P-022 **Selective antibacterial activity of some novel zirconium complex(s)**

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INTRODUCTION

A large number of mixed ligand complexes, bis(β-diketonato)bis(alkoxo)of early transition metal complexes particularly zirconium has attracted special attention due to their antitumour activities which is more than cisplatin as well as act as effective antibacterial, and antifugal agents [Keppler et.al,1991, 1993]. As we can modify the properties of phenol as a ligands by substituting various groups at ortho, para or meta positons. The ligand, 1-phenylbutane-l,3-dionato, (bzac) is an asymmetric β-diketone chelator useful for establishing one key structural feature tbr activity in budotitane[Heim et al, 1989]. In view of this, we herein report a facile synthesis and antibacterial activity of novel phenoxides of dichlorobis(benzoylacetonato) zirconium(IV) using some substituted phenols such as 4-methoxyphenol and 4-nitrophenol so that they may be used as potential chemotherapeutic agents to combat pathogenic microorganisms.

MATERIALS AND METHODS

All chemicals and reagents were purified by standard procedures. All solvents were of AR grade and dried by standard methods.Proton NMR spectra of the isolated complexes were recorded on a BRUKER AVANCE II 400 NMR spectrometer using TMS as an internal standard reference and $CDCl_3$ as solvent. The infrared spectra of the complexes under study were recorded in potassium bromide pallets on PERKIN ELMER Spectrum RXIFT-IR-System (4000–200 cm^{-1}). C and H analyses were performed on a Carlo-Erba 1108 Elemental Analyzer.

Synthesis of [ZrCl(bzac)$_2$(OC$_6$H$_4$–X)] (X=OCH$_3$ and -NO$_2$): To the solution of $ZrCl_2(bzac)_2$ (1.0g; 0.002mol) was made to react with the substituted phenols viz. 4-methoxyphenol (0.256g; 0.002mol) and 4-nitro phenol (0.286g; 0.002mol) in the presence of diethylamine (0.22mL; 0.002) in predetermined 1:1:1 molar ratios, in dry benzene in separate experiments. (Scheme I)

$$ZrCl_2(bzac)_2 + nHOC_6H_4\text{-}X + nEt_2NH \xrightarrow[C_6H_6,\ \text{Reflux}]{\text{Stirring}} nEt_2NH.HCl\downarrow + ZrCl_{2-n}(bzac)_2(OC_6H_4\text{-}X)_n$$

Reaction scheme I

Synthesis of [ZrCl(bzac)$_2$(OC$_6$H$_4$OCH$_3$).L] (L= 2,2-Bipyridyl(bipy) and 1, 10-Phenanthroline (phen)): To a solution of $ZrCl(bzac)_2(OC_6H_4–X)$ aryloxides (0.5g; 0.009mol), in carbon tetrachloride was added equimolar amounts of bidentate amines viz. 1,10-phenanthrolin (0.16g; 0.009mol) and 2,2′-bipyridyl (0.14g; 0.009mol) in predetermined 1:1 molar ratios in separate experiments. Formation of complexes may be rationalized as follows (scheme II):

$$ZrCl_{2-n}(bzac)_2(OC_6H_4\text{-}X)_n + C_{12}H_8N_2 \xrightarrow{CCl_4,\ \text{stirring}} ZrCl_{2-n}(bzac)_2(OC_6H_4\text{-}X)_n.C_{12}H_8N_2$$

$$ZrCl_{2-n}(bzac)_2(OC_6H_4\text{-}X)_n + (C_5H_4N)_2 \xrightarrow{CCl_4,\ \text{stirring}} ZrCl_{2-n}(bzac)_2(OC_6H_4\text{-}X)_n.(C_5H_4N)_2$$

Reaction scheme II

In vitro antibacterial assay: The complexes synthesized during present study were screened *in vitro* for their antibacterial activity on selected bacteria *E. coli* (G^-); *S. aureus* (G^+); *P. aeruginosa* (G^-) and *B. subtilis* (G^+) by disc diffusion method. The bacterial isolates were cultured on Luria broth and nutrient broth separately and incubated for 24 hours at 37°C.

Preparation of Inoculation: A loopfuls of each test microorganisms viz. *E. coli*, *B. subtilis, P. aeruginosa and S. aureus* from culture media were aseptically transferred into 5mL of luria broth (*E .coli , B. subtilis*) and nutrient broth (*P. aeruginosa*, *S. aureus*) separately. The test tubes with above list microorganisms were incubated at 37°C for 24 hour before use with the aim of obtaining microorganisms concentration of 10^5CFU/mL. (Colony Forming Unit)

Minimum Inhibitory Concentration (MIC): The MIC values can be determined by a number of standard test procedures. The most commonly employed methods are the broth dilution method and agar dilution methods. Double fold dilutions are made in nutrient broth (bacterial growth media). The test organisms are added to the dilutions, incubated at 37°C for 24 hours. This procedure is a standard assays for antimicrobial activity. The procedure incorporates the content and intent of the American Society for Microbiology (ASM) recommended methodology. The determination of MIC values using broth dilution method was applied on compounds that proved their high efficacy against test microorganism by disc diffusion method. 5mg of complex dissolved in 7mL of DMSO was used as stock solution for the preparations of dilutions.

RESULTS & DISCUSSIONS

Antimicrobial activity: In the present study the antibacterial activities of some of synthesized compounds were

studies against four human pathogenic bacteria, viz., *E. coli* (G^-); *S. aureus* (G^+); *P. aeruginosa* (G^-) and *B. subtilis* (G^+). The antibacterial activities of compounds assayed at concentration 1000 ppm and 2000 ppm against both gram negative and gram positive pathogenic bacteria. The susceptibility testing was carried out by disc diffusion method. The inhibition zone of diameters was measured and rounded up to the nearest whole number (mm) for analysis after 48 hours of incubation at 37°C. After 48 hours, there was no increase in diameters of zone of inhibition. Thus, the inhibitory effect of complexes at both concentration (1000 ppm and 2000ppm) was measured against these organisms within 48 hours and has been shown in (figures I & II). The screening results indicate that at concentration 2000ppm, complexes $[ZrCl_2(bzac)_2]$, $[Zr(bzac)_2(OC_6H_4OCH_3\text{-}4)_2]$ and $[Zr(bzac)_2(OC_6H_4NO_2\text{-}4)_2]$ has shown average antibacterial activity against *P. aeruginosa* and no activity w.r.t. *E. coli, S. aureus* and *B. subtilis.* Complexes $[Zr(bzac)_2(OC_6H_4OCH_3\text{-}4)_2.C_{12}H_8N_2]$, $[Zr(bzac)_2(OC_6H_4NO_2\text{-}4)_2.C_{12}H_8N_2]$ and $[ZrCl(bzac)_2(OC_6H_4NO_2\text{-}4).(C_5H_4N)_2]$ has significantly inhibit (diameter of inhibition zone ≥15mm) the growth of *P. aeruginosa* but showed moderate activity against *E. coli, B. subtilis* and have no activity w.r.t. *S. aureus* bacterial strain.Whereas the screening results indicate that at 1000ppm concentration complexes, $[Zr(bzac)_2(OC_6H_4OCH_3\text{-}4)_2.C_{12}H_8N_2]$, $[Zr(bzac)_2(OC_6H_4NO_2\text{-}4)_2.C_{12}H_8N_2]$ have shown strong inhibitory effects (diameter of inhibition zone >20mm) against *E. coli, B. subtilis and P. aeruginosa* and moderate antibacterial activity against *S. aureus*. Complex, $[ZrCl(bzac)_2(OC_6H_4NO_2\text{-}4).(C_5H_4N)_2]$ showed moderate antibacterial activity against *E. coli, B. subtilis* and effectively inhibit the growth of the *P. aeruginosa* but showed no activity against *S. aureus*. Complex, $[Zr(bzac)_2(OC_6H_4OCH_3\text{-}4)_2]$ has shown promising antibacterial activity against gram negative bacteria cultured in the present study whereas complex, $[Zr(bzac)_2(OC_6H_4NO_2\text{-}4)_2]$ has shown considerable activity against gram positive bacteria *S. aureus* only.

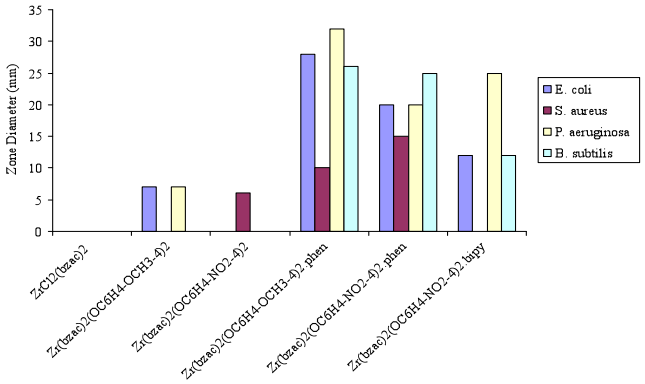

Fig.1 Antibacterial Screening for Complexes Using Disc Diffusion Assay at 1000 ppm.

In general, the antibacterial activity of tested compounds follows the pattern at both the concentrations as:

P.aeruginosa > *E. coli* > *B. subtilis* > *S. aureus*

The complexes containing 1,10-phenanthroline or 2,2'-bipyridyl showed better activity than complexes containing only phenoxides group. This observation strongly suggests enhancement of antibacterial activity as a function of chelation [Sebut et al., 2001].

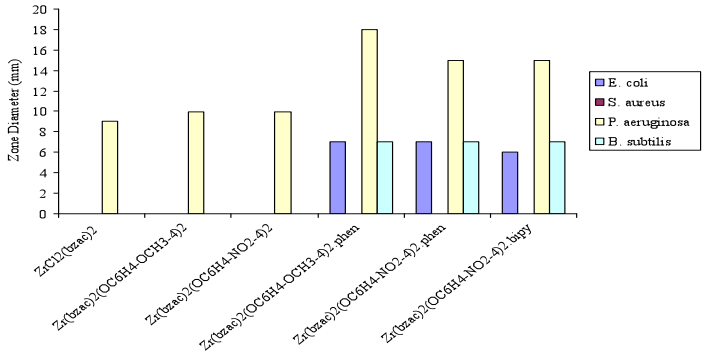

Fig.2 Antibacterial Screening for Complexes Using Disc Diffusion Assay at 2000 ppm

Minimum Inhibitory Concentrations (MICs): MICs are used in diagnostic laboratories to confirm unusual resistance, to give a definitive answer when a borderline result is obtained by other methods of testing, or when disc diffusion methods are not appropriate [Greenwood et al, 1997]. The MICs values confirmed the significant activity against the tested microorganisms. The MICs values ranged from 71-572µg/mL. Overall all the microorganisms *E.coli, B. subtilis, P. aeruginosa and S. aureus* were most susceptible to complex, $[Zr(bzac)_2(OC_6H_4OCH_3\text{-}4)_2.C_{12}H_8N_2]$ displaying values at concentration 142µg/mL.Complex, $[Zr(bzac)2(OC_6H_4NO_2\text{-}4)_2.C_{12}H_8N_2]$ showed MICs values at concentration 286µg/mL.

CONCLUSIONS

It can be concluded from the above study that with the incorporation of chelating ligands, antibacterial activity of complexes increased. These complexes can be used as potential chemotherapeutic agents to combat pathogenic microorganisms.

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