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TESTING THE ACTIVITY OF THE SYNTHETIC COMPOUND DIPHENYLTIN (IV)N- BENZYL METHYL DITHIOCARBAMATE AGAINST BREAST CANCER MCF-7

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Abstract:

Breast cancer is the leading cause of death, especially for women in Indonesia. Compounds that are widely used for anticancer at present are organotin compounds, one of which is dithiocarbamate compounds, with several reports of antifungal, antibacterial, anticancer, anti-alkylation, and apoptosis-inducing activities of metal dithiocarbamate complexes and their mixed ligands. The purpose of this study was to determine the activity test of the synthetic compound Diphenyltin (IV) N-Benzylmethyldithiocarbamate Against Breast Cancer MCF-7 using the MTT Assay (Microculture Tetrazolium Technique) Assay and to determine the functional group and structure of the compound Dibutyltin (IV) N-Benzylmethylditiocarbamate, used Spectrophotometer test FTIR (Fourier Transformed Infrared) and FTNMR (Nuclear Magnetic Resonance). From this study, it was found that the synthetic compound Diphenyltin (IV) N-Benzylmethyldithiocarbamate is a very potent compound in inhibiting the development and growth of MCF-7 breast cancer cells, because the compound has an IC50 value that is smaller than 10 g/ml which is 69, 640ppm.

Keywords: Diphenyltin (IV) N-Benzylmethyldithiocarbamate, FTNMR, MCF-7 breast cancer.

Introduction

Cancer or neoplasm is a disease caused by the growth of cells that grow and multiply incompatible with the nature, shape, and kinetics of normal cells in general (Sukardja, 2000). This cancer is one of the diseases that contribute to the highest mortality rate, especially in Indonesia. The total number of cancer cases in Indonesia according to Globacan (Global Burden of Cancer Study) from WHO (World Health Organization) which recorded cancer cases in 2020 was 396,914 cases with a total with the death of 234,511 cases. In Indonesia, breast cancer contributes to the highest number of cases, namely 65,855 cases or 16.6% of the total 396,914 cancer cases (1). Breast cancer (Carcinoma Mammae) is a disease that attacks breast tissue and develops inside the mammary glands, fatty tissue, and milk ducts. One of the ways breast cancer spreads is with the lymph system, which is characterized by swollen lymph nodes in the yak. Breast cancer can be caused by a family history of cancer, hormonal functions such as getting the first menstruation before the age of 10 years, and age factors, namely women who are over 30 years old have a greater risk, have experienced infections, trauma or impacts, significant weight gain, have received radiation around the breast, the use of hormonal drugs and the use of contraceptives (3) Cancer treatment has been widely carried out such as cancer treatment with chemotherapy using tamoxifen (4) and radiotherapy using radiation beams surgery is carried out aimed at reducing the size of the tumor/cancer, reducing the burden of tumors to increase the response to the treatment carried out and immunotherapy/biotherapy is carried out by increasing the immune system, antibody labeled

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fluorescent, gene therapy and attacking antibody (5) However, the mortality rate is due to Cancer is still high. Therefore, the development of new drugs with various strategies has been carried out, including exploring new compounds that are effective against cancer cells, including organotin compounds

Organotimah compounds are compounds whose tin atoms bind directly to the carbon atoms of the organic group (6)One of the organotin compounds that are often used is the Dithiocarbamate compound, the use of this compound depends on the chelating properties of dithiocarbamate ligands against ions(7). Dithiocarbamate compounds can be classified into three main groups, namely primary, secondary and tertiary(8) Dithiocarbamate compounds are very potent compounds in agriculture, industry, and medicine. According to the latest activity of dithiocarbamate compounds, it can be used as an antifungal, anti-bacterial, anti-cancer, anti-alkylation, and apoptotic-inducing activity of the metal dithiocarbamate complex and its mixed ligands(9) This is known based on research that reports that the synthesis results of the compound Dibyltimah (IV) N-Benzilmethylditiokarbamat have been shown to have antifungal activity against the fungus Candida Albicans by 17 mm (dose 0.050 grams) and 16.58 mm (dose 0.050 grams)(10), dithiocarbamate compounds are also very active against Salmonella typhi and E. Coli bacteria with very small doses and have strong activities (11). Many studies of dithiocarbamate compounds have been carried out on anticancer P-388(8), dithiocarbamate compounds have also been found to be active in anticancer leukemia with active human hepatocarcinoma cells with concentrations below 10 µgr / ml (12).

Based on the results of the research, researchers want to conduct a study on the activity of the N-Benzylmethylmethyldiocarbamate Dibutyltin (IV) compound on one of the breast cancer cells, namely MCF-7 (Michigan Cancer Faoundation-7) cells. MCF-7 is one of the most widely used cancer cell models for in vitro breast cancer research(13). In vitro assay is a cytotoxicity test that uses cell cultures in the evaluation of the acidity of drugs, food additives, pesticides, and cosmetics and to determine the antineoplastic activity of compounds MCF-7 cancer cells are cancer cells that are resistant to the chemotherapy agent Doxorubicin. MCF-7 breast cancer cells have the characteristics of PGP overexpression, and Bcl-2 overexpression and do not express caspase-3 to avoid apoptosis(14)

Testing of MCF-7 cancer cells was carried out using the MTT Assay (Microculture Tetrazolium Technique) Assay method. This MTT Assay method is a technique that uses tetrazolium salt or MTT 3-(4,5-dimethyl thiazole-2-il)-2,5-diphenyl tetrazolium bromide). This method is used to determine whether or not the compounds tested to inhibit the growth of cancer cells(15) This can be known based on the value of the parameter obtained, namely the IC50 value. The IC50 value is a value that indicates a concentration of 50% cell polyference resistance and a value that indicates the toxicity of a compound to cancer cells. The greater the IC50 value, the less toxic the compound is (16)

METHOD

This research was conducted in situ, namely synthesizing the compound Diphenyltine (IV) N-Benzylmethylditiocarbamate, by reacting between carbon disulfide with amines and lead metal directly in Erlenmeyer and stirrer for 2 hours, then the characterization of compounds using FTIR and FTNMR. Furthermore, the anticancer activity test against MCF-7 breast cancer cells using the MTT assay method.

Material and Instrumentation

The tools used are Erlenmeyer, Whatman Filter paper No. 42, analytical scales, hotplate, desiccator, measuring pipette, magnetic stirrer, NMR spectrophotometer (Agilent 500Mhz), and FTIR (PARKIN ELMER), spatial, test tube, incubator, tissue, pipette injection tube, sample bottle, conical tube, flask culture, micropipette, laminar airflow (LAF), microplate 96 bursts, water bath, centrifugation, syringe nonpyrogenic membrane filter, micropipette 20-200 µl and 200-1000 µl, digital camera.

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N-Benzylmethylamine material (Sigma Aldrich), carbon disulfide (CS_2)(Merck), dibutyl amine metal (IV) dichloride(Sigma Aldrich), Methanol (pa), Chlorophorom be deuterium ($CDCL_3$), Rosewell Park Memorial Institute (RPMI) cell media (Gibco), Phosphate Buffer Saline (PBS), Trypsin EDTA 5%, MTT {3-(4,5 dimethyl azole-2il)-2.5 diphenyl tetrazolium bromide}, Trypan Blue Stain 0.4% (sigma), Dimethyl Sulfoxide (DMSO), Alcohol 70%.

PREPARATION AND CHARACTERIZATION

The sample working procedure consists of a primary amine i.e. N-Benzylmethylamine ($C_8H_{11}N$), carbon disulfide (CS_2), and metal Dipyltamine (IV) dichloride $(C_4H_9)_2$ $SnCl_2$, made in situ all dissolved in Methanol (pa) each in 15 ml. Synthesis of Diphenylytin (IV) Compound N-Benzylmethyldithiocarbamate, N-Benzylmethylamine pipette as much as 2.58 ml (0.02 mol) was put in Erlenmeyer, then 15 ml of cold methanol (pa) was added and then a solution of carbon disulfide (CS_2) was added to the 1.2 ml (0.02 mol) di phthalate metal and then a solution of metal diphenyl tin (IV) dichloride $(C_4H_9)_2SnCl_2$ as much as 3.43 g (0.01 mol) in 15 ml of cold methanol was then reacted in situ in Erlenmeyer stirrer for 2 hours formed. The precipitate is then filtered and dried in a desiccator. The resulting sample was in the form of a white powder, odorless, as much as 1.29 grams.

FTIR Spectrophotometer Test FTIR Spectrophotometer is carried out by taking a compound ± 1.00 mg and grinding it in KBr plate powder, after that measure its vibration at wave numbers 4000 cm-1 - 200 cm-1 FTNMR Spectrophotometer Test FTNMR Spectrophotometer Testing by taking pure solids compounds of ± 10.00 mg then dissolved using chloroform deuterium solvent (CDCl3). The sample solution obtained is inserted into the injection tube, then the injection tube is placed in the NMR AGILENT test kit (500 Mhz for ^{1}H-NMR and 125 Mhz for ^{13}CNMR)(18).

The toxicity test uses a microplate of 96 well so the test medium. You do this by taking as much as 100 µl of cell suspense in a serum RPMI medium then inserting it in each tissue culture plate sheet, then incubation using a 5% CO2 incubator at a temperature of 370C for 48 hours to obtain optimal growth (14) After 48 hours the cells will stick to the base of the microplate, then discard the media by reversing the 1800C microplate, then pressed on a tissue to remove the remaining media fluid. A 100 µl PBS solution is inserted into all wells containing cells, then PBS is disposed of by reversing the microplate and then pressed on a tissue so that the remaining PBS fluid is wasted. Media containing 100 µl of Diphenyltamine (IV) N-Benzylmethylditiokarbamat with a concentration of 100; 50; 25; 12.50; 6.25; 3.13; 1.56; 0.78 µg/ml inserted into the microplate well Then 3 (three) repetitions were carried out. Then incubation using a 5 % CO2 incubator at a temperature of 370C for 24 hours. Furthermore, an MTT solution was prepared in a PBS solution (10 mg/ml) and then diluted by adding 10 ml of RPMI culture media for one microplate of 96 burrows (16). After that, remove the Microplate from the CO2 incubator, then the culture medium is discharged again, after which 100 µl of MTT is added to each well including for media control. After that, the cell was

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incubated on a 5% CO2 incubator with a temperature of 370C for 4 hours. Living cells will react with MTT to form a purple-colored formazan, if it stops the cell reaction with MTT as well as dissolving formazan should be added 100 µl SDS 10% in 0.01 N HCl. Then wrap the Microplate with aluminum foil, then incubate it for one night at room temperature. After that, read the absorption by using a spectrophotometer on the ELISA reader device at a wavelength of 595 nm. (15)

Calculation of the percentage of cell death can be used in the MTT Assay method. The presentation of cell death is the difference in the absorbance of the treatment reduced by the absorbance of the media control, divided by the absorbance of the negative control, then reduced by the absorbance of the media control, and then multiplied by 100%. Each of the absorbents has been corrected using the absorbance of the test solution of each level. Cell death is calculated using the MTT Assay method. From the data on the percentage of cell death obtained, data analysis can be used to find out the value of IC50 using Microsoft Excel(16).

RESULT AND DISCUSSION

The result of the synthesis of the compound Diphenyltin (IV) N-Benzylmethylditiocarbamate, the compound obtained is in the form of powder or white colored flour, odorless, and weighs 1.29 grams

Table 1. Fourier Transform Infrared (FTIR) Spectrophotometer Test Results

Range of Absorption Areas (cm^{-1})	**Spectrum results (cm^{-1})**
(C-N) 1420-1550 (C-S) 950-1050 (C-H) 3000-2840 (C-C) 1200-800 (Sn-C) 350-450 (Sn-S) 515-605	1429,25 C- N 993,34 C - S 2931,80 C – H 1091,71 C – C 445,56 Sn – C 549,71 Sn – S

Table 1 shows the results of the FTIR spectrum of the synthesis compound Diphenyltin (IV)N-benzyl methyl dithiocarbamate showing that the peak spectrum of the desired functional group has been achieved such as the functional group C – N at wave number1429.25 cm-1 (s), C – S at wave number 993.34 cm-1, Sn – S at wave number 549.71 cm-1, and peak Sn – C at wave number 445.56 cm-1 This shows that the synthesis compound was successfully formed because the bond between the atoms was not broken. The peak FTIR spectrum yield of the diphenyltin(IV) compound N-benzyl methyl dithiocarbamate can be seen in figure1

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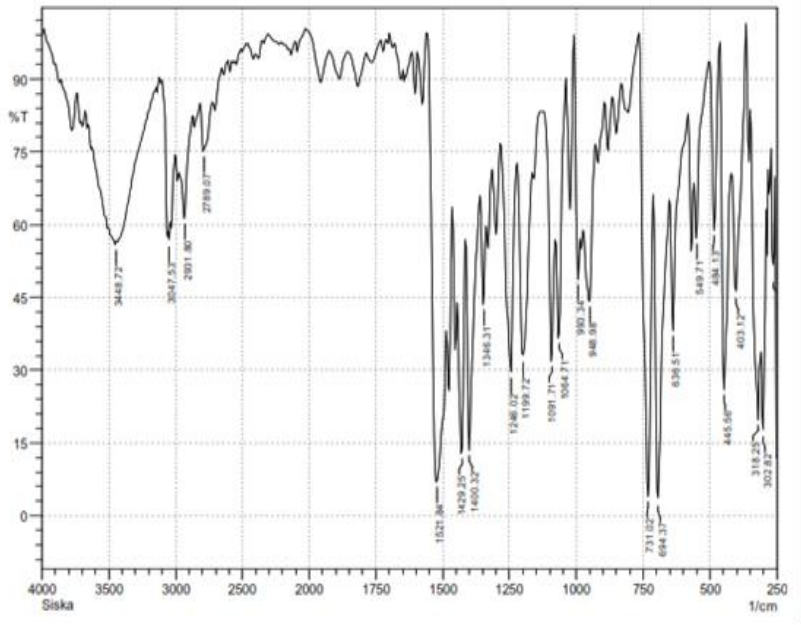

Figure 1. Peak spectrum FTIR Compound Diphenylytine (IV) N-Benzyl methyl dithiocarbamate

In the FTNMR assay proton 1H showed the methyl(-CH_3) molecule appeared at (3.2801 ppm, H in ethyl(-CH_2) appeared at (4.8-5.04 ppm triplet and H in H-phenyl appeared at (7.2854 – 8.0799 ppm, this also corroborates that the synthesized compound has been successfully formed, the results of NMR protons can be seen in Table 2. And the peak of the NMR proton spectrum can be seen in figure 2.

Table 2. FT-NMR proton test results ^1H

Shifting Areas Proton(ppm) (19)	**Analysis Results of Diphenyltin (IV) Compound N-Benzyl methyl dithiocarbamate (ppm)**
0 – 4 ppm	3,2801 ppm s (singlet) (CH_3)
4 – 5 ppm	4,8 – 5,0442 ppm t triplet) (-CH_2-)
6-9 ppm	7,2854 – 8.0799 ppm (H aromatic)

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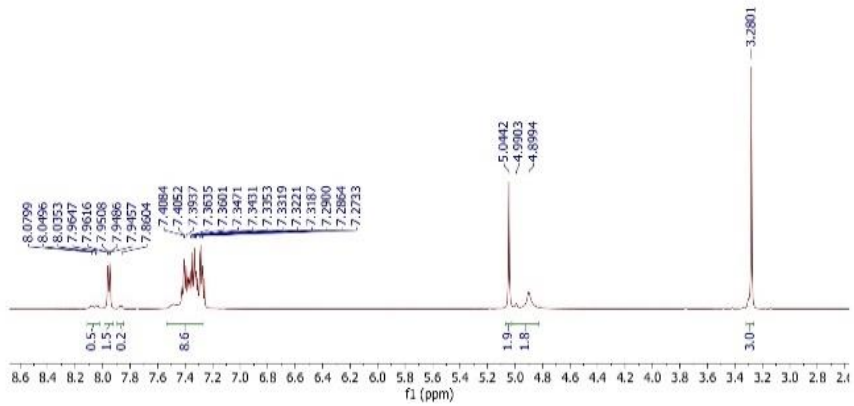

Figure 2. Peak spectrum FTNMR ^{1}H

In NMR ^{13}C testing carbon peaks in methyl appeared at (0 – 20 ppm, peaks –CH2 ethyl appeared at (42.67 ppm singlet, peaks –C – N- appeared at (61.499 ppm singlet, on (127.6517– 135.4453 ppm appeared peaks of C-aromatics(multiplets), while peaks $–S_2C-$ appeared at (201.113879 ppm. Data on the results of the ^{13}C NMR spectrum can be seen in table 3 and the peak spectrum of ^{13}C NMR can be seen in Figure 3. Based on the results of FTIR and FTNMR ^{1}H and ^{13}C the compound diphenyl tine (IV)N-benzyl methyl dithiocarbamate structure can be estimated as in figure 4.

Table 3. Test Results FTNMR ^{13}C Compound diphenyltin(IV)N-benzylmethylditihocarbamate

Carbon Shift Areas (ppm)	**NMR^{13}C Analysis Results δ(ppm)**
0 – 20 ppm $-CH_3$	0–20ppm (s) ($-CH_3$)
20- 45 ppm $–CH_2-$	42,67ppm(s)($-CH_2$)
60 – 70 ppm-CN-	67,4995ppm (s) (CN)
120 – 145 ppm-C aromatic)	127,6517– 135,4453 ppm m (C aromatic)
150 – 200 ppm CS_2	201,113879 (CS_2)

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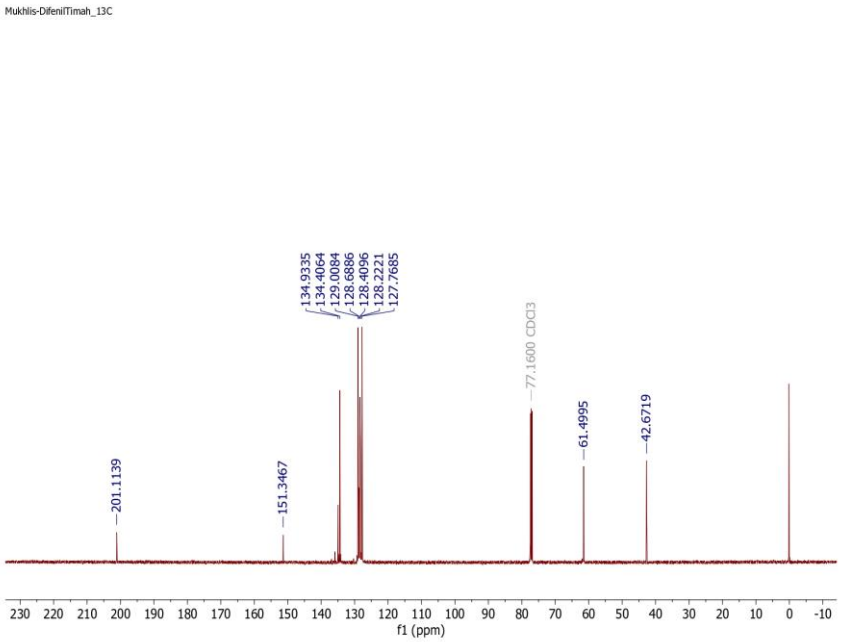

Figure 3. Peak spectrum of results of 13C NMR Compound Diphenyltin(IV)N-benzyl methyl dithiocarbamate

Based on the results of FTIR and FTNMR 1H and 13C analysis so that it can be estimated the chemical structure of diphenyltin(IV)N-benzyl methyl dithiocarbamate compounds as in Figure 4

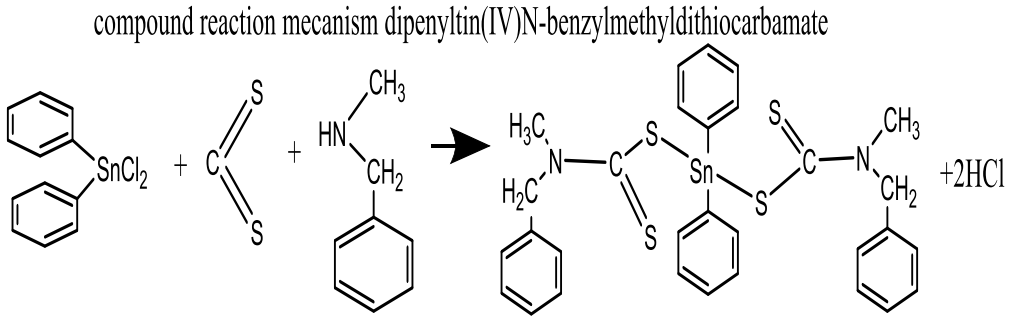

Figure 4. Compound structure of Diphenyl tin(IV) N-benzyl methyl dithiocarbamate

Data on the test results of diphenyltin(IV)N-benzyl methyl dithiocarbamate compounds against breast anticancer using MCF-7 cells can be seen in table 5, the IC-50 value obtained is 69, 640ppm

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Table 4. Data on test results on breast cancer cells (MCF-7)

Concentration, ppm	% Cell Death
100,00	10,759
50,00	83,905
25,00	95,161
12,50	98,086
6,25	99,447
3,13	99,536
1,56	102,032
0,78	95,989

In the test results for breast cancer, the compound Diphenyltin(IV) N-benzyl methyl dithiocarbamate using MCF-7 cells obtained IC-50 results at 69,640 ppm, as shown in figure 5.

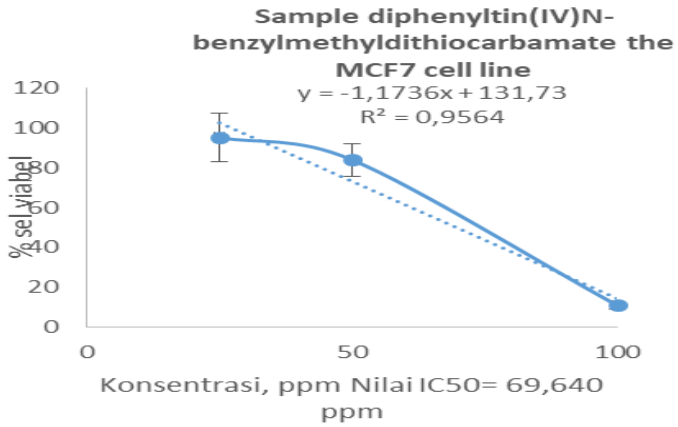

Figure 5. Breast cancer test results of Diphenyltimah (IV) compound N-Benzylmethyldiocarbamate against MCF-7 cells

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CONCLUSION

This study concludes that the synthetic compound Diphenyltine (IV) N-Benzylmethyldithicarbamate is very active in inhibiting and stopping the growth of MCF-7 breast cancer cells. This compound has an IC50 value of 69,640 µg/mL. This compound can be used as an anticancer drug candidate in the future

Appreciation

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A Brief Author Biography

1. Mukhlis Sanuddin (author 1) Mastered chemistry by research as a lecturer in study pharmacy, experienced in research on the synthesis of organotin compounds, both as anti-bacterial, anti-fungi, and anticancer Leukemia and anticancer breast. In this study, he played a role in characterizing compounds with FTIR and FTNMR
2. Indri Meirista (master of pharmacy) experienced in pharmaceutical technology, in this study as an examiner against breast anticancer using the MTT assay method
3. Siska Emilia Nasril (BSc pharmacy) in this study played a role in synthesizing diphenyltin (IV) N-benzyl methyl dithiocarbamate compounds

