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Research Article

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Antimicrobial activity of organotin(IV) alkylisopropildithiocarbamate compounds

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ABSTRACT

*Organometallic compounds have been proved to be capable of acting as biocidal and antimicrobial agents. Organotin(IV) methyl- and ethylisopropyldithiocarbamate compounds are the two series of new compounds that are expected to have biological activities. In this study, evaluation of antimicrobial activity of the compounds was carried out using qualitative disc diffusion method and quantitative broth microdilution method. The compounds were tested on eight bacterial species namely Bacillus cereus, Bacillus subtilis, Staphylococcus aureus, Streptococcus mutans, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium, and Shigella flexneri and three species of fungi namely Aspergillus niger, Candida albicans, and Saccharomyces cerevisiae. Triphenyltin(IV) ethylisopropyldithiocarbamate compound (compound **6**) showed a very active antimicrobial activity with an inhibition zone diameter of greater than 15.0 mm on most of the bacteria and all the fungi tested. MIC values obtained for this compound were better than streptomycin against B. cereus at 0.39 µg/mL, S. aureus at 0.12 µg/mL, and S. mutans at 0.12 µg/mL. Higher MIC values were needed for the Gram-negative bacteria as compared to Gram-positive bacteria, and higher value than nystatin was required to inhibit the growth of fungi. MBC and MFC values obtained showed that compound **6** can act as a bactericidal and fungicidal agent. It could be concluded that compound **6** has a strong inhibition and active antimicrobial activities against the bacteria and fungi tested. This compound also has better antibacterial activities compared to antifungal activities.*

Keywords: Organotin(IV) dithiocarbamate, antibacterial activity, antifungalactivity

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INTRODUCTION

The prevalence of life-threatening infections caused by pathogenic microorganisms is increasing worldwide.These infections arethe causes of morbidity and mortality in developing countries. Although many antimicrobial agents have been discovered, the pathogenic microorganisms are developing resistance against these agents day by day [1]. In recent years, attempts have been made to investigate the drugs against infectious diseases. The synthesis, characterisation, and biological activities of organotin compounds are a continuous field of interest nowadays. In fact, the greater use of organometallic compounds of tin than any other element reflects the broad-spectrum use of organotincompounds in both biological and nonbiological applications [2].

Among the variety of the ligands, organotin complexes of dithiocarbamates have beenextensively studied. Interest in dithiocarbamate complexes of organotin species arises because of the variety in their structural and biological

activities [3]. Organotin compounds are now the active components in a number of biocidal formulations, besides their applications in such diverse areas as fungicides, miticides, molluscicides, marine antifouling paints, surface disinfectants, and wood preservatives [4]. Dithiocarbamaates are also widely used in agriculture as insecticides, fungicides, and pesticides. The reason for such extensive works is the biological activities of both organotin(IV) and dithiocarbamate compounds [3].

In view of the diverse applications of organotin(IV) dithiocarbamate, this studyaims to determine the possible use of two series of new compounds, which are triphenyltin(IV) methyl- and ethylisopropildithiocarbamate. This paper reports the results of antimicrobial activity screening of six organotin(IV) dithicarbamate compounds. Disc diffusion and broth microdilution methods were used for qualitative and quantitative evaluations of the antimicrobial activitity, respectively, against bacteria and fungi.

EXPERIMENTAL SECTION

Materials

Chemicals: Streptomycin sulphate powder and nystatin powder were purchased from Sigma-Aldrich. All the chemicals of organotin(IV) dithiocarbamate were synthesised by Normah Awang, Environmental Health and Industrial Safety Programme, Faculty of Health Sciences, Universiti Kebangsaan Malaysia. The compounds included:

compound **1**: dimethyltin(IV) methylisopropyldithiocarbamate
compound **2**: dibuthyltin(IV) methylisopropyldithiocarbamate
compound **3**: triphenyltin(IV) methylisopropyldithiocarbamate
compound **4**: dimethyltin(IV) ethylisopropyldithiocarbamate
compound **5**: dibutyltin(IV) ethylisopropyldithiocarbamate
compound **6**: triphenyltin(IV) ethylisopropyldithiocarbamate

Microorganisms: The microorganisms were obtained from the culture collection of the Department of Biomedical Science, Faculty ofHealth Sciences, Universiti Kebangsaan Malaysia. The bacteria stock cultures were grown on Mueller-Hinton agar for bacteria and Sabouraud dextrose agar for fungi and yeasts. The microbial strains were *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Shigella flexneri*, *Staphylococcus aureus*, and*Streptococcus mutans*, while the fungal strains were*Aspergillus niger*, *Candida albicans*,and *Saccharomyces cerevisiae*.

Assay for Antimicrobial Activity

Disc Diffusion Method:Antimicrobial activity of the compounds **1-6**was tested using disc diffusion (Kirby-Bauer) method according to Bou (2007), which is a recommended standard of Clinical Laboratory Standard Institute. The discs (6 mm in diameter) were prepared by impregnating them with 10 µL of each compound solution (10 mg/mL), and the solvent was allowed to dry off in an aseptic hood untilthe discs contained 100 µg of compound. The discs were then evenly spaced on the agar surface previously inoculated with the suspension of each microbe (10^6–10^8 CFU/mL) to be tested. Standard discs of streptomycin sulphate (10 µg/disc) for bacteria and nystatin (20 µg/disc) for fungi and yeast were used as positive controls, while 35% DMSO disc was used as a negative control. The plates were incubated at 37°C for 24 h for bacteria and at 32°C for 72 h for fungi. The antimicrobial activity was recorded by measuring the width of the clear inhibition zones around each disc. Each assay in this experiment was repeated in triplicate.

Broth Microdilution Method:The effectiveness of antimicrobial activity of the compound was quantified using the broth microdilution method according to EUCAST (2000) by using 96-well microdilution plates with nominal capacity of approximately 300 µL. In each column, from 1–10 wells, of the microdilution plate dispense 100 µL of compound with different concentration (200–0.39 µg/mL) into 100 µL Mueller-Hinton broth for bacteria and RPMI 1640 for fungi. A hundredmicroliter of culture containing 10^5–10^6 CFU was inoculated in each column and incubated for 24 h at 37°C for bacteria and 72 h at 32°C for fungi. Streptomycin sulphate (10 µg/mL) and nystatin (20 µg/mL) were used as standard antimicrobials for comparison with the activities of the compounds against the microbial species.

RESULTS AND DISCUSSION

The filter paper disc diffusion method is a very convenient and rapid method for screening of antimicrobial activity. The formation of inhibition zone is observed as a result of the diffusion of antimicrobial compounds from the filter paper. The effectiveness of compounds is quantified further by measuring the minimum inhibition concentration that inhibits the growth of microbes compared with the standard antimicrobial [5]. Based on the size of inhibition zone, the compounds are categorised into weak inhibition (1–9 mm), medium inhibition (10–14 mm),or strong inhibition (15–19 mm) [5]. The compounds that inhibit the tested microbe with the size of zone of inhibition ofmore than 15 mm are considered to have an active antimicrobial activity [6, 7].

Table 1 shows the classification of the size of zone of inhibition for compounds **1–6** against the tested bacteria. All of the compounds showed significant antibacterial activities. The size of inhibition zone of compound **1** was 7.0±0.0–14.0±0.0 mm; compound **2**:7.3±0.6–12.3±0.6 mm; compound **3**: 8.7±0.6–13.3±1.5 mm; compound **4**: 8.0±1.0–18.0±0.0 mm; compound **5**: 7.3±0.6–15.3±0.6 mm; and compound **6**: 7.3±0.6–29.3±0.6 mm against the tested bacteria. Based on the size of zone of inhibition, compound **6** showed the strongest inhibition and had the most active antibacterial activity with the size of inhibition zone of ≥15 mm against most of the bacteria except *E. coli* and *S. typhimurium*. Compound **6** also showed a bigger zone of inhibition than streptomycin against *S. aureus* and *P. aeruginosa*. Other than that, compound **4** showed a bigger inhibition size than other compounds against *E. coli* and *S. typhimurium*. Compound **4** showed ahigher activity than streptomycin against *S. typhimurium*.

Table 1: Antibacterial activity of compounds1-6

Strain	Inhibition zone						
	Streptomycin (10 µg/disc)	**1**	**2**	**3**	**4**	**5**	**6**
Gram-positive							
Bacillus cereus	+++	+	+	+	+	+	+++
Bacillus subtilis	+++	+	+	+	++	++	+++
Staphylococcus aureus	+++	+	+	+	+	+	+++
Streptococcus mutans	+++	+	+	+	++	+	+++
Gram-negative							
Escherichia coli	+++	++	+	+	++	+	+
Pseudomonas aeruginosa	+++	+	++	++	++	+++	+++
Salmonella typhimurium	+	++	+	++	+++	++	+
Shigella flexneri	+++	+	++	++	++	++	+++

+ weak inhibition (6–9 mm)
++ medium inhibition (10–14 mm)
+++ strong inhibition (≥15 mm)

Table 2 shows the classification size of zone of inhibition for compounds **1–6** against the tested fungi. Not all the compounds showed antifungal activities. The size of zone of inhibition for compound **1** was 6.0±0.0–6.3±0.6 mm; compound **2**: 6.0±0.0–10.3±0.0 mm; compound **3**: 6.0±0.0–7.7±0.6 mm; compound **4**: 6.0±0.0–14.0±0.6 mm; compound **5**:6.0±0.0–6.3±0.6 mm; and compound **6**: 17.0±1.0–21.7±0.6 mm. Compound **6** showed the strongest inhibition and had the most active antifungal activity against all the fungi.Compound **6** showed a bigger zone of inhibition than nystatin against *A. niger* and *C. albicans* and same inhibition zone size against *S. cerevisiae.*

Table 2: Antifungal activity of compounds 1-6

Strain	Inhibition zone						
	Nystatin (20 µg/disc)	**1**	**2**	**3**	**4**	**5**	**6**
Aspergillus niger	++	-	-	+	+	+	+++
Candida albicans	++	-	++	-	-	-	+++
Saccharomyces cerevisiae	+++	+	+	+	++	-	+++

- no inhibition (0 mm)
+ weak inhibition (6–9 mm)
++ medium inhibition (10–14 mm)
+++ strong inhibition (≥15 mm)

The screening results indicated that not all the compounds exhibited antifungal and antibacterial activities. It can be noted that compounds with phenyl groups showed a greater inhibitory effect against the tested fungi and yeast

compared to other alkyl groups. Thus, the presence of phenyl groups in compound bonded with a tin atom is responsible for the rise of toxicity [8].

Analysis was donefurther to measurethe minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) for compound that showedactive antimicrobial activitiedwith a size of inhibition zone of ≥15 mm [9]. Compounds giving MIC values of between 125 and 250 µg/mL were considered to possess a moderate activity, while those with MIC values of higherthan 250 µg/mL were considered to possess a weak activity [6].

Table 3 shows the values of MIC and MBC of compounds against the tested bacteria. MIC value of compound **6** was 0.39–50.0 µg/mL, i.e., ≤125 µg/mL against all the tested bacteria, thus considered to possess a strong antibacterial activity. Compound **6** showed a better MIC value than streptomycin against most of the Gram-positive bacteria. In terms of bactericidal activity, the compound was better against Gram-positive strains than Gram-negative strains tested. The reason could be due to the difference in the structure of the cell walls. The walls of Gram-negative cells are more complex than those of Gram-positive cells. The lipopolysaccharide forms an outerlipid membrane and contributes to the complex antigenic specificity of Gram-negative cells [4].

Table 3: Minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) of compound6 against tested bacteria

Strain	MIC (µg/mL)		MBC (µg/mL)	
	Streptomycin	Comp. 6	Streptomycin	Comp. 6
Gram-positive				
Bacillus cereus	0.63	0.39	0.63	0.78
Bacillus subtilis	0.16	0.39	0.16	25.0
Staphylococcus aureus	0.63	0.12	2.5	0.78
Streptococcus mutans	0.63	0.12	0.63	3.13
Gram-negative				
Escherichia coli	1.25	12.5	1.25	25.0
Pseudomonas aeruginosa	0.63	25.0	0.63	25.0
Salmonella typhimurium	5.0	50.0	5.0	50.0
Shigella flexneri	1.25	6.25	2.5	25.0

Table 4 shows the values of MIC and MFC of compounds against the tested microbes. MIC value of compound **6** was 1.56–6.25 µg/mL, and it possessed a strong antifungal activity. The resistance of fungal species against compound **6** could be due to their morphological structure. Fungi have thicker cell walls and contain higher percentage of chitin than bacteria. Test microorganisms with low MIC values also showed low concentrations of MBC and MFC. The results showed thatcompound **6** exhibited bacteriostatic or fungiostaticactivities at low concentrations and bactericidal or fungicidal activities at high concentrations. Therefore, the MIC and MBC values are useful as a guideline to choose the appropriate and effective concentrations for therapeutic purposes [10].

Table 4: Minimum inhibition concentration (MIC) and minimum fungicidal concentration (MFC) of compound6 against tested fungi

Strain	MIC (µg/mL)		MFC (µg/mL)	
	Nystatin	Comp. 6	Nystatin	Comp. 6
Aspergillus niger	5.0	6.25	10.0	50.0
Candida albicans	1.25	3.13	2.5	6.25
Saccharomyces cerevisiae	1.25	1.56	2.5	3.13

CONCLUSION

The screening results indicated that all compounds (compounds **1**-**6**) exhibited antibacterial activities but not all compounds exhibited antifungal activities. Compounds with phenyl groups (compound **3** and compound **6**) showed the greatest inhibitory against thetested microbes. However, more studies that focus on mechanism of action, structure activity relationship, and toxicological evaluation are needed. Further studies shouldalso identify the active constituents in the organotin(IV) dithiocarbamate complexes.

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