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Evaluation of Antimicrobial Activities of Organotin (IV) Alkylphenyl Dithiocarbamate Compounds

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ABSTRACT

Antibiotic resistance is a global challenge to the populations and pathogenic bacteria tends to be multiresistant towards a vast majority of antibiotics. The organotin (IV) compounds have proven to have an active biological activity as antimicrobial agent. Two series of a new compounds namely organotin (IV) ethylphenyl dithiocarbamate and butylphenyldithiocarbamate which contained 6 compounds have been tested for their antimicrobial activity using disk diffusion and microdilution tests. These compounds were tested against various microbes namely *Bacillus cereus, Bacillus* subtilis, Methicillin-Resistant *Staphylococcus Aureus* (MRSA), *Staphylococcus aureus, Streptococcus pneumonia, Acinetobacter baumannii, Escherichia coli, Klebsiella* sp., *Shigellasonnei, Vibrio cholerae, Aspergillus fumigatus, Aspergillus niger, Candida* albicans and *Saccharomyces cerevisiae*. Microdilution test was carried out using two-fold dilution with the highest concentration of 5 mg mL^{-1}. Results showed that compound 3 and 6 have the antimicrobial activity towards most of bacteria and fungi tested. The lowest Minimum Inhibitory Concentration (MIC) value was obtained at 39 μg mL^{-1} for compound 3 against *V. cholerae* and compound 6 against *A. baumannii*. Nevertheless, bacteriostatic or fungistatic effect was obtained for all compounds. In conclusion, triphenyltin (IV) dithiocarbamate compounds have a potential to act as an antimicrob agent.

Key words: Organotin (IV), dithiocarbamate, antimicrobial activity, disk diffusion, microdilution

INTRODUCTION

Organotin (IV) complexes and their biocidal properties against bacteria, fungi and cancerous cell lines (Singh *et al*., 2012) have been extensively studied due to their wide applications in industry. The use of organotin (IV) compounds in industry has risen dramatically over the years as a result of their wide range of biocidal and industrial applications (Baul, 2008). Organotin compounds are characterized by a tin-carbon bond and have the general formula $RxSn(L)_{(4-x)}$, where R is an organic alkyl or aryl group and L is an organic (or sometimes inorganic) ligand. However, the organotin moiety is more significant toxicologically as compared to the ligand. Our previous work demonstrated that the organotin (IV) compounds, mainly triphenyltin (IV) exhibited a significant *in vitro* cytotoxicity toward human cancerous cell lines as compared to the diorganotin

(IV) complexes derived from the same series of ligands (Awang *et al.*, 2011). This is probably due to the anionic ligand which only influences physicochemical properties but generally has little or no effect on the toxicology. Because of the influence of the ligand, physicochemical properties and environmental fate modeling derived from them are often uncertain for the organotins (WHO., 2006).

Other than toxicological aspect, many attentions are also given towards the development of new antimicrobial drugs. Bacterial infection is known as one of the major causes of morbidity and mortality among people around the world. For decades, antimicrobial agents have proven to be useful for treatment of bacterial infections. Problem of antimicrobial resistance has become a serious issue in public health. This problem affects the economic and social development of a country (Newman *et al.*, 2011). There are several different classes of antimicrobial agents that are well known and classified based on the mechanism of action. For example, there are several types of antibiotics that can prevent protein synthesis, such as aminoglycoside, chloramphenicol and tetracycline or may interfere with the synthesis of DNA or RNA such as quinolone and rifampin. Other microbial groups that inhibit or damage the cell wall synthesis, such as beta lactamase antibiotics and glycopeptide type, modify the cell wall components such as antibiotics from trimethoprim sulfonamide type (Van Hoek *et al.*, 2011).

The main objective of this study was to perform screening tests for microbial activity of the new series of organotin (IV) ethylphenyl dithiocarbamate and butylphenyldithiocarbamate which contained 6 compounds using synthesis and characterization from several spectroscopic analyses. Microdilution test was also done to determine the Minimum Inhibition Concentration (MIC) of the compounds. This study is expected to contribute to the search for new antimicrobial compounds against microorganisms that are resistant to existing antibiotics.

MATERIALS AND METHODS

Test compounds: The series of organotin (IV) ethyl-and butylphenyldithiocarbamate which contained 6 compounds namely dibutyltin (IV) ethylphenyl dithiocarbamate (compound 1), diphenyltin (IV) ethylphenyl dithiocarbamate (compound 2), triphenyltin (IV) ethylphenyl dithiocarbamate (compound 3), dibutyltin (IV) butylphenyldithiocarbamate (compound 4), diphenyltin (IV) butylphenyldithiocarbamate (compound 5) and triphenyltin (IV) butylphenyldithiocarbamate (compound 6) were synthesized at School of Chemical Sciences and Food Technology, Faculty of Science and Technology, UKM Bangi.

Test cultures

Bacteria: *Bacillus cereus*, *Bacillus subtilis*, Methicillin-resistant *Staphylococcus Aureus* (MRSA), *Staphylococcus aureus*, *Streptococcus pneumonia*, *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella* sp., *Shigellasonnei* and *Vibrio cholerae*; fungi: *Aspergillus fumigatus*, *Aspergillus niger*, *Candida albicans* and *Saccharomyces cerevisiae*. Bacterial cultures were maintained in Mueller-Hinton Agar and fungal cultures were maintained in Sabouraud Dextrose Agar (SDA) slants at 4°C.

Disc diffusion method: A 5 h suspension culture of microorganism containing 1×10^{8} (CFU mL^{-1}) was spread using a sterile cotton swab on the surface of Mueller-Hinton Agar (MHA) for bacteria and Sabouraud Dextrose Agar (SDA) was used for fungi. The compounds 10 μL with the

concentration of 10 mg mL^{-1} was pipetted to the sterile disc and placed on the agar plates and incubated at 37°C for 24 h. Streptomycin (10 μg $disc^{-1}$), imipenem (10 μg $disc^{-1}$) and vancomycin (30 μg $disc^{-1}$) were used as positive controls for bacteria while nystatin was used for fungi (20 μg mL^{-1}). The diameter of zone of inhibition produced by each of disc compounds after incubation was measured and interpreted using the zone size interpretive criteria by standards of the Clinical and Laboratory Standards Institute (CLSI., 2011).

Minimum Inhibition Concentration (MIC): MIC which is defined as the lowest concentration with no visible turbidity observed (i.e., the compound acts as bacteriostatic or fungistatic agent), was carried out if the result of disc diffusion assay exceeded 15 mm of zone of inhibition. The broth microdilution method was used to determine the MIC. This was carried out in 96-well microtitre plates containing 200 μL Mueller Hinton broth for bacteria and RPMI-1640 for fungus with the highest concentration of two-fold dilution at 5 mg mL^{-1}. The positive control was broth with compounds tested and negative control was broth and microorganism inoculums. After 24 h of incubation at 37°C for bacteria and 48 h of incubation at 32°C for fungus, the plates were observed for their turbidity. The least concentration with no turbidity observed was noted as MIC values.

Minimum Bactericidal and Fungicidal Concentration (MBC and MFC): MBC and MFC are defined as the lowest concentration with no observed bacterial or fungal growth, respectively. In this study, the MBC and MFC were determined by subculturing 10 μL of the broth from the MIC well to Mueller-Hinton Agar for bacteria and Sabouraud Dextrose Agar for fungus. Then it was streaked using a sterile wire loop on agar plate and incubated at 37°C for 24 h for bacteria and at 32°C for 48 h for fungal strains. The lowest concentration of the compound with no bacterial growth was recorded as MBC whilst for fungus was recorded as MFC values.

RESULTS

Antibacterial activity: Table 1 shows the antibacterial activity of the standard antibiotics and the compound 1-6.

Table 1: Antibacterial activity of compound for standard antibiotics and organotin (IV) alkylphenyldithiocarbamate (1-6)

	Diameter of zone of inhibition						
	Antibiotic (μg $disc^{-1}$)	1	2	3	4	5	6
Gram positive							
Bacillus cereus	+++	++	+++	+++	++	++	+++
Bacillus subtilis	+++	+	++	+++	+	++	+
MRSA	+++	+	+	++	+	+	+++
Staphylococcus aureus	+++	++	+++	+++	++	++	+++
Streptococcus pneumonia	+++	++	+	+++	++	++	+++
Gram-negative							
Acinetobacter baumannii	+++	+++	+	+++	+++	++	+++
Escherichia coli	+++	+	+	+	+	+	+
Klebsiella sp.	+++	+	-	-	+	-	-
Shigella sonnei	+++	+	+	+	+	+	+
Vibrio cholerae	+++	+++	+++	+++	+	+++	+++

+: ≤11.0 mm, less active, ++: 12.0–14.0 mm, moderately active, +++: ≤15.0 mm active, (CLSI., 2011)

Table 2: Class of antifungal activity of compound for standard antifungal and organotin (IV) alkylphenyldithiocarbamate (1-6)

	Diameter of zone of inhibition						
Fungal strain	Antifungal (μg disc^{-1})	1	2	3	4	5	6
Aspergillusfumigatus	+++	-	-	+++	+	-	+++
Aspergillus niger	+++	+	+	+++	+	+	+++
Candida albicans	+++	+	-	+++	+	+	+++
Saccharomyces cerevisiae	+++	-	-	+++	-	-	+++

+: ≤9.0 mm, less active, ++: 10.0–14.0 mm, moderately active, +++: ≥15.0 mm active

Table 3: Minimum Inhibitory Concentration (MIC), minimum bactericidal or fungicidal concentration (MBC or MFC) of compound 3 (triphenyltin (IV) ethylphenyl dithiocarbamate)

	MIC		MBC or MFC	
Microorganism	Standard antibiotics (μg mL^{-1})	Compound 3 (μg mL^{-1})	Standard antibiotics (μg mL^{-1})	Compound 3 (μg mL^{-1})
Gram-positive bacteria				
Bacillus cereus	10	625	20	2500
Bacillus subtilis	5	78	20	156
Staphylococcus aureus	3	313	10	2500
Streptococcus pneumonia	1	78	5	156
Gram-negative bacteria				
Shigellasonnei	3	625	5	2500
Vibrio cholerae	5	39	10	156
Fungi/yeast				
Aspergillus niger	5	156	20	313

Antimicrobial activity is categorized according to the standard antibacterial susceptibility (CLSI., 2011). Streptomycin was used as the standard antibiotic for all bacteria excluding *Acinetobacter baumannii* and MRSA in which imipenem and vancomycin were used as standard antibiotics, respectively.

Based on the results obtained, at the concentration of 100 μg disc^{-1}, compound 1 and 2 showed a good antibacterial activity against *Acinetobacter baumannii*, *Vibrio cholerae*, *Bacillus cereus* and *Staphylococcus aureus*. Nevertheless, compound 3 showed good antibacterial activity against all Gram-positive bacteria except MRSA, *Acinetobacter baumannii* and *Vibrio cholerae*. Whereas compound 4 showed activity against *Acinetobacter baumannii* and compound 5 has an activity against *Vibrio cholerae*. Finally, compound 6 showed active antibacterial activity against most bacteria tested.

Antifungal activity: Table 2 shows the antifungal activity of the standard antifungal and the compound 1-6. Antimicrobial activity is categorized according to the standard antibacterial susceptibility (CLSI., 2011).

As shown in Table 2, at the concentration of 100 μg disc^{-1}, Compound 3 and compound 6 actively inhibited the growth of all fungi and yeast tested. Nevertheless, other compounds showed very weak activity against most of the fungus tested.

Table 3 shows the values of Minimum Inhibitory Concentration (MIC) and the minimum bactericidal or fungicidal concentration (MBC or MFC) for each microorganism tested using compound 3. The lowest MIC was obtained against bacteria *V. cholerae* at 39 mg mL^{-1} and the lowest MBC was 156 mg mL^{-1} against *B. subtilis*, *S. pneumonia* and *V. cholerae*.

Table 4: Minimum Inhibitory Concentration (MIC), minimum bactericidal or fungicidal concentration (MBC compound 6 (triphenyltin(IV) butylphenyldithiocarbamate)

	MIC		MBC or MFC	
Microorganism	Standard antibiotics (μg mL^{-1})	Compound 6 (μg mL^{-1})	Standard antibiotics (μg mL^{-1})	Compound 6 (μg mL^{-1})
Gram-positive bacteria				
Methicillin-Resistant Staphylococcus Aureus (MRSA)	4	156	16	625
Gram-negative bacteria				
Acinetobacter baumannii	1	39	16	156
Fungi/yeast				
Aspergillusfumigatus	5	1250	20	5000
Candida albicans	20	156	40	313
Saccharomyces cerevisiae	20	313	40	1250

Table 4 shows the values of Minimum Inhibitory Concentration (MIC) and the minimum bactericidal or fungicidal concentration (MBC or MFC) for each microorganism tested using compound 6. The lowest MIC and MBC was 39 and 156 mg mL^{-1}, respectively against *A. baumannii*.

DISCUSSION

In this study, at the concentration of 100 μg disc^{-1}, the antibacterial activity was divided into three categories of streptomycin susceptibility as follows: Resistant to streptomycin with diameter of inhibition zone of ≥11.0 mm, medium resistance to streptomycin with diameter of inhibition zone of 12.0-14.0 mm and susceptible to streptomycin with diameter of inhibition zone of ≥15.0 mm (CLSI., 2011). This study found that different series of organotin (IV) compounds with dithiocarbamate ligands was able to inhibit the growth of Gram-positive and Gram-negative bacteria with different activities against different bacteria.

Results from disk diffusion test revealed that at the concentration of 10 mg mL^{-1}, all of the compounds showed antibacterial activity against most bacteria tested as they were able to produce zones of inhibition with different levels of activity reflected from the diameter of the zones. Compound 3 and 6 showed the largest diameter i.e., ≥15 mm among all compounds against all bacteria tested except *E. coli, Klebsiella* sp. and *S. sonnei*. This is because the effectiveness of the antimicrobial activity of the biocidal agents tested varied depending of the species of the microorganisms and their cell permeability (Shahzadi *et al*., 2008).

In this study, it was found that compound 3 showed active antimicrobial activity to inhibit the growth of most Gram-positive and Gram-negative bacteria. Generally, Gram-positive bacteria are more effective antibacterial target than Gram-negative bacteria. This is because Grampositive bacteria have cell membranes as their external structure that are less complex and could facilitate the entry of any impurities (Pellerito *et al*., 2006).

The type and number of organic groups attached to the organotin (IV) compounds influence the biological activity against bacteria and fungi. The toxicology of organotin (IV) compounds is very complex and has been extensively studied but, its general pattern has been established. Tri-substituted alkyl and aryltin (IV) compounds (TOT) are more toxic than di-substituted organotin (IV) compounds (DOT) while monosubstituted organotin (IV) compounds (MOT) are less toxic (WHO., 2006).

The organotin (IV) compounds with phenyl groups show significant inhibitory effects against various types of fungi compared to the other alkyl groups at the same position. In addition, the antifungal and antibacterial activities of the compounds is in the order of triphenyltin> dibutyltin>trimethyltin (Jamil *et al*., 2009). The compound that shows diameter of zone of inhibition of more than 15.0 mm is classified as active i.e., the compound inhibits the growth of bacteria and fungi. The diameter of inhibition zone produced by the compound 3 against *S. aureus* was ≥15 mm. This result supported the study by Shahzadi *et al*. (2008) who reported that triphenyltin (IV) 4-methylpiperidin dithiocarbamate compound showed active antibacterial activity against *S. aureus* with diameter of zone of inhibition of 15-25 mm. The active mode of antibacterial activity against *S. aureus* was also given by Ph3Sn(2NH2-5-NO2-C6H3COO), a complex derived of 2-amino5-nitrobenzoic acid with 19 mm inhibition zone.

Although it was classified as active, its inhibitory effect was still lower than chloramphenicol, doxycycline and rifampicin (Win *et al*., 2010).

If the compounds producing the diameter zone of inhibition exceeding 15 mm in the disc diffusion test, the compound has to be tested through quantitative testing of microdilution test to obtain the minimum inhibitory concentration of the compounds (Tarafder *et al*., 2002). In this study, only compound 3 and 6 showed the diameter of zone of inhibition of more than 15 mm against the bacteria and fungi tested.

The MIC of ≤125 mg mL^{-1} was considered active antimicrobial activity, 125-250 mg mL^{-1} was considered moderate antimicrobial activity and ≥250 mg mL^{-1} was considered weak antimicrobial activity (Ali *et al*., 1995).

In this study, the value of the minimum inhibition concentration shown by compound 3 was considered active against bacteria *B. subtilis*, *S. pneumoniae* and *V. cholerae*. Compound 6 showed active inhibitory activity against bacteria, *A. baumannii* with MIC value of 39 mg mL^{-1}. The increase in MIC value may indicate the emergence of resistance in a microorganism. This may also indicate that antimicrobial resistance that can cause therapeutic failure (Kumar *et al*., 2011).

For microdilution tests, the results showed that all MBC and MFC values obtained were greater than the MIC values. MBC or MFC value of a compound that is greater than its MIC value indicates that the compound is bacteriostatic or fungistatic i.e., the compound is only able to inhibit the growth of microorganisms, but not able to kill the microorganisms (Scott, 2005). Many infections can be treated effectively by bacteriostatic or fungistatic drugs because they tip the balance in favor of the patient's immune system.

In this study, it was found that the triorganotin (IV) ethylphenyldithiocarbamate compound actively inhibited the growth of Gram-positive bacteria while triorganotin (IV) butylphenyldithiocarbamate compound actively inhibited the growth of fungi. This difference was likely to be due to the difference in the alkyl groups bound to the dithiocarbamate ligand, although according to Pellerito *et al*. (2006), the ligand only serves to support transportation of the complex organostanum (IV) to the target site through the hydrolysis process.

A study by Ahmed *et al*. (2006) found that fungal growth inhibition activity of triorganotin compounds is higher than diorganotin compound and its acid ligand. From the data obtained, the diameter of zone of inhibition of triorganotin (IV) dithiocarbamate compound showed great value than diorganotin (IV) compounds when tested in the fungi and this is in agreement with the study conducted by Ahmed *et al*. (2006). However, the compound still required high concentrations to inhibit the growth of the fungi. In conclusion, the two series of triphenyltin (IV) alkylphenyldithiocarbamate, namely triphenyltin (IV) ethylphenyl dithiocarbamate and triphenyltin (IV) butylphenyldithiocarbamate were active in inhibiting the growth of certain bacteria and fungi.

CONCLUSION

Based on studies that have been conducted, not all organotin compounds (IV) alkylphenyldithiocarbamate surveyed have antimicrobial activity against the tested microorganisms. Compounds 3 (triphenyltin (IV) ethylphenyl dithiocarbamate) have active antimicrobial activity to inhibit the growth of most Gram-positive bacteria such as *B. cereus*, *B. subtilis*, *S. aureus* and *S. pneumonia* with the average of inhibition zone diameter ≥15 mm. The compound 6 (triphenyltin (IV) butylphenyldithiocarbamate) also inhibits the growth of most resistant bacteria and fungus such as Acinetobacter baumannii and MRSA with actively mean inhibition zone diameter of = 15 mm.

MIC values shown by triphenyltin (IV) ethylphenyl dithiocarbamate compounds are active against *B. subtilis*, *Strep. pneumonia* and *V. cholerae* bacteria with the value of ≤125 μg mL^{-1}. While the MIC value shown by triphenyltin (IV) butylphenyldithiocarbamate compounds are active against *Acinetobacter baumannii* with the value of ≤125 μg mL^{-1}.

In conclusion, the compound of triphenyltin (IV) alkylphenyldithiocarbamate are active in inhibiting the growth of certain bacteria and fungi.

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