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Synthesis and *in vitro* anticancer activity of some organotin(IV) benzoate compounds

SUTOPO HADI* and MITA RILYANTI

Department of Chemistry, University of Lampung Bandar, Lampung 35145 (Indonesia).

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ABSTRACT

We have prepared a series of dibutyl-, diphenyl- and triphenyltin(IV) benzoate compounds by the reaction of the dibutyltin(IV) dichloride, diphenyltin(IV) dichloride and triphenyltin(IV) chloride respectively *via* the dibutyltin(IV) oxide, diphenyltin(IV) dihydroxide and triphenyltin(IV) hydroxide with benzoic acid. The compounds synthesized were characterized by ^{1}H and ^{13}C NMR, IR and UV-Vis spectroscopies, and are based on the microanalytical data. The *in vitro* activity showed that triphenyltin(IV) benzoate synthesized exhibit higher antitumor than those of dibutyltin(IV) and diphenyltin(IV) analogous.

Key words: Synthesis, organotin(IV) benzoate, anticancer.

INTRODUCTION

The interest in organotin(IV) compounds are because they display strong biological activity. The compounds normally exhibit high toxicity, even at very low concentration. The current investigations on the coordinating properties of carboxylates toward organotin compounds have led to the isolation of some new organotin(IV) carboxylates and carboxylate derivatives which have shown some interesting biological activities such as antimicrobial[1], antitumor and anticancer[2-4], and antifungal activity[1,5-7].

The antitumor activity study of organotin(IV) as possible antitumor compound has been and is still attracting much attention[2-4]. The study of these compounds with biologically active ligands has attracted particular interest toward the design of potential antitumor agents. Following our interest in the study of organotin(IV) carboxylate compounds[6,7,8], we reported the synthesis and *in vitro* anticancer activity study of dibutyl-, diphenyl- and triphenyltin(IV) benzoates against leukaemia cancer cell, L-1210.

EXPERIMENTAL

Materials

Dibutyltin(IV) dichloride ($[(n\text{-}C_4H_9)_2Cl_2]$), diphenyltin(IV) dichloride ($[(C_6H_5)_2Cl_2]$), triphenyltin (IV) chloride ($[(C_6H_5)_3Cl]$), were obtained either from Sigma, benzoic acid, sodium hydroxide (NaOH) and methanol (CH_3OH) were JT Baker products. All of the reagents used were of AR quality and were used without further purification.

Preparation of organotin (IV) benzoat

The preparation of organotin(IV) benzoate were based on our previously reported method for the preparation of the organotin(IV) carboxylates[6,7] which was adapted from a method applied by Szorcsik *et al.*[9]. An example procedure in the preparation of dibutyltin(IV) dibenzoate is as follows:

3.0383 g (0.01 mol) $[(n\text{-}C_4H_9)_2SnCl_2]$ in 50 mL methanol was added to 0.8 g (0.02 mol) NaOH. The reaction mixtures were stirred for about 45 minutes. Compound 2 was precipitated out as white solid, filtered off and dried *in vacuo* till they are ready for analysis and further reaction. The yield was 2.3508 g (95 %).

To 0.37338 g (1.5 mmol) compound **2** in 50 mL of methanol was added with 2 mole equivalents of benzoic acid and was refluxed for 4 hours at 60 – 70°C. After removal of the solvent by rotary evaporator, the produced compound [(*n*-$C_4H_9)_2Sn(OOCC_6H_5)_2$] was dried *in vacuo* until they are ready for analysis and further use for biological test. The average yields were more than ~ 90 %.

A similar procedure was also adapted in the preparation of diphenyltin(IV) dibenzoate [$(C_6H_5)_3Sn(OOCC_6H_5)_2$] and triphenyltin(IV) benzoate, [$(C_6H_5)_3Sn(OOCC_6H_5)$] where for the last compound one mole equivalent of the benzoic acid was added.

Characterisation techniques

^1H and ^{13}C NMR spectra were recorded on a Bruker AV 600 MHz NMR (600 MHz for ^1H and 150 MHz for ^{13}C). All experiments were run in DMSO-D_6 at 298K. The number of runs used for ^1H experiments were 32 with reference at DMSO signal at 2.5 ppm, while the ^{13}C were 1000-4000 scans with the reference DMSO signal at 39.5 ppm. Elemental analyses (CHNS) were performed on Fision EA 1108 series elemental analyser. IR spectrum in the range 4000-400cm^{-1} was recorded on a Bruker VERTEX 70 FT-IR spectrophotometer with KBr discs. The UV spectra were recorded in the UV region and were measured using a UV-Shimadzu UV-245 Spectrophotometer. Measurements were performed in 1mL quartz-cells. Solutions were prepared using methanol as the solvent with concentration of $1.0x10^{-4}$M.

The *in vitro* anticancer activity test

A known procedure[10] was used in the *in vitro* antitumor activity test which was tested against the leukaemia cancer cell, L-1210. 1 mL of the cancer cells were added into each hole of *multiwell plate tissue culture* containing 20 x 10^5 cell/mL followed by the addition of 10 mL of solution containing the compounds tested in methanol (if the sample was not soluble enough in the solvent used, before it was added, the ultrasonic mixing was done first to homogenize the sample). The sample concentration variations used were 1, 2, 4, 6, 8, 16, 32 mg/mL. As the negative control, the cell was treated with a solution containing 10 mL of solvent was used as comparison. The cell was then incubated for 48 h in 5% CO_2 incubator at 37°C. After being incubated, the sum of cell was counted in microscope using *haemecytometer Fuch Rosental* (0.200 mm x 0.0625 mm^2).

The percentage of inhibition was calculated using the following formula in Eq. (1):

$$\% \text{ inhibition} = 1-\frac{A}{B} \times 100 \quad ...(1)$$

where A is the number of lives cell in medium tested; and B is the number of lives cell in control/blank

The IC_{50} was calculated according to the adaption of Reed and Muench method which is an arithmetic method[11] and as following: the data of all inhibition percentages were plotted into probit table to obtain each probit value of each analysis. The graph between log of concentration (x) and probity value (y) was then created to obtain a linier regression, y = a + bx. By inputting the probit value (y) = 5 (the probit of 50% value), then the value of x (log of concentration) was obtained. The IC_{50} value was then obtained by taking the anti log of x.

RESULTS AND DISCUSSIONS

Table 1 summarizes the microanalytical data of all compounds prepared and all of them are very good and all values obtained are close to the calculated values. The series of organotin(IV) benzoate (3, 6, 9) were successfully prepared from their chlorides [(*n*-$C_4H_9)_2SnCl_2$] (1), [$(C_6H_5)_2SnCl_2$] (4) and [$(C_6H_5)_3SnCl$] (7), respectively, where the reactions in all cases were done *via* [(*n*-$C_4H_9)_2SnO$] (2), [$(C_6H_5)_2Sn(OH)_2$] (5) and [$(C_6H_5)_3SnOH$] (8) respectively similar to those previously reported[6,7].

Based on the IR data as shown in Table 2, the characteristic of the products synthesized was mainly confirmed within the frequency range of 4000 – 250 cm^{-1}. In the starting materials (1, 4, 7), the appearance of the characteristic band of the strong stretching band from the Sn-Cl bond at 390 – 310 cm^{-1}. For example in the spectrum of 1, this bond appeared at a frequency of 334.2 cm^{-1}. The other characteristic bands of this compound appear as stretching band from butyl ligands at 1069 cm^{-1}, and

bending vibration of C-H aliphatic stretch of the butyl at frequency of 2958 – 2864 cm^{-1}.

Once compound 2 formed, the main stretching band of Sn–Cl disappeared and a new strong band at frequency of 417.4 cm^{-1} appeared as one of the main stretching band in 2. This band is characteristic for Sn–O bond in compound [(*n*-$C_4H_9)_2SnO$] (2). The bands for butyls as well as their bending vibrations are still appeared as expected even though their frequencies have changed little bit. The strong asymmetric stretching bands of the benzoates which occurred at *ca.* 1400 cm^{-1} and the symmetric stretch at *ca.* 1600 cm^{-1}, confirming the success of the substitution reaction were observed when dibutyltin (IV) dibenzoate (3) was formed[6,7].

The result of UV spectroscopy analyses is presented in Table 3. Based on Table 3, the λ_{max} of all the synthesized compounds clearly informed us that there was a shifting change in the λ_{max} for each compound in any steps of the reaction. The λ_{max} of 1 was 210.7 nm, while in compound 2, the λ_{max} has shifted lower to 202.9 nm. The shift in wave length to a shorter λ_{max} from 1 to 2 can be caused by either the solvent used or the effect of an auxochrome[12,13]. In this study, however, the solvent used for all measurements was the same (methanol), so this change is caused by auxochrome effect.

For compound 1 and 2, there is a change in the ligand attached to the metal centre, which is oxide group in 2, which has an electron drawing effect bigger than that of chloride group in 1. As a result, the electron transition in 2 doesn't occur easily. Thus, the measured λ_{max} was getting shorter in compound 2 than in compound 1[12,13]. Similar result is also observed for other changes which can be seen from Table 3. In compound 3, the substitution from the oxygen group to C_6H_5COOH which is a chromophore molecule, to the metal centre caused a longer batochromic shift, as a result the electron transition in this molecule will be easier and the energy required is less, thus producing longer λ_{max},

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

Compound	Elemental analysis found (calculated)	
	C	H
[(*n*-$C_4H_9)_2Sn(OOCC_6H_5)_2$] (3)	55.05 (55.61)	5.92 (5.94)
[$(C_6H_5)_2Sn(OOCC_6H_5)_2$] (6)	60.38 (60.62)	3.6 (3.7)
[$(C_6H_5)_3Sn(OOCC_6H_5)$] (9)	63.89 (63.73)	4.30 (4.28)

Table 2: The characteristic and important IR bands of the organotin(IV) compounds (cm^{-1}) synthesized

Compound	3	6	9	References (IR bands)
Sn-O	434.5	595.3	765.8	800-400
Sn-O-C	1032.4	1244.1	1243.9	1050-900
Sn-Bu	675.1	-	-	740-660
CO_2 asym	1420.3	1533.7	1559.2	1600-1400
CO_2 sym	1559.8	1661.2	1631.6	1700-1550
C-H aliphatic	2958 – 2864	-	-	2960 – 2850
Phenyl	-	1468.2; 751.7	1429.6; 729.9	1450, 730

282.6 nm. Similar results are also observed for other compounds prepared. In compound 6 and 9 as the organic ligands attach to metal are phenyl, in addition to the main band on n-π transition, there are two other bands in the regions 200 nm which may be due to the π- π * transition of the benzene ring and the region 400 nm for secondary band of the benzene ring.

The ^{1}H and $^{13}CNMR$ spectra of the compounds prepared are shown Table 4. A number of signals in the spectra recorded have been observed that upon complexation, the chemical shifts have shifted to a higher value. For example, in 4 the chemical shift (δ) of carbons in phenyl was at 125 ppm and has shifted higher in 6 to 129 ppm. Three new peaks were also observed as benzoate bound to Sn which was at 132 and 135 ppm for phenyl carbons and 168 ppm for carbon in the carboxyl group.

We have previously reported the antifungal activity of the compounds reported here[6,7], and it has been shown that the optimal antifungal activity has been associated with the number of carbon atoms present in the organotin(IV) used[14], where in general, the derivative of triphenyltin(IV) carboxylate which contain 18 carbon atoms has smallest minimum inhibition concentration values in the series[6,7]. Similar phenomena were interestingly also observed in this study. The data in Table 5 revealed that triphenyltin(IV) benzoate showed smaller IC_{50} value, while diphenyltin(IV) dibenzoate has IC_{50} value smaller than that of dibutyltin(IV) compounds. This result indicated that the number of carbon atoms present might have a strong effect on the anticancer activity of the organotin(IV) tested. It is also observed that all organotin(IV) benzoates have smaller IC_{50} values compared to the starting materials and the intermediate products.

Table 3: The λ_{max} of the UV spectra of the organotin(IV) compounds

Compound	λ_{max} (nm)		
	π-π*	**n-π**	**benzene ring secondary band**
$[(n\text{-}C_4H_9)_2Sn(OOCC_6H_5)_2]$ (**3**)	-	282.6	-
$[(C_6H_5)_2Sn(OOCC_6H_5)_2]$ (**6**)	201	297.7	407
$[(C_6H_5)_3Sn(OOCC_6H_5)]$ (**9**)	204	300.5	409

Table 4: ^{1}H and $^{13}CNMR$ spectra of the compounds prepared

Compounds	H in butyl or phenyl (ppm)	H in benzoate (ppm)	C in butyl or phenyl (ppm)	C in benzoate (ppm)
$[(n\text{-}C_4H_9)_2Sn(OOCC_6H_5)_2]$ (**3**)	Hα & Hβ:1.4-1.6 (m); Hγ: 1.29 (m); Hδ: 0.93 (t)	7.35-7.89 (m)	Cα: 21.3; Cβ: 26.6; Cγ: 25.9; Cδ: 14.2	130.3; 133.5; 165.9
$[(C_6H_5)_2Sn(OOCC_6H_5)_2]$ (**6**)	7.49 (t); 7.58 (t)	7.94 (d)	129.6	132.2; 135.3; 167.8
$[(C_6H_5)_3Sn(OOCC_6H_5)]$ (**9**)	7.46 (t); 7.55(t)	7.89 (d)	127.4; 136.9	131.9; 134.6; 167.3

Table 5: IC_{50} values of organotin(IV) benzoate tested

Compound	IC_{50} (μg/mL)
$[(n\text{-}C_4H_9)_2Sn(C_6H_5COO)_2]$ (3)	19.6
$[(C_6H_5)_2Sn\ Sn(C_6H_5COO)_2]$ (6)	9.2
$[(C_6H_5)_3Sn\ Sn(C_6H_5COO)]$ (9)	5.33

Therefore, our results reported here are still consistent with a well-known fact that many biologically active compounds become more active upon complexation than in their uncomplexed forms[15]. The actual biological activity of diorganotin compounds of the type RR'SnXY (R and R' = alkyl or aryl; X and Y= anions) is determined solely by the $RR'Sn^{2+}$ moiety. Consequently the group X and Y would only influence the delivery of the active $RR'Sn^{2+}$ ion to the cell, although it is not clear enough how is their mechanism of action[16].

CONCLUSIONS

This study has shown promising results in the *in vitro* anticancer study of organotin(IV) benzoate prepared as they exhibited quite high anticancer activity. The other series of organotin(IV) carboxylates are still being prepared in our laboratory in attempts to find the organotin(IV) carboxylate compounds which might have and show better anticancer activity.

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REFERENCES

1. Mahmood S., Ali S., Bhatti M.H., Mazhar M., Iqbal R., *Turk. J. Chem.*, **27**: 657 (2003).
2. Li Y., Li Y., Niu Y., Jie L., Shang X., Guo J., Li, Q., *J. Bioinorg. Chem.*, **102**: 1731 (2008).
3. Rehman W., Badshah A., Khan S., Tuyet L.T.A., *Eur. J. Med. Chem.*, **44**: 3981 (2009).
4. Siddiqi Z. A., Shahid M., Kumar S., Khalid M., Noor S., *J. Organomet. Chem.*, **694**: 3768 (2009).
5. Ruzika A., Dostal L., Jambor R., Butcha V., Brus J., Cisarova I., Holcapek M., Holecek, *J., Appl. Organomet. Chem.*, **16**: 315 (2002).
6. Hadi S., Irawan B., Efri, *J. Appl. Sci. Res.,* **4**: 1521 (2008).
7. Hadi S., Rilyanti M., Nurhasanah, *Modern Appl. Sci.*, **3**: 12 (2009).
8. Hadi S., Rilyanti M., Ambarwati Y., Qurrata'aini N., Setionugroho R., Submitted to *Arab. J. Chem.,* (2010).
9. Szorcsik A., Nagy L., Gadja-Schrantz K., Pellerito L., Nagy E., Edelmann E.T., *J. Radioanal. Nucl. Chem.*, **252**: 523 (2002).
10. Sumatra M., *Proc. Seminar Indonesian Marine Biotechnology*. Jakarta, 183 (1998).
11. Turner R.A., Screening Methods In Pharmacology. New York, Academic Press, 60-72 (1972).
12. Rao C.N.R. Ultraviolet and Visible Spectroscopy, London, Butterworhs, 1-20 (1961).
13. Sudjadi, The Structure Determination of Organic Compounds. Jakarta, Ghalia Publishers, 327 p (1985) (In Indonesian).
14. Chohan Z.H., Rauf A., *Syn. Reactiv. Inorg. Metal-Org. Chem.*, **26**: 591 (1996).
15. Gershon H., *J. Med. Chem.*, **17**: 824 (1974).
16. Crowe A.J., The antitumour activity of tin compounds. In Metal-based Drugs. Gielen, M. (Ed.). Freund Publishing House, Freund, **1**: 103 (1989).