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Antibacterial activity of organotin(IV) methyl and ethyl cylohexyldithiocarbamate compounds

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ABSTRACT

Antibiotic resistance is becoming a global issue and is threatening the effective prevention and treatment of increasing infections caused by pathogenic microorganisms. Organotin(IV) compounds have been widely used in developing new antimicrobial agents. Two series of organotin(IV) dithiocarbamate namely methyl and ethylcyclohexyldithiocarbamate containing altogether six compounds were used to test their antibacterial activity using disc diffusion and microdilution methods. These compounds were tested against ESKAPE bacteria, which included Enterococcus raffinosus, Staphylococcus aureus, Klebsiella sp., Acinetobacter baumanni, Pseudomonas aeruginosa, and Enterobacter aerogenes. A microdilution test was carried out by using two-fold dilution with the highest concentration of40 mg/mL. The results showed that the compound3 had an antibacterial activity towards most of the bacteria tested with an inhibition range of 10–15 mm. The minimum inhibitory concentration (MIC) of compound 3 was same with the ampicillin, which was 2.5 mg/mL. All the compounds showed bacteriostatic effects as the minimum bactericidal concentration (MBC) values were higher than the MIC values. In conclusion, phenyltin(IV) methylcyclohexyldithiocarbamate has a potential to be developed as an antibacterial agent.

Keywords: Organotin(IV), dithiocarbamate, antibacterial activity, disk diffusion, microdilution

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INTRODUCTION

The widespread deployment of antibiotic is always followed by the evolution of microbial resistance[7].According to WHO, the antimicrobial resistance is defined as the resistance of a microorganism towards the drug that effectively treats the infections caused by the pathogen. Resistance of the microorganisms towards the existing antimicrobial agents in treating infectious diseases has become a global problem due to the death and disability caused.

The presence of ESKAPE bacteria, which are the bacteria capable of escaping the biocidal action of antibiotics and collectively represent new paradigms in pathogenesis, transmission, and resistance [16] cause the antibiotic resistance to become worst. These bacteria have a resistance towards many antibacterial agents. In order to solve this problem, many studies must be done to develop new antibacterial agents[15].

Organotin(IV) compounds are organometallic compounds that have a wide range of applications and have significant biological activities [3]. These compounds have been proven to have a potential as an antibacterial [1], antifungal [13] and antitumour [19]. These compounds has a general formula of RxSn(L)(4−x), with R is an alkyl or aryl group and L is an organic or inorganic ligand. The dithiocarbamate compound attached on the tin makes the

compounds to be balanced. Moreover, these dithiocarbamate compounds can inhibit bacterial activity and growth by disturbing the metabolic process of the bacteria [20].

There are some mechanisms of action of antimicrobial agent against the microorganisms that cause the infections and diseases. According to Black (2005), antimicrobial agents act on the microorganism by inhibiting their synthesis of cell wall by modifying their cell wall components, inhibiting the synthesis of protein, or probably interfering with the synthesis of DNA or RNA. The understanding of these mechanisms of action is important in order to develop new antibacterial agents.

The main objective of this study was to perform a screening test for microbial activity of two new series of organotin compounds, which were organotin(IV) methyl and ethylcyclohexyldithiocarbamate consisting of six compounds using synthesis and characterisation of several processes. This study is expected to contribute to the search of new antibacterial compounds against bacteria that are resistant to the existing antibiotics.

EXPERIMENTAL SECTION

Test compounds: The series of organotin (IV) methyl and ethylcyclohexyldithiocarbamate containing six compounds namely methyltin(IV) methylcyclohexyldithiocarbamate (compound 1), butyltin(IV) methylcyclohexyldithiocarbamate (compound 2), phenyltin(IV) methylcyclohexyldithiocarbamate (compound 3), metyltin(IV) ethylcyclohexyl dithiocarbamate (compound 4), butyltin(IV) ethylcyclohexyldithiocarbamate (compound 5), and phenyltin(IV) ethylcyclohexyldithiocarbamate (compound 6) were synthesised at theFaculty of Sciences and Technology, Universiti Kebangsaan Malaysia, Bangi.

Test cultures:
Bacteria: ESKAPE bacteria including *Staphylococcus aureus, Klebsiella sp., Acinetobacter baumanii*,and *Pseudomonas aeruginosa* were cultured on Mueller-Hinton agar and *Enterococcus raffinosus* ATCC 49464 and *Enterobacter aerogenes* ATCC 51697 were cultured on Tryptic Soy Agar (TSA).

Disc diffusion method: The evaluation of antibacterial activity was done by spreading the suspension of bacteria containing 1×10^8 ($CFUmL^{-1}$) on the surface of Mueller-Hinton agar for *S. aureus, Klebsiella sp., A. baumanii* and *P. aeruginosa* and on TSA for *E. raffinosus* ATCC 49464 and *E. aerogenes* ATCC 51697 by using a sterile cotton swab. 10 µL of the compounds with a concentration of 2 mg/mL was pipetted on the sterile disc and placed on the agar. The agar was incubated at37°C for 24 h. Ampicilin (10 mg/mL) was used as a positive control. The diameter of inhibition zone by each of the compounds was measured and interpreted using Clinical and Laboratory Standard Institute (CLSI 2012).

Microdilution method:
Minimum Inhibition Concentration (MIC): MIC is the lowest concentration with a clear turbidity. The test was done in a 96-well microtitre plate containing 150 µL Mueller-Hinton broth, tested compounds, and microorganism inoculum with the highest dilution concentration of40 mg/mL. The positive control was the broth with the tested compound, while the negative control was an inoculum and the broth. The plate was incubated for 24 h at 37 °C. The lowest concentration with a clear appearance of the mixture was considered the MIC.

Minimum Bactericidal Concentration (MBC): MBC is the lowest concentration with no microorganism growth observed. The MBC was determined by plating and striking the 10 µL of MIC broth into the Mueller-Hinton agar. Then, the agar was incubated for 24 h at 37 °C. The lowest concentration with no growth of microorganism was determined as the MBC.

RESULTS AND DISCUSSION

Figure 1 shows the antibacterial screening of standard antibacterial and compound 1–6 against all the bacteria tested. The antibacterial activity was reflected by the diameter of inhibition zone.

The antibacterial activity was categorised according to the antibacterial susceptibility [6].Ampicillin was used as the standard antibiotic for all the bacteria. In this study, at concentration of 10 mg/ mL, the antibacterial activity was divided into three categories of Ampicillin susceptibility which are resistant to Ampicillin with diameter of inhibition zone ≤13 mm, medium resistance to Ampicillin with diameter of inhibition zone 14-16 mm and susceptible to Ampicillin with diameter of inhibition zone ≥17 mm. Based on the results shown in Figure 1, at the concentration of 2 mg/mL per disc, mostly all the compounds showeda low antibacterial activity on the bacteria tested except for compound 2, which was a monosubtituted organotin compound. Therefore, trisubstituted organotin

compounds were the most toxic, followed by disubtituted organotin compounds and lastly monosubtituted organotin [22].

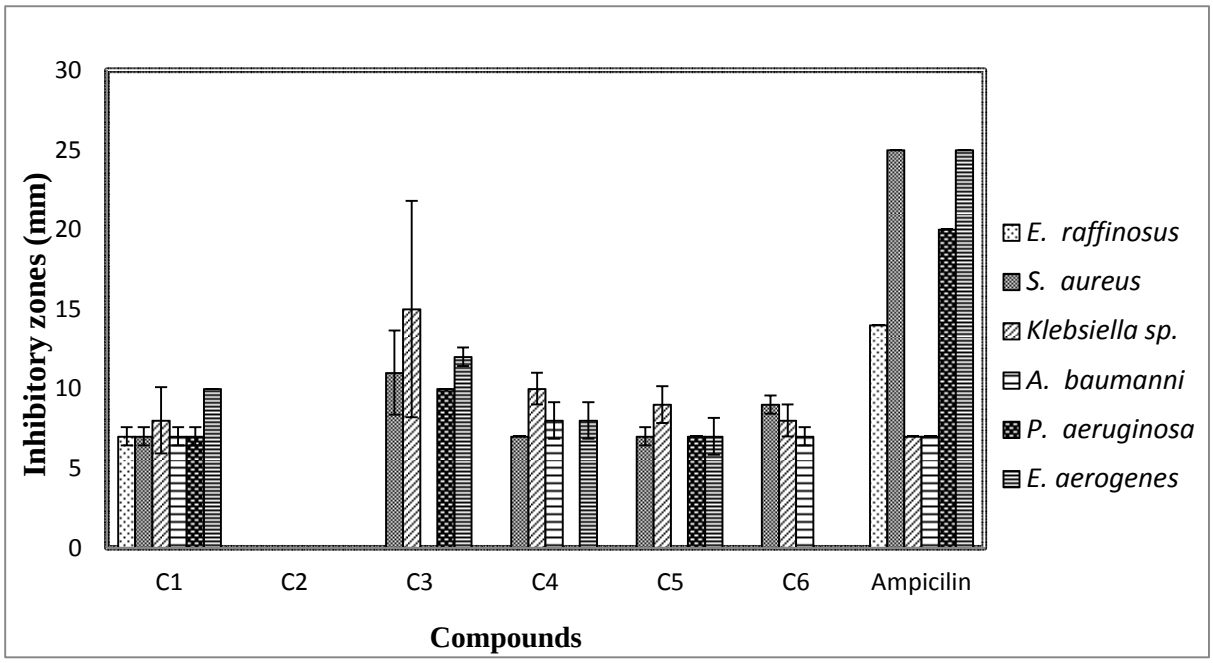

Figure 1: The antibacterial screening of standard antibacterial of organotin(IV) methyl and ethylcyclohexyldithiocarbamate compound 1–6

Most of the compounds showed different antibacterial activities against the bacteria tested based on the different diameters of inhibition zone and only compound 2 did not show an antibacterial activity at all. According to Shahzadi et al. (2006) [21], the different antibacterial activities of the compounds against the bacteria tested depend on the permeability of the cells. Compound 2, which is from the series of organotin(IV) methylcyclohexylditiocarbamates that contain a butyl group, did not show any antibacterial activity. Awang et al. (2011) [3]describe that the series of organotin(IV) methylcyclohexyldithiocarbamate also does not show any antibacterial activities for the compound that has a butyl group even though there are two butyl groups attached to the organotin(IV).

Only compound 1 showed an antibacterial activity on *E. raffinosus*, a Gram-positive bacteria. The resistance of the pathogens to the test compounds can be attributed to the existence of cell wall on the Gram-positive bacteria. The cellular structure reduces the permeability of the tested compounds, and the activity of the compounds against this bacteria may be attributed to their high lipophilicity [8].

Compound 3 showed an antibacterial activity on *S. aureus*, *P. aeruginosa*, and *E. aerogenes*, and only this compound showed a moderate activity on *Klebsiella sp*. with alarger inhibition zone, 15 mm, compared to other compounds. The type and number of organic compounds attached to organotin(IV) influence the biological activities of the organotin compounds towards bacteria and fungi[12]. Compound 3 has a phenyl group attached on the organotin(IV), and according to Jamil et al. (2009) [10], the compound that has a phenyl group has a higher antimicrobial activity on the fungi and bacteria compared to other alkyl groups attached to tin(IV).

Table 1, 2, and 3 show the MIC and MBC values for compound 1, 3, and 4 for certain bacteria that were selectively chosen from the disc diffusion test.

Table 1: MIC and MBC values for compound 1 (methyltin(IV)methylcyclohexyldithiocarbamate)

Bacteria	MIC		MBC	
	Ampicillin (mg/mL)	Compound 1 (mg/mL)	Ampicillin (mg/mL)	Compound 1 (mg/mL)
E. aerogenes	0.625	5	1.25	40

Table 2: MIC and MBC values for compound 3 (phenyltin(IV) methylcyclohexyldithiocarbamate)

Bacteria	MIC		MBC	
	Ampicillin (mg/mL)	Compound 3 (mg/mL)	Ampicillin (mg/mL)	Compound 3 (mg/mL)
E. aerogene	0.625	5	1.25	40
S. aureus	0.312	2.5	1.25	25
Klebsiella sp.	2.5	2.5	2.5	20
P. aeruginosa	0.16	2.5	5	10

Table 3: MIC and MBC values for compound 4 (methyltin(IV)ethylcyclohexyldithiocarbamate)

Bacteria	MIC		MBC	
	Ampicillin (mg/mL)	Compound 4 (mg/mL)	Ampicillin (mg/mL)	Compound 4 (mg/mL)
Klebsiella sp.	2.5	2.5	5	>40

According to Ravoof et al. (2007) [18], microdilution test is conducted for the compounds with a diameter of inhibition zone of >15 mm as these compounds are considered to be actively inhibiting the microorganism. But, for this study, the compounds with an inhibition zone of 10 mm and above were chosen to proceed to the microdilution test. This is according to Nath et al. (2008) [14], who state that for new compounds with a diameter of inhibition zone of 7–13 mm are categorised to be moderately active in inhibiting the microorganism.

As shown in the tables above, all the compounds showed higher values of MIC and MBC compared to ampicillin. This study showed same results with Awang et al. (2015) [4] as the organotin(IV) dithiocarbamate compounds tested in their study showed higher MIC and MBC values against microorganism tested compared to the standard drug.

Moreover, in the present study, compound 1 and 3 showed the highest MIC and MBC values on *E. aerogenes*, which was 5 mg/mL. The high MIC value of certain bacterial strains shows their resistance towards the compound because of the alterations of the outer membrane that causes a poring decrease and lipopolysaccharide modifications [2].The high value of MIC and MBC also means that a high concentration of the compound is needed to inhibit or kill the bacteria.

Compound 3 showed the same MIC and MBC values, which were2.5 mg/mL, against *Klebsiella sp*. This is because of the phenyl group attached to the tin atom that causes the electron delocalisation in chelate ring system, which subsequently increases the lipophilic level of the compounds and causes the compound to be absorbed easily by the bacterial membrane [20].

This study also found that all the compounds can be categorised as bacteriostatic compounds as they showed higher MBC values than MIC values. According to Scott (2005) [9], a compound with higher MBC value than MIC value can be classified as a bacteriostatic compound. This compound only inhibits the bacteria but is not able to kill them. On the other hand, a compound is classified as a bactericidal compound if the MBC and MIC values are same. This means that the compound can kill the bacteria.

CONCLUSION

Based on the study conducted, most of the compounds were less active in inhibiting the growth of the bacteria. Only compound 3, phenyltin(IV) metylcyclohexyldithiocarbamate was moderately active in inhibiting most of the bacteria, with the largest diameter of inhibition zone against *Klebsiella sp*. of 15 mm. The MIC values of phenyltin(IV) metylcyclohexyldithiocarbamate compounds weresame for most of the bacteria except for *E. aerogenes*, and the highest MIC value was 5 mg/mL. But, this compound showed the same MIC value as ampicillin on *Klebsiella sp.* which was 2.5 mg/mL. Nevertheless, all the compounds were categorised as bacteriostatic. In conclusion, phenyltin(IV) metylcyclohexyldithiocarbamate is moderately active in inhibiting the bacteria and has the potential to be an antibacterial agent with some rearrangement in its structure.

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REFERENCES

[1]Alia Basirah, Mohd Faizuddin Abu Hasan, Norliawati Mohd Sidek & Wan M. Khairul, N. I.**2013**. *Journal of Applied Sciences Research* 9(10): 5562-5567.
[2]Aure´lie, Thiolas, Claude, Bollet, Bernard, La Scola, Didier, Raoult, Jean- & Page`s, M. **2005**. *Antimicrobial Agents and Chemotherapy*(4): 1354-1358.
[3] Awang, N., Baba, I., Yamin, B. M., Othman, M. S. & Kamaludin, N. F. **2011**. *American Journal of Applied Sciences* 8(4): 310.
[4] Awang, N., Siti MunirahMohktar, Noraziah Muhammad Zin& Kamaludin, a. N. F. **2015**. *Asian Journal of Applied Sciences* 8(2): 165-172.
[5] Black,J., G. **2005**. Microbiology Principle and Exploration. Ed Ke-6. United state of America: John Wiley & Sons
[6] Clinical Laboratory Standard Institute. **2012**. Performance Standard for Antimicrobial Susceptibility Testing; Twenty-Second Informational Supplement. M100 S22. Ed. Ke 11. Wayne, Pa. CLSI.
[7] Cowen, L. E., Anderson, J. B. & Kohn, L. M. **2002**. *Annual Reviews in Microbiology* 56(1): 139-165.
[8] Ekennia, A. C. **2013**. *Journal of Applied Chemistry* 5(2): 36-39.
[9] Geoff Scott. **2005**. Antibiotic resistance. Medicine 33:3.The Medicine Publishing Company Ltd.
[10] Jamil, K., Bakhtiar, M.,Khan, A.R., Rubina, F., Rehana, R., Wajid, R., Qaisar, M., Danish, M., Awais, M., Bhatti, Z.A., Rizwan, M., Naveed, A., Hussani, M., & Pervez, A. **2009**. *African Journal of Pure and Applied Chemistry* Vol.3 (4): 066-071.
[11] Kumar, R., Shrivastava, S. K. & Chakraborti, A. **2010**. *American Journal BiomeicalSciences* 2(3): 202-208.
[12] Menezes, D., C., Vieira, F., T., de Lima, G., M., Wardell, J., L., Cortes, M., E., Ferreira M., P., Soares, M., A. & Boas, A., V. **2007**. *Applied Organometallic Chemistry*22: 221 - 226.
[13] Menezes, D., Vieira, F., De Lima, G., Wardell, J., Cortés, M., Ferreira, M., Soares, M. & Vilas Boas, A. **2008**. *Applied Organometallic Chemistry* 22(4): 221-226.
[14] Nath, M., Sulaxna, Song, X., Eng, G. & Kumar, A. **2008**. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* 70(4): 766-774.
[15] Pellerito, C., Nagy, L., Pellerito, L. & Szorcsik, A. **2006**. *Journal of Organometallic Chemistry* 691(8): 1733-1747.
[16] Pendleton, J. N., Gorman, S. P. & Gilmore, B. F. **2013**. Clinical relevance of the ESKAPE pathogens.
[17] Powers, J. **2004**. *Clinical Microbiology and Infection* 10(s4): 23-31.
[18] Ravoof, T.B.S.A., Crouse, K.A., Tahir, M. Ibrahim M. Tahir, Cowley, A.R. & Ali, M, A. **2007**. *Polyhedron* 26: 1159-1165.
[19] Ruan, B., Tian, Y., Zhou, H., Wu, J., Hu, R., Zhu, C., Yang, J. & Zhu, H. **2011**. *Inorganica Chimica Acta* 365(1): 302-308.
[20] Shaheen, F., Z.U. Rehman, S. Ali and A. Meetsma, **2011**. *Polyhedron*, 31(1): 697-703.
[21] Shahzadi, S., Ali, S. & Fettouhi, M. **2006**. *Journal of Chemical Crystallography* (38): 273-278.
[22] WHO, **2006**. Mono-and Disubtituted Methyltin, Butyltin and Octyltin Compounds (Concise International Chemical Assessment Documents 73) World Health Organization, Geneva, Switzerland, ISBN-13: 978-9241530736,Pages: 69.