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Antibacterial, Antifungal and Antitumor Studies of Organotin(IV) Hydroxamates

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Abstract

Three Organotin (IV) complexes of N-methyl P-flurobenzohydroxamic acid and N-methyl P-nitrobenzohydroxamic acid were synthesized. The compounds have the general formula R_2SnL_2. R is either butyl or phenyl, while L represents the ligand which may be P-flurobenzohydroxamic acid or P-nitrobenzohydroxamic acid. Antibacterial activities of the compounds were tested against Gram-positive ***Staphylococcus aureus,*** *and Gram-negative* ***Klebsiella, Escherichia Coli, and Pseudomonas.*** *Antifungal activities were evaluated against* ***Aspergillus Niger*** *and* ***Mucor,*** *while antitumor activity against HeLa cell line. Significant results were found against bacterial and fungal strains, while moderate antitumor activity against HeLa cells.*

Keywords: Organotin Hydroxamates, Disc Diffusion Method, IR.

INTRODUCTION

Hydroxamic acid is a unique group of organic compounds, which contain two functional groups in its formula R(CO)N(OH)R'. R and R'

may be alkyl or aryl groups and CO is a carbonyl group. These are amides, in which there is a substituted OH at nitrogen **[1].** When an acyl group substitutes the H atom at the nitrogen of hydroxylamine, as a result, mono-substituted hydroxamic acids are formed **[2].** Due to tautomeric behavior of hydroxamic acids, its derivatives and naturally occurring siderophores act as good chelating agents [3, 4]. The tautomerization gave two anionic forms such as mono-deprotonated (hydroxamate) and bi-deprotonated (hydroxamate) [5].

Hydroxamic acids and their derivatives are usually used as an important inhibitor of metalloenzyme which is continuously increasing the interest of researchers. Hydroxamic acids in the form of siderophore have a very important medicinal role. It is also used as an inhibitor of a large number of enzymes for example peroxidase, hydrolase, urease, lipoxygenase, cyclooxygenase, histone deacetylase, peptide deformylase, etc. Besides this, it has been found that it can be used as anticancer, antimalarial, anti-tuberculosis, and as antifungal agents [6]. Due to high biological activities, hydroxamic acids and their derivatives are used as antibacterial agents and mineral collectors [7]. Moreover, these can be used for the treatment of allergic diseases, restorative cancer, and metal poisoning. Additionally, it has been reported that hydroxamic acids have great potential against HIV, Alzheimer's, and cardiovascular diseases. Recently it has been known that the number of hydroxamic acid derivatives can be used to control tumor growth, hypertension, asthma, inflammation, arthritis, and also as an inhibitor of enzymes like matrix metalloproteinase. Organotin compounds are defined as the compounds in which there is at least one C-Sn covalent bond whereas carbon is being part of an organic group [8]. Oxygen donor ligands like hydroxamic acids interact with organotin moiety either in mono-dentate or bi-dentate fashion and give a variety of compounds [9].

Organotin(IV) derivatives of hydroxamic acids containing two oxygen atoms were studied at a large scale because of their biological importance[10]. The biochemical activities of these compounds mainly depend on the number of organic groups, ligand, the structure of the complex, and the coordination number of tin. Several organotin(IV) hydroxamate polymers have been prepared and characterized against cancer and viruses. The extensive studies of organotin(IV) complexes lead to the discovery of important anti-oxidant, wood preservatives,

and antifouling agents. There is a curiosity among researchers to know about the interaction of organotin moiety of hydroxamates with biomolecules like carbohydrate, nucleic acids, their derivatives, amino acids, and peptides [11].

MATERIAL AND METHOD

Glassware

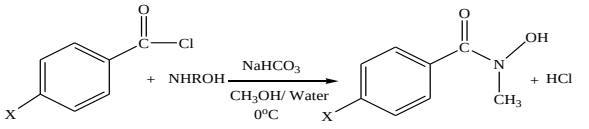
Common glassware in the laboratory (*D*ean and *S*tark water separator, beakers of different sizes, round bottom flask 100 ml and 250 ml, sample bottles and funnels) were used in this study. All these glassware were cleaned in a cleaning solution (chromic acid) overnight. Finally, all these glasswares were cleaned with water and acetone respectively and kept for drying at 72C° in the oven. Before use, all glassware was allowed to cool at room temperature.

Reagents and Solvents

All chemicals in this work were reagent grade, there was no need for further purification: *p*-Nitrobenzoyl chloride (Aldrich), *p*-Fluorobenzoyl chloride (Aldrich), N-methylhydroxylamine Hydrochloride (Aldrich), Ammonium chloride (Fluka), Ammonium solution 30%, Sodium Hydrogen Carbonate (BDH), Zink Dust (BDH), Magnesium Sulphate Dried (R&M), Sodium chloride (BDH), Toluene (Mallinckrodt), chloroform (Fisher Chemicals), Petroleum Ether 60-80 (Fisher Chemicals), Methanol (R&M), Ethanol (R&M), Acetone (R&M), Ethyl Acetate Deionized water, Dibutyltin(IV) Oxide (Fluka), Diphenyltin(IV) Oxide (Aldrich) were used in this work.

Syntheses of Hydroxamic Acid Ligands

N-Methyl *p*-substituted benzohydroxamic acids can be synthesized according to the method which has been discussed by Ulrich & Sayigh (1963). The general method of their preparation is shown in figure No 1.



Where X= F and NO_2; R= CH_3

Figure No 1

Syntheses of *N*-Methyl *p*-nitrobenzohydroxamic acid

Already reported ligand was synthesized according to the method which has been discussed in [13]. For the preparation of *N*-methyl *P*-nitrobenzohydroxamic acid, (4.25 g 50.5 mmol) of sodium hydrogen carbonate and (2.20 g, 25.5 mmol) of *N*-methyl hydroxylamine hydrochloride was taken in 100 ml methanol. *P*-nitrobenzoyl chloride was added in the mixture dropwise with continuos stiring.The temperature of the solution was maintained at nearly 0C°. After the addition of *P*-nitrobenzoyl chloride the mixture was stirred 30 mins more. Filtered and evaporated the solvent at low pressure. Un-necessary materials were removed by the addition of ethyl acetate in the resulting materials. Hydroxamic acids settle down on cooling. To get it in crystal form was left in the fridge overnight and finally dried with the help of silica. Yellow crystals were obtained with 79% yield. IR (KBr cm^{-1}): 3113 (s, br, v O-H); 1719.35 (s, v C=O); 1524 (s, v C-N) 877 (s, v N-O). Melting point: 109-112 C°.).

Syntheses of *N*–methyl *p*-fluorobenzohydroxamic acid

Already reported ligand *N*-methyl *p*-fluorobenzohydroxamic acid was synthesized according to the method which was followed for the preparation of *N*-methyl *p*-nitrobezohydroxamic acid. The *p*-fluorobenzoyl chloride was used instead of *N*-methyl *p*-nitrobenzoyl chloride. Colorless crystals of *N*-methyl *p*- fluorobenzohydroxamic acid were obtained with 83% yield. IR (KBr cm^{-1}): 3178 (s, br, v O-H); 1608 (s, v C=O); 1435 (s, v C-N); 908 (s, v N-O). Melting point (87-89℃).

Syntheses of Diorganotin(IV) Bis(*N*-methyl *p*-Substituted benzohydroxamates)

The methods used by Harrison & Richard (1980) and Das & De (1995) were followed for the syntheses of di-organotin hydroxamates. The general reaction is shown in Figure No 2.

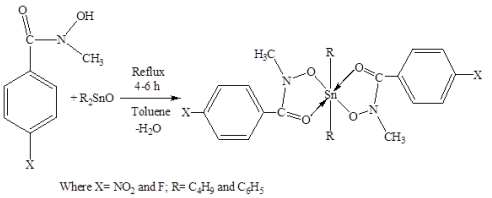


Figure No 2

Syntheses of dibutyltin (IV) Bis(*N*-methyl *p*-fluorobenzohydroxamate)

Dibutyltin(IV) Bis(*N*-methyl *p*-fluorobenzohydroxamate) was synthesized by dissolving free ligand(0.98 g, 5mmol) in hot toluene then adding dibutyltin(IV) oxide (0.41g) to this solution. The solution was stirred for 6hrs and filtered after cooling. The volume of the filtrate was reduced at low pressure and dried by using silica. Hot petroleum ether was added to the precipitates and recrystallized in ethanol as colorless crystals. Yield: 79%. IR (KBr cm^{-1}): 1605 (s, v C=O); 1525 (s, v C-N), 953 (s, v N-O); 478 (s, v Sn-O); 562 (s, v Sn-C). Melting point: 102- 105℃.

Syntheses of Diphenyltin(IV) Bis(*N*-methyl *p*-fluorobezohydroxamate)

Diphenyltin(IV) Bis(*N*-methyl *p*-fluorobenzohydroxamate was synthesized through the procedure followed for the syntheses of Dibutyltin(IV) Bis(*N*-methyl *p*-fluorobenzohydroxamate), but diphenyltin(IV) oxide was used instead of dibutyltin(IV) oxide. White crystals, yield: 81%. IR (KBr cm^{-1}); 1600 (s, v C=O); 1452 (s, v C-N); 950 (s, v N-O); 454 (s, v Sn-O); 563 (s, v Sn-C). Melting point 202-205℃.

Syntheses of Dibutyltin (IV) Bis[*N*-methyl *p*-nitrobenzohydroxamate]

Dibutyltin(IV) Bis[*N*-methyl *P*-nitrobenzohydroxamare] was synthesized by the procedure which was followed for the syntheses of Dibutyltin(IV) Bis(*N*-methyl *p*-fluorobenzohydroxamate). For this synthesis, the ligand was *N*-methyl *p*-nitrobenzohydroxamic acid instead of *N*-methyl *p*-fluorobenzohydroxamic acid. Yellow crystals were obtained with a yield: 82%. IR (KBr cm^{-1}): 1603 (s, v C=O); 1468 (s, v C-N); 937 (s, v N-O); 414 (s, v Sn-O); 597 (s, v Sn-C). Melting point: 152-153 ℃.

Antibacterial assay

Antibacterial activity of synthesized organotin(IV) hydroxamates was tested against Gram-positive ***Staphylococcus aureus,*** and Gram-negative ***Klebsiella Escherichia Coli and Pseudomonas*** bacterial strains using the diffusion disc method. Agar was prepared on Petri

plates and bacterial strains were allowed to grow in 25ml of the medium. For test and stock, solution 100ml was prepared by dissolving the compounds in DMSO (dimethylsulfoxide) with (4mg/ml) concentration. This solution was added to wells and incubated the culture at 37℃ for 24 hours. The antibacterial activity of these compounds was recorded by measuring the diameter of the inhibition zone. Doxycycline (DO 30mg) and DMSO were used as negative and positive control respectively.

Antifungal Assay

The concern fungi ***Aspergillus Niger*** and ***Mucor*** were isolated from their environment and tested against the freshly prepared solution of organotin(IV) hydroxamate complexes in DMSO (0.02mg/5ml). To examine the antifungal activity, the diffusion method was applied. For this activity, these fungal strains were cultured in sabouraud dextrose agar slants. 10-20mg was the final concentration of the solution for fungal cultures that could stay in the wells. After 4, 7, 14 days antifungal activity of these compounds was observed by comparing it with control [12].

Antitumor activity

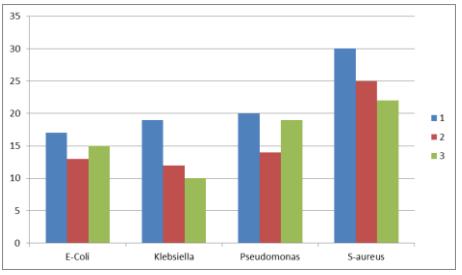
Antitumor activity of compounds was examined in 96-well flat bottomed microplates with MTT (3-[4, 5-diamethylthiazole-2-yl]-2, 5-diphenyl-tetrazolium bromide) as standard in a colorimetric assay. 5% FBS (fatal bovine serum), 100 IU/ml of penicillin, and 100 µg/ml streptomycin were also added in the medium where HeLa cells of cervical cancer were cultured and incubated at 37°C in 5% CO_2. After collecting and counting the cells further diluted with medium. Another culture of these cells was prepared with concentration of 6×10^4cell/ ml. 100µL introduced into each 96-well plate, incubated overnight. After this medium was removed and a fresh medium of 200µL with different concentration of compounds (1-30 µL) was added. After two days, 200µL MTT with 0.5 mg/ml concentration was added in each well further incubated for 4hrs and finally 100µL of DMSO was added to each well. By using micro plate reader the conversion of MTT to formazan was calculated by measuring its absorbance at nearly 570 nm. The concentration which causes 50% growth inhibition was recorded as antitumor activity against HeLa.

RESULT AND DISCUSSION

Antibacterial Activity

Antibacterial activity of synthesized diorganotin(IV) hydroxamates was analyzed against Gram-positive ***Staphylococcus aureus,*** and Gram-negative ***Klebsiella Escherichia Coli and Pseudomonas,.*** Doxycline was used as a standard drug. Disc diffusion and agar well methods were followed to analyze this bioactivity. The inhibition zones were recorded in mm and represented with help of a graph. Below 10mm zone inhibition diameter has been considered as weak zone, 10mm to 16mm as moderate while above 16mm inhibition zone stated as active. Compounds show significant activity against all four bacterial strains but more effective against ***Staphylococcus aureus*** (Gram-positive). It is due to the greater penetration of these complexes and the absence of the outer membrane in ***Staphylococcus aureus*** (Gram-positive). These activities of organotin(IV) hydroxamates were due to the presence of different organic groups attached with tin. The organotin(IV) moiety interacts with deoxyribose nucleic acid (DNA) of bacteria. Three compounds were codded as 1, 2, 3, and the color in Table No 01, blue, red, and green for Dibutyl *N*-methyl *P*-flurobenzohydoxamate, Dibutyl *N*-methyl *P*-nitrobenzohydroxamates and Diphenyl *N*-methyl *P*-flurobenzohydroxamate, respectively.

Table No. 01.



Antifungal Activity

Anti-fungal activities of synthesized compounds were carried out through the diffusion method. Results show strong activity against ***Mucor*** and ***Aspegillus Niger***. All compounds strongly inhibited the fungal activity as shown in the figure. This antifungal activity is due

to the easy penetration of these compounds through the fungal cell wall. The given compounds show strong antifungal activity as compared to antibacterial activity because of differences in the composition of cell walls of bacteria and fungi. All tested compounds completely inhibited the fungal activities as compared to standard drugs. The three compounds were codded as 1, 2, 3 for Di-butyltin(IV) *N*-methyl *P*-flurobenzohydoxamate, Di-butyl(IV) *N*-methyl *P*-nitrobenzohydroxamates and Di-phenyl(IV) *N*-methyl *P*-flurobenzohydroxamate, respectively. The antifungal activity is given in the Table No 02.

Table No. 02.

S. No	*Mucor*	*A Niger*	Total inhibited growth
01	**17mm**	**14mm**	**56%**
02	**13mm**	**20mm**	**37%**
03	**12mm**	**18mm**	**40%**

In vitro antitumor activity

Antitumor activity of synthesized diorganotin(IV) hydroxamates were evaluated against HeLa cell of cervical cancer. Data in Table No 03 presents the activity of these compounds against HeLa cells. The three diorganotin(IV) compounds exhibit important activity against this cell. The compounds show concentration-dependent antitumor activity towards cervical cancer cells. Diphenyl organotin(IV) compounds were found more effective as compared to dibutyl organotin(IV) compounds, due to the presence of phenyl group at the tin atom. This activity was carried out by comparing the antitumor activity of these compounds with doxorubicin. Doxorubicin is used for the treatment of cervical cancer as well as for other types of cancer. This was used as the positive control.

Table No. 03.

Sample Code	Conc.(μM).	%inhibition	IC_{50}
Doxorubicin	30 μm	85.15	1.32μM
DBNMPF	=	40%	1.10μM
DBNMPN	=	39%	1.09μM
DPNMPF	=	47%	1.15μM

Conclusion

By using the two already reported ligands such as *N*-methyl *P*-flurobenzohydroxamic acid and *N*-methyl *P*-nitrobenzohydroxamic acid their three complexes of diorganotin(IV) such as dibulyl *N*-methyl *P*-flurobenzohydrohydroxamate, dibutyl *N*-methyl *P*-nitrobenzohydroxamate and diphenyl *N*-methyl *P*-flurobezohydroxamate were synthesized in the department of chemistry University of Balochistan. Bioactivity of above mentioned three complexes were evaluated against Gram-positive ***Staphylococcus aureus,*** and Gram-negative ***Klebsiella Escherichia Coli and Pseudomonas,*** two fungi (***Mucor and Aspirgellus Niger***) and HeLa cell line of cervical cancer. In antibacterial bioassay, these compounds successfully inhibit the bacterial activity but their antibacterial activity against gram-positive bacteria was more prominent. Similarly, these show antifungal activity against Mucor, A-Niger, and moderate anticancer activity for the HeLa cell line of cervical cancer.

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