Synthesis and Cytotoxicity of 4-Allyl-2 Methoxyphenol Derivatives

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Abstract* – *4-Allyl-2-methoxyphenol derivatives was synthesized through the application of methods of Yamaguchi in one pot with a resulting yield of 80-90%. 4-Allyl-2-methoxyphenol derivatives showed good activity in inhibiting the growth of human breast cancer cell MCF-7.

Index Terms* – *4-allyl-2-methoxyphenol derivative, cytotoxicity, preparation.

INTRODUCTION

Breast cancer is the second leading cause of death in women are caused by cancer. Development of compounds anti-cancer is still being done to get an anticancer drug with high activity and low side effects. Anti-cancer compounds can be derived from the results of the synthesis and isolation of medicinal plants. [1, 2].

Clove (Syzygium aromaticum (L.) Merr. & Perry) is one of the original Indonesian spice used as seasoning with its main compound is 4-allyl-2-methoxyphenol which are known to have activity in inhibits the growth of cancer cells such as human breast cancer cells (MDA-MB-231; MCF-7 and T47-D). 4-allyl-2-methoxyphenyl acetate reported to have activity in inhibiting the growth of cancer cells DU-145 (prostate cancer cells androgen-sensitive) and KB (oral squamous cell carcinoma) [1-5].

Synthesis of 4-allyl-2-methoxyphenyl ester is generally conducted through the reaction between 4-allyl-2-methoxyphenol with various acid chlorides and pyridine [4, 6-8]. Yamaguchi method can be used as an alternative method for the synthesis of 4-allyl-2-methoxyphenyl ester. This method is reported to have successfully applied to the synthesis of enzyme inhibitors Lux-S acid, thiol esters, and a large ring lactone [9-11].

In this paper reported the application of the method of Yamaguchi in the synthesis of 4-allyl-2-methoxyphenol derivatives (Figure 1) and cytotoxicity studies on breast cancer cells MCF-7 in obtaining new anticancer compound that has high activity and low side effects.

MATERIAL AND METHOD

A. General procedure for the synthesis of 4-allyl-2-methoxyphenol derivatives

A solution of 2,4,6-tricholobenzoil chloride (0.75 mmol), carboxylic acid (0.75 mmol), triethylamine (0.75 mmol) in dichloromethane was stirred at room temperature for 1 h. 4-Dimethylaminopyridine (0.75 mmol) and 4-allyl-2-methoxyphenol (0.50 mmol) was added to the solution, and the mixture was stirred further at room temperature for 6 h. The product was extracted several times with dichloromethane. The combined extract was wished subsequently with 5% aqueous hydrochloric acid, 5% sodium hydroxide, 10% aqueous sodium bicarbonate, and water, dried over magnesium sulphate and the solvent was removed under reduced pressure to yield the titled compound.

1) 4-allyl-2-methoxyphenyl propionate ***(a)***. *White solid. Yield: 88% (0.097 g). δ_H (500 MHz, CD_3OD): 1.21 (t, J = 7.8 Hz, 3H, $CH_2C\underline{H_3}$), 2.57 (q, J = 7.8 Hz, 2H, $C\underline{H_2}CH_3$), 3.36 (d, J = 6.5 Hz, 2H, $C\underline{H_2}CH=CH_2$), 3.76 (s, 3H, OCH_3), 5.09-5.14 (m, 2H, $CH_2CH=C\underline{H_2}$), 5.92-6.01 (m, 1H, $CH_2C\underline{H}=CH_2$), 6.75 (d, J = 7.8 Hz, 1H, ArH), 6.87 (s, 1H, ArH), 6.91 (d, J = 7.8 Hz, ArH). δ_C (125 MHz, CD_3OD): 9.55 ($CH_2\underline{C}H_3$), 28.11 ($\underline{C}H_2CH_3$), 41.05 ($\underline{C}H_2CH=CH_2$), 56.33 (OCH_3), 113.92 (ArCH), 116.33 ($CH_2CH=\underline{C}H_2$), 121.66 (ArCH), 123.53 (ArCH), 138.73 ($CH_2\underline{C}H=CH_2$), 139.52 (ArC), 140.52 (ArC), 152.46 (ArC), 174.48 (C=O). MS (EI): m/z 220 (M, 8%), 189 (2), 164 (100), 147 (27), 133 (15), 131 (16), 108 (10), 92 (12), 74 (8), 57 (9). HR-ESI-MS $[M+H]^+$ m/z 221.2643, calculated for $C_{13}H_{17}O_3$, 221.2723.*

2) b) 4-allyl-2-methoxyphenyl butanoate ***(b)***.*Colourless oil. Yield: 86% (0.1008 g). δ_H (500 MHz, CD_3OD): 1.07 (t, J = 7.5 Hz, 3H, $CH_2C\underline{H_3}$), 1.81 (m, 2H, $C\underline{H_2}CH_3$), 2.57 (t, J = 7.5 Hz, 2H, $C\underline{H_2}CH_2CH_3$), 3.39 (d, J = 6.5 Hz, 2H, $C\underline{H_2}CH=CH_2$), 3.81 (s, 3H, OCH_3), 5.09-5.14 (m, 2H, $CH_2CH=C\underline{H_2}$), 5.93-6.01 (m, 1H, $CH_2C\underline{H}=CH_2$), 6.78 (d, J = 8.4 Hz, 1H, ArH), 6.80 (s, 1H, ArH), 6.95 (d, J = 8.4 Hz, ArH). δ_C (125 MHz, CD_3OD): 13.65 ($CH_2\underline{C}H_3$), 18.65 ($\underline{C}H_2CH_3$), 35.95 ($\underline{C}H_2CH_2CH_3$), 40.15 ($\underline{C}H_2CH=CH_2$), 55.81 (OCH_3), 112.76 (ArCH), 116.17 ($CH_2CH=\underline{C}H_2$), 120.70 (ArCH), 122.59 (ArCH), 137.17 ($CH_2\underline{C}H=CH_2$), 138.12 (ArC), 138.90 (ArC), 150.97 (ArC), 171.93 (C=O). MS (EI): m/z 234 (M, 8%), 164 (100), 147 (27), 131 (16), 108 (10), 91 (12), 71 (8), 51 (9). HR-ESI-MS $[M+H]^+$ m/z 235.2335, calculated for $C_{13}H_{17}O_3$, 235.2989.*

3) c) 4-allyl-2-methoxyphenyl isobutanoate ***(c)***. *Colourless oil. Yield: 86% (0.1018 g). δ_H (500 MHz, CD_3OD): 1.34 (d, J = 7.2 Hz, 6H, $CH(C\underline{H_3})_2$), 2.84 (m, 1H, $C\underline{H}(CH_3)_2$), 3.39 (d, J = 6.5 Hz, 2H, $C\underline{H_2}CH=CH_2$), 3.80 (s, 3H, OCH_3), 5.09-5.14 (m, 2H, $CH_2CH=C\underline{H_2}$), 5.93-6.02 (m, 1H, $CH_2C\underline{H}=CH_2$), 6.77 (d, J = 7.8 Hz, 1H, ArH), 6.79 (s, 1H, ArH), 6.94 (d, J = 7.8 Hz, ArH). δ_C (125 MHz, CD_3OD): 19.15 ($CH(\underline{C}H_3)_2$), 34.04 ($\underline{C}H(CH_3)_2$), 40.17 ($\underline{C}H_2CH=CH_2$), 55.89*

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(OCH_3), 112.81 (ArCH), 116.15 ($CH_2CH=\underline{C}H_2$), 120.73 (ArCH), 122.54 (ArCH), 137.22 ($CH_2\underline{C}H=CH_2$), 138.30 (ArC), 138.82 (ArC), 151.04 (ArC), 175.46 (C=O). MS (EI): m/z 234 (M, 8%), 164 (100), 147 (27), 131 (16), 108 (10), 91 (12), 71 (8), 51 (9). HR-ESI-MS $[M+H]^+$ m/z 235.2387, calculated for $C_{13}H_{17}O_3$, 235.2989.

B. Cytotoxicity assay

Breast cancer cells MCF-7 were harvested and seeded at a density 50000 cells/well in 96 well plate. The cells were incubated at 37°C in a fully humidified atmosphere of 5% CO_2 for 24 h. 4-Allyl-2-methoxyphenol derivatives, at six concentration, was added to the cells and incubated further for 24 h. each concentration was tested in triplicate. 100 µL of MTT (0.5 mg/mL) was added per well after incubation. The plates were incubated for an additional 4 h to yield formazan. 5% of SDS in 0.1 N HCl was added, and the plate was covered by aluminium foil, incubated overnight at room temperature in dark room, and read at 570 nm by an ELISA reader. The cytotoxic effect was determined by calculating the absorbance of test resultd as a % of the control wells [12].

RESULT

A. Synthesis of 4-allyl-2-methoxyphenol derivatives

Synthesis of 4-allyl-2-methoxyphenol derivatives the Yamaguchi method was done in two stages. The first stage involved the reaction of carboxylic acid with triethylamine in dichloromethane at room temperature to produce triethylammonium propionate was reacted with 2,4,6-tricholobenzoil chloride produces mixed anhydride. The second stage of the reaction 4-allyl-2-methoxyphenol as nucleophiles with mixed anhydride and 4-dimethylaminopyridine to produce 4-allyl-2-methoxyphenyl propionate, 4-allyl-2-methoxyphenyl butanoate and 4-allyl-2-methoxyphenyl isobutanoate with good yield results is 80-90%.

B. Cytotoxicity assay

Cytotoxicity assay performed with MTT assay, to see the activity of 4-Allyl-2-methoxyphenol derivatives against human breast cancer cells MCF-7 in vitro. Breast cancer cells were given treatment varying concentrations of 4-Allyl-2-methoxyphenol derivatives (6.25, 12.5, 25, 50, 100, 200 µg/mL). From the results obtained that the 4-Allyl-2-methoxyphenyl propionate, 4-Allyl-2-methoxyphenyl butanoate and 4-Allyl-2-methoxyphenyl isobutanoate able to inhibit the growth of human breast cancer cells MCF-7. The IC_{50} were 0.400 µg/mL, 5.73 µg/mL and 1.29 µg/mL for MCF-7, respectively. This indicates that 4-allyl-2-methoxyphenol derivatives has cytotoxicity against human breast cancer cells MCF-7. 4-allyl-2-methoxyphenol derivatives has the best activity better than 4-allyl-2-methoxyphenol (IC50 1.5 µM) [1].

CONCLUSION

4-allyl-2-methoxyphenol derivatives was synthesized through the application of methods of Yamaguchi in one pot with a resulting yield of 80-90%. 4-allyl-2-methoxyphenol derivatives showed good activity in inhibiting the growth of human breast cancer cell MCF-7. The IC_{50} were 0.400 µg/mL, 5.73 µg/mL and 1.29 µg/mL for MCF-7, respectively. The results showed that 4-allyl-2-methoxyphenol derivatives has the potential to be developed as an anti-breast cancer.

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