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Synthesis, characterization and in vitro activity study of some organotin(IV) carboxylates against leukemia cancer cell, L-1210

Abstract: This paper presents successful resynthesizing of several dibutyl-, diphenyl-, and triphenyltin(IV) carboxylate compounds, and their activity against leukemia cancer cell, L-1210. The compounds were synthesized by reacting the dibutyltin(IV) oxide (DBTO) (**1**), diphenyltin(IV) oxide (DPTO) (**3**), and triphenyltin(IV) hydroxide (TPTOH) (**5**) with 3-hydroxybenzoic acid (3-HHBz). Prior to cancer activity tests, the compounds were characterized by UV–Vis, FT-IR, NMR (both ^{1}H NMR and ^{13}C NMR), and microanalysis to determine elemental composition of the samples. The anticancer tests revealed that triphenyltin(IV) 3-hydroxybenzoate (TPTHBz) (**6**) displayed significantly higher activity than those exhibited by dibutyltin(IV) di(3-hydroxybenzoate) (DBTHBz) (**2**) and diphenyltin(IV) di(3-hydroxybenzoate) (DPTHBz) (**4**).

Keywords: anticancer activity test; IC_{50}; leukemia cancer cell L-1210; organotin(IV) 3-hydroxybenzoate.

1 Introduction

Recognizing interesting structural features of organotin(IV) compounds [1–3] application of this group of compounds continuous to grow in various fields, such as antifungal [4], antimicrobial, and antibacterial [2, 5–10], antimalarial [11–13], corrosion inhibitor [14, 15], and anticancer and antitumor agents [1, 16–18]. The main feature that makes organotin(IV) compounds unique is the presence of at least one covalent Sn–C bond, in which the tetravalent Sn can form chemical bond with different numbers of alkyl(R) or aryl (Ar) groups to produce mono-, di-, tri-, and tetraorganotin(IV) compound. The compounds are

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also characterized by the presence of counter anion; with the most anions encountered are chloride, oxide, fluoride, hydroxide, thiolate [3], and carboxylate [4–10].

An important property which attracts continuous interest in exploring organotin(IV) compounds is their powerful biological activity, such as high toxicity even at very low concentration. The primary factors from which the biological activities of these compounds originate are the number and the nature of organic groups attached to Sn atom [3], while the anionic groups function as a secondary factor. Several recent workers on the coordination of carboxylates to organotin compounds have reported successful preparation of new derivatives of organotin(IV) carboxylates and carboxylate, having some promising activities such as antimicrobial [2, 5–10], antitumor, and anticancer [1, 16–18].

In this recent investigation, we reported the results of comparative studies on application and *in vitro* activity tests of several dibutyl-, diphenyl-, and triphenyltin(IV) with ligand of 3-hydroxybenzoic acid to combat leukemia cancer cell, L-1210.

2 Experimental

2.1 Materials

The chemicals used in this works were AR grade purchased from either Sigma or JT Baker chemical supplier, including dibutyltin(IV) oxide ([$(n\text{-}C_4H_9)_2O$]), (DBTO) (**1**), diphenyltin(IV) oxide ([$(C_6H_5)_2O$]), DPTO (**3**), triphenyltin(IV) hydroxide ([$(C_6H_5)_3OH$]), TPTOH (**5**), 3-hydroxybenzoic acid (HHBZ), and methanol (CH_3OH). The chemicals were used as received. The L-1210 cells, were obtained from National Agency of Atomic Energy, Center for Application of Isotopes and Radiation Technology, Jakarta, Indonesia.

2.2 Characterization techniques

The ^{1}H and ^{13}C NMR spectra were produced at 298 K using Bruker AV 600 MHz NMR and using DMSO-d_6 as the solvent. Measurement for ^{1}H NMR was carried out at frequency of 600 MHz with 32 runs and DMSO signal at 2.5 ppm. For, ^{13}C NMR, the frequency used was 150 MHz with 1000–4000 scans and DMSO signal at 39.5 ppm. Elemental composition (CHNS) of the samples was determined using elemental analyser (Fision EA 1108 series). The Bruker VERTEX 70 FT-IR spectrophotometer with KBr discs was applied to produce FTIR spectrum by scanning the sample in the wavenumber range of 4000–400 cm^{-1}. The UV spectrophotometer (Shimadzu UV-245) was utilized to record the UV spectrum. The measurement was conducted 1 mL quartz-cells for the sample with concentration of 1.0×10^{-4} M in methanol as a solvent.

2.3 Preparation of organotin(IV) carboxylates

The procedure applied to synthesize the organotin(IV) carboxylates and similar compounds with different carboxylate ligands has been described in previous works [5–10, 12,

13]. The following steps is an example of the procedure for preparation of dibutyltin(IV) d(4-hydroxybenzoate), DBTHBz (**2**).

A mass of 0.373 g (1.5 mmol) DBTO (**1**) was dissolved in 40 mL of methanol, and then mixed with 2 mol equivalents of 3-HHBz (0.414 g; 3 mmol). The mixture was refluxed at 60–62 °C for 4 h. A Dean–Stark apparatus was used to remove the water formed and rotary evaporator was used to remove the solvent. The product, specified as compound DBTHBz (**2**) was dried *in vacuo* before subjected to analysis and used in biological activity tests. Preparation of DPTHBz (**4**) was conducted with a similar procedure, while for TPTHBz (**6**) preparation, slightly difference procedure was applied, in which only 1 mol equivalent of the 3-HHBz was used instead of 3 mmol.

2.4 Bioassay anticancer activity test against leukemia cancer cell, L-1210

The *in vitro* anticancer activity test against L-1210 cells was performed with the procedure reported in previous works [16, 18]. An aliquot of 1 mL of cells containing 2×10^6 cell/mL was pipetted into each hole of *multiwell plate tissue culture*, followed by addition of 10 µL of solution containing the compounds to be tested dissolved in methanol (if the sample was not soluble enough in the methanol, the sample was homogenized by subjecting it to ultrasonic mixing). The tests were conducted with the samples having varied concentrations of 1, 2, 4, 6, 8, 16, and 32 µg/mL, using the cell was treated with a solution containing 10 µL of solvent as a negative control. The cell was then placed in a 5% CO_2 incubator at 37 °C for 48 h incubation. After the completion of the treatment, the number of the cell was counted using *haemecytometer Fuch Rosental* (0.200 mm × 0.0625 mm^2) microscope.

The performance of the sample defined as percentage of inhibition was calculated using Eq. (1):

$$\% \text{ inhibition} = \left(1 - \frac{A}{B}\right) \times 100 \tag{1}$$

where A is the number of living cells in the medium filed with the sample, and B is the number of living cells in control solution.

The IC_{50} was calculated using arithmetic method developed by Reed and Muench [19]. In this method, the inhibition percentages data were plotted into probit table to obtain the value of each analysis. The graph between log of concentration (x) and probity value (y) was then constructed to obtain a linier regression equation in the form of $y = a + bx$. By subtitling the probit value (y) = 5 (the probit of 50% value), into the equation, the value of x (log of concentration) was obtained, and the IC_{50} value was obtained by taking the anti-log of x.

3 Results and discussion

The re-synthesis of DBTHBz (**2**), DPTHBz (**4**), and TPTHBz (**6**) were successfully performed by reacting the DBTO (**1**), DPTO (**3**), and TPTOH (**5**) with 3-HHBz, respectively, follow the

Table 1: The microanalytical data of the organotin(IV) compounds synthesized.

Compound	Elemental analysis found (calculated)	
	C	H
$(n\text{-}C_4H_9)_2Sn(3\text{-}C_6H_4(OH)COO)_2$ (**2**)	52.3 (52.1)	5.7 (5.6)
$(C_6H_5)_2Sn(3\text{-}C_6H_4(OH)COO)_2$ (**4**)	56.3 (57.0)	3.6 (3.7)
$(C_6H_5)_3Sn(3\text{-}C_6H_4(OH)COO)$ (**6**)	60.7 (61.1)	4.2 (4.1)

preparation of similar compounds previously reported [5–10, 12, 13]. Successful preparation of the samples is supported by the elemental compositions of the samples obtained using microanalytical method which are in agreement with the calculated values as presented in Table 1.

Table 2 is a compilation of the data of the ^{1}H and ^{13}C spectra of the compounds synthesized prepared, sowing that complexation led to a higher value of the chemical shifts of some of signals in the spectra. As an example, in DPTO (**3**) it was found that the chemical shift (δ) of carbons in phenyl shifted from 125 ppm to about 129 ppm. Another feature is the existence of three new peaks in the compounds in which benzoate derivatives bound to Sn. For example, in compound (**7**), the three peaks are located at 132.2 and 135.3 ppm, associated with phenyl carbons, and that at 167.8 ppm associated with carbon in the carboxyl group. Similar data are also observed for other targeted compounds

The important bands of FTIR spectra of the products synthesized are compiled in Table 3. The spectra of the starting materials (**1, 3**, and **5**) are characterized by is the presence of strong band at wavenumber of 417.4 cm^{-1}, resulted from the main stretching vibration of Sn–O bond in compound DBTO (**1**). As expected, the spectra also display the absorption bands due to bending vibrations of butyls, although the position of the bands is slightly shifted. The formation of DBTHBz (**2**) is confirmed by the absorption band at *ca.* 1400 cm^{-1}, resulted from strong asymmetric stretching of the carboxylates, and the band at *ca.* 1600 cm^{-1}, resulted from symmetric stretching of the groups. These FTIR features confirm that substitution reaction has taken place as expected [8–10].

The λ_{max} data of the compounds synthesized produced by UV–Vis spectroscopy analyses are summarized in Table 4. As can be seen in Table 4, there is a shift in the λ_{max} of the compound following the steps of the reaction. For instance, the compound **1** has λ_{max} of 202.9 nm, while compound **2** displays the λ_{max} of 206 and 307.8 nm which indicate a significant change in both $\pi \rightarrow \pi^*$ and n–π^* transitions. These big shifts occur since both the –C=O– and –C=C– bond in the 3-hydroxybenzoate ligand are powerful chromophores. The similar pattern was also observed for compound **4** and **6**.

The activity of diorganotin(IV) and triorganotin(IV) compounds as antifungi is well documented, and for this reason development of new compounds is continuously attempted [4, 20]. Some of the organotin(IV) compounds have also been tested for antitumor activity and showed considerable potentials [1, 16–18].

Table 2: ^{1}H and ^{13}C spectra of the compounds prepared

Compounds	H in butyl or phenyl (ppm)	H in carboxylate (ppm)	C in butyl or phenyl (ppm)	C in carboxylate (ppm)
[(*n*-C_4H_9)$_2$Sn(3-C_6H_4(OH)COO)$_2$] (**2**)	Hα & Hβ:1.4–1.6 (m); Hγ: 1.29 (m); Hδ: 0.93 (t)	7.35–7.85 (m)	Cα: 21.3; Cβ: 26.7; Cγ: 25.9; Cδ: 14.3	130.4; 133.6; 165.8
[(C_6H_5)$_2$Sn(3-C_6H_4(OH)COO)$_2$] (**4**)	7.47 (t); 7.59 (t)	7.94 (d)	129.8	132.3; 135.4; 167.7
[(C_6H_5)$_3$Sn(3-C_6H_4(OH)COO)] (**6**)	7.47 (t); 7.56(t)	7.89 (d)	127.3; 136.5	131.8; 134.7; 167.4

Table 3: The characteristic and important IR bands of the organotin(IV) compound (cm^{-1}) synthesized.

Compound	2	4	6	References
Sn–O	434.5	594.7	765.59	800–400
Sn–O–C	1029.9	1243.4	1243.36	1050–900
Sn–Bu	674.8	–	–	740–660
CO_2 asym	1419.6	1532.9	1558.8	1600–1400
CO_2 sym	1558.7	1660.8	1631.36	1700–1550
C–H aliphatic	2955–2862	–	–	2960–2850
Phenyl	–	1467.6, 751.3	1428.7, 729.64	1450, 730

Table 4: The λ_{max} of the UV–Vis spectra of the organotin(IV) compounds.

Compound	λ_{max} (nm)
[(*n*-C_4H_9)$_2$SnO] (**1**)	202.9
[(*n*-C_4H_9)$_2$Sn(3-C_6H_4(OH)COO)$_2$] (**2**)	206; 307.8
[(C_6H_5)$_2$Sn(3-C_6H_4(OH)COO)$_2$] (**4**)	209; 287.7
[(C_6H_5)$_3$Sn(3-C_6H_4(OH)COO)] (**6**)	210; 302.3

In our previous work to investigate antibacterial activity of the compounds reported here [6], it was found that the optimal antifungal activity is associated with the number of carbon atoms in the organotin(IV) compound [21], where in general, the derivative of triphenyltin(IV) carboxylate containing 18 carbon atoms is acknowledged to possess the smallest minimum inhibition concentration in the series [16, 20]. It is worthy to note that in this current work, the same phenomena was also observed.

In the experimental data presented in Table 5, it can be observed that the derivatives of triphenyltin(IV) compound exhibit smaller IC_{50} value in the series, and the IC_{50} value of the diphenyltin(IV) is less than that of dibutyltin(IV) compound. The IC_{50} values of all synthesized compounds, however, were found to be less active compared to the positive control of anticancer drug, doxorubicin which has IC_{50} of 1.5 µg/mL (Table 5). These results signify the dependence of anticancer activity of the organotin(IV) tested on the number of carbon atoms in the compound. In addition, it is important to note that IC_{50} values of the organotin(IV) carboxylate compounds synthesized are less than those found

Table 5: IC_{50} values of all compounds tested.

Compound	IC_{50} (µg/mL)
[(*n*-C_4H_9)$_2$Sn(3-C_6H_4(OH)COO)$_2$] (**2**)	24.4
[(C_6H_5)$_2$Sn(3-C_6H_4(OH)COO)$_2$] (**4**)	10.3
[(C_6H_5)$_3$Sn(3-C_6H_4(OH)COO)] (**6**)	3.7
Doxorubicin	1.5

for raw materials and intermediate products. In this regards, the results of this current study are consistent with a well-known fact that many biologically active compounds become more active upon complexation compared to their uncomplexed forms [22]. According to Crowe [23] the $RR'Sn^{2+}$ moiety is the only factor which determines the actual biological activity of diorganotin compounds of the type RR'SnXY (R and R′ = alkyl or aryl, X and Y = anions), consequently, the group X and Y would only influence the delivery of the active $RR'Sn^{2+}$ ion to the cell, although the mechanism of their action has not been fully understood.

4 Conclusions

Based on the discussion above, we have reported some promising organotin(IV) 4-hydroxybenzoate compounds which were successfully syntesized and tested *in vitro* anticancer activity. The results indicated that the derivative of triphenyltin(IV) compound is the most active as candidate for anticancer drug. In this respect, further *in vivo* study of the compounds synthesized against human cancer is required in order to evaluate their potential as an anticancer metal base-drug in the future.

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