

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

SAMINA SHAHID^{1,*}, TAHIRA MUGHAL² and HAMID ULLAH SHAH³

¹Department of Chemistry, Queen Mary College, Empress Road, Lahore, Pakistan

²Department of Botany, Lahore College for Women University, Jail Road, Lahore, Pakistan

³Department Nutrition Science, NWFP Agriculture University, Peshawar, Pakistan

*Corresponding author: E-mail: drtahiramughal@gmail.com

(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 lutea*. *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.

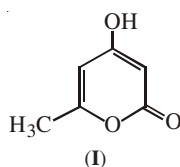
Key Words: Triacetic lactone derivatives, Metal complexes, Antimicrobial.

INTRODUCTION

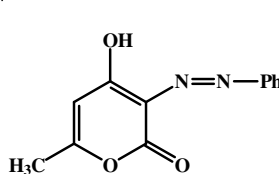
Many complexes by coordination with various metal ions become more active than uncoordinated form. This is due to the facts that these metals are essential for life. These metals cause several diseases occur only when an excess or deficiency of *in vivo* metals appears¹⁻⁴.

2-Pyrones and their derivatives are natural product and their chemistry very interesting. Triacetic lactone (4-hydroxy-6-methyl-2H-pyran-2-one) was originally prepared by previously reported reactions of triacetic lactone with electrophilic reagents⁵ include bromination⁶ nitration acylation⁷ at the 3-position.

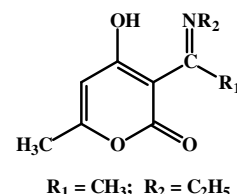
Triacetic lactone (I) and its derivatives not only have important structural variety but also significant biological⁸⁻¹⁰ and pharmacological^{11,12} properties. The oxygen containing hetrocyclic compounds especially the pyrones and their derivatives were used for the preparation of transition metal complexes with hetrocyclic system are studied extensively because of their biological activates.



(I)



(II)



R₁ = CH₃; R₂ = C₂H₅
(III)

The oxygen heterocyclic compounds such as 3-azophenyl 4-hydroxy-6-methyl-pyran-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.

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 °C. 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 products¹⁰.

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 lutea* bacterial strains (Collected from Mayo Hospital Lahore) by using the agar well diffusion method¹³. 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 compounds¹⁴.

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 method¹³. Seven fungal strains were selected for antifungal strains. *Trichoderma viridis* (FCBP# 642) (*T. viridis*) *Aspergillus flavus* (FCBP# 647) (*A. flavus*), *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 Pathology, 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 the 100 µL metal complexes added into non solidified media. Each tube was inoculated with 10⁵ (CFU)/mL⁻¹ fungal spore suspensions of 7 days old culture of fungi. Kept all the tubes at optimum temperature of 28-30 °C 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 method¹³. The structural determinations of these synthesized ligands were done with the help of UV, IR, ¹H NMR and their elemental analysis data.

Due to the excellent chelating properties of oxygen heterocyclic compounds, the derivatives of triacetic lactone (**I**), 4-hydroxy-6-methyl-3-azophenyl-2H-pyran-2-one (**II**), 3-(2-methyl imino ethyl) 4-hydroxy-6-methyl-2H-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.

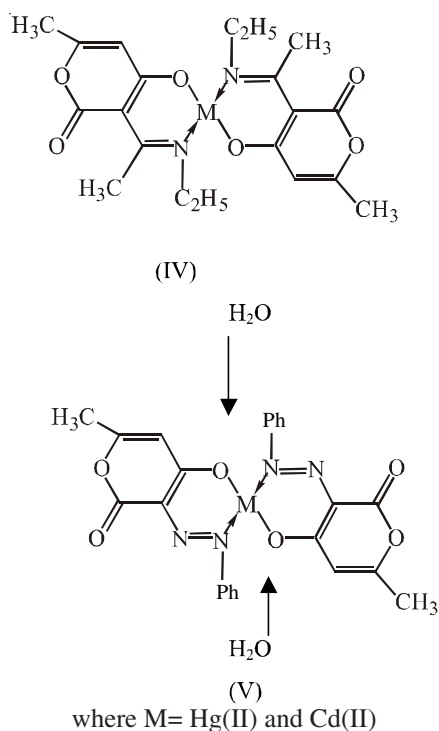
All reactions preceded smoothly and resulted in the formation of coloured products. The products are stable towards air and moisture. Triacetic lactone and its derivatives show sharp melting points. While complexes are in soluble in water but show appreciable solubility in DMF and DMSO but are sparingly 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 ions¹⁵. 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 $\nu(\text{M-O})$ and $\nu(\text{M-N})$ 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 molecules¹⁵. 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.



NMR: On comparing the ¹H 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 spectra¹⁷ 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 H₂O molecules associated with complex formation²⁰. The data obtained from ¹H 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 lutea* (Table-3). The results obtained for the antibacterial potential of Hg(II)(L1)₂ were also good against *B. subtilis* and *S. lutea*. The bactericidal 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 |
| <i>S. aureus</i> | 12 | 13 |
| <i>E. coli</i> | 11 | 14 |
| <i>P. aeruginosa</i> | 9 | 12 |
| <i>S. pneumoniae</i> | 12 | 11 |
| <i>B. subtilis</i> | 11 | 10 |
| <i>S. lutea</i> | 10 | 10 |
| <i>S. mutans</i> | 10 | 12 |

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

| Bacterial strains | Zone of inhibition in mm | |
|--------------------------|--------------------------|----|
| | L1 | L2 |
| <i>A. flavus</i> | 10 | 13 |
| <i>F. laterifum</i> | 13 | 15 |
| <i>A. fumigatus</i> | 12 | 12 |
| <i>C. albicans</i> | 10 | 14 |
| <i>T. mentogrophytes</i> | 08 | 17 |
| <i>M. canis</i> | 12 | 15 |
| <i>T. viridis</i> | 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 |
| <i>S. aureus</i> | 34 | 32 | 45 | 30 |
| <i>E. coli</i> | 28 | 20 | 45 | 17 |
| <i>P. aeruginosa</i> | 22 | 15 | 29 | 22 |
| <i>S. pneumoniae</i> | 38 | 12 | 35 | 15 |
| <i>B. subtilis</i> | 45 | 32 | 36 | 10 |
| <i>S. lutea</i> | 23 | 12 | 38 | 10 |
| <i>S. mutans</i> | 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 |
| <i>A. flavus</i> | 43 | 23 | 46 | 20 |
| <i>F. laterifum</i> | 36 | 34 | 48 | 37 |
| <i>A. fumigatus</i> | 23 | 25 | 24 | 32 |
| <i>C. albicans</i> | 46 | 32 | 45 | 25 |
| <i>T. mentogrophytes</i> | 25 | 32 | 26 | 20 |
| <i>M. canis</i> | 23 | 22 | 28 | 20 |
| <i>T. viridis</i> | 48 | 22 | 27 | 32 |

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

| Bacterial strains | MIC (µg/mL) | | | | | |
|----------------------|-------------|-------------|--------------|-------------|------------|---------------|
| | Ampicillin | Amoxicillin | Levofloxacin | Tetracyclin | Vancomycin | Ciprofloxacin |
| <i>S. aureus</i> | 10 | 50 | 100 | 30 | 1.0 | 5.0 |
| <i>E. coli</i> | 10 | 80 | 150 | 50 | 5.0 | 1.0 |
| <i>P. aeruginosa</i> | – | 50 | – | – | – | 5.0 |
| <i>S. pneumoniae</i> | – | – | 20 | 20 | 5.0 | 5.0 |
| <i>B. subtilis</i> | 250 | 50 | 5.0 | – | – | – |
| <i>S. lutea</i> | 100 | – | 10 | 50 | – | 10 |
| <i>S. mutans</i> | 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 |
| <i>A. flavus</i> | 15 | 50 | – | – | 100 | 500 |
| <i>F. laterifum</i> | – | 10 | 100 | 100 | 500 | 100 |
| <i>A. fumigatus</i> | 250 | 50 | – | – | 500 | 250 |
| <i>C. albicans</i> | – | – | 20 | 250 | 250 | 100 |
| <i>T. mentogrophytes</i> | 50 | 50 | 10 | 50 | 100 | 50 |
| <i>M. canis</i> | 250 | – | 100 | – | – | 50 |
| <i>T. viridis</i> | 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 available 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).

REFERENCES

1. J.N. Collie, *J. Chem. Soc.*, **59**, 179 (1891); **91**, 787 (1907).
2. A. Cowley and B. Woodward, *Platinum Metals Rev.*, **55**, 98 (2011).
3. J.L. Dauglas and T. Money, *Anal. J. Chem.*, **46**, 695 (1968).
4. R.D.H. Murray, *Prog. Chem. Org. Nat. Prod.*, **58**, 83 (1991).
5. I. Musajo and G. Rodighero, *Experimental*, **18**, 153 (1662).
6. G.W. Montgomery, G.B. Martin, L.J. Bars and J.J. Pelletier, *Reprod. Fertil*, **73**, 457 (1985).
7. H. Fukami, M. Nakijama, M. Jacobson, D.G. Crosby, M. Dekker, *Naturally Occurring Insecticides*, New York, p. 71 (1971).
8. E. Atalan, H. Biyik, H. Battal and K. Özdemir, *Asian J. Chem.*, **20**, 25 (2008).
9. N.S. Reddy, K. Gumireddy, M.R. Mallireddigari, S.C. Cosenza, S.C. Bell, P. Venkatapuram, E.P. Reddy and M.V. Ramana Reddy, *Bioorg. Med. Chem.*, **13**, 3141 (2005).
10. W. Kandioller, C.G. Hartinger, A.A. Nazarov, J. Kasser, R. John, M.A. Jakupiec, V.B. Arion, P.J. Dyson and B.K. Keppler, *J. Organomet. Chem.*, **694**, 922 (2009).
11. I.J.S. Fairlamb, L.R. Marrison, J.M. Dickinson, F.-J. Lu and J.P. Schmidt, *Bioorg. Med. Chem.*, **12**, 4285 (2004).
12. T.E. Barman, D.V. Parke and R.T. Williams, *Toxicol. Appl. Pharmacol.*, **5**, 569 (1963).
13. A. Rehman, M.I. Choudhary and J.T. William, *Manual of Bioassay Techniques for Natural Product Research*, Harward Academic Press, Amsterdam, pp. 82-84 (1999).
14. C.J. Bal and Huaserv, *An Introduction to Ligand Field*, McGraw Hill, New York (1962).
15. K. Nakamoto, *Infrared and Raman Spectra of Inorganic and Coordination Compounds, Theory and Applications in Inorganic Chemistry*, John Wiley & Sons (2009).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.