In vitro Antibacterial Activities of Some Ferrocenyl Derivative Complexes

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Abstract

Four ferrocene derivative complexes, designated as compound A, B, C, and D - (Ferrocenyl)- 1,3-dimethyl-1,3-dione di-aqua cobalt (II) , (Ferrocenyl methyl)- 2,2,6,6-tetramethyl-2,5-hexa-dione di-aqua cobalt (II), (Ferrocenyl methyl)-2,2,6,6-tetramethyl-2,5- hexa dione di-aqua nickel (II), and (Ferrocenyl methyl)- 2,2,6,6-tetramethyl-2,5-hexa dione di-aqua copper (II) were synthesized by reaction of ferrocenyl derivative ligands such as (Ferrocenyl methyl)- 1,3- dimethyl propane dione and (Ferrocenyl methyl)- 2,2,6,6-tetramethyl-2,5-hexa -dione with the transition elements Co(II), , Ni(II) and Cu(II) in dichloromethane and water. The prepared four complexes and ferrocenyl lignd were tested *in vitro* for antimicrobial activity against three gram-positive and three gram-negative bacteria using disc-diffusion method. All of the compounds were found active against the test organisms. The minimum inhibitory concentration (MIC) of the active complexes was estimated between 64-128 µg/ml against *Bacillus subtilis* and *Shigella dysenteriae* AL-35587.

Key words: Ferrocenyl deriveative complexes; antibacterial activity

Introduction

Due to evergrowing antibacterial resistance, it necessitates the development of newer and safer antibacterial drugs. Every year thousands of compounds are synthesized with an aim to find a potential chemotherapeutic agent to combat pathogenic microorganisms. But very few compounds are withstood as therapeutic agent for various methodological tests (Alam *et al.*, 2004)

The antimicrobial screening is necessary to find out the suitable candidate of chemotherapeutic agent among the synthesized compounds. Usually many compounds possess antimicrobial properties but have serious toxic effects to the host, therefore in the ideal case, the drug should be highly toxic to the parasite and completely atoxic for the host (Reiners, 1982). In the continuation of our ongoing efforts aimed at finding new compounds for chemotherapy, five new synthesized compounds were selected for antimicrobial screening.

Recently, we have synthesized four complexes of ferrocenyl derivatives such as $[Co\{FcCH_2CH(COCH_3)_2\}_2(H_2O)_2]$, $[Co\{FcCH_2CH(COC(CH_3)_3\}_2\}_2(H_2O)_2]$,$[Ni\{FcCH_2CH(COC(CH_3)_3\}_2\}_2(H_2O)_2]$and $[Cu\{FcCH_2CH(COC(CH_3)_3\}_2\}_2(H_2O)_2]$. These compounds were designated as compound A, B, C, and D, respectively. The compounds contain some functional groups like ferrocene, carbonyl, methyl and some transition metal such as copper and nickel. Those particular moieties are known to contribute antimicrobial activities or toxic activities (Reiners, 1982).

This article describes the antibacterial activity of several newly synthesized ferrocenyl derivative complexes to establish the effectiveness of coordination complexes as a possible potential means for developing new antibacterial principles.

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Materials and Methods

Source of compounds: The compounds used in the present study were synthesized in the inorganic chemistry laboratory of the department of chemistry according to the following general procedure:

Preparation of Ferrocenyl methyl- 1,3-dimethyl propane dione (Ligand): In a typical reaction, 1.1551g of trimethyl ammonium iodide [$FcCH_2N^+Me_3I^-$] (3 mmol) was dissolved in acetonitrile (75 ml), and 0.3662g (3 mmol) of sodium salt acetyl acetonate was added to it and the mixture was refluxed for 16 hours. The mixture was then evaporated to dryness and chromatographed on silica column (23cm×2cm). Elution with ethyl alcohol (C_2H_2OH) gave an orange fraction which on evaporation yielded ferrocenyl methyl- 1, 3-dimethyl propane dione as orange colour. The yield was 1.01g (67%).

Preparation of Ferrocenyl Derivative Complexes (A, B, C,& D): The compound A ([$Co\{FcCH_2CH(COCH_3)_2\}_2(H_2O)_2$]) was prepared by reacting 1mmol of [$FcCH_2CH(COCH_3)_2$] dissolved in 15ml of dichloromethane (CH_2Cl_2) and 0.5 mmol of cobalt (II) acetate [$(CH_3COO)_2Co.4H_2O$] dissolved in 60 ml of water. The other complexes were prepared using the same ratio (1:2) of metal salt and ligand. The compound B is prepared with metal salt cobalt acetate and the ligand (Ferrocenyl)- 2,2,6,6-tetramethyl-2,5- hexa dione, compound C is prepeared with metal salt nickel acetate and the ligand (Ferrocenyl)- 2,2,6,6-tetramethyl-2,5- hexa dione and the compound D is prepeared with metal salt copper acetate and the ligand ferrocenyl methyl- 1, 3-dimethyl propane dione

Test microorganisms: Six microorganisms were used to test the antibiotic activity of the isolates. Three of them were gram-positive and three were gram-negative bacteria. Gram-positive species were *Staphylococcus aureus* ATCC-259233, *Sarcina lutea, and Bacillus subtilis.* Gram-negative strains were *Escherichia coli* FPFC-1407, *Pseudomonas aeruginosa*, and *Shigella dysenteriae* AL-35587. They were maintained in nutrient agar slants at 4ºC.

Antibacterial Screening: In vitro antibacterial activities the complexes were performed by standard disc diffusion technique (Al-Bari, 2005). The discs (300 µg/disc) were prepared by dissolving the compounds in DMSO and applied on the sterilized filter paper discs (5mm in diameter) with the help of a micropipette in an aseptic condition and allowed to leave these discs for a few minutes in an aseptic hood for complete removal of the solvent. Kanamycin (30µg/disc) was used as standard antibiotic. As a negative control, a blank disc impregnated with solvent followed drying off was used. Briefly, in this study, the test discs, standard discs and blank discs were placed in a petridish with a particular bacterium and then left in a refrigerator at 4°C for 12-18 hour in order to diffuse the compounds from the discs to the surrounding media in the Petri dishes. The Petri dishes were then incubated at 37°C for overnight to allow the bacterial growth. The antibacterial activities of the pure compounds were then determined by measuring the respective zones of inhibition (ZOI) in mm.

Determination of minimum inhibitory concentration (MIC): The Minimum Inhibitory Concentration (MIC) was determined using broth-dilution method (Reiner, 1982, and Tyler *et al.*, 1988). The MIC was determined against two pathogenic bacteria- *Bacillus subtilis* and *Shigella dysenteriae.*

Results and Discussions

Antimicrobial activity: The complexes showed moderate antibacterial activates at the concentration of 300 µg/disc against a series of gram-positive and gram-negative pathogenic organisms (Table 1). Among them, complex C, ([$Ni\{FcCH_2CH(COC(CH3)_3\}_2\}_2(H_2O)_2$]) and complex D, ([$Cu\{FcCH_2CH(COC(CH_3)_3\}_2\}_2$]) were more active than others. The zone of inhibition (ZOI) against the bacterial pathogens were in the range of 9-16 mm. Gram-positive bacteria were found to be more sensitive than gram-negative bacteria.

Our present study supports the previous results of antibacterial activity for metal co-ordination complexes (Biswas *et al.*, 2002; Pratt *et al.*, 1979 and Bauer *et al.*, 1966).

Minimum inhibitory concentration (MIC): The minimum inhibitory concentration of the complex C, ([$Ni\{FcCH_2CH(COC(CH3)_3\}_2\}_2(H_2O)_2$]) and D, ([$Cu\{Fc CH_2CH(COC(CH_3)_3\}_2\}_2$]) were determined against *Bacillus subtilis* and *Shigella dysenteriae* by serial dilution technique. The MIC levels of the complex C were found 128µg/ml against *Bacillus subtilis* and *Shigella*

dysenteriae, respectively. The MIC of complex D against these two pathogens was 64 µg/ml (Table 2).

The pattern of antibacterial activities of the complexes exhibited a structure activity relationship. The complex C and D were more active than other three complexes. Compound C contains nickel as transition metal with coordination bond and twelve methyl groups, whereas the complex D contains the element copper with same number of methyl. The compounds A and B contain cobalt. Thus, the difference in the presence of transition metals may be a contributing factor for varying antimicrobial activity. Moreover, the complex A and B are octahedral whereas the complex C and D are square planar. So, according to our study, the square planar compounds are more active than the octahedral compounds. Therefore, the position and attachments of the groups are playing an important role to contribute to the biological activity. These results correspond to our previous findings (Zakaria *et al.*, 2000, and Zakaria *et al.*, 2001). During antibacterial screening of some other ferrocene derivative compounds, we observed that the complex -(Ferrocenylmethyl) triphenylphosphonium iodide- showed higher activity than other complexes. This complex has three phenyl groups with phosphorus. The other compound that had second highest activity contained only one phenyl group. In this report, we have observed that the difference in activity among the complexes is due to the presence of different elements.

Table 1. *In vitro* antibacterial activity of co-ordiantion complexes

Bacterial strains	Zone of inhibition in mm					
	Complex A (300µg/disc)	Complex B (300µg/disc)	Complex C (300µg/disc)	Complex D (300µg/disc)	Complex E (300µg/disc)	Kanamycin (30µg/disc)
Gram-positive						
B. subtilis	10	9	16	14	0	32
S. aureus	10	9	14	16	0	31
S. lutea	9	8	15	14	0	30
Gram-negative						
E. coli	9	10	15	14	0	30
S. dysenteriae	9	8	15	13	0	32
P. aeruginosa	8	9	14	14	0	31

In another study, the MIC of titanium-based complexes was found to be 64 µg/ml against *Bacillus subtilis* which is similar to our results (Seikh *et al.*, 2004). It has also been reported that the MIC of cobalt µ-peroxo complexes and zirconium µ-peroxo complexes were in the range of 32-64 µg/ml (Alam *et al.*, 2004). These results indicate that these complex compounds exhibit almost similar inhibitory activities.

Table 2. Minimum inhibitory concentration of the compound C and D against *Bacillus subtilis* and *Shigella dysenteriae*

Complexes	MIC values (µg/ml)	
	Bacillus subtilis	*Shigella dysenteriae*
Complex C	128	128
Complex D	64	64

In this study, we have studied only the antibacterial potential of ferrocenyl derivative complexes. As reported by Hossain *et al.*, in 2004, 2-aminobenzoic acids and 2-aminophenol and their complexes showed antifungal and cytotoxic activities. Our complexes should also be screened against the fungal pathogens. Further acute toxicity and other pharmacological tests of the complexes are also necessary to confirm their potential as chemotherapeutic agent.

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