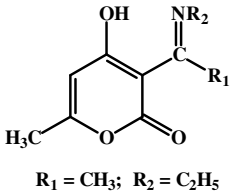
The oxygen heterocyclic compounds such as 3-azophenyl 4-hydroxy-6-methyl-pyrane-2-one (**II**) have been prepared and used as ligands for the formation of Hg(II) and Cd(II) complexes.

EXPERIMENTAL

All the chemicals and solvents used were of analytical grade (Merck). Mueller-Hinton agar was used; all metal(II) salts are used as chlorides. IR spectra were recorded on a Philips Analytical PU 9800 FTIR spectrophotometer. NMR spectra were recorded on a Perkin-Elmer 283B spectrometer. UV-Visible spectra were obtained in DMF on a Hitachi U-2000 double-beam spectrometer. Elemental analysis of C, H and N were determined on a Coleman automatic analyzer. Magnetic measurements were carried out on solid complexes using Gouy's method. Melting points were recorded on a Gallenkamp (UK) apparatus.



(**II**)



$R_1 = CH_3$; $R_2 = C_2H_5$

(**III**)

Preparation of L1

3-Azophenyl-4-hydroxy-6-methyl-pyran-2-one (II): Benzene diazonium chloride (50 mL) was added to triacetic lactone (10 g) dissolved in 10 % aqueous solution of sodium carbonate (200 mL) at 50 ºC. After 1 h, the reaction mixture was acidified with acetic acid. The resulting yellow precipitate was filtered, dried and crystallized from ethanol as yellow solid.

Preparation of L2

3-2-Methyl Iminoethyl) 4-hydroxy 6-methyl-pyran-2-one (III): Dehydroacetic acid (10 g) was treated with ethylamine (70 %, 12 mL) and heated on a steam bath for 30-40 min. Reaction mixture on cooling gave yellow solid product. This on recrystallization from *n*-hexane gave light yellow product.

General method for the preparation of metal complexes (1-4): A solution of corresponding metal(II) salt (0.01 M) in ethanol (20 mL) was added to a solution of appropriate ligand (0.02 M) in alcohol and finally with ether. Then the products were dried. The solid products were obtained immediately in most of the cases; otherwise the reaction mixture was concentrated to get the products[10].

Antimicrobial activity: The synthesized ligands L1 and L2 and their Cd(II) and Hg(II) complexes (**1-4**) were screened *in vitro* for their antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, *Bacillus subtilis*, *Streptococcus mutans* and *Sarcina lutae* bacterial strains (Collected from Mayo Hospital Lahore) by using the agar well diffusion method[13]. Nutrient agar media was used for antibacterial activity. Nutrient broth media was used to grow the culture media. 10 mL of broth were inoculated with test strains of bacteria in all culture tubes. Mixed the 0.6 mL of broth culture in 60 mL of the molten agar media, mixed well and poured in Petri dishes. After solidifying, 3 mm holes are cut by using the sterile cork borer. Test samples of metal complexes were dissolved in sterile DMSO to serve as stock solution. Different concentrations were prepared from the stock solution of the metal complexes. 100 µL of the metal complexes were poured in these holes of all concentration. The minimum inhibitory concentration was determined using the agar well diffusion technique by preparing concentration containing 10, 25, 50 and 100 µg/mL of the compounds[14].

DMSO was used as negative control. Other wells were supplemented with reference compound *i.e.*, ampicillin, amoxicillin, levofloxacin, tetracycline, vancomycin and ciprofloxacin as positive control. All the antibacterial activities were carried out in triplicate and their means are recorded.

Antifungal activity of plants extract was determined by the agar tube dilution method[13]. Seven fungal strains were slected for antifungal strains. *Trichoderma viridis* (FCBP# 642) (*T. viridis*) *Aspergillus flavus* (FCBP# 647) (*A. flavusi*), *Fusarium laterifum* (FCBP# 624) (*F. laterifum*), *Aspergillus fumigatus* (FCBP# 474) (*A. fumigatus*) *Candida albicans* (FCBP# 478) (*C. albicans*) were obtained from the Department of Mycology and Plant Patholgy, University of the Punjab, Quaid-e-Azam Campus, Lahore. Two identified fungal strains trichophyton mentogrophytes and microsporum canis were obtained from the Main Microbiology Laboratory of Mayo Hospital, Lahore.

Test samples of metal complexes were dissolved in sterile DMSO to serve as stock solution. Different concentrations were prepared from the stock solution of the metal complexes. Sabouraud dextrose was used to grow the fungal strains in test tube. All media took in the tubes, after sterilization the100 µL metal complexes added into non solidified media. Each tube was inoculated with 10^5 (CFU)/mL^{-1} fungal spore suspensions of 7 days old culture of fungi. Kept all the tubes at optimum temperature of 28-30 ºC for growth for 7-10 days. DMSO was used as control as negative control and ketoconazole, econazole, nystatin, amphotericin, clotrimazole and miconazole used as positive control. After the incubation for 7-10 days the test tubes with no visible growth of the microorganism was taken to represent the zone of inhibition of the test sample which was expressed in µg/mL. The test was carried out in triplicate and their means were recorded.

RESULTS AND DISCUSSION

The ligands L1 and L2 were prepared by reported method[13]. The structural determinations of these synthesized ligands were done with the help of UV, IR, ^{1}H NMR and their elemental analysis data.

Due to the excellent chelating properties of oxygen hetrocyclic compounds, the derivatives of triacetic lactone (**I**), 4-hydroxy-6-methyl-3-azophenyl-2*H*-pyrane-2-one (**II**), 3-(2-methyl imino ethyl) 4-hydroxy-6-methyl-2*H*-pyran-2-one (**III**) have been prepared and used as ligand for the formation of Hg(II) and Cd(II) complexes.

A solution of the appropriate ligand on treatment with Cd(II) or Hg(II) salt in ethanolic or methanolic solvent in presence of sodium carbonate gave the respective complexes in appreciable yields. In order to ensure the completion of the reaction, the reaction mixture was also heated on steam bath and then cooled to get the products.

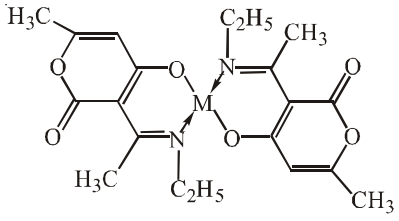
All reactions preceded smoothly and resulted in the formation of coloured products .The products are stable towards air and moisture. Triacetic lactone and its derivatives show sharp melting points. While complexes are in soluble in water but show appreciable solubility in DMF and DMSO but are sparingly soluble or even insoluble in other solvents such as ethanol, methanol, chloroform, ether, acetone and carbon disulphide.

The polydentate nature of ligands provides a number of sites for coordination. However, in the basic medium, all this ligands are invariably expected to lose the proton of -OH at 4-position to produce the anion which provides the preferential point for the attachment of the ring. However, the coordination of the oxygen of the carbonyl group at the 2-position cannot be ruled out.

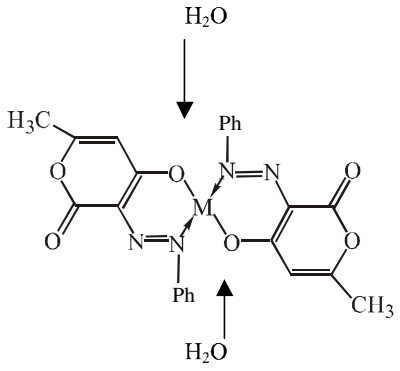
IR Spectroscopy: The tentative structures assigned to the metals complexes (**1-4**) are supported by infrared spectra of ligands and complexes are compared. Ligands gave a strong band due to OH group 3114-3100 cm^{-1}. In all the complexes, the absence of characteristic band of -OH group indicates the participation of phenolic oxygen in bonding with metal ions[15]. No change is observed in the absorption of carbonyl group (1700-1730) stretching.

The C=N stretching bands are observed in 1652-1698 and 1434-1432 peaks at 2884-2847 and 722-720 fit very well in the expected ranged of the C-H in all complexes and also showed strong band of C-N in 1575-1576. Two new bands were observed at 688-667 and 564-550 for all metal complexes, which did not exist in the spectrum of the free ligand. It could be sign to ν(M-O) and ν(M-N)[15]. The inferred band assignment though its nitrogen and oxygen atoms. The presence of broad at 3600-3200 cm^{-1} in the same complexes is attributed to the -OH of the coordinated with water molecules[15]. According to the above data suggests the ligands behaved as bidentate towards all metals. On the basis of analytical data and spectroscopic evidence, the molecular formula proposed for the complexes in general is M:L (1:2).

The electronic spectra of ligands and complexes in DMSO are also studied. The solutions of each complex were noted. The λ_{max} for each complex were noted. The λ_{max} of complexes are compared with λ_{max} of the respective ligands. The shift in the λ_{max} confirms the formation of metals complexes.



(IV)



(V)

where M= Hg(II) and Cd(II)

NMR: On comparing the ^{1}H NMR spectra of complexes with ligands in DMSO-d_6 using TMS as internal standard. The enolic OH signal observed at 16.5-16.6 ppm in the spectra[17] of L1 and L2 ligands, disappeared on complex formation confirming that the bonding of metal (**II**) ion to the ligands take place through a proton displacement from the enolic OH group. A new signal observed in spectra of complexes at 3.6-3.77 is not present in the spectra of free ligands can be assigned H_2O molecules associated with complex formation[20]. The data obtained from ^{1}H NMR of the complexes are in accordance with the results obtained from IR spectra. This is the sporting the mode of bonding between the metal (**II**) ion and (L1 and L2) ligands.

The zone of inhibition of L1 and L2 as control study against all test bacterial strains (Tables 1 and 2). The $Hg(L2)_2$ showed highest activity against, *S. aureus*, *E. coli* and *S. mutans* while it showed moderate bactericidal activity against *B. subtilis* and *Sarcina lutae* (Table-3). The results obtained for the antibacterial potential of $Hg(II)(L1)_2$ were also good against *B. subtilis* and *S. lutae*. The bacterialcidal activity of $Cd(II)(L1)_2$ showed poor result against all the bacterial strains except *S. aureus*. The result of $Cd(II)(L2)_2$ activity also very poor result as compared to ligands activity even (Table-3).

TABLE-1
ZONE OF INHIBITION OF LIGANDS (CONTROL STUDY)

Bacterial strains	Zone of inhibition (mm)	
	L1	L2
S. aureus	12	13
E. coli	11	14
P. aeruginosa	9	12
S. pneumoniae	12	11
B. subtilis	11	10
S. lutae	10	10
S. mutans	10	12

TABLE-2
ZONE OF INHIBITION OF LIGANDS (CONTROL STUDY)

Bacterial strains	Zone of inhibition in mm	
	L1	L2
A. flavus	10	13
F. laterifum	13	15
A. fumigatus	12	12
C. albicans	10	14
T. mentogrophytes	08	17
M. canis	12	15
T. viridis	13	11

TABLE-3
ZONE OF INHIBITION OF METAL COMPLEXES OF L1 AND L2

Bacterial strains	Zone of inhibition (mm)			
	$Hg(II)(L1)_2$	$Cd(II)(L1)_2$	$Hg(II)(L2)_2$	$Cd(II)(L2)_2$
S. aureus	34	32	45	30
E. coli	28	20	45	17
P. aeruginosa	22	15	29	22
S. pneumoniae	38	12	35	15
B. subtilis	45	32	36	10
S. lutae	23	12	38	10
S. mutans	45	13	47	20

The $Hg(L2)_2$ showed highest activity against, *A. flavus*, *C. albicans* and *T. viridis* while it showed moderate bactericidal activity against *F. laterifum*, *A. fumigatus*, *T. mentogrophytes* and *M. canis*. The results obtained for the antifungal potential of $Hg(II)(L1)_2$ were very enhancing against *A. flavus*, *F. laterifum* and *C. albicans* (Table-4).

TABLE-4
ZONE OF INHIBITION OF METAL COMPLEXES OF L1 AND L2

Bacterial strains	Zone of inhibition (mm)			
	$Hg(II)(L1)_2$	$Cd(II)(L1)_2$	$Hg(II)(L2)_2$	$Cd(II)(L2)_2$
A. flavus	43	23	46	20
F. laterifum	36	34	48	37
A. fumigatus	23	25	24	32
C. albicans	46	32	45	25
T. mentogrophytes	25	32	26	20
M. canis	23	22	28	20
T. viridis	48	22	27	32

TABLE-5
MIC VALUE OF DIFFERENT ANTIBIOTICS (POSITIVE CONTROL)

Bacterial strains	MIC (μg/mL)					
	Ampicillin	Amoxicillin	Levofloxacin	Tetracyclin	Vancomycin	Ciprofloxacin
S. aureus	10	50	100	30	1.0	5.0
E. coli	10	80	150	50	5.0	1.0
P. aeruginosa	–	50	–	–	–	5.0
S. pneumoniae	–	–	20	20	5.0	5.0
B. subtilis	250	50	5.0	–	–	–
S. lutae	100	–	10	50	–	10
S. mutans	50	30	10	–	1.0	5.0

TABLE-6
MIC VALUE OF DIFFERENT ANTIBIOTICS (POSITIVE CONTROL)

Bacterial strains	MIC (μg/mL)					
	Ketoconazole	Econazole	Nystatin	Amphotericin	Clotrimazole	Miconazole
A. flavus	15	50	–	–	100	500
F. laterifum	–	10	100	100	500	100
A. fumigatus	250	50	–	–	500	250
C. albicans	–	–	20	250	250	100
T. mentogrophytes	50	50	10	50	100	50
M. canis	250	–	100	–	–	50
T. viridis	10	30	50	–	–	500

The activity of $Cd(II)(L1)_2$ and $Cd(II)(L2)_2$ showed mordant results against all the tested fungal strains. The result of antifungal activities of metal complexes compared with commercially available common fungicidal drugs; the metal complexes of $Hg(II)(L1)_2$ and $Hg(II)(L2)_2$ are more effective (Table-4).

This data compared with commercially avaible common antibiotics; the metal complexes of $Hg(II)(L1)_2$ and $Hg(II)(L2)_2$ are more effective as compared to these metals. These metal complexes showed very good potential against the infections disease caused by the tested bacterial strains (Tables 5 and 6).

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