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RESEARCH ARTICLE

# Hydroxytriazenes and their Cobalt (II) Complexes as Bioactive Agents

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## ABSTRACT

The biological activity of hydroxytriazenes and their complexes of Co(II) have been evaluated against E. coli, klebseillla pneumoniae, bacillus by pathogen city tests. These tests were carried out by cup or agar well assay method. The antibacterial activity of metal ligand complexes revealed enhanced activity of complexes as compared to corresponding ligands.

## KEYWORDS

Hydroxytriazenes, Cobalt (II) complexes, Antibacterial activity

## INTRODUCTION

A number of hydrotriazenes and their metal complexes have been reported to be antifungal[1-3], antibacterial[4-6], insecticidal[7-12], analgesic[13] and anti-inflammatory[14-15] and wound healing agents[16]. In light of above facts the present works has been devoted to studies of biological activities of Co (II) complexes of hydroxytriazenes and compare them with the biological activities of pure ligands.

## Experimental

### *Synthesis of Hydroxytriazenes & their Cobalt (II) Complexes*

Synthesis of hydroxytriazene involves preparation of aryl hydroxylamine and their aryl diazoniun salts and coupling them at the temperature between 0-5°C under suitable pH range 4-6 using this method the compounds have been synthesized.

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### *Step (a): Preparation of Hydroxylamine*

Synthesis of hydroxylamine: In a one liter beaker (0.1mol) of nitroaryl benzene or substituted nitro aryl benzene, 0.1mol of $NH_4Cl$, 50 ml water and 25 ml $C_2H_5OH$ were mixed, stirred mechanically and cooled to 0° C by surrounding the beaker with ice salt mixture, 20 gm Zn dust was added in small lots such that the temperature of reaction mixture maintained between 50 to 60° C. The reaction mixture was stirred mechanically for another 15 min. The solution was filtered and filtrate was taken in a beaker and kept in freezer and used as such for coupling with diazotized product.

### *Step (b): Diazotisation of Sulphanilic acid*

In a 500 ml beaker 0.1 mol of sulphanilic acid was dissolved in warm mixture of 25 mL of concentrated HCl and 25 mL of water and in another beaker 0.1mol of sodium nitrite was dissolved in 20 mL of distilled water. The beaker which contained sulphanilic acid solution was put in an ice bath to maintain temperature between 0 to 5º C. To this sodium nitrite solution was added drop by drop with

continuous stirring. The diazotized product so obtained was directly used for coupling.

***Step (c): Coupling***

The hydroxylamine obtained in step (a) was coupled with the diazotized product at 0 to 5°C under mechanical stirring with occasional addition of sodium acetate solution for maintaining the pH close to 5 during coupling process. The compound was obtained as light yellowish needle shape crystals after recrystallization from distilled water and checked for purity by TLC.

***Synthesis of Cobalt (II) Complexes of Hydroxytriazenes***

For the synthesis of complex requisite quantity of hydroxytriazene (0.02 Mole) was dissolved in minimum quantity of water in a 100 mL of beaker. Similarly requisite quantity of cobalt (II) nitrate hexahydrate of A.R. grade was dissolved in minimum quantity of double distilled water. The pH of both the solutions were adjusted to respective pH range as studied early each one of the complex. Solution of hydroxytriazene after adjustment of suitable pH was kept on a magnetic stirrer in a beaker. Cobalt (II) solution was added drop by drop to hydroxytriazene solution along with magnetic stirring. During this period temperature of the solution was maintained between 40-50°C. After complete addition of cobalt (II) solution, stirring was continued for another fifteen minutes.

Finally, the solution was kept aside for attaining room temperature. In each case yellowish-brown colored microcrystals separated out. The complex so formed was filtered under suction and washed with double distilled water to remove unreacted cobalt (II). It was finally recrystallized with suitable solvent.

## MATERIALS AND METHOD

Borosil and corning glassware's were used for the experimentation. All the glassware's used during experiment were cleaned by Chromic acid and then distilled water. They were dried before being autoclaved in hot air oven at 180°c for 2 hours.

The pure culture of Pathogenic bacteria used for antibacterial activity was sub cultured and characterized by standard method of identification.

***Growth Medium Preparation for Bacteria***

To culture all the bacteria nutrient agar medium was used. The composition of nutrient agar was-

Peptone = 10 gm

Yeast extract = 10 gm

Beef extract = 6 gm

Agar = 30 gm

Distilled water = 2000 ml

The above mentioned quantities of Peptone, beef extract and agar were mixed with two liter of double distilled water. The $p^H$ of this medium was adjusted at 6.8 with the help of 0.1N hydrochloric acid and 0.1N sodium hydroxide. This medium was then transferred into conical flask, plugged and autoclaved at 121$^O$C for 15 minutes.

**Pathogenicity Test**

Pathogenicity tests were carried out by Cup or agar well assay method[17]. These tests were carried out in 90 ml petriplates, which were thoroughly sterilized before their use. In each petriplate 30 ml of molten growth medium developed for bacteria was transferred and it was allowed to solidify at room temperature. A 0.1 ml of bacterial culture was evenly spread over the whole surface of growth medium in petriplate. Thereafter a well of 10 mm diameter was dug in the growth medium with the help of presterilized cork borer and it was filled with 200 ppm solution of test compound in DMF for antibacterial study. Petriplates so prepared were incubated in BOD at 37°c for 72 hours. After that zone of inhibition caused by test compounds around the well was measured in mm. Same procedure was repeated for DMF and standard drugs used. Results obtained in antibacterial studies against *E. coli, Klebsiella, Bacillus* are given in table no.1 and 2.

Table 1: Study of antibacterial activities of hydroxytriazene at 250 ppm

| HT* No. | Name of compound | Zone of inhibition in mm. for | | |
|---|---|---|---|---|
| | | *E.coli* | *Klebsiella* | *Bacillus* |
| (i) | 3-hydroxy-1,3- diphenyltriazene | 13 | 6 | 11 |
| (ii) | 3-hydroxy-3-phenyl -1-p-sulphonato (sodium salt) phenyltriazene | 17 | 5 | 3 |
| (iii) | 3-hydroxy-3-o-tolyl-1-p-sulphonato (sodium salt) phenyltriazene | 16 | 15 | 11 |
| (iv) | 3-hydroxy-3-m-tolyl-1-p-sulphonato (sodium salt) phenyltriazene | 17 | 12 | 7 |
| (v) | 3-hydroxy-3-p-tolyl-1-p-sulphonato (sodium salt) phenyltriazene | 17 | 6 | 4 |
| (vi) | 3-hydroxy-3-p- acetophenyl-1-p-sulphonato (sodium salt) phenyltriazene | 16 | 16 | 15 |
| (vii) | 3-hydroxy-3-p- tolyl-1-p-acetanalide phenyltriazene | 8 | 8 | 8 |
| | Ciprofloxacin | 27 | 26 | 25 |

**HT= Hydroxytriazene**

Table 2: Study of antibacterial activity of cobalt (II) complexes of hydroxytriazenes at 250 ppm

| Complex No. | Cobalt (II) complex with hydroxytriazene | Zone of inhibition in mm. for | | |
|---|---|---|---|---|
| | | *E.coli* | *Klebsiella* | *Bacillus* |
| C-(i) | 3-hydroxy-1,3- diphenyltriazene | 17 | 12 | 7 |
| C-(ii) | 3-hydroxy-3-phenyl -1-p-sulphonato (sodium salt) phenyltriazene | 17 | 16 | 10 |
| C-(iii) | 3-hydroxy-3-o-tolyl-1-p-sulphonato (sodium salt) phenyltriazene | 17 | 15 | 10 |
| C-(iv) | 3-hydroxy-3-m-tolyl-1-p-sulphonato (sodium salt) phenyltriazene | 11 | 10 | 2 |
| C-(v) | 3-hydroxy-3-p-tolyl-1-p-sulphonato (sodium salt) phenyltriazene | 11 | 16 | 10 |
| | Ciprofloxacin | 27 | 26 | 25 |

## RESULTS AND DISCUSSION

Antibacterial studies were carried out by cup or agar well assay method. All Hydroxytriazenes and cobalt complexes were screened for their antibacterial activity against four bacterial strains *E. coli, Klebsiella, Bacillus*. For the sake of comparison same process was repeated with standard drugs namely Ciprofloxacin. Antibacterial studies were carried out in water solution of compound at 250 ppm. In each case a blank was run only with solvent to exclude activity due to solvent. However no antibacterial activity was shown by blank. It is interesting to note that in case of activity against *E. coli* all hydroxytriazenes has marginally better as compared to standard drugs. All the five complexes have shown better or equal activity.

Thus further investigation is required to be done for the use of complexes of Cobalt (II) with hydroxytriazenes and hydroxytriazenes for their use as antibacterial compounds.

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